AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION

A. PRIMARY: Agriculture — AF30-0120-0000

B. SECONDARY: Cereal Crops

2. TITLE AND SUBTITLE

High quality protein maize; proceedings

3. AUTHOR(S)

(101) CIMMYT-Purdue Int. Sym. on Protein Quality in Maize, El Batan, Mex., 1972

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1975	533 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Agronomy Department, Purdue University, LaFayette, Indiana 47967

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

Published by Dowden, Hutchinson, & Ross, Inc., 523 Sarah Street, Stroudsburg, Pennsylvania 18260

9. ABSTRACT

This volume brings together the papers presented at the International Maize and Wheat Improvement Center (CIMMYT)-Purdue Symposium on protein quality in Maize held at El Batan, Mexico in 1972. An overview of some of the world's most critical food needs is described, along with strategies for developing more nutritive cereals and making them available to undernourished populations. Emphasis has been placed on maize as a source of protein and it is considered from breeding, production, chemical, biological, social, and economic viewpoints. Other topics discussed are: progress in breeding high quality protein in other cereals; breeding, chemical, biological, and analytical techniques, the introduction of improved varieties at the farm level, and an overview of world food needs.

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-AAB-534	
12. DESCRIPTORS	13. PROJECT NUMBER
	14. CONTRACT NUMBER: CSD-2809 Res.
	15. TYPE OF DOCUMENT

AID 890-1 (4-74)

HIGH-QUALITY PROTEIN MAIZE

Dowden, Hutchinson & Ross, Inc.
STROUDSBURG, PENNSYLVANIA

Distributed by
HALSTED PRESS A division of John Wiley & Sons, Inc.

Library of Congress Catalog Card Number: 74-23463
ISBN: 0-470-15800-X

77 76 75 1 2 3 4 5

Manufactured in the United States of America.

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA

CIMMYT-Purdue International Symposium on Protein
Quality in Maize, El Batán, Mexico, 1972.
High-quality protein maize.

Includes index.
1. Maize breeding--Congresses. 2. Maize--Congresses. 3. Proteins--Congresses. 4. Grain breeding--Congresses. I. International Maize and Wheat Improvement Center. II. Purdue University, Lafayette, Ind. III. Title.
SB191.M2C18 1972 633'.15'3 74-23463
ISBN 0-470-15800-X

Exclusive distributor: Halsted Press,
A Division of John Wiley & Sons, Inc.

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HIGH-QUALITY PROTEIN MAIZE

PREFACE

The first symposium on high-quality protein maize was held at Purdue University in June 1966. It was primarily a national conference with only two of the 25 speakers from outside the United States. The majority of the research papers on opaque-2 maize were contributed by the Purdue staff. Research findings were limited, since the discovery of the effect of the opaque-2 gene had been reported by our group only two years earlier.

The need for another symposium on high-quality protein maize was apparent early in 1972 at a joint planning conference held in El Batán, Mexico, by the maize research groups at Purdue and CIMMYT. Fortunately, strong financial support for the maize program at Purdue had been initiated in 1970 by the U.S. Agency for International Development[1] (AID) and at CIMMYT by the United Nations Development Program.[2] AID agreed to provide an additional grant to cover part of the conference expenses at CIMMYT.[3]

The CIMMYT–Purdue symposium was truly international, with less than one-half of the papers contributed from the United States. The reports indicate that opaque-2 maize is now a part of agricultural research on every continent, and that plant breeders worldwide are attempting to improve the protein quality not only of maize, but of other cereals as well.

[1]U.S. Agency for International Development, Contract 2809, "Inheritance and Improvement of Protein Quality and Content in Maize," with Purdue University.

[2]United Nations Development Program (UNDP), Global 1 Contract on Maize Protein Quality.

[3]U.S. Agency for International Development, Grant AID/CM/ta-G-73-2 to Purdue University.

All agricultural scientists agree that more efficient use of the earth's land resources is required, if world food production is to keep pace with world population. One way to use the land more efficiently is to replace present cereal grains with grains of higher protein quality. The use of a high-quality protein cereal such as opaque-2 maize in the diet of humans provides protein previously available only from a good mixture of normal cereals and legumes, or from meat, milk, and eggs. The latter three require much larger areas of land per calorie of food, and legumes are usually lower-yielding than the cereal grains.

If the scientists gathered at this symposium can develop for the world's farmers acceptable varieties of high-quality protein cereals such as opaque-2 maize, they will make a substantial contribution to the global production of good-quality protein in a form ideal for human consumption.

E. T. MERTZ

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INTRODUCTION TO SYMPOSIUM

The *CIMMYT–Purdue International Symposium on Protein Quality in Maize* brought together over 150 scientists from every continent, each equipped with his latest research findings and a great deal of tenacity and foresight. Their purpose: to discuss the progress and plan strategy for future development of high-quality protein maize.

The symposium participants gathered at the International Center for the Improvement of Maize and Wheat (CIMMYT), December 4–8, 1972, at El Batán, Mexico. The meeting came less than a decade after the discovery of the enhanced quality of opaque-2 maize by Mertz, Bates, and Nelson (at Purdue), a discovery that has fired the imagination of maize breeders, biochemists, nutritionists, and others intent on bettering nutritive status of foods for the world's undernourished families. The Purdue findings signaled a breakthrough in maize breeder's efforts to develop a high-lysine maize, and were thus a major advance toward higher protein quality.

Since the early part of the twentieth century, scientists have sought to breed a more nutritive maize. Although cereals supply over 70% of mankind's protein, they are lacking in protein quantity and quality as a food. In terms of nutritive value, maize's most serious deficiency is the low content of tryptophan and lysine—essential amino acids that man and other single-stomach animals do not synthesize and must obtain from their food.

The Purdue investigations showed that a remarkable gene, opaque-2, could approximately double the kernel's lysine content when introduced

into a normal strain of maize. A similar capacity was discovered shortly afterward for the floury-2 gene.

The new maize was tested by scientists throughout the world, but early results were discouraging; there were problems of reduced yield and unattractive and lusterless grain. However, some scientists persevered, mainly those represented at the symposium. And, more recently, financial support from international and national funding agencies has stimulated a renewal of interest and a quickening of the research pace.

Progress over the past 3 or 4 years has been outstanding. We now know that there need be no reduction in yield, we know that normal crystalline grains of high nutritive quality are attainable, and we have examples for proof.

An outstanding contribution to the universal availability of this unique nutritious food source has been the conversion of a large number of genetically diverse and variable maize populations to a high-quality protein form. Selection in many environments, for a wide range of characteristics, is providing the germ-plasm base for the development of quality protein varieties and hybrids for worldwide use.

To document some of this recent work, these proceedings have been organized in much the same pattern as the symposium — to provide a detailed look at (1) the latest breeding efforts and nutritional studies on high-quality protein maize, (2) commercial production aspects of this maize, (3) chemical and biological evaluations of maize protein quality, (4) economic implications of high-quality protein, (5) progress in the breeding of high-quality protein in other cereals, and (6) results of the symposium, workshops (breeding methodology, chemical and biological analytical techniques, methods of introducing improved protein varieties at the farm level, and economic and social factors in the acceptance of improved protein varieties). An opening section provides an overview of world food needs as presented by A. M. Altschul and R. G. Anderson. Questions and answers were obtained following each presentation, and some pertinent reports that were not available at the symposium are also presented.

One central aim of the symposium has been to present new dimensions that have evolved in the research on quality protein in maize. Whereas earlier research reflected only reactions in inbred lines and hybrids, it is hoped that these proceedings will show the results of more recent emphasis, such as the valuable contribution of populations with greater genetic variability.

The symposium reports have been edited here for consistency of style, and some have been slightly reorganized and condensed. However, every effort has been made to retain each author's emphasis and philosophy.

Part I

WORLDWIDE NEEDS FOR QUALITY PROTEIN

Aaron M. Altschul *Georgetown University School of Medicine, Washington, D.C.*

Numerous attempts have been made to quantify the disparity between protein supply and *human need.* In a sense, this paper is an inquiry into the many meanings of the word *need* as it has been related to protein in food.

Most quantifiers, particularly those dealing with less-developed countries, assume a *physiological need* based on the best available medical and nutritional consensus, with the protein supply then related to such requirements. These calculations and projections therefore reflect physiological need and projections of population size.

Another approach, suitable for populations enjoying per capita economic growth, is to estimate the demand for more protein foods generated by higher income. Such a *demand* is not necessarily related to *physiological need.* Actually, the affluent countries include more protein in the diet than is considered necessary to meet physiological need. Such drives or demands are not nutrition-based, but seek to satisfy sensory desires for meat and other animal-protein products.

This essay is concerned with models of protein need as related to political support, reflecting my previous interest in models of protein need that constitute a political imperative and generate political support (1). First, however, I shall present one example of the classical approach used to quantify the disparity between protein supply and need. Other models will represent cases that either do — or might — generate political support, including examples of (1) the increased need for meat generated by higher

income, (2) the political liability resulting from failure to maintain the protein–calorie ratio at the existing level, and (3) the opportunities for improving protein supply that arise from new technologies, plus the political advantage of maximum utilization of new technologies.

CLASSICAL CALCULATION OF PROTEIN NEED

Perhaps one of the best analyses of the protein problem is by Autret (2). It follows the pattern of the various analyses reported by the Food and Agriculture Organization of the United Nations (FAO) (5). First, assumptions are made of physiological requirements; then supplies are calculated to meet either (1) the requirements of 50% of the population, (2) the requirements of 97.5% of the population, or (3) the need to supply 10 to 12% of the calories as protein. Adjustments are made in the definition of protein requirements to take into account the quality of protein in a national diet. For the latter two requirements there is now a deficit of protein in the less-developed countries.

If we consider population growth and demands due to economic gain, India, for example, would require 60 and 95% more protein by 1975 and 1985, respectively. Data for the developed and less-developed regions of the world are given in Tables 1 to 3. For the less-developed regions, 80 million tons of *additional* protein will be required by 1985 to meet the physiological needs of 97.5% of the anticipated population; this increase alone is almost double the present supply (Table 3). Additional modest increases would be required to support anticipated needs generated by economic gains.

Present trends in agricultural production permit little optimism that these increased goals can be reached. The major stress will be on efforts to maintain adequate total food supplies, hopefully mostly as grain. These

TABLE 1 Total protein requirements and supplies in 1963-1965. (million metric tons per annum)

	World	Less-developed regions	Developed regions
Supplies	80	48	32
Requirements			
1[a]	89	56	33
2[b]	83	50	33
Deficit	9	8	1
U.N. population 1965 (millions)	3,289	2,251	1,038

Source: References 2, 5.

[a] Requirements for balanced diet providing ratio of calories from proteins/total calories >10%.

[b] Practical requirements = average (retail level) + 20% (practical allowance) considered adequate to cover 97.5% population.

TABLE 2 Total protein supplies compared to animal-protein supplies. (million metric tons per annum)

	World	Less-developed regions	Developed regions
Total protein supplies	80	48	32
Animal-protein supplies	25	10	15
U.N. population 1965 (millions)	3,289	2,251	1,038

Source: References 2, 5.

efforts will compete for land now planted in legumes and reduce the supply of this protein-rich source.

The excesses of grain upon which animal production in developed countries is based are not available in the less-developed countries. There is no conventional way, therefore, by which the protein deficit can be made up. Nor is any serious effort being made toward producing unconventional sources of protein in any of the major less-developed countries. They still seek the conventional goal of more legumes or more animal protein, each is more difficult to achieve at the present and at anticipated levels of resources as related to population size.

In theory, considering the surfeit of protein in most developed countries and the deficit in the less-developed countries, the total world supply presents a much better picture of protein supplies as related to total world protein needs. But the required redistribution of protein supplies to achieve a better balance seems no more likely to occur than any other major redistribution of wealth. Actually, the pressures could be in the opposite direction: as affluent nations seek to increase their animal protein consumption commensurate with improved income, they will compete with less-developed countries for shrinking grain supplies. And this force may constitute the most demanding pressure for more high-quality protein. This issue will be elaborated in the next section.

TABLE 3 Projected increase in protein production required by 1985.[a] (million metric tons per annum)

Protein production required by 1985	World	Less-developed regions	Developed regions
Increase	120	80	40
Total (present supplies + increased production)	200	128	72
U.N. population 1985 (millions) (medium variant)	4,933	3,658	1,275

Source: References 2, 5.

[a] Practical requirements for 97.5% of population.

CONDITIONS UNDER WHICH NEED FOR PROTEIN CLEARLY GENERATES POLITICAL SUPPORT

Increased consumption of meat is a sure sign of improved income. In the United States, which had a high animal protein intake at the beginning of this century, animal protein consumption increased from half the total protein in 1910 to two thirds in 1970. In the 22 years since 1950, annual per capita consumption of meat in the United States increased from 66 to 86 kg, poultry more than doubled, and fish consumption remained almost constant. The yearly sum total consumption of meat, poultry, and fish increased from 82 to 115 kg/capita in that 22-year period.

In the U.S.S.R. meat production rose from 10.7 to 12.3 million tons from 1966 to 1970, and is targeted to reach 16 million tons in 1975. Milk production rose in that period from 76 to 82.9 million tons, with a planned 100 million tons in 1975.

Japan's meat consumption is increasing drastically, also. And so it goes, wherever incomes are increasing. This point is shown clearly in a paper by Pérrisse et al. (4).

The Russian government intends to increase annual supplies of milk and meat protein in the 5-year period from 1970 to 1975 by 1.2 million tons of high quality protein. Let us suppose that the diet of the Russian people, who now have available 36 g/capita/day of animal protein, were to achieve the protein consumption features of the American diet with over 3,000 kcal and 70 g of animal protein/capita/day. Then it can be shown that the U.S.S.R. would require an additional 3 million metric tons of animal protein for its

TABLE 4 Animal protein supplies and calculated increases in production required if standards were raised

	(1) Animal protein content of food supply (1968)		(2)	(3) Increased production required if standards were raised (million metric tons per annum)	(4) Total production columns (1B) + (3) (million metric tons per annum)
	(A) g/capita/day	(B) metric tons per annum	Population 1971 (millions)		
U.S.A.	69.6	9.5	208		
U.S.S.R.	35.8	9.2	244	U.S.S.R. to U.S.A., 3	6.2
Japan	29.5	1.1	104	Japan to U.S.S.R., 0.2	1.3
Italy	57.8	0.7	55		
Mexico	15.1	0.3	51	Mexico to Italy, 0.4	0.7

present population of 244 million. The U.S.S.R.'s present goals for 1975 probably are one half the 3 million metric tons. The level of 3 million tons may seem somewhat far-fetched, however, any other goal can be taken—the principle is the same. The Russian example, and others, are summarized in Table 4.

Achieving such goals is becoming increasingly more expensive. If new technologies are introduced into the picture, however, the situation could change drastically. For example, it has been estimated that by 1980 textured vegetable protein from soybeans will replace from 10 to 20% of the meat in the diet in the United States. This amounts to a reduction in the cost of meat to the American public. Perhaps even more significantly, it puts part of the cost on nonagriculture sectors, the manufacturing sector. Therefore, it constitutes a further reduction in demand on agricultural resources than a comparison of costs of meat and meat analogs would show.

The cost of animal protein can be put into terms of total equivalent plant calories required to feed humans and the animals that supply the protein. An example of such calculations is shown in Table 5. The actual available calories and the equivalent plant calories do not differ greatly in the less-developed countries, thus the caloric cost is comparatively lower. However, the total caloric cost of feeding an individual in the United States is much higher, requiring 11,000 total equivalent grain calories compared to 3,000 calories actually available to be eaten.

Increases in animal protein intake within a country will not uniformly benefit all inhabitants. In any given country, distribution of animal protein and total caloric consumption are based on income. The nature of the distribution will vary from country to country. As the wealth of a country

TABLE 5 Grain caloric equivalents estimated as required to supply caloric intake and level of animal protein consumption for selected countries

		Per capita per day		
Country	Year	Animal protein (g)	Calories	Grain equivalent calories
U.S.A.	1967-1968	69.6	3,240	11,040
Sweden	1968-1969	54.1	2,800	8,960
Italy	1968-1969	37.8	2,960	7,180
U.S.S.R.	1966	35.8	3,150	7,170
Japan	1968	29.5	2,160	5,760
Mexico	1967	15.1	2,600	4,300
Philippines	1966	14.9	2,010	4,250
Tunisia	1966	10.7	2,190	3,990
India	1966	6.1	1,900	2,580

Source: Reference 6.

Note: Calculations are based on assumptions given in Reference 7; animal protein is converted to grain equivalent by using the ratio 28:1.

increases, the demand for higher-quality food will increase. There will be some improvement in the diets of the poor, but not as much as the figures would seem to indicate. Major improvements in protein consumption are likely to occur among those people with increased incomes who are better able to meet their desires for more high-quality protein.

CONDITIONS UNDER WHICH NEED FOR PROTEIN MIGHT GENERATE POLITICAL SUPPORT

Previous examples have dealt with problems of increased income and a need "driven by the desire for higher aesthetics in protein." But what about countries where there is a deficiency in protein? The clearest case where protein need could probably be transferred to political action is when the food situation becomes worse. Certainly, there is no question that failure to satisfy minimum requirements for food will generate social instability. Even steep increases in the price of bread in countries where bread is the major food source can stimulate social unrest. No government can survive when there is chronic widespread starvation.

Consider the case where grain supplies have increased and are relatively adequate for a population because of improved technology and the expansion of resources at the service of agriculture. This is the case where the Green Revolution has been successful. However, there can be circumstances where the price paid for the Green Revolution could be a reduction in the acreage reserved to protein concentrates. This seems to be the situation in India, where the acreage of wheat has increased and the acreage of pulses, the main source of protein concentrate, has decreased. The FAO estimates that the percentage of calories from protein will decrease through 1980 in Asia and Central America (Table 6).

Regardless of how one argues about the adequacy of the protein content of the diets in these areas, the fact that a major element of the quality of the diet is decreasing could constitute a political issue and arouse action to try to rectify it.

One could calculate that the additional protein required to maintain the protein–calorie ratio at the 1972 level, assuming a constant calorie availability, will be 1.6 million metric tons/year. This represents more expensive protein than that in the normal diet: from fortified or specially bred grains, from legumes, from oil seed, from fish or microorganisms, or from animal protein. The amount of additional protein in this calculated deficit represents a substitution of 2% of the calories as more legumes, or 2.3% of the calories as more meat. But this is a serious strain on an already strained dietary. And since distribution of food in the family is not necessarily related to need, a modest reduction such as this will be reflected first in poorer nutrition of the vulnerable groups — infants, children, and pregnant and nursing women.

TABLE 6 Trend of the protein-calorie ratio relative to projected supplies (%)

	1962	1975	1985
Zone C	10.6	10.4	10.3
South America	10.6	10.5	10.6
Central America	10.2	9.9	9.7
Africa south of the Sahara	10.9	10.8	10.9
North Africa	11.3	11.3	11.2
Near East	12.1	12.0	12.0
Asia	10.3	10.1	9.9

Source: Reference 5, p. 510.

PROPERTIES OF THESE MODELS

The models discussed can well cover all the population classes. The first group of models, dealing with those whose incomes are increasing, includes two types of demands on protein: increased income and increased population. The second group deals primarily with demands of increased population. The demands based on increased income in the second group are minor compared to the demands based on increased population.

In all these considerations it is quite clear that one cannot treat the calorie problem and the protein problem as independent problems. Just as in the case of nutritional consideration, it is more proper to utilize *protein–calorie malnutrition* as the umbrella to define the various ranges of nutritional problems encompassed by this term. In an economic and resource sense the two problems are indistinguishable.

Each nation, if it has the choice, can trade off protein and calories. It can insist on a high-meat proportion in its diet and thereby consume, in effect, a higher proportion of calories. Or it can subsist on a smaller meat proportion and consume less plant-equivalent calories. Or it can develop new technological models, such as analogs of meat, and thereby consume less equivalent plant calories.

A nation on the borderline of having adequate calories can decide to shift to an agricultural practice that maximizes the caloric production per acre—at the expense of crops that contain more protein, but produce less calories per acre. In this instance, caloric sufficiency, or at least caloric intake, is maintained, but at the expense of protein intake. Or the nation can decide to emphasize new technologies to improve the protein quality of the grain. And there are many variants in between.

Now let us consider the chances of improving the protein quality of a diet by some means faster than increasing income. I already have shown that the protein content or quality of the diet improves concurrently with increased income. When a population starts at a low income, improvement requires a

high income increment. François (3) has shown in an elegant way that a laissez-faire approach depending entirely on increased income would delay improvement in the nutritional value of diets several generations, or might delay it altogether. Hence, those who have been interested in improving the nutrition of a population have striven to develop a public policy that would give nutrition a higher priority than general development.

And good arguments can be made for such decisions. The best case can be made for infants and preschool children. Even in India, where there is considerable controversy at the moment as to whether the problem is calories or protein, there is general admission that present protein intake for children is insufficient.

I shall mention briefly some arguments favoring emphasis on infants and children. Because they are in an active state of growth, their needs for nutrients are more demanding. Failure to meet nutritional requirements means impaired growth and lower resistance to disease, and is reflected in high infant mortality. Worse yet is the accumulating evidence that infant malnutrition may result in permanent mental impairment and hence aggravate the problems of poverty. It is not only the children who are involved; data are appearing which indicate that the nutrition of the mother affects the status of the infant. Certainly, low birth weight constitutes a high risk to the infant's chances for survival and normal development.

Preschool children in poor countries have less to eat, and the percentage of protein is less. In published reports from South India, Thailand, and Guatemala, the percentage of protein calories ranges from 7.4 to 11%. In the United States among poverty groups the percentage of protein calories is as low as 12.8% for a rural, low socioeconomic group. But generally, all children, poor and nonpoor alike, eat a diet ranging from 14.5 to 16.1% protein calories. The difference in protein content also reflects a difference in quality, because higher-quality protein is required to bring the protein–calorie percentage of 14%. Hence, the difference in protein impact between the rich and poor countries is all the greater. The dietary standards in the United Kingdom, for example, require that 14% of the calories be protein for infants, children, and pregnant and nursing women, and 10% for all others. Some among the poor infants in the United States have less than the required protein calories. But, clearly, most infants in the lower socioeconomic groups in the less developed countries mentioned have less than the British standard of protein. Increasing the total amount of food will help, but it will not change the protein picture.

Is this argument sufficient to generate political support? In the rhetoric, perhaps. And here and there, perhaps, some faltering steps. But in a world of contracting resources, the nutrition argument will probably not be sufficient to generate political support.

MODELS BASED ON IMPROVED TECHNOLOGY

The availability or invention of a new technology will generate political action to improve the protein quality of the diet of children, or of an entire population, and more rapidly than will occur through increased income alone. In this instance, the increments of increased protein content are determined more by the technology than by the perceived need.

Institutional Technology

When the infrastructure of a country reaches a point where the potential for utilizing certain delivery systems is achieved, one can consider using this institutional technology as a means of delivering extra protein. Hence, as a specific political function, it is very proper to undertake to provide school lunches when a significant proportion of the population is in school. One could calculate the extra protein needed if all the children in school were to receive a lunch. The same calculations could be made for day-care centers and for maternal and child health centers. The limiting factors on the amount of extra protein that can be given are the delivery system and the resources.

New Protein Technology

New protein technology is also a generator of new opportunities for providing protein and can be a basis for a political decision to improve nutrition faster than increased income could. The invention of an infant food that is equal in nutritional value to milk, but costs less and is attractive and accepted, could generate increased consumption of that product by infants. The government could support such a venture at far less cost than improving diets by conventional means.

The discovery and development of high-quality protein maize is obviously a major generator of thoughts about improving nutrition. One can calculate the additional protein that would be added to the diet of a country like Colombia, if all the maize grown were high-quality protein; this would be a significant increment to the protein supply of the country. The chances of doing this would depend on the cost of producing this kind of maize as compared to normal maize and, therefore, the cost to the government for encouraging this approach.

A similar approach involves the possibility of increasing the protein supply in countries where most of the calories are obtained from a single major staple, such as wheat, maize, or rice, wherein the staple is improved and its protein impact increased by proper fortification with amino acids. Here

again calculations could be made of the increased protein supply by this technological modification.

One disadvantage of fortification or genetic improvement could be that it improves the supply of food for the entire population, even for those who presumably are not in need of additional protein. This may not really be a disadvantage, since it may be cheaper to do this than to reach a specific target population.

These considerations point out the importance of field trials of the various alternative approaches to increased protein supply, especially when the improvement is not visible, as is true for all fortification or genetic procedures. There is need to develop concrete evidence that the improved nutrition is truly reaching the population and constitutes the least-cost approach.

An alternative generator of increased protein supply is an increased trend toward the processing of major foods. For example, more and more bread is becoming available. Prepared tortilla mix is becoming increasingly available.

People are not relying exclusively on one cereal, but are reducing the proportion of each cereal and increasing the number of cereals in their diet. In certain parts of South America, for example, wheat as bread is invading what was exclusively a maize-eating area. Introducing convenience foods provides another opportunity for a government to consider adding protein to the diet in the form of fortifying the bread or the tortilla mix with the necessary ingredients to improve its impact.

Combination of New Protein Technologies and Institutional Technologies

Perhaps the best generator of opportunities for introducing protein into the diet, faster than economic development, is to combine a new protein technology with institutional technology.

It is one matter to agree to furnish an additional glass of milk to everyone under 15 years of age, let us say, as has been suggested or attempted in Chile. But here the use of distributional technology is limited by the cost, in this instance in terms of foreign exchange, of the nutrient. It would be far more reasonable to combine a distribution technology with new protein technologies. One that comes to mind is that of introducing CSM or Incaparina in institutional programs, particularly in schools and in infant and maternal health-care centers.

Modifying the rules governing nutrients in the U.S. school lunch program has permitted the introduction of textured soybean, thereby reducing the cost of the lunch to the school authorities. This is another example of a combination of an institutional technology (the school lunch) and a new protein technology (textured vegetable protein).

If governmental efforts to introduce more protein into the diets of children were based on a product like Duryea (now being sold privately in

Colombia), this surely would (1) cost less and (2) provide a more substantial opportunity for developing milk analogs, than would conventional food items.

Applications to Affluent Countries

The combinations of new protein technologies with and without institutional technologies are not limited in their applications to less-developed countries. They have equal application to affluent countries, and, indeed, in many instances are progressing faster in those countries. Wherever food is eaten, cost is still a factor. If it is possible to maintain a level of meat-type foods in the diet, or even increase it, with less increase in meat production and, hence, less increase in cost, the economic and political benefits will be enormous.

The concept of convenience, which is a contribution of new food technologies and institutions, can benefit special groups. For example, the elderly and the poor probably need convenience more than the affluent. Ordinarily, convenience foods have been designed without any particular emphasis on nutrition. But this need not be so.

A new force, not yet appreciated but being looked at seriously, is the role of new protein technologies in providing more options for changing nutrition based on medical needs. For example, if a person has a desire to increase his meat consumption, he also will increase his consumption of a saturated animal fat, total fat, and cholesterol. Now, however, food technology makes it possible to separate the concept of protein aesthetics from the other nutrients that tag along with aesthetic protein foods, such as the saturated fats and cholesterol. Hence, by the new technologies it is possible to provide greater options to the people who want to retain the aesthetics and yet would like to modify the intake of other constituents. This concept is certainly not yet a political force, but it is tempering the opposition to changes introduced by fabricated foods, because of the possible medical – nutritional benefits that could be gained.

SUMMARY AND COMMENTS

1. Protein supplies will increase more slowly than energy supplies. It is more expensive to synthesize protein than calories, and animal proteins are the most expensive of all. I do not consider shortages of vitamins and minerals in the same category; these can come from nonagricultural sources.

2. The competition for protein will intensify between the *haves*, who want more animal protein, and the *have-nots*, who need to conserve their present, albeit meager, supplies of energy and protein.

3. There is always the desire among some planners to return to simple solutions — solutions that maintained an ecological balance at much lower

population levels. This is a romanticism coupled with nostalgia for the "good old times." The Green Revolution represents the application of the most sophisticated and advanced agricultural methodology to solve the basic food problems of less-developed countries. Further advances in the efficiency of producing energy will require engineering a basic change in the photosynthetic process itself. To combine increased yields of energy with higher protein impact is the most difficult and basic biological challenge of all.

4. The greatest chance of redressing the balance between protein and calories lies in the adoption of new technologies. Such new technologies can lessen the competition between *haves* and *have-nots*. Where there is some general economic improvement, these technologies can act to improve nutrition faster than general development. Complete reliance on agriculture will not suffice. Pure agriculture, that is, an agriculture in ecological balance with renewable energy sources, disappeared 100 years ago. Now, a proper balance between agricultural and industrial technologies will do better than either alone.

5. Political support of new technologies to transform a need for more protein into a practical social response will require demonstrations to the public of the value of any new technology. Field trials become more than a scientific experiment; they are part of the social apparatus leading to action.

6. The world must reassess the balance between population and resources. Otherwise, the only outcome, whatever the technology, is disaster.

7. As is true in many other instances, an investigation into the need for high-quality protein becomes basically an investigation into the fundamental nature of social concerns, into aspects of poverty and wealth.

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MEETING WORLD FOOD NEEDS

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Man's search for adequate food has been a principal endeavor for centuries. In the beginning, he lived literally from hand to mouth, and his very existence as a species was precarious indeed. However, the invention of agriculture about 9,000 years ago enabled man to settle, to cease his wanderings, and thus to develop the first awakenings of civilization.

Today, in the supermarket abundance of the developed world, the urban public often fails to link their well laden tables with the fields and farmers who grow this food. The greatest danger of food shortage in such an affluent society lies in the failure of systems to deliver the food or the failure of the economic system to deliver the food to those in want.

Such a comfortable picture is not reflected in many developing countries. Privation has been a constant specter for years in many of these countries and remains today the outstanding critical problem. Isolated bright spots have appeared in agricultural progress, which collectively have been referred to as the Green Revolution. Although these advances have set aside a disaster that might now have been upon us, they are a temporary palliative to the problem. We have to recognize that the Green Revolution must spread to many countries. And even then, at best, this contribution is temporary and leaves us no time to rest.

Before turning to the question of how to meet world demands for food, I should like to philosophize about the origins of these problems. How did it happen that large areas of the world failed to move ahead as others did? And

why is change so late in coming? Many theories have been presented to account for this lack of development: politics, poor educational systems, and so forth. In the lowland tropical areas of the world, however, it is probable that some other factors also were involved.

TWO WORLDS

In temperate regions only one crop can be grown in the course of a year. The crop must be husbanded and stored for use during winter months. Thus, in these areas, from very early times, there was a need to ensure that the crop was saved. Furthermore, there was a real pressure to ensure that the supply was adequate, so that life would persist over these nonproducing periods. This very necessary preoccupation with obtaining sufficient food would account for the relatively late appearance of civilized societies in these regions.

In the tropics, on the other hand, crops could be harvested year-round. Pressures to grow and store surplus were largely removed. Furthermore, the market to absorb excess production was limited under such conditions. Because of a lesser need for clothing and housing, the sale of products to supply these needs was not nearly as essential as in the less endowed temperate areas. We must not forget that it is more difficult to store food in the tropics also. The need to produce beyond immediate needs, therefore, was less urgent in these societies. The situation was aggravated further by the usual tenure of land system whereby the landlord received most of the increased production. I do not mean to infer that life was easy — for life was hard — but there seemed little chance for improvements under existing conditions. The preceding is undoubtedly an oversimplification of the whole problem, but, in my opinion, it is significant.

TWO TYPES OF EMERGING SOCIETIES

Thus, we find two types of societies emerging. Circumstances forced the temperate peoples to ensure an adequate food supply. They had to develop strong research and increase the efficiency of production. As this movement gained momentum, the newly developing efficiency was exploited to build industrialized societies. Today, only 4 to 5% of the U.S. population is directly involved with primary food production.

In the more tropical regions, where societies remained agrarian, research was given little support. Although food was inadequate in the nutritional sense, there was sufficient food in terms of the market's ability to absorb production. There ever were exports of food on a regular basis.

Following World War II, broad scale health improvements contributed to a dramatically lower death rate in most countries, leading to a marked increase in the average life expectancy. In many countries life expectancies

doubled, due to sharp decreases in infant mortality. Populations began to rise rapidly.

In most developing countries, governments have been unable to build a system of social security into their economics. Parents in their old age depend on children for care. In earlier days of widespread plagues, of 10 children born, perhaps 3 survived. Rapid improvements in disease control and prevention outpaced many countries' ability to adapt to the new situation; families have continued to be large. Each new set of married partners again needed their young for "social security," and populations soared. The immediate multiplier effect is obvious.

CRITICAL POPULATION LEVELS

It is difficult for most people to comprehend the truly frightening aspects of the gigantic wave of population about to engulf us. In India, it is estimated that births exceed deaths by something in excess of 1 million persons each month. Let us assume a monthly figure of 1 million, which represents a daily increase of over 33,000 persons. Let us further assume that a like number of persons die each day, which in a population of 560 million is quite feasible. Thus, we can calculate that over 70,000 Indian children are born each day. Consider this growth rate in terms of education. If we assume 35 children per classroom, the daily requirement is 2,000 classrooms and 2,000 teachers to provide even a minimal education to the children born each day in India.

Aside from education, think of the implications of providing health care, work opportunities, and even standing space in the long term. This is the situation in India, where the problem is now critical. Could even the richest government in the world hope to contend with this burden on its economy? With the present birth rates, other countries can anticipate a similar problem in a few years.

Thus, in any discussion of food needs for the world — with all of our philosophizing and crystal gazing in which we foresee unlimited expansion of food production through research—all seems insignificant in the face of this relentless population increase. Each of us is interested in food and nutrition. However, I say categorically that, unless methods are found quickly to stop this onslaught of people, our best combined efforts will be of no avail; we can wage only a rearguard action against hunger. In 28 years, at present rates, the population will double. Each new mouth will demand food. How are we to cope?

PLAN NEEDED

I have purposely painted a dismal picture, because we should suffer no illusions as to the severity of the situation — this problem *must* have the

highest priority. However, I am an optimist, basically, and I refuse to accept the position that we will be unable to survive as a species.

We *must* start with the assumption that we can control the population increase. Folding our hands and awaiting the inevitable without a fight is not what nature had in mind when it endowed man with the will to survive. Man's struggle upward from natural animal to the socially conscious animal that he is today demanded fiercely competitive battles.

Valiant efforts are being made to solve the population aspect of our dilemma, and very substantial sums of money are being invested in research on the subject. We, as agriculturalists, have our part to play. Our role is to buy a maximum of time for the population-limiting efforts.

What weapons do we have in our arsenal and what are the prospects? We now have the necessary technology to provide food for double the present population if we could assume (1) unlimited supplies of inputs, (2) noninterference by people who know nothing of agriculture (I refer here to the rabid environmentalist), and (3) a rapid and extensive adoption of this technology throughout present agricultural lands of the world. Since tillable land areas are not easily expanded without tremendous investment in rechanneling of water supplies, most of the food increase will have to come from existing lands. We must, therefore, concentrate our efforts toward raising genetic capacity for yield; stabilizing yields through disease, insect, and drought resistance; and maximizing yields through appropriate agronomic treatments. At this conference, we are considering nutrition. This factor is of utmost importance. We sometimes forget that the effect of raising nutritive value is to increase yield, since lesser quantities of food can deliver the essential proteins, calories, and vitamins.

DEFINITION OF QUALITY

Discussions at this conference prompt me to ask if we have defined clearly our requirements for quality. As a plant breeder, I suggest that we need an easily applied method which will show us what a particular line or family will produce in the way of growth. I am not specifically concerned with protein level, or value. I am concerned with the end product, with the sum of all characteristics for enhancing growth possessed by a line or family as it may compare with others.

It seems to me that the cereal should be tested for how it works as it is and for its value per unit consumed, rather than in a diet that has been modified to a certain protein level or fat or carbohydrate level. In those countries which we serve, balanced rations for humans or animals are unlikely for many years. Thus, it is the cereal itself we should test, not some artifact we produce.

In the developed countries the problem is much less urgent. Education is generally high, efficiency of food production is high, and technological

research is well developed if not always efficient in operation. In general, these countries can produce beyond their needs. For them, population increases have stabilized, and the social security aspect is being managed. I would predict that for most of these countries food supply will continue to keep pace with population increases until the end of the twentieth century, providing that plant protection measures are continued. These economies are generally strong and can afford the needed inputs.

MANAGING AGRICULTURAL PRODUCTION

Since World War II, efficient production in developed countries has been used to make up the food shortfall in many developing countries. On the one hand, critics say that this usage benefits the agricultural community of the developed countries. Perhaps this is so; nevertheless, this exportation represents a flow of treasure from the contributing countries and, in this light, a humanitarian gesture of a magnitude never equaled. Famine would have destroyed millions without this trade. However, gifts of this type or sales on a long-term low-interest loan basis can create an artificial condition. When such foods are made available, internal food supplies are likely to remain relatively stable. Thus, governments of importing nations are able to set aside the evil day when they might be forced to face the inevitable: that their own agriculture must be made profitable and self-sustaining. They forget that under food duress—where there is dependence on another nation or nations for supplies—governments are not fully masters in their own house.

INDIA AND PAKISTAN PRODUCTION EFFORTS

Some countries recognized the need for change earlier than others. I think it is fair to say that it was no accident that India and Pakistan were two of the first. The shortage of food supplies had passed the critical mark, and they were fast approaching the "ship-to-mouth" conditions that finally arrived in 1966–1967. A difficult and necessary decision was made by the governments of those two countries. As a beginning, they asked for and received the assistance of the Ford and Rockefeller Foundations, in Pakistan and India, respectively, to assist with their research program reorientation. They imported new seeds, created an atmosphere of national emergency, and backed this by effective government action. Many other agencies asisted in the production drive for maize, wheat, rice, and sorghum production. The combined efforts paid good dividends.

I do not wish to dwell on this aspect, since much has been written of the agricultural change that has come to be known as the Green Revolution. Although much of the writing is inaccurate, I do not intend to deal with such inaccuracies, since this would be the subject of a week's discussions. Certain

basic steps were taken in India and Pakistan, which can serve as a partial blueprint of the approaches that might be used profitably in stepping up food production in any country.

BLUEPRINT FOR FOOD PRODUCTION

What were these factors and what are the procedures? I will assume that varieties are available which are superior to existing cultivars, as was the case for some of the crops used in the India–Pakistan areas. The general outline of the situation in these countries and the remedial programs developed are as follows:

1. Both countries were in a crisis condition. Governments were perennially short of money and only crisis needs were met day by day. This factor affects not only the basic programs, but also the timing and planning of the component parts within a program. Thus, production of agricultural products was the chief concern of government and received its backing. Without this *national* thrust, production programs will advance slowly, if at all.

2. Based on a new approach to research formulated by the maize program in India, the system of research in both countries was remodeled for other crops. Research thus became coordinated and crop-oriented at the national level, and not discipline-oriented as in the past. Crop teams operated under a national coordinated program. Unlike pilot projects, they had the full force of government action behind their effort, since decisions were applied throughout the country. Research was designed to give first priority to studies of disease-resistant varieties and agronomic practices. Other quality variables and disease and insect surveillance were integrated into the picture later. The evolution from an early focus of effort on quantity to a later consideration of quality was a justified strategy in this crisis condition.

The importance of remodeling research to a crop-oriented coordinated program cannot be overstressed. Institutionalizing research within developing countries is a major need if crop production is to be self-sustaining and effective. I venture to say that a similar system in the developed countries would bring about greater efficiency in research.

But what about countries with few scientifically trained personnel? The same principle applies, but a somewhat longer building period is needed. This does not mean that production must await education. However, preliminary steps must be taken in national planning to set up the necessary educational system. This system should produce, as a minimum, sufficient numbers of young scientists at the Ingeniero or Bachelor's level to man the research teams. This training should receive top priority.

There are international opportunities for graduate work in other countries, and the brightest young men should be selected and so educated. However, it is not necessary to have Ph.D. to be an effective scientist, and the impression should not be created that this degree is a requisite for applied

scientific work. Funding agencies must realize that the extreme dearth of scientific manpower will push the first trained personnel quickly into administration positions. While these moves would seem to misuse the scientists receiving advanced education, the training process must be continued until the demands for scientists are fully met. It is very important, therefore, that the first people selected for training be the best available. If the scientist has no initiative, he will be a poor leader. If well motivated, he will encourage the young men within his administration.

3. The results of research must be translated to the farmer through demonstrations on his farm. I have no regard for the excuse that the farmer will not accept change because he lacks education. *Intelligence and education are not synonymous terms.* The peasant farmer is normally very intelligent concerning his farming practices, in spite of his inability to write. Were he not intelligent, he would not have persisted and might have died out as a subspecies.

My experience suggests that the first successful demonstrations must be made by the research personnel who know the varieties and the best package of agronomic practices. Furthermore, these workers must have convinced themselves that the system will work. This is no reflection on extension personnel; however, for the most part, extension workers simply know little about such demonstrations. Because of their own lack of confidence, extension workers often are unable to convince their client farmers of the value of new techniques. The extension workers usually have many duties to perform; in addition, they work under a system in which failure means losing one's position or authority. Maintaining the status quo, on the other hand, is the safe route, since no upward or downward change leaves the situation static and their position secure. As soon as these workers are shown the way, however, they too will become an effective educational arm.

As a teaching device, the farmer-to-farmer flow of information is often very underrated, yet it is one of the most important methods of extension. Time and again a farmer will tell you that he found out about a new idea from another farmer.

4. The government must ensure that the relationship between the cost of inputs and price of the product is in balance. The farmer must make a profit. Furthermore, if a particular crop is being fostered, he must be able to make more profit from that crop than can be made from a competing crop which might be of less value to the economy at that particular time. If rice brings more net profit than maize in the monsoon season in India, the farmer is not likely to grow maize. Similarly, if safflower and cotton bring more returns than does wheat in Sonora state in Mexico, wheat will not be grown. Priorities, therefore, must be set. Ideally, all crops should have the same body of research behind them, so that efficiency of production will be relatively equal for all. Unfortunately,

this is never the case, and price manipulations are the means most often used in the market to encourage change in production patterns. Market-price manipulation is a very delicate procedure and can throw the entire economy out of balance if care is not exercised in its application. A floor price must be set that provides the necessary incentive for production in the crop most needed, and it must be scrupulously defended by government, which must buy all grain of acceptable quality offered at this floor price. Failure to do so hurts the small farmer in particular, since he is unable to hold stocks for the later price rises expected in the preharvest market of the next year. He would have to sell at harvest when deliveries are high, and prices — without support — would plummet. The consumers' advantage comes from the greater price stability resulting from the use of government stocks to buffer the market.

5. As the new technology begins to take hold on a broad scale, the government must simultaneously endeavor to meet the input needs. Fertilizer supplies initially will have to be imported. But consider for a moment that 1 ton of fertilizer will produce 16 to 20 tons of grain if correctly used, or one shipload of fertilizer will produce 16 to 20 shiploads of grain. Think of the reduction in port handling and the increase of wealth within the country. As quickly as possible, fertilizer plants must be brought into domestic production. Such plants may be public or private, and their construction may be subsidized by government or not. In any case, prices must be controlled at a profitable level. Manufacture of agricultural chemicals, such as weedicides, fungicides, and insecticides, must be developed rapidly within the country. Where irrigation is possible, pumps, casings, and so on, must be available. Other agro-industries, such as machinery supply, will begin to develop as buying power is generated in the agricultural sector of the economy.

6. If the government is to intervene in the grain-handling market, marketing facilities are essential. Storage also is essential, but, consistent with the theme that financing is critical, it should not be expected that governments will build storage until production forces its construction. As any government administrator will tell you, it is impossible to find financing for new storage facilities when present facilities are empty. Such storage will entail some loss in the beginning, but these losses cannot be avoided.

7. As self-sufficiency is approached and adequate buffer stocks are produced, a downward adjustment in floor price can be expected and should be made if excessive surplus is to be avoided.

MECHANIZATION

I should like to comment briefly on mechnization. In my opinion, only certain activities should be mechanized in many countries having a large

rural population. Of course, the substitution of tractor for animal power will free land, formerly used to feed animals, for the production of human food. This change probably will operate most satisfactorily on a hire basis in a country of small farms. Mechanical seed drilling is important in ensuring stands. Poor stands are responsible for severe yield reductions in many countries. Harvesting can be left to hand labor in a plentiful labor market. Threshing, however, must be mechanized rapidly to release the power animals for field work. This becomes particularly critical in a multiple-cropping situation.

These points I have mentioned are important segments within the composite picture of raising production and providing the backstopping to ensure a continuation of research. I would venture that these suggestions will work in most situations and, with certain modifications, are universally applicable.

DIRECT FOOD PRODUCTION

Now let me return to the direct food question. Using the preceding and similar approaches, what gains have been made? I will deal chiefly with wheat, since I know this crop best, but the story is similar for other crops receiving research support.

In India, with government backing, new varieties and new technology for wheat, maize, and rice have shown remarkable increases in production in the past few years. Maize has risen from 4.1 million tons in 1961 to 7.4 million in 1971; milled rice from 34.6 million tons in 1961 to 42.4 million in 1971; and wheat, with a decade (1956–1966) average of 10.3 million tons, rose to an estimated 26.5 million in 1972. Pakistan raised wheat production from a 1966 average of about 4.0 million tons to about 7.2 million tons in 1970. Political dismemberment, war, and other factors have tended to keep Pakistan's production static at between 6.8 million and 7.2 million tons. Alleviation of these factors would ensure production increases to about 10 million tons. Rice and maize have also advanced rapidly in that country.

Algeria has moved in 3 years from an original 5,000 hectares (ha) of wheat with improved varieties and technology (1969–1970) to 140,000 ha (1970–1971) to 325,000 ha (1971–1972). This program is receiving tremendous government support. Self-sufficiency was reached this year after a series of deficit years. Tunisia also reached self-sufficiency in this crop season. Morocco has improved considerably and this year established a new record in the bread wheats. Similar, if less spectacular, results have been obtained with wheat or other crops in many Afro-Asian countries and certain of the Latin American countries.

However, tremendous potential remains for increasing yields of food crops in the tropics, particularly in Asia and Africa. In Latin America, in

addition to yield increase possibilities, there are still vast land areas to be exploited for agricultural production.

Tremendous advances in total production will come from suitable multiple-cropping rotations. In certain countries where land is limited, as many as five crops per year are taken by planting the succeeding crops between the rows of the previous crop near harvest. Without going to this extreme, several countries report the increased practice of harvesting three or four crops in successive plantings of suitable season crops and suitable varieties within crops. Wheat production on the lower Ganges in India is one example of the practice. West Bengal grew 100,000 acres of wheat for many years. In 1972 the figure was about 1.2 million acres. This increase was made possible by the introduction of an early maturing wheat, which could be sown after late-harvested paddy rice. The practice did not replace existing crops, but made land productive that had previously lain fallow in the winter.

Chemical and cultural control of weeds can add tremendously to yield in all agricultural lands. Good water management in the drylands, coupled with judicious use of fertilizer, can double present yields in virtually all such areas. Wider use of varieties with genetic drought resistance and disease and insect resistance will result in better production.

Introduction of animal–grain rotation through the use of legume forage crops in rotation with grain can lead to greater agricultural stability.

Salinized land in present irrigation projects must be drained without delay. Much of the water that now runs into the sea must be harnessed for agricultural production.

By means of such measures, the present acreage of agricultural lands in both hemispheres can be made to produce two times the present level of food, but we must apply known technology and continue to provide suitable varieties in the research programs. This will require a vast international marshaling of resources, and this is achievable if we have the will.

WARNING SIGNALS

On the other side of the coin, we are witnessing some very ominous signs. In our search for sustained availability of food we are not yet able to depend on full stability of production. So we must continue to overproduce and store for the lean years.

In recent months, the world stocks of food have been largely depleted. This is particularly true in the case of wheat. Yet only a few years ago the exporting countries were bemoaning these surpluses. Land area sown to some agricultural crops was allotted on a quota basis, and phenomena such as the land bank in the United States and later in Canada were introduced. These brakes on production were essential to their economies. It is a comment on our lack of organization as a species that, on the one

hand, we are restricting production, while on the other we are searching for means of increasing production. This economic factor of distribution, however, is not easily solved.

To return to the theme, the present reduction in stocks occurred because of tremendous winter wheat losses in the Soviet Union, leading to imports of about 18 million tons. Presumably, China's wheat crop also was damaged, since their imports have been large.

Viewed in this context, what if India, Pakistan, Afghanistan, Iran, Turkey, and North Africa also had suffered crop failures this past year? Obviously, supplies would not have been available to offset widespread famine.

I have recently received a letter from Dr. Borlaug indicating that in Brazil frosts came this year (1972) in late August and early September. Yields of wheat were cut by an estimated 50%. Continuous rains followed, and another 50% of the remaining crop was destroyed by *Septoria*. Borlaug said that Brazil will be lucky to harvest 800,000 tons of chicken feed from the 2.4 million ha sown. Paraguay is reported to have suffered a similar disaster, and Argentina will be affected at least in part. Brazil and Paraguay are likely to be in the international market to buy and further push the price upward.

In the summer of 1972, India suffered a substantial loss of food in the Kharif or monsoon crops. This loss has been estimated as high as 15 million tons. To meet this shortfall, India embarked on a crash program to sow all available land to wheat, hoping to increase production from the present 26.5 million to 33 million tons in this winter season. They are attempting to do this with about 30% less fertilizer than they should have. The failure of the monsoon also lowered water supplies for irrigation and hydroelectric power to operate well pumps.

From conversations here, I understand that a heavy rainfall fell over a wide area in December 1972, which will hopefully alleviate the situation to some degree. The only thing that staved off famine in 1972 was a buffer stock of wheat and rice, which had been planned for and accumulated in the spring. The 7 to 9 million tons of insurance is standing them in good stead during this crisis. Droughts from India to Lebanon will put pressures on all supplies.

These shortages work against the developing nations in another way. The increased demand has pushed prices from $1.40/bu to $2.15/bu on the international market; in import costs this represents a 54% increase over the previous price. In view of the impending shortages, it will be necessary for all exporting nations to produce at a higher than normal level during this season to ensure that widespread famine does not come in 1973.

From our viewpoint as research scientists, we must continue to explore all possibilities of achieving stability of yield. The widely adapted variety is

one control, and resistance to disease in its broadest sense must be enhanced. Better regulation of our water resources is essential both for irrigated and nonirrigated production. Recovery of salinized land through drainage and extension of irrigation potential must receive top priority.

In closing, I repeat that population increase is our greatest single problem as a species. Food is important; but if we are to ensure a world at peace, its people also must have opportunity. Failure means extinction. Territorial needs are essential to man, and destruction of nation by nation will ensue if population pressures become too great. I would predict that food needs are unlikely to be the immediate cause of widespread social strife. Lack of opportunity to earn, lack of schooling, and lack of equality are likely to force the issue earlier than lack of food.

Food is needed for life, but life is something to be enjoyed, not endured. We can buy time and must buy time. As I said, I am an optimist. I do not think the reasoning animal man has struggled this far only to meet extinction. We need to organize internationally and nationally to marshal the resources and technology necessary to do the job. I am convinced we can provide the time, but there must be no slacking.

Part II

QUALITY PROTEIN MAIZE IN HUMAN NUTRITION

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In the Central Andean region of South America, maize seems destined to remain important in human nutrition. As in maize production throughout the world, the methods of use and quantities consumed vary greatly from one country to another. In Latin America, maize and other cereals are combined with legumes, mainly beans (*Phaseolus vulgaris*).

Within this setting, the Green Revolution is increasing the production and the consumption of cereals, but could reduce the quality of food utilized, unless this revolution is associated with a *quality revolution* in cereals. An example of this problem of nutritional unbalance was reported by Han and Yu (3), who described the results of increased rice consumption in Korea. Even though positive protein balance could be achieved by the ingestion of

increased amounts of poor-quality protein in adults (6), this balancing becomes uneconomical because of losses through urine and feces (2).

Potential dietary solutions, such as enrichment of staple foods, vegetable mixtures, or new sources of proteins, are often proposed. However, despite the fact that changing habits is sometimes relatively easy, as demonstrated in some industrialized societies, the communication and marketing systems for low socioeconomic groups are often limited. Most technological improvements never reach these underprivileged groups. It seems more feasible to present people with improved quality staple foods that are not very different in phenotype from the food grains normally used.

Previous experience has clearly demonstrated the limited impact of any given discipline working alone to overcome hunger and malnutrition. Maize in the Andean region is no exception. Research and development interests must cooperate to increase availability and acceptance of maize with improved quality. Against a background of urgency, Colombia has pushed the production of maize with a modified-protein endosperm. Maize is one source of vegetable protein that is widely used and represents a basic staple for many regions of the country. The achievements to date have been possible only by close cooperation among Centro Internacional de Agricultura Tropical (CIAT), Instituto Colombiano Agropecuario (ICA), the Rockefeller Foundation, Universidad del Valle, and industry. Summaries of several studies will be presented in this paper.

EXPERIMENTAL RESULTS

In August 1964, only 2 months after Mertz et al. published their results on opaque-2 maize (9), seed carrying the opaque gene was obtained, and Palmira researchers began to explore the use of this discovery in the tropical conditions of the Cauca Valley. Realizing that the opaque-2 gene would produce a maize with a soft endosperm unlike that usually accepted in the markets, two initial decisions were made:

The first decision was to transfer the gene to inbred lines of two locally adapted hybrids as quickly as possible to obtain a large volume of seed for extensive testing.

The second determination was to have the maize available in the best-adapted genetic background. The white maize double-cross hybrid D.H. 253 and yellow double-cross hybrid ICA H. 207 were chosen as base materials. They seemed logical choices since industry was already using these hybrids, and it would be possible to test the processing qualities of the opaque and normal versions. Also, by using these hybrids the yield and other agronomic characteristics of the new material could be evaluated. It was known that, if this maize were to be taken to the public, the best possible first impression should be created.

TABLE 1 Amino acid composition of normal (H. 253, H. 207) and opaque-2 modified maize (H. 255, H. 208) endosperm

	Normal		Opaque-2 modified	
	H. 207	H. 253	H. 208	H. 255
Lysine	2.0	2.2	5.0	4.07
Leucine	19.5	16.1	12.7	11.60
Isoleucine	5.0	5.0	4.4	5.0
Phenylalanine	6.5	5.4	5.3	4.6
Methionine	3.2	2.8	3.2	2.8
Threonine	4.1	3.5	3.7	4.5
Valine	5.3	5.4	6.67	6.15
Tryptophan	0.55	0.6	1.10	0.99

Experiment 1: Including the Opaque-2 Gene in Local Hybrids

In 1967, after 2 years of intensive plant breeding, it was possible to begin pilot production of the high-quality protein maize for nutritional research. After analytical testing of the different crosses, it became clear that the opaque-2 gene was present in the two hybrids (Table 1) (11). Hybrid yields approached those of the original after the third backcross (12). In commercial harvests, segregating kernels with a hard endosperm were present, especially in the yellow hybrids. Several collections of the different types, from 100% hard (flint) to the 100% soft (floury) endosperm, were analyzed for the two hybrids. The results demonstrated that both lysine concentration and aminograms of the protein were essentially equal (Table 2) for the hard and soft types (12).

After animal testing of the two modified hybrids (4, 8), human studies were begun. Two severely malnourished children (5 and 6 years old) were admitted in the metabolic unit. For one child, the treatment diet was made with 80% of the protein from opaque-2 maize endosperm and 20% from milk. For the other child, the 80% opaque-2 base was used, also, with the remainder of the protein derived from low-quality vegetable sources. The

TABLE 2 Protein, lysine, and tryptophan content of soft and hard endosperm of opaque-2 modified maize

	Protein (%)	Lysine (g/100 g of protein)	Tryptophan (g/100 g of protein)
H. 255			
Hard	10.9	5.2	0.83
Soft	10.7	5.4	0.90
H. 208			
Hard	10.2	4.9	1.00
Soft	9.4	4.6	1.10

two patients achieved normal physical recovery and demonstrated excellent absorption and retention during the recuperation period (11). These results encouraged the testing of this protein in a different manner.

Experiment 2: Response of Malnourished Children to Opaque-2 Maize Endosperm

Male children classified as severely malnourished by dietary, anthropometric, and biochemical criteria were selected for these studies; their ages were between 28 and 60 months (12). Weight and height did not exceed the fiftieth percentile for a 24-month-old normal child. Each patient had been screened for other pathological conditions, and only patients free of the debilitating diseases frequently associated with malnutrition were admitted. The first week after admission was used for elimination of parasites and administration of a complex of biochemical tests to assess nutritional status. The diet during this period was calculated to provide the same amount of nutrients the patients had received at home.

During the second week, the patients were placed on standard metabolic balance procedures for quantitative collection of data related to duplicate diets, food refusals, and analysis of feces and urine for nitrogen, fat, calcium, phosphorus, magnesium, urinary creatinine, and dietary amino acids. Blood was drawn at weekly intervals for hemoglobin, plasma proteins, and amino acids. All methods used are standard and previously reported in the literature. Daily weight was recorded.

Each diet was calculated to supply the calories, protein, and vitamins recommended by the Food and Nutrition Board of the U.S. National Research Council for this age group. Mineral content was adequate. Diets offered as a liquid formula and bread made of maize (*arepa*) supplying 110 cal/kg of body weight/day, with a net dietary calorie percentage from protein (N × 6.25) ranging between 8 and 10%, carbohydrates between 48 and 53%, and fats between 38 and 43%. All protein was derived from the opaque-2 endosperm.

The weight data in Figure 1, plotted in a chart to classify malnutrition (First, second, and third degree), demonstrate the catch-up syndrome of children recovering from malnutrition, using either animal protein or that from opaque-2 endosperm. If growth is plotted as a function of developmental age, this syndrome is seen to be no more than the normal growth of a child of this developmental age. Urinary creatinine, plasma albumin, and essential – nonessential amino acid ratio increased steadily once the experiment was started (Table 3). The recovery was as expected, being somewhat slower than therapeutic diets containing higher levels of milk protein (120 to 150 days), since total nitrogen intake was limited. These findings suggested that opaque-2 endosperm protein provides adequate amounts of essential

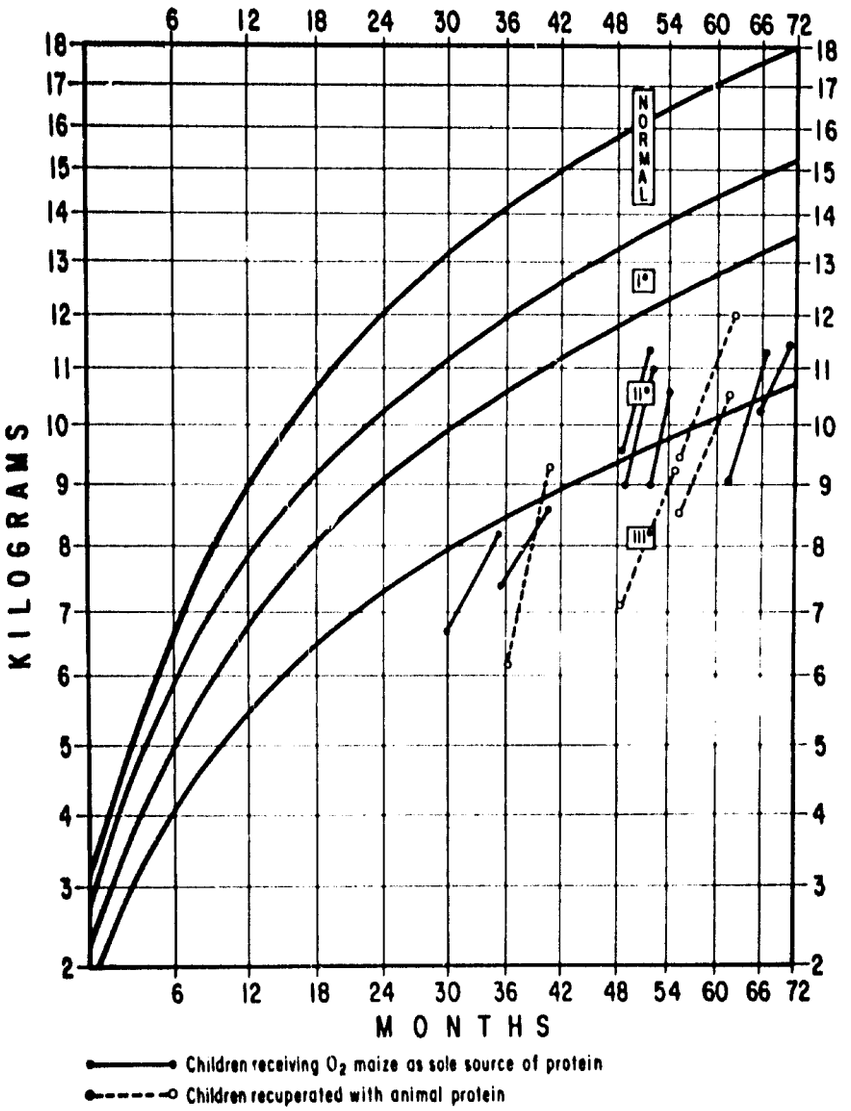

FIGURE 1 Catch-up syndrome in children recovering from malnutrition (only admission and discharge weights are plotted).

amino acids and total nitrogen for efficient protein repletion in malnourished patients and for the basic diet of a normal infant of the same developmental age.

TABLE 3 Plasma amino acid ratio (AAR) by circular chromatography, 24-hour urinary creatinine excretion (UC), and serum albumin (A) in six children receiving opaque-2 endosperm as the sole source of protein[a]

AAR	2.5	2.1	1.4	1.3
UC (mg)	82	105	143	182
A	1.8	2.4	2.6	2.8

[a] Data are averages for the samples taken every 4 weeks.

Experiment 3: Effect of Replacement and Addition of Nonessential Nitrogen to Opaque-2 Maize Diets

Experiments with rats and with children were done in the metabolic unit (10). The purpose was to determine the availability of essential amino acids from opaque-2 maize endosperm protein in relation to the proportion of nonessential nitrogen (NEN) from other sources. Three different diets were prepared with various amounts (0, 25, and 50%) of NEN, using glycine and diammonium citrate, and compared to a standard 100% casein diet. A total of 10% of the calories of the diet was derived from opaque-2 maize endosperm protein (N = 6.25).

The results obtained in weanling Sprague-Dawley rats are shown in Table 4. Decreases of 25 and 50% in the amount of opaque-2 protein which were replaced by NEN, produced a striking decrease in the growth rate. This finding was reflected also in lower protein efficiency ratio (PER) values obtained with the dilution, suggesting that at that level (10%) of protein intake the proportion of essential amino acids supplied by opaque-2 maize cannot be reduced without impairing nitrogen utilization.

Similar diets were used for six children. Nitrogen intake was supplied at various levels. At each level, opaque-2 endosperm nitrogen was replaced by 25 and 50% of NEN (glycine and diammonium citrate). As shown in Table 5, net nitrogen retention did not change significantly with 25 and 50% replacement, except when opaque-2 nitrogen was less than 155 mg/kg of body weight/day. This value, plus or minus 10 mg, was fairly constant in the six patients studied. Absorption increased steadily, as expected, with the

TABLE 4 Protein efficiency ratio values of opaque-2 maize when different proportions of nonessential nitrogen[a] were added

	Diet (%)	NEN (%)	Weight gain	Protein intake (g)	PER[b]
Opaque-2	50	50	13.0	12.9	0.82
Opaque-2	75	25	33.0	22.8	1.42
Opaque-2	100	0	103.2	37.4	2.74
Casein			72.8	25.9	2.95

[a] Nonessential nitrogen and unessential nitrogen are used interchangeably in this paper, and are applied to the moiety consisting of unessential amino acids and ammonium compounds.
[b] PER values were computed according to the method of Campbell.

TABLE 5 Nitrogen retention when opaque-2 maize protein is diluted with nonessential nitrogen (observations on six children)

N (source opaque-2)[a]	190	142	95	230	175	115	280	210	140	310	232	155
NEN[a]	–	47	95	–	65	115	–	70	140	–	77	155
Absorption	130	140	145	120	160	165	190	190	210	220	220	180
Retention	80	(-)40	-80	80	20	-50	80	20	(-)50	50	40	60

[a] Mg/kg body wt/day.

higher levels of NEN, and so did urinary excretion, leaving the net retention with very similar values for the three diets.

An amount of 9.7 g of opaque-2 maize endosperm per kilogram of body weight per day is sufficient for the maintenance of children with developmental ages of 18 to 24 months. Intakes below that amount, regardless of total nitrogen intake, produced negative balances. This could imply that the amounts of lysine, tryptophan, and other essential amino acids present in this quantity of endosperm are readily available for human utilization and could represent the minimal requirements for this age group.

Experiment 4: Opaque-2 Endosperm Protein Mixtures with Other Staples

Four different modifications of high-protein commercial formulas (HPCF) were tested in six 5- to 6-year-old malnourished children, and observations were made before recovery. Isocaloric and isonitrogenous diets were maintained during the whole period of observation. Three days of a nonprotein diet were included at the beginning of the study. Based on endogenous fecal and urinary losses, nitrogen intake, and total urinary and fecal losses, the biological value (BV), net protein utilization (NPU), digestibility, and net retention were calculated.

The nutrient composition of HPCF is quite similar to whole milk, except for the fat content. However, the total calories furnished by all formula diets were adjusted to the same level in order to keep constant the daily caloric intake. As shown in Table 6, the determinations of protein quality are similar in the seven diets except for nitrogen retentions found in formula A (soybean + common maize + milk), D (soybean + opaque-2 maize), and MY (opaque-2 + yuca or cassava), which induced the greatest nitrogen retention per kilogram of body weight. Formulas A, D, and MY have a nutritive value similar to milk, perhaps the most acceptable food for infant feeding.

It is possible to suggest two beneficial effects of the use of opaque-2 maize in weaning foods: (1) wide acceptance of the product through the prestige of this type of maize, and (2) the stimulus to growers by the presence of a market to sell their production.

Tests also were made on albino weanling rats with bread and spaghetti, prepared by the Technology Research Institute, Bogotá, in which 25% of

TABLE 6 Protein quality of vegetable mixtures containing opaque-2 maize (observations in 5- to 6-year-old children weighing 14 to 16 kg)

	A	B	C	D	EM	MM	MY
Intake	175	175	175	175	175	175	160
Digestibility	87	87	81	86	85	86	91
BV	68	59	59	63	74	81	84
NPU	61	51	50	53	59	65	76
N retention	115	102	97	114	111	127	121

Notes: A, nonfat milk + normal maize + soybean; B, normal maize + soybean; C, nonfat milk + opaque-2 maize + soybean; D, opaque-2 maize + soybean; EM, evaporated whole milk; MM, modified milk; MY, opaque-2 maize + yuca (cassava).

the wheat was replaced by opaque-2 maize endosperm. The results demonstrated a twofold increase in nutritive value. The beneficial effects of this replacement are clear by the nutritional improvement and its impact in lowering the price of the product.

Experiment 5: Comparison of Other Types of High-Quality Maize with Opaque-2 Maize

Recuperated children, weighing 12 to 14 kg, were used for this test (4). Each diet was tested in four children for 9 days, and results were pooled to obtain mean values. The diet was maintained at 1.5 g of protein/kg of body weight. Each maize sample was given for 2 days of adaptation and three 3-day test periods. White and yellow normal (H. 253 and H. 207) and opaque-2 modified (H. 255 and H. 208) maizes were tested. Floury-2 modified maize obtained from Purdue University and a sample (high fat, high lysine) sent by D. E. Alexander from the University of Illinois were compared with a test diet of casein. A 3-day nitrogen-free diet for quantification of endogenous values was used at the beginning of the experiment.

The results in Table 7 show that the protein quality of every modified maize sample tested is significantly superior to that of normal varieties (H. 207 and H. 253). Values found in the modified types are 85 to 95% of the values obtained for casein.

TABLE 7 Biological testing of opaque-2 (H. 208, H. 255), floury-2, high lysine high fat (Ill.), and normal maize (H. 207, H. 253) in four children

	H. 208	H. 255	H. 207	H. 253	Ill.	Floury-2	Casein
Protein intake							
NEN	0.077				0.085	0.080	
Protein N	0.227	0.160	0.225	0.200	0.255	0.240	0.150
Digestibility	95.0	95.0	76.9	66.0	88.2	84.9	98.0
BV	76.5	80.0	20.9	17.2	75.6	61.9	85.0
NPU	61.6	86.0	16.1	11.3	66.7	54.2	83.0
Retention	0.140	0.120	0.030	0.023	0.170	0.130	0.130

Experiment 6: Protein Quality of Hard-Endosperm Phenotypes of Opaque-2 Modified Maize

The soft endosperm of opaque-2 maize has limited its acceptability in the middle altitudes and lowlands of Colombia (1). There is an immediate and critical need for developing a maize that would have (1) enhanced protein quality combined with a vitreous endosperm, (2) ready acceptance by the consumer, and (3) easy passage through market channels.

Early in 1966, observations of commercial harvests of double-cross opaque-2 hybrids revealed a range in endosperm type from the predominant class of soft kernels through intermediates to almost completely flint types. Analytical studies (5) demonstrated a high lysine content of these segregating kernels. On an illuminated board, the soft grains were separated from the essentially vitreous flint types. Protein content as well as lysine and tryptophan concentrations were not significantly different in the two types (Table 2). Diets were prepared with these types of opaque maize, as well as with normal H. 207 maize and with casein. Standard protein efficiency ratio analyses were made in rats, and nitrogen balance studies were made in normal 5- to 6-year-old children, using 1 g of protein/kg of body weight.

The results presented in Tables 8 and 9 show that hard kernels which contain the opaque-2 gene are almost identical in biological value to the maize we classify as opaque (soft). The results demonstrate that an opaque

TABLE 8 Average gain, feed consumed, and protein efficiency ratio values in rat feeding study

	H. 208 opaque	H. 208 flint	H. 207 normal	Casein
Gain (g)	84	62	26	69
Feed consumed (g)	330	288	216	311
Actual protein (%)	7.9	8.6	8.4	6.0
Protein consumed (g)	26.1	21.9	18.2	18.7
PER	3.21	2.81	1.43	3.68
PER, standard[a]	2.18	1.91	0.99	2.50
Casein (%)	87.2	76.4	39.4	100

[a] Value of PER standardized casein as 2.50.

TABLE 9 Comparative nitrogen balances in three children, using different protein sources[a]

	H. 208 soft	H. 208 hard	H. 207 normal	Casein
Intake	175	175	175	175
Digestibility	91	87	78	98
NPU	69	65	36	75
BV	76	75	47	77
N/retention/day	1.52	1.50	0.93	1.81

[a] Calculated diet included 1.0 g of protein and 100 cal/kg of body weight/day.

(soft) endosperm is not completely and irrevocably tied to the increased lysine and tryptophan contents, that is, reduced zein. It appears that modifier genes can be located which will effectively modify the endosperm type without reducing nutritive value (7).

SUMMARY

The findings confirm the higher nutritional value of opaque-2 maize endosperm compared to that of normal maize. For the children tested, 9.7 g of opaque-2 maize/kg body weight/day supplies minimal essential amino acids. Regardless of total nitrogen intake, lesser amounts gave negative balances. The average intake of maize in urban communities in Colombia is 36.7g/person/day with a range from 6.8 to 113 g. Acceptance of this maize could be increased if hard and soft endosperm phenotypes could be offered to the consumer. Results presented suggested it would be possible to locate modifier genes for endosperm type. The utilization of this maize could decrease the price of products such as bread and spaghetti by decreasing the need for imported wheat, and also could increase nutritional value.

Supported by The Rockefeller Foundation, 65071.

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IMPROVING MAIZE DIETS WITH AMINO ACID AND PROTEIN SUPPLEMENTS

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It is well established that the poor quality of normal maize protein is due to deficiencies of two main amino acids, lysine and tryptophan (11, 18), as well as minor ones, such as isoleucine (11, 25). Furthermore, excessive amounts of leucine reduce the protein quality of maize to some unknown extent (19).

Various approaches to improve the protein quality of maize-based diets have sought a better amino acid balance through (1) amino acid supplements, (2) genetic means, (3) protein supplements, and (4) high protein quantity and quality mixtures. All these approaches have been evaluated at the Institute of Nutrition of Central America and Panama (INCAP). The purpose was not to discriminate among them, but to find the easiest practical solution that would provide greater nutritional benefit for people whose diets require better-quality protein.

Thus, our research was focused upon this final objective, rather than upon the study of the individual merits of any procedure. The results presented here are centered around this primary task and include summary evaluations of maize protein improvements by the four indicated approaches, their effect as components of low-quality diets, and the progress achieved in the application of the research findings.

IMPROVING THE PROTEIN QUALITY OF MAIZE

Amino Acid Supplementation

In most cases, the results to be presented were obtained with children aged 2 to 5 years, fed maize protein exclusively. Experimental animal work has repeatedly given similar results. Evaluation was by the nitrogen-balance technique, which gives the amount of nitrogen retained after nitrogen intake is corrected for losses in feces and urine.

Results in Table 1 indicate that maize protein quality is improved by adding lysine and tryptophan. Also, a further improvement is obtained when iscleucine is added in the presence of lysine and tryptophan (1). The data indicate that the effect was obtained through a decrease in urine nitrogen losses, since fecal nitrogen remained quite constant.

The results shown were obtained at various levels of protein intake. Nitrogen balance increased as protein intake increased from 1.5 to 3.0 g of protein/kg of body weight/day. Also, adding the two amino acids increased nitrogen retention at all levels of protein intake, with greater increases when intake of maize protein was equal to 3g/kg/day.

Isoleucine addition increased nitrogen balance at all levels of intake. This finding was of interest and was attributed to the high level (about 53 to 55%) of zein protein in the maize fed. Zein is an unbalanced protein with deficiencies in lysine and tryptophan and excesses of leucine. It was felt that zein could explain such results, since maize varieties with low levels of protein (and, therefore, low levels of zein) give better nitrogen balances. This relationship holds, even at low levels of protein intake, as is shown in Table 2. These results show that the low protein-containing maize gave a low, but positive, nitrogen balance at an intake of 1.25 g/kg/day. Nitrogen-retention values ranged from 10 to 55 mg/kg/day, with an average value of 30 mg/kg/day. Milk protein retention values were about 2.5 times higher at

TABLE 1 Effect of the addition of amino acids to lime-treated maize at various levels of nitrogen intake

Amino acids added to maize[a]	Protein intake 3.0 g/kg/day		Protein intake 2.0 g/kg/day		Protein intake 1.5 g/kg/day	
	Intake	Retention	Intake	Retention	Intake	Retention
None	469	14	326	-5	238	-10
Lysine	482	38	335	24	239	-4
Lysine + tryptophan	461	83	328	36	239	30
Lysine + tryptophan + isoleucine	475	108	335	40	240	46
Milk	458	70	364	73	–	–

Source: Bressani (1).

[a] Levels used: L-lysine HCl, 0.56%; DL-tryptophan, 0.35%; DL-isoleucine, 0.45%.

TABLE 2 Nitrogen balance of children fed a high-zein maize and a low-zein maize

Type of maize	Protein intake (g/kg/day)	Nitrogen balance (mg/kg/day)				
		Intake	Fecal	Urine	Absorbed	Retained
High zein	1.50	238	58	190	180	-10
Low zein	1.25	192	48	114	144	30
High zein + lysine + tryptophan	1.50	239	47	162	192	30
Milk	1.25	195	38	82	157	75

Source: Viteri et al. (26).

equal levels of intake (26). The low (7%) protein-containing maize used has less zein (about 35%) and, therefore, more lysine and tryptophan than maize with 10% protein.

Results were pooled to calculate the nitrogen-balance index of the unsupplemented and amino acid supplemented protein (3, 4). This index is equivalent to a biological value that represents the amount of nitrogen actually retained from the nitrogen absorbed.

Figure 1 summarizes (on the left) the results for unsupplemented maize, and (on the right) maize supplemented with lysine and tryptophan. The line represents nitrogen intake versus nitrogen balance. Regression equations relating nitrogen balance (NB) to nitrogen intake (NI) and nitrogen absorbed (NA) were calculated (Figure 1). The index of biological value for the

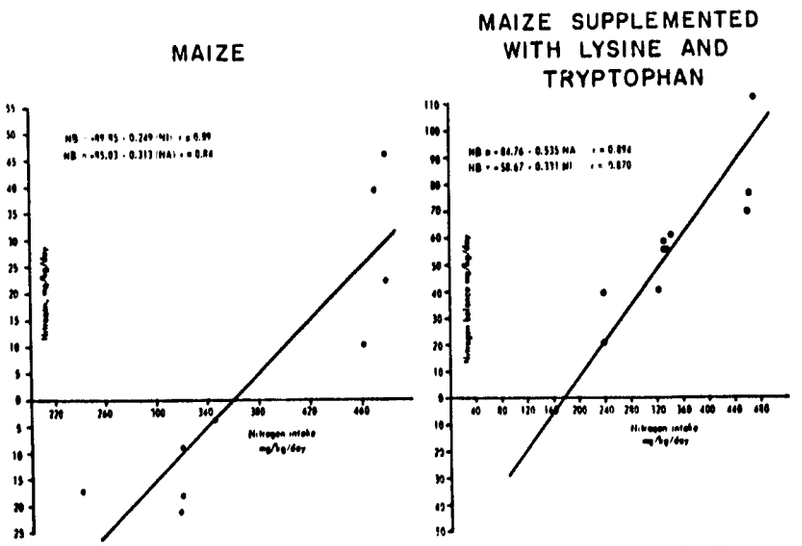

FIGURE 1 Nitrogen balance of children fed various levels of protein from maize and from maize supplemented with lysine and tryptophan.

maize used is the coefficient of regression (0.313, or 31.3%). The same kind of analysis was made when maize was supplemented with lysine and tryptophan. In this case, the coefficient of regression was 0.535, and the biological value was 53.5%, a significant increase over the unsupplemented maize. Probably a higher value would be obtained if the same calculation were made when maize is supplemented with isoleucine, also.

Genetic Procedures

As is well known, increased protein quality has been introduced into maize by incorporating various genes, among which the opaque-2 gene proved to be very effective (22).

The studies reported have tested the protein quality of opaque-2 maize developed at Purdue University in 1964. Through the collaboration of E. T. Mertz, a batch of opaque-2 maize grown in the United States and received at INCAP in 1965 was tested, with children as subjects. The maize was lime-cooked, d' ied, and analyzed before feeding it to children as the sole protein source. As before, the protein quality was measured by the nitrogen-balance method. The results are shown in Figure 2, where nitrogen absorbed is

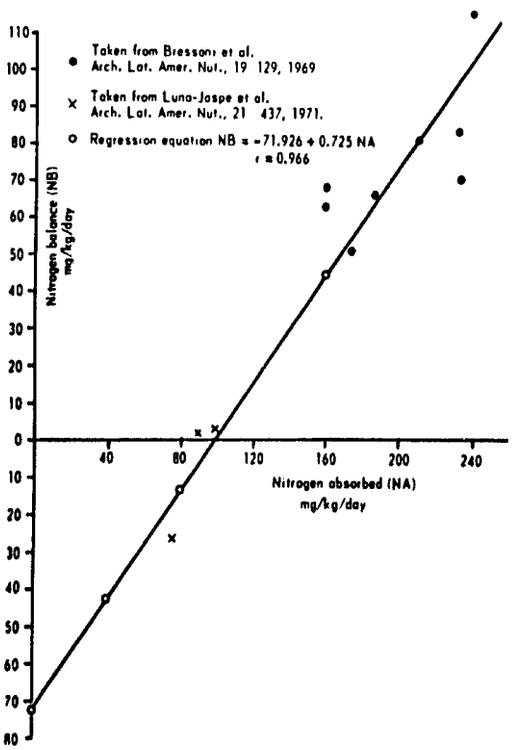

FIGURE 2 Nitrogen-balance index of opaque-2 maize protein.

plotted against nitrogen balance (12). Figure 2 also includes results published in 1971 by Luna-Jaspe et al. (20), using a Colombian opaque-2 maize. These figures were included, since they fall along the regression line calculated previously from our results (4, 12). The regression equation for all the results is shown in Figure 2, and indicates that the nitrogen-balance index of opaque-2 maize for children is 0.725. Using this method of nitrogen-balance analysis, the calculations for milk protein at various levels of intake gave a nitrogen-balance index of 0.80. This suggests that opaque-2 maize proteins have 90% of the protein value of milk proteins. Using the true protein digestibility of opaque-2 maize and the nitrogen-balance index value, a biological value for opaque-2 maize of 87% was calculated. The graph indicates that 100 mg of absorbed nitrogen gives nitrogen equilibrium, which for maize is about 125 g/day. An intake of about 180 g of opaque-2 maize would ensure a highly positive nitrogen balance.

The values shown in Table 3 were calculated from the results presented in Figures 1 and 2. This table shows the nutritional advantage of amino acid-supplemented normal maize or opaque-2 maize over normal maize. Using these data, it was estimated that nitrogen equilibrium in children was possible when nitrogen intake was equal to 300 mg/kg/day from normal maize, in contrast to 176 mg from normal maize supplemented with lysine and tryptophan, and to 135 mg of nitrogen derived from opaque-2 maize. In terms of nitrogen absorbed for nitrogen equilibrium, the above values correspond to 272, 158, and 99 mg/kg of body weight/day for normal, amino acid-

TABLE 3 Analysis of the results of the protein quality of normal, lysine- and tryptophan-supplemented, and opaque-2 maize

	Normal maize		Maize + lysine + tryptophan		Opaque-2 maize	
N intake at N equilibrium (mg/kg/day)	360		176		135	
N absorbed at N equilibrium (mg/kg/day)	272		158		99	
Biologic value (%)	31		53		72	
Utilized N at N equilibrium (mg/kg/day)	84		84		71	
	Amino acids in nitrogen (mg/kg/day)					
Amino acids	Absorbed	Utilized	Absorbed	Utilized	Absorbed	Utilized
Arginine	52	16	30	16	41	29
Histidine	41	13	24	13	19	13
Isoleucine	74	23	43	23	24	17
Leucine	203	63	118	63	61	43
Lysine	47	14	43	23	29	21
Total sulfur amino acids	59	18	34	18	21	15
Total aromatic amino acids	150	46	87	46	52	37
Threonine	65	20	38	20	24	17
Tryptophan	4.9	1.5	14	7.6	9	6.6
Valine	76	23	44	23	34	24

supplemented, and opaque-2 maize, respectively. The biological value of the three protein sources has been calculated to be 31, 53, and 72%, respectively (Table 3). Since biological value is defined as the amount of nitrogen retained from that which has been absorbed, it is possible to estimate how much of the nitrogen absorbed is actually utilized for metabolic function. These estimates correspond to 84, 84, and 71 mg of nitrogen/kg/day for normal maize, maize supplemented with lysine and tryptophan, and opaque-2 maize, respectively.

The absorbed and utilized essential amino acids were estimated from the absorbed and utilized nitrogen values. Under the column for the three sources of maize protein, the values are quite similar, with low values for lysine and tryptophan. These represent minimum maintenance amounts for children of the age used in the studies. The data also indicate the wastefulness of nitrogen when provided as normal maize, since 300 mg are required to give basically the same essential utilizable amino acid pattern as that from 176 mg of nitrogen from maize supplemented with lysine and tryptophan or from 135 mg of opaque-2 maize.

Protein Supplementation

A third possible way to improve the protein quality of maize is protein supplementation, which consists of adding small amounts of proteins that are rich sources of the amino acids deficient in maize. Various materials have been tested as possible supplements, and some results are presented in Table 4. The column in the middle indicates the amount used to obtain the improvement indicated in the last column. With maize having a protein efficiency ratio (PER) value of 1.0, the listed supplements increased protein quality up to 2.5 times. This effect is due to the contribution the protein supplements make in lysine and tryptophan, and in protein content (9). Whereas improvement with synthetic amino acids increases protein quality only, improvement with protein supplements increases both protein quality and quantity. Therefore, protein supplementation provides greater amounts of usable protein than does the addition of amino acids.

TABLE 4 Optimum levels of various proteins to supplement lime-treated maize

Protein source	Optimum level % in diet	PER
None		1.00
Fish protein concentrate	2.5	2.44
Soybean flour	8.0	2.25
Cottonseed flour	8.0	1.83
Torula yeast	2.5	1.97
Casein	4.0	2.21
Egg protein	3.0	2.24

Source: Bressani and Marenco (9).

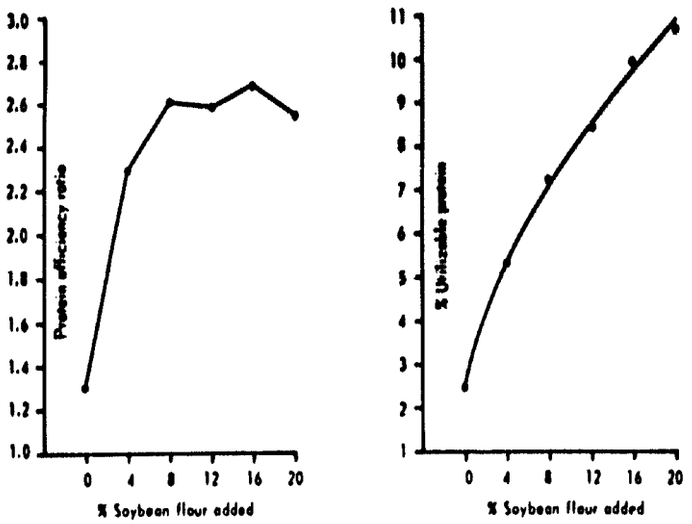

FIGURE 3 Soybean flour supplementation of maize.

Figure 3 shows the increases in usable protein for maize supplemented with soybean flour (5, 6). Supplements of up to 20% soybean flour were added to 70% maize diets. The curve on the left shows the effect on PER, suggesting an optimum level of about 8% soybean flour. Calculations in terms of usable protein were made and the results plotted, as shown on the graph (on the right). Usable protein which includes both protein quality and protein concentration, increased almost linearly, from about 2.5% for maize alone, to 7.2% when 8% soybean flour was added, to 10.6% when 20% soybean flour was supplemented. Owing to technological considerations, plus organoleptic aspects, the level chosen for further testing was 8% soybean flour (2).

Previous work indicates that the optimum levels of lysine and tryptophan needed for maximum improvement are from 0.30 to 0.40% L-lysine and from 0.05 to 0.10% DL-tryptophan (5, 1). Since 8% soybean supplementation was slightly deficient in providing all the lysine, although it provided all the tryptophan, maize was supplemented with 8% soybean flour and 0.10% lysine. Maize supplemented in this way was then tested for nutritional value in children, and compared with milk, maize, and maize with beans *(Phaseolus vulgaris)* (87% maize and 13% cooked black beans) (26). The average results of nitrogen balance in the children are shown in Table 5. Protein intake was essentially the same, as indicated by the nitrogen-intake column. Nitrogen retention, shown in the last column for maize supplemented with soybean flour and lysine, was as high as that from milk; maize alone or maize with black beans gave the lowest values. Therefore, these results confirm the results obtained with experimental animals. They

TABLE 5 Nitrogen balance of children fed various protein sources at a protein intake of 1.25 g/kg/day

Protein source	No. of children	Nitrogen balance (mg/kg/day)		
		Intake	Absorbed	Retained
Milk	7	195	157	75
Maize	6	192	144	30
Maize + beans	4	207	150	36
Maize + protein supplement (soybean flour + 0.15% L-lysine HCl)	6	197	154	63

Source: Viteri et al. (26).

indicate that maize supplemented with 8% soybean flour and 0.10% lysine has a protein quality approximately 90% that of milk.

Protein Complementation

When maize is consumed in large amounts, as in various Latin American or African countries, it provides relatively high levels of total protein, along with levels of nonprotein dry matter, which is mainly carbohydrate. This is obviously due to the relatively low level of protein in maize, a level that becomes of nutritional significance in relation to the total dry matter intake for children. As an improvement, food mixtures of maize with other ingredients have been developed, which provide both a higher quality and a higher quantity of protein. These are known as the INCAP vegetable protein mixtures, or Incaparina formulations (2, 6, 7).

Table 6 summarizes the analysis of nitrogen-balance results of three such mixtures in comparison with milk and egg protein, as tested in children (13).

TABLE 6 Correlation and regression equations between nitrogen intake (NI) and nitrogen retention (NR), and between nitrogen absorbed (NA) and nitrogen retained (NR)

Protein source	Correlation coefficients	Regression equations
Milk	NI vs. NR = 0.66	NR = -35.7 + 0.55 NI
	NA vs. NR = 0.80	NR = -33.0 + 0.69 NA
Vegetable mixture 9	NI vs. NR = 0.56	NR = -23.8 + 0.28 NI
	NA vs. NR = 0.81	NR = -30.3 + 0.50 NA
Vegetable mixture 14	NI vs. NR = 0.89	NR = -52.0 + 0.55 NI
	NA vs. NR = 0.94	NR = -39.3 + 0.63 NA
Vegetable mixture 15	NI vs. NR = 0.75	NR = -55.4 + 0.48 NI
	NA vs. NR = 0.73	NR = -39.5 + 0.53 NA
Whole egg	NI vs. NR = 0.85	NR = -52.5 + 0.59 NI
	NA vs. NR = 0.87	NR = -43.3 + 0.64 NA

Source: Bressani et al. (13).

The regression equation between nitrogen absorbed and nitrogen balance is shown in the last column. The coefficients for milk, mixtures 9, 14, and 15, and egg are 0.69, 0.50, 0.63, 0.53, and 0.64, respectively. Therefore, all mixtures have a protein quality close to that of high-quality reference proteins, such as milk and egg. The mixtures contain about 25% protein and are made from 38% cottonseed or soybean and 58% maize for formulas 9 and 14, and 19% cottonseed, 19% soybean, and 58% maize for formula 15. This approach to improve maize protein includes both total protein concentration and protein quality.

ADVANCES IN APPLYING THE VARIOUS APPROACHES

Applying laboratory findings involves such problems as the physical and organoleptic properties of the fortified food, which must be equal to or improved over the natural product. The four approaches discussed here meet such demands completely or to a very large extent. However, two additional problems of application are found in societies that (1) produce their own food, and (2) have traditional and well-implanted methods of food preparation. Methods to overcome these barriers are being studied. An additional problem is price, but this will not concern us at this time.

Synthetic Amino Acids

In our laboratories, the use of synthetic amino acids is being studied by infusion techniques. By adjusting infusion conditions, lysine has been incorporated at levels up to 6.2 g/100 g of kernels. By using other conditions, tryptophan levels have reached 6.94 g/100 g (17). The infused kernels are shown in Figure 4. The lysine-infused kernels look quite normal. However, the tryptophan-infused kernels have a darker color, owing to the conditions of infusion.

Since the infused kernels lose about 50 to 60% of the infused lysine or tryptophan during wet processing of maize (16), the amino acid-rich kernels may be added to maize when it is being ground in the mill shown in Figure 5. To avoid adding raw kernels, those which are to be used for infusion should be precooked. As they go through the mill, they are ground and mixed with the rest of the cooked maize. Any further processing does not destroy or inactivate the added amino acids (15). The effect of improving protein quality of normal maize with infused kernels is shown in Table 7. Diet 2 was made with 0.31% synthetic lysine and 1% tryptophan-infused maize to give a tryptophan level of 0.05% of that of diet 5. Similarly, diet 3 contained 0.05% synthetic tryptophan and 3.875% lysine-infused maize to give a lysine level of 0.31% of that of diet 5. The results clearly show no difference in protein quality when the lysine and tryptophan were added in the synthetic form, or as infused kernels (16).

The results show the potential of this technique, even though it still has

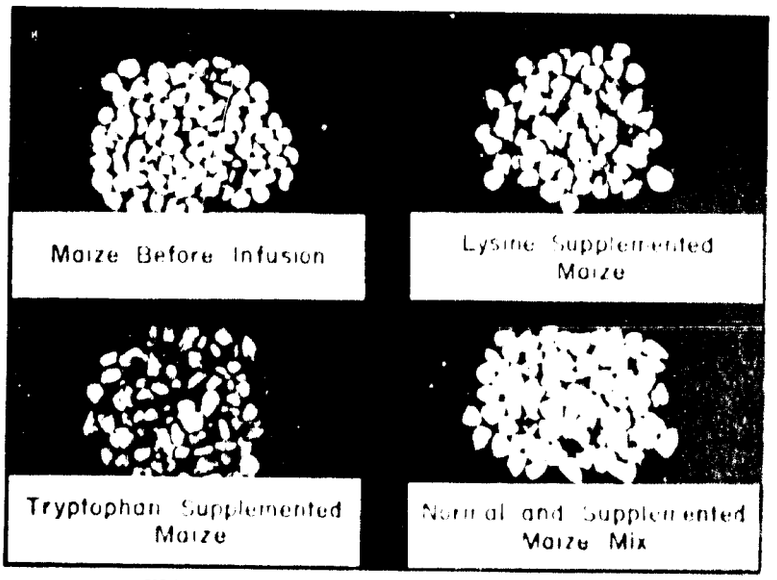

FIGURE 4 Appearance of infused kernels

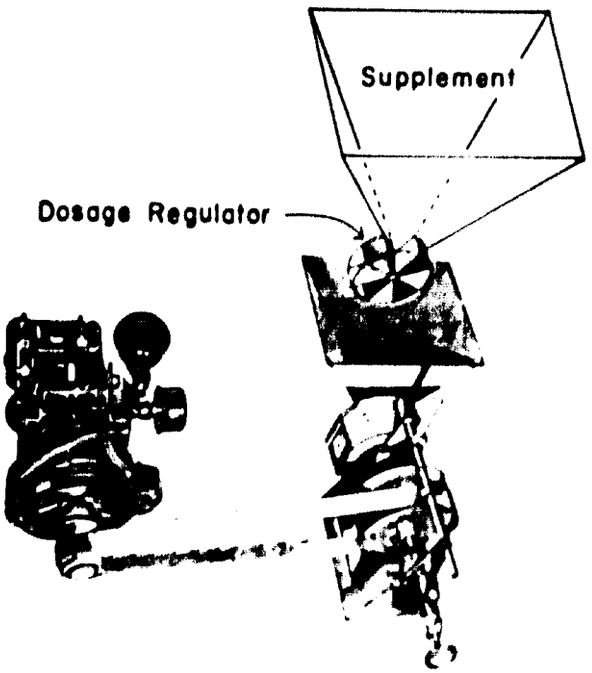

FIGURE 5 Nixtamal mill for grinding maize.

TABLE 7 Effect of lysine- and tryptophan-infused maize kernels on the protein quality of maize

Dietary treatments	Protein in diet (%)	Avg wt gain (g)	PER
1. Maize	8.6	30 ± 3.5	1.22 ± 0.08
2. Maize + synthetic lysine + maize (tryptophan)	9.0	92 ± 8.0	2.42 ± 0.10
3. Maize + synthetic tryptophan + maize (lysine)	8.9	100 ± 11.1	2.59 ± 0.12
4. Maize + maize (lysine) + maize (tryptophan)	8.8	91 ± 9.3	2.38 ± 0.12
5. Casein	10.4	135 ± 6.8	2.73 ± 0.07

some limitations, particularly regarding cost. Cost is 30 to 40% of the price of maize, owing mainly to the high price of tryptophan.

Protein Supplements

As indicated previously, a second way in which maize protein can be improved nutritionally is through protein supplementation. Various fortifying mixtures were developed and tested. Their composition and the amounts giving improved protein quality when added to maize, without interfering with organoleptic, physical, or functional properties (2, 14, 15), are shown in Table 8. The vitamins were added because they were found deficient in diets consumed by people in Central America, and also because

TABLE 8 Formulations of supplements for maize

Ingredients	Formulations 1	2	3
L-lysine HCl	6.000	1.25000	1.25000
DL-tryptophan	2.000		
Maize	91.844		
Skim milk powder		98.65250	
Soybean flour (soybean flour 200 M)			98.65250
Riboflavin	0.0060	0.00375	0.00375
Thiamine	0.0400	0.02563	0.02500
Niacin	0.0600	0.03750	0.03750
Vitamin A (g)	0.0500 [a]	0.03750	0.03750
	100.0000	100.00000	100.00000
% of addition	5	8[b]	8[c]

[a] Equivalent to 25,000 IU of vitamin A or retinol. Vitamin A acetate (500,000 IU/g). This amount supplies the half-level recommended of 750 μg of retinol per 100 g of tortilla.

[b] Amount of skim milk that supplies 0.04% L-tryptophan and 2.8 g of protein.

[c] Amount of soybean flour that supplies 0.05% L-tryptophan and 4.0 g of protein/100 g.

lime cooking of maize causes extremely large losses, particularly in riboflavin (9, 10).

The mixture chosen was the formula with soybean flour as the protein source. When added at the rate of 8% to maize, this formula covers all the tryptophan deficiency in the cereal and almost all the lysine deficiency. To cover all the lysine deficiency in maize, 0.15% of L-lysine HCl is also a part of the fortifying mixture.

The soybean supplement is presently being tested in a field study involving 1,300 families in rural Guatemala, even though it was developed to be used with industrially prepared, cooked maize flours. At present, it is added as a flour to lime-cooked whole maize just before grinding (21). Other forms of addition being considered are as pellets in the shape of maize or cylindrical, as shown in Figure 6 (2, 23).

The field test was started in May 1972. Therefore, it is still too early to determine the nutritional effects. About 60% of the population is now consuming maize with the supplement. Only a few problems have been encountered; some were completely unexpected and suggest how challenging it is to bring about nutrition improvement with any of the new foods produced either by technological techniques or by genetics. One such problem concerns the use of insect-damaged kernels, which rural people have to consume just before the new harvest comes in. These kernels are almost free of endosperm. Therefore, cooking procedures change, which has an impact on the properties imparted by the supplement. A second problem concerns the use of immature maize, resulting also from the lack of the cereal just before the next crop comes in. These problems have been solved, but indicate the difficulties of applying nutritional intervention techniques.

Protein Mixtures

Protein-rich food based on cooked maize supplemented with 0.25% L-lysine HCl and human-grade cottonseed flour has been on the Guatemala

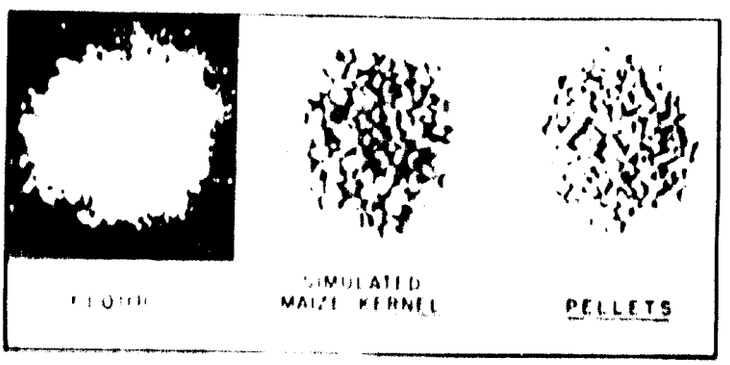

FIGURE 6 Forms of soybean protein supplement.

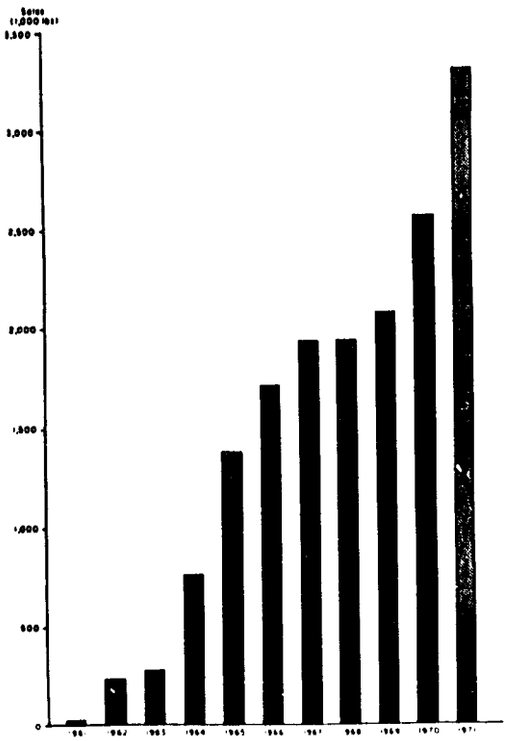

FIGURE 7 Incaparina sales in Central America and Panama (1961 – 1971).

market under the name Incaparina for about 12 years. Sales of the product are increasing slowly (Figure 7). During 1964–1967, the yearly sales tended to reach a plateau. However, from 1967 – 1971, annual increases have been about 30%. The product is not reaching all population sectors for which such protein foods were developed. However, plans are already being implemented to increase production and diversify the product with a modern plant of 4- to 5-ton production capacity/hour.

IMPROVING RURAL DIETS WITH IMPROVED MAIZE

The diet consumed by most Latin American rural residents consists mainly of maize and beans (about 72% maize and 8% beans). The remaining 20% consists of tubers, some vegetables, and sugar. Such diets are deficient in quality protein and in calories. Since most of the dietary protein comes from maize, it would be expected to be deficient in the same amino acids tha limit maize, it would be expected to be deficient in the same amino acids that limit quality when supplemented with lysine and tryptophan. Results along these lines are shown in Table 9.

TABLE 9 Effect of lysine and tryptophan supplementation on the protein quality of a maize-bean diet (72.4% maize + 8.1% cooked black beans)

Dietary treatment to maize in diet	Avg wt gain[a] (g)	PER
None	65 ± 3.7	2.02 ± 0.06
+ 0.10% lysine[b]		
+ 0.025% tryptophan[b]	80 ± 9.3	2.36 ± 0.17
+ 0.050% tryptophan	94 ± 2.8	2.49 ± 0.06
+ 0.20% lysine		
+ 0.025% tryptophan	108 ± 4.0	2.76 ± 0.03
+ 0.050% tryptophan	110 ± 5.7	2.78 ± 0.08
+ 0.30% lysine		
+ 0.025% tryptophan	105 ± 9.8	2.77 ± 0.13
+ 0.050% tryptophan	102 ± 3.1	2.79 ± 0.04

Source: Elías and Bressani (14).
[a] Average initial weight: 48 kg.
[b] L-lysine HCl; DL-tryptophan.

These results show that a 92% maize + 8% bean diet is improved when supplemented with 0.20% L-lysine HCl and 0.025% DL-tryptophan (14). Optimum levels for corn were 0.30 to 0.40% L-lysine HCl and 0.05 to 0.10% DL-tryptophan (11), which indicates that beans are supplying about 33% of the lysine needed. Remember that protein quality is just one aspect in the improvement of maize–bean diets. The significance of this is shown in Table 10. In this study, the 72% maize + 8% bean diet was supplemented as shown in the first column. As indicated, the amino acids added without other nutrients were ineffective in improving the quality of the diet. Actually, the animals died from severe vitamin, and probably mineral, deficiencies (14, 15). These results indicate, therefore, the need to also consider that most nutrients are deficient in such diets, nutrients needed by the animal to make efficient use of an improved essential amino acid pattern.

TABLE 10 Effect of the individual addition of groups of nutrients on the protein quality of a maize-bean diet (72.4% maize + 8.1% cooked black beans)

Dietary treatment to basal diet	Avg wt gain[a] (g)	PER
None	26 ± 2.3	1.09 ± 0.07
+ 0.20% L-lysine HCl	26 ± 2.5	1.10 ± 0.08
+ 0.025% DL-tryptophan		
+ Vitamins[b] + minerals[b]	70 ± 2.1	1.90 ± 0.04
+ Vitamins + minerals	107 ± 4.9	2.55 ± 0.06
+ lysine + tryptophan		

Source: Elías and Bressani (14).
[a] Average initial weight: 44 g.
[b] Recommended levels for laboratory rats.

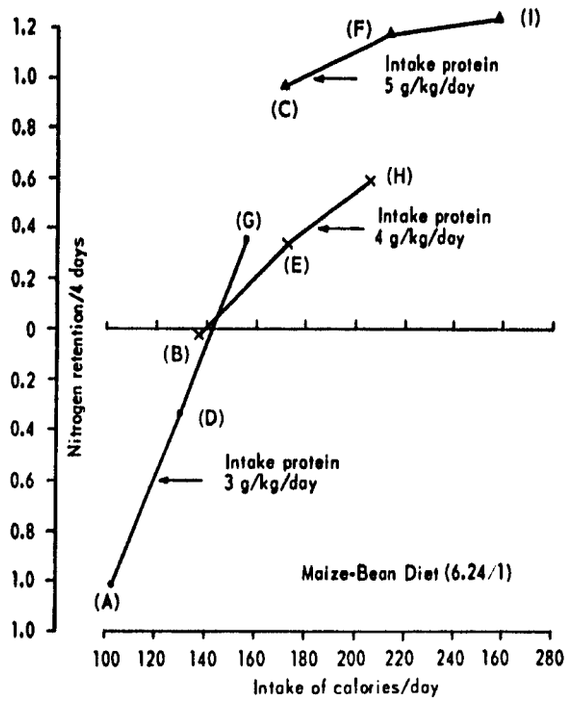

FIGURE 8 Effect of supplementation with calories (fat) on nitrogen balance of young dogs fed maize–bean diets.

As indicated before, these maize–bean diets do not provide all the calorie needs of young people, probably because of the diets' relatively high bulkiness. Adding calories as fat improves utilization of the protein of such diets (Figure 8). In the results (24) shown, young dogs were fed 3, 4, and 5 g of protein/kg of body weight/day of a maize–bean diet. Each level of dietary protein intake was supplemented with 25 and 50% additional calories added as oil. Nitrogen retention without additional calories (points A, B, C) increased as protein intake increased. The additional 25% calories (points D, E, F) improved protein utilization at all levels of protein intake, but the improvement was lower at the higher levels of protein intake. Similarly, 50% additional calories increased protein utilization (points G, H, I) mainly when protein intake was equivalent to 3 and 4 g/kg/day. These results indicate that additional calories are needed and that they cannot be derived from additional maize because (1) this will increase bulk and (2) a higher intake with the same level of beans will result in a diet with a protein quality value lower than the one used at present. Therefore, it is essential to improve the protein quality of maize.

Opaque-2 maize, when used to replace normal maize in maize–bean diets, is also effective in improving the protein quality of such diets. Representa-

TABLE 11 Nitrogen retention of young dogs fed a maize-bean diet alone or supplemented with amino acids (average: six dogs and three balance periods of 4 days each)

Treatment on basal diet	Nitrogen balance (mg/kg/day)				
	Intake[a]	Fecal	Urine	Absorbed	Retained
Normal maize	399	152	206	247	41
Normal maize + lysine + tryptophan	374	156	143	218	75
Normal maize	357	157	165	200	35
Opaque-2 maize	407	165	127	242	115

Source: Bressani and Elías (8).
[a] Protein intake: 2.5 g/kg/day. Calorie intake: 100 kcal/kg/day.

tive results are shown in Table 11. When the basal diet fed provided 2.5 g protein/kg of body weight/day, retention of nitrogen varied from 35 to 45 mg, or about 10% of intake. Adding lysine and tryptophan increased retention to 75 mg/kg/day (about 20%), and using opaque-2 maize produced retention of about 28%. In general, results of various studies similar to this one show that nitrogen retention of the maize–bean diet supplemented with amino acids or using opaque-2 maize has resulted in about the same increase in protein quality over the basal level (8, 16).

Maize supplemented with 8% soybean flour and lysine also improves the protein quality of maize–bean diets. An example of this is shown in Table 12. Three protein supplements to the maize–bean diet were compared with the effect of synthetic amino acids (14, 15). First, diets made with protein-supplemented maize contained more protein than the control diets. Adding amino acids improved weight gain, an improvement that was higher when the diet was supplemented with protein. Protein efficiency increased to about the same level, whether the maize–bean diet was supplemented with amino acids or protein. The last column shows the amount of usable protein in the diet. The values indicate that higher amounts are utilized from the

TABLE 12 Improvement of the protein quality of a maize-bean diet by the use of amino acids or a protein supplement

Treatment to maize in basal diet[a]	Protein (%)	Avg wt gain (g)	PER	Utilizable protein (%)
None	8.3	52	1.75	4.36
+ Lysine + tryptophan[b]	8.5	80	2.48	6.32
+ 8% Soybean flour + 0.15% L-lysine HCl	10.9	120	2.50	8.17
+ 8% Skim milk powder + 0.10% L-lysine HCl	9.8	111	2.63	7.73

Source: Elías and Bressani (14).
[a] 72.4% maize + 8.10% cooked black beans.
[b] 0.31% L-lysine HCl + 0.10% DL-tryptophan.

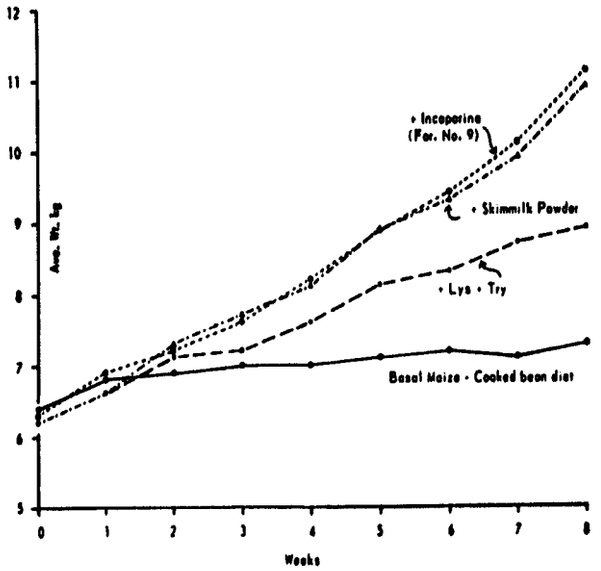

FIGURE 9 Weight gain of young pigs fed on maize–bean diets with and without supplements.

protein-supplemented than from the amino acid-supplemented maize–bean diet (14, 15).

Similar results may be obtained when the normal maize–bean diets are supplemented with protein-rich foods, either of animal origin or high-protein vegetable mixtures. Figure 9 summarizes one experiment in which the basal maize–bean diet was supplemented with lysine and tryptophan, powdered skim milk, and Incaparina 9. The results show that the two supplements were equally efficient and superior to lysine and tryptophan. However, the difference between the results from the supplements as compared with those from lysine and tryptophan is due to a higher protein concentration in the protein-supplemented diet.

All findings indicate that it is highly desirable to increase protein concentration in such diets, as well as protein quality. This is also evident in the results shown in Figure 10. In this case, the basal diet of maize and beans was supplemented with an additional 25% calories from fat. The dietary protein was replaced by 10, 20, 30, and 40% milk protein or equivalent amounts of lysine, tryptophan, and methionine (24). The nitrogen-balance results show that introducing whole protein produced higher values than did amino acids. Adding whole proteins probably provides other amino acids that might be deficient in the maize–bean diet, giving a better overall amino acid balance.

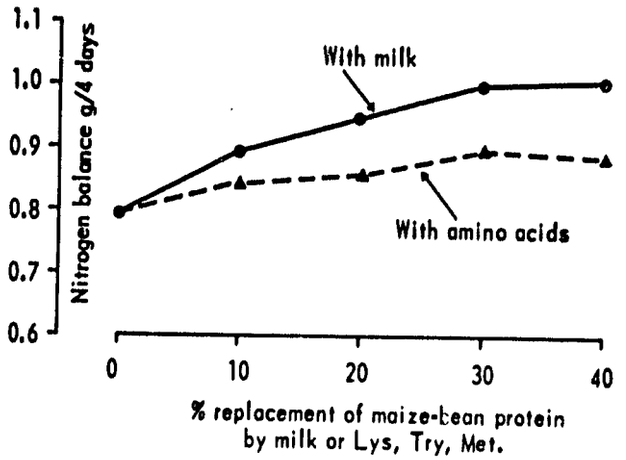

FIGURE 10 Effect of the substitution of maize–bean protein by milk proteins or equivalent amounts of lysine, tryptophan, and methionine.

The preceding findings provide the following conclusions:

1. Protein quality of normal maize can be improved by amino acid supplementation, by protein supplementation in low or large amounts, and by genetic means.

2. Protein quality of maize improved by any of the methods indicated is significantly higher than that of normal maize. In terms of total usable protein, protein-supplemented maize is superior in quality to amino acid-supplemented or opaque-2 maize.

3. Diets based on maize and small amounts of other foods are improved in their protein quality by using maize of higher protein value. They are improved in both protein quality and quantity when the maize used is protein supplemented with small or relatively large amounts of good-quality foods, such as milk, soybean flour, and others.

4. These diets are also deficient in calories, vitamins, and minerals. Therefore, protein-supplemented maize might be superior to amino acid-supplemented maize or opaque-2 maize. A higher calorie intake from maize is not recommended because of increased bulk and because the overall quality of the diet would decrease. The deficient vitamins and minerals are more easily provided in the diet when maize is protein supplemented than by the use of amino acid supplemented or opaque-2 maize.

5. Although there is no field evidence, it appears that maize of improved protein quality, whether obtained by genetic means, amino acid addition, or by protein supplementation, is capable of increasing the overall nutritional quality of maize-based diets. All efforts should be continued to put all these developments in the hands of people who need better-quality protein.

[*A discussion of this paper can be found on pp. 483–484 of* **Questions and Answers.**]

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QUALITY PROTEIN MAIZE IN SWINE NUTRITION

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It has long been recognized in swine nutrition research that the constituents of protein are the critical elements rather than the protein itself. Actually, swine do not require protein in the diet. Although about 20 amino acids have been recognized in animal feeds, only about 10 are considered essential for swine nutrition.

If a source of nitrogen is present, the nonessential amino acids can be synthesized by the pig's body tissue; therefore, they are not required in the diet. The nonessential amino acids cystine and tyrosine can partly satisfy the need for the essential amino acids methionine and phenylalanine, respectively. The essential amino acids of the pig are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Nonessential amino acids are alanine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, proline, serine, and tyrosine.

The value of any protein in swine nutrition can be determined by its ability to supply amino acids in the proportions and at the levels required by the animal. Since different life processes, such as maintenance, growth, gestation, and lactation, require different quantities of amino acids, the value of a specific feed or feed ingredient will vary with its ability to supply the necessary level and proportion of amino acids. It is well established that baby pigs and growing pigs require higher levels of amino acids than do older pigs being finished for the market. Also, it has been found that lactating sows

TABLE 1 Amino acid needs of the pig at various stages of development (percent of diet)

		Stage of development; weight of pig (kg)		
		Baby 4.5	Weanling 13.6	Finishing 45
Amino acid	Level of dietary protein (%):	22	16	12
Arginine		0.37	0.25	0.15
Histidine		0.34	0.23	0.14
Isoleucine		0.76	0.52	0.35
Leucine		0.98	0.67	0.40
Lysine		1.20	0.74	0.50
Methionine[a]		0.73	0.50	0.30
Phenylalanine[a]		0.79	0.54	0.32
Threonine		0.66	0.45	0.27
Tryptophan		0.18	0.12	0.07
Valine		0.67	0.46	0.28

Source: Becker et al. (2).

[a] Cystine can satisfy 40% of the total need for methionine, and tyrosine can satisfy 30% of the total need for phenylalanine.

must be supplied with adequate quality protein for high lactation performance.

Table 1 presents an example of different requirements of various ages and sizes of pigs. Each increase in age and size reflects a reduced level of amino acids required for normal performance. Figure 1 provides a graphic explanation for at least some of the different nutrition requirements from weaning to market. Each increase in pig body weight corresponds with an increase in body fat and a reduction in total protein. During the finishing period, the pig is accumulating more fat than protein, thus requiring less total protein in the diet.

Swine producers feed their animals a basic grain source and an adequate level of protein supplement to supply the total level and balance of amino acids. A high-quality, 20 to 22% protein diet generally is fed to young pigs (5 to 18 kg), a 16% protein diet for growing pigs (18 to 50 kg), and a 12 to 13% protein diet for finishing pigs (50 to 90 kg). Although gestating sows are less demanding in their requirements, lactating sows producing large quantities of milk also require a good-quality, 15 to 16% protein diet to meet their daily nutrient needs. These requirements are adequately furnished by normal maize supplemented with soybean meal, fish meal, or combinations of these with other high-protein supplements.

The value of high-quality protein maize, such as opaque-2 maize, is associated with its ability to substitute for part of the supplemental protein. In other words, it requires less supplemental protein to produce results equal to those obtained with adequately supplemented normal maize.

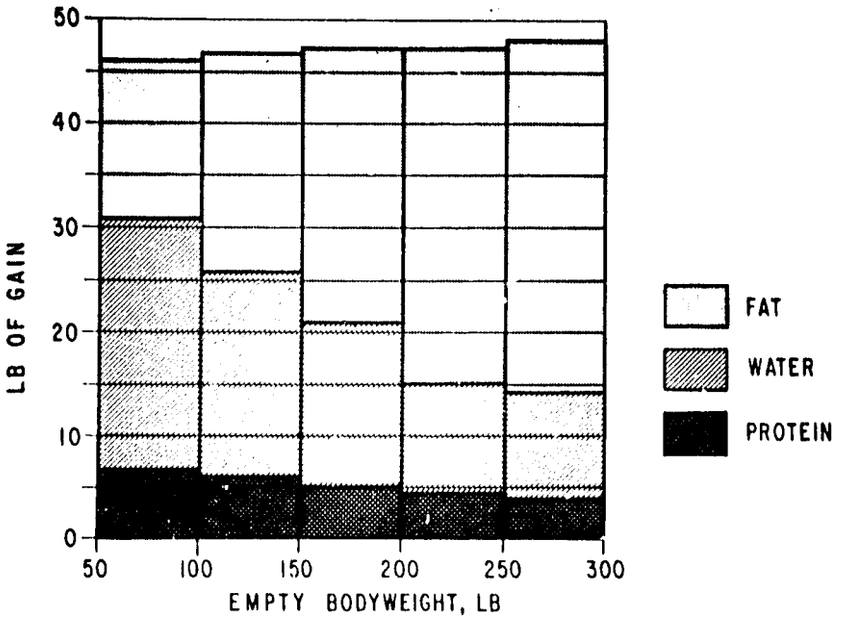

FIGURE 1 Amounts of fat, protein, and water in the gain of pigs at various stages of development.

For many decades, maize has been used as the basic grain for both human nutrition and for swine feeding. However, as early as 1914, nutritionists noted the poor quality of maize protein when Osborne and Mendel (30) showed that zein, the major protein of maize, is nearly devoid of lysine and tryptophan. Their work indicated that diets containing zein as the only protein cannot support growth in young rats. However, good growth was produced when both lysine and tryptophan were added as a combination in zein diets. Their studies demonstrated clearly for the first time that these amino acids are essential dietary components.

More recently, Edwin Mertz of Purdue University began to screen maize varieties for lower levels of zein in the endosperm. He knew that the zein fraction of maize was very low in lysine and tryptophan, that the biological value of this protein was very low, and that the glutelin fraction was a more complete and higher-quality protein. In 1963, L. Bates, a student of Mertz, working with Mertz and O. E. Nelson, identified a variety with an unusually high level of lysine, which was an opaque-2 mutant. Although the mutant had been identified and studied some 30 years before by Emerson et al. (7) of Cornell University, it was not until 1963 that the biological potential of this mutant was realized. The reevaluation of the opaque-2 mutant gene and discovery of the improved amino acid patterns of the endosperm protein (27) stimulated a reappraisal of maize as an animal feed.

TABLE 2 Protein and amino acid composition of Colombian normal, opaque-2, and floury-2 maize

Amino acids	Normal (g/100 g protein)	Opaque-2 (g/100 g protein)	Floury-2 (g/100 g protein)
Essential			
Arginine	4.6	6.2	4.3
Histidine	2.9	2.7	1.1
Isoleucine	5.0	3.6	3.1
Leucine	16.1	8.0	10.5
Lysine	2.8	4.0	2.3
Methionine	3.0	4.5	0.8
½ Cystine	1.9	1.8	2.7
Phenylalanine	5.4	3.8	4.0
Threonine	3.5	3.3	3.0
Tryptophan	0.6	1.0	0.7
Valine	7.2	5.6	4.4
Nonessential			
Alanine	9.4	5.9	6.8
Aspartic acid	6.7	8.6	6.6
Glutamic acid	20.6	15.2	15.7
Glycine	4.1	5.3	2.9
Proline	8.9	7.2	7.5
Serine	4.6	3.9	4.0
Tyrosine	4.9	3.5	3.1
Ammonia	2.9	2.0	0.5
Protein	10.0	10.3	9.8

Source: Maner et al. (26).

Table 2 presents the protein and amino acid composition of normal, opaque-2, and floury-2 maizes. Table 3 compares the amino acids supplied by each in an all-maize diet (95.8% maize and 4.2% vitamins and minerals) to the requirements of a baby pig, a growing pig, and a finishing pig. These tables show that normal maize is limiting in lysine and tryptophan for all age groups and deficient in threonine and isoleucine for both the baby and growing pig. The deficiency of isoleucine may be complicated by a large excess of leucine. Opaque-2 maize is deficient in lysine for all age groups and contains adequate tryptophan, threonine, and isoleucine to meet the needs of only finishing pigs. The Colombian floury-2 maize contains amino acid levels similar to normal maize.

OPAQUE-2 MAIZE

The initial opaque-2 maize work with pigs was reported by Beeson et al. (3) and Pickett (32) of Purdue University. They showed that pigs between 13.8 and 25.7 kg grew 3.6 times faster on opaque-2 maize than on normal maize. Pigs fed opaque-2 maize grew at a rate equal to that of pigs fed a diet of normal maize and soybean meal that supplied the same quantity of crude

TABLE 3 Quantity of amino acids supplied by an all-maize diet as compared with the requirement for a 13.6 kg pig

	Total requirement as % of diet (kg)			In 95.8 kg of maize		
Amino acid	4.5	13.5	45	Normal (kg)	Opaque-2 (kg)	Floury-2 (kg)
Arginine	0.37	0.25	0.15	0.44	0.61	0.40
Histidine	0.34	0.23	0.14	0.28	0.27	0.10
Isoleucine	0.76	0.52	0.35	0.48	0.55	0.29
Leucine	0.98	0.67	0.40	1.56	0.79	0.99
Lysine	1.20	0.74	0.50	0.27	0.59	0.22
Methionine	0.44	0.30	0.18	0.29	0.44	0.08
Cystine	0.29	0.20	0.12	0.18	0.18	0.25
Threonine	0.66	0.45	0.27	0.33	0.33	0.28
Tryptophan	0.18	0.12	0.07	0.06	0.10	0.07
Valine	0.67	0.46	0.28	0.69	0.55	0.41
Phenylalanine	0.55	0.38	0.22	0.52	0.37	0.38
Tyrosine	0.24	0.16	0.10	0.47	0.35	0.29

protein (Table 4). Finishing pigs, with an average initial body weight of 59 kg, gained weight 50% faster on opaque-2 maize than on normal maize, a rate of gain similar to that of pigs fed normal maize – soybean meal diets containing 13.0% protein.

In later studies at Purdue (5), 10- to 13-kg pigs were fed opaque-2 maize containing 104% more lysine and 67% more tryptophan than a normal maize. When these two maizes were compared, pigs consuming opaque-2

TABLE 4 Nutritional value of opaque-2 maize for growing and finishing swine

	Treatments			
	1 Normal maize	2 Opaque-2 maize	3 Normal maize + soybean meal	4 Normal maize + soybean meal
Growing pigs				
Protein level (%)	8.6	11.6	11.6	
Avg initial wt (kg)	14.0	13.8	13.7	
Avg final wt (kg)	17.3	25.8	25.6	
Avg daily gain (kg)	0.12	0.43	0.42	
Avg daily feed (kg)	0.82	1.41	1.37	
Feed/gain ratio	6.88	3.32	3.23	
Finishing pigs				
Protein level (%)	8.9	11.2	11.2	13.0
Avg initial wt (kg)	59.5	59.4	59.0	60.4
Avg final wt (kg)	73.8	81.0	84.3	85.2
Avg daily gain (kg)	0.68	1.03	0.88	1.09
Avg daily feed (kg)	3.21	3.70	3.29	3.97
Feed/gain ratio	4.71	3.59	3.74	3.65

Source: Brown et al. (3).

maize as the sole source of protein and energy gained 4.3 times faster than pigs receiving normal maize (0.26 versus 0.06 kg/day).

Since the opaque-2 maize contained more total protein than the normal maize (11.6 versus 9.1%), nonessential nitrogen was added as diammonium citrate, glutamic acid, and glycine to the normal maize diet to make it isonitrogenous with the opaque-2 maize diet.

The nonessential nitrogen did not improve the performance of pigs fed normal maize. Neither lysine nor tryptophan supplementation significantly affected performance of pigs fed normal maize. However, either (1) combining the two amino acids as an addition to the normal maize diets at levels calculated to provide total lysine and tryptophan equal to that in opaque-2 diets or (2) adding soybean meal at a level to provide total protein equal to the opaque-2 maize diet improved performance to the level of that supported by the opaque-2 maize diet.

Since these initial pig trials, many studies have been reported from many countries that demonstrate the value of opaque-2maize in swine diets (1, 4, 6, 8–12, 16, 20–22, 24–26, 28, 29, 31, 35, 37). Although it is behond the scope of this paper to make a complete summary of all published papers, adequate information will be given to demonstrate the value of opaque-2 maize in swine nutrition during all phases of the life cycle.

Baby Pigs

Baby pigs, because of their requirement for higher levels and quality of protein (Table 1), provide a sensitive and critical test for assessing the value of the opaque-2 maize protein. Thirty-five-day-old pigs averaging 8.9 kg were fed diets in which all the protein was supplied by either normal maize or opaque-2 maize (24). The groups fed only maize were compared with groups fed standard control diets. The control diets contained a constant proportion of maize and soybean meal, but at differing levels of protein (Table 5); protein level was varied by diluting the protein with sucrose.

The differences in pig response to each diet were striking (Table 6 and Figure 2). Each decrease in protein level in the control maize–soybean diets, from 16 to 10 to 7%, was accompanied by corresponding decreases in daily feed consumption and average daily gain, with increases in feed required to produce 1 kg of gain.

Baby pigs performed very poorly when fed normal maize as the sole source of protein, averaging gains of only 21 g/day during the entire 130-day experimental period and requiring 35.19 kg of feed to produce 1 kg of gain. The low level of feed consumed (0.74 kg/day) indicates the nutritional unpalatability caused by the very poor balance of essential amino acids in the normal maize.

As the experiment progressed, the pigs fed only normal maize demonstrated the classical symptoms of protein deficiency, and some died a few days after the experiment was terminated at 130 days (35). All animals were

TABLE 5 Comparison of normal maize-soybean, normal maize, and opaque-2 maize diets fed at different protein levels to pigs from 35 to 165 days of age

Ingredients	Diet 1	2	3	4	5	6	7
Opaque-2 maize	–		–	–	91.00	64.00	–
Normal maize	72.70	45.45	31.43	91.00	–	–	91.00
Soybean meal	16.00	10.00	7.00	–			–
Sucrose	6.65	39.90	56.92	4.35	4.35	31.35	4.35
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Calcium carbonate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-Lysine HCl					–	–	0.28
L-Tryptophan							0.04
	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Protein (%)	16	10	7	10	10	7	10
Calcium (%)	0.87	0.85	0.84	0.83	0.83	0.83	0.83
Phosphorus (%)	0.65	0.54	0.48	0.60	0.60	0.53	0.60

Source: Maner et al. (24).

autopsied and their tissues examined histologically by both light and electron microscopy. Changes of the stomach, duodenum, and jejunum were characterized by growth arrest and atrophy of the lining epithelium and development arrest of essential organelle formation necessary for normal cellular function. The ribs and long bones indicated a distinct cessation of skeletal-system development. The livers of all the pigs of this group were fatty and friable, with microscopically lobular fatty accumulations.

In contrast, pigs fed the opaque-2 maize diet performed much better than the normal maize-fed pigs (Figure 3). Their growth rate was 254 g/day, 12

TABLE 6 Performance of pigs fed opaque-2 maize, normal maize, and normal maize-soybean diets at different protein levels from 35 to 165 days of age

Treatment	Avg daily gain (kg)[1]	Feed efficiency[1]	Daily feed consumed (kg)
1. Normal maize-soybean, 16%	0.722^{a}	3.27^{e}	2.36
2. Normal maize-soybean, 10%	0.506^{b}	3.64^{e}	1.84
3. Normal maize-soybean, 7%	0.158^{d}	5.85^{c}	0.92
4. Normal maize, 10%	0.021^{e}	35.19^{a}	0.74
5. Opaque-2 maize, 10%	0.254^{c}	4.43^{d}	1.13
6. Opaque-2 maize, 7%	0.076^{de}	8.80^{b}	0.67
7. Normal maize + lysine + tryptophan, 10%	0.143^{d}	5.29^{c}	0.76

Source: Maner et al. (24).

[1] a, b, c, d, e values in the same column with different superscripts are statistically different.

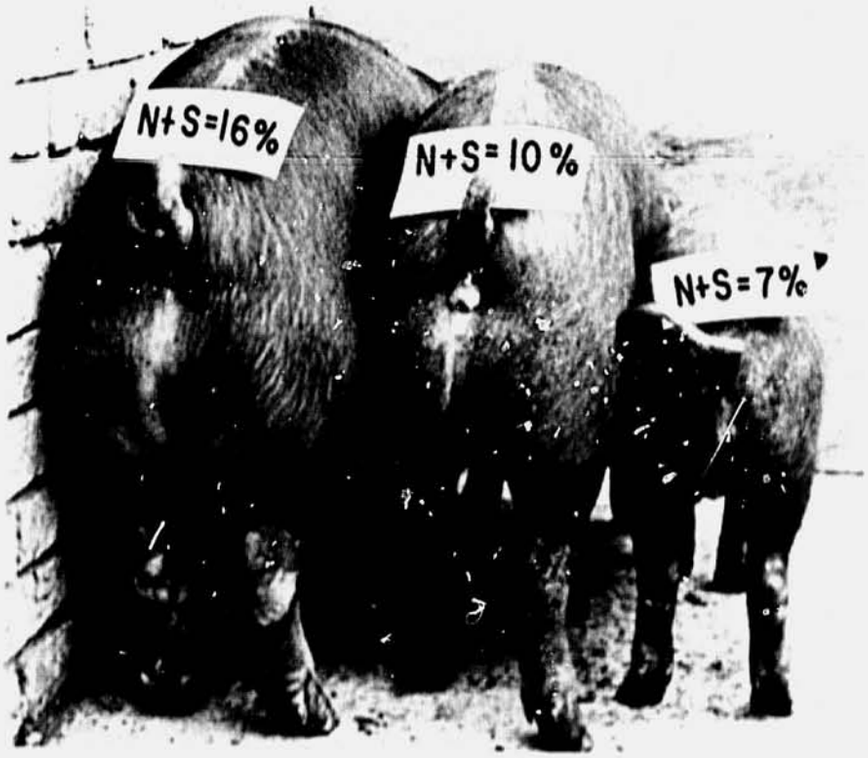

FIGURE 2 Effect of protein level on the performance of pigs from 35 to 165 days of age.

times greater than those fed normal maize. These pigs consumed 53% more feed, but required only 4.43 kg/kg of body weight gain. The performance of pigs fed on opaque-2 diet was much inferior, however, to that of pigs fed an equal level of protein supplied by maize and soybean meal. This lesser performance of the opaque-2 diet indicated that, although the level and balance of essential amino acids of the opaque-2 maize is much better than that of normal maize, the proportion and level of essential amino acids in that diet does not support a level of growth equal to that produced by the maize–soybean control diet containing an equal level of protein but a superior balance of the essential amino acids.

Although the opaque-2 diets showed neither a growth rate nor feed conversion ratio equal to that produced by the control diet with equal protein, the pigs fed the opaque-2 maize demonstrated an overall histopathological soundness and a general absence of gross protein deficiency symptoms. The level of amino acids and nitrogen supplied by opaque-2 maize permitted maintenance of the cellular organelle structures, but the total epithelial structure still did not equal the quantity observed in normal, protein-adequate animals, nor did opaque-2 maize alone completely prevent fatty changes of the liver.

FIGURE 3 Comparison of pigs fed either opaque-2 or normal maize from 35 to 165 days of age. N, normal maize; O, opaque-2 maize.

The pigs demonstrated improved gains, feed conversion efficiency, and general soundness when fed the normal maize diet supplemented with 0.28% L-lysine HCl and 0.04% L-tryptophan. However, the performance of the pigs on the normal maize diet was inferior to both the control and opaque-2 diets.

When it was found that (1) the opaque-2 maize failed to supply a level and balance of amino acids that would support performance equal to that of the control (maize–soybean) diet with equal protein, and (2) that supplementing normal maize with both lysine and tryptophan failed to improve the quality of normal maize to a level observed in opaque-2 maize, both these maizes were studied for comparative responses to amino acid supplementation (10, 11). All treatments were based on opaque-2 or normal maize basal diets (Table 7) to which 0.28% L-lysine HCl was added. L-tryptophan (0.04%), L-threonine (0.08%), and DL-methionine (0.10%) were added to each maize source in a 2 by 2 by 2 factorial arrangement of treatments so that each amino acid appeared alone, and in all possible combinations. A normal maize–soybean meal control diet containing an equal level of protein was included as positive control.

One hundred and thirty-six pigs averaging 9 kg were fed the 17 dietary

TABLE 7 Composition of diets utilized to compare response of 9-kg pigs to amino acid supplementation of opaque-2 or normal maize

Composition of diet	Diet 1: Maize-soybean (9.9% protein)	Diet 2: Maize test diet
Maize	45.45	91.00
Soybean meal	10.00	–
Sucrose	39.90	–
Bone meal	3.00	–
Vitamin premix	1.00	–
Mineral premix	0.40	–
L-lysine HCl	–	0.28
Amino acids	–	+++[1]

[1] Addition of amino acids listed in Table 8 to basal maize test diet to form experimental diets.

treatments for 21 days. The performance data from these treatments are shown in Table 8. Pigs fed normal maize plus lysine responded only to tryptophan supplementation. No individual or additional response was observed to either threonine or methionine supplementation.

When opaque-2 maize was used with lysine, a significant improvement in both growth and feed efficiency was observed when both tryptophan and threonine were added. Not one of the supplemental amino acids alone

TABLE 8 Comparative response of pigs fed normal or opaque-2 maize supplemented with lysine, tryptophan, threonine, and methionine singly or in combinations

Treatments	Avg daily gain (kg)	Feed/gain ratio
1. Normal maize (NM) + 0.28% lys	0.02	16.02
2. NM + lys + try	0.15	4.08
3. NM + lys + thre	0.02	18.63
4. NM + lys + met	0.03	12.68
5. NM + lys + try + thre	0.16	3.66
6. NM + lys + try + met	0.18	3.65
7. NM + lys + thre + met	0.03	13.32
8. NM + lys + try + thre + met	0.16	3.75
9. Opaque-2 (OM) + 0.28% lys	0.22	3.72
10. OM + lys + try	0.20	3.80
11. OM + lys + thre	0.18	3.64
12. OM + lys + met	0.19	3.62
13. OM + lys + try + thre	0.32	2.89
14. OM + lys + try + met	0.21	3.55
15. OM + lys + thre + met	0.17	3.52
16. OM + lys + try + thre + met	0.28	2.93
17. Normal maize–soybean (9.9% protein)	0.19	3.45

Source: Gallo et al. (11).

Note: Amino acids were added at the following levels: L-lysine (lys), 0.28%; L-tryptophan (try), 0.04%; L-threonine (thre), 0.08%; and DL-methionine (met), 0.10%.

elicited a response, nor did the addition of methionine improve growth rate over that observed when both tryptophan and threonine were added to the basal (maize plus lysine) diet.

Normal maize plus lysine and tryptophan supported performance equal to that of the (maize – soybean, 9.9%) control, as did opaque-2 maize supplemented with lysine. Supplementing opaque-2 maize with lysine, tryptophan, and threonine (diet 13) in this study produced results superior to those produced by the maize – soybean control fed at the same level of protein (diet 17).

Drews et al. (6) fed increasing levels of soybean meal with normal maize and opaque-2 maize to 120 pigs averaging 25 days of age and 5.187 kg. At levels of up to 28.7% soybean meal in the diet (19.5 and 20.5% protein for normal and opaque-2 maize, respectively), they reported that the pigs receiving the opaque-2 maize averaged greater gains and required less feed per unit of gain than pigs fed normal maize. However, a reversal occurred at higher levels of soybean meal supplementation. At suboptimal protein levels, early-weaned pigs gained significantly more with lower feed – gain ratios when opaque-2 maize replaced an equal amount of normal maize in the diet (Table 9).

Growing Pigs

The protein and amino acid levels of opaque-2 maize are not adequate to support satisfactory growth of pigs at an initial weight of 9 kg; however, this maize provides a more adequate source of protein for larger pigs owing to their lower total protein and amino acid requirements. Studies (23) show that when opaque-2 maize is the only source of protein and energy (Table 10) for pigs weighing between 18 and 50 kg, it supports growth equal to 75% (0.455 versus 0.610 kg) of that obtained with the 16% protein (maize – soybean) control diet. Similarly, opaque-2 maize will support growth equal to 364% of that obtained when such animals are fed normal maize (0.455 versus 0.125 kg) as the sole source of protein. The opaque-2 maize was

TABLE 9 Performance of baby pigs fed varying levels of soybean meal with either normal or opaque-2 maize

Level of soybean meal (%)	Normal maize			Opaque-2 maize		
	Level of protein (%)	Total gain (kg)	Feed-gain ratio	Level of protein (%)	Total gain (kg)	Feed-gain ratio
18.7	15.5	9.07	2.03	16.5	10.76	1.87
23.7	17.5	9.74	1.90	18.5	9.67	1.66
28.7	19.5	10.88	1.77	20.5	11.28	1.68
33.7	21.5	10.18	1.74	22.5	8.99	1.62
38.7	23.5	11.24	1.66	24.5	7.06	1.80

Source: Drews et al. (6).

TABLE 10 Performance of growing pigs (18 to 50 kg) fed either normal maize, opaque-2 maize, or a mixture of maize-soybean meal

Diet	1 Maize-soybean (16% protein)	2 Opaque-2 (9.7% protein)	3 Normal maize (9.7% protein)
Average daily gain (kg)	0.610	0.455	0.125
Feed-gain ratio	2.69	3.46	7.26
Days to 50 kg	56	79	112[a]

Source: Maner and Gallo (25).
[a] At the termination of the study, pigs weighed an average of only 31.7 kg.

inferior to the adequate maize–soybean diet in both gain and feed conversion, and the pigs required 41% longer to reach 50 kg body weight; however, when fed as the only source of protein, it was almost four times more productive than normal maize.

Since opaque-2 maize cannot be utilized as the only source of protein during the growing period, studies were conducted to determine at what level of substitution the opaque-2 maize could be utilized to reduce the total level of dietary protein. Table 11 compares diets containing 16, 12, and 9.7% protein. These diets were based on either normal maize or opaque-2 maize supplemented with soybean meal where necessary to supply the required level of protein.

The results from feeding these diets (Table 12) clearly demonstrate that, for growing pigs, there are no advantages in using opaque-2 maize to substitute for normal maize in diets containing adequate (16%) levels of protein. However, when opaque-2 maize was utilized in a 12% protein diet, growth was obtained equal to that provided by optimum diets (16%), even though the supplemental protein (soybean meal) was reduced by 66%. At this level of substitution, slightly more feed was required to produce 1 kg of gain, but growth was not significantly different. This finding was in contrast to that for the 12% normal maize–soybean diet, which supported lesser rates

TABLE 11 Diet composition of basal diets used in opaque-2 maize studies

Composition of diet	% Protein		
	16	12	9.7
Normal maize	80.75	90.42	96.25
Soybean meal	15.75	5.88	
Bone meal	2.00	2.50	2.50
Calcium carbonate	0.50	0.20	0.25
Vitamin premix	0.60	0.60	0.60
Mineral premix	0.40	0.40	0.40
Amino acids			***[a]

[a] Addition of amino acids listed in Table 8 to basal maize test diet to form experimental diets.

TABLE 12 Performance of pigs fed opaque-2 and normal maize during the growing period

Treatment	Avg. daily gain[1] (kg)	Feed-gain ratio[1] (kg)
1. Normal maize-soybean, 16%	0.88^{a}	2.40^{w}
2. Opaque-2 maize-soybean, 16%	0.79^{abc}	2.45^{w}
3. Normal maize-soybean, 12%	0.69^{cd}	5.02^{y}
4. Opaque-2 maize-soybean, 12%	0.80^{ab}	2.66^{wx}
5. Normal maize, 10%	0.56^{e}	4.08^{z}
6. Opaque-2 maize, 10%	0.64^{d}	2.94^{xy}
7. Normal maize + lysine + tryptophan, 10%	0.66^{d}	5.09^{y}
8. Opaque-2 maize + lysine + tryptophan, 10%	0.75^{bcd}	2.80^{xy}
9. Normal maize + lysine + D-tryptophan, 10%	0.64^{d}	5.15^{y}

Source: Gallo et al. 18.

[1] a, b, c, d, w, x, y, z values in the same column with different superscripts are statistically different.

of growth and efficiency of feed conversion than those obtained with either the control (16%) or 12% opaque-2 maize – soybean meal rations.

When opaque-2 maize was used as the only source of protein, performance was similar to the 12% normal maize – soybean diet, but significantly inferior to either the 16% controls of the 12% diet based on opaque-2 maize. On the other hand, opaque-2 maize alone supported growth rates double those of unsupplemented normal maize diets. Normal maize, but not opaque-2 maize, was significantly improved by adding 0.28% L-lysine and 0.04% L-tryptophan.

A summary of other published work on the value of opaque-2 maize for growing pigs is presented in Table 13. In each case, the superiority of opaque-2 maize over normal maize is demonstrated. Generally, the opaque-2 maize requires a lower level of supplemental protein to equal gains obtained with normal maize. Lysine is the first limiting amino acid of opaque-2 maize. Studies have indicated that, although opaque-2 maize is superior to normal maize for growing pigs (18 to 50 kg), it will not supply the level of amino acids required to support growth equal to that provided by an adequate control diet. Hence, the nutritive values of opaque-2 and normal maize, with and without amino acid suplementation, were compared to that of an adequate 16% protein basal maize – soybean diet. Amino acid levels were adjusted for each maize source in an attempt to equalize the amino acid adequacy of each. Previous studies had shown that lysine, tryptophan, and threonine supplementation improves growth. Isoleucine was added in addition to these three supplements.

All amino acids were added in the L form and were calculated to provide equal levels of total lysine, tryptophan, threonine, and isoleucine. The

normal maize diet was supplemented with 0.56% L-lysine, 0.08% L-tryptophan, 0.12% L-threonine, and 0.06% L-isoleucine. The opaque-2 maize diet was supplemented with 0.28% L-lysine, 0.04% L-tryptophan, 0.08% L-threonine, and 0.06% L-isoleucine. These diets were compared to maize–soybean diets containing 16 and 9% protein and to those containing only opaque-2 or normal maize.

Table 14 presents the performance data obtained from this 21-day trial. Normal maize fed as the only source of protein supported only poor growth. Supplementation with the four amino acids improved growth to a level obtained with either opaque-2 maize or the 9% maize–soybean control (diet 2). However, both growth and feed conversion of animals fed this amino acid supplemented diet were inferior to those obtained with the adequate 16% control.

Opaque-2 maize alone supported a level of performance similar to and not statistically different from the 9% maize–soybean control, but its performance was inferior to the 16% maize–soybean control. When the opaque-2 maize diet was supplemented with the four amino acids, overall performance improved to a level not significantly different from that of the 16% protein maize–soybean meal control. These data indicate that the level of protein and amino acids (other than the four added) are adequate for optimal pig performance, and that supplementations with the four amino acids enable the opaque-2 maize protein to support a level of growth equal to that of the adequate control.

Finishing Pigs

The finishing pig (50 to 90 kg) requires less protein and has a lower requirement for amino acids than the growing pig. As shown in Table 15 (8, 9), opaque-2 maize fed as the only source of protein in the diet is adequate to support optimal growth. Pigs fed this diet grew as rapidly and as efficiently as those receiving the control 16% maize–soybean diet. However, those fed normal maize as the protein source had slower and less efficient gains. When 0.28% lysine and 0.04% tryptophan were added as supplement to the normal maize diet, gains were improved to a level equal to the control or opaque-2 maize diet. Therefore, opaque-2 maize alone can serve as the only source of protein and energy for the finishing pig.

In similar studies (12), an opaque-2 maize–soybean meal diet containing 11% protein produced growth and feed conversion equal to the 13% protein normal maize–soybean meal diet. Similar results were produced by normal maize diets containing 11% protein supplemented with soybean meal and lysine. However, a normal maize diet supplemented only with soybean meal to produce an 11% diet was inferior to all other diets. Since this study did not include opaque-2 maize as the only source of protein, a direct comparison of results with other studies is not possible.

TABLE 15 Comparative value of opaque-2 and normal maize for the growing pig

University (U.S.A.)	Treatment[1] (protein level, %)	Initial weight (kg)	Avg daily gain[2] (kg)	Avg daily feed[2] (kg)	Feed to gain ratio[2] (kg)	Source
Purdue	OP (11.2)	10	0.26[a]	0.91[a]	3.46[a]	Cromwell et al. (5)
	NM (8.8)	10	0.06[b]	0.64[b]	9.88[b]	Cromwell et al. (5)
	NM + N (11.2)	10	0.06[b]	0.65[b]	9.98[b]	Cromwell et al. (5)
	NM + N + L (11.2)	10	0.06[b]	0.54[b]	8.52[b]	Cromwell et al. (5)
	NM + N + L + T (11.2)	10	0.21[a]	0.87[a]	4.16[a]	Cromwell et al. (5)
	NM + SBM (11.2)	10	0.21[a]	0.91[a]	4.41[a]	Cromwell et al. (5)
Purdue	NM + SBM (16)	15	0.44[a]	1.14[a]	2.57[a]	Cromwell et al. (5)
	OP + SBM (16)	15	0.58[b]	1.39[b]	2.40[a]	Cromwell et al. (5)
	NM + SBM (14)	15	0.42[a]	1.11[a]	2.67[a]	Cromwell et al. (5)
	OP + SBM (14)	15	0.40[a]	1.08[a]	2.67[a]	Cromwell et al. (5)
	NM + SBM (11.8)	15	0.31[a]	1.08[a]	3.49[b]	Cromwell et al. (5)
	OP + SBM (11.2)	15	0.35[a]	1.14[a]	3.41[b]	Cromwell et al. (5)
Minnesota	NM + SBM (16)	15.5	0.66[f]	1.38	2.09	Pick and Meade (31)
	OP + SBM (15)	15.5	0.66[f]	1.35	2.04	Pick and Meade (31)
	OP + SBM (13.5)	15.5	0.59[ef]	1.31	2.22	Pick and Meade (31)
	OP + SBM (11.9)	15.5	0.57[ef]	1.36	2.39	Pick and Meade (31)
	OP + SBM (10.3)	15.5	0.51[e]	1.31	2.57	Pick and Meade (31)
	OP (8.7)	15.5	0.38[c]	1.23	3.23	Pick and Meade (31)
Purdue	CC (8.5)	15	0.15	0.88	5.90	Klein et al. (16)
	OP (11.5)	15	0.36	1.12	3.10	Klein et al. (16)
	OP (8.5)	15	0.22	1.06	4.80	Klein et al. (16)
	CC + AA (9.1)	15	0.31	1.02	3.30	Klein et al. (16)

TABLE 13 (cont.)

University (U.S.A.)	Treatment[1] (protein level, %)	Initial weight (kg)	Avg daily gain (kg)	Avg daily feed (kg)	Feed-gain ratio (kg)	Source
Virginia Polytechnic Institute (VPI)	NM + PM (16)	24	0.26	1.30	5.00	Thomas and Kornegay (37)
	NM + PM + L (16)	24	0.48	1.47	3.28	Thomas and Kornegay (37)
	NM + PM (14)	24	0.24	1.32	5.50	Thomas and Kornegay (37)
	NM + PM + L (14)	24	0.37	1.46	3.95	Thomas and Kornegay (37)
	OP + PM (14)	24	0.36	1.51	4.20	Thomas and Kornegay (37)
	OP + PM (16)	24	0.37	1.47	3.97	Thomas and Kornegay (37)
	NM + PM (16)	18	0.25	1.18	4.81	Thomas and Kornegay (37)
	NM + PM + L (16)	18	0.42	1.57	3.71	Thomas and Kornegay (37)
	OP + PM (16)	18	0.35	1.36	3.90	Thomas and Kornegay (37)
	OP + PM + L (16)	18	0.55	1.75	3.16	Thomas and Kornegay (37)
Purdue	NM + SBM (16)	20	0.63	1.99	3.16	Gipp and Cline (12)
	NM + SBM (14)	20	0.60	2.08	3.46	Gipp and Cline (12)
	NM + SBM + L (14)	20	0.65	2.03	3.12	Gipp and Cline (12)
	OP + SBM (14)	20	0.64	1.91	2.98	Gipp and Cline (12)
	OP + L (11)	20	0.57	1.88	3.29	Gipp and Cline (12)

[1] Opaque-2 maize (OP), normal maize (NM), nonessential nitrogen (N), lysine (L), tryptophan (T), soybean meal (SBM), amino acids (AA), peanut meal (PM).

[2] a, b, c, e, f values in same column with different superscripts are statistically different.

TABLE 14 Performance of pigs fed same total level of amino acids with opaque-2 or normal maize

Treatment[a]	Avg daily gain (kg)	Feed-gain ratio
1. Normal maize + soybean, 16%	0.73	2.50
2. Normal maize + soybean, 9%	0.47	3.33
3. Normal maize (NM), 9%	0.22	4.88
4. Opaque-2 maize (OM), 9%	0.52	2.76
5. NM + 0.56% lys + 0.08% try + 0.12 thre + 0.06% iso	0.45	4.00
6. OM + 0.28% lys + 0.04% try + 0.08% thre + 0.06% iso	0.68	2.64

Source: Gallo, J. T., and J. H. Maner. Unpublished data.
[a] Lysine (lys), tryptophan (try), threonine (thre), and isoleucine (iso).

Other studies by Maner et al. (26) confirmed earlier findings, clearly demonstrating that the protein level and quality of the opaque-2 maize diet is completely adequate for finishing pigs.

Growing–Finishing

If 18- to 29-kg pigs are fed similar diets (Table 11) during the entire growing and finishing period (18 to 90 kg), the overall results are similar to those obtained at 59 kg (Table 16). Neither 0.28% lysine added to the opaque-2 maize diet nor 0.28% L-lysine plus 0.04% L-tryptophan added to an all-maize protein diet was adequate to support levels of performance equal to those obtained with the 16% protein control. The lysine and tryptophan supplementation of the normal maize diet, however, did significantly improve both growth and efficiency of feed utilization (23).

Brazilian studies (17) showed results similar to those reported here, except that the diet of normal maize as the only source of protein showed better results because the pigs were started at a heavier weight (30 versus 18 kg). These studies indicate that opaque-2 maize fed to growing–finishing pigs is inferior to an adequate 16% protein diet, but is significantly superior to the diet with normal maize as the only protein source.

Purdue University studies (12) indicate that a diet consisting only of opaque-2 maize can substitute for 16 to 13% protein maize–soybean meal diets (for growing–finishing pigs), if the opaque-2 diet is supplemented with lysine or a little soybean meal.

Gestation

Different levels of protein fed during complete gestation (13, 15, 18, 19) produce similar litter size, pig birth weight, and weaning weight at different ages. Pregnant swine exhibit a remarkable capacity to provide nutrients to the developing fetuses despite severe protein restriction. No deleterious effects on offspring at birth were observed when sows were fed 5% protein

TABLE 15 Comparative value of opaque-2 and normal maize for finishing pigs

Country	Treatment[1] (protein level, %)	Pig wt. (kg)	Ave daily gain[2] (kg)	Avg daily feed[2] (kg)	Feed-gain ratio[2]	Source
Colombia	NM + SBM (16)	50-90	0.79[a]	2.65	3.35[b]	Gallo et al. (9)
	NM (10)	50-90	0.62[b]	2.84	4.58[z]	Gallo et al. (9)
	OP (10)	50-90	0.81[a]	2.97	3.67[b]	Gallo et al. (9)
	NM + L + T (10)	50-90	0.81[a]	2.89	3.57[b]	Gallo et al. (9)
U.S.A. (Purdue U.)	NM + SBM (13)	53-92	0.75[c]	3.16	4.21[a]	Gipp and Cline (12)
	OP + SBM (11)	53-92	0.73[a]	3.16	4.33[a]	Gipp and Cline (12)
	NM + SBM (11)	53-92	0.62[b]	3.04	4.90[a]	Gipp and Cline (12)
	OP + L (11)	53-92	0.79[a]	2.99	3.79[b]	Gipp and Cline (12)
	NM + SBM + L (11)	53-92	0.69[ab]	3.22	4.67[a]	Gipp and Cline (12)

[1] Normal maize (NM), soybean meal (SBM), opaque-2 maize (OP), lysine (L), tryptophan (T).

[2] a, b, c, y, z values in same column with different superscripts by same author are statistically different.

TABLE 16 Comparative value of opaque-2 and normal maize for growing-finishing pigs

Country	Treatment[1] (protein level, %)	Pig weight (kg)	Avg daily gain[2] (kg)	Avg daily feed (kg)	Feed-gain ratio[2] (kg)	Source
Brazil	NM (10.5)	30-20	0.358[a]	1.78	4.98[a]	Kronka et al. (17)
	OP (10.7)	30-100	0.628[b]	2.46	3.91[b]	Kronka et al. (17)
	NM + SBM (15.3)	30-110	0.712[b]	2.55	3.58[b]	Kronka et al. (17)
	OP + SBM (16.1)	30-109	0.705[b]	2.62	3.71[b]	Kronka et al. (17)
U.S.A. (Purdue U.)	NM + SBM (16-13)	20-90	0.69	2.33	3.37	Gipp and Cline (12)
	OP + SBM (14-11)	20-90	0.69	2.55	3.69	Gipp and Cline (12)
	NM + SBM (14-11)	20-90	0.61	2.57	4.22	Gipp and Cline (12)
	OP + L (11-11)	20-90	0.68	2.43	3.58	Gipp and Cline (12)
	NM + SBM + L (14-11)	20-90	0.67	2.63	3.92	Gipp and Cline (12)
Colombia	NM + SBM (16)	18-90	0.66[a]	2.13	3.22[a]	Gallo and Maner 1969
	NM (10)	18-32	0.12[b]	0.87	7.26[b]	Gallo and Maner 1969
	OP (10)	18-90	0.47[c]	1.65	3.52[a]	Gallo and Maner 1969
	NM + L + T (10)	18-90	0.40[c]	1.48	3.69[a]	Gallo and Maner 1969
	OP + L (10)	18-90	0.53[c]	1.78	3.36[a]	Gallo and Maner 1969
Colombia	NM + SBM (16-16)	20-90	0.78	2.37	3.04	Maner et al. (25)
	NM + SBM (16-12)	20-90	0.79	2.49	3.15	Maner et al. (25)
	OP + SBM (16-10)	20-90	0.76	2.46	3.24	Maner et al. (25)

[1] Normal maize (NM), opaque-2 maize (OP), soybean meal (SBM), lysine (L), tryptophan (T).
[2] a, b, c values in same column with different superscripts by same author are statistically different.

diets during pregnancy (4, 34). Even when fed an essentially protein-free diet from day 24 of pregnancy to parturition, the sows produced normal-sized litters with normal pig birth weight (33, 36).

Opaque-2 maize has been fed as the only source of protein and energy to gestating sows without altering normal production (1, 13, 14). Control sows fed only normal maize, or maize–soybean meal diets containing from 11.2 to 20% crude protein, during gestation, farrowed litters of similar size and pig birth weight as did those fed the opaque-2 maize diet. However, number of pigs weaned per litter, litter gain, and total litter weaning weight were superior from gilts fed the opaque-2 maize diets, as compared with those fed the normal maize diet (1).

Lactation

Mahan and co-workers (20,21) at the University of Illinois fed opaque-2 maize supplemented only with vitamins and minerals to first- and second-litter sows, and compared their performance to other sows fed maize–soybean meal diets containing dietary protein levels of 10 to 18%. As Table 17 indicates, litters from sows fed opaque-2 maize gained at rates between those produced by 12 and 14% protein diets, but similar to those obtained with the 10% protein diet. When the litter size was held constant at 8 pigs for the first litter and 10 pigs for the second, with feeding level controlled to ensure approximately equal intake of all sows, litter gains of sows fed opaque-2 maize were inferior to the maximum litter gains under 16 and 18% dietary protein levels. The sows fed opaque-2 maize diets were in negative nitrogen balance during lactation, as were sows fed only 10% dietary protein

TABLE 17 Effect of protein level and opaque-2 maize on sow and litter performance

Criteria	Dietary protein					
	Maize-soybean meal diet (%)					Opaque-2 (%)
	10	12	14	16	18	9.7
First-litter sows						
Sow farrowing weight	156.4	149.3	163.5	154.4	153.5	163.6
Sow weight loss, 0-28 days	-26.9	-15.3	-13.5	-12.8	-11.0	-25.3
Sow feed consumption, 0-28 days	86.6	94.6	97.0	97.8	96.0	92.2
Litter weight, 28 days	45.9	49.2	51.4	54.6	53.0	49.8
Avg pig weight, 28 days	5.7	6.2	6.4	6.8	6.6	6.2
Second-litter sows						
Sow farrowing weight	188.4	195.0	187.9	197.9	186.4	184.8
Sow weight loss	-25.6	-20.8	-16.5	-15.6	-13.9	-22.0
Sow feed consumption	109.0	111.8	107.1	114.7	116.0	114.1
Litter weight, 28 days	49.6	49.8	50.7	53.8	56.5	48.9
Avg pig weight, 28 days	5.0	5.0	5.1	5.4	5.7	4.9

Source: Mahan et al. (21).
Note: All weights in kilograms.

TABLE 18 Production of lactating sows fed opaque-2 maize diets with suboptimum protein levels

Criteria	Dietary protein		
	Normal maize, soybean meal[1] (16% protein)	Opaque-2 maize, soybean meal[1] (12% protein)	Opaque-2 maize[1] (9.8% protein)
Number of sows	23	26	25
Avg weight, 24-h postpartum (kg)	175.6	184.6	174.2
Avg weight, 35 days postpartum (kg)	188.3	195.0	172.0
Avg sow gain or loss (kg)	+12.8[a]	+10.4[a]	-2.2[b]
Avg daily feed, 0-35 days (kg)	4.63	4.85	4.57
Avg pigs born alive	9.9	9.6	10.0
Avg pigs at 35 days	7.4	7.4	8.0
Avg birth weight (kg)	1.3	1.3	1.3
Avg weaning weight, 35 days (kg)	6.1	6.1	6.1

Source: Adapted from Maner et al. (25).

[1] a, b values in same row with different superscripts are statistically different.

from maize – soybean meal (21). A marked decrease in milk production resulted when sows were fed either the opaque-2 maize diets or a maize – soybean diet containing 16% protein (22).

Maner and Colombian co-workers (25) compared opaque-2 maize diets containing suboptimum levels of protein (9.8 and 12%) with a control normal maize – soybean diet containing 16% protein. The 9.8% protein diet contained only opaque-2 maize, vitamins, and minerals. In addition to these ingredients, the 12% diet contained 5.64% soybean meal. After feeding these diets to lactating sows for 35 days, no differences in litter size or weight were observed (Table 18). Sows fed the 9.8% protein opaque-2 maize diet lost significantly more weight than the other two treatment groups.

SUMMARY

In summary (Figure 5), it can be concluded that opaque-2 maize can be used as the only dietary source of protein during the finishing, pregestation, and gestation periods of a pig's life cycle without reducing pig performance. This finding is substantiated by comparisons with diets based on National Research Council requirements (Figure 4). Opaque-2 maize alone is not adequate for baby pigs, growing pigs, or lactating sows. It must be supplemented with some protein and or amino acids to produce optimum and maximum performance. The advantage of the opaque-2 maize diet is that it contains an improved balance of amino acids, especially lysine and tryptophan. As compared to the normal maize diet, the opaque-2 maize diet produces equal performance with less supplemental proteins. A recommended life-cycle feeding system based on opaque-2 maize is presented in Figure 5.

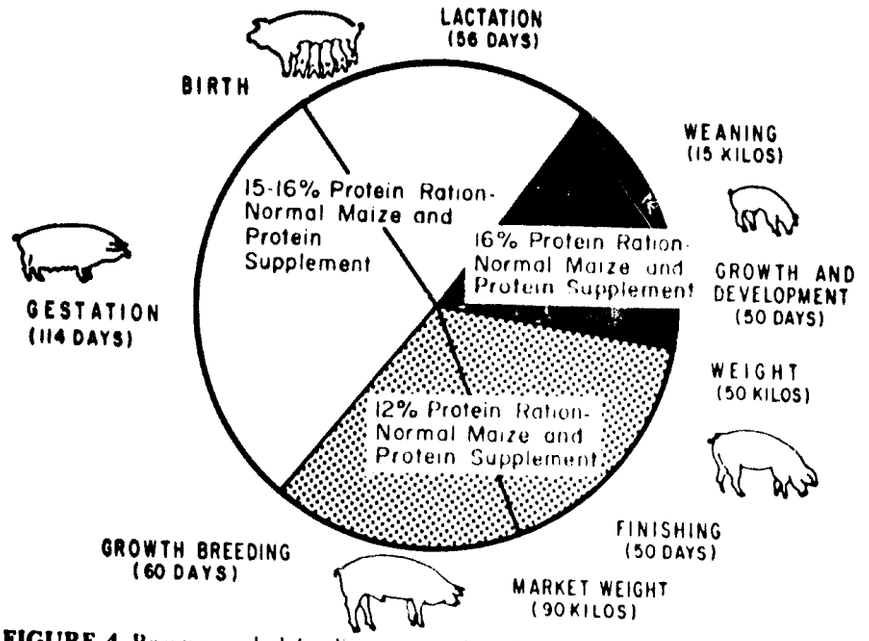

FIGURE 4 Recommended feeding system based on normal maize and soybean meal.

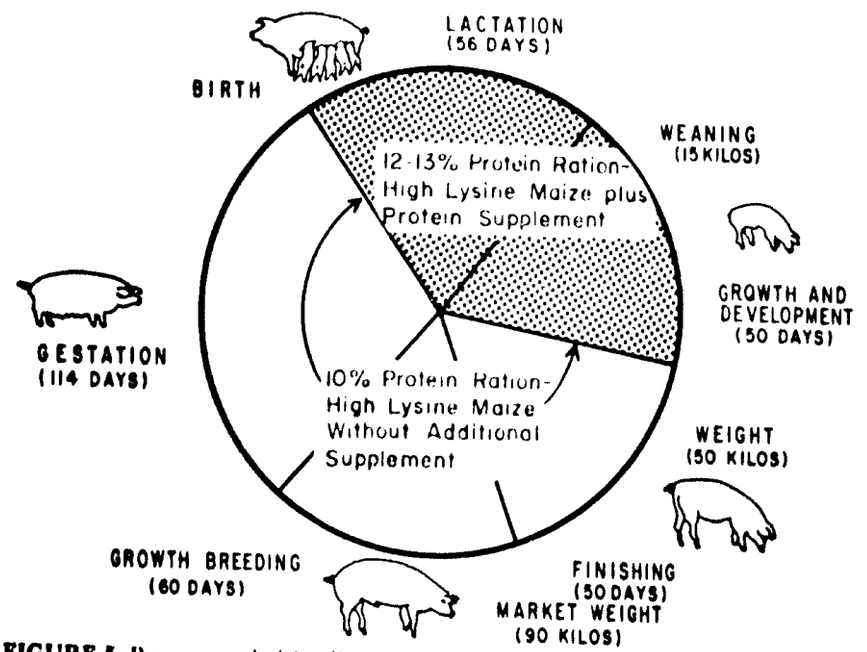

FIGURE 5 Recommended feeding system based on opaque-2 maize.

[*A discussion of this paper can be found on pp. 484–485 of* **Questions and Answers.**]

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Part III

BREEDING FOR PROTEIN QUALITY IN MAIZE: CURRENT ISSUES AND PROBLEMS

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Six and one-half years ago, many of us met at Purdue University to learn about and to report the early research on modified-protein maize. Some of us were there to prophesy. Among the seers was a U.S. Senator, who said something that I believe has been substantiated by the research of the intervening 6 years: ". . . in my judgment, this conference marks one of the great breakthroughs of the twentieth century in the most important war of our age — the war against human hunger and want."

SHOULD MODIFIED-PROTEIN MAIZE BE BRED?

Since the 1966 conference, however, some doubt has been expressed about the wisdom of attempting to modify the protein quality of cereals by genetic means. These reservations were well stated by J. R. Aitken in the April – May 1969 issue of the American Society of Agronomy's popular journal *Crops and Soils*:

> There is no economic justification for trying to make cereal grains into more complete feedstuffs. Deficiencies in protein quality and quantity can be corrected more economically by supplementation with non-grain protein supplements or with synthetic amino acids, which are likely to drop in price with the passage of time. In

> the development of new feed grain varieties, the proper study of the plant breeder is to cut the cost per calorie by increasing the yield of available nutrient energy per acre.

Needless to say, Aitken's note stimulated several responses, most of them disagreeing with his view. Among those who disagreed, and responded, was the maize breeding group at the University of Illinois, who said:

> Several economists have calculated that regular opaque-2 corn competes favorably with the ordinary corn and soybean meal diet in swine feeding providing the yield reduction is no greater than 4 to 6 percent. The question then is simply whether modified protein corn yields now are below this minimum and whether they are likely to remain below that of ordinary corn in the future.
>
> The question of supplementation of low-quality corn protein with synthetic amino acids can best be answered by a recent comment of E. T. Mertz, co-discoverer of the opaque-2 effect: "Assuming no yield reduction, the opaque-2 corn plant can produce its extra endosperm lysine and tryptophan for approximately 2 cents a pound. It is doubtful that any industrial process can equal this low cost of production."
>
> It seems to us that some perspective is useful in evaluating the likely course that events might take. Plant breeding is an evolutionary process — not a revolutionary one. Dent and flint types, as well as some floury ones, have been selected by man for 10,000 or more generations. Gene complexes that favorably modify these endosperm types have slowly been accumulated. It would be most surprising to find that in a few generations of breeding the opaque-2 allele in a genetic background of modifiers favoring the dent allele would produce a satisfactory product. The evidence now at hand suggests that today's breeders are much nearer to breeding agronomically satisfactory corns that can satisfy calorie–protein requirements of 70-pound pigs and young humans than breeders were to producing successful corn hybrids in 1908.
>
> In summary, we believe that breeders should devote attention to the improvement of modified protein corns. To do otherwise would be to ignore the reality that endosperm proteins need not be of low biological quality.

UNITED STATES PRODUCTION

Relatively little modified-protein hybrid seed has been offered for sale to farmers in the United States, and almost all of that now for sale carries the opaque-2 gene. We asked four well-known hybrid producers about their sales and their future plans for opaque-2 maize. On the basis of their response, we estimated that no more than 254,545 tons of commercial

opaque-2 maize was produced in the United States in 1972, about 0.2% of the total crop.

All the seed producers pointed out that yields were almost always less than from their normal dent hybrids. One reported that his company had converted essentially all their commercial maize hybrids to opaque-2 maize versions and that only 2 to 3% appeared to equal the performance of normal maize counterparts.

Thus, U.S. commercial producers of hybrid seed are not optimistic about the future of opaque-2 maize, although most apparently have adopted a "wait-and-see" attitude and continue modest conversion and testing programs. Hybrids are being produced and offered for sale, particularly to swine feeders.

CURRENT STATUS OF MODIFIED-PROTEIN MAIZE BREEDING

In 1966, I posed three questions that I believed to be central to the problems then confronting maize breeders:

1. Does the opaque-2 gene adversely affect agronomic performance?
2. Do epistatic effects exist, do modifiers of opaque-2 genes influence lysine concentration or phenotypic appearance of the kernel?
3. Should breeding work be conducted at the 9 to 10% protein level exclusively, or should higher-protein types be bred as well?

I believe that the first two questions have been well answered in these 6 years. Perhaps the third question cannot be answered as decisively, but evidence will be presented that the effort should be entered with a great deal of reservation.

PRESENT STATUS AND FUTURE PROSPECTS OF BREEDING FOR BETTER PROTEIN QUALITY IN MAIZE THROUGH OPAQUE-2

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Although the opaque-2 gene favorably alters the amino acid spectrum in maize, it has several shortcomings that limit its widespread commercial use. Comparisons between normal and opaque-2 maize types have been made by many workers for numerous important agronomic traits. The significant points emerging from these comparisons are as follows:

1. *Grain yield.* In general, opaque-2 maize varieties have 10 to 15% lower grain yields than do normal maize varieties (18, 28, 33). The lower grain yield of opaque-2 maize has been ascribed to lower test weight. Opaque-2 grains are 9 to 15% lighter in kernel weight (2, 4, 12, 14, 18, 22, 25, 33). The lower grain weight can be attributed to loose packing of starch particles in the endosperm (9).

2. *Moisture content.* Opaque-2 varieties have been shown (18, 25) to have 1.8 to 4.2% more moisture in grains at harvest than their comparable normal types. Higher moisture content requires additional drying after harvest.

3. *Grain appearance.* Opaque-2 kernels are chalky and dull in appearance in contrast to the shiny transparent kernels of traditionally grown flint or dent varieties.

4. *Susceptibility to diseases and pests.* Opaque-2 kernels have been found to

be more vulnerable to attack by *Sitophilus oryzae* (13) than normal-type kernels (Table 1), both in terms of infestation and loss in weight of grain. The genotype × version interaction (opaque-2 or normal) was statistically significant for weevil infestation as was moisture level. Opaque 2 versions also were more susceptible to seed rots caused by *Cephalosporium acremonium* and *Fusarium moniliforme* (4) (Table 2). Opaque 2 varieties have also been reported to be more susceptible to *Helminthosporium* (8).

5. *Other plant characters.* Available information does not support any significant relation of the opaque-2 gene to changes in ear length, ear diameter, number of kernel rows, number of kernels per row, and shelling percentage (4). Even though some increase in kernel number per ear (31, 33), ear length, ear diameter, and shelling percentage was recorded for opaque-2 versions (Table 3), the differences were not significant. Only one published report (31) shows significant reduction in leaf length and stem diameter and increase in time of anthesis in opaque-2.

6. *Amino acid balance.* Various workers have provided adequate proof that opaque-2 varieties have a better amino acid balance and 60 to 130% more lysine and tryptophan than does normal maize, plus a 12 to 40% reduction in leucine (1, 5, 21, 23, 30, 35).

These comparisons between opaque 2 maize and normal maize should be viewed against the background of materials and procedures used in the development of opaque-2 maize varieties. A critical evaluation of the background is necessary to formulate future action.

In most of the studies referred to, the opaque-2 conversions were made by using Purdue–Illinois opaque 2 stocks as donors into locally well-adapted lines or varieties. The donor opaque 2 stocks were not subjected to any rigorous selection; thus, the stocks would be expected to have low gene

TABLE 1 Relative susceptibility of normal and opaque 2 versions of maize to *Sitophilus oryzae* L.

Variable	Version	Mean[1]	Range	C.D.[2]% (transformed data)
At 30% kernel moisture level				
Number of insects/100 g of kernel	Normal	280.2 (16.7)		1.3
	Opaque-2	390.5 (19.8)	245.9-353.1	
Weight loss (%)	Normal	12.8 (19.8)	5.0-26.8	1.45
	Opaque 2	30.8 (33.5)	21.1-45.9	
At 12% kernel moisture level				
Number of insects/100 g of kernel	Normal	19.9 (4.5)		0.56
	Opaque 2	51.0 (7.1)	15.6-29.5	
Weight loss (%)	Normal	5.2 (12.9)	3.5-7.1	2.2
	Opaque 2	12.4 (20.5)	11.1-13.7	2.2

Source: Unpublished data of D. Sivasankar and V. L. Asnani, 1972.

[1] Figures in parentheses are transformed data.

[2] C.D., critical difference.

TABLE 2 Relative susceptibility of normal and opaque-2 versions of maize to *Cephalosporium acremonium* and *Fusarium moniliforme* (percentage of seed rot)

Character and genotype	Versions[1] Normal	Opaque	C.D.[2] at 5% (transformed data)
C. acremonium			
CM 109	22.2	74.2	
CM 112	26.2	59.9	
CM 113	28.4	60.2	
CM 201	20.2	64.9	
Mean	24.3 (29.4)	64.7 (54.0)	3.6
F. moniliforme			
CM 109	17.6	41.3	
CM 112	25.3	60.2	
CM 113	18.2	36.0	
CM 201	12.2	51.8	
Mean	18.4 (25.2)	43.0 (43.4)	2.0

Source: Unpublished data of D. Sivasankar and V. L. Asnani, 1972.
[1] Figures in parentheses are of transformed data.
[2] C.D., Critical difference.

frequency for those genes (1) conferring resistance to various diseases and pests and (2) transmitting high yield. The donor stocks in particular were very poorly adapted to the tropical maize-growing regions of the world.

In most of the studies cited, opaque-2 types were recovered after two or three backcross generations, retaining 7 to 13% of the genotype of the nonrecurrent parent. Moreover, no selection has been made in the backcross recovered populations which retained considerable variation for various characters. Backcross as a procedure (2) has several limitations, which

TABLE 3 Yield and yield components of normal and opaque-2 versions

Character	Version	Mean	Range	C.D.[1] at 5%
1,000-kernel weight (g)	Normal	236	201-293	6
	Opaque-2	209	171-250	
Ear length (cm)	Normal	16.0	12.8-18.3	1.4
	Opaque-2	14.9	12.2-18.4	
Ear diameter (cm)	Normal	3.7	3.5-4.0	0.3
	Opaque-2	3.7	3.1-4.0	
Shelling percentage	Normal	70	63-77	5
	Opaque-2	71	63-76	
Kernel rows/ear	Normal	12.9	12.0-13.8	0.8
	Opaque-2	13.6	9.9-15.0	
Grain yield (g)/plant	Normal	56.6	33.7-72.8	21.5
	Opaque-2	56.0	28.0-74.4	

Source: Asnani and Gupta (4).
[1] C.D., critical difference.

are partially responsible for limiting the recovery of the desired genetic background in which opaque-2 would be expressed ideally.

The question is still open as to whether the observed adverse effects are due to direct or indirect pleiotropic effects of the opaque-2 gene, or are due to the closely linked undesirable genes received from the donor parent. Another important finding is that improved amino acid balance resulting from the opaque-2 gene is associated with soft endosperm (2, 17). More critical data are needed for analyzing adverse effects of the opaque-2 gene.

In most comparisons between normal and opaque-2 maize types, marked differences were observed in the actual level of performance for various characters. These variations have been attributed to the genotypes of the recurrent parent. Even though opaque-2 varieties, in general, have given 10 to 15% lower yield, several cases have been reported (4, 18, 33) where opaque-2 varieties were equal to or even better than their normal counterparts (Table 4). Also, the test weight of opaque-2 maize in certain genotypes is comparable to that of normal maize (2, 18, 28). Variation in the relative susceptibility of opaque-2 varieties to ear rot incidence, weevil attack, and borer damage has been observed (13, 27). The possibility of obtaining higher kernel weight — comparable to or higher than normals — plus low susceptibility to diseases and insect pests in certain genetic backgrounds suggests that wise selection of parental varieties to be converted to opaque-2 may provide a way to control the undesirable effects of the opaque-2 gene.

Besides the problems enumerated, the dull, chalky appearance of opaque-2 kernels may hinder their acceptability in regions where shiny flint and dent varieties traditionally are grown. Efforts have been directed to developing opaque-2 types with near-normal appearance (26). Modified opaque kernels with varying degrees and patterns of vitreousness are frequently found in backcross programs. The frequencies of occurrence of various grades to near normal kernels vary, depending on the genetic background of the recurrent parent. Until recently, most backcross programs retained only completely opaque kernels, while modified opaques were discarded.

TABLE 4 Some populations having better performance in opaque-2 version

Population	Version	Yield (g/plant)	Ear length (cm)	Ear diameter (cm)	Shelling (%)
Cuba 11J	Normal	45.3	14.8	3.5	64
	Opaque-2	50.3	16.1	3.5	66
Cuba V 66	Normal	49.1	17.2	3.8	67
	Opaque-2	58.1	13.3	4.0	70
Waimea Dent	Normal	60.7	18.3	3.6	70
	Opaque-2	74.4	18.4	4.0	72
C.D.[1] at 5%		21.5	1.4	0.3	5

Source: Asnani and Gupta (4).

[1] C.D., critical difference.

The vitreous appearance in opaque-2 kernels apparently is due to the complementary effects of several modifiers. Precise information is not available on the number and nature of these modifiers, which, in the presence of the opaque-2 gene, provide considerable variability for protein, kernel density, tryptophan, lysine, and so on. Where selection for semiopaque and flint kernels was made (Table 5), this variability was observed in composites

TABLE 5 Chemical composition of modified opaque-2 lines selected from composites Shakti, Rattan, and Protina

Pedigree	Protein in defatted endosperm (%)	Tryptophan in defatted endosperm (%)	Tryptophan in protein (%)	Specific gravity
Rattan				
2	12.24	0.069	0.53	1.24
5	8.09	0.076	0.94	1.23
27	8.89	0.064	0.72	1.22
34	9.47	0.050	0.52	1.24
36	8.89	0.050	0.56	1.26
42	10.51	0.074	0.70	1.17
43	12.28	0.082	0.67	1.16
57	11.90	0.056	0.47	1.27
64	8.32	0.061	0.73	1.19
65	12.36	0.053	0.43	1.27
70	7.28	0.066	0.91	1.26
110	17.27	0.057	0.33	1.15
114	10.86	0.040	0.37	1.34
116	11.78	0.048	0.41	1.27
119	12.93	0.045	0.35	1.27
123	11.32	0.040	0.35	1.29
127	9.93	0.039	0.39	1.15
Shakti				
319	8.66	0.045	0.52	1.18
323	10.74	0.057	0.53	1.25
326	8.55	0.054	0.63	1.30
346	9.47	0.079	0.83	1.16
201	6.70	0.050	0.74	1.24
210	12.47	0.064	0.51	1.26
235	8.32	0.087	1.04	1.14
376	11.67	0.067	0.57	1.31
362	8.31	0.033	0.40	1.30
387	12.13	0.064	0.53	1.12
388	10.28	0.041	0.40	1.25
405	10.63	0.052	0.49	1.33
409	11.20	0.050	0.45	1.16
Protina				
496	15.36	0.077	0.50	1.11
508	10.51	0.045	0.43	1.16
534	10.05	0.060	0.60	1.18

Source: Unpublished data of M. L. Lodha, H. O. Gupta, and J. Singh.

Shakti, Rattan, and Protina. Selections with high protein (17.27%) and low tryptophan (0.33%), and low protein (8.32%) and high tryptophan (1.04%), were observed in the limited number of samples analyzed. Flinty opaque-2 selections are comparable to chalky types, as shown in Table 6. In the modified opaque-2 population the association was very low between protein and tryptophan in the sample (Table 7). This finding is in contrast to the significant positive association observed in chalky opaque populations.

The opaque-2 gene influences several quality traits that are quantitative in inheritance. Any effective improvement program requires adequate variability, information on the heritability and nature of the gene action, knowledge of the genotype – environment interaction and the association among various pertinent quality characters. The choice of selection procedure should depend on such precise information, plus a careful determination of available resources and specifications of the nature of the end product.

For normal maize, considerable information is available about the nature of variability, heritability, genotype – environment interaction, and gains made by different selection schemes for various quality traits, particularly protein, oil, and essential amino acids. However, relatively little is known about opaque-2 maize. In fact most presently available information relates to the synthetic or composite populations that have been developed through

TABLE 6 Comparison of modified opaque-2 kernel phenotype and opaque-2 segregants from S_1 families

	100-kernel weight (g)		Protein (%)		Tryptophan in protein (%)	
Family	Modified phenotype	Opaque-2	Modified opaque-2	Opaque-2	Modified opaque-2	Opaque-2
166-7	27.9	25.8	7.2	8.0	0.92	0.99
169-2	28.0	27.6	7.7	8.5	0.87	0.71
163-4	23.4	22.0	7.9	7.6	0.81	0.81
152-6	22.8	21.8	8.9	8.8	0.78	0.79
164-6	19.3	15.8	7.1	8.0	0.68	0.67

Source: Unpublished data of Asnani and Vasal.

TABLE 7 Correlation coefficients between protein percentage, tryptophan percentage, tryptophan as a percentage of protein, and specific gravity in modified opaque-2 selections of Shakti, Rattan, and Protina

	Tryptophan % sample	Tryptophan % protein	Specific gravity
Protein	0.0802	0.5831**	-0.1922
Tryptophan % sample		0.7348**	-0.4559**
Tryptophan % protein			-0.2450

Source: Unpublished data of M. L. Lodha, H. O. Gupta, and J. Singh.
**Significant at 1%.

two or three backcrosses, with no detailed knowledge about recurrent and nonrecurrent parents.

Table 8 shows the concomitant variability for several quality characters available in the Yellow Opaque-2 Composite developed from four opaque-2 composites (J_1, Cuba 11J, Doeto, and Antigua 2D). Data are based on 320 half-sib families selected from this composite (Figure 1). The coefficients of variation for protein, oil, 100-grain weight, and seed density were 12.7, 18.2, 11.1, and 4.5%, respectively. It is expected that, with prior knowledge of various traits involved, parents can be combined to generate still greater variability. Data on the nature of variability in three opaque-2 synthetics also have been reported (10), suggesting the presence of adequate variation for most of the quality traits.

Much higher variability for individual quality characters has been recorded in other studies. Sixty-five cycles of selection in Burr White at Illinois have resulted in the selection of lines with 25% protein. The higher protein content of Illinois High Protein has been considered an artifact due to lower starch synthesis (3, 24). Even though this may suggest that high yield and protein cannot be combined, it at least indicates that maize selections can be developed with up to 25% protein.

Most available opaque-2 maize varieties average 3.5 to 4.5% lysine as a percentage of protein. However, a recent study (29) shows that double mutants involving the opaque-2 gene and other endosperm mutant genes (bt_1, bt_2, fl_1, su_1, su_2, and others) can raise the lysine level by 50%. Lysine level also can be raised by using multialeurone layer stocks (37).

Marked variability for 100-grain weight, kernel density, reaction to ear rot, and weevil attack has also been shown (10, 18, 28).

Genetic analyses of quality characters involving opaque-2 varieties have been made (10, 33). Additive genetic variance was 10 to 17 times higher than nonadditive genetic variance for kernel weight, kernel number, grain yield, lysine percentage in sample, and lysine yield; both additive and nonadditive

TABLE 8 Variability in Yellow Opaque-2 Composite (based on 320 half-sib families)

Characters	Mean	S.E.[1]	Range	Coefficient of variation
100-grain weight (g)	12.3118	±0.0758	8.47	11.08
100-grain volume (mm)	11.5742	±0.0758	8.50	11.79
Protein (%)	10.8315	±0.0765	7.24	12.71
D.B.C.[2]	0.2268	±0.0018	0.39	14.23
Oil (%)	4.7822	±0.0484	7.55	18.21
Specific gravity	1.0567	±0.0026	0.696	4.51

Source: Unpublished data of A. K. Kaul and J. Singh.

[1] S.E., standard ear.

[2] D.B.C., dye-binding capacity.

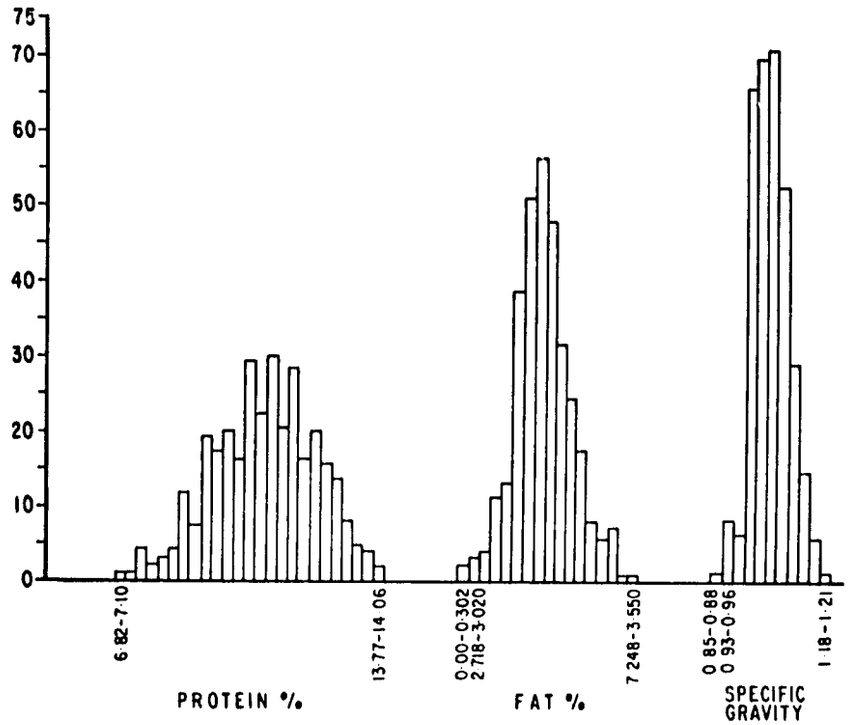

FIGURE 1 Frequency distribution for protein percentage, fat percentage, and specific gravity in Yellow Opaque-2 Composite.

variances were important for protein percentage and protein yield. Based on the average of four composites, heritability values for protein percentage, oil percentage, kernel density, and moisture percentage were high (66, 72, 63, and 77%, respectively). For lysine percentage, grain yield, and lysine per plot, the values were intermediate to low (41, 49, and 44%, respectively), and low (41%) for lysine as a percentage of protein (10).

For the simultaneous improvement of various characters, it is necessary to have information on how they are associated. Several workers have reported correlations among various quality characters (10, 31, 33). Correlations among characters in 45 opaque-2 composites, and within a brachytic-2 opaque-2 composite, are recorded in Tables 9 and 10, respectively. The data show significant positive correlations between the percentage of tryptophan in the sample and protein. Correlation between protein and tryptophan as a percentage of protein was negative and significant. These observations agree with other published reports (10, 33). The Yellow Opaque-2 composite showed low positive association (Table 11) between kernel density and percentage of tryptophan in the sample (0.0022) and kernel density and tryptophan as a percentage of protein (0.0144). Low negative association

TABLE 9 Correlation coefficients among various characters involving 45 opaque-2 composites

	D.B.C.[1]	Tryptophan % sample	Tryptophan % protein	Phosphorus	Potash	Zn	Mn	Cu
Protein (%)	0.7504**	0.3054*	-0.5656**	0.3709**	0.3648*	0.3939**	-0.0798	0.0266
D.B.C.		0.4828**	-0.2067	0.3563*	0.3922**	0.1997	-0.1140	0.0427
Tryptophan % sample			0.6074**	0.0709	0.1799	0.3272*	0.0245	0.1325
Tryptophan % protein				-0.2333	-0.1497	-0.0662	0.0729	0.0675
Phosphorus					0.4137**	0.0439	-0.2223	0.1830
Potash						0.0112	0.0138	-0.0959
Zn							0.0364	0.1308
Mn								0.0209

Source: Unpublished data of M. L. Lodha, H. O. Gupta, and J. Singh.

[1] D.B.C., dye-binding capacity.

*Significant at 5%.

**Significant at 1%.

TABLE 10 Correlation coefficients among characters involving 65 lines of brachytic-2 opaque-2 populations

	Tryptophan % sample	Tryptophan % protein	Lysine in sample	Lysine % protein
Protein %	0.6469**	-0.1781	0.5774**	-0.1792
Tryptophan % sample		0.6304**	0.5416**	0.0934
Tryptophan % protein			0.1199	0.3137*
Lysine % in sample				0.6879**

Source: Unpublished data of M. L. Lodha, H. O. Gupta, and J. Singh.
*Significant at 5%.
**Significant at 1%.

between lysine and kernel density of varied magnitudes has been reported elsewhere (10). A strong positive association between the lysine and tryptophan content of the endosperm suggests that selection could be based on either of the characters (16).

Like other quantitative characters, protein, lysine, tryptophan, and other essential amino acids are influenced considerably by various environmental factors, such as level of nitrogen and location. Other management practices, nitrogen applications at low levels of simazine use, for example have produced marked changes in protein and various amino acids in normal maize varieties (15, 32, 34). Studies with normal maize varieties have shown strong genotype – location interaction for protein, lysine, methionine, isoleucine, and tryptophan. The variance due to locations and the location – variety interaction for various quality traits accounted for 15 to 69% and 15 to 56% of total variance, respectively. The variance due to varieties was much smaller than for locations and location – variety interaction. Based on the relative importance of locations, it has been suggested (6) that "selection of varieties based on nutrient content must be undertaken at the local level." No published reports are available on the magnitude of genotype – environment interaction in opaque-2 maize.

In a recent study, 45 opaque-2 composites along with 4 normal maize varieties were tested in India at Delhi, Dholi, Ludhiana, and Coimbatore. This study indicated that (1) variance due to replications for tryptophan was not significant; (2) variance due to varieties and locations was significant for the percentage of tryptophan in the sample, percentage of protein, and percentage of tryptophan in protein; (3) variance due to location was much higher than that due to variety, being 30, 5.3, and 5.9 times greater for protein, percentage of tryptophan in protein, and percentage of tryptophan in the sample, respectively; (4) stability analysis (11) for the percentage of tryptophan in the sample indicated a very wide range in regression coefficient values for various varieties. This suggested that different varieties with comparable mean values varied in relative stability.

These observations agree with those reported for normal maize (6), and

TABLE 11 Correlation coefficients between various characters in 320 half-sib families of Yellow Opaque-2 Composite

	Volume (100 grain)	Protein %	D.B.C.[1]	Tryptophan	Oil %	Specific gravity	Tryptophan/ protein
Weight (100 grain)	0.9072	-0.0401	-0.1187	0.0224	-0.0392	0.1273	0.0158
Volume (100 grain)		0.0021	-0.0736	0.0211	-0.0163	-0.2991**	0.0099
Protein %			0.3524**	-0.0049	0.0078	-0.0998	-0.1742*
D.B.C.				0.1142	0.0346	-0.0956	0.0386
Tryptophan					0.0098	0.0022	0.9668**
Oil %						-0.0605	-0.0018
Specific gravity							0.0144

Source: Unpublished data of A. K. Kaul and J. Singh.

[1] D.B.C., dye-binding capacity.

*Significant at 5%.

**Significant at 1%.

clearly show the considerable need for studying opaque-2 populations within different environments. However, current information suggests that (1) adequate variability is available for various quality traits and desired agronomic characters, (2) relative dominance is in the partial-to-complete dominance range, (3) heritability for most characters is medium to high, (4) correlations are moderate to low (even though significant, suggesting no serious linkage problems), and (5) the quality characters are susceptible to location or environmental effects.

Using the preceding information, any selection scheme capitalizing on additive genetic variance would be effective. Schemes like mass selection, recurrent selection for general combining ability, and other recurrent selection schemes have been suggested (33) for improving quality. These selection schemes are fairly simple and inexpensive, but are normally performed at only one location. Since previous data emphasized the importance of location, it is likely that improvement will be most effective if selection is practiced at more locations. Probably the most efficient would be the modified ear-to-row augmented design (19). This design differs from modified ear-to-row only in that materials are planted in an augmented design at each location; thus, each location can be handled individually, and data from various locations can be pooled to recover the desired information. This scheme should be more efficient since it provides (1) simultaneous evaluation of materials at three or more locations, (2) possibilities for interprogeny and intraprogeny selection, (3) completion of one cycle of selection within a year's time, and (4) simultaneous selection for more than one character. Populations improved after a few cycles of selection can be used as they are or as the base population for developing elite lines for use in the hybrid program.

Improvements in quality traits at each cycle of selection should be supported with chemical analysis to sort out superior stable genotypes. Chemical analysis is even more important when selecting for modified opaques, where lysine or tryptophan content cannot be ascertained visually (26). For efficient ear-to-row selection, chemical-analysis facilities must be able to handle material from three or four locations.

Opaque-2 varities have been developed and released for general cultivation in Colombia, Mexico, the United States, Brazil, India, and Nigeria. Moreover, several experimental materials are available in other countries. Even though developed through standard backcross procedure, most populations retain considerable variability, which could be used to improve for yield, disease and pest resistance, and protein content. If adequate variability exists, selection can be made for modified types, also.

Countries initiating new programs must develop suitable opaque-2 base populations. With the availability of the opaque-2 gene in high-yielding, better-adapted backgrounds, it is no longer necessary to use the Illinois or Purdue stocks, or to follow the standard backcross program, particularly in

the tropics. The opaque-2 gene can be incorporated conveniently by growing and detasseling a few rows of opaque-2 maize in the middle of a high-yielding, well-adapted variety in isolation. The population, once stabilized, can be taken up for further improvement.

NEED FOR ADDITIONAL INFORMATION

Before initiating a quality improvement program, it is necessary to determine program objectives based on probable uses of the materials. Animal nutritionists also can help considerably (7). Breeders urgently need information on the balance of protein, tryptophan, and lysine. A basic question is should breeders devote their efforts (1) toward increasing the lysine and tryptophan content in presently available high-yielding varieties with relatively low protein levels (8 to 10%), or (2) toward improving the protein content at the present level of lysine (3.5 to 4.5%)? Combining relatively high levels of protein and lysine in more productive backgrounds seems a desirable goal.

Quality traits of opaque materials shows considerable genetic variation in relative stability to various environments, and stable varieties should be identified. It also is necessary to determine which will be more stable, high-lysine, medium-protein genotypes or medium-lysine, high-protein genotypes.

The opaque-2 gene has been shown to increase the lysine content in the endosperm, whereas relatively little improvement has been found in the chemical composition of the embryo. However, an increase in embryo size has been recorded by several research workers. Most studies have been made on only a few varieties. Varieties with better embryo composition must be identified. Such types would help raise the protein and lysine level of the whole kernel.

Most breeders are now using tryptophan analysis (16) as a measure of improved protein quality. Although a relatively fast technique, it is expensive, and its pace restricts the number of samples analyzed. Development of devices similar to those used for oil analysis, like wide-line nuclear magnetic resonance (NMR), would greatly improve the rate of progress. Improvements in microstaining techniques (17) may help distinguish high-lysine genotypes. More efficient, simpler, and relatively less expensive techniques will be welcomed by breeders.

A final and urgent need is for a basic understanding of the mode of operation of the opaque-2 gene itself. How does a single gene simultaneously affect several characters? How does the gene increase lysine and tryptophan content, decrease leucine, produce dull grain appearance, make endosperm soft, and increase sugar content? Is opaque-2 a complex locus? Attempts have been made to answer these questions (20, 36), and future findings will help in developing varieties with even higher lysine and tryp-

tophan content. Such basic information would also aid development of rapid and more efficient methods of screening more desirable genotypes.

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SELECTION SYSTEMS FOR INCREASING PROTEIN QUALITY WITH OPAQUE-2 GENE IN FLOURY MAIZE

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Ninety percent of the maize grown in the highlands of Bolivia, Ecuador, and Peru is a soft floury type used for direct human consumption without any transformation or leavening. To the Andean people, maize represents a basic food, with maize and potatoes sharing a prime importance in their diets. At least 50% of the area's population lives in the highlands, and these people have the countries' lowest income per capita. High-quality-protein goods are expensive and not easily available.

Most highland people are subsistence farmers who consume most of their own farm production. Increasing crop yields is not the entire solution for these families, since overproduction relative to their needs will lead only to reduced crop areas in the next season. Improving the nutritional quality of present Andean food crops seems the best way to help these people raise healthy children, thus enabling them to deal with the cultural changes they will experience as communication increases and development proceeds.

The majority of the Andean floury maize varieties have the floury-1 gene, and the phenotype of the grain in many varieties is identical to that of the opaque-2 mutant. This complicates the conversion process, since the simplest and most familiar scheme depends on visual observation of opaque segregants in a normal background. Conversion of floury maize to opaque-2

maize is usually accomplished using test crosses to evaluate progeny, and these must be supported by laboratory analyses to assure progress toward protein quality. Several schemes for conversion are presented, and the advantages and disadvantages of each are summarized.

SELECTION SCHEMES FOR CONVERSION OF HIGHLAND FLOURY MAIZE

Owing to the difficulties mentioned in recognizing high-quality segregants or individual kernels during the backcrossing program, special genetic manipulations and laboratory testing of progeny are essential to successful conversions of floury maize to opaque-2 maize. Six schemes for conversion of these highland maize varieties are presented here. The first three related methods involve testcrossing during each backcross generation. Primary differences in these three methods are found in the desired end product and the handling of the floury-1 gene. Two other schemes depend on visual selection, and on the phenotype and genetic background of the recurrent parent that is used in the conversion. These first five schemes require a laboratory input only to test progress and to assure that the quality of the opaque-2 material is maintained.

The sixth system which is highly dependent on a continuous laboratory input and requires a sophisticated single-grain analysis, is the most positive in terms of identification, but requires an input of technology and resources that are available in only a few places. The references and other information sources available to the authors are listed for each system.

Following the presentation of these six schemes, a general genetic approach is suggested that (1) incorporates the best features of the several systems, and (2) has alternative final products and ways of developing them.

Conversion of Floury Varieties or Hybrids to Opaque-2 Without Reference to the Floury Locus

The simplest system (A) for converting floury lines or varieties to opaque-2 requires a known opaque-2 tester during each advanced backcross generation, and this tester must be in a clearly expressed flint background. This kind of system, presented by Crane (3), involves only the opaque-2 locus, and for this reason it is less complicated than the two systems that follow it in our discussion. Table 1 outlines the system as used to convert floury lines.

The original cross of the line or variety to be converted with the best available source of opaque-2 material (best adapted to the highland conditions) may be made in either direction. Following this initial cross, the recurrent local parent normally is used as male pollinator for the heterozyg-

TABLE 1 Scheme A: Conversion of floury lines or varieties using an opaque-2 tester in a known flint background

	Recurrent parent		Female parent	Testcross results[a] $\varnothing$ (ω_2/ω_2)
Original cross	+/+	X	ω_2/ω_2	
			↓	
First backcross	+/+	X	$+/\omega_2$	
			↓	
Second backcross + testcross	+/+	X	+/+	Normal
			$+/\omega_2$	1 normal : 1 opaque segregates[a]
			↓	
Third backcross	+/+	X	+/+	$+/\omega_2$ normal
			$+/\omega_2$	1 normal : 1 opaque segregates[a]
			↓ (to further cycles)	

Source: Crane (5).

[a] In each cycle, select the female backcrossed plants whose respective testcross showed a ratio of approximately 1 normal : 1 opaque-2. Recovery of the homozygous genotype at any step may be accomplished by self-pollinating the tested heterozygote and making a testcross to an opaque-2 tester.

ous female plants selected in each cycle. If it is easier to make the first backcross in the opposite direction, this will not change the procedure.

Seed from the first backcross is planted, and individual plants are pollinated from the recurrent parent. Numbered testcrosses are made onto the opaque-2 tester with pollen from these same individuals. On the basis of the testcross ears, female backcrossed plants are selected. Those individuals which give a normal testcrossed ear are discarded; those which give a segregating testcross ear are retained for the next backcross. Using this system, one can attain a backcross in each generation.

Following the second backcross, it is advisable to begin selfing several of the heterozygous progeny to produce an opaque-2 line or variety at this level of conversion. A laboratory test of the opaque-2 kernels assures that quality is maintained. The self-pollination also provides seed of a converted opaque-2 variety (or line that can be used to form hybrids), which can be tested immediately on the experiment station and with farmers. Depending on (1) the degree of adaptation of the opaque-2 source, (2) the heterosis between this source and the variety or line to be converted, and (3) the results of yield tests of early generations of the conversion, this procedure may require from two to five generations of backcrossing.

Whereas this system is the simplest of the conversion and testing schemes for soft-endosperm highland maize, it disregards the floury locus of the recurrent parent and the final product. With rare exceptions, the final product will be homozygous for both floury-1 and opaque-2.

The primary disadvantage in this scheme may come from the commercial planting of the double-mutant maize varieties in a zone where the predominant maize in farmers' fields is also floury-1, but normal for the opaque-2 locus. There will be no way to detect contaminants in the field, since both the high-quality maize kernels and the contaminated grains pollinated by plants that are normal at the opaque-2 locus will be phenotypically alike and floury, and the high-quality-protein characteristic conferred by opaque-2 will soon disappear. The following schemes avoid this problem in the field, and allow separation and elimination of contaminated kernels.

Conversion of Floury Varieties or Hybrids to Opaque-2 with the Final Objective a Single Recessive Maize

A second scheme (B), proposed by Bauman and Crane (1971) and outlined in Table 2, is similar to the first but controls the floury gene. As in the previous scheme, the original cross should be made to the best-adapted opaque-2 source known. This cross may be made in either direction, depending on flowering dates, plant vigor, or potential seed production. Introduced material often does not do well in the first cycle, and may successfully produce pollen even though it does not produce a good ear.

Following this initial cross, the first backcross must be made with the recurrent parent as male and the F_1 heterozygote as female. Seed from this backcross is planted, and individual plants are pollinated with a mixture of pollen from the recurrent local variety or adapted line. Numbered testcrosses are made with pollen carried to the opaque-2 tester from the same individual plants. Selection of double heterozygote ($+/fl_1\ +/o_2$) females is based on the examinztion of both the backcross and the testcross ears. As shown in Table 2, those backcross individuals are selected which segregate 1 normal:1 floury kernels in the backcross ear pollinated by the recurrent parent and the testcross ear to which pollen was carried. This accelerated system gives a continuous backcrossing with one cycle per planting season.

Starting with the second backcross planting, half the genotypes can be eliminated by planting only the normal kernels from the selected segregating ears in the backcross. These normal-phenotype kernels will be a mixture of ($+/fl_1\ +/+$) and ($+/fl_1\ +/o_2$), and it is not possible to distinguish between the types at this point. However, this process does eliminate the unnecessary burden of carrying unneeded material into the next generation.

Following the second backcross cycle, it also is important to begin to isolate the desired mutants for laboratory and field testing. Self-pollination and testcrossing, followed by laboratory analysis, will assure either the (fl_1/fl_1 o_2/o_2) or the ($+/+\ o_2/o_2$) genotype, depending on the tests and selection, and corresponding high-quality protein. Logically, the selfed ears that are eventually selected should be tested in the laboratory before including each ear in the bulked opaque – floury ($fl_1/fl_1\ o_2/o_2$) population or the opaque population ($+/+\ o_2/o_2$).

TABLE 2 Scheme B: Conversion of floury lines or varieties using an opaque-2 tester, with either $(+/+\ o_2/o_2)$ or $(fl_1/fl_1\ o_2/o_2)$ maize as final objective

	Recurrent parent		Genotype of backcross plants		Backcross phenotypes	Testcross phenotypes ♀ $(+/+\ o_2/o_2)$
Original cross	fl_1/fl_1 +/+	x	+/+	o_2/o_2		
			↓			
First backcross	fl_1/fl_1 +/+	x	$+/fl_1$	$+/o_2$		
			↓			
Second backcross + testcross	fl_1/fl_1 +/+	x	fl_1/fl_1	+/+	floury	normal
			fl_1/fl_1	$+/o_2$	floury	1 normal : 1 opaque
			$+/fl_1$	+/+	1 normal : 1 floury	normal
			$+/fl_1$	$+/o_2$ [a]	1 normal : 1 floury	1 normal : 1 opaque [b]
			↓			
Third backcross + testcross	fl_1/fl_1 +/+	x	$+/fl_1$	+/+	1 normal : 1 floury	normal
			$+/fl_1$	$+/o_2$ [a]	1 normal : 1 floury	1 normal : 1 opaque [b]
			↓			
			(to further cycles)			

Source: Bauman and Crane, 1971.

[a] Beginning with the second backcross, selection of only the normal kernels from this segregating backcross ear will eliminate half the progeny in the next planting and half the unnecessary materials from the program.

[b] In each cycle, select the female backcrossed plants whose testcross showed segregation approximately 1:1 for normal to opaque *and* whose own backcrossed phenotype segregated 1 normal : 1 floury.

The same considerations outlined in scheme (A) are pertinent in determining the number of generations of backcrossing needed before commercial use of these converted materials. Field results from yield trials can best indicate progress and how far to carry the conversion.

Scheme B (Table 2) requires no more work in the conversion than scheme A (Table 1), and we find that the control of the floury-1 locus is a major advantage of this method. There also may be certain organoleptic properties of a product with the homozygous floury-1 gene that make this maize desirable to the consumer. Another advantage of scheme B is the potential to recover either the single mutant ($+/+$ o_2/o_2) line and/or the double mutant (fl_1/fl_1 o_2/o_2) line in the last step, to be compared in yield trials and laboratory tests.

Conversions of Floury Varieties or Hybrids to Opaque-2 with the Final Objective a Single-Recessive Maize

Modification of the backcross and testing system has also been proposed by Beingolea and Sevilla (1). Their scheme (C) also assumes that floury varieties in the Andean zone are floury-1, with a dosage effect where the $fl_1fl_1/+$ endosperm has a floury phenotype. Conversion scheme C is outlined in Table 3.

Floury local is used as male and crossed to the opaque-2 source. The reverse may be used in the original cross if this facilitates the operation. The heterozygous F_1 is crossed as female to the recurrent parent local floury (fl_1/fl_1 $+/+$). Backcross-1 (BC_1) kernels will segregate 1 normal:1 floury. The genetic constitution of the normal kernel embryos are ($+/fl_1$ $+/o_2$)and ($+/fl_1$ $+/+$). These are planted, and at flowering time they are selfed and also backcrossed to the recurrent parent as female to obtain BC_2.

At harvest, all BC_2 ears are floury, owing to the dosage effect of floury-1. Selfed ears of BC_1 plants are of two types: the ears carrying the opaque-2 gene will segregate 6 normal:10 floury; the ears that do not carry the opaque-2 gene will segregate 1 normal:1 floury. Only the BC_2 ears which received pollen from plants that segregated 6 normal:10 floury are selected for further backcrossing. The same procedure is followed for BC_3, but now the number of pollinations must be doubled, since there are four different genotypes from BC_2, from which only one will be selected. The same procedure as in BC_3 may be followed for further backcross generations.

Recovery of a homozygous opaque-2, single-mutant variety ($+/+$ o_2/o_2) is obtained by selfing the last backcross generation (Table 4). In the 6 normal:10 floury segregating ears, the floury kernels are selected and planted. In each plot at least 30 plants are self-pollinated. Those plants with completely floury selfed ears are planted in a detasseled ear-to-row field with the recurrent parent as pollinator. All lines may be increased ear-to-row in another block. The desired lines are those with uniformly normal (flint) ears in the detasseled field. The increased seed of these lines is planted in an

TABLE 3 Scheme C: Conversion of floury varieties or lines to opaque-2 with a single-recessive maize as the final objective

	Recurrent parent		Genotype of backcross plants		Backcross phenotypes	Self-pollination phenotypes
Original cross	fl_1/fl_1 +/+	X	+/+	o_2/o_2		
			↓			
First backcross	fl_1/fl_1 +/+	X	$+/fl_1$	$+/o_2$	1 normal : 1 floury	
			↓		plant normal (flint) kernels	
Second backcross	fl_1/fl_1 +/+	X	$+/fl_1$	+/+	floury	1 normal : 1 floury
+ self-pollination			$+/fl_1$	$+/o_2$	floury	6 normal : 10 floury[a]
			↓		plant BC_2 from plants with 6 normal:10 floury ratio	
Third backcross	fl_1/fl_1 +/+	X	fl_1/fl_1	+/+	floury	floury
+ self-pollination			fl_1/fl_1	$+/o_2$	floury	floury
			$+/fl_1$	+/+	floury	1 normal : 1 floury
			$+/fl_1$	$+/o_2$	floury	6 normal : 10 floury[a]
			↓			
			Plant BC_3 from plants with 6 normal : 10 floury. (to further cycles)			

Source: Beingolea and Sevilla (1).

[a] In each cycle, select the female backcrossed plants from numbered selfed plants that gave a 6 normal:10 floury segregation.

TABLE 4 Fixation of desired genotype of lines or varieties from the third conversion scheme (c) after the desired number of backcross cycles

BCx plants		Self-pollinated ears	
fl_1/fl_1 +/+	⊗	floury	
fl_1/fl_1 $+/o_2$	⊗	floury	
$+/fl_1$ +/+		1 normal : 1 floury	
$+/fl_1$ $+/o_2$		6 normal : 10 floury	
			Testcross to ($+/+$ o_2/o_2)
$+/fl_1$ +/+		1 normal : 1 floury	normal
$+/fl_1$ $+/o_2$	⊗	6 normal : 10 floury	1 normal : 1 opaque
$+/fl_1$ o_2/o_2	⊗	floury[a]	opaque
fl_1/fl_1 +/+	30 plants	floury	normal
fl_1/fl_1 $+/o_2$	(+ testcross)	floury	1 normal : 1 opaque
fl_1/fl_1 o_2/o_2		floury[a]	opaque
+/+ o_2/o_2		floury[a]	opaque
		testcross result with (fl_1/fl_1 +/+)	
$+/fl_1$ o_2/o_2[b]		segregates	
fl_1/fl_1 o_2/o_2[b]	testcross as ♀ with	floury	
+/+ o_2/o_2[b]	(fl_1/fl_1 +/+)	normal	

[a] Select floury self-pollinated ears that also give a completely floury (opaque) ear when testcrossed to ($+/+$ o_2/o_2). This testcross reduces the population required in the third generation and permits recovery of both ($+/+$ o_2/o_2) and (fl_1/fl_1 o_2/o_2).

[b] Increase seed from each ear row at the same time the testcross is made on several plants as females. This will save one season in the increase and use of a new variety or line.

isolated mixing plot. The genetic constitution of the new floury variety is ($+/+$ o_2/o_2). When this variety is planted by the farmer, any contamination with local floury varieties that do not carry opaque-2 (fl_1/fl_1 $+/+$) will be easily detected in each harvest, since the crossed kernels ($+/+/fl_1$ $o_2/o_2/+$) will have flint normal endosperm. A disadvantage of this system is that, in some genetic backgrounds, sufficient difficulty in classifying individual kernels may be encountered so that it will not be possible to distinguish between a 1:1 and 6:10 ratio.

Modifying scheme (C) as originally described includes a testcross to opaque ($+/+$ o_2/o_2) during the second step of fixing the desired genotypes to allow reducing the number of lines to be carried to the final step and recovery of ($fl_1/fl_1 o_2/o_2$) (Table 4). Further modifications suggested by Crane and Bauman include the possibility of split pollinations on the ears of segregating generation plants and the use of aleurone markers to identify desired individuals homozygous for either ($+/+$ o_2/o_2) or for (fl_1/fl_1 o_2/o_2). In addition to the testcross with pollen from each plant to a ($+/+$ o_2/o_2) tester, the second ear (or about one third the silks on the main ear) is pollinated with ($+/+$ $+/+$) pollen from a purple aleurone stock. The main

ear (or remainder of the main ear) is self-pollinated a day or two later. From the testcross results to the (+/+ o_2/o_2) all plants not homozygous o_2/o_2 are discarded; among those that are o_2/o_2, if the purple kernels are *all* floury on a particular plant, the nonpurple kernels form the new (fl_1/fl_1 o_2/o_2) line or variety. If the purple kernels are *all* normal on a particular plant, the nonpurple (selfed) kernels from that plant will form the new line, or will be pooled into the new variety, which is (+/+ o_2/o_2).

Visual Selection of Opaque-2 Segregants within a Floury Background

Although most conversions of high-altitude Andean maize varieties must depend on the sophisticated selection and testing schemes just outlined, there are some highland materials in which the opaque-2 phenotype can be distinguished from the floury types being converted. In the Colombian national program, Daniel Sarria, Enrique Arias, and Manuel Torregroza are converting a series of maize varieties at Tibaitatá, near Bogotá. Among these varieties there is a sufficient difference in color, or sheen, between the floury (homozygous or heterozygous) kernels and the opaque-2 segregants in the selfed ears to allow visual selection of families in the classical conversion manner. This procedure is outlined briefly in Table 5 (scheme D). If this procedure is successful (and it has proved valuable in Colombia), the conversion is identical to that in flint or dent-type maize. After the first backcross generation, selfed plants may be examined visually for segregating kernels

TABLE 5 Scheme D: Visual selection of opaque-2 segregants within a floury background

	Recurrent parent		Female parent	Selfed progeny
Original cross	+/+	×	o_2/o_2	
			↓	
First backcross	+/+	×	+/o_2	
			↓	
Second backcross	+/+	×	+/+ ⊗ →	floury segregates
			+/o_2	3 floury : 1 opaque[a]
			↓	
Third backcross	+/+	×	+/+ ⊗ →	floury segregates
			+/o_2	3 floury : 1 opaque[a]
			↓	
			(to further cycles)	

[a] Depends on visual separation of the floury kernels from the opaque-2 segregants or by sectioning, staining, and examination with microscope to identify the types low in zein (assumed to be high quality).

that are homozygous (o_2/o_2), and these materials are increased for laboratory analysis and field testing. Scheme D is identical in every detail to the well-known conversion system for flint maize, and certainly is a recommended approach if the varieties or lines to be converted have this type of endosperm. This method may prove dangerous, however, since the opaque-2 gene seems to react with some genetic backgrounds to produce a deleterious effect. It is likely that this negative effect could be associated with some visual expression (such as reduced sheen). This deleterious effect on the endosperm might later be associated with reduced yield or other performance criteria.

A better scheme for visual selection of high-quality segregants, at least in the initial screening tests, is a method reported by Pradilla et al. (4) at the Biological Congress in Cali, and previously used by Wolf et al. (5) and Choe et al. (2). This method entails sectioning the kernel with freezing microtome, staining a section with hemotoxilin dye, and examining this preparation under a high-resolution microscope. Kernels that are high in zein content (poor quality) show a concentration of darkly stained red zein bodies in the spaces between starch granules, as well as staining of the margin between each two granules. On the other hand, kernels that are low in zein show only limited staining of the few zein particles found in the spaces, and staining is almost absent from the marginal lines between granules. This distinction between the two types is so striking and clear that no previous experience with maize is needed for a laboratory technician to recognize and classify the contrasting types. It is probably possible to test individual kernels and plant the same kernels for the next generation of backcrossing, testing, or seed increase, if the hand section method presented by Pradilla et al. (4) is employed. This method does not require freezing of the test material.

Conversion of Local Highland Varieties Phenotypically Similar to Floury Maize

After many centuries of selection in the highlands of the Andean zone, man has created a great diversity of maize races and biotypes. It is interesting and valuable to note that most of the soft-kernel varieties have a flint counterpart, which is used to prepare other types of foods. Indians prefer the soft floury type for most typical preparations and direct consumption, but the flint varieties have been preserved and are still planted in many areas.

Introducing the opaque-2 gene into these flint counterparts represents no problem to the breeder. The procedure is identical to the one outlined previously and to the scheme currently used with flint varieties. This last approach is being used for highland varieties in the maize program in Peru.

Laboratory-Analysis-Dependent System for Conversion of Floury Varieties or Lines to Opaque-2

Scheme E (Table 6) is very similar to the one used to convert dent or flint maize to opaque-2. Since visual selection in the self-pollinated ears is not possible, a single-kernel analysis will show which kernels within the floury ears are carrying or segregating for the opaque-2 gene. Table 6 outlines the procedure. Starting with the first backcross, the breeder may select only the floury kernels, and have fl_1 in the homozygous condition. On the other hand, if only normal (flint) kernels are selected, he can maintain the Fl_1 (+/+) in the system and finally get a soft maize, owing to the opaque-2 gene, but which can show contamination when pollinated with floury varieties. In each cycle, analyses should be performed only in those ears with clearly 6 normal (flint):10 floury segregation to confirm the presence of opaque-2. This will halve the work in the laboratory.

In any cycle, laboratory germination of the high-quality kernels of self-pollinated ears could be used to increase seed and allow testing at this level of conversion. There is some doubt about the speed and efficiency of this laboratory testing scheme when other genetic systems are available that do not require such a heavy dependence on the laboratory. However, there is no doubt about the importance of the laboratory in checking progress during the backcrossing cycles and in checking each line for quality before producing a hybrid or pooling material in a composite. This system is currently being used in the CIMMYT program.

GENERAL GENETIC SCHEME FOR THE CONVERSION OF FLOURY MAIZE TO OPAQUE-2

Schemes A to E as presented by their original authors, have provided a valuable genetic background on which to build a system adapted to each national program and maize to be converted and suited to the laboratory facilities available. Our discussion now turns to a general genetic scheme (F) for conversion. Using the best features of each system previously discussed, scheme F points out the variations available at each step, depending on the final objective of the program, the type of maize to be converted, and the availability of a laboratory input.

The general scheme, as presented in Table 7, is essentially method A of Bauman and Crane (1971); it has a backcross each cycle with the recurrent parent as male and a testcross to the original or other opaque source (+/+ as o_2/o_2) or double mutant (fl_1/fl_1 o_2/o_2) in the final product, and which route to follow for the two alternatives shown. Kernels may be selected from BC_1 for each route. If there is doubt about which product will be desired or most acceptable to the consumer, the correct route to follow is to select normal kernels to control both loci so that both (+/+ o_2/o_2) and (fl_1/fl_1 o_2/o_2) may be recovered in the final step.

TABLE 6 Scheme E: Conversion of floury varieties or lines to opaque-2 with help of protein-quality laboratory

	Recurrent parent		Female parent	Backcross results	Self-pollinated ears: Phenotypes	Self-pollinated ears: Laboratory results
Original cross	fl_1/fl_1 +/+	x	+/+ o_2/o_2			
			↓			
First backcross	fl_1/fl_1 +/+	x	+/fl_1 +/o_2	1 normal : 1 floury		
			↓			
			Normal Kernels from Backcross			
Second backcross + self-pollination	fl_1/fl_1 +/+	x	+/fl_1 +/+	floury	1 normal : 1 floury	All low
			+/fl_1 +/o_2	floury[a]	6 normal : 10 floury	Some high[a]
			Floury Kernels from Backcross			
	fl_1/fl_1 +/+	x	fl_1/fl_1 +/+	floury	floury	All low
			fl_1/fl_1 +/o_2	floury[a]	floury	Some high[a]
			↓			
			Floury Kernels from Normal			
Third backcross	fl_1/fl_1 +/+	x	+/fl_1 +/+	floury	1 normal : 1 floury	All low
			+/fl_1 +/o_2	floury[a]	6 normal : 10 floury	Some high[a]
			fl_1/fl_1 +/+	floury	floury	
			fl_1/fl_1 +/o_2	floury	floury	
			Floury Kernels from Floury			
	fl_1/fl_1 +/+	x	fl_1/fl_1 +/+	floury	floury	All low
			fl_1/fl_1 +/o_2	floury[a]	floury	Some high[a]
			↓			
			(to further cycles)			

[a] Laboratory tests of floury kernels from the segregating ears will reveal some kernels (one-fourth) that are high in quality, based on analysis of either lysine or tryptophan. Floury kernels from those backcrossed are ears then selected for further cycles.

TABLE 7 Scheme F: General genetic scheme for conversion of floury maize to opaque-2 maize

	Recurrent parent		Genotype of backcross plants	Backcross phenotypes	Testcross phenotypes ♀ (+/+ o_2/o_2)
Original cross	fl_1/fl_1 +/+	x	+/+ o_2/o_2		
			↓		
First backcross	fl_1/fl_1 +/+	x	+/fl_1 +/o_2	1 normal : 1 floury	
			↓		
			Normal Kernels from Backcross[a]		
Second backcross + testcross	fl_1/fl_1 +/+	x	+/fl_1 +/+	1 normal : 1 floury	normal
			+/fl_1 +/o_2	1 normal : 1 floury	1 normal : 1 opaque[a]
			Floury Kernels from Backcross[b]		
			fl_1/fl_1 +/+	floury	normal
			fl_1/fl_1 +/o_2	floury	1 normal : 1 opaque[b]
			↓		
			Normal Kernels from Backcross[a]		
Third backcross + testcross	fl_1/fl_1 +/+	x	fl_1/fl_1 +/+	floury	normal
			fl_1/fl_1 +/o_2	floury	1 normal : 1 opaque
			+/fl_1 +/+	1 normal : 1 floury	normal
			+/fl_1 +/o_2	1 normal: 1 floury	1 normal : 1 opaque
			Floury Kernels from Backcross[b]		
			fl_1/fl_1 +/+	floury	normal
			fl_1/fl_1 +/o_2	floury	1 normal : 1 opaque[b]
			↓		

TABLE 7 (cont.)

	Recurrent parent		Genotype of backcross plants	Backcross phenotypes	Testcross phenotypes ♀ (+/+ o_2/o_2)
			Normal Kernels from Backcross		
Fourth backcross + testcross	fl_1/fl_1 +/+	X	fl_1/fl_1 +/+	floury	normal
			fl_1/fl_1 +/o_2	floury	1 normal : 1 opaque
			+/fl_1 +/+	1 normal : 1 floury	normal
			+/fl_1 +/o_2	1 normal : 1 floury	1 normal : 1 opaque[a]
			Floury Kernels from Backcross		
			fl_1/fl_1 +/+	floury	normal
			fl_1/fl_1 +/o_2	floury	1 normal : 1 opaque[a]
			↓ (to further cycles)		

[a] If desired maize is (+/+ o_2/o_2), or both (+/+ o_2/o_2) and (fl_1/fl_1 o_2/o_2), select normal kernels from backcross-2 (or later backcross cycle) ears in these rows.

[b] If desired maize is (fl_1/fl_1 o_2/o_2), select floury kernels from backcross-2 (or later backcross cycle) ears in these rows.

In the case of the double mutant (fl_1/fl_1 o_2/o_2), verification of the genotype requires no special procedure if a backcrossing procedure was used in scheme F. As shown, the selected backcross progeny in each cycle on the basis of testcross results is already of the desired genotype. Verification of the single recessive(+/+ o_2/o_2) was outlined in Table 4, and this is recommended as the most efficient procedure.

PROGRESS IN FLOURY MAIZE CONVERSION IN HIGHLAND ZONES

The principal activity in conversion of these high-altitude maize varieties is concentrated in the Andean zone and Mexico. Progress in these conversion programs is slow owing to the long growing season, which generally limits breeding to one cycle per year. The complicated nature of the conversion process also has caused lost generations in some situations.

Colombia

The national program in Colombia is now in the third backcross of floury maize materials at Tibaitatá, the highland research center near Bogotá. Primary emphasis has been on visual selection, although in some varieties testcrossing schemes are needed to separate families that carry the opaque-2 gene.

Ecuador

In Ecuador the first backcross is now in the field; current plans for selection are dependent on the new protein laboratory that has just been installed in Santa Catalina near Quito.

Peru

The third backcross has been produced in three flint backgrounds and three floury maizes. A second backcross is in the field in another two flint highland types. Further progress in these materials depends on testcrossing and laboratory analyses in the next cycle.

Mexico

In Mexico, conversion of Cacahuacintle and other floury types is planned for the near future; some opaque-2 composites are currently available from CIMMYT (Composite I).

Bolivia

The first backcross has been made in Bolivia with self-pollinations and testcrosses made during the winter months. Two generations are produced per year in Cochabamba in this way, and the selfing and testing are considered necessary to preserve a maximum of adaptation and grain acceptability while searching for the desired (+/+ o_2/o_2) genotype. Yield is also a prime objective in the Cochabamba conversion and improvement scheme, since introduction of a new variety in the zone is thought to be unacceptable if protein quality is the only difference between the new type and the traditional variety.

In addition to the time required for each generation in the conversion process, most programs have been limited by a lack of prompt and reliable laboratory results on which to base decisions and further selections. With the installation of laboratories in La Molina (Peru), Santa Catalina (Ecuador), Cochabamba (Bolivia), and CIAT (Colombia), and the continuous check systems of standards proposed by CIMMYT and Purdue, this problem should lessen in the near future. The laboratory input is helpful to monitor progress in these floury materials.

CONCLUSIONS AND RECOMMENDATIONS

The conversion schemes presented are self-explanatory, and more details about each scheme may be requested from the sources listed. The advantages and disadvantages of the procedures are detailed also. The decision to choose a particular system will depend on the maize available, the final objective of the conversion, and the laboratory facilities available.

If there is a reasonable way to utilize the schemes that are based on visual selection in the selfed ears, this route is simple and requires a low-level laboratory input, although genetic testing should be incorporated into the scheme to assure opaque-2 quality in the final product.

When it is impossible to distinguish the opaque-2 segregants from the floury background, one of the other systems is necessary. The two modified systems (schemes A and B) give control of the floury gene as well as identification of the high-quality opaque-2 segregants. Choice of a scheme depends on the final objective. Because of the ease in identifying contaminated kernels in farmers' fields, we recommend conversion of local highland varieties to an opaque-2 single-mutant genotype (+/+ o_2/o_2). This will prevent loss of identity and quality in the new material after it is introduced to the farmer and after he selects his own seed for a few generations. Laboratory analyses logically should be used to test the products of any of these schemes, especially the procedures that have a double-mutant maize as the final objective (fl_1/fl_1 o_2/o_2) and those based on visual selection.

Rapid conversion of the highland floury-type maize is essential to improved nutrition in highland zones. Development efforts such as the Puebla Project in Mexico, the Cajamarca Project in Peru, and the Rionegro Project and others in Colombia, are all located in medium-altitude or highland zones. To date, no opaque-2 maize or other maize with improved quality has been available for general use in the project areas.

Since these programs of extension and integrated development are concentrating on improving human nutrition, a maize variety with an improved quality is an essential component in the production package to be introduced. Each national program has a responsibility to move ahead as rapidly and efficiently as possible with the conversion of varieties and testing under farm conditions. International and regional centers such as CIMMYT and CIAT must provide the necessary field and laboratory training and laboratory sample testing needed to implement these programs in the field.

Much progress can be made by converting maize in one region for use in another zone or country, and this exchange may give an initial solution to the need for a high-quality maize variety. The conversion of plant types adapted to a specific climatic zone, and of a special large-grain type preferred for consumption in one region, make these conversion schemes essential to each national program where floury maize is important in the diet. Only by introducing this new maize with improved quality can we hope to significantly improve the nutritional levels in many areas. The methodology and new laboratory facilities now available will give a new boost to this work, and soon we should have varieties available to the Andean zone farmer.

The suggestions of P. L. Crane and L. M. Bauman of Purdue University and S. K. Vasal, CIMMYT, and F. Poey, Mexico, were especially valuable in the preparation of the manuscript.

The authors are also indebted to the following scientists for their personal communications relative to the conversions of floury materials: Enrique Arias and Daniel Sarria, Programa Nacional de Maiz y Sorgo, C.N.I.A. Palmira, ICA, Apartado aéreo 233, Palmira, Valle, Colombia; Gonzalo Avila, Director, Granja Modelo Pairumani, Casilla 128, Cochabamba, Bolivia; Luis Beingolea and Ricardo Sevilla, Programa de Maiz, Universidad Agraria La Molina, Apartado 456, Lima, Peru; and Paul Paz, Programa de Maiz, Estación Experimental Santa Catalina, INIAP, Apartado 2600, Quito, Ecuador.

[*A discussion of this paper can be found on pp. 486–487 of* **Questions and Answers.**]

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GENETIC IMPROVEMENT OF MODIFIED PROTEIN MAIZE

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With the discovery that lysine and tryptophan levels in maize endosperm protein could be dramatically increased by the recessive gene opaque-2 and the semidominant floury-2 gene, a key was discovered for improving the diets of monogastric animals, including man.

In countries where maize is a staple food, the implications of these findings are obvious and have been pointed out repeatedly. However, in the United States, as in many other countries, the primary use of maize is for livestock feeding. Hence, widespread use of modified protein hybrids depends on their economic competitiveness with normal dent maize plus soybean meal supplement.

The key questions originally considered when modified protein breeding work was initiated in Illinois were as follows:

1. Can acceptable yields of modified protein hybrids be obtained (1) from backcross derivatives of currently used lines, or (2) by isolating new lines from populations homozygous for opaque-2 or floury-2 that have been selected for improved agronomic fitness?

2. Can the level of lysine in opaque-2 or floury-2 populations be in-

creased? (This question was important because early feeding trials established that, whereas the level of tryptophan in opaque-2 or floury-2 hybrids was adequate for diets in most rations, the level of lysine, although drastically improved, was below optimum for finishing swine.)

3. Can the problems inherent in the soft-endosperm texture of the opaque-2 and floury-2 genotypes be overcome while maintaining improved protein quality?

Attempting to answer these questions, we initiated research in three major areas:

1. Developing and testing backcross-derived opaque-2 and floury-2 hybrids.
2. Developing broad-based germ-plasm pools homozygous for either opaque-2 or floury-2, followed by recurrent selection for yield and lysine percentage.
3. Isolation and genetic analysis of modifier genes that would increase kernel density in an opaque-2 background without detrimental effects on protein quality.

This paper describes results from this research and their implications in the development of modified-protein maize.

PERFORMANCE OF OPAQUE-2 HYBRIDS

When reading papers dealing with modified protein maize, one often encounters the truism, "Yield must be improved before wide adoption will occur." We believe that this is an oversimplification. Problems in the United States, for example, are very different from those in Eastern Europe, where high-quality-protein supplements for animal feeding are scarce, or in parts of Latin America where maize is widely used as human food. The acceptable loss of calories per acre as a trade-off for increased amounts of high-quality protein is a function of demand for the two components. In the United States, soybean meal has, traditionally, been relatively inexpensive. Hence, lower maize yields to improve protein quality have been unacceptable.

However, the economics of protein–calorie production may be changing. Soybean meal is now (November 1972) selling for $120/ton in the United States, an increase of 76% in the last year. Thus, in regions where an adequate supply of good-quality protein does not exist, and where maize is an acceptable food, yield may be a secondary consideration.

We believe that yields of opaque-2 maize hybrids need not be inferior to yields of standard dents. Over the years, we have observed a definite improvement in performance in our trials. In 1966, near-isogenic versions of the same hybrids were tested at Urbana (4). The opaque-2 versions yielded 85% of the normal counterparts, on the average, although only 5 of the 17 hybrids yielded significantly less than the normal version. Backcross-4 recoveries of the same 7 lines were tested in 1967. The opaques yielded, on the average, 92% of the normal maize counterparts.

We have continued testing hybrids of backcross lines derived from opaque-2 synthetics with mounting optimism. The 1972 performance of the eight highest-yielding opaque-2 hybrids and T sterile versions of commercial dent hybrids is shown in Table 1. The T dents were planted at random among the opaque-2 hybrids and were pollinated by them. Damage from the T race of *H. maydis* was minimal and had little or no effect on yield.

The eight higher-yielding hybrids averaged yields of 7,600 kg/ha, 99% of that of the six commercial dents. The highest-yielding opaque-2 hybrid was only 7% below the highest-yielding dent. To add perspective, commercial 1 is the "hybrid to beat" in the central United States corn belt.

POPULATION IMPROVEMENT

Although backcrossing has been successful in producing opaque-2 inbred lines and hybrids that are commercially acceptable, progress from this approach is limited. To increase the diversity of opaque-2 and floury-2 germ plasm, and to provide random mating populations for studying the genetic potential for improving populations with improved protein quality, the opaque-2 and floury-2 alleles were independently introduced into two maize synthetics [Iowa super stiff stalk, (SSSS) and Synthetic Disease-Oil (DO)]. The procedure for developing opaque-2 and floury-2 versions of these synthetics has been described (2). In brief, each synthetic was crossed to a source of opaque-2 and floury-2. The F_1 was backcrossed twice to the appropriate synthetic. For SSSS – o_2 and DO – o_2, the BC_2F_1 was random mated; then homozygous o_2o_2 kernels were selected and plants from these kernels were random mated. In the floury-2 conversion program, plants grown from floury kernels selected from the BC_1F_1 random-mated genera-

TABLE 1 Performance of opaque-2 and dent commercial hybrids: Urbana, 1972 (experiment 402)

Pedigree	Yield		H_2O in grain	Lodged
	bu/acre	kg/ha	(%)	(%)
93160 X 98176[a]	149	9,354	24	0
98176 X 98168[a]	142	8,914	25	2
N28(C123 X Mo17)	139	8,726	26	0
N28(W64A X H49)	138	8,663	28	0
Va43(C123 X Mo17)	136	8,538	25	5
R177(C123 X Va35)	136	8,538	25	1
98153 X 98191[a]	135	8,475	23	0
R177(W64A X H49)	133	8,349	26	13
Commercial 1[b]	161	10,106	24	2
$\bar{x}$, six commercials[c]	140	8,789	25	3

[a] Purdue hybrids.
[b] Highest-yielding dent hybrid (T sterile).
[c] T sterile dent hybrids.

tion were selfed, and kernels from ears homozygous fl_2fl_2 were selected to produce the plants that were random mated.

In each of the four populations (SSSS – o_2, SSSS – fl_2, DO – o_2, and DO – fl_2), a modified ear-to-row selection scheme (5) was initiated. In each synthetic, 200 ears from the second generation of random mating were planted ear-to-row in three replications of a blocks-in-replications design. Within each replication, entries were grouped into 10 blocks of 20 entries. The same 20 entries were included in a block in each replicate. Plots were single rows, 1 by 5m in 1969 and 0.76 by 3.80 m thereafter. Population density was approximately 54,000 plants/ha.

A fourth replicate was planted in isolation to provide seed for the next generation. A single row of each of the 200 families was included. Two pollinator rows planted to a bulk lot of seed obtained by compositing equal quantities of seed from each of the 200 ears were planted every four rows. The 200 rows from individual ears were detasseled and five ears selected from each row.

From the yield trial, the weight of shelled grain and moisture percentage were determined and a sample was saved for chemical analysis. Plots were hand harvested in 1969 and 1970 and machine harvested in 1971 and 1972. For each plot the following chemical analyses were made:

1. Percentage of protein (Kjeldahl N × 6.25).
2. Percentage of lysine in the whole kernel (microbiological assay using *Leuconostoc mesenteroides*, 1969 and 1970 (3); modified trinitrobenzene sulfonic acid method (TNBS), 1971 (7).
3. Percentage of oil (nuclear magnetic resonance).

In addition, kernel density was measured in 1969 and 1970 as grams per 162 cm^3. The original generation was grown in 1969; the first, second, and third cycles of selection were grown in 1970, 1971, and 1972, respectively.

Selection was based on kilograms of lysine per hectare, obtained by multiplying yield in kilograms per hectare by the percentage of lysine. The highest four families within each block were selected, with the restriction that the percentage of lysines be greater than the block mean. Thus, in each synthetic, 5 ears per family from 40 families were selected to provide the 200 entries for the next cycle.

After the initial generation, one plot of an appropriate hybrid check and one of the original population were included in each block. Thus, there were 30 hybrid check plots and 30 plots of the original synthetic in each experiment.

Analyses of variance and covariance were made on a within-block basis, using the pooled entries in blocks mean square, and the pooled error for replications times entries in blocks, for example, to test for significant differences among entries. Heritability values were calculated on the basis of selection within blocks from one location grown in 1 year with three replications. Phenotypic and genotypic correlations were calculated using entry means on a pooled-within-block basis for each synthetic.

Predicted response from selection of the upper 20% of families was calculated as $R = i\sigma_p h^2$, where i is 1.4, σ_p is the phenotypic standard deviation of the character selected, and h^2 is heritability. Correlated response was calculated as $C = i\sigma_p h_y h_x r_{Gxy}$, where i is 1.4, σ_p is the phenotypic standard deviation, h_y and h_x are square roots of heritability of the selected character and the correlated character, and r_{Gxy} is the genetic correlation between the two characters. Response is reported as a percentage of the population mean. Response was calculated for each synthetic in each cycle and averaged over three cycles to give average response per cycle.

Because the selection experiments were grown only 1 year per cycle, estimates of genotype – environment interaction were not possible. A second experiment included six opaque-2 hybrids grown at 10 locations in Illinois in 1969. Two replications were grown at each location. Approximately 10 plants per replicate were selfed, and a bulk sample of seed from each plot was analyzed for percentage of lysine (amino acid analyzer) and percentage of protein (Kjeldahl N × 6.25).

RESULTS

Selection Experiments

The primary effect of selection was to increase yield of grain and kilograms of lysine per hectare (Table 2). With the exceptions of the percentage of lysine in cycle 2 of DO–o_2 and DO–fl_2 and the percentage of protein in cycle 1 of SSSS – fl_2 and DO – fl_2, none of the comparisons of cycle means with the mean of cycle 0 grown in the same year was significant for percentage of lysine, grams of lysine per 100 g of protein, or percent protein. In the four cases of significant differences just cited, the magnitude of the difference was so small as to be of no practical importance. The greatest increase in yield and kilograms of lysine per hectare occurred in cycle 1 in all four synthetics. Only SSSS – o_2 showed an increase in cycle 2 over cycle 1. Although DO–o_2 consistently outyielded cycle 0 by from 6 to 7% in cycles 1, 2, and 3, no individual comparison of a selected cycle with the original population was significant. The largest gain for both yield of grain and yield of lysine occurred in SSSS – o_2, which had an average increase of approximately 4.7% per cycle for yield (three cycles) and 7.4% per cycle for kilograms of lysine per hectare (two cycles) (Table 7). At the end of two cycles, gain in yield for SSSS – o_2 was 6.6% per cycle. Thus, the difference in gain per cycle between grain and lysine yield when three cycles are compared with two cycles will probably be narrowed when lysine data are available for cycle 3 of SSSS – o_2.

The marked gain from selection in grain yield and kilograms of lysine per hectare, and the lack of progress in percentage of lysine, resulted from (1) higher heritabilities of grain yield than percentage of lysine, and (2) higher

TABLE 2 Means for each cycle (absolute value and as a percentage of cycle 0 grown in the same year) for several traits in two opaque-2 and two floury-2 synthetics

	Cycle						
	0	1		2		3	
Population	Mean	Mean (%)	Cycle 0[a]	Mean (%)	Cycle 0	Mean (%)	Cycle 0
				kg Lysine/ha			
SSSS-o$_2$	20.8	23.2	109.4[b]	22.5	114.8[b]		
SSSS-fl$_2$	15.2	16.0	109.6[b]	16.0	108.3[b]		
DO-o$_2$	22.8	20.0	109.3	20.2	108.0[b]		
DO-fl$_2$	17.8	18.7	111.3[b]	16.4	106.5		
				% Lysine			
SSSS-o$_2$	0.400	0.407	100.0	0.424	100.7		
SSSS-fl$_2$	0.305	0.303	99.0	0.323	100.0		
DO-o$_2$	0.414	0.385	100.3	0.378	102.4[b]		
DO-fl$_2$	0.377	0.344	100.0	0.334	97.1[b]		
				Yield (kg/ha)			
SSSS-o$_2$	5,216	5.725	109.7[b]	5,300	113.1[b]	6,053	114.5[b]
SSSS-fl$_2$	5,002	5,295	110.7[b]	4,954	110.0[b]	5,287	110.1[b]
DO-o$_2$	5,508	5,154	107.8	5,358	105.6	5,378	107.2
DO-fl$_2$	4,786	5,438	111.2[b]	4,909	109.3	5,275	113.7[b]
				gL/100 gP[c]			
SSSS-o$_2$	3.77	3.91	100.5	4.18	102.2		
SSSS-fl$_2$	2.72	2.86	97.9	2.97	98.7		
DO-o$_2$	3.83	3.62	102.0	3.52	101.4		
DO-fl$_2$	3.25	3.26	101.9	3.02	98.0		
				% Protein			
SSSS-o$_2$	10.6	10.3	99.0	10.2	99.0		
SSSS-fl$_2$	11.2	10.6	101.0[b]	10.8	100.9		
DO-o$_2$	10.8	10.9	100.9	10.7	100.9		
DO-fl$_2$	11.6	10.6	99.1[b]	11.1	99.1		

[a] Percentage of cycle 0 check grown in same year.

[b] Significantly different from cycle 0 at the 0.05 probability level as measured by F test in the analysis of variance.

[c] In Tables 2 to 13, gL = grams of lysine, and gP = grams of protein.

genetic correlations of lysine per hectare with grain yield than with percentage of lysine (Tables 3, 6, and 7). In all four synthetics in all cycles, the genetic coefficients of variation (CV) were consistently higher for yield than for percentage of lysine (Table 4). The same trend was evident for heritabilities in cycles 1 and 2.

The floury-2 synthetics were consistently lower in percentage of lysine, grams of lysine per 100 g of protein, yield of grain, and kilograms of lysine per hectare than their opaque-2 counterparts, but were higher in percentage of protein (Table 2). With the exception of grain yield, these results are consistent with unpublished data from our laboratory on backcross-derived floury-2 and opaque-2 inbreds and hybrids. Preliminary yield tests of floury-2 hybrids indicate that yields of floury-2 genotypes may not be as

TABLE 3 Heritability values (%) for five traits measured in each cycle of two opaque-2 and two floury-2 synthetics

Population	Cycle 0	Cycle 1	Cycle 2	Cycle 0	Cycle 1	Cycle 2	Cycle 3
		% Lysine			*Yield*		
SSSS-o_2	35[a]	37[a]	7	34[a]	65[a]	59[a]	58[a]
SSSS-fl_2	65[a]	35[a]	45[a]	62[a]	59[a]	63[a]	52[a]
DO-o_2	39[a]	30[a]	47[a]	40[a]	60[a]	68[a]	60[a]
DO-fl_2	32[a]	35[a]	46[a]	79[a]	62[a]	61[a]	65[a]
		kg lysine/ha			*gL/100 gP*		
SSSS-o_2	40[a]	51[a]	50[a]	29[a]	14	5	
SSSS-fl_2	64[a]	47[a]	62[a]	35[a]	10	26[a]	
DO-o_2	43[a]	39[a]	64[a]	23[a]	2	23[a]	
DO-fl_2	59[a]	53[a]	56[a]	7	28[a]	16	
		% Protein					
SSSS-o_2	69[a]	66[a]	63[a]				
SSSS-fl_2	86[a]	47[a]	77[a]				
DO-o_2	73[a]	72[a]	69[a]				
DO-fl_2	91[a]	64[a]	73[a]				

[a] Genetic variance component significant at the 0.05 probability level as measured by F test in an analysis of variance.

TABLE 4 Genetic coefficient of variation (%) for each cycle of two opaque-2 and two floury-2 synthetics

Population	Cycle 0	Cycle 1	Cycle 2	Cycle 0	Cycle 1	Cycle 2	Cycle 3
		% Lysine			*Yield*		
SSSS-o_2	3.7	4.4	1.3	4.2	8.8	10.1	9.8
SSSS-fl_2	8.1	4.8	3.1	10.7	8.0	9.0	8.7
DO-o_2	3.6	3.9	3.3	4.8	9.3	11.5	11.3
DO-fl_2	4.8	4.2	3.2	16.4	7.4	9.6	10.9
		kg lysine/ha			*gL/100 gP*		
SSSS-o_2	5.9	8.4	9.6	3.1	2.3	1.1	
SSSS-fl_2	12.7	8.7	9.8	4.1	2.3	2.2	
DO-o_2	6.2	7.4	11.5	2.4	0.8	2.0	
DO-fl_2	13.8	8.4	9.3	1.9	3.5	1.6	
		% Protein					
SSSS-o_2	3.1	3.2	2.6				
SSSS-fl_2	5.8	2.8	3.6				
DO-o_2	3.2	4.0	3.1				
DO-fl_2	6.2	3.2	3.6				

greatly reduced from normals as are opaque-2 hybrids. The higher yield of opaque-2 synthetics reported here is probably because partially converted inbred lines were used as sources of the opaque-2 gene, whereas the source of the floury-2 gene was a genetic stock with poor agronomic qualities.

Although no significant progress in the percentage of lysine has been

observed, significant genetic variation was detected in each cycle of each synthetic, except for cycle 2 of SSSS o_2 (Table 3). The heritability values are large enough to suggest that progress from selection for percentage of lysine should be possible if selection were based solely on the percentage of lysine. The fact that genetic coefficients of variation for percentage of lysine are generally as high as those for percentage of protein, even though heritabilities are lower, suggests that sufficient genetic variance is present to allow for successful selection for percentage of lysine if the environmental variance can be reduced sufficiently.

The low heritability values for grams of lysine per 100 g of protein are partly the result of large error variance resulting from the fact that error variance for grams of lysine per 100 g of protein includes error associated with the measurement of both percentage of protein and percentage of lysine.

Coefficients of variation (CV) were very similar between synthetics and between opaque-2 and floury-2 versions of the same synthetics (Table 5). The shift from the use of the microbiological assay to the TNBS method for lysine determinations in cycle 2 resulted in a significant reduction in CV's for percentage of lysine and grams of lysine per 100g of protein. The change to machine harvesting instead of hand harvesting in cycle 2 had no appreciable effect on the CV's.

Phenotypic correlations were very similar in the four synthetics (Tables 6 and 7). Phenotypic correlations of the percentage of lysine with grams of lysine per 100 g of protein and grain yield with lysine per hectare were high and positive, as were those of percentage of lysine with percentage of protein

TABLE 5 Coefficients of variation (%) for each cycle for several traits in two opaque-2 and two floury-2 synthetics

	Cycle			Cycle			
Population	0	1	2	0	1	2	3
		% Lysine			*Yield*		
SSSS-o_2	8.7	9.0	7.8	10.1	10.4	13.9	13.8
SSSS-fl_2	10.3	10.8	5.8	14.4	10.8	11.3	13.5
DO-o_2	7.8	9.8	5.8	10.3	12.4	13.0	15.0
DO-fl_2	12.2	9.6	5.8	14.8	9.9	12.5	13.2
		kg lysine/ha			*gL/100 gP*		
SSSS-o_2	12.5	13.1	15.9	8.6	9.1	7.8	
SSSS-fl_2	16.6	15.2	12.8	9.8	11.6	6.1	
DO-o_2	12.2	15.1	14.3	7.7	9.5	6.1	
DO-fl_2	19.9	13.3	13.5	12.3	9.5	6.1	
		% Protein					
SSSS-o_2	3.6	3.6	3.3				
SSSS-fl_2	4.0	4.9	3.2				
DO-o_2	3.4	4.0	3.3				
DO-fl_2	3.4	3.8	3.6				

TABLE 6 Phenotypic (above diagonal) and genotypic (below diagonal) correlations among several traits in opaque-2 and floury-2 versions of SSSS

	Cycle	% Lysine		gL/100 gP		% Protein		Yield		Lysine/ha	
		o_2	fl_2	o_2	fl_2	o_2	fl_2	o_2	fl_2	o_2	fl_2
% Lysine											
	0			0.81[a]	0.79[a]	0.41[a]	0.72[a]	-0.02	-0.15	0.64[a]	0.50[a]
	1			0.84[a]	0.84[a]	0.55[a]	0.42[a]	-0.24[a]	-0.10	0.39[a]	0.56[a]
	2			0.77[a]	0.57[a]	0.33[a]	0.54[a]	-0.12	-0.00	0.24[a]	0.39[a]
gL/100 gP											
	0	0.60	0.74			-0.20[a]	0.15	0.17	-0.07	0.56[a]	0.45[a]
	1	0.72	0.89			0.01	-0.12	-0.07	0.06	0.45[a]	0.60[a]
	2	-1.44	0.11			-0.34[a]	-0.38[a]	0.01	0.15[a]	0.28[a]	0.36[a]
% Protein											
	0	0.57	0.87	-0.32	0.31			-0.30[a]	-0.16	0.05	0.30[a]
	1	0.90	0.91	0.34	0.61			-0.34[a]	-0.29[a]	0.02	0.03
	2	1.01	0.78	-1.21	-0.53			-0.21[a]	-0.17[a]	-0.08	0.06
Yield											
	0	0.22	-0.16	0.72	-0.10	-0.47	-0.14			0.75[a]	0.77[a]
	1	-0.39	-0.18	-0.17	0.13	-0.43	-0.41			0.80[a]	0.76[a]
	2	-0.57	0.01	-0.05	0.28	-0.27	-0.17			0.93[a]	0.92[a]
Lysine/ha											
	0	0.74	0.51	0.81	0.42	0.04	0.42	0.82	0.77		
	1	0.12	0.39	0.21	0.64	0.02	0.12	0.87	0.83		
	2	-0.47	0.37	-0.30	0.32	-0.13	0.11	0.99	0.94		

[a] Phenotypic correlation significant at the 0.05 probability level.

TABLE 7 Phenotypic (above diagonal) and genotypic (below diagonal) correlations among several traits in opaque-2 and floury-2 versions of Syn DO

		% Lysine		gL/100 gP		% Protein		Yield		Lysine/ha	
	Cycle	o_2	fl_2	o_2	fl_2	o_2	fl_2	o_2	fl_2	o_2	fl_2
% Lysine											
	0			0.76[a]	0.67[a]	0.51[a]	0.54[a]	-0.06	-0.33[a]	0.57[a]	0.12
	1			0.76[a]	0.83[a]	0.57[a]	0.37[a]	-0.29[a]	-0.07	0.30[a]	0.57[a]
	2			0.67[a]	0.54[a]	0.59[a]	0.61[a]	-0.07	-0.19[a]	0.26[a]	0.19[a]
gL/100 gP											
	0	0.48	-0.61			-0.17	-0.26[a]	0.14	0.02	0.58[a]	0.33[a]
	1	0.04	0.68			-0.09	-0.20[a]	-0.09	0.01	0.36[a]	0.52[a]
	2	0.42	-0.03			-0.26[a]	-0.33[a]	0.09	0.15[a]	0.32[a]	0.35[a]
% Protein											
	0	0.77	0.92	-0.20	-0.90			-0.27[a]	-0.46[a]	0.11	-0.22[a]
	1	1.00	0.55	-0.08	-0.23			-0.34[a]	-0.14	0.00	0.12
	2	0.81	0.90	-0.19	-0.46			-0.20[a]	-0.35[a]	-0.03	-0.12
Yield											
	0	0.05	-0.67	0.62	-0.08	-0.37	-0.49			0.78[a]	0.89[a]
	1	-0.60	-0.09	-0.51	0.05	-0.49	-0.19			0.82[a]	0.78[a]
	2	-0.14	-0.31	0.16	0.50	-0.26	-0.50			0.94[a]	0.92[a]
Lysine/ha											
	0	0.62	-0.47	0.74	-0.41	0.19	-0.25	0.81	0.97		
	1	-0.23	0.42	-0.64	0.38	-0.10	0.12	0.92	0.86		
	2	0.14	0.03	0.29	0.52	-0.04	-0.21	0.96	0.94		

[a] Phenotypic correlation significant at the 0.05 probability level.

and percentage of lysine with lysine yield. Correlations of grams of lysine per 100 g of protein with lysine yield were positive and intermediate, whereas the correlations of yield with percentage of protein were negative and intermediate in magnitude. The genotypic correlations were similar in sign and magnitude to the phenotypic correlations, except for the correlations of percentage of lysine with grams of lysine per 100 g of protein, percentage of protein, and lysine per hectare. The genotypic correlations of percentage of lysine with percentage of protein were all higher than the phenotypic correlations. In contrast, the genotypic correlations of percentage of lysine with grams of lysine per 100 g of protein and kilograms of lysine per hectare were not consistent between synthetics, and were generally lower than the phenotypic correlations.

Average observed response to selection for kilograms of lysine per hectare was approximately half the predicted response in all synthetics except SSSS o_2, where observed and predicted response were equal (Table 8). No significant change in the percentage of lysine as the result of selection was observed or predicted. Significant increases in yield occurred in all synthetics, with the possible exception of DO – o_2, although the observed responses were always less than the predicted correlated response. Although data are not shown, no significant shifts were observed in the percentage of oil, percentage of moisture at harvest, or density in any of the synthetics.

In a plant breeding program, selection may be based solely on yield, percentage of lysine, or percentage of protein, depending on the circumstances. Predicted increases in the percentage of lysine per cycle, if selection were based solely on percentage of lysine, ranged from 2.3 to 5.2% of the

TABLE 8 Average predicted and observed change per cycle in percentage of the population mean for four modified protein synthetics when selection was based on kilograms of lysine per hectare

	Change (% of mean)			
	SSSS-o_2	SSSS-fl_2	DO-o_2	DO-fl_2
kg Lysine/ha				
Observed	7.4	4.2	4.0	3.2
Predicted	7.4	10.8	8.0	10.7
% Lysine				
Observed	0.4	0.0	1.2	-1.4
Predicted	0.8	2.5	0.6	-0.2
Yield[a]				
Observed	4.7	3.0	1.9	3.9
Predicted	6.4	8.0	7.5	10.8
% Protein				
Observed	-0.05	0.4	0.4	-0.4
Predicted	-0.01	1.1	0.0	-0.7

[a] Observed values based on three cycles for yield, two for kg lysine/ha, % protein and % lysine. P Predicted values are averages of three cycles.

population mean (Table 9). More rapid progress could be made by changing the breeding system to one such as selection among S_1 families, where more of the additive variance would be available for selection. Predicted correlated responses suggests that selection for percentage of lysine alone will result in a yield reduction of 1.0 to 4.0% of the mean per cycle and an increase in the percentage of protein of 1.6 to 3.4% of the mean per cycle.

If facilities are not available for measuring the percentage of lysine, a possible alternative would be to select for the percentage of protein, because the genetic correlations between the two are high, and the heritability of the percentage of protein is higher than for lysine. If selection is based on the percentage of protein only, predicted response for the percentage of lysine is slightly higher in each synthetic than when selection is based on the percentage of lysine alone (Table 9). However, predicted yield reduction ranged from 2.2 to 5.9% of the mean per cycle, nearly twice the reduction expected if selection is based on the percentage of lysine. Predicted increases in the percentage of protein ranged from 3.2 to 5.2% of the mean per cycle.

If facilities for chemical analysis are not available to the breeder, selection based on yield alone would be expected to reduce lysine by less than 1.0% of the population mean per cycle, except in DO – fl_2 (Table 9). Predicted yield increases ranged from 7.7 to 12.7% of the mean per cycle; predicted decreases in the percentage of protein ranged from 0.9 to 2.1% of the mean per cycle.

Since predicted responses were generally greater than those observed (and particularly since the genetic variance estimates on which these are based included any genotype – environment interaction), it seems possible to increase yield in opaque-2 or floury-2 populations with negligible reductions in the percentage of lysine by selecting for yield alone. Similarly,

TABLE 9 Predicted change in percentage of the population mean if selection had been based on percentage of lysine, yield, or percentage of protein in four modified protein synthetics

	Change (% of mean)			
	SSSS-o_2	SSSS-fl_2	DO-o_2	DO-fl_2
Response for		*Selection Based on % Lysine*		
% Lysine	2.3	5.2	3.0	3.3
Yield	-1.4	-1.0	-1.9	-4.0
% Protein	1.6	3.4	2.4	2.9
		Selection Based on % Protein		
% Lysine	2.6	5.3	3.7	4.1
Yield	-3.1	-2.2	-3.5	-5.9
% Protein	3.2	4.8	3.9	5.2
		Selection Based on Yield		
% Lysine	-0.6	-0.3	-0.9	-1.8
Yield	7.7	9.8	8.8	12.7
% Protein	-1.1	-0.9	-1.3	-2.1

selection for a few generations for the percentage of lysine alone should have only minor effects on yield. However, selection for the percentage of protein, which might give as great an increase in the percentage of lysine as selection for the percentage of lysine alone, could result in an appreciable reduction in yield.

Genotype–Environment Interaction

Only limited data are available on the magnitude of genotype – environment interaction for the percentage of lysine, grams of lysine per 100 g of protein, and percentage of protein. Six opaque-2 hybrids grown at 10 locations in Illinois in 1969 showed significant differences among hybrids and among locations for all three characters, but no significant genotype – environment interactions were found. Location means ranged from 0.450 to 0.552 for percentage of lysine, 4.39 to 4.81 for grams of lysine per 100 g of protein, and 10.2 to 11.9 for percentage of protein (Table 10). Hybrid means, averaged over locations, ranged from 0.477 to 0.583 for percentage of lysine, 4.34 to 5.16 for grams of lysine per 100 g of protein, and 10.3 to 11.9 for percentage of protein (Table 11). For two hybrids, R801 × R802 and R801 × Oh7N, the percentage of lysine was 0.5 or greater at each location.

These limited data suggest that genotype – environment interactions are not great, but locations may differ widely for all three characters. Also, the data suggest that it should be possible to develop hybrids that consistently produce above 0.5% lysine in the grain.

VITREOUS-ENDOSPERM OPAQUE-2 HYBRIDS

The opaque-2 mutant segregates in a monohybrid fashion, providing it is in an appropriate genetic background. Opaque-2 is a "good" gene in the terminology of maize geneticists, because it segregates into discrete classes.

TABLE 10 Location means, average of six opaque-2 hybrids, grown in 1969

Location	gL/100 gP	% Lysine	% Protein
Aurora	4.41	0.450	10.2
Decatur	4.73	0.497	10.5
DeKalb	4.45	0.513	11.5
E. St. Louis	4.39	0.520	11.9
Granville	4.47	0.494	11.0
Jacksonville	4.81	0.552	11.5
Mason City	4.71	0.500	10.6
Momence	4.75	0.518	10.9
Piper City	4.65	0.492	10.6
Urbana	4.77	0.515	10.8

TABLE 11 Means and ranges of location means for six opaque-2 hybrids grown in Illinois in 1969

	% Lysine		gl/100 gP		% Protein	
	Mean	Range	Mean	Range	Mean	Range
R802 X Oh7N	0.482	0.467-0.503	4.68	4.35-4.89	10.30	9.68-10.84
R802 X R803	0.477	0.414-0.544	4.34	3.38-4.74	11.06	10.09-12.00
R802 X Oh43	0.478	0.445-0.540	4.91	4.52-5.24	10.44	9.43-11.31
R801 X R802	0.583	0.504-0.640	4.59	4.02-4.88	11.91	11.06-12.37
R801 X Oh7N	0.571	0.503-0.725	5.16	4.51-5.86	11.07	9.93-12.37
Oh43 X W64a	0.440	0.360-0.495	4.02	3.54-4.48	10.97	10.12-11.62

However, early breeding efforts met with classification problems in some backgrounds, notably United States inbreds C103 (1) and perhaps lines related to T8, although many others doubtless fall into the "difficult" category. We suspect that many breeders ignored the exceptional cases and selected segregates that clearly behaved in the expected way.

The harder, vitreous segregates could not long be thrust aside, because it became obvious that machine harvesting and multiple handling of the soft opaque-2 kernels damaged them severely. Acceptability would be improved if high-quality-protein maize of dent- or flint-like texture could be bred.

We have struggled with the genetics of the opaque-2 modifier complex since 1966. Several independent sources of modifiers have been found, notably in inbreds C103, Mo5, and Va35, and from Syn DO – o_2 and SSSS – o_2. As all opaque-2 maize breeders know, the modifiers, have effects ranging from small islands of hard vitreous starch ("saddle" or "crown" hard starch), to hard, vitreous, flint-like types virtually indistinguishable from normal maize. We have developed several tentative genetic models, all unsatisfactory when tested in depth.

However, S. K. Nigam of our laboratory has been working on a modifier complex (01716) isolated from DO – o_2, which produces kernels almost indistinguishable from regular dents, but in which the endosperm protein quality is similar to standard opaque-2 stocks (Tables 12 and 13). Still under development is a model involving two tentative loci, probably independent,

TABLE 12 Composition of dent, standard opaque-2, and vitreous opaque-2 stocks ($\bar{x}$ of 20 ears)

	Whole kernel			Endosperm		
	OH43+/+	Oh43o_2/o_2	01716o_2/o_2	Oh43+/+	Oh43o_2/o_2	01716o_2/o_2
Lysine (%)[a]	0.26	0.40	0.49	0.16	0.26	0.31
Protein (%)	9.74	9.95	13.0	9.32	8.79	10.3
gL/100 gP	2.70	4.04	3.75	1.69	3.02	3.03

Source: After Nigam (6).
[a] Percentage of dry matter.

TABLE 13 Endosperm composition of F_3 segregates of $Oh43o_2/o_2$ X $01716o_2/o_2$ (vitreous) ($\bar{x}$ of 20 ears)

	Vitreous	Partial vitreous	Standard opaque-2
Lysine (%)[a]	0.23	0.24	0.25
Protein (%)	7.91	8.05	7.92
gL/100 gP	2.97	2.99	3.14

Source: After Nigam (6).
[a] Percentage of dry matter.

in which the triple recessive endosperm genotype ($o_2o_2o_2$ $vt^1vt^1vt^1vt^2vt^2vt^2$) has vitreous endosperm. We have found that either or both of the modifier loci exhibit dosage. Vitreous endosperm is produced by two doses of the recessive alleles at the two presumed loci. Two doses of the dominant alleles, possibly at both loci, result in the classical opaque-2 soft endosperm, although small islands of vitreous starch occur in some segregating populations.

It seems probable that other modifiers exist in the maize which are not allelic to those considered by Nigam, and that conversion of varieties, synthetics, or inbreds will uncover more of them. This would not be particularly surprising to maize geneticists who have used other single-gene mutants in their studies and have encountered backgrounds in which the allele becomes difficult to classify. Therefore, if the soft opaque-2 endosperm is an "aberration" that appears only if specific modifiers exist in the same cell, then the creation of breeding pools containing various modified opaque-2 stocks and desirable germ plasm seems to be advisable. Thus, breeding high-quality-protein maize might be based on standard polygene schemes, and maize with flint- or dent-like attributes would result.

Maize breeders are tempted to assume that the lower yield of classical opaque-2 maize is associated with the low density of its endosperm, and that flinty sorts such as Nigam's 01716 strain will be higher yielding than ordinary opaque-2 materials. We have a great deal of reservation about drawing this conclusion now. In earlier work (2) we found that yield per plant and kernel density were independent in three opaque-2 synthetics. Admittedly, the synthetics did not carry modifier complexes in a frequency that could have affected the correlations appreciably. Hence, the question of density and yield relationship remains unanswered from our research.

SUMMARY AND CONCLUSIONS

1. Opaque-2 hybrids with acceptable yield and other agronomic traits have been developed for the U.S. corn belt from backcross-derived lines.

2. Because of generally lower lysine levels, the floury-2 gene is inferior to opaque-2 as a tool for improving maize protein quality.

3. Two cycles of selection for kilograms of lysine per hectare in two opaque-2 and two floury-2 synthetics increased kilograms of lysine per hectare and yield, without changing the percentage of lysine (whole kernel), percentage of protein, or grams of lysine per 100 g of protein.

4. Based on predicted response calculated from three cycles of selection in two opaque-2 and two floury-2 synthetics,

   a. Selection for yield alone would reduce the percentages of lysine and protein slightly.

   b. Selection for percentage of protein alone would increase the percentage of lysine as rapidly as selecting for percentage of lysine, and would reduce yield approximately 3% per cycle.

   c. Selection for percentage of lysine alone would increase the percentage of lysine, would reduce yield only half as much as selection for percentage of protein, and would increase the percentage of protein.

5. Modifier gene complexes exist that will allow development of opaque-2 homozygotes with high lysine content and hard kernels. It is not necessary to develop such types to provide usuable opaque-2 hybrids for the U.S. market, although they would be less damaged by machine harvesting and subsequent handling and, thus, be preferred.

[*A discussion of this paper can be found on pp. 487–489 of* **Questions and Answers.**]

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Part IV

BREEDING FOR PROTEIN QUALITY IN MAIZE: CURRENT ISSUES AND PROBLEMS

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Many new plant materials challenge plant breeders of today as a result of the identification of a high-quality-protein factor in maize by E. T. Mertz, O. E. Nelson, and L. S. Bates in 1963. These materials, when properly utilized, have the potential for solving the world's need for a cheap source of high-quality protein, particularly in the tropical and subtropical countries where maize is used mainly for human consumption.

Many maize breeding programs throughout the world, using several different breeding techniques, have incorporated a higher lysine content into adapted varieties or hybrids. However, simply converting normal maize into opaque-2 or floury-2 types is not and should not be our only objective. Total acceptability of this new type of maize by the growers and consumers should be our final goal.

This goal can be achieved if we can offset some undesirable characteristics associated with opaque-2 or floury-2 maize and make them comparable to, or better than, the present normal maize varieties or hybrids. Records show that yield is generally reduced with the incorporation of the opaque-2 or floury-2 gene into normal maize types. An average decrease of about 10% in kernel weight was reported by several breeders. The decrease in yield seems

to be associated with a low density of the grain and loosely packed starch granules, producing the opaque appearance characteristic of these high-quality-protein maizes.

Several alternatives for improving the yield of opaque-2 maize are available to plant breeders. There are indications that yield reduction resulting from incorporating the opaque-2 gene into normal corn varies with the genetic background. Thus, yields of high-quality protein maize can be improved by incorporating the opaque-2 gene into different genetic backgrounds. J. Singh from India reported some opaque-2 populations with superior yields and kernel weight. Likewise, J. W. Dudley from the University of Illinois reported some opaque-2 hybrids that yielded as much as commercial hybrids. Enough genetic variability can be found in opaque-2 populations developed at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and national programs to make improvement possible. With different breeding approaches, we can improve the opaque-2 populations.

Another means of improving the yield, and even the texture, is by taking advantage of modifier genes that affect the endosperm phenotype of opaque-2 maize. The modifications involve the appearance of varying amounts of flint or hard fraction in an otherwise soft and chalky endosperm. A flinty fraction improves kernel weight and texture. Studies indicate that the modified character is heritable and can be fixed in the population with proper breeding techniques.

Chemical analyses of the grain indicate that it is possible for one line to have both good kernel weight and superior protein quality. Efforts by CIMMYT and national programs in the tropical countries now are being concentrated on developing high-quality protein varieties with hard endosperm. Some of their breeding work will be presented in this symposium.

Acceptability of opaque-2 or floury-2 maize is a major problem, especially in countries where farmers are used to growing yellow flint or white flint varieties and hybrids. In the Philippines, for example, maize is used mainly for human consumption as grits. With opaque-2 maize, the milling recovery is very low. Thus, our efforts are concentrated on developing hard-endosperm materials with high-quality protein.

Other effects of the opaque-2 and floury-2 genes on the plant have not been studied thoroughly. There are claims that opaque-2 kernels are more vulnerable to stored-grain pests and diseases. These claims are based on the presence of softer and chalky textured kernels in opaque-2 maize, compared to the hard kernels of normal flints. Problems of this sort are greater in the humid tropics, owing to the corn weevil and molds (*Aspergillus* sp. and *Pencillium* sp.). However, some modified opaque-2 materials show resistance comparable to that of their normal counterparts. A. Ortega from CIMMYT

discusses this in his paper. Sources of resistance to various pests and diseases are available, and with the right breeding procedure they can be incorporated into opaque-2 maize.

Another claimed disadvantage of opaque-2 maize is its slow drying rate. The claim is that opaque-2 ears must remain in the field longer than normal ears, thus leaving them more exposed to the attack of pests and diseases. Genetic differences could produce differences in rate of drying. If such differences exist, it should be possible for us to select these opaque-2 populations that dry fastest. David Sperling discusses this in his paper.

I have tried to enumerate some of the desirable characteristics that opaque-2 maize must possess to gain universal acceptance. It is evident that improved protein quality alone, although very desirable, does not guarantee acceptance. When a farmer chooses his crop, profit is still his main concern. Therefore, we must see that opaque-2 maize or any superior-protein quality maize can measure up to the farmers' and consumers' criteria for desirability.

The major task before us is to incorporate the desirable traits that will make high-quality protein maize more acceptable, because only then will the benefit from the opaque-2 mutant be fully utilized. After looking at the papers presented in this symposium, I feel this goal is within our reach.

BREEDING SYSTEMS FOR RAPID DEVELOPMENT OF QUALITY PROTEIN VARIETIES

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The purpose of plant breeding is to develop varieties of plants that best suit man's needs for food and fiber. Since man's needs are constantly changing, plant improvement becomes an integral part of the continuous process of agricultural development. When we focus an analytical lens on the elements of this process, they seem to form an interlocking pattern:

Focus 1. The rapidly increasing world population demands more and more food. And, as science and technology develop improved quality characteristics of different food crops, diets will be altered and new methods found for processing, storing, and preparing foods.

Focus 2. As mechanization of agriculture progresses, crop plants must be of more uniform size and more uniform maturity, better able to withstand machine handling and so on. Meeting the demands for more and more food will require greater production per unit of land area. Similarly, greater use of fertilizers must be made to obtain high yields, and to grow sequences of crops for more efficient total use of the land, and for varieties of crops that respond to fertilizers and more intensive management practices.

Focus 3. As population increases, larger amounts of tillable land will be diverted to roads, buildings, and other nonagricultural purposes. A greater population implies less land for production.

Focus 4. Even more complex considerations arise within the public's concern for the natural environment of the world. Strong pressures are brought against the use of fertilizers, insecticides, and other chemicals that help grow

better crops. As these pressures restrict or change the chemicals that may be used, crop varieties must be developed to perform adequately within the "crop-production" practices that are permitted.

Focus 5. As crop varieties are developed that respond to fertilizers and give higher yields, the microenvironments of the fields in which these crops are grown become modified owing to the increased density of the plant communities. This, in turn, alters the nature of the insect and disease problems in these new environments (4). Usually, the denser crop growth means greater insect and disease problems. Therefore, more chemical controls are indicated, or crops will require greater varietal resistance as a control.

Focus 6. Concern with the nutritional qualities of our crop plants is beginning to become an important consideration. Man requires certain minimum amounts of a wide array of complex amino acids, vitamins, and other nutrients to maintain health and growth. Many of the world's families have inadequate diets because of the high cost and unavailability of proper foods. Major nutritional improvement for large groups of people may become possible through developing crop varieties (like opaque-2 maize) that contain better-balanced nutritional attributes.

Focus 7. The discovery at Purdue University (13) of the relationship of the opaque-2 and floury-2 mutants to the relative content of lysine and tryptophan in maize grain generated tremendous interest and enthusiasm. The potential contribution to human nutrition represented a major new concept in how to confront the growing food problems of the world.

Focus today. Our presence here today is evidence of continuing preoccupation with the nutritional quality of our cereal grains, especially as it pertains to maize. Our concern is not only to evaluate the progress made thus far in utilizing high-quality protein grain, but also to take a sober look at the difficulties and limitations encountered thus far and perhaps project new ideas on how to proceed from here.

VARIABILITY: AN ESSENTIAL CONCEPT

Plant breeding is a never-ending process of fitting plants to better serve human needs, of rearranging the variability of our crop plants. Variability is a characteristic of living things. The individuals within a group in any living species vary among themselves in all sorts of ways. These differences, in some cases, are quite obvious and distinct, as between yellow and white maize grains. In other cases, the differences must be expressed on some measured scale and may be almost imperceptible, such as the differences found in plant height, maturity, resistance to lodging, and many other traits whose distributions of slight differences form continuous curves.

Individual plants can be characterized by a wide array of attributes, such as height, maturity, color of seed, color of flower, resistance to diseases and

insects, leaf width, number of leaves, protein content, sugar content, yield, and many others, limited only by the ability to enumerate traits and to measure them. The concept of genetic variability is prerequisite to an understanding of plant improvement, and no plant improvement is possible in the absence of genetic variability. Plant breeding is based upon the ability to detect and measure genetic differences, and new varieties are constructed from the building blocks of genetic variability, or genetic differences (6).

Genetic or Environmental Differences?

An organism develops within the limits of its genetic potential to the extent that its environment will permit. If the differences observed in certain traits are the results of environmental factors, one cannot expect those differences to be transmitted to the progeny of such individuals. It is essential, therefore, to be able to distinguish between genetic and environmental differences.

Many different breeding schemes, all based on the ability to distinguish and measure these genetic differences, have been devised to combine genetic differences to form new varieties of crops (10). New and different varieties of plants can be produced by any procedure or breeding method that allows identification and measurement of these genetic differences.

New Dimension

Our conference deals with an intriguing new dimension in maize breeding: the problem of linking protein quality to a modified kernel appearance has been added to the multitude of agronomic trait problems in maize.

The genetic mechanism of a given attribute may consist of one or more genes. Countless crop-plant studies have estimated how many genes may be involved in the determination of identifiable attributes (2, 4, 6, 8, 14 – 16, 18). Single-gene characteristics are often spoken of as major genes with large, easily identifiable differences among individuals, which enable rapid classification into contrasting classes for the character. This is the case with the opaque-2 mutant, which segregates into the classical 3 to 1 ratio. Reports of these one-gene or few-gene characters seem to have an interesting and revealing tendency in nearly all cases. Almost invariably, the authors include a comment suggesting the presence of "modifying genes," which either altered the expected ratios or made it difficult to classify ratios that should have been straightforward.

A long, impressive list of genetic characters, each with relatively simple inheritance ratios, has been compiled for maize (1, 5, 14, 18). However, upon contemplating the combination of resistance to *Puccinia sorghi*, *Helminthosporium turcicum*, and *Ustilago maidis*, for example (and perhaps one or two other simply inherited characters), the picture begins to look a bit complicated. As each single gene is added, a population four times larger is

required to recover the desired recombinations in the segregating progenies resulting from intercrossing sources of these characteristics.

Acceptability

Too often, great efforts are devoted to isolating a particular trait of tolerance to a certain disease or insect, or to demonstrating that a certain procedure enables one to modify a selected trait, whereas all other attributes are ignored in the meantime. All too frequently yield is the only factor considered in selection, and other important economic traits are ignored, although they may be required for farmer acceptance of a variety. Farmers want varieties with as many desirable attributes as possible — those which will grow and produce better. They are not interested in a variety that resists a certain disease or insect but lodges or fails to yield as well as their previous varieties. Truly superior varieties must be developed that are better suited to the farmers' needs.

BREEDING PROGRAMS

Well-managed breeding programs decide in what ways varieties should be improved and then set about doing it. The first step in setting up a breeding program is to define the objectives of the program. This includes enumerating the characteristics of resistances to diseases and pests for a given area, as well as the attributes of adaptation to ecological conditions. The planned varieties must fit into available growing periods, whether limited by ecological factors or by cropping sequence practices.

The second step is to select the materials with which to work. Whether the work is to proceed with the opaque-2 character, floury-2 mutant, or other genetic variant, it is essential that the appropriate genetic source material should be utilized. Any breeding program based on using known inferior germ plasm has built in an unnecessary disadvantage. Choosing the right materials to begin with is probably the biggest single step that can be taken to arrive at superior varieties. Years can be wasted developing materials that might have been available at the start.

To the extent possible, all desirable attributes that are contemplated as characteristics of the projected varieties or hybrids should be assembled into the working materials. Evaluation of material for possible inclusion in breeding work should be a continuing phase of all breeding programs.

If two or more varieties or sources of traits are to be utilized in the selection program, they should be crossed and mixed for several generations before selection pressure is imposed. Basic to any long-range program of improvement is the concept of raising the performance level of populations as such, regardless of whether or not hybrids are to be developed. Fundamental to population improvement are (1) using adequate numbers of plants

among which to select, and (2) using recurrent selection procedures that may require many selection cycles to achieve the desired combinations of characters. The importance of recognizing the existence of complex multigenic systems that determine plant characteristics cannot be overemphasized (3, 4, 6 – 8, 15 – 17). The growth of the maize plant and its harvest of usable products involves an enormously complex series of chemical processes that can be modified in kind, intensity, sequence, and final points, depending on the genetic complement of the plant.

Important Criteria

Probably one of the most serious defects in tropical maize varieties generally is excessive plant height. The defect might be amplified to include the relationship of grain produced to the total plant dry matter. From the standpoint of intensified management of a crop plant, excessive height is undesirable. Consideration must be given to resistances to field hazards of diseases such as *Helminthosporium* spp., *Puccinia* spp., other foliage diseases, stalk and ear rots, insects that feed on the leaves and bore holes in the stalks, plus the interactions of these organisms. The biological ecology of maize production in tropical areas is a rigorous and complex one. Breeding programs must be sufficiently comprehensive to deal effectively with a whole series of plant characteristics. It is not enough to consider single traits by themselves, if improved varieties are to be developed. The purpose of this paper is to consider a breeding program intended to develop varieties or populations with improved agronomic traits and with adaptation over as wide an area as practicable. High-priority traits are (1) shorter plant height, (2) resistance to *Helminthosporium* spp., (3) resistance to *Puccinia* spp., (4) resistance to stalk and ear rots, (5) resistance or tolerance to insect stalk borers and foliage feeders, (6) resistance to lodging, (7) tolerance to high plant canopy density, (8) higher yields, and (9) quality protein. These are not listed in order of priority, because not all can be selected for with equal intensity in a given planting. These are important criteria to be considered simultaneously when selection conditions permit, and individually when conditions are less favorable. Furthermore, as modification occurs in a given trait, emphasis can be shifted from one trait to another as appears desirable.

As far as possible, preference is to be given to general resistance rather than to single-gene resistances. The well-known history of wheat stem rust races and the never-ending search for another gene to confer resistance to a new race of rust illustrate the reason for choosing general resistance in reference to both diseases and insects. Acceptable levels of resistance are achievable only by testing under different environments and after repeated cycles of selection and recombination. Materials for development are to be chosen largely from those already important in commercial use and with reasonably good levels of productivity. Continuous interchange

of materials should be made with various areas of the world to detect desirable attributes.

The enumerated traits for priority work are assumed to be under multigenic control in nature, with the genes widely scattered and at relatively low frequency (2, 4, 6, 8, 10, 15, 16). Gene action is presumed to be largely additive. The number of selection cycles required to materially modify the expression of a given trait will vary, since more than one character must be considered at a time in the improvement program.

Relatively low gene frequency assumed for the attributes under selection implies the need for large numbers of plants in the selection procedure. It is important to initiate any selection program in an adequately broad genetic base. Many maize germ plasm bank collections originate from only a very few ears, and this may limit selection progress because of limited sample size. Selection schemes should be applied to composites of two or more variety samples.

For any given trait, two individuals may exhibit equal expression of that trait and yet contain differing genetic complements with respect to that trait. In other words, each may contain the same number of additive genetic factors but have few identically in common. In a heterozygous maize population, two such individuals, when crossed, could give rise to a range of progeny individuals differing for the trait both above and below the level exhibited by the two particular parents.

PROCEDURE

Obviously, it is impossible to review all the many breeding methodologies in one short paper. We shall describe one procedure briefly, along with possible variations. Although the method described here was intended primarily to modify plant height, it can be utilized for other characters as well. Initially, heavy emphasis is on reducing plant height. The method chosen at CIMMYT to select for shorter plant height also allows a great deal of flexibility in selecting for other attributes during each cycle. A cycle of selection is completed each time the crop is grown. The procedure used is basically that of full-sib selection.

Full-sib family rows are defined as those resulting from the cross of two individual plants so that all individuals in the progeny row have both parents in common. Open-pollinated ears may be used to begin the process, or hand pollinations may be made in the population to be modified. Many full-sib family rows are used to represent a population, and selection is carried out among and within families.

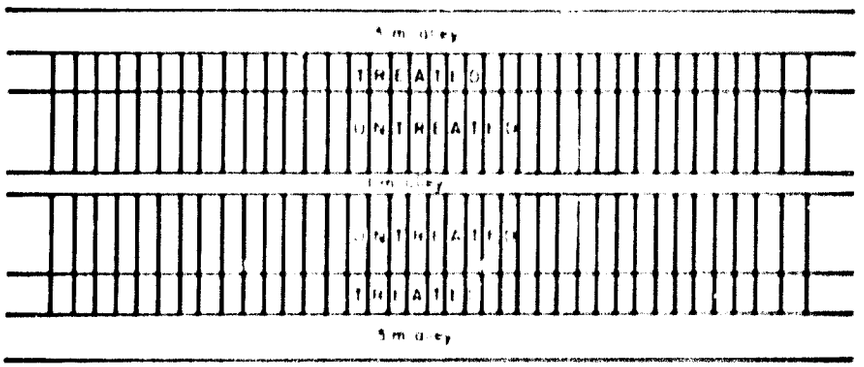
The number of rows necessary is set arbitrarily; 400 to 500 rows is considered adequate. Each ear is planted in a 5-m row. Rows are arranged in double ranges with 1-m alleys separating ranges and with double ranges separated by alleys of 2 m or more. Granulated insecticide is

applied as protection to the three or four hills in each row adjacent to the wide alleys. The large central portion of the double range receives no insecticide treatment (Figure 1).

The entire nursery is artificially inoculated with the pathogens appropriate to the location and season. Stalks are inoculated with stalk rots, and suspensions of ear rot pathogens are sprayed on silks of developing ears. As more complete facilities for infestation with insects and other diseases are developed, these will be utilized.

Approximately half the family rows are selected each cycle for pollination (Figure 2). Rapid visual selection is made as nearly as possible to the time of pollination. A form of continuous, "moving-average" estimate is used to mark the rows that display average or better performance for the trait under selection. Each row thus selected as a family is subjected to individual-plant-within-family selection. About five selected plants per row are then crossed to other similarly selected plants in other rows, each plant with a different row. This results in approximately 2.5 times as many individual pollinations as there are rows. Diseased, lodged, too tall, too late, or other undesirable type families can be avoided at pollination time. Insofar as possible, pollinations should be made with plants in the part of the row unprotected by insecticide.

Two and one-half times as many pollinations as rows gives reasonable margin for losses due to poor pollination and miscellaneous other mishaps in the field. This process also allows a chance for selection at harvest. All family rows are completely harvested, and pollinated ears are placed on the pollinating bags at the head of each row. Thus, the full production of all rows is visible, alongside the plants from which they came. Again maintaining the moving average concept of performance, families are



Treat only part of each row with insecticide (see diagram)

Lines represent family rows

FIGURE 1 Outline of procedure to include resistance to insects as a criteria of selection in nursery

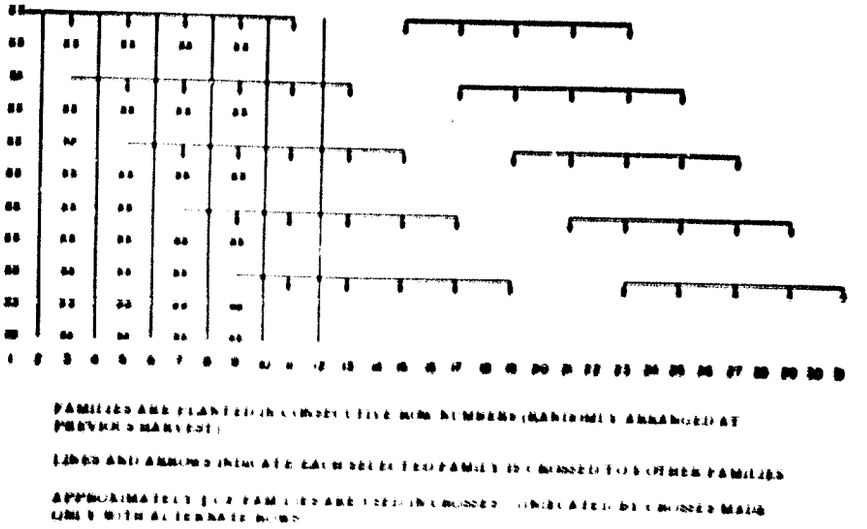


FIGURE 2 Crossing pattern among selected families in full sib continuous selection

judged on the basis of production, ear rots, stalk rots, and insect damage. The poor families and their crosses are discarded, while again saving approximately the same number of ears as was used in the previous cycle.

If certain families prove particularly desirable at harvest and were not included in the pollinations, their reserve seed is put back as rows in the following cycle. In this way, two separate selections (at flowering time and at harvest) are practiced each time the material is grown. At both times, the family is the principal basis of selection, followed by within family individual plant selection. The harvested, selected ears are randomly arranged and numbered consecutively so that related crosses are unlikely to occur closely together in the subsequent planting. Envelopes in seed storage are marked with the family cross of the previous cycle, but for simplicity this does not appear in the field book. It would thus be possible to go back to reserve seed and remake any given family to family cross.

The numbers assigned to the harvested ears are used to designate family rows in the subsequent planting. Thus bookkeeping and pedigree listing are minimized. Selections are made each cycle, and there are no intervening "missing" generations. The process is continuous. Preliminary data following several cycles of selection using this procedure to reduce plant height are shown in Table 1.

The trends of plant height reduction and the response in lodging and maturity are illustrated in Figure 3. It is obvious that major change occurred in plant height. The solid tendency is equally significant since the tests were conducted at standard plant densities of about 45,000

TABLE 1 Yield of grain in kilograms per hectare and measures of agronomic characteristics following cycles of selection in three populations of maize (combined data for two planting seasons, Poza Rica, Veracruz, 1971)

No. of Recombination	Pedigree	Cycle	Days to Flower	Height Plant (cm)	Height Ear (cm)	Lodging[a] Root	Lodging[a] Stalk	No. of ears[b]	Yield (kg/ha)	% of check
1	Tuxpeño Criollo 1 8% Bmp	C_0	73	291	161	3.8	2.0	50	3,691.5	88.6
2	Tuxpeño Criollo 1 8% Bmp	C_1	71	273	145	3.0	1.8	55	3,717.6	88.4
3	Tuxpeño Criollo 1 8% Bmp	C_2	69	248	127	1.8	1.5	55	3,915.9	92.5
4	Tuxpeño Criollo 1 8% Bmp	C_3	66	229	124	1.8	1.3	57	4,286.6	101.4
		$\bar{x}$	70	260	136	2.6	1.7	54	3,902.9	92.2
5	Eto Blanco 8% Bmp	C_0	70	243	123	2.8	1.6	28	3,818.2	82.6
6	Eto Blanco 8% Bmp	C_1	67	256	134	2.2	1.5	50	3,218.5	72.3
7	Eto Blanco 8% Bmp	C_2	66	229	114	1.9	1.2	55	3,122.2	71.7
8	Eto Blanco 8% Bmp	C_3	65	210	110	1.8	1.4	57	3,415.7	78.7
		$\bar{x}$	67	250	114	2.2	1.4	55	3,343.7	76.3
9	(Nic II × Coll Capo. II) Eto 8%	C_0	69	259	144	2.6	1.8	54	3,748.7	88.1
10	(Nic II × Coll Capo. II) Eto 8%	C_1	68	244	127	2.2	1.6	54	4,088.0	95.7
11	(Nic II × Coll Capo. II) Eto 8%	C_2	66	235	117	1.8	1.8	57	4,497.1	104.3
12	(Nic II × Coll Capo. II) Eto 8%	C_3	65	212	104	1.2	1.4	57	4,000.1	92.5
		$\bar{x}$	67	238	123	2.0	1.5	56	4,082.2	95.2
13	Tuxpeño Cr. II 8% Bmp	C_0	72	256	161	3.4	2.0	52	3,778.6	88.9
14	Eto Blanco 8%	C_1	67	235	129	2.7	1.9	55	3,400.4	79.8
15	(Nic II × Coll Capo. II) Eto 8%	C_2	68	274	145	3.3	1.9	55	4,042.3	96.5
16	[illegible] (Check)		72	262	167	2.4	1.7	55	4,270.0	100.0
		$\bar{x}$	70	273	146	3.0	1.9	55	3,872.8	91.3

[a] Lodging is measured on a numerical scale of 1 to 5, with 1 being a quasi-absence of lodging.

[b] Number of ears refers to the actual number of ears harvested from a perfect population of 40 plants.

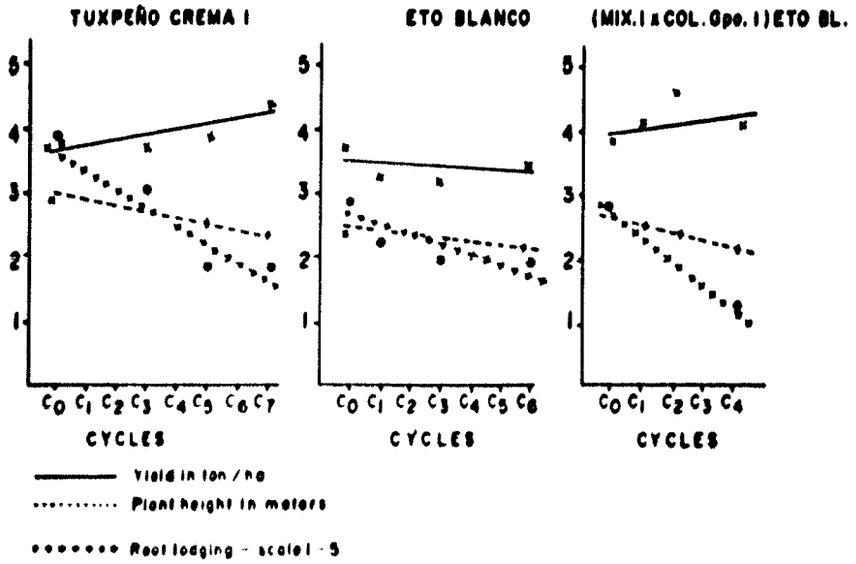


FIGURE 5 Response to selection for plant height and the effects on grain yield and root lodging in three populations of maize.

plants ha. The reduced plant size should permit intensified management of greater stand density and should allow increased fertilizer applications with corresponding yield improvement.

DISCUSSION

A maize variety can be thought of as a population of individual plants, each the result of a cross between two gametes that probably were produced by different plants. Thus, each plant might be thought of as a hybrid and the variety itself as a population of hybrid plants. To the extent that selection pressure is effective in favoring or discriminating against certain genotypes, gene frequency will be modified. This is precisely what is attempted in plant breeding, so the most desirable procedures are those that permit this modification as rapidly, simply, and easily as possible.

When several ears are shelled together, the planting made from such a mixture contains a random distribution of the progeny. When the kernels from individual ears are planted together in a row, the plants are arranged in a progeny row of related individuals which tend to resemble each other because of their relationship. Family traits are thus recognizable, and differences can be distinguished visually on a family basis that cannot be done on an individual plant basis in the field. Fall-ab (both

parents in common) similarities are even more obvious than half-sib (one parent in common) similarities.

The systematic arrangement of a variety in the field on the basis of full-sib family rows gives the maximum opportunity (1) to recognize similarities and differences among families and (2) to make matings among those families which most nearly appear to meet the goals established as characterizing the variety under development. Maintaining many families assures reasonable precaution against too rapid narrowing of the germ-plasm base and possible ill effects from inbreeding.

Genetically, the full-sib families are the equivalent of conventional double-cross hybrids. Presumably, each family has approximately similar chances to perform, subject to the variability of the environment in the field. For example, one can visualize the nursery as an unreplicated yield trial and make selection for height at flowering time and selection for yield at harvest. This scheme can utilize a moving-average concept for plant height and yield, together with approximately 50% selection of the families planted. Admittedly, this must be regarded as a mild selection pressure for yield. However, it can become quite significant through several cycles.

Pressure for yield selection can be increased by planting yield trials of the same families that go into the nursery. These may be planted at the same time as the nursery, or even before the nursery; and they may be planted in several locations, even different countries. Great flexibility exists here when plantings are not limited to a single growing cycle per year. Pollination and selection procedure can be modified to make four or five pollinations with each of nearly all the rows. Then discards can be made on the basis of yield trial results. If yield trial results become available too late to be taken into account in the selection cycle, reserve seed can bebe used to replace families that may have been missed in previous cycles.

Using this procedure at a single location carries the hazard of selecting those characteristics required only at that specific location. A simple modification can be added by making up the pollinations in one planting season and growing yield trials of these families in several locations in the next season. Thus, the selection of which families are to be included in the following pollination cycle can be based on results in several different environments, and variety testing becomes an integral part of varietal development. The single ears provide enough seed for two replications of single-row plots at each of five locations. If additional seed is required, the pollinations can be made reciprocally.

As a matter of procedure, it is suggested that two or more populations be worked simultaneously, never use just one population. These two or more populations may be different original varieties or composites that combined well as varieties in a variety cross. Or they may simply be two

nearly equal halves of the same base population that have been selected simultaneously, but independently, and maintained apart.

Flexibility and Uses

The procedure outlined allows for a great deal of flexibility in the breeding nursery and for rapid seed increase when this is desired. Insect and disease problems do not occur with sufficient reliability and uniformity of intensity to enable good selection for such traits in every cycle. One must be prepared at each cycle to select for those resistances for which adequate infestations occur. It is important to intensify overall selection by including several traits simultaneously. Consideration of traits visible at flowering time and intercrossing selected components of the population are powerful means of rapidly modifying such visibly identifiable traits.

It is not implied here that the procedure outlined is the only one that should be used; alternative procedures might be more desirable under different circumstances.

Modified ear-to-row mass selection for yield (9) among half-sib families is especially useful where available quantities of pollinating bags, supplies, and labor may seriously limit the number of hand pollinations that can be made. Undesirable plants in male rows of such programs should be detasseled. A program of half-sib selection of this kind can be initiated at any desired cycle in the procedure outlined of full-sib selection.

The procedure described, the half-sib selection (9) for yield, and mass selection (7) are very convenient procedures from the standpoint of being able to supply relatively large amounts of seed very quickly when this is required. By growing blocks in isolation there is automatically a large seed increase each cycle.

To add hybrid development to the procedure, two approaches might be used. The first would be to make cryptic hybrids (12) each cycle between selected families from different populations in the regular population improvement nursery. The other approach would be to select a few of the best families in two or more populations at harvest, and to plant these out separately in the following cycle in order to make hybrids among such families. Relatively few families would need to be selected in each cycle to produce several potential hybrids in a short time.

With relatively little extra effort, a continuous hybrid combination sampling of this kind can be made along with the population improvement. Within a population, outstanding family combinations can be identified and approximately repeated by going back to reserve seed of the families used to make the cross in the previous cycle.

An array of systematically arranged hybrids can be found in the field in every cycle. Identifying a few superior families each cycle should provide good hybrids with minimum effort. By intercrossing a few of the outstanding families from each of two or more unrelated developing populations, one can develop patterns of behavior of certain materials and concentrate efforts on those which consistently give best results.

The number of characters that can be handled under selection pressure at any one time is a matter of judgment. Obviously, the greater the number of attributes under simultaneous selection, the more difficult progress becomes for each (2, 4, 6, 10, 17). Emphasis may be shifted from one attribute to another as progress is achieved, so that modification of traits becomes a stepwise process. It must be recognized that certain traits, such as earliness and plant height, tend to be correlated (3, 5, 17). Whether this is desirable or not depends on the goals established; in any case, a continuous recycling process over generations is a tremendous tool for modifying such relationships.

Any single-gene mutant, such as opaque-2 or floury-2, can be incorporated into populations under selection by planting a few rows as a subplot adjacent to that population. In one season the opaque source would be crossed to the population with the F_2 obtained by sibbing in the next planting cycle. The recovered mutant kernels would then be planted again as a subplot for backcrossing. Thus, simultaneous conversion to a mutant type can be carried along with the regular selection work. In the case of the modified hard-kernel opaque endosperm, it is not quite clear whether it would be possible to carry it as a subpopulation because of the necessity of concentrating the modifier genes for hard endosperm. However, selection for modified hard kernels would be ideally suited to the described full-sib process. Laboratory analyses would then be a necessary part of the selection criteria.

Much attention in the work on quality protein is being focused on the opaque-2 mutant. Apparently, results with the floury-2 gene have been less consistent and less satisfactory. Whether other mutants with similar effects are discovered remains to be seen. When and if they are, and if any find widespread use, it is well to keep in mind the biological hazards intrinsic in genetic uniformity in a crop. The near catastrophe resulting from the use of Texas male sterile cytoplasm to produce hybrids is a striking example of what can happen. One or all of these single gene mutants might produce a similar occurrence; there is no guarantee otherwise. Perhaps no such problem will ever be encountered, but it is certainly good judgment to be on the alert.

Even though these single genes appear to offer the best hope of achieving high-quality protein in maize, the possibilities of modifying both pro-

tein levels and amino acid profiles through recurrent selection utilizing multigenic systems should be investigated more thoroughly. Any improvements should be useful alone and in combination with the single-gene mutants, and would represent an alternative to complete dependence on single-gene mutants.

[*A discussion of this paper can be found on pp. 489–490 of* **Questions and Answers.**]

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AGRONOMIC ASPECTS OF PRODUCING QUALITY PROTEIN MAIZE

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Staff discussions at Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) generated several questions about opaque maize production that could not be resolved without additional information. Staff opinions differed as to whether (1) opaque maize was more susceptible than normal maize to ear rots, (2) opaque grain dried more slowly in the field, (3) the reported yield reduction caused by the soft opaque endosperm was significant in tropical varieties and, if so, (4) the modified hard endosperm would compensate for this yield reduction.

The experiment to be described was designed as a result of these discussions to answer these more specific questions:

1. Are there differences in susceptibility among normal, opaque, and modified opaque varieties to ear rots, particularly *Diplodia* spp. and *Gibberella* spp.?

2. Are there differences among these three phenotypes in the rate of drying of grain in the field, particularly after physiological maturity?

3. If they exist, are the differences in questions 1 and 2 related?

Secondary questions were

1. Do the cob weights of normal and opaque phenotypes differ, as has been reported elsewhere?

2. Are there differences in the rates of grain dry-matter accumulation between normal and opaque grain types?

3. If they exist, are differences in rates of drying associated with differences in morphology of the ears or grain?

4. Are differences in yield associated with differences in the morphology of the ear?

Previous studies, using single-cross hybrids, demonstrated that rates of grain dry-matter accumulation of 175 kg/ha/day were possible. Rates were not reported for opaque-2 maize. Rates of moisture loss of husked ears had been studied extensively under controlled conditions, but the only field study indicated that husk cover was more important than characteristics of the grain. All the work had used temperate-region material, and the findings could be applied to tropical varieties only indirectly.

MATERIALS AND METHODS

The varieties were studied in two groups. Group A contained the normal and opaque counterparts of five varieties from the opaque-2 conversion program. As such, these varieties were relatively unselected for yield. Group B contained opaque and modified opaque counterparts selected from common seed lots of each of four other varieties that had been in yield-improvement programs. All varieties used are listed in Table 1.

The two groups of varieties provided a treatment set of 18 entries: 5 normal, 9 opaque, and 4 modified opaque phenotypes. An additional treat-

TABLE 1 Grain yields of normal, opaque, and modified opaque materials in groups A and B (kg/ha)

Group A	Normal[1]	Opaque[1]	Difference (N-O)
Nicaribo	5,440[b,c]	4,587[c]	853[c]
Tuxpeño X Ant. Gpo. 2	6,987[a]	5,175[a,b]	1,814[c]
La Posta	6,027[b]	5,280[a]	747
Francés Largo	4,640[d]	4,747[c]	107
Fobracción Cristalina	5,653[b,c]	4,907[b,c]	746[c]
Mean	5,707	4,960	

Group B	Opaque	Modified opaque	Difference (O-MO)
Thai Opaco	5,222[a]	4,427[a]	906[c]
Compuesto K	4,853[b]	4,320[a]	533[c]
Compuesto CIMMYT	5,387[a]	4,747[a]	640[c]
Ver.181 X Ant. Gpo. 2	5,067[a,b]	4,427[a]	640[c]
Mean	5,175	4,480	

[1] Comparisons are valid only within columns for each class of entries. Means with the same letter as superscript are not significantly different at the 0.05 level.

[2] Significant difference at the 0.05 level.

[3] Significant difference at the 0.01 level.

ment contrast, inoculation with ear rots versus protection with fungicides, was applied to the set of 18 entries, resulting in an 18 × 2 set of treatments replicated four times. The inoculum, a suspension of spores of *Diplodia* spp. and *Gibberella* spp., was sprayed on the silks 10 days after 75% silk emergence. The inoculation technique was not successful; therefore, the experiment was continued as 8 replicates of the original 18 entries. Thus, this report will only describe the dry weight and moisture changes in the grain and cob of these materials. In subsequent experiments, answers were sought to the questions of differences in susceptibility to disease. Ortega reports the results of these experiments in his paper.

Each plot consisted of ten 5-m rows, 75 cm apart. Hills were 25 cm apart within the row. The plots were planted December 22, 1971, at the rate of two seeds per hill. Three weeks after, the stand was thinned to one plant per hill.

Controlled pollination was considered impractical owing to the large amount of material used. Therefore, the phenotypes were grouped into blocks: normal, opaque, and modified opaque. The opaque and the modified opaque phenotypes were isolated from the normal entries by using a minimum of 10 rows of opaque or modified opaque border.

Since comparisons were confounded with blocks in the field, a modified form of analysis was used in which comparisons were made only between varieties within a phenotype of a group.

Ear samples were taken weekly, starting at mid-silk and continuing until 3 weeks past physiological maturity. The sample consisted of the ears, with husks intact, from each of eight plants. The ears were then taken to the laboratory where the husks were removed. The ears were weighed, and the ears and grain were oven dried to determine their dry weight. Measurements made on the dried ears included the number of kernel rows, length and width of ear, width of cob, and kernel depth.

The time at which the black layer was fully developed was determined, and the filling period was defined as the number of days from mid-silking until 50% black-layer formation.

RESULTS

Dry-Matter Changes in the Grain

The grain yields (kilograms per hectare) presented in Table 1 are the average of harvests 7 to 10 in this experiment. Yields of the same varieties were ranked similarly in the 1970–1971 CIMMYT Report, indicating that confounding effects, if present, were of a low magnitude. Therefore, differences between varieties of differing grain phenotypes would be due to inherent varietal differences, not to field location effects. Significant yield differences occurred in all classes except the modified opaque. The normal entries significantly outyielded their opaque counterparts, with the exception of La Posta and Francés Largo. The average yield reduction for the

opaque class was 747 kg/ha, or 13% of the normal. All the opaque entries in group B significantly outyielded their modified opaque counterparts. The average yield reduction of the modified opaque was 693 kg/ha, or 13.4% of the opaque.

Grain yield is determined by the rate of dry-matter accumulation and the length of time that the rate is in effect. Differences in grain yield can be examined in terms of these two parameters. The curves of grain weight for the four classes of entries presented in Figure 1 graphically describe such an analysis. The rates of dry-matter accumulation in the grain were generally similar. Regression analysis indicated no differences between rates of dry-matter increase within classes or between variety counterparts. Increases in dry weight occurred until harvest 7 for all classes except the normal, which showed a slow increase after harvest 7. Dry weights decreased after harvest 7 for the three opaque classes. However, this decrease was due to the sampling effect, since harvest 7 was taken from near the edge of the plot (border effect), and thus was not an actual reduction in yield.

Therefore, the difference in yield between the opaque and normal classes of group A seems to be a result of a longer duration of dry-matter increase. On the other hand, a similar response in dry-weight curves was obtained, but a 13% difference in final yield occurred. During the early harvest, the modified opaque had a slower dry-matter accumulation than the opaque

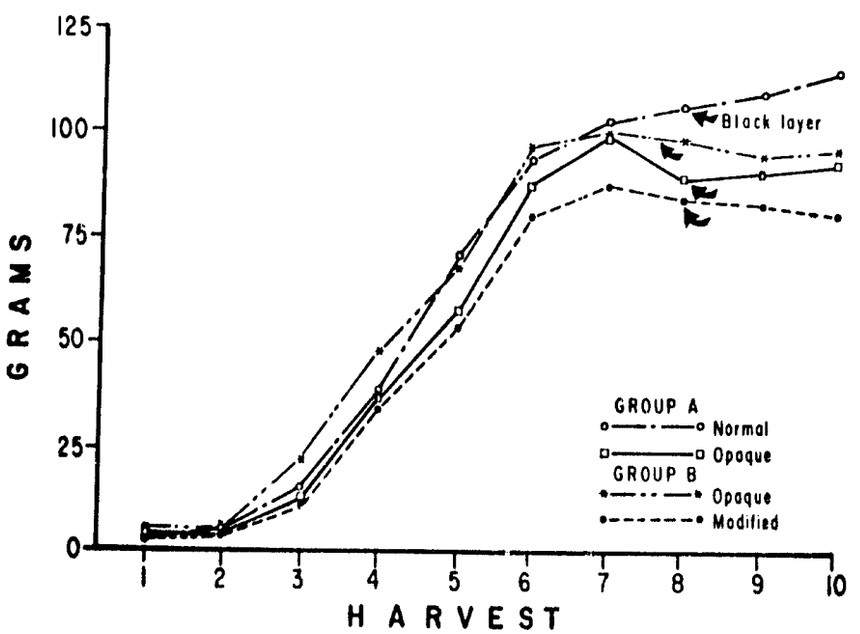

FIGURE 1 Changes in weight of grain (g/plant).

counterparts; however, after harvest 3, the dry-matter increase in modified opaque paralleled that of the opaque. Therefore, the yield reduction of the modified opaque class of group B can be attributed to a reduced rate of dry-matter accumulation prior to the third harvest.

Only small differences were noted in the length of the filling period (silking to black-layer formation), as indicated by the arrows in Figure 1. The two classes of group A and the modified opaque class of group B had an average filling period of 59 days, which coincided with harvest 8. The opaque class of group B had a filling period of 56 days, which terminated 3 days before harvest 8. These differences were not statistically significant.

Black-layer formation occurred about 2 weeks after the termination of rapid rates of dry-matter accumulation in the grain, whereas the dry-weight curves stabilized about 7 days after the termination of rapid dry-weight increase.

Dry-Matter Changes in the Cob

Recent findings have indicated a reduced cob weight of opaque varieties as compared with normal counterpart varieties, but the significance of these results is not readily apparent. Cob weight increased rapidly until harvest 3 and more slowly until harvest 6 (Figure 2). Average cob weights per plant were presented in Table 2. Differences in cob weight occurred in all classes except the modified opaque. The normal entries of Nicarillo, La Posta, and Población Cristalina had significantly greater cob weight than did their opaque counterparts. The cob weights of the Tuxpeño × Ant. Gpo. 2 counterparts were equal, and the cob weight of the Francés Largo opaque entry was significantly greater than that of the normal entry. The difference between the two classes was 0.6 g, with the normal class greater. A marked reversal of cob weights was noted for the variety Francés Largo, which in the

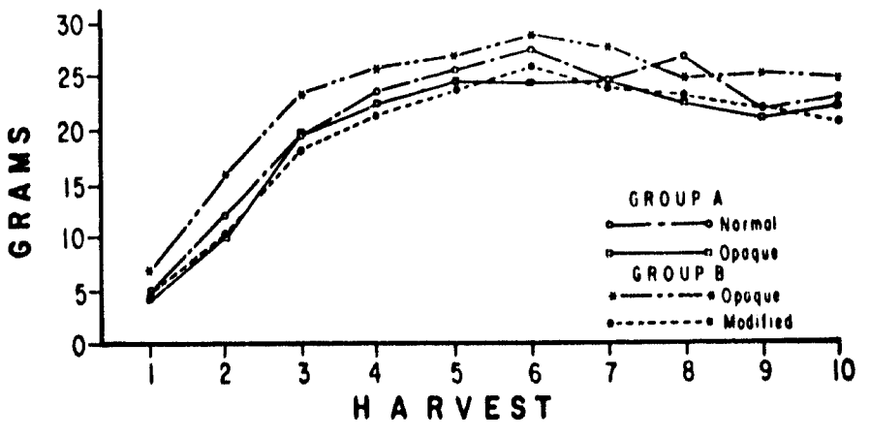

FIGURE 2 Changes in weight of cob (g/plant).

TABLE 2 Cob weight per plant for normal, opaque, and modified opaque materials

Group A	Normal[1]	Opaque[1]	Difference (N-O)
Nicarillo	26.1[a,b]	22.5[a,b]	3.8[2]
Tuxpeno X Ant. Gpo. 2	24.3[c]	24.5[a]	-0.2
La Posta	25.1[b]	21.5[b,c]	3.6[2]
Francés Largo	11.8[d]	19.8[c]	-8.0[2]
Población Cristalina	26.5[a]	22.7[a,b]	3.8[2]
Mean (-Francés Largo)	(25.5)	(22.7)	

Group B	Opaque	Modified opaque	Difference (O-MO)
Thai Opaco	27.1[a]	23.1[a]	4.0[3]
Compuesto K	24.1[a,b]	21.7[a]	2.4
Compuesto CIMMYT	23.1[b]	22.8[a]	0.3[3]
Ver. 181 X Ant. Gpo. 2	26.9[a]	21.5[a]	5.4
Mean	25.3	24.2	

[1] Comparisons are valid only within columns for each class of entries. Means with the same letter as superscript are not significantly different at the 0.05 level.
[2] Significant difference at the 0.01 level.
[3] Significant difference at the 0.05 level.

opaque version had lost a characteristically diminutive cob. If this variety is excluded, the means are 25.5 g for normal and 22.7 g for opaque, an average reduction of 2.8 g, or 11% of normal. In group B a significant reduction in cob weight of the modified opaque was noted for Thai Opaco and Ver. 181 × Ant. Gpo. 2. The average reduction in cob weight for the modified opaque was 0.9 g, a 3.5% decrease. The reduction observed for the modified opaque is not large, but the cob weight decrease observed for the opaque in group A is of the same magnitude as the reduction in grain yield.

If the reduction in cob weight was proportional to the decrease in grain weight, there should be no difference between counterpart varieties in the grain weight–cob weight ratio. The ratios are presented in Table 3. Significant differences were found between entries in all classes. However, significant differences between counterparts were found for only two varieties in group A.

The significant difference in Francés Largo could be attributed to marked differences in cob size, whereas the difference in ratios for Tuxpeño × Ant. Gpo. 2 was due to marked differences in grain yield.

The general lack of significant differences between variety counterparts in grain weight–cob weight ratio indicates that reduction in cob weight is proportional to the reduction in grain yield. Thus, the weight of the entire ear of opaque varieties is reduced.

TABLE 5 Grain weight/cob weight ratios for normal, opaque, and modified materials

Group A	Normal[a]	Opaque[a]	Difference (N-O)
Nacarillo	5.9[a]	5.8[b]	0.1
Tuxpeño 8 Aut. Gpo. 2	5.8[b]	4.9[b]	1.5[b]
La Posta	4.9[a]	4.5[a]	0.3
Tremio Largo	7.8[a]	4.5[a]	2.9[b]
Poblanito Criollo	5.9[a]	5.8[b]	0.1
Mean	5.0	4.1	

Group A	Opaque	Modified opaque	Difference (O-MO)
Thai Opaque	5.7[b]	5.5[b]	0.2
Composite K	5.7[b]	5.7[a,b]	0.0
Composite CIMMYT	4.8[a]	5.3[a]	0.5
Vera 101 8 Aut. Gpo. 2	5.5[b]	5.3[a]	0.2
Mean	5.6	5.7	

[a] Comparisons are valid only within columns for each class of entries. Means with the same letters as superscripts are not significantly different at the 0.05 level.
[b] Significant difference at the 0.01 level.

Changes in Moisture Content of Cob, Grain, and Ear

Figure 3 shows the curves of the percentage of grain moisture for each of the four classes of entries. The decrease in moisture percentage is nearly linear for the entire period. It was supposed that the phenomenon chosen for

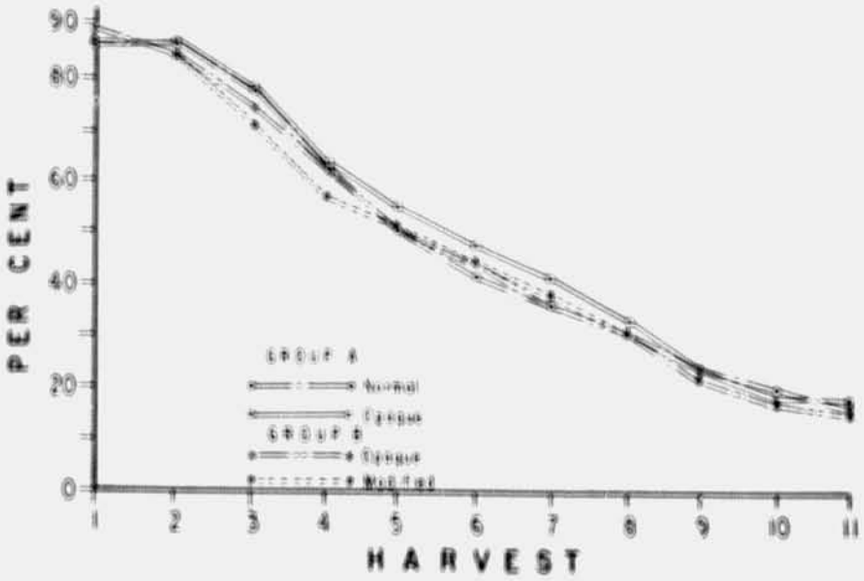

FIGURE 3 Changes in grain moisture content

this study would vary in rates of moisture loss, yet the most striking point of the graphs is that all classes lost moisture at a similar rate. Regression analysis confirmed the similarity in rate of moisture loss.

The weights of water in the cob, the grain, and the ear were calculated and are presented in Figures 4, 5, and 6, respectively. The weight of water in the cob (Figure 4) increased rapidly as the weight of the cob increased from the first to the third harvest, reaching a maximum of 60 to 80 g. Thereafter, the weight in the cobs decreased until the final harvest.

The grams of water in the grain for each class (presented in Figure 5) increased to a maximum of 62 to 75 g at harvest 6, depending on the class. After harvest 6, the weight of the water in the grain decreased for all classes, with a rapid decline occurring after harvest 7. No differences were found in rates of moisture loss after physiological maturity, which occurred at or just before harvest 8. The graphs clearly demonstrate that moisture loss from the grain did not occur in this study until after harvest 6, or 45 days after silking. Therefore, a drying process, in the sense of moisture loss, did not commence until after the period of rapid dry-matter increase in the grain had ended (see Figure 1). The decrease in the percentage of grain moisture observed during this period seems due to dry matter increasing more rapidly rather than to water in the grain.

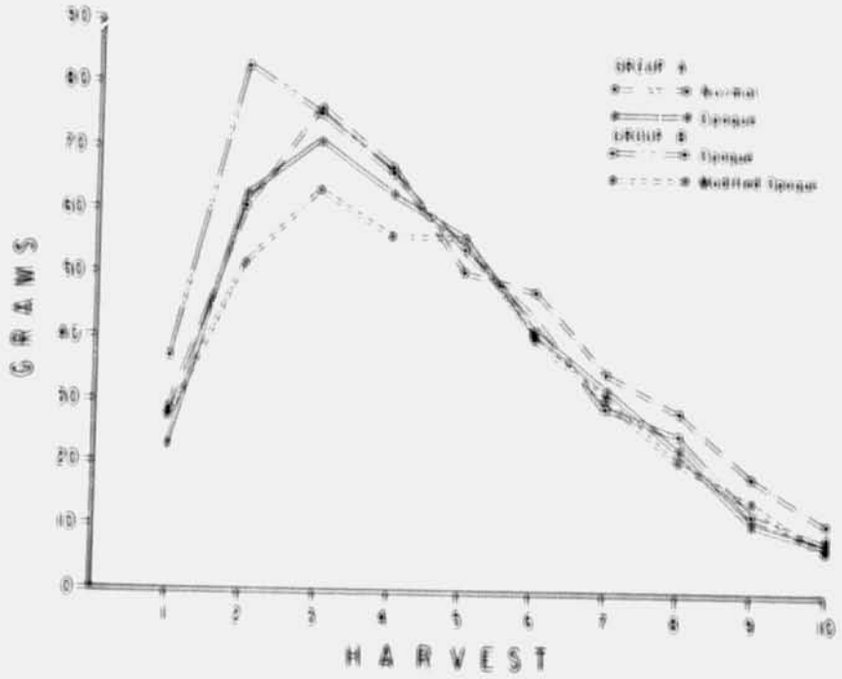

FIGURE 4 Grams of water per plant in cob for the four classes of sweeps from harvest 1 to 10.

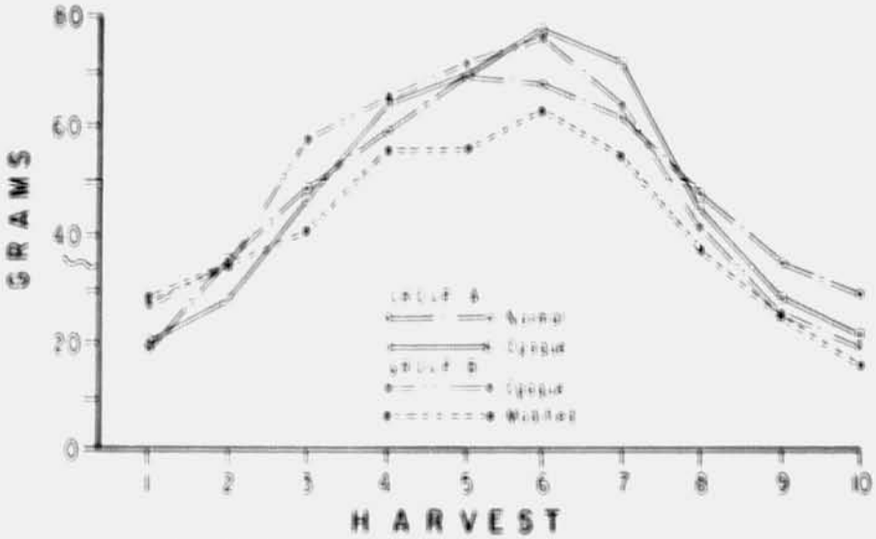

FIGURE 5 Grams of water per plant in grain

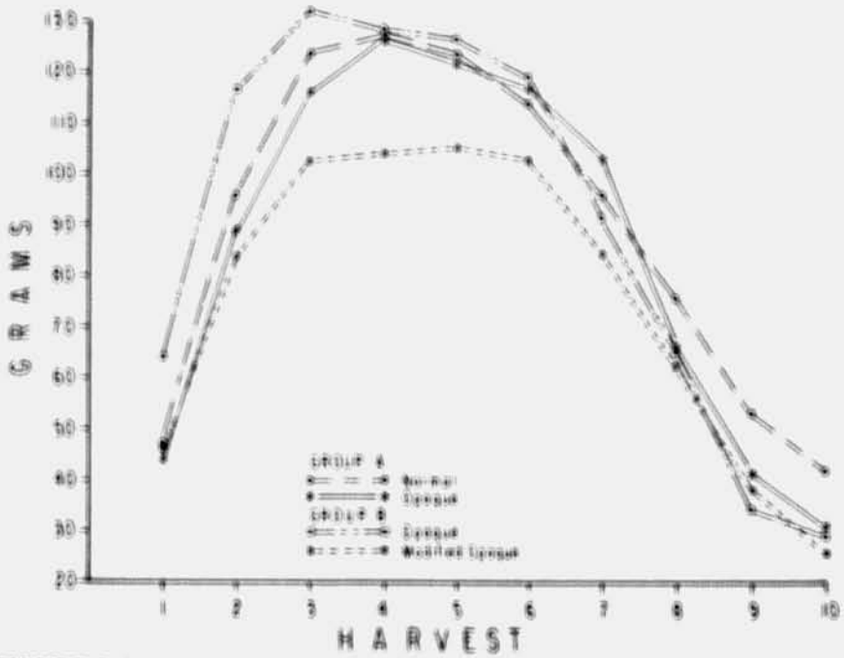

FIGURE 6 Grams of water in straw per plant for the four classes of moisture from harvest 1 to 10

The changes in the water content of the ear (Figure 6) result from the combined changes in water content of the cob and the grain. Thus, the initial increase in water in the ear seems due to increases in water in the cob. However, in harvests 3 through 6, the relatively uniform maximum weight of water in the ear was due to a decrease in water in the cob and an increase in the weight of water in the grain. After harvest 6, the water content in the ear declined as the grain and cob dried.

As a matter of curiosity, calculations were made as to the amount of water lost each week and the number of calories needed to vaporize that quantity of water (Table 4). The number of calories available from solar radiation was calculated. It was assumed that 8 h of solar radiation at 1 cal cm^{2} min was striking 40% of the surface of an ear 6 cm in diameter and 15 cm long. The ear had a 1-cm husk covering with a thermal conductivity of 0.0005. The surface area was assumed to be the mean of the surface calculated as a cone and as a cylinder. Under these conditions, 146 cal/week were available to vaporize moisture in the ear. We calculated that solar radiation supplied 1 to 5% of the calories needed, indicating that most of the water was lost by advection, a process in which relative humidity and wind movement play a large role. The importance of advection would explain the observed variation in length of drying in areas with bright, sunny weather.

Relation of Ear Parameters to Yield

Correlation analysis was used to study the relationship between yield and the ear parameters of kernel row number, ear length, cob width, and kernel depth. Significant correlations were obtained between cob diameter and kernel row numbers (0.541), and between yield and both cob diameter (0.478) and ear length (0.489). These correlations suggest that an expansion of kernel number will increase yield. However, a highly significant correlation (0.738) was obtained between yield and kernel depth, indicating that

TABLE 4 Water loss from grain and calories required to vaporize this water loss

	Group A		Group B	
Harvest period	Normal	Opaque	Opaque	Modified opaque
	Grams of Water per Plant Lost from Grain			
7-8	18.5	26.5	22.2	16.4
8-9	12.4	5.8	16.0	11.2
9-10	6.0	7.0	5.0	5.4
	Calories Required to Vaporize Water Lost by Grain[1]			
7-8	8,410	15,570	12,876	9,512
8-9	7,018	3,364	9,280	6,496
9-10	3,480	4,292	2,900	3,452

[1] 580 cal/g water at 50°C.

54% of the variation in yield was due to differences in kernel depth. Kernel depths demonstrate differences of 20 and 15% in the normal and opaque classes of group A and 7% in both classes of group B. Further evidence for varietal differences in rates of dry-matter accumulation in the grain is found in the fact that kernels varying in size by 15 to 20% were filled in the same length of time.

DISCUSSION

The yield reported for varieties in this study varied from 6,987 to 4, 640 kg/ha for normals, from 5,333 to 4,587 kg/ha for opaques, and from 4,747 to 4,320 kg/ha for modified opaques. Clearly, the three phenotypes overlap one another in yielding ability; but the mean yield for each phenotype indicates that normal grain types yield 13% more than opaques, and that opaque grain types yield about 13% more than modified opaque grain types. Such data are consistent with data reported for temperate-region materials. The overlap of the different phenotypes and the great variation within each phenotype suggest that increased yield should be possible in all phenotypes when under selection. Certainly, tropical varieties of normal grain type have shown yield improvement due to recurrent selection (CIMMYT Report 1970–1971, p. 75). At present, there is no reason to doubt that similar yields can be obtained in the opaque and modified opaque populations. In fact, the dry-weight curves of Figure 1 show that the improved opaque entries of group B had a rate of dry-matter increase as great as the normal entries of group A.

The ability to transfer stalk carbohydrate appears to be limited or lacking in the opaque varieties. No clear explanation is known for this phenomenon. If, in fact, this ability is lacking (and it appears to be), then selection against such a response would increase the yielding ability of opaque maize. Such selection could be accomplished by harvesting half the replicates of a yield trial 1 week after all leaf area had senesced, and then harvesting the remaining replicates after black-layer formation. The entries giving an increase in yield between the first and the second harvest would be saved for further selection.

The reduction in cob weight was greater in the opaque–normal comparison than in the opaque–modified opaque comparison. The reduction in cob weight was of the same proportion as that for grain-weight reduction. This suggests that a generalized reduction in ear dry-matter accumulation is associated with the opaque genotype.

The increase in grain moisture until the loss of leaf area at harvest 6 suggests that moisture loss does not occur until after loss of the leaf area. Decrease in moisture percentage until this time was the result of a faster increase in dry matter than in water in the grain. Thus, it seems likely that moisture loss before the loss of the functioning leaf canopy has the recip-

rocal of dry-matter accumulation as a causative factor, rather than moisture loss.

The relatively larger amount of water in the opaque grain will require longer to dry to a given percentage of grain moisture than will normal grain if the environment is limiting. This may be the case in the temperate areas. However, in an environment favorable to drying, the rate of moisture loss (weight per unit time) would be greater for opaque than for normal grain types. Such a response is illustrated in Figure 4. In an environment limiting moisture losses, the extended period of relatively high levels of moisture and the nutritionally improved substrate of the opaque grain may favor fungal growth.

The opaque and modified opaque class of group B were intermediate in percentage of grain moisture between the normal and opaque classes of group A. Genotype differences between the groups do not allow definite conclusions; however, the improvement of opaque varieties as to yield or endosperm texture would seemingly reduce the relatively higher grain moisture levels observed for opaques in group A.

CONCLUSIONS

Among the three grain phenotypes studied, the normal yielded more than the opaque, which, in turn, yielded more than the modified opaque. However, the differences in yield between the different phenotypes were not large. Therefore, recurrent selection for improved yield should produce opaque and modified opaque varieties that approach or equal the yields of normal maize.

The percentage of grain moisture was similar for all the phenotypic classes at each harvest. Therefore, weight of moisture in the ear was more dependent on the dry weight of the grain than on the percentage of grain moisture. The environment at Hallizapan was very favorable for drying; however, differentials in rates of drying might appear in less favorable environments.

Since no serious ear rot infestations occurred in this study, subsequent experiments were conducted to determine differences in disease susceptibility. If, in these studies, no differences in rates of drying are found and differences in ear rots do occur, then ear rots would be associated with factors other than the rate of grain drying.

[*A discussion of this paper can be found on p. 490 of* **Questions and Answers.**]

SOME GROWTH AND YIELD CHARACTERISTICS OF TROPICAL MAIZE

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For millions of people living in tropical countries whose diet is deficient in protein, maize is a staple food. For these people, opaque-2 maize can provide a source of urgently needed high-quality protein. First, however, the opaque-2 gene associated with the high-quality-protein characteristics must be introduced into new or traditional maize varieties that the people can grow. Then the quantities produced will depend on the yield capability of the converted materials.

The Centro Internacional de Mejoramiento Maiz y Trigo (CIMMYT) maize breeding program is developing high-yielding varieties that will be useful over a wide range of environments throughout the world. The opaque-2 gene has been introduced into these varieties and study has begun on (1) how the growth and development of tropical maize varieties are affected by the environment, and (2) factors that limit yield. This is to assist breeders to select for increased yield.

This report describes some of the characteristics of tropical maize varieties and provides examples to indicate the scope of research interest.

ADAPTATION TO ENVIRONMENT

An adapted variety is one that is efficient in the environment in which it is grown. An important part of adaptation, under rain-fed conditions, is the ability to grow and produce grain when water is available. Within tropical latitudes, evidence suggests that temperature variations with season and

altitude are probably more important in determining the length of the growing period for a variety than is variation in day length.

The CIMMYT breeding program uses the broad range of environments available in Mexico to develop varieties with wide adaptation. Breeding and selection are done at several sites, representing a range of altitudes and latitudes, and the progenies are moved from one location to another. Thus, the varieties developed are adapted to a wider range of environments than are the component populations from which they were derived.

Figures 1 and 2 show the seasonal temperature and rainfall distribution for three of the main work sites.

GROWTH AND YIELD

If a crop is examined as a system for producing grain, it is convenient to think of it as a *source* of assimilates and a series of *sinks* where the assimilate is used for growth. The *source* is the photosynthetic surface and (for the studies considered here) can be described in terms of its *size* (the leaf area index) and its efficiency (dry weight production per unit of leaf area).

The sink of most interest to us here is the grain. The *size* of the sink determines the potential grain yield and, for present purposes, can be measured conveniently as the number of fertile florets per unit of land area.

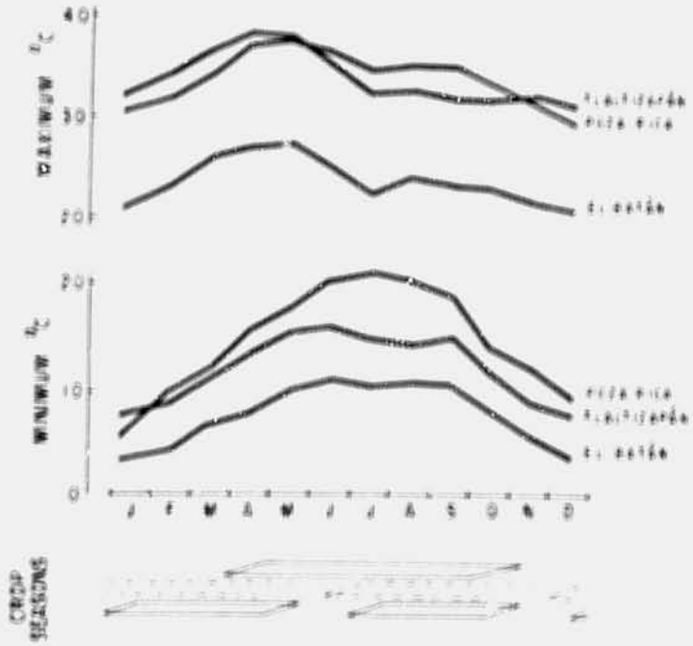

FIGURE 1 Temperature range. Three experiment sites in Mexico.

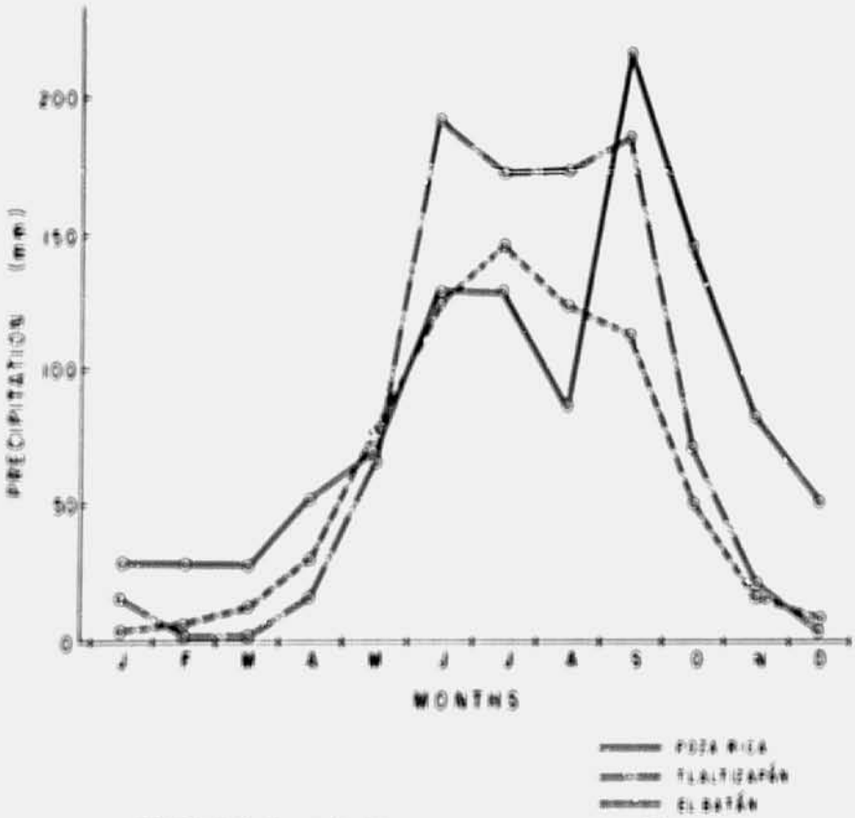

FIGURE 2 Rainfall. Three experiment sites in Mexico

In an ideal grain crop, the vegetative growth period should be as short as possible, while allowing for the leaf area development needed to maintain a high rate of dry-weight production after anthesis, and a sufficiently large number of fertile florets to accommodate the assimilate that is translocated to the grain. Ideally, all the dry-weight increase after flowering then should go toward grain production. Examples of two such nearly ideal crops are shown in Figures 3 and 4.

The following examples of tropical maize crops have been chosen to illustrate how crop growth and yield are affected by environment. They indicate the kind of studies being made to determine factors limiting yield and to what extent our present materials fall short of the ideal.

HIGHLAND VERSUS LOWLAND CROPS

The first example is an analysis of tropical maize growth and is taken from work that will be reported more completely elsewhere by J. Yamaguchi. It

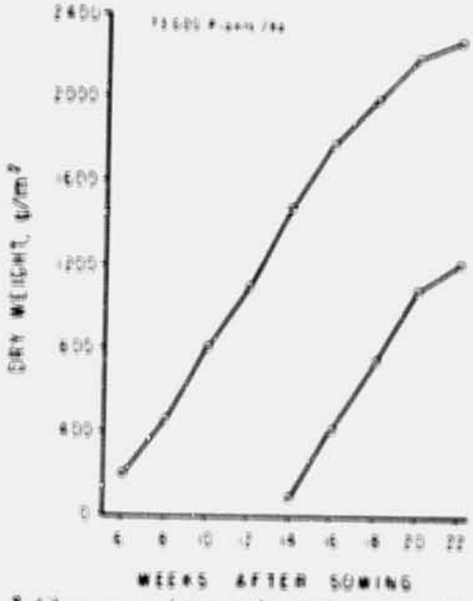

FIGURE 3 Change in dry weight and grain yield of Ireland maize Saladours (1)

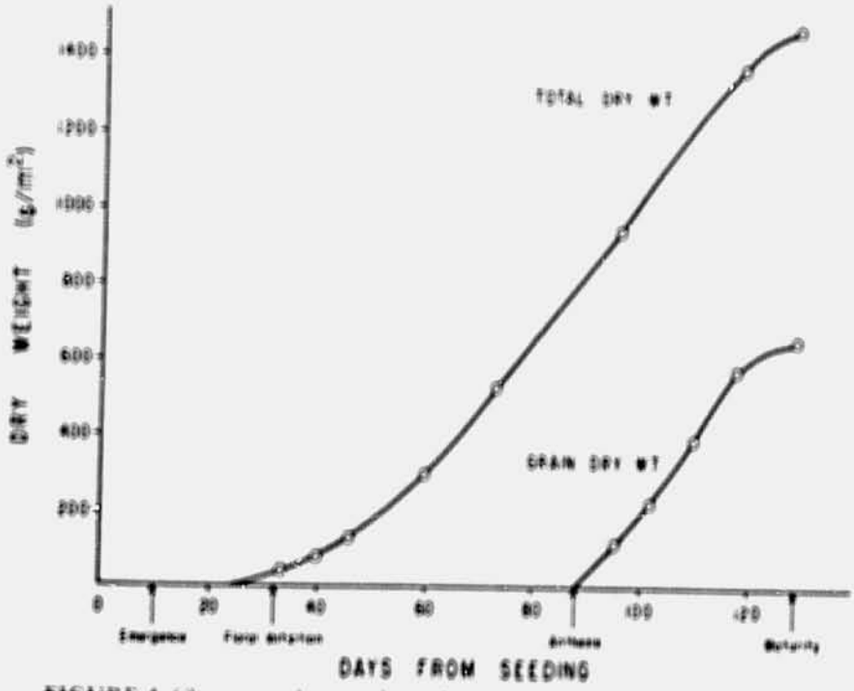

FIGURE 4 Change in dry weight and grain weight of Yecora wheat (2)

illustrates the contrasts in pattern of growth and yield occurring within a range of environments, and compares a highland hybrid (H.125) grown at El Batán (2,250 m) and a lowland hybrid (H.507) grown at Poza Rica (60 m).

Grain Yield

The yield of H.125 at El Batán was much larger than that of H. 507 at Poza Rica (Table 1). The difference in yield between the two crops was completely accounted for by a difference in grain size. The number of grains per square meter was the same in both crops. The larger number of ears per unit area of H. 125 compensated for the smaller number of grains per ear.

Dry-Weight Formation

The highland hybrid (H.125) produced much more dry weight than H. 507 (Figure 5a). The crop growth rate was less at El Batán than at Poza Rica, but the period of growth was much longer in El Batán's lower temperature (193 days compared with 112 days). During the first part of the grain-filling period, the increase in grain weight in the highland hybrid and the lowland hybrid was less than the increase in total dry weight, indicating that only a fraction of the dry weight produced went to the grain. Subsequently, there was a loss in dry weight while the grain weight continued to increase. This growth pattern does not follow the growth curves of the ideal crops described earlier.

Leaf Area

Maximum leaf area indexes were attained 60 and 91 days after sowing (Figure 5b). The most significant difference between the leaf area development was the difference in leaf area duration after silking. Whereas the leaf area index declined rapidly after silking at Poza Rica, at El Batán it maintained almost its maximum value for an additional 45 days before it began to decline rapidly.

At Poza Rica, the leaf area duration (LAD) after silking was 134 days compared to 385 days at El Batán. This difference in leaf area duration accounts for the differences in dry weight and yield of the two crops.

Since the assimilate for grain production in maize comes largely from photosynthesis after flowering, the weight of grain produced per unit leaf

TABLE 1 Grain yield, highland and lowland maize

Site	Variety	Grain (tons/ha)	1000 kernels (g)	No. kernels M-2	No. ears M-2	No. kernels/ear
El Batán	H. 125	9.6	372	2,590	6.0	431
Poza Rica	H. 507	5.9	228	2,590	5.3	486

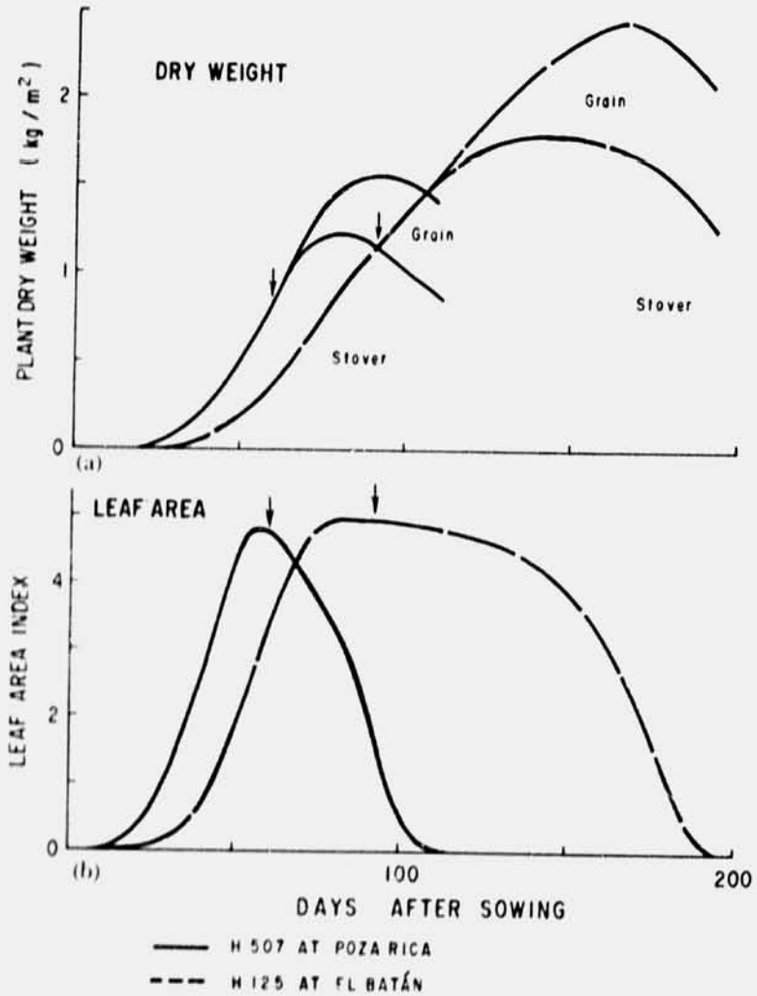

FIGURE 5 Growth of highland and lowland maize.

area duration after silking (grams of grain per day) is an index of the efficiency of the leaves for producing grain. It is interesting to note that the grain weight formed per unit LAD was larger at the lowland site (4.40 g/day) than at El Batán (2.49 g/day). Furthermore, in terms of grain produced per hectare per day, the lowland tropical crop (5.9 tons/ha in 110 days = 52.8 kg/ha/day) was at least as efficient as the upland crop (9.6 tons/ha in 193 days = 50 kg/ha/day). However, the dry-matter production after silking and the grain yield were much greater at El Batán than at Poza Rica, due to the much longer grain-filling period at El Batán.

Since the total dry weight (TDW) of the crop decreased at both sites

during the final stages, the ratio of grain weight to TDW at harvest (harvest index) only partially indicates biological efficiency. Values for both crops were lower (Table 2) than those reported for temperate maize (0.5).

COMPARISON OF TWO LOWLAND TROPICAL VARIETIES

The second example is a comparison of the growth and yield of two lowland tropical varieites. Tuxpeño planta baja and Tuxpeño × Eto planta baja (*planta baja* means short plant).

Grain Yield

The grain yield of both varieties was larger at Tlaltizapán than at Poza Rica. Tuxpeño planta baja yielded more than the variety cross at both sites.

Grain yield increased with plant population up to 150,000 plants/ha, or three times the population at which breeders normally carry out their selection. The largest yield (8.3 tons/ha oven-dried grain) was from the highest plant population of Tuxpeño planta baja at Tlaltizapán (Figure 6).

Grain yield is the product of the number of grains per square meter and the size of the grains. The graphs show that grain yield was proportional to grain numbers per square meter (2,500 to 4,000/m²); thus, plant population had little effect on grain size.

The number of grains per square meter is the product of the number of ears per square meter and the number of grains per ear. At high plant populations, both varieties produced fewer ears than plants, indicating that

TABLE 2 Grain yield and yield components of four Tuxpeños

		Variety			
	Plants/m²	CR.1	PB	BR	BRPB
Grain yield	2.5	3.81	–	–	–
(tons/ha)	5	5.81	4.82	3.57	3.67
	10	–	5.61	4.02	3.97
Weight of (g)	2.5	296	–	–	–
1,000 kernels	5	282	237	242	229
	10	–	229	242	220
Kernels/m²	2.5	1285	–	–	–
	5	2057	2039	1480	1601
	10	–	2451	1651	1802
Kernels/ear	2.5	514	–	–	–
	5	457	429	333	327
	10	–	331	296	265
Ears/m²	2.5	2.5	–	–	–
	5	4.5	4.8	4.5	4.9
	10	–	7.4	5.6	6.8

Notes: CRI, Tuxpeño crema 1; PB, Tuxpeño short plant selection; BR, Tuxpeño brachytic; BRPB, Tuxpeño brachytic short plant selection.
Source: J. Yamaguchi (3).

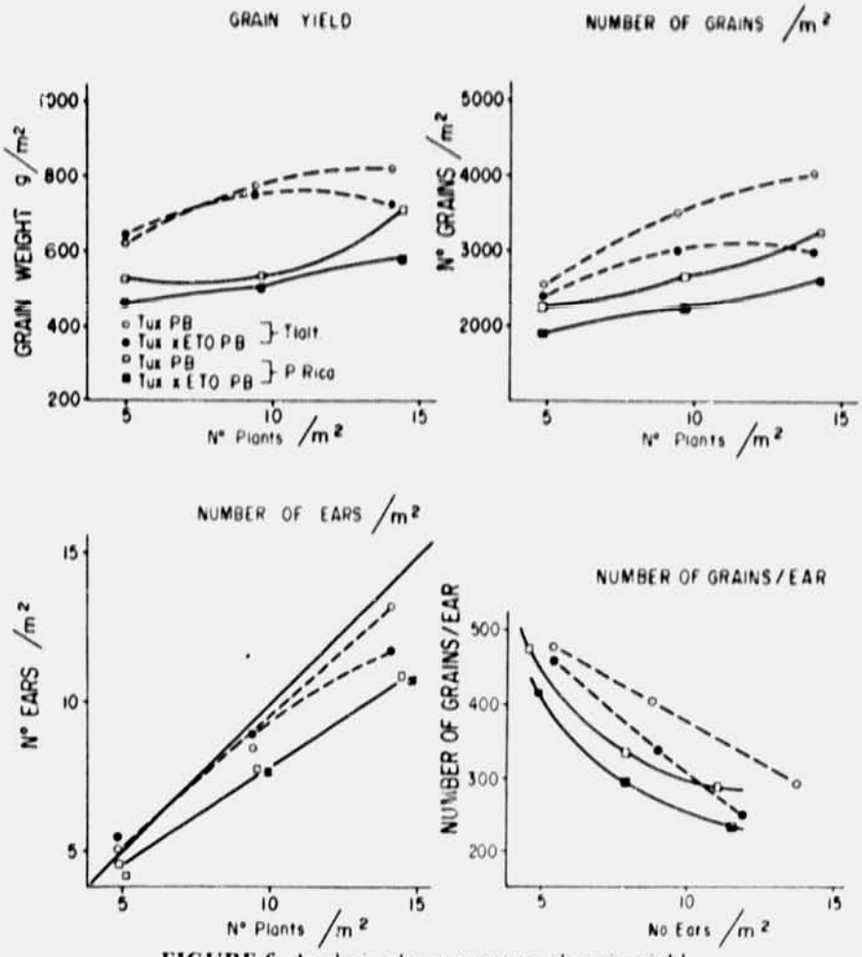

FIGURE 6 Analysis of components of grain yield.

some plants were not contributing to yield. There were fewer barren plants at Tlaltizapán than at Poza Rica; and at the greatest population level at Tlaltizapán, Tuxpeño planta baja produced more ears than did the variety cross.

As the number of ears per square meter increased with plant population, the number of grains per ear decreased; but at each population level, the number of grains per ear was larger in Tuxpeño planta baja than in Tuxpeño × Eto. These differences accounted for the larger number of grains per square meter and larger yield of Tuxpeño planta baja.

Dry-Weight Formation

The dry-weight production of these crops is shown in Figure 7. Interesting points in these graphs are the following:

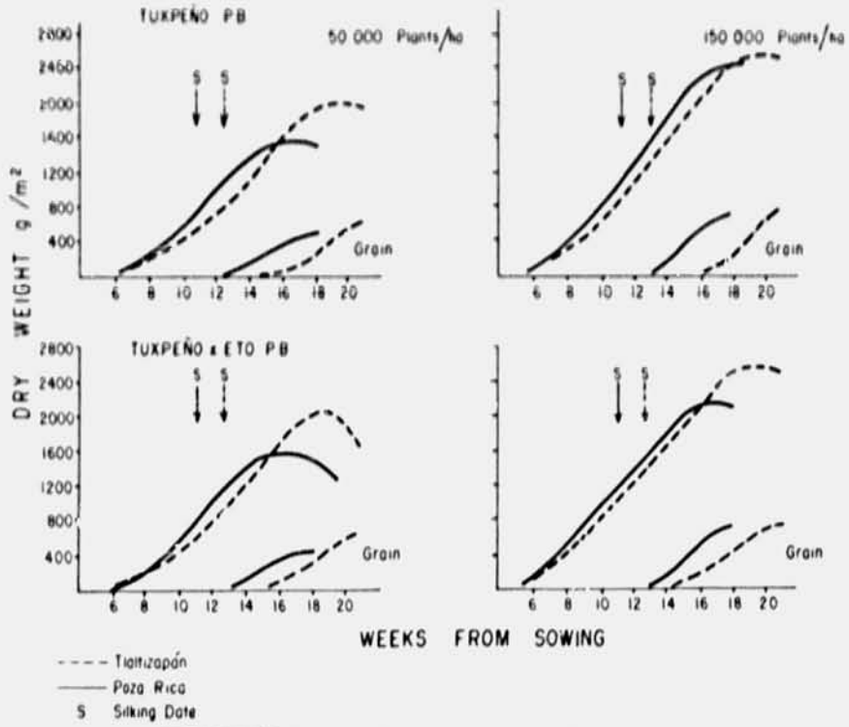

FIGURE 7 Dry weight and grain yield of maize.

1. The total dry weight produced increased with an increase in plant population. The duration of growth was unaffected by population, and the increase in dry weight was a result of an increase in the rate of dry-matter production (the crop growth rate, C). The dry weights at Poza Rica were less than those at Tlaltizapán because the growth period was shorter.

2. The crop growth rate at Poza Rica was as high as or higher than at Tlaltizapán. Therefore, there is little evidence from these experiments to support the contention that grain yields in the lowland tropics are reduced because of the limiting effects of high night respiration losses on crop growth rates.

3. After silking, in both varieties and populations, the rate of increase in grain dry weight is smaller than the rate of increase in total dry weight. As in the previous example, only a fraction of the dry weight produced after silking goes into the grain. In an efficient grain crop, all the dry weight after anthesis goes into the grain.

4. A large portion of the crop dry weight is vegetative growth, which is only indirectly related to grain yield. For example, 15 weeks after sowing (when accumulation of dry weight in the grain began), Tuxpeño planta baja at

Hialtizapán with a population of 150,000 plants/ha had produced between 16 and 20 tons of dry matter /ha.

5. The comparatively small difference in temperatures at the two sites greatly affected the rate of development, and thus the growth curve. At Hialtizapán the interval from sowing to silking was about 2 weeks longer than at Poza Rica, mainly as a result of the lower night temperatures at Hialtizapán.

Leaf Area

As indicated previously, differences in crop dry weight are associated with differences in leaf area index. Thus, the increase in crop growth rate with corresponding increase in plant population is associated with a more rapid development of leaf area and a larger leaf area index. Similarly, the longer growth period at Hialtizapán is associated with a longer duration of leaf area than at Poza Rica. This difference in leaf area duration was partly an effect of temperature and partly a result of leaf diseases (Figure 8).

COMPARISON OF TALL AND SHORT TUXPEÑO

A third example shows the differences in yield and yield components between tall and short plant forms of Tuxpeño and Tuxpeño brachytic (Table 2). These data also are to be reported in more detail elsewhere by J. Yamaguchi.

Tuxpeño Crema 1 is tall and, because of its susceptibility to lodging, was

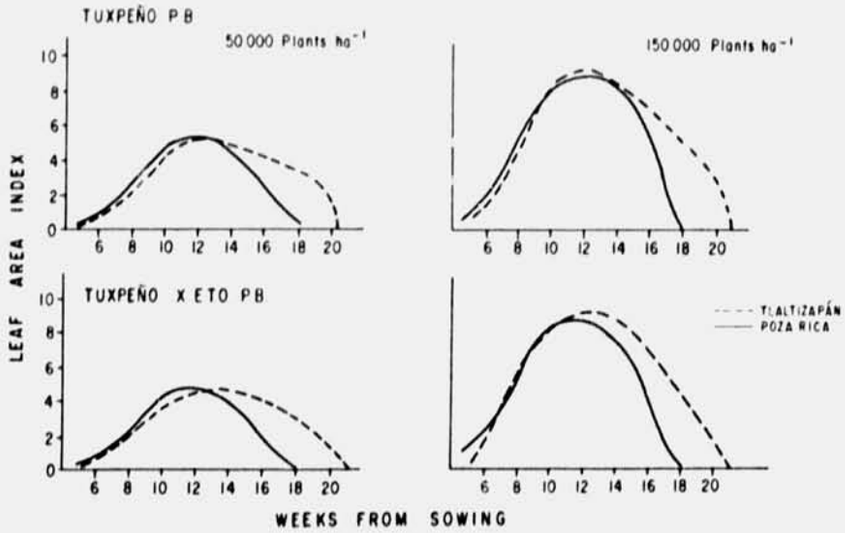

FIGURE 8 Leaf area index.

grown at lower plant densities (25,000 and 50,000 plants/ha) than the short plant selections or the brachytic forms (50,000 and 100,000 plants/ha).

Grain Yield and Its Components

Tuxpeño Crema 1 at 50,000 plants/ha gave the largest yield (5.81 tons/ha). The yield of the Tuxpeño short plant selection at 100,000 plants/ha was only slightly less (5.61 tons/ha). Crema 1 grains were larger than those of other varieties. The difference in yield between Crema 1 and the short plant selections at 50,000 plants/ha was accounted for mainly by this difference in grain size; the other yield components were similar in the two varieties. The yield of the short plant selections was larger than that of either of the brachytic varieties for both population levels.

In all varieties, grain yield increased with an increase in plant population, but the difference was greater in the short plants than in the brachytics. Plant population had little effect on grain size; and since the grain size in the short plant selections and the brachytics was similar, differences in yield were determined mainly by differences in the number of grains per square meter. At 50,000 plants/ha, the number of ears per square meter in short plant selections and the brachytics was similar. Differences in the number of grains per square meter were accounted for mainly by the larger number of grains per ear on the short plants.

At the higher population (100,000 plants/ha), the differences between varieties in the number of grains per ear were small. However, the short Tuxpeño produced more ears per square meter than either of the brachytics. Even so, 26% of the plants of the short Tuxpeño were not contributing to yield at this population; for the brachytics, the number was even larger: 44 and 32%.

The more detailed report of the experiments will provide an examination of the differences in yield in terms of dry-weight production and distribution of these crops, as illustrated in the previous examples.

SUMMARY

By manipulating plant populations and leaf area index, comparatively high crop growth rates (35 g/m²/day) can be attained in the lowland tropics. It seems unlikely, from evidence available, that crop growth rate per se limits tropical maize yields. The problem seems to be the distribution of dry weight and, in the lowland tropics, the comparatively short period of dry-weight accumulation in the grain.

In the present materials, much of the dry weight produced is vegetative growth that is only indirectly related to yield. After flowering, only a fraction of the dry-weight increase is as grain. Thus, there seems to be considerable research scope for developing plants with growth and development patterns

such that a much larger portion of the resources available are used for grain production.

The capacity of the crop to accumulate carbohydrate in grain is determined mainly by the number of grains per square meter. The varieties described, at populations of about 50,000 plants/ha, usually produce about 2,500 grains/m^2. The number of grains can be increased by increasing plant population; but with the materials available and at the plant populations needed to produce 4,000 grains/m^2, a large proportion of the plants are barren. Therefore, plant breeders and agronomists need to join together to find ways of eliminating this loss and the loss associated with the decrease in the number of grains per ear as the number of plants per square meter is increased.

Increasing the capacity of the sink will be useful only if a sufficiently large source (leaf area) can be maintained to fill it. Senescence of leaves occurs more rapidly at high temperatures, and this is almost certainly a major factor determining the comparatively short leaf area duration of lowland tropical varieties. However, it is interesting to note that an indirect effect of the selection for shorter plants in Tuxpeño appears to have been an increase in the leaf area duration after flowering. The patterns of leaf area development and senescence are similar, but because the short plants flower about 5 days earlier than the materials from which they were selected, a larger fraction of the dry weight is produced after silking. These and other differences indicate that there is ample scope for further improving the yield of tropical maize. Drought and disease apart, there is little reason to believe that the physical environment in the tropics currently limits grain yield.

From what we know of the varieties that have been described, such as the Tuxpeño, it seems reasonable to suppose that, without any other changes, yields of 10 tons/ha could be attained by modifying the pattern of dry-weight distribution. With the combined effort of agronomists and breeders these increased levels can be achieved.

DISEASE–INSECT INTERACTIONS IN QUALITY PROTEIN MAIZE

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G. Granados *CIMMYT, Mexico*
S. K. Vasal *CIMMYT, Mexico*

The reaction of opaque-2 maize materials to insect pests and pathogens has received only limited documented study. However, present information suggests that the soft opaque-2 maize endosperm may be associated with susceptibility to *Fusarium* ear rots and vulnerability to stored-grain insect pests.

For valid test comparisons of such associations, the opaque-2 gene should be present in similar genetic backgrounds. Recently, such maize populations have been made available, which are suited to high-altitude valleys, subtropical, and tropical environments. The maize varieties discussed in this report have, on the average, undergone three backcrosses, and the modified types have been selected for at least three cycles.

FIELD PESTS AND DISEASES

A set of 15 maize varieties, including the normal and its opaque version in each case, was tested in humid tropical, semiarid tropical, and subtropical environments (respectively, Poza Rica, Obregón, and Tlaltizapán). Also, a group of opaque-2 converted varieties and their normal counterparts were observed in two high-altitude environments, at El Batán and Toluca.

In addition, another group of maize varieties, including the normal, opaque-2, and modified opaque-2 types in each case, was used to determine the rate of ear drying and the reaction of each type to different fungi and ear-feeding insects in a humid tropical environment (Poza Rica) and a subtropical environment (Tlaltizapán). Field trials consisted of 5-m-row plots replicated four times in a split plot design, with the maize variety as main plot and the endosperm version as subplots: normal, opaque, and modified opaque.

Our observations were based on the following fungi and insects:

Ear rots:	*Fusarium moniliforme*
	Fusarium roseum
	Diplodia maydis
	Diplodia macrospora
Earworms:	*Diatraea saccharalis*
	Heliothis zea
Rusts and blights on foliage:	*Puccinia sorghi*
	Puccinia polysora
	Helminthosporium turcicum
	Helminthosporium maydis
Insects on foliage:	*Spodoptera frugiperda*
	Diatraea saccharalis
Stalk rots:	*Fusarium moniliforme*
Stem borer:	*Diatraea saccharalis*
Stored-grain fungi:	*Penicillium* spp.
	Aspergillus spp.
Stored-grain insects:	*Sitophilus zeamais*
	Sitotroga cerealella

The reaction of the maize varieties was observed in the field under natural incidence of insect pests and pathogens, except in the case of rust (at El Batán), *Diplodia* ear rot, stalk rots, and stored-grain fungi and insects, which were placed artificially on the appropriate plant parts.

Discussion of the pests and pathogens generally follows the preceding list.

Ear Rots

Tables 1 to 3 summarize the reaction of the varieties to *Fusarium* in five different environments. The incidence of *Fusarium* in the set of 15 materials was highest in the humid tropical environment of Poza Rica. The mean reaction of the varieties indicated that *Fusarium* affected about 50% of the harvest of the opaque versions, whereas infection of the normal versions was below 30%. The data also show an extreme degree of susceptibility in some genetic backgrounds; the differences between normal and opaque versions are not so great in other backgrounds. Several populations showed similar reactions in the other environments.

TABLE 1 Natural infection by *Fusarium* ear rot on normal and opaque-2 versions of 15 maize populations: Poza Rica, Obregón, and Tlaltizapán (1972)

Maize populations	Endosperm versions		Maize population means
	Normal	Opaque	
	Poza Rica, Veracruz		
Francés Largo	8	46	27.0
Compuesto Blanco Caribe	19	48	33.5
La Posta	13	55	34.0
Antigua Gpo. 2	17	53	35.0
Compuesto K	38	35	36.5
Población Cristalina	18	56	37.0
PD(MS)6 X Granos Amarillos	38	41	39.5
(Mix 1 X Col. Gpo) Eto Bl.	27	53	40.0
Compuesto Grano Duro	27	54	40.5
Puerto Rico Gpo. 1	36	51	43.5
Nicarillo	40	48	44.0
Tuxpeño X PD(MS)6	42	47	44.5
Ver. 181 X Ant. Gpo. 2	33	48	44.5
Compuesto L (ME)C_1	35	54	44.5
Tuxpeño X Antigua Gpo. 2	40	61	50.5
Endosperm version means	28.7	50.0	
	Obregon, Sonora		
Nicarillo	7	28	17.5
Tuxpeño X PD(MS)6	7	32	19.5
(Mix 1 X Col. Gpo. 1) Eto	7	37	22.0
Compuesto Blanco Caribe	7	38	22.5
Compuesto Grano Duro	9	37	23.0
Población Cristalina	9	37	23.0
PD(MS)6 X Granos Amarillos	13	34	23.5
La Posta	7	41	24.0
Ver. 181 X Ant. Gpo. 2	6	43	24.5
Tuxpeño X Antigua Gpo. 2	8	51	29.5
Compuesto K	28	36	32.0
Antigua Gpo. 2	9	56	32.5
Compuesto L (ME)C_1	10	63	36.5
Puerto Rico Gpo. 1	24	70	47.0
Endosperm version means	10.7	43.0	
	Tlaltizapán, Morelos		
Compuesto Blanco Caribe	4	13	8.5
Ver. 181 X Ant. Gpo. 2	5	15	10.0
Francés Largo	11	16	13.5
Tuxpeño X PD(MS)6	8	20	14.0
PD(MS)6 X Granos Amarillos	6	23	14.5
Compuesto Grano Duro	6	22	15.0
La Posta	10	20	15.0
Nicarillo	12	18	15.0
(Mix 1 X Col. Gpo. 1) Eto	12	19	15.5
Puerto Rico Gpo. 1	10	21	15.5
Compuesto L (ME)C_1	16	19	15.5

TABLE 1 (Continued)

Maize populations	Endosperm versions: Normal	Endosperm versions: Opaque	Maize population means
Compuesto K	16	20	18.0
Antigua Gpo. 2	7	30	18.5
Población Cristalina	12	25	18.5
Tuxpeño X Antigua Gpo. 2	15	26	20.5
Endosperm version means	10.1	20.4	

Note: Differences between means were established on the transformed values (arcsin $\sqrt{\text{percentage}}$) at the 5% level. Data presented are actual percentages. Means covered by the same line are not significantly different. At Obregón, the France's Largo was eliminated because of poor stand.

The second highest incidence of *Fusarium* ear rot was in the irrigated semiarid tropical environment of Obregón (Table 1). Again, the infection for opaque versions was significantly higher than that for the normal types. On the average, about 40% of the opaque ears had *Fusarium*, compared with about 10% found in the normal ears.

In the subtropical environment of Tlaltizapán (Table 1), about 20% of the

TABLE 2 Natural infection (percentage of damaged ears) by *Fusarium* ear rot on normal and opaque-2 versions of several maize populations: El Batán and Toluca (1972)

Maize populations	Endosperm versions: Normal	Endosperm versions: Opaque	Maize population means
El Batán, Mexico			
Compuesto J	10	24	17.0
Mexico Gpo. 10	15	25	20.0
Hidalgo 8	8	38	23.0
Puebla Gpo. 1	12	42	27.0
CIPA	15	40	27.5
Compuesto I	20	37	28.5
Compuesto Celaya	18	41	29.5
Mexico Gpo. 10 X Hidalgo 8	18	53	35.5
Endosperm version means	14.5	37.5	
Toluca, Mexico			
Compuesto I	14	14	14.0
Mexico Gpo. 10	10	19	14.5
Hidalgo 8	9	22	15.5
Mexico Gpo. 10 X Hidalgo 8	16	22	19.0
Endosperm version means	12.2	19.2	

Note: Differences between means were established on the transformed values (arcsin $\sqrt{\text{percentage}}$) at the 5% level. Data presented are actual percentages. Means covered by the same line are not significantly different.

TABLE 3 Natural infection (percentage of damaged ear) by *Fusarium* ear rot on normal, opaque 2, and modified opaque 2 versions of four maize populations: Tlaltizapan and Poza Rica (1972)

Maize populations	Endosperm versions			Maize population means
	Normal	Opaque	Modified	
	Tlaltizapán, Morelos			
Compuesto Blanco Caribe	16	16	12	14.6
Compuesto Blanco Caribe	11	18	19	16.0
Ver. 181 × Ant. Gpo. 2 × Ven.	15	20	20	18.3
Compuesto K	15	19	23	19.0
Compuesto CIMMYT				
Endosperm version means	14.2	18.2	18.5	
	Poza Rica, Veracruz			
Ver. 181 × Ant. Gpo. 2 × Ven.	32	53	60	48.3
Compuesto K	23	62	64	49.6
Compuesto Blanco Caribe	50	44	63	52.3
Compuesto CIMMYT	34	68	56	52.6
Endosperm version means	34.7	56.7	60.7	

Note: Differences between means were established on the transformed values (arcsin $\sqrt{\text{percentage}}$) at the 5% level. Data presented are actual percentages. Means covered by the same line are not significantly different.

opaque ears were affected by *Fusarium*, compared with about 10% of the normal ears.

At El Batán (Table 2), located in the Valley of Mexico, the susceptibility of the tested populations also was evident. More than 30% of the opaque ears were damaged by *Fusarium*, whereas about 15% of the normal ears were affected. The infection was the lowest at Toluca, the other high-altitude environment. However, the opaque versions also presented a significantly higher infection by *Fusarium* ear rot.

At three out of the five highly contrasting environments (Sonora, Tlaltizapán, and El Batán), significant differences were found among populations. The materials with the lowest *Fusarium* ear rot infection, on the average, were the normal versions of Compuesto Blanco Caribe, La Posta, and Ver. 181 × Ant. Gpo. 2, and the opaque-2 versions of Nicarillo, Compuesto K, Compuesto Blanco Caribe, and Tuxpeño × PD(MS)6. Among the high-altitude maize populations, Compuesto J and Mexico Gpo. 10 were the least susceptible.

When normal, opaque, and modified versions were compared at Poza Rica (Table 3), the opaque-2 and modified opaque-2 materials again showed significantly higher *Fusarium* infection. In Tlaltizapán there were no significant differences, but the percentage of ears infected was slightly higher in the opaque versions.

There was practically no difference among the normal, opaque, and modified versions with respect to infection by *Diplodia* ear rot.

In summary, the preceding data seem to confirm previous reports. The

opaque-2 versions showed a significantly higher infection by *Fusarium* ear rots than their normal counterparts in all cases, regardless of genetic background or environment. However, variations in reaction in space and time also have been reported. Thus, the agronomic conditions under which the crop is grown and the prevalence of a given race of the fungus may accentuate differences between opaque and normal materials.

Earlier we indicated that there seems to be a clear association between opaque-2 endosperm and infection by *Fusarium* ear rot. We attempted to learn how the known higher moisture content of opaque materials might contribute toward this higher susceptibility. Observations were conducted at a humid tropical and a subtropical environment. Weekly samplings were initiated 2 weeks after tasseling. Plots consisted of four rows, 10 m long, replicated four times. The infection by *Fusarium* and damage by earworms were estimated by an index reflecting a weighted mean of ears, with values of 1 (no damage) to 5 (severe damage).

In spite of a low incidence of *Fusarium* (Figure 1), differences in susceptibility are apparent for Tlaltizapán and Poza Rica, where the opaque-2 types appeared more damaged than the normals.

Patterns of ear drying were similar in most varieties in both environments. Most of the normals were 2 to 3% lower in ear moisture content than were the opaques, and the modified versions were intermediate between these versions (Figure 2).

It is not known whether differences in ear moisture content of 1 to 3% could be biologically significant, or if they favor a higher infection by *Fusarium* ear rot in the opaque maize types. However, our data suggest that there might be an association. On the other hand, infection by *Fusarium* also seems to be associated with damage by earworms. This relationship was particularly evident in Poza Rica.

Higher susceptibility of opaque-2 varieties to *Diplodia* ear rot has also been reported. Findings from the humid tropical environment of Poza Rica do not seem to support this view. Our reports are based on ears that were artificially inoculated at silking time (2.5×10^6 spores/ml). Data show that the fungus was growing on the ears 4 weeks after pollination. The average infection and fungus growth were higher in the normal types, intermediate in the opaque versions, and lowest in the modified versions. This finding seems to suggest a negative association with ear moisture content.

Earworms

In general, the data suggest that damage by earworms (*Heliothis zea* and *Diatraea saccharalis*) was heavier in the opaque versions than in the normal versions in the four environments sampled. Again, the range in reaction among the tested materials suggests that the degree of susceptibility varies in different genetic backgrounds. Data in Table 5 illustrate this point. Of the 15 maize materials, four entries showed significantly less injury, seven entries

showed intermediate injury, and four entries reflected the highest damage. A close association between earworm damage and *Fusarium* was observed in most environments. The materials with lowest *Fusarium* infection, such as Compuesto Blanco Caribe, Nicarillo, La Posta, and Ver. 181 × Ant. Gpo. 2, also registered the lowest earworm damage. In the group of varieties where the three types (normal, opaque, and modified) were observed, the same tendency was evident.

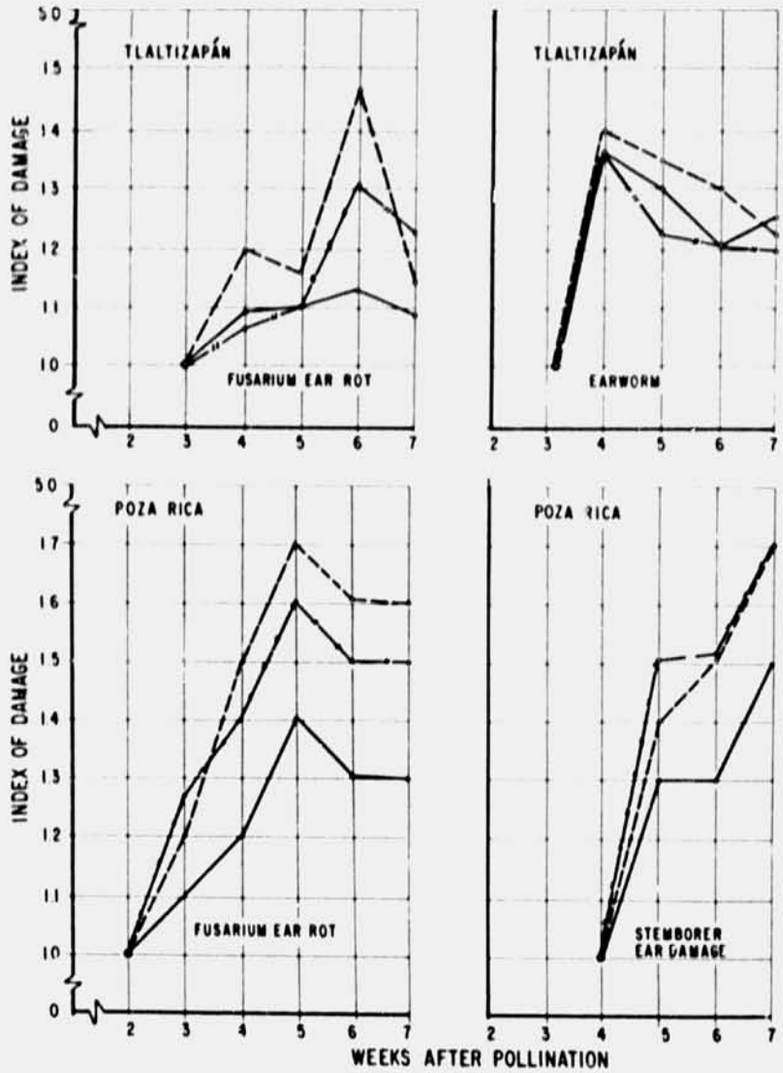

FIGURE 1 Average reaction of five maize populations and their opaque-2 and modified opaque versions under natural incidence of indicated agents (1972). See Figure 2 for key.

At Poza Rica, earworm ear injury (Table 4) was about 15% greater than at Tlaltizapán, and earworm damage (Table 5) was about 6% greater in the modified types than in the normal. The damage on the opaques was intermediate between the other two types (Figure 2). At the high-altitude environments of El Batán and Toluca there were no significant differences.

Levels of foliar damage by the budworm and by the stem borer were similar in normal, opaque, and modified types. Normal and opaque versions did not appear to differ with regard to infection by *Fusarium* stalk rot and stem borer damage on the stalks. The observed differences among maize materials could be associated with a known reaction to pests, rather than with the opaque-2 gene.

The possible association of endosperm character with the reaction to foliar- or stem-damaging agents has not been determined. Obviously, a

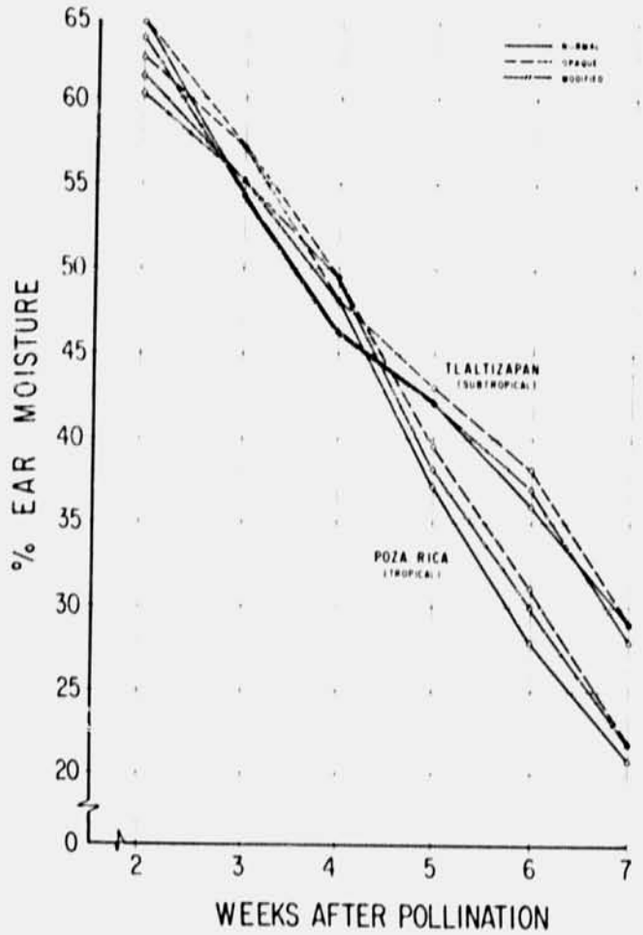

FIGURE 2

TABLE 4 Natural attack by the stem borer *Diatraea saccharalis* on (percentage damaged) ears from normal, opaque-2, and modified opaque-2 versions of several maize populations: Poza Rica (1972)

Maize populations	Endosperm versions		Maize population means
	Normal	Opaque	
Nicarillo	22	20	21.0
Población Cristalina	20	27	23.5
Antigua Gpo. 2	12	37	24.5
La Posta	20	32	26.0
PD(MS)6 X Granos Amarillos	27	32	29.5
Compuesto Grano Duro	23	39	31.0
Francés Largo	26	36	31.0
Compuesto Blanco Caribe	24	42	33.0
Ver. 181 X Ant. Gpo. 2	33	33	33.0
Puerto Rico Gpo. 1	29	40	34.5
Tuxpeño X Ant. Gpo. 2	33	38	35.5
Compuesto L(ME)C_2	27	44	35.5
Compuesto K	39	37	38.0
Tuxpeño X PD(MS)6	50	36	43.0
(Mix. 1 X Col. Gpo. 1) Eto	29	64	46.5
Endosperm version means	27.6	37.1	

Maize populations	Endosperm versions			Maize population means
	Normal	Opaque	Modified	
Ver. 181 X Ant. Gpo. 2	25	42	32	33.0
Compuesto Blanco Caribe	43	45	54	47.3
Compuesto K	33	51	61	48.3
Compuesto CIMMYT	44	47	55	48.6
Endosperm version means	36.2	46.2	50.5	

Note: Differences between means were established on the transformed values (arcsin $\sqrt{\text{percentage}}$) at the 5% level. Data presented are actual percentages. Means covered by the same line are not significantly different.

higher susceptibility of opaque-2 converted materials could be expected in the early generations if a nonadapted opaque-2 donor is used as the nonrecurrent parent.

Rusts and Blights

Rusts and blights are among the important foliar maize pathogens. Our findings did not reveal differences in infection by these pathogens among normal, modified, or opaque maize types in any of the environments sampled. Differences in susceptibility or resistance were observed among maize populations.

TABLE 5 Natural attack by the earworm *Heliothis zea* on normal, opaque-2, and modified opaque-2 versions of several maize populations: Tlaltizipán (1972)

Maize populations	Endosperm versions: Normal	Endosperm versions: Opaque	Endosperm version means
PD(MS)6 X Granos Amarillos	7	9	8.0
Nicarillo	11	8	9.5
Compuesto Blanco Caribe	8	13	10.5
Tuxpeño X PD(MS)6	10	11	10.5
Francés Largo	12	10	11.0
La Posta	11	12	11.5
Población Cristalina	14	10	12.0
Compuesto Grano Duro	12	14	13.0
(Mix. 1 X Col. Gpo. 1) Eto	12	14	13.0
Compuesto L(ME)C_2	17	9	13.0
Ver. 181 X Ant. Gpo. 2	8	19	13.5
Tuxpeño X Ant. Gpo. 2	18	13	15.5
Antigua Gpo. 2	13	20	16.5
Puerto Rico Gpo. 1	19	15	17.0
Compuesto K	15	23	19.0
Endosperm version means	12.4	13.3	

Maize populations	Endosperm versions: Normal	Endosperm versions: Opaque	Endosperm versions: Modified	Endosperm version means
Compuesto Blanco Caribe	15	12	13	13.3
Compuesto CIMMYT	14	12	18	14.6
Ver. 181 X Ant. Gpo. 2 X Ven.	8	19	18	15.0
Compuesto K	14	19	24	19.0
Endosperm version means	12.7	15.5	18.2	

Note: Differences between means were established on the transformed values (arcsin $\sqrt{\text{percentage}}$) at the 5% level. Data presented are actual percentages. Means covered by the same line are not significantly different.

Stored-Grain Fungi

The maize varieties with their normal, opaque-2, and modified opaque-2 versions used in the field work were also tested in the laboratory to measure their reaction to stored-grain fungi. These varieties were: Compuesto K, Ver. 181 × Ant. Gpo. 2, Compuesto CIMMYT, Yellow hard-Endosperm Composite, and Compuesto Blanco Caribe.

Moisture in the samples was uniformly raised to 16% by adding a predetermined amount of water containing spores of *Aspergillus* spp. and *Penicillium* spp. Then the samples were placed in closed environments where the relative humidity (RH) had been adjusted to 75 or 85% by means of

oversaturated NaCl or KCl solutions, respectively. All treatments were arranged in split plot designs and replicated three times. In the design, main treatments included the maize populations, and subtreatments included the two or three versions within each maize population.

Samples from all treatments were taken at 20-day intervals, and standard procedures were used to analyze for moisture, seed viability, and number of *Aspergillus* and *Penicillium* colonies.

Variations in seed moisture are shown in Table 6. No statistical analysis was made, since only one replicate was considered. However, Table 6 shows that at 75% RH (when all grain samples reached moisture-content stability) there was a slight tendency for the opaque and modified versions to maintain a moisture level slightly higher than the normal types. However, differences in moisture content were negligible among the different populations under study. Similar tendencies were observed at 85% RH with a rapid moisture increase in the initial stages of the experiment. Data show that all treatments were similar in tending to reach a certain moisture stability at uniform levels.

The effects of the two different moisture environments on seed viability of the normal, opaque, and modified versions of maize of the same populations were investigated. At 75% RH, no statistical differences in germination were found among the six samplings. At 85%, some differences were detected in both the initial and subsequent samplings. However, no consistent differences in germination could be detected among normal, opaque, and modified versions of the five maize populations under study (Table 7).

In another set of materials, all varieties reacted approximately the same throughout the experiment in terms of germination of normal and opaque maize versions. However, in the final stages it was evident that the normal and opaque versions of Población Cristalina, Tuxpeño × Ant. Gpo. 2, and La Posta showed the lowest germination values. In the last sampling of this experiment, the differences in germination between versions were consistently significant at the 5% level (Table 8).

TABLE 6 Variation in percentage of grain moisture of normal, opaque, and modified versions of five maize populations held at 75% and 85% relative humidities: El Batán (1972)[a]

	75% RH		85% RH	
	Sampling no.		Sampling no.	
Version	1	6	1	6
Normal	15.8	15.0	16.9	17.0
Opaque	16.6	14.9	17.5	17.2
Modified	16.4	15.3	17.5	17.3

[a] Values from one replication.

TABLE 7 Variation in seed viability of normal, opaque, and modified versions of five maize populations held at 75 and 85% relative humidities: El Batán (1972)[a]

Version	85% RH Sampling no. 1	85% RH Sampling no. 6
Normal	95	38
Opaque	91	45
Modified	93	40
LSD 5%	3	N.S.

[a] No differences between treatments at 75% R.H.

TABLE 8 Variation in seed viability in normal and opaque versions of ten maize populations held at 75 and 85% relative humidities: El Batán (1972)

Version	75% RH Sampling no. 1	75% RH Sampling no. 6	85% RH Sampling no. 1	85% RH Sampling no. 6
Normal	94	83	94	59
Opaque	93	75	92	45
LSD 5%	N.S.	4	N.S.	4

The increase of *Aspergillus* colonies (among normal, opaque, and modified varieties) was measured by culturing on a malt–salt–agar medium and recording the logarithms of the actual number of colonies. Table 9 shows that, in the earlier samplings at 75% RH, the opaque versions offered a significantly better substratum for *Aspergillus*, allowing more fungous growth than the normal or modified versions. However, in the last sampling, all versions from all five populations reacted similarly, indicating that the three versions in all populations became similarly susceptible after 6 months of unfavorable storage conditions.

Early samplings of these same treatments at 85% RH did not show clear-cut differences, and apparently they were all equally susceptible. However, in the sixth and final sampling, the normal versions had a significantly higher fungous development than did the opaque and modified versions.

Variation in the rate of development of *Aspergillus* in normal and opaque versions of the same populations is shown in Table 10. At 75% RH the opaque versions showed significantly less fungous growth during early samplings. Later on, no statistical differences were detected among versions. At 85% RH no differences could be detected between normal and opaque versions in all populations.

TABLE 9 Variation[a] of *Aspergillus* spp. colonies in normal, opaque, and modified versions of five maize populations held at 75 and 85% relative humidities: El Batán (1972)

	75% RH		85% RH	
	Sampling no.		Sampling no.	
Version	2	6	2	6
Normal	4.91	4.66	6.44	6.38
Opaque	5.27	4.56	6.45	6.12
Modified	4.97	4.76	6.29	6.16
LSD 5%	0.18	N.S.	N.S.	0.21

[a] Values expressed in logarithms of number of colonies.

TABLE 10 Variation[a] in the number of *Aspergillus* spp. colonies in normal and opaque versions of 10 maize populations held at 75 and 85% relative humidities: El Batán (1972)

	75% RH		85% RH	
	Sampling no.		Sampling no.	
Version	1	6	1	6
Normal	4.37	4.96	4.22	7.29
Opaque	4.22	5.05	4.22	7.27
LSD 5%	0.11	N.S.	N.S.	N.S.

[a] Values expressed in logarithms of number of colonies.

The rate of growth of *Penicillium* in normal, opaque, and modified versions was measured by the method described for *Aspergillus*. At 75% RH no statistical differences could be detected among normal, opaque, and modified versions throughout the experiments (Table 11).

At 85% RH statistical differences among normal, opaque, and modified versions were observed in the early samplings (second, third, and fourth); opaque versions consistently were most damaged. The fifth and sixth samplings showed no differences among versions, as was observed for *Aspergillus*.

In evaluations of *Penicillium* growth for only normal and opaque versions at 75 and 85% RH, no statistical differences were found between normal and opaque versions.

In summary, considering the average performance of the maize populations, at the 85% RH level all maize versions showed a gradual increase in moisture before reaching moisture stability. This gain was greatest in the modified versions, intermediate in the opaque types, and lowest in the normals.

TABLE 11 Variation[a] of *Penicillium* spp. colonies in normal, opaque, and modified versions of five maize populations held at 75 and 85% relative humidities: El Batán (1972)

	75% RH		85% RH	
	Sampling no.		Sampling no.	
Version	2	6	2	6
Normal	4.17	4.76	6.45	7.49
Opaque	4.19	4.66	6.60	7.52
Modified	4.22	4.61	6.30	7.56
LSD 5%	N.S.	N.S.	0.19	N.S.

[a] Values expressed in logarithms of number of colonies.

At the 75% RH level the modified types retained a slightly higher amount of moisture than either the normal or opaque versions.

On the average, the development of *Penicillium* was essentially the same in all three endosperm versions at both moisture levels.

The increase of *Aspergillus* was slightly higher on the opaque types at both moisture levels; normal and modified versions were alike in their reactions.

Only a slight decrease in germination took place in the three endosperm types at the 75% moisture level. At the 85% moisture level, the germination decreased about 45% in all three versions.

Relationships among moisture of the grain, development of the fungi, and reduction in germination were not clear when the data from each population were considered separately. However, the observations suggest that fungous development in some maize populations may be closely associated with moisture level, rather than with endosperm type.

Stored-Grain Insect Pests

There is considerable evidence indicating that the soft endosperm associated with the opaque-2 gene renders the kernels more vulnerable to stored-grain insect pests.

In the test reported in Table 12, the degree of vulnerability was estimated by recording the numbers of insects emerging from 100 kernel samples. Vulnerability to the weevil *Sitophilus zeamais* varied among types within varieties: Compuesto K was the least damaged population regardless of type; Ver. 181 × Ant. Gpo. 2 × Ven. registered the highest number of weevils and the heaviest damage.

The pattern of vulnerability to weevils seems to be clear. In every case, the normals were the least damaged. Compuesto K and Compuesto CIMMYT showed insect emergence twice as high on the modified versions as compared to the normal. There was practically no difference between normal

TABLE 12 Percentage of emergence of maize weevils and angumois grain moths from normal, opaque, and modified endosperm versions of four maize populations: El Batán (1972)

Maize population	Endosperm version			Maize population means
	Normal	Modified	Opaque	
	Weevil, *Sitophilus zeamais*			
Compuesto K	5	13	15	11.0
Yellow Hard Endosperm Composite	17	18	29	21.3
Compuesto CIMMYT	8	19	56	27.6
Ver. 181 X Ant. Gpo. 2 X Ven.	16	20	55	30.3
Endosperm version means	11.5	17.5	38.7	
	Moth, *Sitotroga cerealella*			
Compuesto K	65	72	74	70.3
Compuesto CIMMYT	70	66	79	71.7
Yellow Hard Endosperm Composite	83	60	74	72.4
Ver. 181 X Ant. Gpo. 2 X Ven.	69	87	93	83.0
Endosperm version means	71.7	71.2	80.0	

Note: Average of four replications.

and modified versions in Ver. 181 × Ant. Gpo. 2 and Yellow Hard Endosperm Composite. Weevil emergence was, on the average, three to four times higher in the opaque versions than in the normal. Differences in weevil damage and numbers between normal and modified types may not be significant.

With the exception of the Yellow Hard Endosperm, moth emergence *(Sitotroga cerealella)* was slightly higher on the opaque versions than in the modified or normal. On the average, there were no differences in moth emergence between the normal and modified types.

CONCLUSION

We are aware of the need to breed for resistance against *Fusarium* ear rot and earworms in opaque types that seem to be susceptible to these pests.

Vulnerability to some of the most important stored-grain insect pests may be reduced considerably as progress is made in reducing the soft endosperm portion of the opaque-2 types.

No association seems to be present between opaque-2 converted materials and their reactions to foliar and stem diseases or insect pests.

[*A discussion of this paper can be found on pp. 490–492 of* **Questions and Answers.**]

Part V

BREEDING FOR PROTEIN QUALITY IN MAIZE: CURRENT ISSUES AND PROBLEMS

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In considering the possibilities for utilizing high-quality protein maize, we can note that there are three different stages of investigation. We are now in the first of these stages, which may be characterized briefly as (1) utilizing the available material and technology to make high-quality-protein maize available as rapidly as possible, (2) considering all available means of increasing the lysine level (stated as a percentage of the dry weight) above that currently found in opaque-2 maize, and (3) investigating other means of increasing the content of limiting essential amino acids in plants.

This conference is concentrating, properly, on the first of these objectives in considering the steps that should be taken now to make high-quality protein maize a practical reality and to induce people to utilize such improved strains.

One has only to think back 6 years to the first conference on high-quality protein maize to realize how much has been accomplished by plant breeders and investigators in associated fields. Very importantly, several investigators are reporting that they have developed opaque-2 hybrids with yields within the range of the better nonopaque types. There also has been a sharper focus on the problems that may be encountered in producing opaque-2 maize. We now understand a great deal more about which difficulties are trivial. We also understand more about those which must be considered important, such as the apparent greater susceptibility to *Fusarium* and the grain weevil as reported by A. Ortega.

In surveying progress in the productivity of high-quality protein maize, I wish to remind you that the course of yield improvement in these years since the last conference has followed quite closely the predictions of D. E. Alexander. In 1966, he indicated his confidence that the difficulties would be overcome at a time when many plant breeders professed to believe that the difficulties were insurmountable.

Since most discussion at this meeting will be devoted to means for rapid utilization of high-quality protein maize as we now know it, I wish to discuss the other two stages of investigation: the possibility of raising the level of limiting essential amino acids within the major genotypes already identified, and the possibility of being able ultimately to increase the content of only a single amino acid. We want to raise lysine levels even further, since they are not yet completely adequate for weanling humans or nonruminant domestic animals.

Several strategies have been suggested. One is selection for higher protein content, and I do not feel that we have completely explored the possibilities of this avenue.

The second strategy is the possible utilization of the floury-2 mutation, which is of considerable interest for several reasons. First, lysine as a percentage of protein in floury-2 strains depends on the background in which the gene is placed. However, adequate lysine (stated as a percentage of protein) can be found in floury-2 strains. There is also a very significant increase in protein content in some backgrounds. We have seen protein contents above 16% in some floury-2 inbreds. Additionally, substituting the floury-2 allele for its normal counterpart always produces higher methionine levels. This is significant since methionine tends to be the limiting essential amino acid for growth in all diets based on a mixture of a cereal and a legume. It is apparent from these observations that floury-2 strains or hybrids might be bred which would have lysine contents (stated as a percentage of dry weight) higher than any of the opaque-2 hybrids that have been synthesized. Indeed, several years ago I succeeded in breeding some floury-2 hybrids having lysine contents (as a percentage of dry weight) that were 50% higher than the opaque-2 hybrids being tested.

E. T. Mertz tested these hybrids and found that these higher-protein floury-2 hybrids would not sustain a growth rate for weanling rats that was higher than that sustained by an opaque-2 hybrid with only 67% as much lysine and tryptophan. The reason(s) for the biological value of the floury-2 hybrids not equaling the value predicted from chemical analyses of the limiting essential amino acids is still a mystery. B. O. Eggum in Copenhagen has found that the floury-2 hybrid utilized in these feeding trials has equal availability of amino acids and equal metabolizable energy when compared with the opaque-2 hybrid. These observations now make it unclear whether using the floury-2 gene is the answer to raising the total lysine level in high-quality protein maize.

Still another approach toward improvement is the incorporation of genetic factors that change the proportion of tissues within the seed, where these tissues have quite different protein compositions. For example, in stocks that have seeds in which the embryo proportion can be increased through genetic factors, there will be an increase in the content of lysine and tryptophan. We also are exploring the genetic backgrounds that condition the development of alternate florets, providing greater surface area per unit weight and a greater amount of protein-rich aleurone tissue. A third possibility of changing the proportions of the various tissues may be found in the dominant factor conditioning multiple aleurone layers in maize, as reported several years ago by M. J. Wolf and M. S. Zuber. In our recent work we have found that this factor reinforced the effect of the opaque-2 gene, but not to the extent that we had hoped.

In the third stage of investigation into improving protein quality, we can assess the feasibility of selecting for an enhanced content of one amino acid alone. Let me amplify briefly on this particular point. When the level of a particular amino acid is altered by a major gene (opaque-2 or floury-2) or selection for polygenes with less pronounced effects, we may be selecting for a target amino acid, but the contents of several other acids may vary. Almost all the amino acids are protein-bound, and the amino acid sequence for a protein is specified by the structural gene for that particular protein. A mutational event may alter the amino acid at a particular position in a polypeptide chain, but this is not capable of having a significant effect on the overall amino acid composition of the strain.

We have known for several years that the net effect of the floury-2 or the opaque-2 genes is to depress the synthesis of the lysine- and tryptophan-poor zein fraction. As a secondary consequence of this repression of zein synthesis, there is a concomitant enhancement of other fractions that have greater quantities of lysine and tryptophan. At the same time, because of the change in proportion of several proteins, these mutants show an increase in the amoung to aspartate, arginine, and glycine that is present and a decrease in the amount of leucine, alanine, tyrosine, and phenylalanine. So, although our attention has been focused on lysine and tryptophan, the limiting essential amino acids, there has been a change in content of several other acids within these mutants.

The finding that the free amino acid pool in seeds is low indicates that the synthesis of these amino acids is regulated by the requirement for their participation in protein synthesis, and in the other reactions in which they may participate. The mechanisms that regulate the content of these amino acids are those of feedback inhibition and/or repression of enzyme synthesis. What would happen, however, if the regulatory mechanisms on the biosynthetic pathway leading to a particular amino acid were disrupted? Assume that this disruption worked so that synthesis of the amino acid would not shut off when a sufficient quantity was available for

incorporation into the proteins being synthesized. In effect, the plants would be overproducers of that particular amino acid. Such variants have been found in bacteria and recently have been observed in higher plants.

There is active interest in investigating the possibility that such mutations might have nutritionally significant consequences in higher plants. Brock, Friederich, and Langridge in Australia have initiated an investigation of the possibility of lysine overproduction in plants and have utilized a very interesting approach to the problem. Higher plants synthesize lysine by the same pathway as does the bacterium *Escherichia coli*. Therefore, using *E. coli* as a model system and employing a growth-inhibitory lysine analog as a selective device, they have asked how many mutations are needed to develop strains that are overproducers. Their tentative conclusion is that two mutations are needed to effect this sort of change. These are mutations in the genes coding for the aspartate kinase allozyme that is specific to lysine and the dihydrodipicolinic acid synthetase. Two mutational events are necessary because of the branched pathway leading to lysine synthesis. With this background garnered from studies of the *E. coli* system, Brock and his colleagues now will proceed to use the same selective sieve to investigate possible lysine overproducers in barley.

At the recent 12th International Symposium in Basic Biology in Cali, Colombia, Peter Carlson reported the isolation of three methionine overproducers in cell cultures of *Nicotiana tabacum*. These methionine overproducers also were selected by their resistance to a methionine analog. Carlson reports the reconstitution from these cells of plants that are viable and apparently healthy. This is a most important demonstration, since it has not been apparent that such disruptions in the biosynthetic mechanism of a plant would be viable, or, if viable, that they would be thrifty plants.

These are welcome efforts to utilize the most modern techniques to improve protein quality in plants, but must still be seen as future considerations. For the present, we have means for markedly improving maize protein quality. The immediate challenge is to make these improvements available to the people who can benefit from such changes.

USE OF GENETIC MODIFIERS TO OBTAIN NORMAL-TYPE KERNELS WITH THE OPAQUE-2 GENE

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Worldwide interest has been generated by the finding that normal maize endosperm protein quality can be upgraded genetically, using the opaque-2 gene to enhance the levels of two limiting essential amino acids, lysine and tryptophan. Although a very wide range of genetic materials has been converted to opaque-2 in recent years in different parts of the world, commercial production of opaque-2 maize has moved slowly because of specific problems associated with this type of maize. Two major problems hampering progress are (1) the reduced grain yield of converted materials and (2) nonacceptability of the floury-type endosperm of opaque-2 kernels.

It has been found that the problem of low grain yield of opaque-2 conversions can be dealt with speedily and effectively through breeding approaches, such as (1) extensive screening of better genetic materials in which opaque and their normal counterpart kernels weigh about the same, (2) practicing selection for better kernel test weight during backcrossing and segregating generations, and (3) selection for modifiers that affect kernel test weight (4) and phenotype of opaque-2 kernels (6). Using any of the approaches alone or in combination, it seems likely that opaque-2 varieties and hybrids can be developed that can compete economically with any commercial variety.

The second problem, the floury phenotype of opaque-2 kernels, varies in different parts of the world, depending upon the preference of the

people for a particular grain texture. In the Andean region, where farmers already grow varieties with a floury texture, opaque-2 conversions of these floury materials should not pose any acceptance problems. However, wherever farmers have a special preference for growing hard flint, yellow or white maize varieties, the opaque-2 kernel's dull, chalky, and lusterless appearance will probably be a major hurdle in gaining acceptance of this maize type. However, it is felt that this problem can be solved by capitalizing on modifier genes of opaque-2 locus, which change the floury phenotype of opaque-2 kernels to a more vitreous and translucent one.

The first report on modified phenotype of opaque-2 kernels was published by Paez et al. in 1969 (6). They reported that the lysine content of modified kernels did not differ from completely opaque-2 kernels. Also, the opaque and vitreous fractions of modified-phenotype opaque-2 kernels did not differ in lysine content. Similar modified phenotype has been reported by Pollacsek et al. (9), but they found about 20% superiority in the lysine of opaque-2 lines compared with vitreous normal counterparts. Modified phenotypes have also been reported by Vasal (10), by Poey and Villegas (7, 8), and by Annapurna and Reddy (2).

Maize breeders at Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) have observed modified-phenotype opaque-2 kernels since 1969 and are attempting to exploit this variability by investigating fundamental problems relating to kernel appearance from the acceptance point of view and gathering basic information for the breeding program. The following discussion provides brief mention of some of our observations over the past 2 years.

VARIATION FOR KERNEL PHENOTYPE IN OPAQUE-2 CONVERTED POPULATIONS

We have observed considerable variation in opaque-2 phenotype during conversion of normal maize varieties to opaque-2 varieties, and also during seed increases of opaque-2 materials, depending on the degree and distribution of the hard-endosperm fraction in the endosperm of opaque-2 kernels. Two broad classifications can be made within this variation of phenotypes, regular and irregular.

In the regular grouping, the hard fraction is located toward the top, or crown, of the kernel, with the opaque fraction located nearer the base. For the regular group we can make two additional classifications: (1) vitreous or translucent fractions, which are clear and distinct, and (2) those fractions which are dull and shabby. We have observed all grades of continuous variation in the regular group. Vitreousness ranged from a very small fraction on the top of the kernel to almost normal looking kernels having no opaque fraction identity at the base.

Within the irregular group, the vitreous fraction may be located irregularly in different parts of the kernel. This fraction may be described as (1) *scattered* when the vitreous starch granules are found as small specks throughout the floury endosperm, (2) *banded* when the vitreous fraction appears as a small central transverse band in the center while the top and basal fractions are completely opaque, (3) *bridge or saddle* when the vitreous fraction is limited to sides of the kernel, or (4) *vitreous base* when the vitreous fraction is located toward the base and the soft opaque fraction is located toward the top.

In general, the regular hard-endosperm pattern is more common, although irregular patterns do appear occasionally in some materials.

VARIATION IN FREQUENCY OF MODIFIED-PHENOTYPE OPAQUE-2 KERNELS IN DIFFERENT GENETIC BACKGROUNDS

We have observed that genetic materials differ considerably in their ability to show opaque-2 modifiers. Some materials produce modified kernels with a high frequency; others produce few or none. In CIMMYT's breeding program, some promising materials that merit special mention are PD(MS) 6, Tuxpeño-Ant. Gpo. 2, Ver. 181-Ant. Gpo. 2 × Ven. 1 opaco-2, Cuban materials, Composite K, CIMMYT opaque-2 composite, Thai opaque-2 composite, and Nicarillo.

This differential behavior in throwing varying frequencies of vitreous kernels suggests that, during evolution, these maize varieties may have accumulated varying numbers of modifying gene complexes that would be less desirable in a homozygous recessive population for opaque-2 gene (1).

RELATIONSHIP OF KERNEL VITREOUSNESS TO PROTEIN, TRYPTOPHAN, AND LYSINE CONTENT IN OPAQUE-2 CONVERTED MATERIALS

Opaque and modified-phenotype kernels from 20 half-sib segregating ears were analyzed to determine the relationship of kernel vitreousness with protein and tryptophan content. The results are presented in Table 1. Modified kernels generally had higher protein than opaque phenotype counterparts. The percentage of tryptophan in protein, however, was superior in opaques as compared to modified types, and the superiority ranged from 1.26 to 41.89%. However, in some families the decline was almost negligible, and these families should be of great interest to breeders.

The relationship of the percentage of kernel vitreousness with the percentage of protein and the percentage of tryptophan in protein was inves-

TABLE 1 Comparison of percentage of protein and tryptophan in protein of completely opaque and modified phenotype opaque-2 kernels selected from different half-sib families of tropical opaque-2 composite

Family number	% Protein in endosperm			% tryptophan in protein		
	Opaque	Modified	Difference %	Opaque	Modified	Difference %
Half sib-63	9.88	9.75	+1.33	0.89	0.86	3.49
67	9.13	8.94	+2.13	0.82	0.80	2.50
69	9.00	9.75	-8.33	0.84	0.66	27.27
79	9.19	10.63	-15.67	0.89	0.66	34.85
125	7.63	10.19	-33.55	1.05	0.74	41.89
144	7.00	7.56	-8.00	0.96	0.99	-3.12
147	8.25	9.00	-9.09	1.09	0.91	19.78
151	7.75	8.13	-4.90	0.90	0.87	3.45
159	6.44	7.25	-12.58	0.98	0.87	12.64
162	9.19	9.75	-6.09	0.97	0.82	18.29
164	8.88	9.38	-5.63	0.72	0.69	4.35
175	8.38	8.25	+1.58	0.80	0.79	1.26
196	8.50	9.25	-8.82	1.01	0.90	12.22
250	9.13	12.88	-41.07	0.72	0.53	35.84
278	8.50	8.31	+2.28	0.88	0.84	4.76
279	8.38	11.38	-35.80	0.93	0.73	27.40
292	11.25	11.50	-2.22	0.63	0.60	5.00
297	9.13	9.38	-2.74	0.78	0.70	11.43
118	10.38	11.13	-7.22	0.84	0.74	13.51
101	9.75	10.50	-7.69	1.07	0.78	37.18

tigated further, using five categories of kernels, ranging from category 1 (100% vitreous) to category 5 (0.0% vitreous). These categories were made from 10 different materials and then analyzed for protein and tryptophan content. The results are presented in Table 2. In some materials there was a consistent trend: as vitreousness increases, the percentage of protein increases correspondingly. The other materials failed to show any regular trend, but modified kernels (categories 1 to 4) consistently had higher percentages of protein than did category 5. The percentage of tryptophan in protein showed a consistent decline as vitreousness increased in Composite K. Other materials, however, failed to show any regular trend. The percentage of tryptophan in category 5, however, was superior to categories 1 to 4 in all materials.

These kinds of differences suggest that the vitreous sectors of categories 1 to 4 do not differ in the percentage of tryptophan in protein. Whatever differences occur within categories 1 to 4 can probably be attributed to the percentage of vitreousness in the kernel, rather than to differences between vitreous fractions among categories.

TABLE 2 Percentages of protein and tryptophan in protein of different categories of modified-phenotype opaque-2 kernels selected from different opaque-2 populations

No.	Material	% Protein in various kernel categories					% Tryptophan in protein in various kernel categories				
		1	2	3	4	5	1	2	3	4	5
1	Composite K	8.79	8.29	7.75	7.59	7.47	0.65	0.68	0.75	0.79	0.88
2	PD(MS)6-Gr. Amar.	11.07	10.50	10.25	10.44	9.94	0.87	0.79	0.85	0.85	0.93
3	Composite Blanco Caribe	10.29	10.25	9.58	9.25	8.88	0.70	0.84	0.81	0.78	0.80
4	Tuxpeno-Ant. Gpo. 2-#-#	9.99	9.84	9.65	9.08	.	0.79	0.79	0.82	0.85	–
5	CIMMYT Opaque-2 Composite	9.36	8.56	9.07	8.69	8.05	0.55	0.72	0.71	0.77	0.87
6	Thai Opaque-2 Composite	8.58	8.50	8.43	8.82	7.86	0.82	0.80	0.83	0.82	0.93
7	Ver. 181-Ant. Gpo 2 X Ven. 1 opaco-2	9.04	8.72	9.06	8.90	8.71	0.62	0.63	0.70	0.74	0.75
8	Yellow hard-endosperm Composite	7.82	7.16	7.36	7.88	7.33	0.92	1.00	0.94	0.91	0.98
9	Ant. Gpo. 2-1-3-2-#	8.98	8.80	9.24	9.07	.	0.76	0.87	0.88	0.84	–
10	PD(MS)6-Eto-Cuba 11J-Pob. Crist. 1(A)-2-1-#	9.08	9.34	8.87	9.80	8.53	0.73	0.79	0.88	0.86	0.83

Note: Category 1, more or less normal; 2, 75% translucent, 25% opaque; 3, 50% translucent, 50% opaque; 4, 25% translucent, 75% opaque; 5, 0% translucent, 100% opaque.

TABLE 3 Protein lysine and tryptophan content in whole endosperm and opaque-2 lines

No.	Line	% Protein			
		Whole endosperm	Fraction		Difference (%)
			Hard	Opaque	
1	PD(MS)6 Eto-Cuba-1 LJ-Pob. Crist. 1(A)-1-#-#	9.88	9.99	7.69	29.90
2	PD(MS)6 Eto-Cuba-1 LJ-Pob. Crist.-1-1-#-#	8.75	8.49	9.13	-7.54
3	Pob. Crist.-1-#-#-#	9.42	10.21	8.43	21.11
4	(Tropical opaque-2 Comp. 163 6 1 1) × PD(MS)6-#-#-#-#	10.92	11.57	11.25	2.84

PROTEIN AND AMINO ACID COMPOSITION OF HARD AND SOFT FRACTIONS OF MODIFIED-PHENOTYPE OPAQUE-2 ENDOSPERM

Hard and soft fractions of endosperm in four modified-phenotype opaque-2 lines were analyzed separately for protein and amino acid composition. The results are presented in Tables 3 and 4. Table 3 shows that the hard fractions of endosperm had higher protein than did the opaque fractions in three out of four lines, and that hard fractions ranged in superiority from 2.84 to 29.90%. The percentage of tryptophan in protein of the soft fractions was superior by percentages ranging from 12.98 to 56.52%. The percentage of lysine in protein of the opaque fractions was superior to that in hard fractions by percentages ranging from 22.48 to 38.27%.

A complete amino acid analysis of hard and soft fractions is presented in Table 4. Some of the amino acids, such as glutamic acid, alanine, leucine, and tyrosine, showed an increase in the hard fractions over the soft fractions.

Our data indicate that hard and soft fractions differed in the percentage of protein, and in some amino acids, within these four lines. However, an assumption may be made that more variation can be expected in heterozygous populations; thus, probabilities are greater for picking up families showing no differences in hard and soft fractions.

PERCENTAGE OF PROTEIN FRACTIONS IN NORMAL, OPAQUE, AND MODIFIED-PHENOTYPE OPAQUE-2 ENDOSPERM

Normal, opaque, and modified-phenotype samples in two populations were analyzed for the percentage of protein fractions in the endosperm. The results are shown in Table 5.

in hard and opaque fractions of the endosperm separately in four modified-phenotype

% Tryptophan in protein				% Lysine in protein			
Whole endosperm	Fraction		Difference (%)	Whole endosperm	Fraction		Difference (%)
	Opaque	Hard			Opaque	Hard	
0.70	0.83	0.63	31.75	2.67	2.88	2.22	29.73
0.87	0.87	0.77	12.98	2.81	3.30	2.60	26.92
0.73	0.72	0.55	30.91	2.73	3.36	2.43	38.27
0.76	0.85	0.63	34.92	2.87	3.65	2.98	22.48

The opaque and modified opaque samples had more acid-soluble and glutelin fractions than did the normal samples in the two populations. Zein fraction was less in the opaque and modified opaque samples than in the normal sample in both the populations. Comparison of the opaque and modified samples further indicates that for the modified types the acid-soluble and glutelin fractions decreased, while zein fraction increased, in both materials.

TABLE 4 Amino acid composition of endosperm protein in hard and opaque fractions separately in four modified-phenotype opaque-2 lines

Amino acids in protein	(1) Fraction		(2) Fraction		(3) Fraction		(4) Fraction	
	Opaque	Hard	Opaque	Hard	Opaque	Hard	Opaque	Hard
Lysine	2.88	2.22	3.30	2.60	3.36	2.43	3.65	2.98
Histidine	3.06	3.75	3.41	3.78	3.38	3.52	3.70	3.61
Arginine	3.94	3.81	4.46	4.12	4.09	3.95	5.15	4.24
Aspartic acid	6.26	5.75	7.36	7.95	7.49	7.98	7.58	7.57
Threonine	3.26	3.67	3.45	3.54	3.42	3.61	4.01	3.68
Serine	3.83	4.52	4.04	4.29	3.84	4.32	4.58	4.39
Glutamic acid	16.09	20.59	17.25	21.68	16.70	25.24	17.55	20.62
Proline	9.29	10.61	8.97	10.39	9.05	10.98	9.79	10.37
Glysine	3.77	3.72	3.92	3.99	3.81	3.70	4.62	3.90
Alanine	5.20	6.40	5.54	6.74	5.20	7.14	5.88	6.42
Cisteine	1.38	0.77		0.48	0.24	1.42	1.11	0.84
Valine	3.43	4.30	5.02	5.14	4.82	4.86	9.40	4.71
Methionine	1.56	1.26	1.19	1.18	1.51	1.32	1.57	1.30
Isoleucine	2.00	2.82	3.30	3.47	3.02	3.43	3.06	3.16
Leucine	8.00	10.98	9.17	10.95	8.28	12.32	9.62	11.23
Tyrosine	2.60	2.77	1.91	3.05	2.24	3.41	2.85	2.59
Phenylalanine	3.25	3.94	3.78	4.12	3.45	4.32	3.99	4.12
Tryptophan	0.70	0.63	0.82	0.93	0.85	0.65	0.84	0.72

Note: Column (1) PD(MS)6-Eto-Cuba-Pob. Crist. 1(A)-1-#-#; (2) PD(MS)6-Eto-Cuba 11J-Pob. Crist.-1-1-#-#; (3) Pob. Crist.-1-#-#-#; (4) (Tropical opaque-2 composite 163-6-1-1) X PD(MS)6-Gr. Amar. #-#-#-#.

TABLE 5 Percentage of protein fractions in normal, opaque, and modified-phenotype opaque-2 samples of two populations

Population	Type of sample	% Protein fractions in endosperm					
		Acid soluble		Zein		Glutelins	
		Actual	% of normal	Actual	% of normal	Actual	% of normal
1. Ver. 181-Ant. Gpo. 2 X Ven. 1	Normal	27.0	100.0	42.3	100.0	19.8	100.0
	Opaque	39.7	147.0	24.2	57.2	31.3	158.1
	Modified	35.0	129.6	26.3	62.2	29.3	148.0
2. White composite	Normal	32.5	100.0	45.2	100.0	19.0	100.0
	Opaque	35.0	107.7	25.4	56.2	31.4	165.3
	Modified	33.5	103.1	26.7	59.1	28.5	150.0

Five categories of kernels, ranging from completely opaque to completely vitreous, also were analyzed in two populations for the percentage of protein fractions in the endosperm. The results are shown in Table 6.

Acid-soluble fractions in the first population showed a gradual decrease as vitreousness increased (except kernels with 25% vitreousness in Ver. 181 – Ant. Gpo. 2 × Ven. 1 opaco-2, which had about the same acid-soluble fraction). In the white hard-endosperm composite population, the categories had similar acid-soluble fractions.

Zein fractions did not show any regular trend, although all the vitreous categories had higher zein fractions than did the opaque phenotype in the white hard-endosperm composite.

Kernels with a varying percentage of kernel vitreousness were lower in the glutelin fraction than opaques, but these kernels showed no regular trend with increased vitreousness in the white hard-endosperm composite. In Ver. 181 – Ant. Gpo. 2 × Ven. 1 opaco-2 population, only the 100% vitreous class was low when compared to 0.0% vitreous kernels, whereas all other vitreous classes were higher in glutelins.

RELATIONSHIP BETWEEN KERNEL VITREOUSNESS AND TEST WEIGHT IN MODIFIED-PHENOTYPE KERNELS

The 100-grain test weight of opaque and modified-phenotype opaque-2 kernels with 50 to 75% vitreous fraction was obtained in several materials. The results are shown in Table 7. In general, there was an increase in the test weight of modified kernels over the opaque phenotype, but the percentage of increase varied considerably from one material to another, ranging from 0.73 to 10.58%. The data suggest that selection for vitreousness may help overcome the yield barrier in some genetic materials, but not in others. Also, it appears that some modifiers may influence only phenotype, while others may affect phenotype as well as kernel density. There also is a possibility that there may be a separate group of modifiers affecting only kernel density, as has been suggested by Bauman (3).

RELATIONSHIP BETWEEN KERNEL VITREOUSNESS AND GERM SIZE

Table 8 shows the results of incorporating the opaque-2 gene in normal maize materials, an increased percentage of contribution of germ to the total kernel protein, with this increase varying tremendously among materials. The increase in the percentage of contribution of germ to the total kernel protein can be attributed to the increased germ size.

The percentages of contribution of germ to the whole-grain protein in opaque and modified-phenotype opaque-2 kernels are shown in Table 9.

TABLE 6 Percentage of protein fractions in opaque and modified phenotype opaque-2 kernels with varying percentage of vitreous endosperm

Population	% Vitreous fraction in kernel	% Protein fractions in endosperm					
		Acid soluble		Zein		Glutelin	
		Actual	% of normal	Actual	% of normal	Actual	% of normal
1. Ver. 181-Ant. Gpo. 2 X Ven. 1	0.0	39.7	100.0	24.2	100.0	31.3	100.0
	25.0	39.8	100.2	22.6	93.4	34.0	108.6
	50.0	37.5	94.4	23.6	97.5	33.8	108.0
	75.0	36.8	92.7	25.7	106.2	33.3	106.4
	100.0	36.0	90.7	27.0	111.5	30.0	95.8
2. White composite	0.0	35.0	100.0	25.4	100.0	31.4	100.0
	25.0	36.0	102.8	27.0	106.3	30.0	95.5
	50.0	34.2	97.7	30.3	119.3	30.8	98.1
	75.0	35.2	100.6	26.5	104.3	30.0	95.5
	100.0	34.6	98.8	27.9	109.8	29.3	93.3

TABLE 7 One-hundred-grain test weight of opaque and modified phenotype opaque-2 kernels selected from different opaque-2 converted materials

No.	Material	Family or ear	100-grain test weight (g) Modified	Opaque	% Increase
1	PD(MS)6-Eto-Cuba 11J-Pob. Crist.	3	23.10	22.73	1.62
2	PD(MS)6-Eto-Cuba 11J-Pob. Crist.	4	30.30	30.00	1.00
3	164-3 Cat. 1(ii)-2-1	2	29.00	27.83	4.20
4	La Posta-6-1	1	20.00	18.92	5.70
5	Flint Comp. Amarillo-6	1	28.11	25.42	10.58
6	Nicarillo-#-#	1	28.70	26.49	8.34
7	Composite K	514	25.71	25.45	1.02
8	Composite K	515	28.46	27.08	5.09
9	Composite K	517	23.04	22.07	4.39
10	Composite K	518	27.30	26.59	2.67
11	CIMMYT Opaque-2 Composite	–	28.89	28.68	0.73

TABLE 8 Contribution of germ to the total kernel protein in different maize varieties and composites

No.	Material	% Contribution of germ to total kernel protein: Opaque	Normal	Difference
1	Vijay	19.8	10.4	9.4
2	Composite A_2	25.6	10.5	15.1
3	J_1	27.7	10.9	16.8
4	Usatigua	20.5	10.6	9.9
5	Cupurico X Flint Compuesto	27.1	8.2	18.9
6	Centralmex	22.5	7.2	15.3
7	Compuesto Blanco Caribe	24.5	10.6	13.9
8	UPCA–Variety 2	29.8	9.3	20.5
9	B_{16} (yellow)	27.9	10.9	17.0
10	Compuesto grano duro	35.3	20.2	15.1
11	Mix. 1–Col. Gpo. 1 X Eto	20.4	11.5	8.9
12	Granada Gpo. 2	34.3	9.5	24.8
13	Cuba 11J	24.7	15.2	9.5
14	Ver. Gpo. 41	16.0	11.5	4.5
15	Nicarillo	29.0	23.6	5.4
16	Iowatigua	21.5	18.2	3.3
17	Tuxpeño F.F.	12.8	9.7	3.1
18	Tuxpeño Cr. 1	13.3	10.6	2.5
19	Amagaceño	11.1	7.4	3.7
20	Composite AC	9.8	10.4	-0.6

The percentage of contribution of germ to the total kernel protein showed no regularity. The data may suggest that germ size also may be influenced by a modifying gene complex.

The preceding data allow these conclusions:

1. Kernel vitreousness in floury opaque-2 endosperm is influenced by genetic modifiers.

2. Modified kernels with varying degrees of vitreousness in the endosperm generally show an increase in the percentage of protein, with a slight decline in the percentages of tryptophan and lysine in protein.

3. The percentage of tryptophan in protein in vitreous fraction seems to be independent of the degree of vitreousness, and those differences occurring between kernels with varying fractions of hard endosperm may be due to differences in the ratio of hard and soft endosperm fraction.

4. Hard fractions of modified-phenotype opaque-2 endosperm differ from soft fractions in having more protein and less tryptophan and lysine. However, variation does occur, suggesting that still greater variation may be found between these fractions.

5. Decreases in lysine and tryptophan found in modified types as compared to opaque types seem to be due to increased zein fractions. Amino acids, such as glutamic acid, leucine, alanine, and tyrosine, showed an increase also. These findings seem to indicate that the mode of action of modifiers is opposite to that of the opaque-2 gene, at least in materials where lysine and tryptophan tend to decrease.

6. Increased vitreousness due to modifiers shows no regular association with increased test weight of the kernels.

7. Germ size seems to be influenced by modifying gene action.

TABLE 9 Effect of selection for hard endosperm on the contribution of germ to the whole-kernel protein in opaque-2 converted materials

No.	Material	Ear or family no.	% Contribution of germ to whole-grain protein		
			Opaque	Modified	Difference
1	Composite K	118	22.01	17.92	+4.09
2	Composite K	4	20.55	14.41	+6.14
3	Ver. 181–Ant. Gpo. 2 X Ven. 1 opaco-2	8	33.37	26.13	+7.24
4	PD(MS)6-Gr. Amar.-2-1	1	22.00	14.67	+7.33
5	Thai Opaque-2 Composite-1	1	17.56	13.38	+4.18
6	Nicarillo	Self 1	24.75	18.01	+6.74
7	Flint Compuesto Amarillo	1	28.08	24.38	+3.70
8	Eto Blanco	1	24.17	18.37	+5.80
9	Composite K	1	13.59	15.07	-1.48
10	Composite K	101	28.21	32.10	-3.89
11	Ver. 181-Ant. Gpo. 2 X Ven. 1 opaco-2	3	11.87	18.73	-6.86
12	Nicarillo	1	25.99	33.55	-7.56
13	Antigua Gpo. 2	2	24.31	25.78	-1.47
14	Ver. 181-Ant. Gpo. 2 X Ven. 1 opaco-2	4	15.54	17.85	-2.31
15	Thai Opaque-2 Composite	4	17.07	18.03	-0.96
16	Thai Opaque-2 Composite	2	16.41	17.31	-0.90

INHERITANCE OF OPAQUE-2 MODIFIERS AFFECTING KERNEL VITREOUSNESS

The variation observed for kernel vitreousness follows a continuous pattern, suggesting that the mode of inheritance of these genetic modifiers is complex and under polygenic control. An inheritance pattern of kernel vitreousness controlled by a modifying gene complex was developed by choosing eight parents and crossing these among themselves to produce all possible 28 F_1 crosses. The mean kernel vitreousness ratings of parents and the 28 F_1 crosses are presented in a diallel table (Table 10). Kernel vitreousness in modified-phenotype opaque-2 kernels was rated on a scale of 1 to 5 (1 indicates more or less normal; 5 indicates completely opaque).

As suggested by Hayman (4, 5), various statistics were calculated and used to draw a $V_r - W_r$ graph and to estimate different components of genetic variance.

The $V_r - W_r$ graph is shown in Figure 1. The regression coefficient deviated significantly from zero, but not from unity. The observed regression line cut the W_r axis above the point of origin, indicating partial dominance in the expression of kernel vitreousness. The order of array points along the observed regression line indicated the relative proportion of dominant and recessive alleles. The points with lower values of V_r and W_r and close to the point of origin will have a maximum number of dominant alleles. The points toward the far end of the regression line will have a maximum number of recessive alleles. Parents P_3 and P_8 seem to have a maximum number of dominant genes; P_1 and P_6 have a maximum number of recessive genes.

The estimates of different components of genetic variance are shown in Table 11. The additive variance seems to be more important than dominance variance (H_1 and H_2). The F value (an indicator of relative frequency of dominants versus recessive alleles in the parents) was negative, indicating

TABLE 10 Parental, F_1 and array means for kernel vitreousness of opaque-2 endosperm in eight lines

Parents	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	Array mean
P_1	4.32	4.78	3.76	4.48	3.78	4.22	3.74	4.05	4.14
P_2		4.50	4.52	4.44	4.04	3.92	4.02	4.12	4.29
P_3			3.81	3.86	3.85	3.55	3.98	3.95	3.91
P_4				3.89	3.99	3.81	3.44	3.94	3.98
P_5					3.32	3.20	3.26	4.00	3.68
P_6						4.02	3.12	3.82	3.71
P_7							3.23	3.72	3.56
P_8								3.61	3.90

more recessive alleles than dominant alleles. The mean degree of dominance was 0.9135, calculated by the formula $(H_1/D)^{1/2}$, again showing partial dominance. The ratio of $\frac{1}{4}(H_2/H_1)$ estimates the average frequency of negative versus positive alleles in the parents. If the distribution is equal, the ratio should be 0.25; if unequal, it will be smaller. The calculated value was 0.1555; thus, it was below 0.25, suggesting that the parents probably did not have equal distribution of positive and negative alleles.

MATERNAL INFLUENCE ON THE EXPRESSION OF OPAQUE-2 MODIFIERS

Reciprocal crosses were made between opaque and modified-phenotype opaque-2 materials. The ears with different phenotypes were counted, and their percentage of frequency is presented in Table 12. A greater frequency of ears with a completely opaque phenotype or segregating type occurred when P_1 or P_6 (opaque phenotype) was used as the female parent. In reciprocal crosses, however, when modified parents were used as female parents, ears with modified and segregating type were counted more frequently. The differences between reciprocal crosses suggest maternal influence on the expression of kernel vitreousness. This could be explained by the double contribution from the maternal side in the

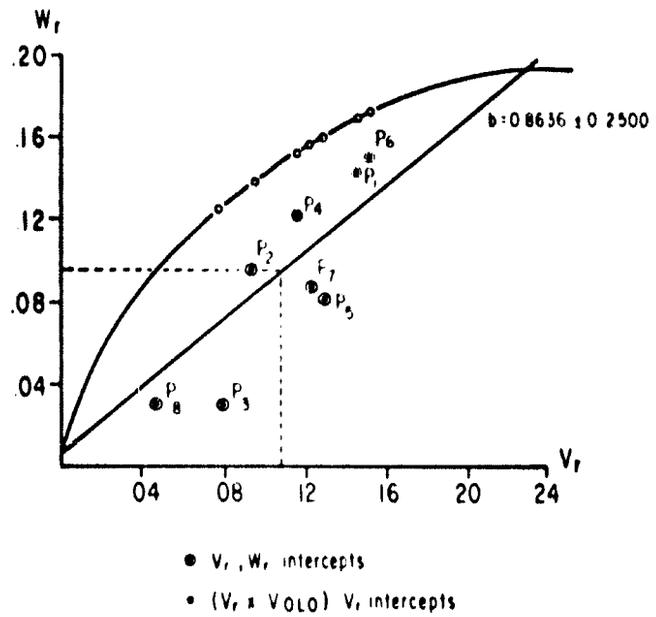

FIGURE 1 V_r – W_r graphical analysis (kernel vitreousness in modified — phenotype opaque-2 endosperm).

TABLE 11 Estimates of components of genetic variance

D	=	0.1379
F	=	-0.0715
H_1	=	0.1151
H_2	=	0.0716
$\sqrt{H_1/D}$	=	0.9135
$H_2/4H_1$	=	0.1555

TABLE 12 Frequency of ears with different phenotypes in reciprocal crosses between opaque and modified-phenotype opaque-2 materials

		% Frequency of ears with different phenotypes		
No.	Material	Opaque	Segregating	Modified
1	$P_1 \times P_3$	56	40	1
	$P_3 \times P_1$	–	70	30
2	$P_1 \times P_5$	94.4	5.6	
	$P_5 \times P_1$	–	63.2	36.8
3	$P_1 \times P_4$	8.3	58.3	33.3
	$P_4 \times P_1$	–	11.8	88.2
4	$P_1 \times P_5$	9.1	63.6	27.3
	$P_5 \times P_1$	–	21.4	78.6
5	$P_6 \times P_3$	75.0	16.7	8.3
	$P_3 \times P_6$	–	75.0	25.0
6	$P_6 \times P_5$	69.4	30.6	
	$P_5 \times P_6$	38.5	46.2	15.4
7	$P_6 \times P_4$	42.1	57.89	
	$P_4 \times P_6$	10.0	55.0	35.0
8	$P_6 \times P_5$	78.6	21.4	
	$P_5 \times P_6$	9.1	63.6	27.3

Note: P_1, Ver. 181-Ant. Gpo. 2 X Ven. 1 opaco-2 (opaque phenotype); P_3, Ver. 181-Ant. Gpo. 2 X Ven. 1 opaco-2 (modified phenotype); P_5, Thai Opaque-2 (modified phenotype); P_4, Composite K (modified phenotype); P_5, PD(MS)6 Gr. Amar.-6-# (modified phenotype); P_6, Thai Opaque-2 (opaque phenotype).

makeup of the triploid endosperm compared with a single contribution from the paternal side.

POSSIBLE APPROACHES TO DEVELOP HARD-ENDOSPERM OPAQUE-2 MATERIALS

The dull, chalk-like phenotype of opaque-2 kernels can be improved substantially by capitalizing on genetic modifiers that change the soft, floury texture of opaque-2 kernels to a vitreous one. The kernels improve in phenotypic appearance considerably and look more like normal kernels. However, the problem is complicated because all vitreous kernels do not maintain protein quality equivalent to that of the opaque phenotype. This complication requires a backup chemical laboratory to help the

breeder in the screening of modified-phenotype opaque-2 families, or lines that have acceptable protein, lysine, and tryptophan content.

Also, since modifying genes affecting kernel vitreousness show partial dominance, it seems likely that breeding schemes that can exploit additive genetic variance, such as full-sib, half-sib, and S_1, would be effective in accumulating frequency of favorable alleles in the population.

Three different general approaches have been used at CIMMYT to develop hard-endosperm opaque-2 materials, including the following:

1. *Reconstitution of vitreous lines into populations.* Hard-endosperm lines selected from a wide array of genetic materials, and shown to be superior in protein content and quality, can be combined to develop hard-endosperm opaque-2 composite. Using this approach, we have developed two populations: White hard-endosperm opaque-2 composite, and Yellow hard-endosperm opaque-2 composite. The recombined population thereafter can be handled through any one of the population improvement schemes, coupled with laboratory analysis.

2. *Intrapopulation selection for hard endosperm in opaque-2 converted populations.* This approach can be used effectively in opaque-2 converted populations that have a fairly high frequency of modified-phenotype opaque-2 kernels. As a first step, ears can be selected that are modified, or at least segregating for modified kernels. Modified kernels can then be selected from each ear separately and planted on an ear-to-row basis. Also, a sample of 10 kernels can be sent for laboratory analysis. If laboratory analyses are available before pollination, those families can be discarded which are not in the acceptable range of protein and tryptophan values. Plant-to-plant pollinations may be restricted between selected families to develop full-sibs. At harvest, only good-looking modified ears should be selected. Kernels from each selected ear again are sorted on an illuminated glass screen, evaluated in a chemical laboratory, and planted in a field on an ear-to-row basis for making a new set of full-sibs. We have used this approach with Composite K, CIMMYT Opaque-2 Composite, and Ver. 181 – Ant. Gpo. 2 × Ven. 1 opaco-2.

3. *Crosses of hard endosperm opaque-2 materials with normal populations.* This is a simple backcross approach. Hard-endosperm segregates are selected from each segregating generation, evaluated for protein quality, and used for making crosses to normal in each backcross. We crossed about 15 normal populations to different sources of modifiers, and in F_2 we have been able to recover modified kernels from each cross. This approach should work very well.

We have accumulated a fairly high frequency of modified kernels in all populations handled by the first two approaches, but there is still too much variation in protein and tryptophan content. Scatter diagrams (Figures 2, 3, and 4) show this variation for percentage of protein and percentage of tryptophan in protein from three populations: White hard-

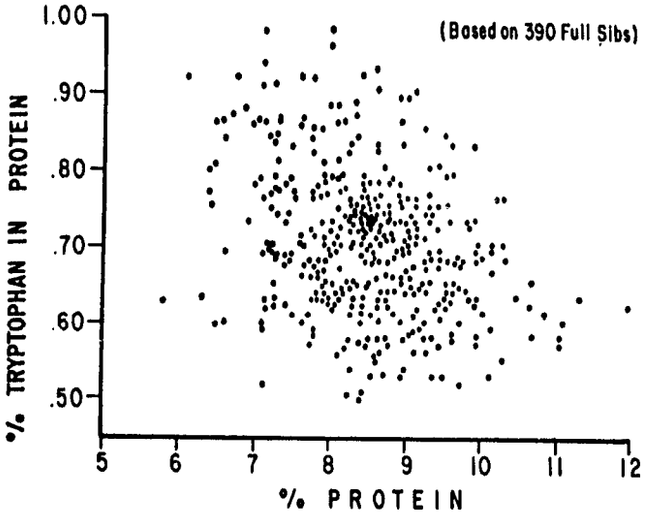

FIGURE 2 Scatter diagram of percentages of protein and tryptophan in protein in hard-endosperm opaque-2 populations: Ver. 181–Ant. Gpo. 2 × Ven. 1 opaco-2.

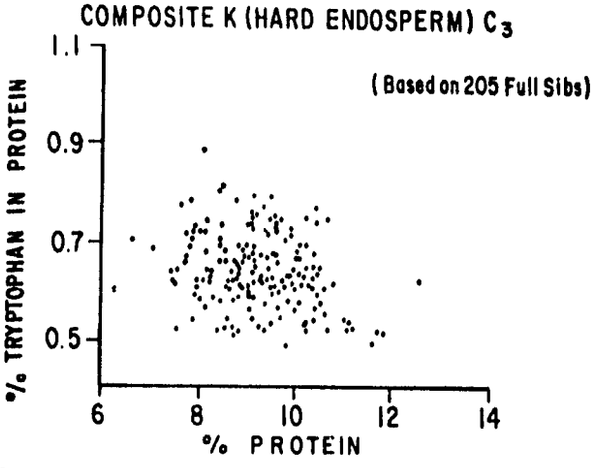

FIGURE 3 Scatter diagram of percentages of protein and tryptophan in protein in modified-phenotype opaque-2 population: Composite K (hard endosperm) (C_3).

endosperm composite, Composite K, and Ver. 181–Ant. Gpo. 2 × Ven. 1 opaco-2. The correlation coefficient *(r)* in all three populations was negative, but was significant in Composite K and Ver. 181–Ant. Gpo. 2 × Ven. 1 opaco-2. Table 13 shows the range and mean values for protein and tryptophan and the percentage of frequency of acceptable families

TABLE 13 Ranges in protein and tryptophan values and correlation coefficient (r) in some of the hard-endosperm opaque-2 populations undergoing improvement

No.	Population	Cycle of selection	No. of full-sib families	Range in protein values	Mean protein (%)	Range in tryptophan in protein (%)	Mean tryptophan in protein (%)	Frequency of acceptable families (%)	Correlation coefficient (r)
1.	Ver. 181-Ant. Gpo. 2 X Ven. 1 opaco-2	3	390	5.12-11.88	8.48	0.51-0.96	0.70	58.97	-0.4675[a]
2.	Composite K	3	205	6.56-12.50	9.19	0.51-0.80	0.65	55.12	-0.1460[a]
3.	White hard-endosperm (opaque-2) composite	1	153	6.31-10.63	8.76	0.51-1.17	0.75	77.77	-0.1150

[a] Significant at 0.05 probability.

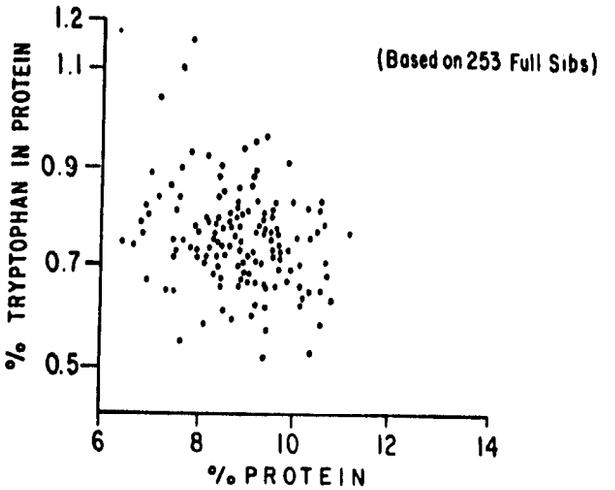

FIGURE 4 Scatter diagram of percentages of protein and tryptophan in protein in modified-phenotype opaque-2 population: white hard-endosperm opaque-2 composite (C_1).

TABLE 14 Protein lysine and tryptophan in whole grain of opaques and modified opaques in two populations

Sample No.	Material	Phenotype	% Protein	% Tryptophan in protein	% Lysine in protein
1	Composite K C_3 (hard endosperm)	Opaque	9.63	1.05	4.51
		Modified	10.38	1.02	4.27
2	Ver. 181-Ant. gpo. 2 X Venezuela 1 opaco-2 (hard endosperm)	Opaque	9.88	0.97	4.71
		Modified	9.69	0.97	4.72

(protein, 7.50% and above; tryptophan, 0.65% and above) from the last cycle of selection in three populations. Through further selection, it seems possible to build the frequency of favorable modifiers still higher and to increase the frequency of families with acceptable protein and tryptophan values.

Table 14 shows the analyses of bulk samples from the last cycle of selection of two populations for protein, tryptophan, and lysine. Modified versions were fairly similar to opaque counterparts in the percentages of protein, tryptophan, and lysine.

We have concluded that through selection for opaque-2 modifiers breeders should be able to change the dull phenotype of opaques to more normal looking ones without sacrificing protein quantity and quality

[*A discussion of this paper can be found on pp. 492–494 of* **Questions and Answers.**]

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GERM AND ENDOSPERM VARIABILITY, MINERAL ELEMENTS, OIL CONTENT, AND MODIFIER GENES IN OPAQUE-2 MAIZE

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Since Mertz et al. (7) discovered that the opaque-2 gene improves protein quality in maize, there has been only limited progress toward utilization of maize types with improved protein quality. The primary reason for this is lower yield; but other factors involved are soft endosperm not acceptable for some human food uses, greater vulnerability to ear rots and grain insects, and harvest and storage problems. Initial approaches involved continuous backcrossing procedures to rapidly develop hybrids or varieties with the opaque-2 gene.

Breeders soon found that there are definite genotype interactions with opaque-2 maize, and these interactions have received limited use in producing opaque-2 hybrids or varieties that are reasonably acceptable for some areas. However, there is no question that further improvements can, and should, be made in the acceptability and protein quality of opaque-2 varieties.

This paper summarizes some studies of the opaque-2 gene interactions and effects in relation to yield, maturity, cob weight, chemical characteristics, and modifier genes. The reader is urged to exercise the usual caution in extending or generalizing this information to other populations or germ plasm.

GRAIN DRY-MATTER ACCUMULATION OVER TIME, COB WEIGHT, AND MATURITY

Makonnen (6) studied an opaque-2 diallel involving six inbred parents and the normal counterpart diallel in a replicated split-plot experiment. Kernel dry-matter accumulation curves were determined based on harvests made at 7-day intervals, starting 28 days after pollination and continuing through 63 days. Analysis of variance revealed significant differences between genotypes (opaque-2 versus normal maize), among hybrids, and among harvest dates for kernel weight. All interactions were significant for kernel yields, indicating the importance of genetic background.

The opaque-2 gene performed differently in the various hybrids over harvest dates. For B14 × B37 (Figure 1), the opaque-2 hybrid had a kernel yield quite similar to the normal counterpart at the first and second harvest dates (28 and 35 days postpollination, respectively). In contrast, with A239 × A545 (Figure 2) a marked difference was found between kernel yields of opaque-2 and normal hybrids at the two early harvest dates.

In general, the reduced accumulation of kernel dry matter in opaque-2

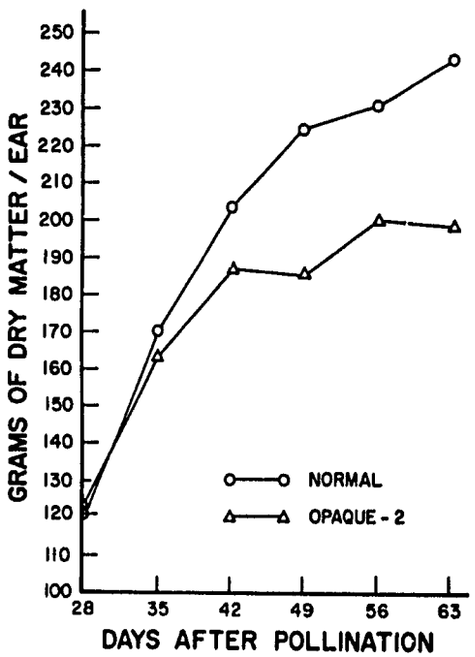

FIGURE 1 Comparison of kernel weight of opaque-2 and normal versions of B14 × B37.

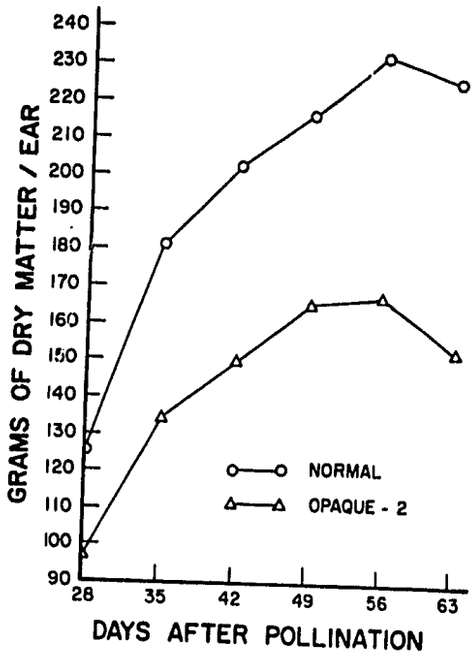

FIGURE 2 Comparison of kernel weight of opaque-2 and normal versions of A239 × A545.

hybrids was accompanied by retention of more moisture. At physiological maturity the opaque-2 hybrids averaged 3.5% higher moisture content than did the normal. The black layer (indicator of physiological maturity) formed at about the same time in the opaque-2 and normal hybrids, despite the earlier nearly complete cessation of dry-matter accumulation in the opaque-2 hybrids.

The opaque-2 gene had a pronounced and consistent effect on cob weight, with normal hybrids having about 10% greater cob weight. If this effect is generally true for various germ plasms, it would produce a slight bias against opaque-2 varieties if yield is based on ear weight.

INTERRELATIONSHIPS AMONG PROTEIN, LYSINE, OIL, AND CERTAIN MINERAL ELEMENTS

Goodsell (4) has reported that opaque-2 kernels contain more potassium than normal. Therefore, evaluations were made of the relationships of protein and lysine to potassium and other mineral elements. Concentrations of lysine, protein, oil, potassium (K), phosphorus (P), magnesium (Mg), iron (Fe), and zinc (Zn) were determined for normal and opaque-2 kernels from (1) 60 segregating ears from a heterozygous population, and (2) 62 ears from a homozygous opaque-2 population (1).

Correlations between the percentage of protein and the mineral elements were all positive and significant for both kernel types in the heterozygous population (Table 1). In the homozygous opaque-2 population, protein concentration was positive and significantly correlated with P, Mg, and Zn.

Lysine concentration was significantly correlated with P and Zn in the homozygous population, and with P, Mg, Fe, and Zn in the opaque-2 kernels from the heterozygous population (Table 2). The low or nonsignificant correlations between K and protein and lysine were disappointing. Oil concentration was significantly correlated with protein percentage ($r = +0.25$) and lysine ($r = +0.39$) only for the homozygous population.

The coefficients of determination for second-order multiple regression equations (Table 3) indicate that protein and the combination protein and oil, as expected, account for a fairly high amount of the variation in lysine, particularly in the homozygous population. The individual correlations and the combinations of P, K, Mg, Fe, and Zn as related to the

TABLE 1 Correlation between protein concentration and concentration of mineral elements

	Homozygous population		Heterozygous population
	Kernel type		
Mineral element	Opaque	Normal	Opaque
K	0.17	0.26*	0.32**
P	0.65**	0.59**	0.45**
Mg	0.42**	0.53**	0.53**
Fe	0.18	0.29*	0.37**
Zn	0.61**	0.36**	0.48**

* and ** denote significance at the 5% and 1% levels, respectively.

TABLE 2 Correlation between lysine concentration and concentration of mineral elements

	Homozygous population		Heterozygous population
	Kernel type		
Mineral element	Opaque	Normal	Opaque
K	0.01	0.33**	0.20
P	0.43**	0.35**	0.25*
Mg	0.24	0.23	0.36**
Fe	0.19	0.43**	0.30*
Zn	0.49**	0.25*	0.33**

* and ** denote significance at the 5% and 1% levels, respectively.

TABLE 3 Coefficients of determination for second-order multiple regression equations (percentage of lysine is a dependent variable)

Independent variable(s)	Homozygous population		Heterozygous population
	Kernel type		
	Opaque	Normal	Opaque
Protein	0.68	0.25	0.51
Protein and oil	0.75	0.29	0.52
P, K, Mg, Fe, Zn	0.52	0.53	0.38

variation in lysine certainly warrant further investigation of these mineral elements.

VARIATION AND CORRELATION INVOLVING PROTEIN AND LYSINE IN GERM AND ENDOSPERM

Thirty kernel samples from 44 ears (random) from opaque-2 Syn HA were analyzed for oil content. The kernels then were dissected into germ and endosperm portions, and protein, lysine, and oil content were determined. Surprisingly, large variations were observed for all characteristics (Table 4). Both environmental and genetic variation are involved. Should some attention be given to variation in the germ portion for maximum nutritional improvement? Interestingly, the quantities and variations of lysine in the endosperm and germ are nearly equal.

Some of the more pertinent correlations among these germ and endosperm characteristics (Table 5) showed high and significant values. The percentage of oil in the kernel was positively correlated with the percentage of protein in the kernel and endosperm and with the percentage of

TABLE 4 Variation in germ and endosperm of 44 opaque-2 maize ears

Variable	Mean	Range
Germ		
Percent	16.5	13.0 - 21.9
Oil, %	32.4	25.1 - 42.5
Protein, %	26.7	22.9 - 31.2
Lysine, % of protein	5.8	5.0 - 6.9
Lysine, %	1.5	1.2 - 1.9
Lysine, mg in 30 germs	19.4	10.2 - 28.4
Endosperm		
Protein, %	10.3	6.4 - 14.1
Protein, mg/30 endo.	657	386 -1003
Lysine, % of protein	3.34	2.7 - 5.0
Lysine, %	0.34	0.25- 0.42
Lysine, mg/30 endo.	21.2	13.0 - 29.9

TABLE 5 Correlations for several characters measured on 44 opaque-2 maize ears

Characters	Correlation coefficient
Oil (% of kernel) and protein (% of kernel)	+0.48**
Oil (% of kernel) and protein (% of endo.)	+0.42**
Oil (% of kernel) and lysine (% of kernel)	+0.41**
Oil (% of kernel) and lysine (% of endo. prot.)	-0.33*
Oil (% of germ) and protein (% of germ)	-0.43**
Protein (% of kernel) and lysine (% of kernel)	+0.70**
Protein (% of kernel) and lysine (% of ker. prot.)	-0.52**
Protein (% of kernel) and protein (% of endo.)	+0.97**
Lysine (% of kernel) and lysine (% of endo.)	+0.76**

* and ** denote significance at the 5% and 1% levels, respectively.

lysine in the kernel, but was related negatively to lysine as a percentage of endosperm protein. The percentage of oil in the germ was negatively correlated with the percentage of protein in the germ. The high correlation (r = +0.97) between the percentage of protein in the kernel versus the percentage in the endosperm indicates that analyses based on endosperm would be quite accurate.

RELATIONSHIP OF OPAQUE-2 WITH GERM AND OIL CONTENT

Opaque-2 maize generally has a higher oil percentage than normal maize, as shown by Lambert et al. (5) and our tests at Purdue University. This is primarily because the germ constitutes a greater proportion of the kernel, owing to reduced endosperm weight. The report of Sreeramulu et al. (10) and data in Table 6 indicate that germ weight may actually be greater; this may be a compensatory effect because of reduced endosperm weight. However, the percentage of oil in the germ may be less in opaque-2 maize (Table 6), which would tend to negate the effect that increased germ weight might have on the percentage of oil in the kernel.

Data in Table 5, from the report of Dudley et al. (3), and other data at Purdue University indicate a significant positive correlation between the percentages of oil and protein. Since the germ contains a high percentage of good-quality protein, and the oil percentage is heritable and easily

TABLE 6 Characteristics of opaque and normal kernels from nine segregating ears

	Opaque	Normal	Percent of normal
Germ as % of kernel	16.25	13.15	123.6
Percent oil in germ	31.43	35.70	88.0
Germ weight	0.82	0.73	112.3
Kernel weight	24.7	27.4	90.2

measured, the effect of oil selection on protein level and quality was investigated in an opaque-2 synthetic.

Ruschel (9) selected for increased percentage of oil in opaque-2 Syn HA using half- and full-sib (two cycles each) and S_1 progeny selection (one cycle); two subselection procedures were applied in the second cycle of the half-sib method. When the five selected populations as a group were compared with the base population, the increase in the percentage of oil (0.52%) was significant, with a significantly correlated increase in the percentage of protein of 0.46%. Selection did not affect yield or grams of lysine per 100 g of protein. These findings are regarded as preliminary, and further selection is underway. It may be feasible to develop high-oil opaque-2 varieties, particularly for specialty uses.

VITREOUS (MODIFIED) OPAQUE-2 TYPES

Paez et al. (8) described vitreous opaque-2 types and indicated the possibility of using these types as an approach for correcting the yield disadvantage due to reduced endosperm weight. Bauman and Aycock (2) briefly discussed this approach, and other breeders, including Vasal and co-workers at CIMMYT and workers in our project, have used this approach to develop opaque-2 varieties that are more acceptable in terms of yield, grain quality, and human food uses.

Some general observations concerning the vitreous types are (1) the degree of vitreousness is influenced by environment (for example, populations are more modified in the winter nursery); (2) populations may differ in frequency of vitreous types; (3) generally more than one gene is involved; and (4) vitreous types tend to have poorer protein quality, and selection pressure must be applied.

Urbano Ribeiral, a Purdue graduate student, degermed kernels of six families and then separated the endosperm into vitreous and opaque portions. Analyses (Table 7) reveal a distinct difference between the portions,

TABLE 7 Comparison of protein and lysine values from opaque and vitreous portions of modified kernels from opaque-2 families

	Protein percent			Lysine g/100 g protein		
	Opaque portion	Vitreous portion	Whole endosperm	Opaque portion	Vitreous portion	Whole portion
1	9.2	11.5	10.5	4.82	2.55	3.03
2	10.2	13.3	12.7	3.28	2.08	2.36
3	8.8	12.2	10.9	4.56	2.34	2.80
4	8.4	12.1	11.2	4.73	2.44	2.83
5	8.0	12.9	12.0	5.14	2.33	2.75
6	7.6	12.1	10.0	4.40	2.30	2.62
Mean	8.7	12.4	11.2	4.49	2.34	2.73

with the vitreous being higher in percentage of protein and lower in lysine when compared to the opaque portion. Admittedly, this is a small sample and is in contrast to the data of Paez et al. (8), but the findings suggest the need for caution in adequately sampling the entire endosperm for analysis.

Ribeiral also studied a diallel among vitreous opaque inbreds. In a split-plot design, the two treatments involved sib pollination and outcrossing to normal (+/+) pollen. This permits yield comparisons of the vitreous F_1's with their potential yield as "normals" (assuming $+/o_2/o_2$ is comparable to +/+/+). These vitreous inbred parents have been intercrossed and will be available as a germ-plasm modifier source population after intercrossing in our winter nursery. This material would be of interest primarily to breeding programs in the temperate zones. Above-average rainfall delayed harvest in the 1972 tests involving vitreous types, so only limited or preliminary data are available at this time.

Dejene Makonnen, another Purdue graduate student, determined kernel dry-matter accumulation curves over the maturation period for two vitreous single crosses and a high-yielding nonvitreous opaque hybrid

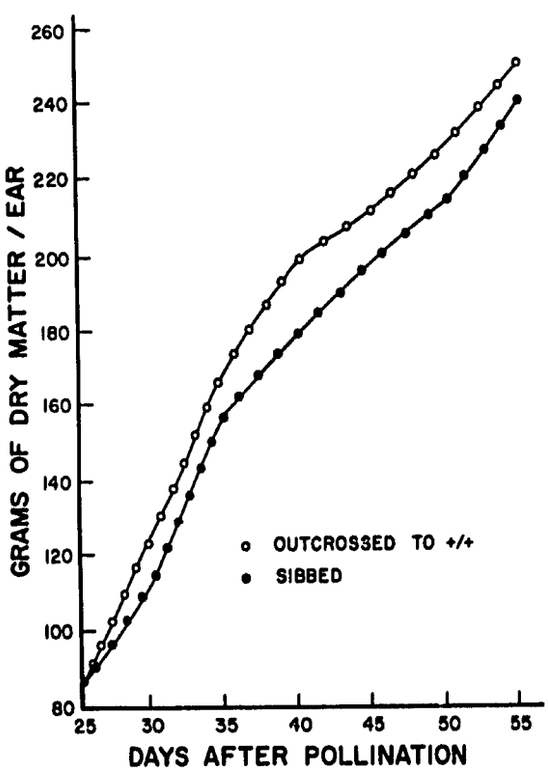

FIGURE 3 Kernel yields of sibbed and outcrossed versions of vitreous experimental 21 during maturation.

(Figures 3 to 6). These hybrids were sibbed and outcrossed to normal (+/+) pollen to provide an estimate of their yield potential in relation to "normal." The two vitreous hybrids 21 and 25 were quite similar to their "normal" counterparts at all harvest dates (Figures 3 and 4). The vitreous opaque approach appears quite promising with these two hybrids.

The grain yield of the high-yielding nonvitreous hybrid 16 was 96% of its "normal" counterpart (Figure 5). More typical grain-yield accumulation curves are shown for opaque and normal versions of W64A × B37 (Figure 6). Unfortunately, in this experiment some of the hybrids may not have attained physiological maturity at 55 days after silking because of below-average temperatures.

In recent tests, experimental and commercial nonvitreous opaque-2 hybrids produced yields quite competitive with the better normal commercial hybrids. These hybrids were relatively free of ear rots, even during the excessive rainfall of the 1972 fall season.

One might question the necessity for developing vitreous opaque-2 types for the more temperate areas. Nonvitreous opaque-2 types also would be more desirable if the reported advantage of opaque-2 maize for cattle feeding is related to greater digestibility because of the softer endosperm.

The development of vitreous opaque-2 hybrids is not a simple breeding procedure. Initially at least, the vitreous modifier genes must be intro-

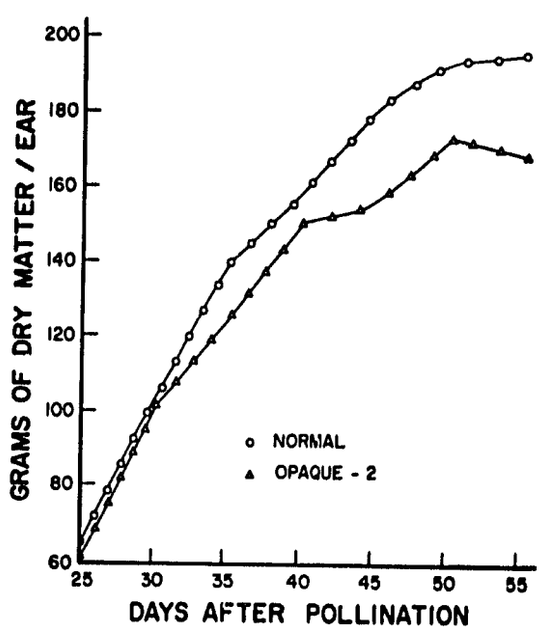

FIGURE 4 Kernel yields of sibbed and outcrossed versions of vitreous experimental 25 during maturation.

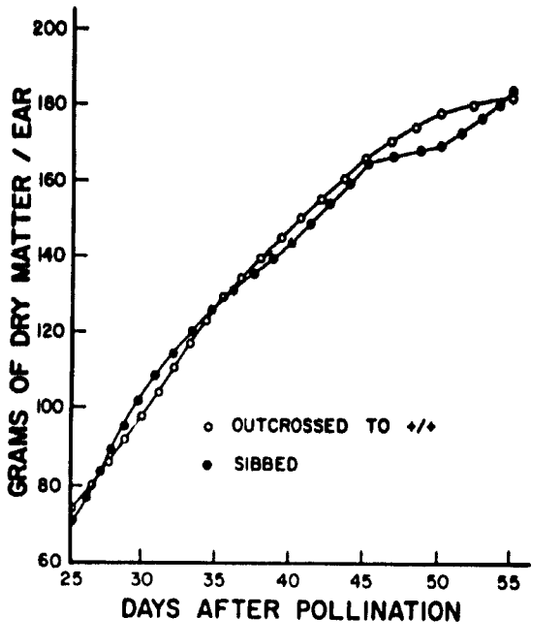

FIGURE 5 Kernel yields of sibbed and outcrossed versions of opaque experimental 16 during maturation.

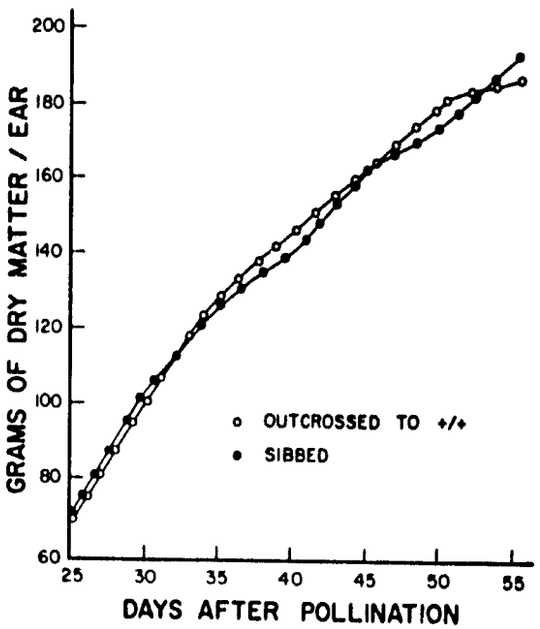

FIGURE 6 Kernel yields of normal and opaque versions of W64A x B37 during maturation.

duced into elite inbreds by a limited number of backcrosses with alternate generations of selfing to identify vitreous types in the backcross progenies, and by applying selection for protein quality. Development of vitreous opaque-2 populations or varieties for direct use by the farmer should be a relatively easier task with less time required.

Based on research information and progress to date, we are confident that acceptable opaque-2 hybrids and varieties can be developed for any maize-growing area.

This article is Journal Paper No. 5041, Purdue University Agricultural Experiment Station. The work was supported by the U.S. Agency for International Development under Contract AID/Csd-2809, titled "Inheritance and Improvement of Protein Quality and Content in Maize."

[*A discussion of this paper can be found on p. 494 of* **Questions and Answers.**]

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GENETICS OF ENDOSPERM MUTANTS IN MAIZE AS RELATED TO PROTEIN QUALITY AND QUANTITY

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Many mutants in maize (*Zea mays* L.) produce striking differences in textural properties, form, and amount of endosperm. Several mutants influence gelatinization temperatures, birefringent end point, starch granule digestibility, and carbohydrate storage products (such as amylose, amylopectin, water-soluble polysaccharides, and sugars).

The effects of opaque-2 (o_2) and floury-2 (fl_2) and the interaction of these two mutants in double-mutant combination have been known for several years (10, 12, 13). Nelson (12) reported the high lysine content of the double-mutant fl_2o_2. Surprisingly, the phenotype of Nelson's double-mutant was not floury; it was nearly indistinguishable from normal. Although variation in flintiness exists in fl_2o_2 endosperms, it has been observed that most are soft floury types in dent maize backgrounds.

Since unexpected interactions occur quite often between mutant genes affecting endosperm characteristics, genetic materials have been developed

to rigorously explore the o_2 gene interactions with several other endosperm mutant genes in double combinations. In cooperation with the Department of Biochemistry, our objective is to find genotypes that may have potential for genetic improvement or alteration in (1) amino acid profile, (2) total energy content as carbohydrates, protein, and oil, (3) digestibility of the grain, and (4) perhaps other nutrients to improve the food value of maize. There is potential for obtaining maize types having desirable starch characteristics and adequate amounts of nutritionally balanced protein for humans and animals.

Several mutants are known to affect starch synthesis in the endosperm of maize. The effects of gene substitutions on carbohydrate storage products have been reported for waxy (wx) (15), sugary-1 (su_1) (16), shrunken-2 (sh_2) (7), brittle-1 (bt_1) and brittle-2 (bt_2) (3), and dull (du) (2). All these mutants, with the exception of wx, result in the synthesis of less starch. In some mutants, such as sh_2 and bt_2, only a small proportion of the starch is synthesized. The effects of mutants and double-mutant combinations on polysaccharide synthesis have been summarized (4). None of these include opaque-2.

The wx, sugary-2 (su_2), du, and amylose-extender (ae) mutants also result in changes in the relative proportions of amylose to amylopectin. The starch of the wx endosperm is entirely amylopectin (15). The starches synthesized in du, su_2, and ae endosperms all have higher percentages of amylose than normal, with ae containing about 60% amylose (5).

It has been suggested that the digestibility of commercial strains of maize may be improved by using specific gene mutations like wx and su_2 (14). These genes may be important in improving the nutritional value of maize for humans and animals.

EFFECT OF ENDOSPERM MUTANTS AND DOUBLE-MUTANT COMBINATIONS WITH OPAQUE-2

Discussion here is limited to portions of our data concerning selected endosperm mutants and their double-mutant combinations with opaque-2 in near-isogenic subline comparisons (1, 11) and in hybrids. Initial phases of the work involved backcrossing the mutants to selected inbred backgrounds and recovering near-isogenic subline materials within an inbred.

Single Endosperm Mutants and Double-Mutant Combinations with Opaque-2 in an Inbred Background

Data are shown for the isogenic comparisons among several mutant recoveries (o_2, fl_2, su_2, wx, ae, du, bt_1, bt_2, su_1, sh_2, fl_1, and h) in inbred Oh43 after six backcrosses, and their double-mutant combinations with opaque-2 and the normal counterpart.

Tables 1 and 2 show the lysine and tryptophan concentration in the single endosperm mutants and double-mutant combinations. Compared to their normal counterparts, all the mutants show increases in the concentration of lysine and tryptophan. The ae and du mutants show increases in lysine and tryptophan equal to those of fl_2, while su_2 shows less. All mutants showed some decreases in leucine. Cystine was increased in ae, and methionine was increased in wx and du, although not to the level found in fl_2.

The endosperm proteins of bt_2, bt_1, su_1, and sh_2 have notable increases in the concentrations of lysine and tryptophan (Table 2). In addition, each shows a decrease in the concentration of leucine. Brittle-2 and su_1 have methionine levels approaching the concentration found in fl_2. Compared to their normal isogenic counterpart, many of these mutants may be classified as maize mutants with high lysine content (1, 11). For example, bt_2 is as effective in altering the amino acid pattern of endosperm protein as o_2.

Each endosperm mutant increased lysine and tryptophan concentrations in the endosperm proteins when the gene was combined with o_2 (Tables 3 and 4). Table 3 shows that the double-mutant combinations su_2 o_2, wx o_2, ae o_2, and du o_2 cause notable increases in concentrations of lysine and tryp-

TABLE 1 Amino acid composition of defatted endosperms in near-isogenic Oh43 sublines

	Amino acid concentration (g/100 g protein) in						
Amino acid	+	o_2	fl_2	su_2	wx	ae	du
Lysine	1.6	3.5	2.3	1.9	1.7	2.3	2.3
Tryptophan	0.3	0.8	0.5	0.5	0.4	0.5	0.6
Protein (%)	11.6	10.1	11.5	11.5	10.0	11.7	11.0

TABLE 2 Amino acid composition of defatted endosperms in near-isogenic Oh43 sublines

	Amino acid concentration (g/100 g protein) in					
Amino acid	+	o_2	bt_2	bt_1	su_1	sh_2
Lysine	1.6	3.5	3.3	2.6	2.3	2.7
Tryptophan	0.3	0.8	0.7	0.5	0.3	0.7
Protein (%)	11.6	10.1	13.4	12.3	12.4	20.5

TABLE 3 Amino acid composition of defatted endosperms of double-mutant combinations in Oh43

	Amino acid concentration (g/100 g protein) in						
Amino acid	+	o_2	fl_2 o_2	su_2 o_2	wx o_2	ae o_2	du o_2
Lysine	1.6	3.5	2.8	4.0	3.7	3.9	3.7
Tryptophan	0.3	0.8	0.8	1.0	0.8	0.9	1.0
Protein (%)	11.6	10.1	11.3	10.7	9.6	10.9	9.7

TABLE 4 Amino acid composition of defatted endosperms of double-mutant combinations in Oh43

Amino acid	Amino acid concentration (g/100 g protein) in					
	+	o_2	bt_2 o_2	bt_1 o_2	su_1 o_2	sh_2 o_2
Lysine	1.6	3.5	5.3	4.8	3.9	4.2
Tryptophan	0.3	0.8	1.3	1.4	0.8	1.2
Protein (%)	11.6	10.1	14.9	10.3	13.3	18.7

tophan to levels above that in o_2. Leucine decreases to 12.4, 11.3, 12.0, and 9.5%, respectively, as compared to 16.4% in the normal counterpart. The fl_1 o_2 and h o_2 combinations (not shown) also indicate some increased differences in amino acid content. In contrast to these mutant combinations, no enhanced effect is observed when fl_2 is combined with o_2.

When bt_2, bt_1, su_1, and sh_2 are combined with o_2, each such combination substantially increases lysine and tryptophan concentrations (Table 4). Leucine decreases considerably in these double-mutant combinations from between 9.9% in su_1 o_2 to 7.9% in sh_2 o_2. Tables 5 and 6 show the zein concentration (fraction II) (6) and the zein-to-glutelin-3 (fraction V) ratio for each of the single endosperm mutants and their double-mutant combinations. With the introduction of any one of the mutant genes, the zein content drops and the major glutelin fraction (glutelin-3, fraction V) increases. Zein synthesis is further repressed when the endosperm mutants are combined with o_2 in the double mutants (Table 6). This synergistic effect is most pronounced in the mutants bt_2 o_2, bt_1 o_2, and sh_2 o_2 that characteristically synthesize small amounts of starch when compared to normal. For example, zein content drops to 3.2% in bt_2 o_2 and the major glutelin fraction increases to 52.8%. P. S. Misra's report discusses the pro-

TABLE 5 Zein concentration and zein to glutelin ratio of defatted endosperms in Oh43

Genotype	Zein (fraction II)[1]	Ratio of zein to glutelin (fraction V)
+	60.8[2]	4.28
o_2	29.2	0.92
fl_2	50.7	2.23
su_2	48.7	2.75
wx	51.5	3.43
ae	38.0	1.74
du	42.7	2.14
bt_2	28.9	0.93
bt_1	37.1	1.31
su_1	29.2	1.19
sh_2	32.8	1.25

[1] Fractionation sequence D:Landry and Moureaux (6).
[2] Percentage of total soluble nitrogen, data corrected to 100% recovery.

TABLE 6 Zein concentration and zein to glutelin ratio of defatted endosperms of double-mutant combinations in Oh43

Genotype	Zein (fraction II)[1]	Ratio of zein to glutelin (fraction V)
+	60.8[2]	4.28
o_2	29.2	0.92
fl_2 o_2	28.0	1.05
su_2 o_2	18.5	0.52
wx o_2	23.7	0.70
ae o_2	20.1	0.56
du o_2	14.6	0.36
bt_2 o_2	3.2	0.06
bt_1 o_2	2.9	0.05
su_1 o_2	3.2	0.07
sh_2 o_2	1.3	0.03

[1] Fractionation sequence D:Landry and Moureaux (6).
[2] Percentage of total soluble nitrogen; data corrected to 100% recovery.

tein distributions and characterization of the fractions extracted from each of these samples.

The germ proteins and lysine concentrations did not differ greatly in these mutants and the double-mutant combinations. The germ protein contents of o_2 and fl_2 (28%) were the highest compared to normal, with 25% protein. Lysine concentrations ranged from 5.4 to 6.2% of total protein.

Within the single-mutant lines and the o_2 double combinations, significant differences were found among genotypes for the per kernel mineral element content of phosphorus, potassium, magnesium, copper, and iron. An interesting observation was that su_2 and its double combination su_2 o_2 were consistently high in most of the mineral elements. It was also noted that su_2 had a greater kernel density in the Oh43 background and was superior to the normal. Sugary-2 also interacted with o_2 to improve the kernel density of the o_2 in the double combination.

L. Bartolome of our laboratory evaluated the relative *in vitro* digestibility characteristics of these endosperm mutants and their double-mutant combinations with o_2 in the inbred Oh43. Her data show that su_2, su_2 o_2, and wx o_2 were consistently superior in producing the highest digestion rates in both uncooked raw-starch and whole-kernel sample preparations. Opaque-2 was superior to the normal counterpart and tended to improve the digestibility rates of the double-mutant samples when compared to the single nonopaque mutant counterpart.

Single Endosperm Mutants and Double-Mutant Combinations with Opaque-2 in Hybrid Backgrounds

Some data are available on a few selected mutants and their double-mutant combinations with o_2 from preliminary experiments in hybrid back-

grounds. A study being conducted by G. A. Tosello has four nearly isogenic subline sets (inbreds Oh43, W64A, B37, and C103), each with the single mutants o_2, fl_2, su_2, wx, ae, du, and fl_1 and their double combinations with opaque-2. These sets were crossed in a diallel series and together with their normal counterparts were grown in 1971 in a split-plot randomized complete block design. The data reflect the mature grain characteristics of six hybrids of the su_2, wx, ae, du, fl_2, and o_2 sets and their double combinations with o_2. The du and fl_1 and their double combinations with o_2 were evaluated in three hybrids.

Figure 1 shows the mean percentage of protein in the endosperm fraction of the single-mutant lines and double-mutant combinations with o_2 of all hybrids tested. The percentage of protein in the o_2 single-mutant hybrid and all the o_2 double-mutant combination hybrids was significantly below the mean of the comparable single nonopaque hybrids, thus showing the effect of the o_2 gene. However, none of the o_2 double combinations in the hybrids was significantly lower in the percentage of endosperm protein than the o_2 hybrids themselves. The fl_2 hybrids were superior in concentration of endosperm protein on a percentage basis, as has been found in other research. Considered on the basis of quantity of protein per endosperm, the single-mutant wx and ae hybrids (32.2 and 31.4 mg of protein/endosperm, respectively) did not differ from their normal counterpart (32.6 mg of protein/endosperm). The su_2 (29.6 mg of protein/endosperm) and fl_2 (28.2

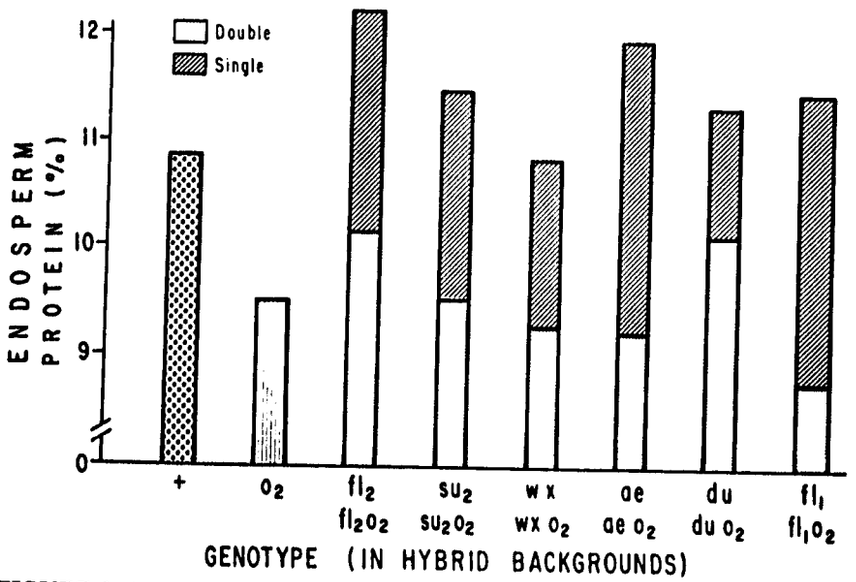

FIGURE 1 Average percentage of protein for several single endosperm mutants and their double-mutant combinations with opaque-2 over hybrids (Indiana, 1971).

mg of protein/endosperm) single-mutant hybrids were lower in protein quantity, but not substantially so. Opaque-2 and most of the double-mutant combination hybrids showed decreased protein quantity in the endosperm fraction.

Figure 2 shows mean lysine as a percentage of protein values for all hybrids. The single endosperm mutant and double-mutant combination hybrids showed mean significant increases in lysine concentration when compared with their normal counterparts. Next to the o_2 hybrids, fl_2 showed the most pronounced increase (53%) over normal hybrids, followed by su_2 with a 24% increase. Note the enhanced effect on lysine concentration of combining the endosperm mutants su_2, wx, ae, and du with o_2 in the double-mutant combination hybrids, whereas in the fl_2 o_2 or fl_1 o_2 hybrids, no enhancement of lysine as a percentage of protein occurred. Compared on a lysine quantity per endosperm basis (milligrams of lysine per endosperm), the su_2 o_2 and ae o_2 hybrid yields were significantly greater ($P < 0.01$) than that of o_2 hybrids, with each of the former producing a 7% increase in lysine yield. The fl_2 and wx o_2 hybrids were 3% greater in lysine yield than the o_2 hybrids, but not significantly greater.

Compared with the mean of the normal hybrids (Figure 3), the means of the single- and double-mutant combination hybrids showed decreased 100-kernel weight. Waxy and fl_1 were reduced the least, followed by su_2 and ae. The 100-kernel weight means of all double-mutant combination hybrids

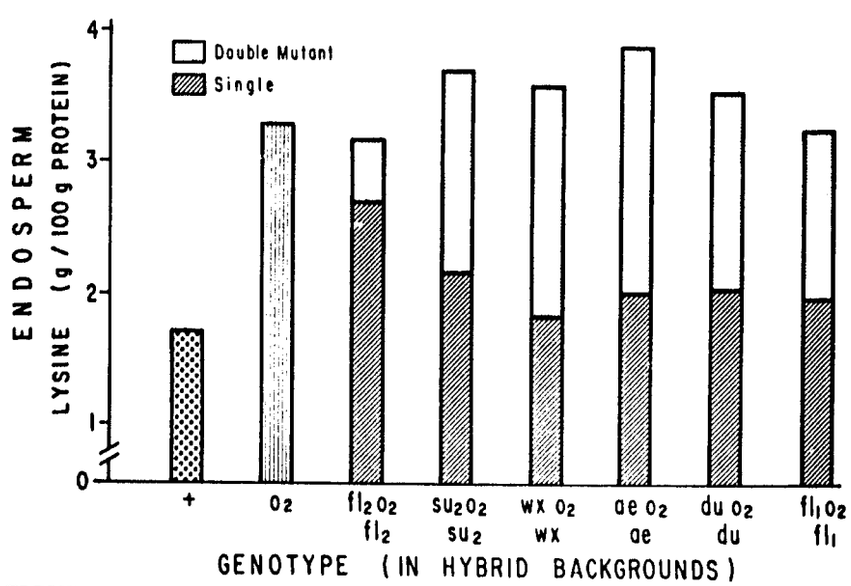

FIGURE 2 Average lysine as a percentage of protein for several single endosperm mutants and their double-mutant combinations with opaque-2 over hybrids (Indiana, 1971).

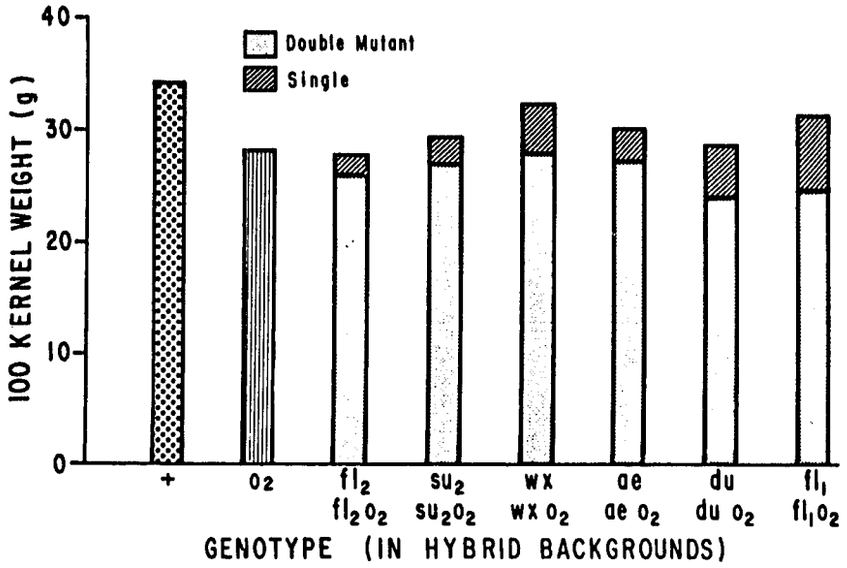

FIGURE 3 Average 100-kernel weights for several single endosperm mutants and their double-mutant combinations with opaque-2 over hybrids (Indiana, 1971).

were reduced by the o_2 gene, when compared to the corresponding mean of the single nonopaque hybrids.

The mutant gene su_2 showed the most pronounced effect on kernel density in the hybrids (Figure 4). The mean of the su_2 o_2 double-mutant combination hybrids was significantly superior in kernel density ($P < 0.01$) to the mean of the o_2 hybrids and other o_2 double-mutant combination hybrids. Apparently, su_2 combined with o_2 improves the kernel density in the double-mutant su_2 o_2 to nearly that of the normal counterpart.

Figure 5 illustrates the mean proportion of germ in the whole seeds of the hybrids. The effect of the o_2 mutant in producing seeds with larger germs was shown once again in these o_2 hybrid results. And certainly the effect of the o_2 gene was pronounced in increasing the percentage of germ in the double-mutant combinations. The su_2 o_2 and wx o_2 hybrids were the only double combinations in which the mean values for the percentage of germ of the whole seeds were not significantly different from that of the opaque-2 hybrids.

From our previous analysis of the mutant endosperm materials in the near-isogenic background of inbred Oh43, we had observed that the su_2 gene was increasing the percentage of oil of the whole seeds without a concomitant increase in the proportion of germ. We analyzed for the percentage of oil of the whole kernels from the su_2, su_2 o_2, o_2 hybrids and their normal counterparts. A comparison among the genotype means of the

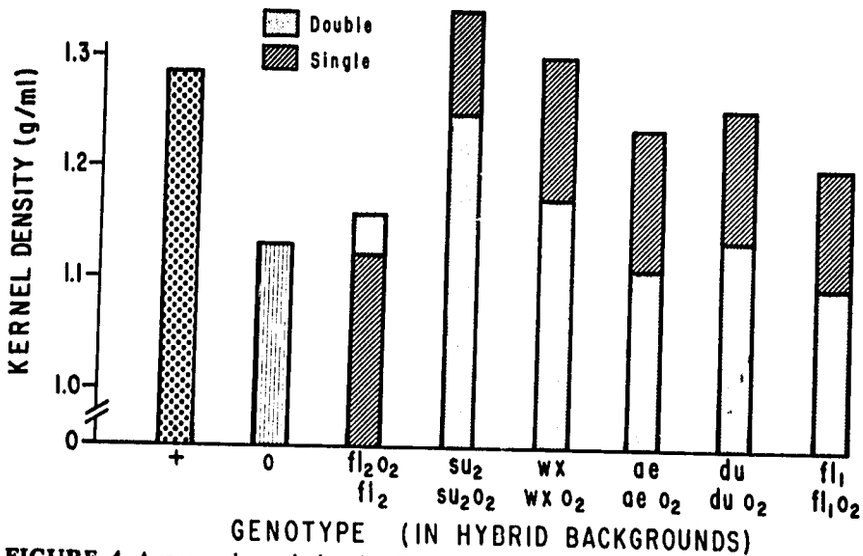

FIGURE 4 Average kernel density for several single endosperm mutants and their double-mutant combinations with opaque-2 over hybrids (Indiana, 1971).

hybrids for percentage of oil and oil content per whole seed is given in Table 7. It is apparent that the su_2 gene significantly increased the percentage of oil. Even more interesting is the finding that actual oil quantity per kernel

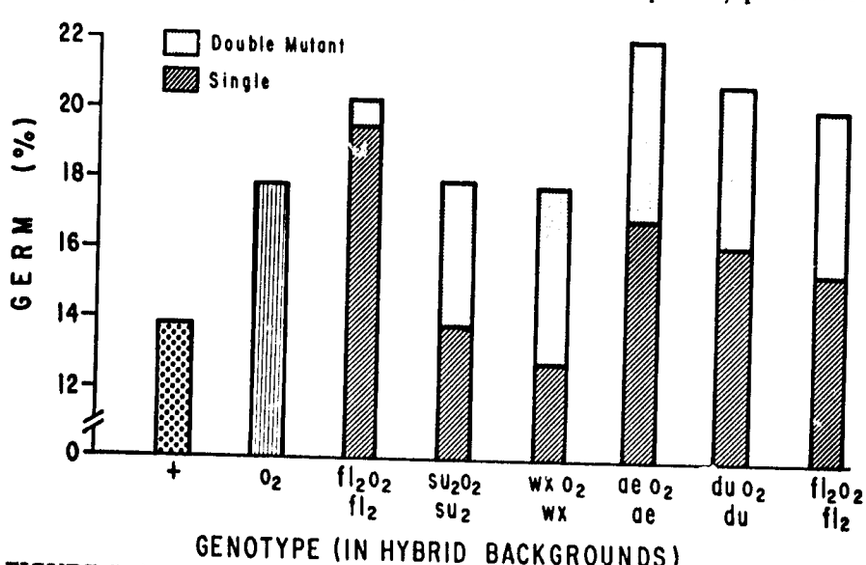

FIGURE 5 Average percentage of embryo fraction of whole kernel for several single endosperm mutants and their double-mutant combinations with opaque-2 over hybrids (Indiana, 1971).

TABLE 7 Mean oil content of whole kernels of opaque-2, sugary-2, and sugary-2:opaque-2 hybrids and their normal counterparts (1971)

Genotype	Oil[2] (%)	Oil/kernel (mg)
+[1]	4.21[D]	14.63[B]
o_2	5.05[C]	14.58[B]
su_2	5.57[B]	16.78[A]
su_2 o_2	6.36[A]	17.19[A]

[1] Means of six hybrids and four replications.
[2] Means within column followed by a common superscript do not differ significantly at the 1% level (DMRT).

(milligrams of oil per kernel) in the su_2 and su_2 o_2 hybrids was significantly ($P < 0.01$) increased over both normal and opaque-2 counterparts, while the proportion of germ in the whole seed of su_2 hybrids remained the same (Table 8). The su_2 and su_2 o_2 hybrids were better in oil yield per kernel than their normal counterpart by 15 and 18%, respectively. Even though the percentage of oil was significantly increased in o_2 hybrids, the actual oil quantity per kernel was not different from the normal hybrids.

These results in the single-cross hybrid backgrounds should be viewed as preliminary in nature, but they do support findings in the near-isogenic inbred Oh43 materials. The endosperm mutant genes exert a similar effect in the repression of zein synthesis. Several mutants (su_2, wx, ae, du, bt_2, bt_1, su_1, and sh_2), when combined with the o_2 gene, enhance the increased lysine concentration by reducing zein levels to an even greater extent. In contrast to most of the endosperm mutants just mentioned, no enhanced effect on lysine concentration was observed when, for example, fl_2 was combined with o_2.

These endosperm mutants and their double combinations with opaque-2 should be examined cautiously. Mutant and mutant combinations that suppress starch synthesis considerably (bt_1, bt_2, su_1, and sh_2) are agronomically

TABLE 8 Mean percent germ and endosperm lysine (g/100 g pro.) of opaque-2, sugary-2, and sugary-2:opaque-2 hybrids and their normal counterparts (1971)

Genotype	Germ[2] (%)	Endosperm lysine (g/100 g protein)
+[1]	13.99[B]	1.74[D]
o_2	18.14[A]	3.30[B]
su_2	13.99[B]	2.16[C]
su_2 o_2	18.10[A]	3.70[A]

[1] Means of six hybrids and four replications.
[2] Means within column followed by a common superscript do not differ significantly at the 1% level as determined by Duncan's multiple range test.

undesirable from the standpoint of mature grain characteristics, even though they do contain the most favorable zein-to-glutelin ratios. The double-mutant combinations of these genes with o_2, however, do have value as edible types in the green-maize stage of development, because of their favorable carbohydrate and protein quality. Perhaps they might have value as a special food for infants, depending upon the economics of production and the development of the food preparation.

Only preliminary data are available for the endosperm mutants su_2, wx, ae, du, fl_2, and fl_1 and their combinations with o_2 in the hybrids. Although potential is indicated in special modified endosperm starch types for improving protein quality, digestibility characteristics, density of the grain, and perhaps energy content and mineral levels in such types as su_2 o_2 and wx o_2, there are some uncertainties concerning the nutritional significance of some of these mutant combinations. We do not have adequate information on the feeding value and biological efficiency of these modified types. Nor do we know how well they will meet the calorie–protein requirements of either nonruminants or ruminant subjects.

Initial tests of whole ground kernels of su_2 o_2, wx o_2, ae o_2, bt_2 o_2, and bt_2 in the Oh43 inbred background were fed to rats for 10 days at an amount that was 95% of the ration. Although the results must be considered as very preliminary, they did suggest that the extra lysine and tryptophan of the double mutants are available for growth purposes. There is some indication from feeding trials with steers and lambs at the University of Illinois (E. E. Hatfield and W. L. Braman) that waxy maize provides improved feeding efficiency. Increased gains also have been obtained by feeding opaque-2 maize to cattle. High-oil maize feeding trials with swine have shown that feed efficiency is substantially greater with high-oil maize than with normal maize. Maybe the wx o_2 and su_2 o_2 double combinations, for example, might contribute to an improved feed and/or food value of maize for ruminants and nonruminants alike.

Data from our very preliminary tests suggest that yielding ability of the wx o_2 and su_2 o_2 double-combination hybrids may be more promising than other combinations, since starch synthesis is reduced the least in these genotypes.

Other Mutants That Affect the Opacity of the Endosperm

Several other mutants affect the opacity of the endosperm as do the opaque-2 and floury-2 materials that we have analyzed. McWhirter (9) identified a floury endosperm mutant that occurred spontaneously in W22 stock. He reported that it is high in lysine and linked on chromosome 10. This mutant has been designated opaque-7 (o_7). We obtained seeds of o_7 and the double-mutant o_2 o_7 (F_3 segregates from the cross W23/L317 o_2/o_2; +/+ × W22 +/+; o_7/o_7) from McWhirter. We have recovered the o_7 mutant from

intercrosses with several inbred backgrounds and isolated o_2 o_7 and fl_2 o_7 double-mutant combinations in several backgrounds. The double-mutant combinations o_2 o_7 and fl_2 o_7 are not readily identifiable by phenotypic expression of the endosperm. Furthermore, the single-gene o_7 recovery in various backgrounds is subject to considerable modification and does not give clear expressivity in many cases.

Federico Poey recovered a mutant similar to opaque-2 in Cuban flint (O Poey). From materials given to us by Poey, it has been determined that this mutant is a recessive allele at the fl_1 locus on the short arm of chromosome 2. The gene, designated as opaque-4 (o_4), is a recessive allele also at the fl_1 locus. Therefore, these two mutants are allelic to the fl^a gene originally described by Mazoti and reported to be similar to the opaque-2 gene in lysine concentration (8). Our preliminary data show that none of these three mutants is substantially different in lysine content from its normal counterpart.

We have obtained numerous samples of putative opaque mutants from a search in Colombian and other germ plasm. One mutant was found to be allelic to o_2 and another allelic to o_1. Many of these mutants which affect the opacity of the endosperm have presented interesting genetic problems with regard to expressivity of "opaqueness," but so far none has shown any practical nutritional advantage.

Table 9 shows the lysine concentrations for the spontaneous o_7 mutant in the W22 inbred background; the o_2 o_7 double-mutant received from McWhirter; the o_7, o_2 o_7, and fl_2 o_7 recoveries from crosses to several inbreds; and fl^a. The o_7 (W22 inbred) mutant had a value of 3.8 lysine as a percentage of protein compared to a lysine content value of 2.3 in its normal counterpart (11). The o_7 recoveries within the four different backgrounds show increased concentrations of lysine, but not to the extent originally reported (8, 11). The W22 o_7 materials are very low in protein. The lysine concentrations of the double-mutants o_2 o_7 and fl_2 o_7 are increased. However, these are not rigorous comparisons. It appears that the o_7 gene and fl_2 have similar influences on lysine levels.

The fl^a mutant material was obtained from A. V. Paez (Pioneer Hi-Bred International, Inc.). The lysine concentration of this mutant possibly is

TABLE 9 Amino acid composition of defatted endosperms

	Amino acid concentration (g/100 g protein) in						
	W22			Inbred backgrounds			
	+	o_7	o_2 o_7	o_7[1]	o_2 o_7[1]	fl_2 o_7[1]	fl^a[2]
Lysine	2.3	3.8	3.5	2.8	3.6	3.3	2.4
Protein (%)	8.5	7.3	7.6	11.4	10.4	12.0	12.0

[1] Mean of four backgrounds.
[2] One background.

greater than normal. Once again, a rigorous comparison could not be made, because backgrounds were not comparable.

Journal Paper No. 4997, Purdue University Agricultural Experiment Station. This research was supported by the U.S. Agency for International Development, Contract AID/Csd-2809 with Purdue University, titled "Inheritance and Improvement of Protein Quality and Content in Maize."

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APPROACHES TO IMPROVING PROTEIN QUALITY IN MAIZE WITHOUT THE USE OF SPECIFIC MUTANTS

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Improvement of protein quality in maize should prove of great value in solving many of the world's nutrition problems. Maize is the main part of the human diet in many countries and an important source of food for many monogastric animals, such as swine.

As early as 1914, Osborne and Mendel (9) and others had reported that maize is deficient in lysine and tryptophan. However, early research workers studying protein quality lacked rapid and efficient methods of determining amino acid contents. More recently, new automated equipment has speeded the analysis of many samples.

Variation in the amino acid composition of maize has been reported by Tello et al. (12), Aguirre et al. (1), Bressani et al. (3), and numerous others. They found significant differences among maize varieties for several amino acids, including lysine, tryptophan, and methionine. Their analyses were assumed to be made on normal endosperm maize and on a whole-kernel basis. Flynn et al. (4), Frey et al. (5), and Miller et al. (8) found significant differences in protein and amino acids for U.S. corn belt maize. However, these studies did not indicate whether any of these maizes with higher lysine

and tryptophan content were superior as food for monogastric animals, when compared with maize of lower content.

The big breakthrough on protein quality was the Mertz et al. (7) report that the opaque-2 gene nearly doubled the lysine content of endosperm tissue. Their findings stimulated research interest in upgrading the protein quality of maize and the other cereal grains. Alexander (2) reported that opaque-2 maize segregates usually weighed less than normal maize siblings, but in certain genetic backgrounds opaque kernels weighed the same as normal siblings. Comparisons of normal and opaque-2 hybrids by Lambert et al. (6) also showed that opaque-2 hybrids were lower than their normal counterparts in grain yield and kernel weight, but were higher in grain moisture, cracked kernels, and total oil content. Similar results have been reported in numerous studies.

Alexander (2) mentioned possible modifier genes that tended to alter the opaque-2 phenotype in certain background genotypes. Later, Paez et al. (10, 11) reported genotypic opaque-2 kernels with endosperms that were phenotypically half translucent and half opaque. They found that the two fractions did not differ in lysine content. These results encouraged a search for translucent endosperm types among opaque-2 kernels. Types now have been identified with lysine values equal to opaque-2 materials. The translucent types have not yet been evaluated for test weight or yield, but the kernel definitely has a harder texture. Hopefully, the agronomic performance of these translucent types will equal that of their nonmutant counterparts.

Improving protein quality in normal dent maize without major endosperm mutants is another possibility. Surveys by several investigators —Aguirre et al. (1), Tello et al. (12), and Paez et al. (10, 11)—indicated a considerable range in lysine content among different races and strains of maize. Because those lysine determinations were made on a whole-kernel basis, the question has been raised whether the range in lysine reported may have been due to varying germ-to-endosperm ratios. Because germ tissue is high in lysine content, the lysine content would be highest in kernels with a larger proportion of germ tissue.

OBJECTIVES

In the study reported here, the main objectives were to determine (1) whether lysine content of nonmutant maize could be increased through recurrent selection, and (2) whether an association exists between germ– endosperm ratios and lysine content.

MATERIALS AND METHODS

Three open-pollinated varieties were selected on the basis of their higher-than-average lysine values obtained from the survey of maize strains

for lysine content reported by Paez et al. (11). The three varieties chosen were Midland Yellow Dent (PI222609), Logan County Composite (PI232 666), and Reid Yellow Dent (PI213698).

Approximately 75 plants in each variety were selfed in 1967. Lysine content was determined on S_0 ears on a whole-kernel basis. Thirty kernels were sampled from each ear and ground with a micromill. Lysine content was determined by the automated lysine decarboxylase method, as described by White and Gauger (13). Total protein was determined on the same sample. Ten percent of the ears with the highest lysine content were selected for initiating the first cycle. Equal amounts of kernels were removed from the selected ears, bulked, and planted to give approximately 150 plants. These plants were intercrossed by using pollen mixtures. Two generations of intermating were followed by a selfing generation. A random 100 ears from over 400 selfed ears were analyzed for lysine and protein. Each cycle thus requires a minimum of 2.5 years. Methods of selection, sampling procedures, and chemical determinations were the same for both the first and second cycles and for each of the three populations. Oil analysis was obtained by wide-line nuclear magnetic resonance (NMR) through the courtesy of the Illinois Agricultural Experiment Station.

RESULTS AND DISCUSSION

The ranges in protein and lysine values for the first and second cycles of the three populations are shown in Table 1. All three populations followed a similar pattern. The protein range for the second cycle was not as great as for the first. Nor did the highest protein value for any of the three populations change materially between the first and second cycles. The range in lysine content between the two cycles was about the same, but the lower and upper values increased from the first to the second cycle, suggesting that selection was effective in increasing lysine content for each of the three populations.

TABLE 1 Range, mean, and percentage of change for protein and lysine content for two cycles of recurrent selection for lysine content on a whole-kernel basis

Variety	First cycle Range	First cycle Mean	Second cycle Range	Second cycle Mean	% Change
			Protein		
Midland	9.4-17.8	13.4	10.4-16.5	13.6	+1.5
Logan	8.5-16.8	13.1	11.0-16.5	13.7	+5.0
Reid	8.1-17.0	12.3	10.5-15.0	12.3	None
			Lysine		
Midland	0.19-0.32	0.26	0.26-0.43	0.35	+34.6
Logan	0.19-0.38	0.27	0.34-0.49	0.40	+48.2
Reid	0.18-0.33	0.26	0.28-0.38	0.34	+30.8

The frequency distribution for the percentage of lysine content on a whole-kernel basis for each of the two cycles is shown in Figures 1, 2, and 3. The frequency distribution for the percentage of protein on a whole-kernel basis for each of the two cycles for each population is shown in Figures 4, 5, and 6. Mean protein values for the first and second cycles were essentially the same, and the frequency distributions followed similar patterns. The frequency distributions indicated no material change in protein content between the two cycles.

It is possible that some of the increase in lysine between the two cycles was due to the effect of environment, because the first and second cycles for each population were grown in different years. However, the same pattern was observed in the increase in lysine between the two cycles for each of the three populations, with no essential change in protein between cycles, thus suggesting that the environmental effects may have been minimal. A future study will investigate these environmental effects by growing the unselected, first-cycle and second-cycle populations at the same time and place.

Recurrent selection for lysine on a whole-kernel basis appeared to be effective (and probably was not due to any change in the level of protein); thus, the question arose as to whether any change in the amount of lysine per

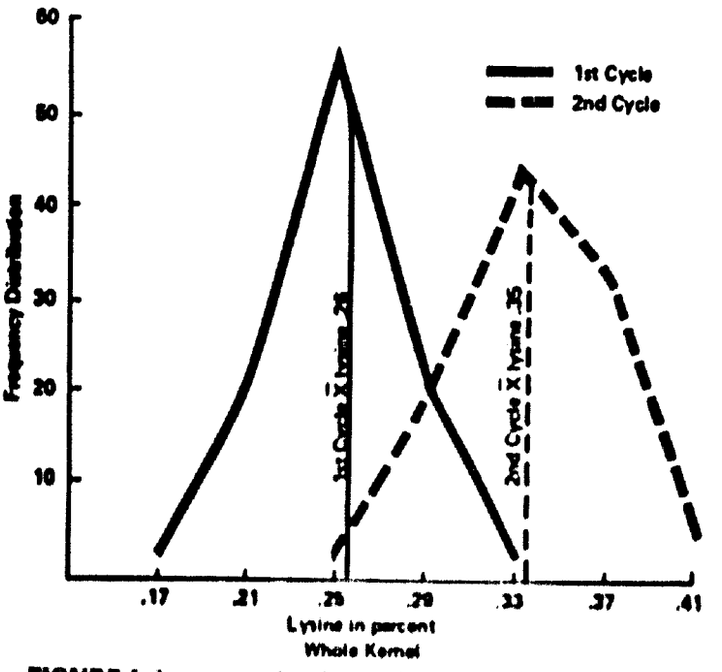

FIGURE 1 Frequency distribution for first and second cycles of recurrent selection for high lysine on a whole-kernel basis in Midland Yellow Dent

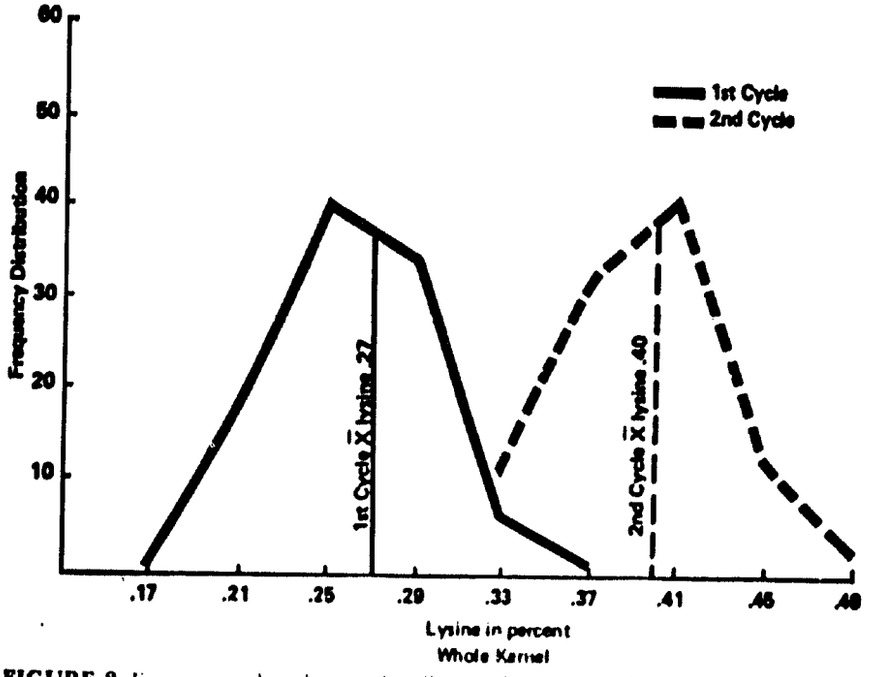

FIGURE 2 Frequency distribution for first and second cycles of recurrent selection for high lysine on a whole kernel basis in Logan County Composite.

100 g of protein had occurred between the two cycles. The frequency distribution for the number of grams of lysine per 100 g of protein is shown in Figures 7, 8, and 9. Frequency distributions for the three populations followed a similar pattern, with the second cycle showing a substantial increase in the amount of lysine per 100 g of protein.

Previous studies had shown that dent maize inbred lines with above-average whole-kernel lysine content failed to produce hybrids with above-average lysine content. We assumed that the decrease in lysine content for these crosses was due to a change in the germ–endosperm ratios. Inbred lines had proportionally more germ tissue, whereas hybrids had proportionally more endosperm tissue. The question then is: Were the increased lysine contents associated with proportionally larger germs?

We assessed germ and endosperm weights by hand separation and computed the germ–endosperm ratios for all selections for each population from the second cycle. We also calculated whole kernel oil content for each selection, assuming that any change in germ size would be reflected by a concomitant change in oil content. Because wide line NMR is nondestructive, we were able to obtain germ–endosperm ratios on the actual samples used for oil analysis.

Table 2 shows the correlation coefficients between lysine and other ratio-

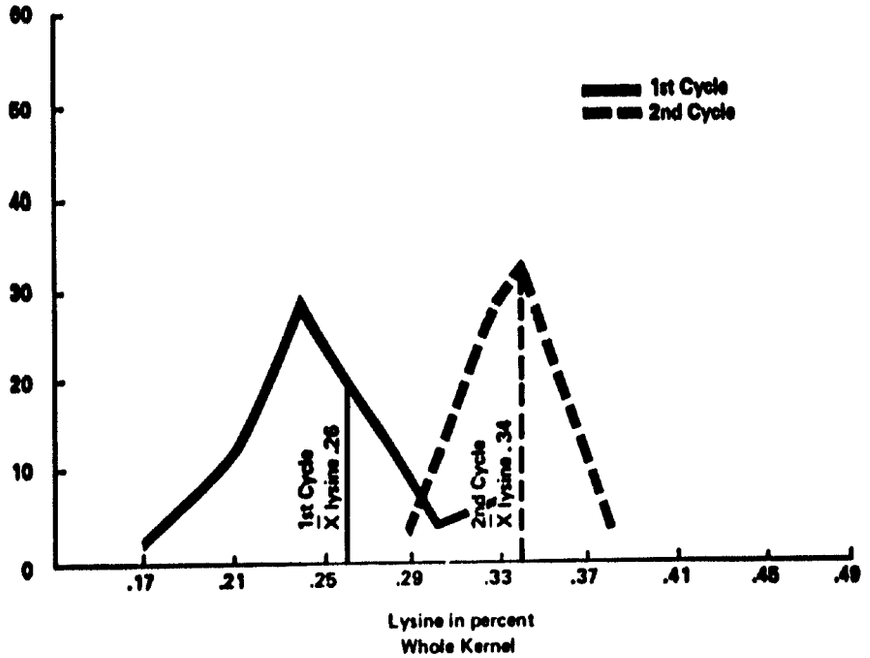

FIGURE 3 Frequency distribution for first and second cycles of recurrent selection for high lysine on a whole kernel basis in Reid Yellow Dent.

TABLE 2 Correlation coefficients of lysine, oil, and protein with other variables for the second cycle of recurrent selection for lysine content on a whole-kernel basis

Component	Midland Yellow Dent	Logan County Composite	Reid Yellow Dent
Lysine versus protein	0.49[a]	0.57[a]	0.41[a]
Lysine versus oil	0.03	0.04	-0.19
Lysine versus kernel weight	-0.09	0.16	0.03
Lysine versus endosperm weight	0.21[b]	-0.19	-0.06
Lysine versus germ weight	0.26[b]	0.07	0.09
Lysine versus endosperm germ ratio	0.02	-0.07	-0.05
Oil versus endosperm weight	0.05	-0.28[a]	0.13
Oil versus germ weight	0.53[a]	0.28[a]	0.17
Oil versus endosperm germ ratio	-0.59[a]	-0.51[a]	-0.22[b]
Protein versus endosperm weight	0.16	0.12	0.05
Protein versus germ weight	0.22[b]	-0.04	-0.18
Protein versus endosperm germ ratio	0.12	-0.15	0.13

[a] Significant at $p = 0.01$.
[b] Significant at $p = 0.05$.

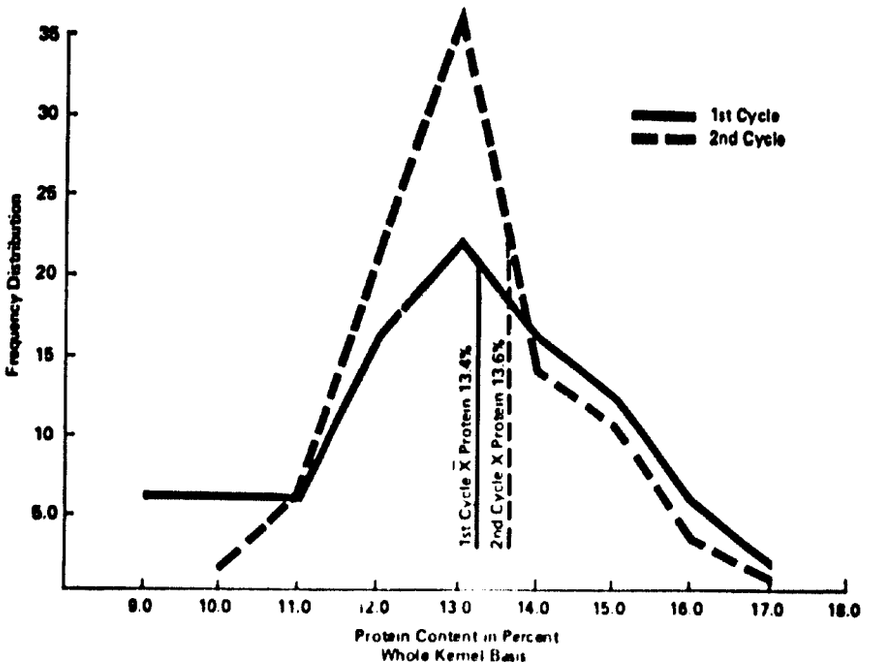

FIGURE 4 Protein content frequency distribution for first and second cycles of recurrent selection for high lysine on a whole-kernel basis in Midland Yellow Dent.

ables for Midland Yellow Dent, Logan County Composite, and Reid Yellow Dent. Lysine was significantly correlated with protein, endosperm weight, and germ weight in Midland Yellow Dent. Lysine was significantly correlated with only protein in Logan County Composite and Reid Yellow Dent. The magnitude of these correlations with oil or germ weight suggests that germ size accounted for very little of the variation in lysine content. Oil content and germ weight were not significantly correlated in Reid Yellow Dent, which is puzzling, because oil versus endosperm germ ratio was significant and negative as expected.

Comparative coefficients of determination (R^2) and b values between the first and second cycles of the selection are shown in Table 3. The R^2 values between lysine and protein ranged from 0.340 to 0.490 for the first cycle, but apparently decreased for the second cycle. This reduction in the size of the R^2 values from the first to the second cycle is not understood. The b values were statistically significant for protein versus lysine for the three populations and for both cycles. The R^2 values between protein and oil, and between lysine and oil, were low and nonsignificant.

Although the results from these first two cycles suggest that selection for lysine content has been effective, we must investigate the biological value of the higher lysine content of the second-cycle selections.

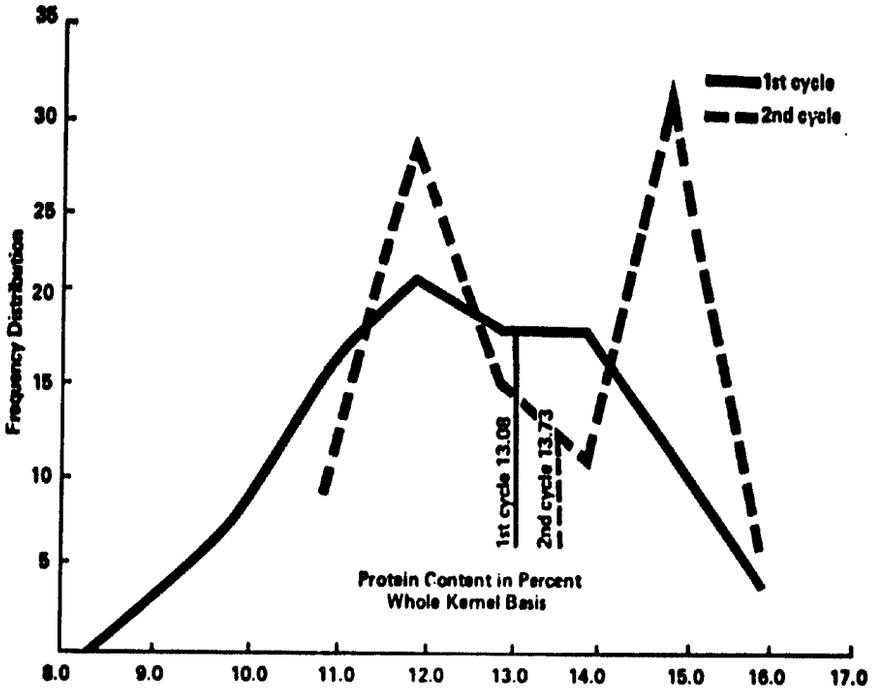

FIGURE 5 Protein content frequency distribution for first and second cycles of recurrent selection for high lysine on a whole-kernel basis in Logan County Composite.

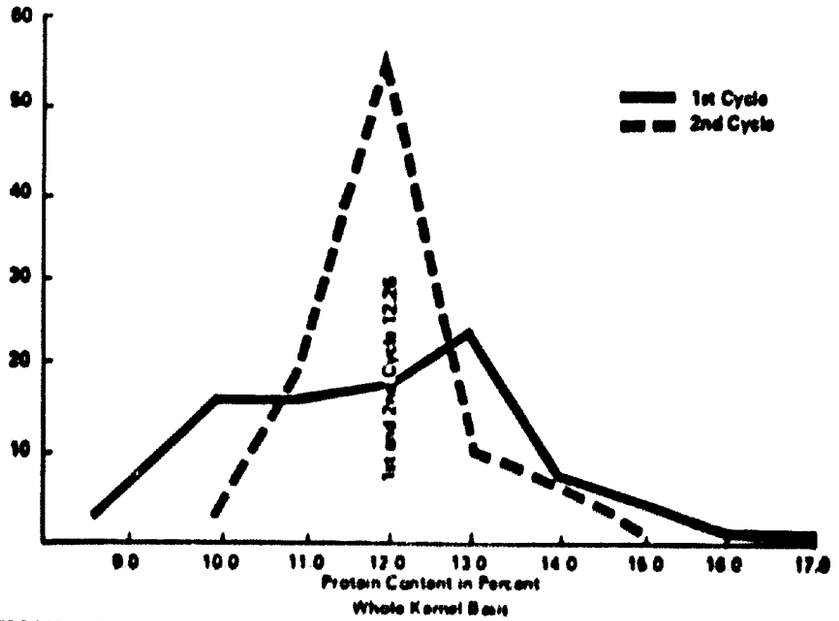

FIGURE 6 Protein content frequency for first and second cycles of recurrent selection for high lysine on a whole-kernel basis in Reid Yellow Dent

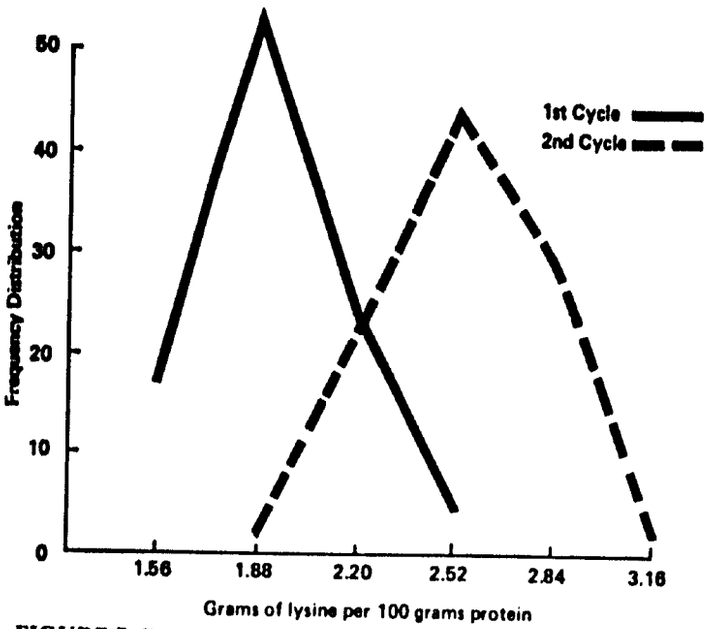

FIGURE 7 Frequency distribution for grams of lysine per 100 g of protein on a whole-kernel basis for the first and second cycles of recurrent selection in Midland Yellow Dent.

Because lysine content was successfully increased by recurrent selection for three different populations, we have speculated on a logical genetic basis for this increase. Our results suggest that many genes affecting lysine synthesis could be involved. We are investigating the effect of the high-lysine selections from these nonmutant populations when crossed with the

TABLE 3 Coefficient of determination and estimates of *b* values for protein versus lysine, protein versus oil, and lysine versus oil on a whole kernel basis

Component	First cycle R^2	First cycle *b* values	Second cycle R^2	Second cycle *b* values
		Logan		
Protein versus lysine	0.490	0.01199*	0.314	0.0134*
Protein versus oil			0.002	-0.0776
Lysine versus oil			0.001	0.0022
		Midland		
Protein versus lysine	0.420	0.00847*	0.240	0.0118*
Protein versus oil			0.028	0.4104
Lysine versus oil			0.001	0.0016
		Reid		
Protein versus lysine	0.510	0.00993*	0.165	0.0101*
Protein versus oil			0.012	0.2126
Lysine versus oil			0.035	-0.0091

* Significant at $p = 0.05$.

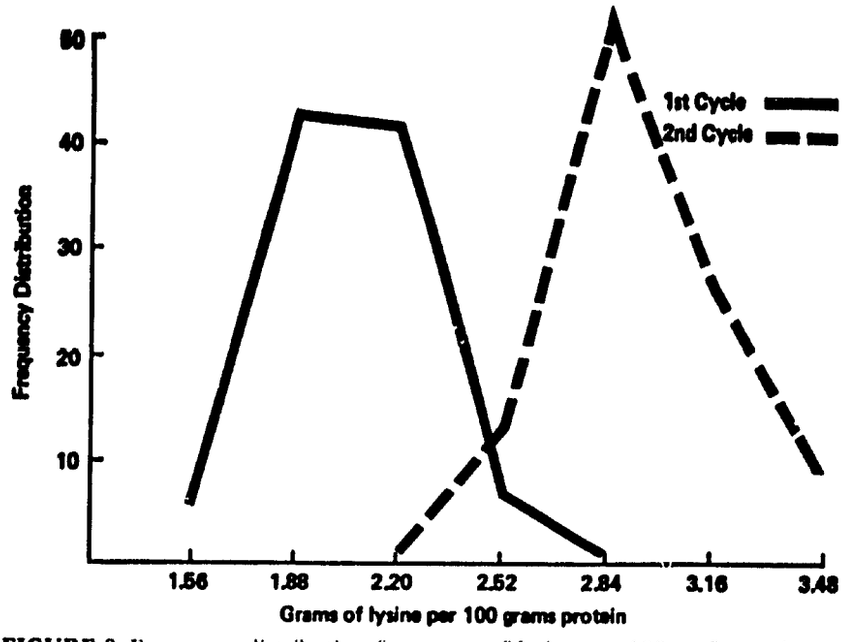

FIGURE 8 Frequency distribution for grams of lysine per 100 g of protein on a whole-kernel basis for the first and second cycles of recurrent selection in Logan County Composite.

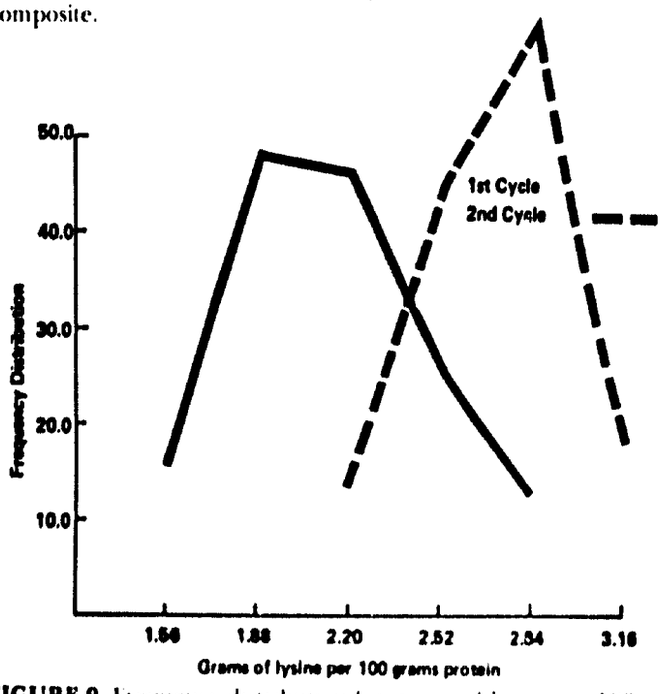

FIGURE 9 Frequency distribution for grams of lysine per 100 g of protein on a whole-kernel basis for the first and second cycles of recurrent selection in Red Yellow Dent.

opaque-2 mutant and the mutant is recovered in segregating generations. If the genetic system involved for the increase in lysine in the nonmutant populations is additive and complementary to the opaque-2 gene, we may be able to increase the lysine values for the opaque-2 mutant.

In the recurrent selection for lysine content in these three nonmutant populations, we confined our selection to lysine content on a whole-kernel basis. We paid no attention to protein, because we assumed that lysine and protein synthesis involved two different genetic systems. We thought the best approach would be to first increase the lysine level, if our assumptions were valid. The significant positive correlation, although small in magnitude, suggests that any increase in lysine should be accompanied by an increase in protein.

[*A discussion of this paper can be found on pp. 495–497 of* **Questions and Answers.**]

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Part VI

COMMERCIAL PRODUCTION OF QUALITY PROTEIN MAIZE: CURRENT ISSUES AND PROBLEMS

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Presentations at this symposium center around fundamental aspects of maize breeding and the chemical and nutritional evaluation of protein quality in maize. These technical papers come from the highly specialized world of nurseries, laboratories, and hospitals, where the scientific method is applied and results are measured objectively. Success in these specialized fields has been quite evident during the last few years, prompting institutions in some countries to promote commercial seed production and encourage marketing of opaque-2 maize for direct and industrial use. However, the commercial production of opaque-2 maize is related to the world of business and marketing traditions, in that world results and progress are measured in more subjective parameters, such as profits and consumer acceptance.

The problems related to the commercial production and utilization of opaque-2 maize can be traced to the soft or floury structure of the endosperm, which is associated with the improved protein quality provided by the opaque-2 gene. This soft structure implies lower kernel weight, increased vulnerability to insect and disease damage, and consumer resistance to accepting a nontraditional maize.

The higher nutritional value of opaque-2 maize, however, has motivated official and private institutions to seek answers for the problems involved. Strategies being used in the United States, Colombia, and Brazil will be discussed next. These three countries offer widely different conditions for maize production and utilization. The United States uses maize mainly for

animal feeding and industrial processing for human consumption and industrial use. In Colombia, maize is consumed principally by humans, and in Brazil most maize is fed to animals. Close cooperation between government and private institutions in these countries has made it possible to apply the newest technology and to establish convenient market policies, which seem necessary to promote the commercial use of opaque maize.

Efforts to promote maximum cooperation between official and private sectors demand priority attention if the difficult, but urgent, task of making high-quality protein maize available to undernourished people is to be accomplished soon. This cooperation can be sought for seed production as well as marketing.

In attempting the commercial production of high-quality protein maize in any country, we must first look into the seed production and distribution aspects. These aspects face quite different conditions and problems in each country. Government policies have a tremendous influence on these activities, both in developed and developing countries. This influence ranges from practically no control on seed production and distribution, in some countries, to a strict and exclusive monopoly of these activities by official agencies. Private enterprises, on the other hand, range from highly capitalized organizations that include efficient breeding programs to small firms that operate on a local basis, and usually on small budgets.

A new type of international institution, such as Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), is contributing to breeding programs, regardless of their financing or nationality. In this field, we notice more progress in the introduction of opaque-2 maize in countries with closer relationships between private, official, and international institutions.

Regarding the commercial utilization of opaque-2 maize, one important distinction can be made according to the way maize is consumed. For direct consumption, the pure opaque-2 maize is discriminated against in maize-consuming communities, which traditionally have used harder kernel types. This discrimination tends to be stronger in the more underdeveloped communities, generally coinciding with the more protein deficient population sectors. When transformed by some industrial or intermediate process, the soft texture of opaque-2 maize does not seem to affect its acceptance unless some other characteristic of the end product is also affected. Whereas tradition appears to be the most important element in direct consumer decision to accept high quality protein maize, the cost-profit relation of an industrial process seems to be the main element of decision for a food-processing operation.

The use of high quality protein maize in the food industry represents an important channel for the utilization of opaque-2 maize. Industry can make all-maize products or partially supplement or substitute them within other products. This is already being done with bread and pastas, for example,

when wheat flour is replaced partially by opaque-2 maize flour. Opaque-2 maize also has found its way into some other popular food products.

The following papers will present views and experiences on seed production, commercial introduction, and industrial use of high-quality protein maize. Although experience may be relatively brief in this area, I am sure, the views expressed will aid in our search for ways to introduce high-quality protein maize on a commercial scale in many parts of the world.

The objectives set for this session cannot be fully obtained, however, unless an active participation is taken by the distinguished international audience meeting here today. I encourage you to voice your questions and opinions.

I am confident that this session will make a lasting contribution to finding practical and urgently needed mechanisms for the application of our research.

WORLDWIDE SEED INDUSTRY EXPERIENCE WITH OPAQUE-2 MAIZE

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Seed industry experience with opaque-2 maize has been extremely limited, both in the United States and abroad. It was less than 10 years ago that the group at Purdue University demonstrated the effects of the mutant opaque-2 gene on the amino acid balance of the maize endosperm. This discovery touched off a flurry of attempts among breeders to introduce the opaque-2 gene into desirable genotypes. Little was then known about how the opaque-2 gene might interact with other factors influencing the physical and biochemical composition of the maize endosperm.

Opaque-2 hybrids, and varieties possessing most of the desired traits demanded by modern agriculture, require time to develop, even though the transfer of a single gene through successive backcrossing is relatively simple. Four or five backcrosses to desirable genotypes usually do not result in lines that when crossed, produce hybrids capable of competing with the best hybrids available. Even though some opaque-2 hybrids produced in this manner have been marketed, the seed industry only now is beginning to release hybrids whose parental lines have had sufficient backcrossing to permit critical comparisons of performance with their normal counterparts. Thus, industry experience with adequately tested and proved opaque-2 hybrids has been extremely limited.

Some breeding work with opaque-2 maize is being done in most countries where maize is of importance; however, the significant commercial use of opaque-2 hybrids and varieties apparently has been limited to three coun-

tries, Brazil, Colombia, and the United States. Brazil is reported to have produced 400,000 kg of opaque-2 maize seed in 1971 and Colombia, in the same year, produced approximately 250,000 kg. The amount of opaque-2 maize seed currently being produced in the United States remains an insignificant percentage of the required total of maize seed. These quantities of seed and additional details relative to the U.S. experience are discussed later in this report. The current status of opaque-2 hybrids and varieties in Brazil and Colombia will be presented in reports that follow. My general discussion now turns to the seed industry's experience with opaque-2 maize in three categories: (1) breeding, (2) seed production and (3) farmer or grower experience.

BREEDING

Undesirable kernel characteristics and relatively low yields associated with opaque-2 endosperm mutants have hindered widespread acceptance of this type of maize where it has had to compete with normal types. Therefore, the use of such maize has been limited mostly to special contract production operations and to regions where its price is supported at levels profitable to the grower.

For these reasons, most breeders have turned their attention to (1) selection for high test weight within opaque-2 phenotypes, (2) selection for modified endosperm types with kernel characteristics similar to normal kernel phenotypes and having the high nutritive value of opaque-2 and (3) selection of double-mutant endosperm combinations.

Most breeders working with opaque-2 maize have observed (in some backgrounds) segregation for endosperm types that depart from the 100% opaque phenotype. Mottling or sectoring of the endosperm tissue occurs, with the types ranging from a slight departure from the typical opaque phenotype to full normals. Alix Paez has isolated several such lines in our laboratory and has been working with them. When three families of segregating progenies were subjected to chemical analysis for protein and lysine, the best 15 lines ranged in lysine content from 0.37 to 0.47%, compared to 0.45% for the standard opaque-2 check. Lysine as a percentage of total protein in these lines ranged from 3.97 to 5.12%, compared to 4.73% for the opaque-2 control. Several of these lines have been tested in hybrid combinations, with the better combinations comparing favorably in percentage of lysine with standard opaque-2 phenotypes.

These results suggest the possibility of selecting high-lysine lines with normal, translucent endosperm tissue. If this can be done, it should then be possible to develop high-quality protein hybrids that do not have the reduced yields or low test weight associated with the conventional opaque-2 phenotype. In other words, the opaque-2 trait itself appears not to be a requisite for high-quality protein maize. It should be pointed out, however,

that there is great variation in the occurrence of normal and seminormal endosperm types segregating from crosses of opaque-2 × normal genotypes, which apparently depends upon the genetic background of the particular normal line used in such crosses. Segregants of modified endosperm type should not be expected to emerge from all crosses of opaque-2 × normal genotypes.

Another promising approach to developing high-quality protein, vitreous-endosperm maize involves the use of double-mutant endosperm combinations. In 1966, Nelson (2) reported that the double mutant fl_2/fl_2 o_2/o_2 reconstitutes a dense endosperm with an amino acid balance similar to that of fl_2/fl_2. He suggested that the use of such a combination might well overcome the yield loss, inherent in soft-endosperm types, that results from the use of either gene separately.

However, Lambert and Cochran (1) have reported that the double-mutant combination exhibits translucent endosperm with normal kernel weight only in some genetic backgrounds. Paez's results (3) have confirmed these findings. Furthermore, he has hypothesized that the apparent interaction of opaque-2 and floury-2 genes to produce a modified endosperm phenotype may be due instead, to the operation of other unidentified genes carried along in the floury-2 background.

In addition to opaque-2 and floury-2 genes, certain other endosperm mutants, when combined with opaque-2, produce interesting phenotypes and amino acid profiles, which may offer further opportunities for improving the nutritive value of maize. These include the mutants ae, du, and sugary-2. Discussion here is limited to Paez's results obtained with the double-mutant su_2/su_2 o_2/o_2. The stock of this particular gene combination (for which we have the most data) is characterized by a translucent endosperm of hard texture. When fully developed, this endosperm has corneous tissue extending from the pericarp to the embryo, and the kernel density and weight compare favorably with normal dents and semidents.

Chemical analysis of several of these double mutants indicates that lysine content is as high or higher than the lysine content of opaque-2 stocks used as checks or reported in the literature. Lysine percentages of whole-kernel maize in this material ranged from 0.50 to 0.62%, compared to 0.45% for opaque-2 maize. Lysine as a percentage of protein in these lines ranged from 4.99 to 6.00%. In comparison, lysine as a percentage of protein in an opaque-2 maize check was 4.73%. The double-mutant su_2/su_2 o_2/o_2 has been testcrossed to several homozygous opaque-2 lines, confirming the presence of the opaque-2 gene in the double-mutant stock.

These preliminary data suggest, at least, that the interaction of the opaque-2 and sugary-2 genes in some genetic backgrounds might produce translucent segregants of high kernel weight with lysine percentages which may exceed those obtained through the use of either gene separately. Furthermore, the starch of sugary-2 endosperms appears to have a higher

degree of digestibility than does that of normal maize, thus enhancing the potential nutritive value of sugary-2, opaque-2 combinations.

The factors delaying general acceptance of opaque-2 maize appear to be directly or indirectly associated with the soft, floury endosperm characteristic of this genotype. The floury endosperm of the opaque-2 hybrids and varieties probably contributes to their reduced yield, greater vulnerability to insect attack, and perhaps to greater disease susceptibility. Certainly, the opaque-2 phenotype is more susceptible to mechanical damage during harvesting and seed processing.

For these reasons, breeding emphasis is now aimed at altering the physical characteristics of the endosperm while maintaining an amino acid balance comparable to that of opaque-2 maize. These goals are being accomplished either through selection for modified endosperm types among progenies of crosses of opaque-2 × normals or through the use of double-mutant endosperm combinations.

SEED PRODUCTION

Experience with seed production of opaque-2 maize has been on such a limited scale and has involved so few hybrids that anything said today relative to matters of seed production could well be proved wrong tomorrow. Recognizing these limitations, some general observations can be made.

The practicality of hybrid seed production depends upon numerous traits of the hybrid parents. Many, but not all, of these are the same traits that farmers consider when choosing a hybrid. For example, the yield of the seed parent of the hybrid is of major importance, for obvious reasons. The seedsman prefers seed parents that are resistant to insects, diseases, and lodging, and male parents that shed adequate pollen under a wide range of environmental conditions. Inherently good germination and seedling vigor, plus reasonable resistance to mechanical damage to the kernel during seed processing, are also important factors.

COMPARISON OF TRAITS

Seed yields of the parents of opaque-2 hybrids appear to be well within the range of acceptability for seed production. Although the average seed yields of opaque-2 parents are probably 10% below yields of their normal endosperm counterparts, this reduction is not sufficient to preclude their use. Actually, the difference between the yields of opaque-2 and normal versions of a particular genotype is probably not as great as the difference between the seed yields of several normal endosperm hybrids of similar maturity. In my opinion, seed yields of opaque-2 hybrids have been, and will continue to be, satisfactory.

The apparently greater susceptibility of some opaque-2 genotypes to

some insects and diseases can create problems in seed production. Some opaque-2 genotypes are obviously more susceptible to ear molds caused by *Fusarium moniliforme* and other pathogens than are their normal endosperm counterparts. However, genotypes differ markedly in this trait; it seems premature to assume that all opaque-2 hybrids are susceptible to ear molds. Nonetheless, this trait is likely to limit the use of opaque-2 maize unless particular attention is given to selection for resistance to ear mold organisms.

A more serious problem in tropical, subtropical, and southern temperate zones of the world is grain damage resulting from insect infestation. The floury endosperm of opaque-2 maize seems to be especially attractive to numerous grain weevils and stored-grain insects. Field infestation before harvest can be a serious problem where grain weevils are prevalent. Under such conditions, some opaque-2 genotypes are attacked much more heavily than are some maize types with more vitreous endosperms. This may limit seed production of opaque-2 hybrids and the commercial use of this type of maize in some parts of the world.

The recessive nature of the opaque-2 gene suggests that fields used for producing opaque-2 hybrid seed may require more stringent isolation requirements than those used for production of normal seed. Again experience has been limited, but from the information available it appears that the usual isolation requirements for producing normal single-cross hybrids will probably be adequate for producing opaque-2 hybrids. Opaque-2 seed has been produced using isolation distances similar to those required for normal single-cross production, and the results have not indicated significant contamination from outside pollen.

Perhaps the most serious problem associated with seed production of opaque-2 hybrids and varieties is that of maintaining seed viability comparable to that of normal endosperm types. Some opaque-2 genotypes tend to have cold-test germination percentages that are lower than comparable lots of normal seed by as much as 20%. Warm-test germination rate is frequently 5 to 10% below normal types. It is assumed, although not proved, that mechanical injury during seed handling causes the differences in seed viability between opaque-2 and normal maize. Probably the floury endosperm is more susceptible to kernel damage during the handling procedures for normal seed. If this problem persists, and if the use of opaque-2 hybrids becomes widespread, special handling methods might be necessary for this type of seed. However, it might be easier to alter the endosperm constitution of opaque-2 genotypes through selection than to devise more gentle methods of seed handling applicable to large-scale production.

A survey was conducted among the major hybrid maize seed producers in 1972, in an attempt to obtain specific information on the quantity of opaque-2 and/or floury-2 hybrids currently being produced and utilized in the United States. The questionnaire asked for the percentage of 1972 seed sales devoted to opaque-2 and/or floury-2 hybrids, the estimated production

of opaque-2 and floury-2 hybrids by 1975, and the distribution of opaque-2 or floury-2 hybrid seed outside the United States. Answers to these questions were amazingly uniform. The amount of opaque-2 and/or floury-2 hybrid seed distributed in 1972 ranged from none to less than 1% of total hybrid seed sales. Similarly, the amount of opaque-2 seed produced in 1972 (for distribution in 1973) averaged less than 1% of the total seed produced. Among the firms reporting, none were distributing opaque-2 seed outside the United States.

It is obvious from these reports that opaque-2 and floury-2 maize have not become a significant part of the total U.S. maize production. It also was apparent that the individuals responding to these questions were not optimistic about the short-term future of this type of maize in the United States. The estimates for production of opaque-2 or floury-2 maize by 1975 ranged from none to a maximum of 1% of the total maize seed produced. These estimates suggest that not more than 5,250,000 kg of opaque-2 seed will be produced by 1975, which should provide seed to plant only about 240,000 ha of the approximately 24 million ha of maize grown annually in the United States.

The general feeling among those responding to the questionnaire was that maize types with improved amino acid balance would likely play a more important role in areas of the world where protein sources are limited than in the United States, where soybeans provide ample quantities of high-quality protein.

FARM EXPERIENCE WITH OPAQUE-2 MAIZE

As might be expected, the limited farm experience with high-lysine maize has varied from good to poor. In the United States, most production has been used in swine rations on farms where the maize is grown. Where comparisons have been made, the superior nutritive value of opaque-2 has been demonstrated, thus confirming the results of earlier feeding tests with swine. In some locations, farmers have experienced significant yield loss and reduced grain quality owing to severe damage from ear mold. Among those farmers, there is little interest in continuing to use opaque-2 hybrids.

Some growers, particularly in the northern U.S. corn belt have had difficulty establishing satisfactory stands of opaque-2 hybrids. Other growers noted that germination and seedling survival rates of opaque-2 hybrids did not compare favorably with the normal hybrids to which they were accustomed, while other growers had no difficulty in this respect.

There are no provisions in the U.S. grain-marketing channels for the special handling or pricing of opaque-2 maize. Thus, the generally lower yielding capacity of opaque-2 types, as compared to normal maize, limits its use to those growers who are also swine producers. This situation is likely to continue in the United States until high-quality protein hybrids are de-

veloped with yields more comparable to normal maizes, or until the feed trade is willing to pay for the improved feeding value of such maizes. There is no indication that the latter is likely to occur.

It is important to recognize that the factors which have limited the widespread acceptance of opaque-2 maize in the United States are not necessarily the same factors which limit its use in other parts of the world. In the United States, maize is used primarily as a source of feed. A small percentage of the total production is consumed as human food. Conversely, in many countries of Latin America and Africa, and in parts of India, maize is an important element in the human diet, and little of the crop reaches feed channels. Man is a creature of habit, and his food habits change very slowly, even though a change might be to his advantage nutritionally. Therefore, in parts of Latin America, Africa, and Asia, the decision to use opaque-2 maize will depend largely upon consumers' attitudes toward it as a food.

Pinstrup-Andersen's report (4) indicated that the majority of Colombian housewives queried would not use opaque-2 maize for making *mazamorra* and *arepa* simply because it was not the type of maize customarily used for those purposes. Similarly, opaque-2 maize may be better accepted in the high elevations of Latin America, where the ordinary maize is of floury endosperm texture, than in the lowlands where flints and semiflints are most common. Preferences are well established with respect to endosperm color also. It would seem foolish to introduce yellow opaque-2 maize into areas where only white maize is used for food, for example.

Despite his personal strong food preferences, man seems to be completely objective in his selection of feed for livestock. The critical factor here is economics, and it is my impression that the farmer will use almost anything for livestock feed which is economically profitable.

A second factor worth recognizing when introducing opaque-2 maize is the fact that the crop will be produced by farmers. Their acceptance of the new crop will depend primarily upon whether it is more profitable than the one being grown. Although they may recognize the need for protein-enriched maize, the decision to produce it will depend upon relative profit, and not upon the nutritional needs of the consumer. Therefore, in the absence of special price supports, the yields of opaque-2 or other types of maize with modified amino acid balance will have to be comparable to those of varieties that the farmer is accustomed to growing. This is, and will continue to be, a fact of life, irrespective of the nutritional merits of new introductions.

The tremendous potential value of opaque-2 and floury-2 maize to human and animal nutrition is well recognized today and is in itself a significant contribution to human welfare. It would seem, however, that the great amount of research on the nutritive value of cereals now in progress, which was stimulated by the early work with maize endosperm mutants, may be of even greater potential value. Those engaged in the breeding of most

cereals today are giving attention not only to further improvement in yield potential, but to improvement in quality as well.

Although much of this report may indicate a somewhat disappointing degree of acceptance of the opaque-2 maize now available, the future seems optimistic for the use of this and other similar modifications of maize. Considering the short time in which breeders have been working with opaque-2 maize, it is not surprising that few, if any, varieties are available today which compare favorably in most traits with the best normal hybrids now in use. It should be remembered that the latter have been bred intensively for almost a half century. The newer materials emerging from breeding programs around the world make me confident that much maize of the future will be of types possessing improved nutritional value. This is said while recognizing that much work remains to be done, and that the physical characteristics of the endosperm that we associate with high-protein quality maize today almost surely will be replaced with more acceptable types.

SUMMARY

1. Opaque-2 hybrids and varieties with adequate backgrounds of selection and testing are just now emerging from breeding programs. These are much superior to those hybrids which were in limited use 2 to 3 years ago.

2. Undesirable kernel characteristics and reduced yielding capacity (relative to normal maize) are still major deterrents to the widespread acceptance of opaque-2 maize. To overcome these problems, breeders are now concentrating on (a) selecting modified endosperm types of higher test weight and (b) selecting double-mutant endosperm combinations. Both approaches appear to offer considerable promise.

3. Greater susceptibility of some opaque-2 genotypes to disease and insect damage is a problem that needs to be constantly monitored and selected against.

4. There appear to be no inherent problems in seed production of opaque-2 maize that cannot be overcome. Reduced seed viability and seedling vigor can be a problem in some opaque-2 genotypes.

5. In the United States, opaque-2 maize has not yet become a significant part of total maize production. It has been estimated that by 1975 not more than 1% of the total U.S. production will be opaque-2 maize. This percentage could change rapidly with the appearance of better hybrids. In parts of Latin America, interest in opaque-2 maize has been stimulated through subsidy payments that encourage production.

6. Expanded research on the nutritive value of cereals is an important by-product of the original work with opaque-2 and floury-2 mutants. The new materials coming from breeding programs today indicate that much of the maize of the future will possess improved nutritional value.

The author acknowledges, with thanks, the use of much unpublished data kindly provided by Alix V. Paez of the Department of Corn Breeding, Pioneer Hi-Bred International, Inc.

[*A discussion of this paper can be found on pp. 497–498 of* **Questions and Answers.**]

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PRODUCTION, PRODUCTIVITY, AND THE USE OF OPAQUE MAIZE IN COLOMBIA

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For the last 4 years (1968–1972), the total maize production area in Colombia remained stable at about 700,000 ha annually. Use of improved varieties stabilized also, reaching 25% of the total maize cultivation in 1968 and remaining at that level. These improved varieties have characteristics similar to the maize traditionally grown in Colombia.

It does not seem likely that the area devoted to production of opaque-2 maize will increase soon. Rather the area planted in opaque-2 maize probably will remain constant, at a low level, following the pattern of other improved maize varieties. It is necessary to emphasize that Colombia is a mountainous country, where 43% of the total maize production is planted on sloping terrain, using traditional agricultural methods. This usage pattern partially explains the leveling off in the amount of improved maize being planted.

DEVELOPMENT OF OPAQUE-2 IN COLOMBIA

Two commercial hybrids (ICA H 208 and ICA H 255) have been developed in the process of transforming Colombian varieties, which are adapted to hotter climates, to high-quality protein maize. This work was initiated in 1965, and the first two hybrids were distributed to farmers for commercial production in 1969. The two hybrids produced an experimental yield of 5.6 tons of grain/ha, scarcely 10% lower than that of their normal counterparts, ICA H 207 and ICA H 253.

A favorable governmental attitude for promoting the use of opaque-2 maize in Colombia was created by the excellent results shown by opaque-2 maize in nutrition trials with undernourished children, as well as in trials with swine, poultry, and in industry. In November 1968, a presidential decree created the Committee to Promote Opaque-2 Maize. The first promotion plan was devised at that time by the Director of the Maize and Sorghum Program of Instituto Colombiano Agropecuario (ICA). The plan included regional production trials, the use of opaque-2 maize as a human food and for swine feeding and the use of radio, television, and regional seminars for information dissemination.

In 1968, ICA produced 48 tons of opaque-2 maize, the first sizable commercial production. This was distributed free for industrial, human, and animal nutrition tests.

Production of improved seed was left to the initiative of private and semiofficial enterprises. They distributed 15 tons of seed to farmers in 1969, and 199.6 tons in 1971. Commercial production was 48 tons in 1968 and 2,925 tons in 1969, the only years for which statistics are available. The areas planted in opaque-2 maize increased from 975 ha in 1969 to 12,974 ha in 1971. Plantings in the first half of 1972 increased again; 64 tons of seed were sold to farmers and approximately 14,160 hectares were planted.

In April 1969, an agreement was signed between ICA and Centro Internacional de Agricultural Tropical (CIAT) for conducting regional trials of opaque-2 maize. The trials were to determine the area of adaptation of the opaque-2 maize varieties, their acceptance, and profitability, and to make comparisons with local maizes and the best hybrid for the region, using different technologies. Good performance was shown at altitudes from sea level up to 1,700 m. The average yields of the regional trials (1969–1971) varied from 3.2 to 3.7 tons/ha showing a superiority of at least 1.0 ton/ha over the local maize.

MAIZE UTILIZATION

Production of opaque-2 maize has been concentrated largely in the areas of the Cauca Valley, the Atlantic Coast, and the Santanderes and the Arauca region on the eastern plains. Production from the Cauca Valley is destined principally for industrial processing; the other areas produce maize for human consumption or animal feeding. People of the Atlantic coast and the Santanderes regions traditionally have eaten floury maize.

Maize is consumed by the people in several forms, principally *arepas* and *mazamorra*. The Institute of Family Welfare (Instituto de Bienestar Familiar) studied the traditional forms of maize consumption using opaque-2 maize, and published a bulletin entitled *Manual de Recetas con Maiz Opaco* (Handbook of Recipes Using Opaque Maize) in 1971. An industrial product called Derivca has been marketed nationally since 1970 and is recommended for

several uses, including infant feeding. The Institute of Technological Investigations has been studying mixtures of opaque-2 maize and wheat flour for bread making. It has been found that opaque-2 maize flour can be substituted for 30% of the wheat flour without impairing baking quality, while increasing the nutritional value of the bread.

SUMMARY

Since there is a tendency for the production of opaque-2 maize to level off, more aggressive campaigns must be initiated in areas where the consumption of floury maize has been a tradition, such as in the Santanderes and the Atlantic Coast regions.

There is a need to produce flint maize with a high nutritional value and a yield equal to or better than that of the improved maize now distributed. With this objective, ICA started to analyze flint segregants from the opaque-2 hybrids ICA H-208 and ICA H-255 in 1970. Inbred lines derived from these hybrids have been developed that are flint type and superior in protein quality.

[*A discussion of this paper can be found on pp. 498–499 of* **Questions and Answers.**]

PRODUCTION AND ACCEPTANCE OF OPAQUE-2 MAIZE IN BRAZIL

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Maize, Brazil's largest grain crop, must play two major roles in both satisfying the actual food deficit and feeding Brazil's ever-increasing population. Despite the recent remarkable advances within the country's total economy, a gigantic task lies ahead in order to keep food production abreast of growth in overall gross national production. The immensity of this task is indicated by Brazil's population, which reached 100 million people in August 1972 and is now growing at the rate of about 2.7% annually, for a net increase of nearly five persons every minute. At this rate, the population will probably double by the end of this century.

Brazil's total maize production for the last 2 years was about 13.5 million metric tons yearly, the second largest maize production in the Western Hemisphere.

Maize is produced throughout the country, although 80% comes from the central-southern states between 16 and 32° latitude and at altitudes ranging from sea level to 1,000 m. The total area devoted to maize production is about 10 million ha, more area than to any other crop. Only one third of this area is planted with improved seed. The average yield is about 1,350 kg/ha.

Maize utilization data averaged for the last 3 years indicate that about 65% of the grain was consumed on the farm by domestic animals (hogs, chickens, dairy cattle, working horses, and mules), about 25% was milled for food and feed; and about 10% was exported.

OPAQUE-2 MAIZE

The Federal University of Viçosa in the state of Minas Gerais brought the first opaque-2 maize seed into Brazil in 1965, from Purdue University. Maize breeding work at Viçosa, the College of Agriculture at Piracicaba, and at the Agronomic Institute of Campinas has produced improved populations bearing the opaque-2 gene. Agroceres (a private seed company), breeding two generations a year, has developed high-yielding opaque-2 hybrids that are well adapted to tropical conditions and quite well accepted by farmers. Other public institutions have joined the four pioneers for continuing research.

Opaque-2 maize now has attracted the attention of federal and state agencies. The Extension Service has become keenly interested as results of experiments and demonstrations made by universities and private organizations have shown the excellent feeding value of opaque-2 maize protein, particularly for human infants and hogs.

The remainder of the paper will focus on some particular developments and problems related to opaque-2 maize production and utilization in Brazil.

Seed Production

Both Viçosa and Campinas are selling improved opaque-2 seed, the latter through the State Secretariat of Agriculture of São Paulo. Viçosa's 1972 production is small, although about 690 tons of improved seed has been made available to farmers by the secretariat.

The first 100 tons of the Agroceres hybrid opaque-2 seed was sold in 1970, mainly to farmers who produced feed for hogs on their own farms. In 1971, Agroceres' production increased to 400 tons, and 900 tons was on the market in 1972, as compared to 23,000 tons of normal hybrid seed. This may seem a rather modest start, but, by comparison, it took Agroceres 6 years to match this amount in the early days of hybrid seed maize introduction in Brazil.

Even though opaque-2 seed costs 17% more for Agroceres to produce than does normal hybrid seed, it is sold at a 15% higher price.

Farmers' Production Problems

There have been three rather universal restrictions to the commercial production of opaque-2 maize: (1) lower yields, (2) increased susceptibility to ear rots, and (3) increased vulnerability to weevils, both in the field and in storage.

Fortunately, the first two restrictions have not been encountered in Brazil, at least with Agroceres hybrids. Farmers are finding opaque-2 maize to yield quite satisfactorily. Because most farmers evaluate yield by volume rather

than by weight, some even declare that opaque-2 hybrids are yielding as well or slightly better than normal hybrids.

To check this condition, several experiments have been conducted. In one of them, three of the best normal hybrids and two Agroceres opaque-2 hybrids were tested in six different localities. The results are shown in Table 1. Statistical analysis showed no significant yield differences among hybrids. Ag-504 ranked second in ear rot resistance. Its excellent husk protection decreased the risk of attack by weevils and rots while the crop was drying in the field.

Storage Problems

Under typical farm conditions in Brazil, storing opaque-2 maize presents a serious problem because it is more vulnerable to insects than normal maizes. However, earlier harvesting and proper treatment, which also should be used on normal maize, have provided satisfactory results.

Appearance

The unusual appearance of opaque-2 kernels is a potentially serious marketing problem. If there is large-scale production of opaque-2 maize for general use before its superior feeding value is properly appreciated, its different appearance could have an overall negative effect on commercialization. To avoid this pitfall, Agroceres' strategy has been to sell seed to selected farmers who raise hogs.

Despite this selective introduction procedure, the maize oil and food industries have started production contracts for opaque-2 maize offering a 15% premium above the price paid for normal maize. It seems that selective introduction has been successful and that the general commercial demand for opaque-2 maize can be expected to increase.

TABLE 1 Performance of two opaque-2 hybrids and three normal hybrids (average of six localities, 1971-1972)

Hybrid	Yield	Ears per 100 plants	Lodging[a] (%)	Rotten ears (%)	Moisture at harvest (%)
H 7974 (N)[b]	4,683	99.6	18.9	5.0	11.8
Ag-504 (O)	4,533	106.3	16.0	3.2	12.6
Ag-152 (N)	4,385	83.6	21.9	8.3	13.7
Ag-251 (N)	4,330	100.5	15.2	2.5	12.6
Ag-502 (O)	4,274	94.1	16.8	7.5	12.6

[a] At one location a windstorm increased lodging.

[b] (N) normal; (O) opaque-2.

STRATEGIES FOR EXPANDING OPAQUE-2 MAIZE USE

Hog Feeding

Brazil's total hog herd, a very important source of protein and fat for its population, numbers about 68 million head. More than two thirds of this herd is fed almost entirely on maize.

During the past 3 years, several experiments and demonstrations have compared weight gains of pigs when fed opaque-2 maize, with improvements ranging from 50 to 500% when minerals and vitamins were provided with the grain in both treatments. This is the main reason for inferring that the most efficient way of introducing opaque-2 maize to farmers is by concentrating on those farmers producing maize to feed their own herds.

Considering the importance of pork in the typical Brazilian's diet, plus the government's high-priority policy of liberating as much beef as possible for export, hog feeding should represent an extremely large market for opaque-2 maize. Agroceres is capitalizing on this situation by establishing many opaque-2 maize feeding demonstrations at the farm level with Extension Service cooperation. The goal is to compare opaque-2 maize performance with that of normal maize when fed to hogs under local conditions and with the farmers' direct participation. As a side benefit of this work, farmers are being taught to use minerals and vitamins, which previously were unfamiliar to them. Agroceres' experience has shown that it is easier to introduce a new technology by providing two or more complementary inputs in the same package, rather than separately. As a corollary, as soon as farmers see the results of opaque-2 maize, they seem to become more receptive to other improvements, such as better housing and overall production management.

Along with opaque-2 maize promotion, Agroceres also is breeding high-quality, meat-type hogs and selling them to farmers to improve overall herd performance. This, we hope, will also contribute to wider acceptance of opaque-2 maize by farmers.

Rural Nutrition Project

An interesting rural community project was launched last year, starting with 61 farmers in Vicosa county. It is sponsored by the U.S. Agency for International Development (USAID), under the supervision of the Extension Service with the assistance of the Federal University of Viçosa. This pilot project is an attempt to evaluate the economic expansion of rural nutritional resources through the utilization of opaque-2 maize as a substitute for normal maize.

The geographical isolation of rural populations, low educational levels, and low purchasing power are well-known obstacles in fighting malnutri-

tion, an unfortunate fact of life for virtually all tropical countries. Brazil is no exception. Because of these factors, any program designed to fill nutritional needs in rural communities constitutes a difficult undertaking.

However, the introduction of opaque-2 maize as a substitute for normal maize seems to avoid most of the problems inherent in conventional nutritional programs for rural areas. Conventional programs usually involve an industrial process and theaddition of amino acids, minerals, and vitamins, thus generating transportation and packing expenses. An additional problem is involved in the need for changing peoples' eating habits. However, when the community traditionally plants and consumes maize as in the Viçosa project, the simple substitution of the seed will provide production *and* use of a high-quality-protein grain at the farm level at very low cost.

Data from this project, in its first year, have not been fully evaluated. However, preliminary information from the 61 farmers involved indicates that:

1. The opaque-2 seed (Ag-504) yielded better than normal hybrids planted by neighbors.

2. Opaque-2 maize was more vulnerable to weevils when cribbed. (This problem requires more research.)

3. Opaque-2 maize milled twice as fast as normal maize and produced a finer, faster-cooking meal.

4. Both children and pigs showed preference for opaque-2 maize when offered a choice.

5. Most of the traditional recipes (using maize meal as the main ingredient) tasted better and cooked faster when opaque-2 maize meal was used.

For 1972–1973, a new agreement was signed to establish the same project, on about the same basis as at Viçosa, in eight different counties covering four widely different regions of the state of Minas Gerais. Hopefully, this project will develop a low-cost model program to improve rural nutrition in maize-consuming areas of Brazil and, perhaps, in tropical countries around the world.

Food

Very little human nutrition study has been done in Brazil using opaque-2 maize as a protein source. However, in Patos de Minas, state of Minas Gerais, Paulo J. Amorim has accumulated interesting data on protein deficiency recovery in children, using opaque-2 maize as the only source of protein.

Although maize is not as important a staple food for Brazil as it is for neighboring countries on the Pacific Coast, a fairly large portion of Brazil's population consumes maize, either fresh on the cob or in dishes made from maize meal. Thus, opaque-2 maize also is important for direct human consumption. More research must be done in this field to disclose the virtues and uses of this grain.

Last year the National Campaign for School Lunches (CNAE) spent over $30 million (U.S.) on food consumed by 12 million school children. No

opaque-2 maize was used. Its introduction in the program—as a source of protein as well as energy—might mean food for 1.5 to 2.0 million more children for the same budget.

Public hospitals and nurseries are using rather expensive ingredients, like powdered milk, to obtain the necessary protein for baby feeding. Opaque-2 maize would stretch their budget allocations much farther, without jeopardizing quality. It is our opinion that, with strong support from the government, opaque-2 maize would receive rapid, general acceptance for human consumption.

Food companies are becoming interested in opaque-2 maize. A maize oil factory, for instance, despite difficulties in separating the embryo from the endosperm of opaque-2 maize, is paying a 15% premium for commercial opaque-2 maize. This company found that a particular opaque-2 hybrid has 30% more oil than normal maize. They sell the degermed and ground endosperm, also at a premium, to a large baby food factory.

Another company is about ready to market eight different high-protein maize products—dehydrated soups, soft drinks, macaroni, cookies, and candy—mainly aimed toward school lunch use. The basic formula for these products has undergone careful chemical and biological tests and appears to have a protein of higher quality than that of milk. Extensive acceptability tests involving school children are underway and, so far, results are encouraging. A low-cost, well-accepted product is exactly what the official lunch program is seeking.

Feed

The feed industry is increasing steadily in importance, particularly for poultry, swine, and dairy cattle. Theoretically, this increase should mean an important potential market for opaque-2 maize. However, most feed factories base their business on producing and selling protein concentrates with minerals and vitamins, which the users mix with normal maize meal. New concentrate formulas would be required to be mixed with opaque-2 maize. Therefore, opaque-2 maize represents a challenge to the traditional method of animal feeding in Brazil.

CONCLUSION

Considering what has been accomplished in Brazil over the last 7 years, since the first opaque-2 maize seeds were introduced, the balance is highly positive. Private enterprise has played, and is playing, an important role in the production and use of opaque-2 maize. And if research does not find an even better mutant, opaque-2 maize will grow in importance very rapidly in Brazil. With a few exceptions (where maize might have special uses, as in popped maize and hominy), little can be done with normal maize that opaque-2 maize could not do better. The long-term possibilities for opaque-2 maize in Brazil are practically limitless.

UTILIZING OPAQUE-2 MAIZE IN FOOD PRODUCTS

Denis Robinson *Best Foods Research Center, Union, New Jersey*

The utilization of opaque-2 maize in commercial food products has been limited for at least two basic reasons: first, it has not been economically feasible; and, second, the grain has been evaluated against standards for normal varieties and found lacking in the field, in the villages, and at the mill.

Many of the alleged drawbacks to this high-quality-protein maize have been discussed at this symposium, and I will repeat some of them briefly to set the stage.

Agriculture sector: Some objections to opaque-2 maize from the farmers' point of view are poor germination, higher moisture at maturity, vulnerability to ear rot, brittle kernels, soft endosperm, lower density, and lower test weights. All these factors add up to lower yields and costs increase by as much as 30% above those of normal maize, thus deterring farmers from growing the high-quality protein maize.

Mills: In the processing industry, when the high-quality protein varieties are submitted to conventional dry milling procedures, they produce a more undesirable split of fractions than normal maizes. Thus, they are not of commercial interest, irrespective of initial cost.

When processed by wet milling techniques, the higher content of soluble protein in the opaque-2 varieties generates excessive amounts of steepwater, thus making recovery of gluten more expensive and reducing interest to processors.

Farm and village: The major opponents to acceptance of these new varieties, and yet the key to their future, are the villagers and subsistence farmers. To them, opaque-2 maize is virtually unacceptable for use in such staples as *tortillas* and *arepas*. This maize does not conform to their customary practices.

The preceding objections seem to be the prime motivating forces pushing so much research effort into breeding programs to develop flinty varieties of high-quality-protein maize.

Maybe the time has come to devise ways to grow and use the varieties already available, rather than to wait until further genetic development creates the ideal variety. Must we continue to deny the needy the benefits of high-quality protein maize because they are inhibited by tradition at the village level; and are we tied to past practices in creating foods? Maybe we should spend more effort promoting the product and teaching people how to use it.

EFFECTIVE PROTEIN VALUE

Let us review the situation then, starting with the agricultural aspects, and see whether we can present an argument favoring the spread and use of currently available high-quality protein maize.

First, we must recognize the true food value of these varieties, looking beyond the quantitative aspect of yield and considering the *effective protein value* of the crop. What contribution can this maize make to the total food supply? These considerations should become prime motivating forces. Figure 1 is a simplified explanation of the significance of effective *protein value*, comparing high-quality protein varieties with normal maizes.

The data used in this diagram were provided by E. Mertz from research done in several laboratories within the past year, and are based on the protein efficienty ratios (PER) established by research collaborators, using the PER of casein as a standard.

Issues of yield differences, even on the order of 30%, are dwarfed by the importance of this protein-value factor. The effective protein value of

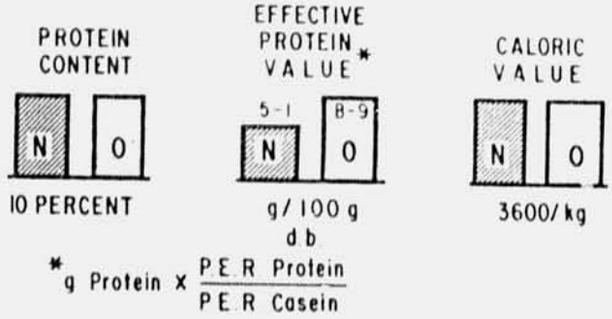

FIGURE 1 Comparative food value of normal and opaque maize.

opaque-2 maize is, on the average, almost 75% greater than normal maize and, as seen in Figure 2, presents the interesting possibility for getting more food value per hectare of maize.

Sufficient evidence has been presented by nutritional researchers to prove the effectiveness of this protein for children in maize-eating communities. Ricardo Bressani of the Institute of Nutrition of Central America and Panama (INCAP) has reported that a 5-year-old child consumes only 130 g of maize, or about one third of that child's requirements to obtain positive nitrogen balance if the diet were normal maize. However, 130 g of opaque-2 maize would not only be adequate, but would also give nitrogen-retention values close to those of milk protein.

Nevin Scrimshaw and co-workers at the Massachusetts Institute of Technology equated the protein quality of opaque-2 maize with milk powder in the diets of young adults.

Using these data, let us now project their significance into the total food situation in Mexico. Table 1 indicates that if all maize produced were opaque-2 maize, we would get much more food value per hectare of maize at much lower cost, and could make a greater contribution to the food needs of the people.

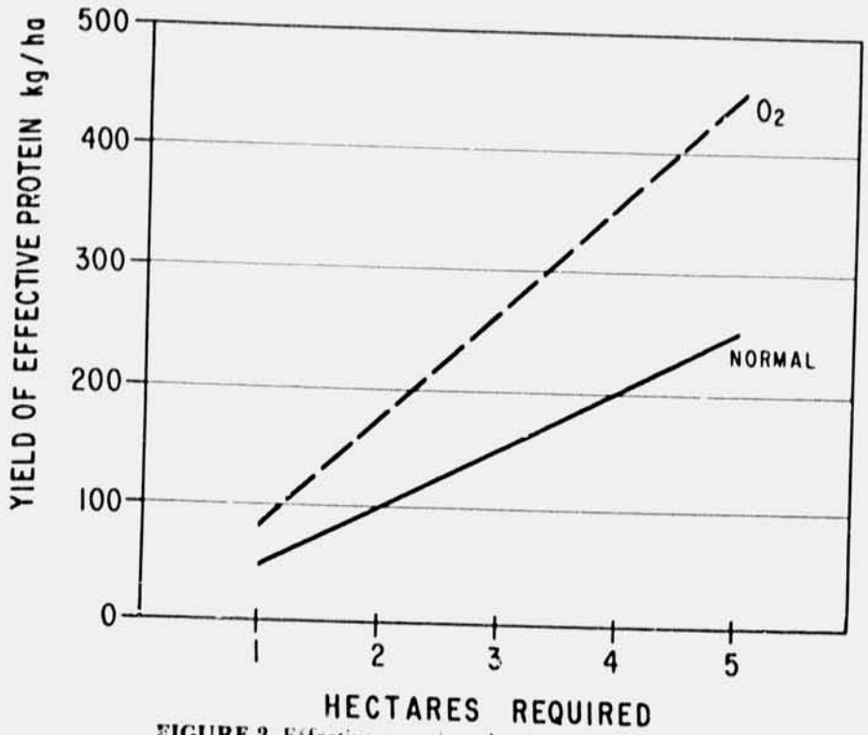

FIGURE 2 Effective protein value in agriculture.

TABLE 1 Effect if opaque maize replaced normal maize for *tortillas* in Mexico

Annual	Millions of tons
Average crop	8
Average use as human food	5.6
Used in *tortillas*	4.5
Opaque maize	2.5

Another way to evaluate this protein value appears in Table 2, which shows the economic value of opaque-2 maize protein compared with milk protein.

Regrettably, the favorable nutritional aspects of the opaque-2 maize product are reduced during normal milling procedures. Few commercial millers grind their maize as whole meal, and must rely on better yields of oil and grits to make their business profitable. Table 3 shows the reactions of opaque-2 maize under factory conditions, and indicates more clearly why millers object to it.

Table 4 indicates the dilution of the protein in those fractions which are considered most useful by commercial millers, and Table 5 depicts the shift of the lysine in the process.

The sad truth emerging from these data is that those fractions of opaque-2

TABLE 2 Value of opaque maize versus skim milk powder

Skim milk powder	≠	opaque maize
Price so recognizes		
\$0.90/kg		\$0.15/kg
Protein in skim milk	≠	protein opaque
35%		10%

But 35/10 is less than 90/15 at 3.5 < 6

This fails to recognize extra calories from maize.

More realistically we should consider

1 part skim milk + 2.5 parts low-cost carbohydrate source (e.g., molasses sugar).

\$0.90 + 2.5 × \$0.18 = \$1.35

3.5 kg opaque = \$0.525

Opaque maize is 2.5 times the better buy when we talk = calories = protein.

TABLE 3 Typical milling yields in U.S. systems: normal versus opaque maize (percentage of yield)

	Germ	Grits	Flour	Feed
Normal	14	48	22	16
Opaque	18	30	56	16

TABLE 4 Protein levels in opaque maize fractions after milling (%)

Germ	Grits	Flour	Feed
15	7.5	6.4	10

TABLE 5 Lysine distribution in opaque maize fractions after milling

	Germ	Grits	Flour	Feed
Grams of lysine/100 g	0.85	0.25	0.25	0.58
% Total lysine	37.5	10.5	36.0	16

maize evolved from dry milling (grits and flour) are certainly least attractive nutritionally. However, the germ fraction and the animal feed elements are nutritionally better. This is as it was before man in his economic evolution discovered (1) that the oil from the maize germ was valuable and (2) that oil was the cause of rancidity in whole meal after milling.

Therefore, Best Food's laboratories are directing their research to the use of opaque-2 maize in whole meal form as an ingredient in prepared foods. It made its debut in Duryea, an infant weaning food first available in Colombia. The whole meal form will probably not be utilized on a larger scale or in other commercial products until there is a greater production of opaque-2 maize.

Meanwhile, our research seeks to learn how to make more effective use of this excellent food source if and when opaque-2 maize becomes more popular and readily available.

Let us now discuss some other ways in which opaque-2 maize can be used, remembering we are recommending that it always be as a whole grain or meal. Table 6 shows the major food categories, other than on-the-cob, in

TABLE 6 Utilization of maize

Product categories	Form of maize
Soups[a]	Whole or ground
Breads[a]	Whole or ground
Casseroles	Whole or ground
Relishes	Whole or ground
Cereals	Whole, ground, or extruded
Cereals for infants	Ground or extruded
Desserts	Ground or extruded
Puddings	Ground or extruded
Beverages	Ground or extruded
Candy	Ground or extruded
Snacks	Whole or extruded

[a] Village developments—now.

which maize kernels, grits, meal, or flour are normally used. A variety of foods is included, and there is no dish currently made with normal maize that cannot be made better with opaque-2 maize. We can truly say "from soup to nuts." But we repeat, it will take much time and effort to show people how to use the new types.

A prime example of maize utilization is, of course, *tortillas*. In countries where they are consumed as the principal diet, this food category must become the starting point for real acceptance of new maize types. Figure 3 shows the classical procedure for making *tortillas*. Using the opaque-2 maize, one brings the mixture of maize, lime, and water up to a boil, then removes it from the fire, covers it, and allows it to soak as normal. The only change necessary, is to eliminate the 50-minute boiling. Everything else remains the same, and it works.

This method is equally applicable to commercial operations for *tortillas*, *tortilla*-type flours, or maize chips, and permits production of products equal in texture and appearance to those made with normal maize and usually superior in acceptance for flavor.

We recommend using very simple reduction methods for making maize meal from the softer maize types. Stone *mase* mills work well. Good results can also be obtained on a larger scale by making an initial break in a hammer-type mill, followed by further reduction in pin or micromills. Excellent results have been obtained with these procedures, even without hull removal. If a more refined flour, without fiber, is desired, it can be produced by chemically removing the hull with acid or alkali. Whole kernels treated this way also have been further processed into nut-like snacks with high consumer acceptability. New approaches using extrusion techniques

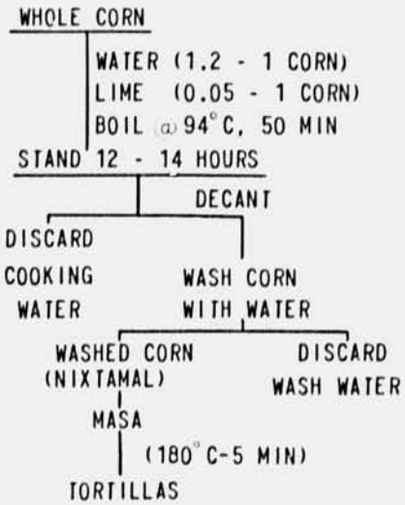

FIGURE 3 Classical method for preparing *tortillas*

are opening up wider areas of potential future use for opaque-2 maize in the industrial sector. However, the main thrust must still be to encourage use of opaque-2 maize at the village level. Then, when greater amounts are produced, industry will be ready to convert it into products for use in the cities. At this time, however, it simply is not economically viable.

Another use for opaque-2 maize has emerged as a result of experiments in the United States. A high-quality-protein maize meal cereal is being used successfully as the principal diet for phenylketonurics. This was reported initially by S. Coburn of Fort Wayne State Hospital during the Ninth International Congress of Nutrition in Mexico City in September 1972, and I have had the pleasure of cooperating with him in the program.

Purely as a result of random genetic error, these unfortunate children (phenylketonurics) are generally unable to metabolize phenylalanine into tyrosine, a condition that leads to severe mental retardation and poor behavioral patterns. Treatment through dietary therapy with proprietary foods is necessary to prevent or reduce brain damage and is a very costly procedure. The high-quality protein maize preparation looks very encouraging as a substitute and is, of course, much lower in cost than proprietary foods. Preliminary studies also have shown marked behavior improvement in patients given high-quality protein maize, leading to the belief that this may be the best treatment yet found for older institutionalized patients.

Other uses for opaque-2 maize will emerge, but I repeat that we ought to spend more time and money showing people how to use the varieties already available and be less concerned about genetic alterations. The sooner this is done, the sooner millions of people consuming maize as their staple food will emerge from the constant threat of malnutrition.

[*A discussion of this paper can be found on p. 499 of* **Questions and Answers.**]

Part VII

CHEMICAL AND BIOLOGICAL EVALUATION OF MAIZE PROTEIN QUALITY: CURRENT ISSUES AND PROBLEMS*

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Better methods for chemical and biological evaluation of cereal protein quality are needed for further improvements of high-quality protein maize, and other cereals as well.

Since the exciting discovery of high-lysine maize genotypes by Mertz, Nelson, and Bates (8, 11), research has proliferated in seeking to breed agronomically successful lines and to investigate their benefits in foods and feeds. However, faster, less-expensive, small-sample chemical methods of protein quality evaluation in maize are needed for the increasingly large numbers of breeding selections to screen for better lysine content and other attributes. And when high-quality protein maize enters commercial channels, simple procedures will be needed to distinguish it from other kinds of maize, especially if vitreous grain types of high-quality protein maize are developed and grown commercially.

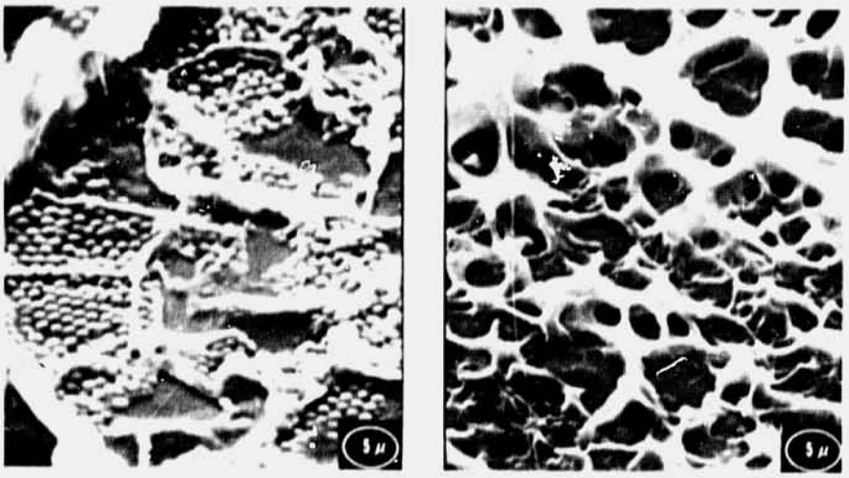
*Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Biological testing is also essential for evaluating actual protein utilization. As kernel characteristics and protein types in maize are altered, changes in the digestibility of its protein may occur. Although not found in current selections of high-lysine maize, possible harmful levels of antinutritional factors, such as trypsin inhibitors or phenolic compounds that impair protein utilization, could be detected by biological tests. Furthermore, one must be certain that processing grains for foods or feed does not reduce the nutrient value of the proteins.

Finally, to understand these problems better, we must fully appreciate the nature of maize proteins, and we must investigate the differences in proteins due to genetic selection that can influence amino acid content and nutrient availability. Perhaps future breeding programs can be better directed, with a clearer concept of the genetic factors that govern the synthesis of maize protein.

LOCATION OF MAIZE PROTEINS

The scanning electron micrographs in Figure 1 show impressive differences in protein-rich subcellular components of normal and high-lysine endosperm sections. The starch granules were removed by enzymic treatment so that the insoluble proteins could be seen (21). In a normal maize



Normal High Lysine

Maize Protein

FIGURE 1 Scanning electron micrographs of sections of endosperm from normal and high-lysine (opaque-2) maize (starch granules removed by treating section with amylase).

section, strands of matrix protein engulf large numbers of prominent protein bodies. By gradient ultracentrifugation, we have been able to separate protein bodies from matrix protein in homogenates of the immature endosperm (1). Based on amino acid and starch gel electrophoretic analysis, we verified that the matrix protein is composed of glutelin (the alkali-soluble fraction of maize proteins). As reported earlier by Duvick (3), the protein bodies were shown to consist almost entirely of zein (the alcohol-soluble, lysine-deficient maize protein fraction.)

In contrast, the protein bodies in the high-quality-protein maize section (Figure 1) are so small as to be almost obscured by the larger amount of glutelin matrix. The differences in structures of subcellular particulates were demonstrated by Wolf et al. (22). Earlier studies by Mertz et al. (8) and Mosse and co-workers (10) showed that normal and opaque-2 maize differed in the quality of maize protein, owing mainly to a decrease in the proportion of zein to glutelin, albumin, and globulin proteins; however differences in glutelin composition between genotypes were reported. Until recently, the causes of glutelin protein variability remained a mystery, because glutelin is insoluble in most protein-dispersing agents, and is destroyed by alkaline solvents.

GLUTELIN PROTEINS

The tough, insoluble glutelin matrix binds the endosperm starch granules and protein bodies together. Foster et al. (4) and Mertz and Bressani (7) reported that most maize proteins could be solubilized by alkali, and also by such reducing agents as sulfite or mercaptoethanol in the presence of dissociating agents like surfactants. As shown in Figure 2, we found that glutelin consists of several different polypeptide chains linked by disulfide bonds (due to the amino acid cystine) to form an insoluble three-dimensional matrix (12). After the disulfide bonds were cleaved by the reducing agent (β-mercaptoethanol), the polypeptide chains were liberated and could be stabilized by alkylating the sulfhydryl groups. The different polypeptides could then be separated by gel filtration chromatography on Sephadex G-200 columns with 6 *M* guanidine hydrochloride, as shown in Figure 3 (13).

The separated polypeptides differed in molecular size, electrophoretic mobilities, and amino acid composition. Certain of the smaller polypeptide chains eluted at a volume near the elution position of zein. Unlike the other polypeptides, they were deficient in lysine and had low electrophoretic mobilities. They could be dissolved in and extracted by 70% ethanol, as are zein proteins, but these proteins were higher than zein in methionine and cystine content.

As shown in Table 1, the alcohol-soluble subunits constitute an appreciable proportion of the glutelin protein of normal maize (14). When added to the zein obtained by direct extraction of meal with 70% ethanol–0.5% sodium acetate, this protein further contributes to the low nutrient value of

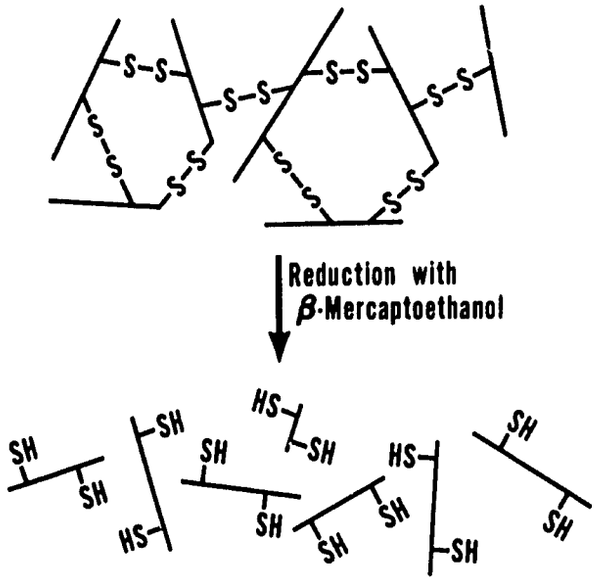


FIGURE 2 Pathway of disulfide bond cleavage through glutelin structure.

normal maize protein. In contrast, the larger amount of glutelin in opaque-2 maize contains a smaller proportion of this zein-like protein (14).

The observation that an alcohol-soluble fraction of glutelin can be extracted from reduced glutelin was also made independently by Landry and Moureaux (6). They further separated the remaining reduced glutelin into two additional fractions, one soluble in alkaline buffer and the other soluble in the surfactant, sodium dodecyl sulfate. Differences between normal and opaque-2 maize in amounts of alcohol-soluble glutelin subunits were confirmed by Sodek and Wilson (17).

Possibly, the opaque-2 gene favors production of more glutelin at the expense of zein, and also may reduce the content of components low in lysine in glutelin.

CHEMICAL EVALUATION OF PROTEIN QUALITY

The relative changes in amounts of different proteins in opaque-2 and floury-2 genotypes are reflected in a better balance of essential amino acids, especially lysine, tryptophan, and leucine (8, 11). Direct analysis of one of these amino acids is the most obvious way in breeding programs to survey protein quality in maize grain. Since lysine is usually the limiting amino acid in cereal-rich human diets, much attention has been given to its determination. The automated amino acid analyzer which separates amino acids in hydrolyzates by chromatography and determines them colorimetrically is by

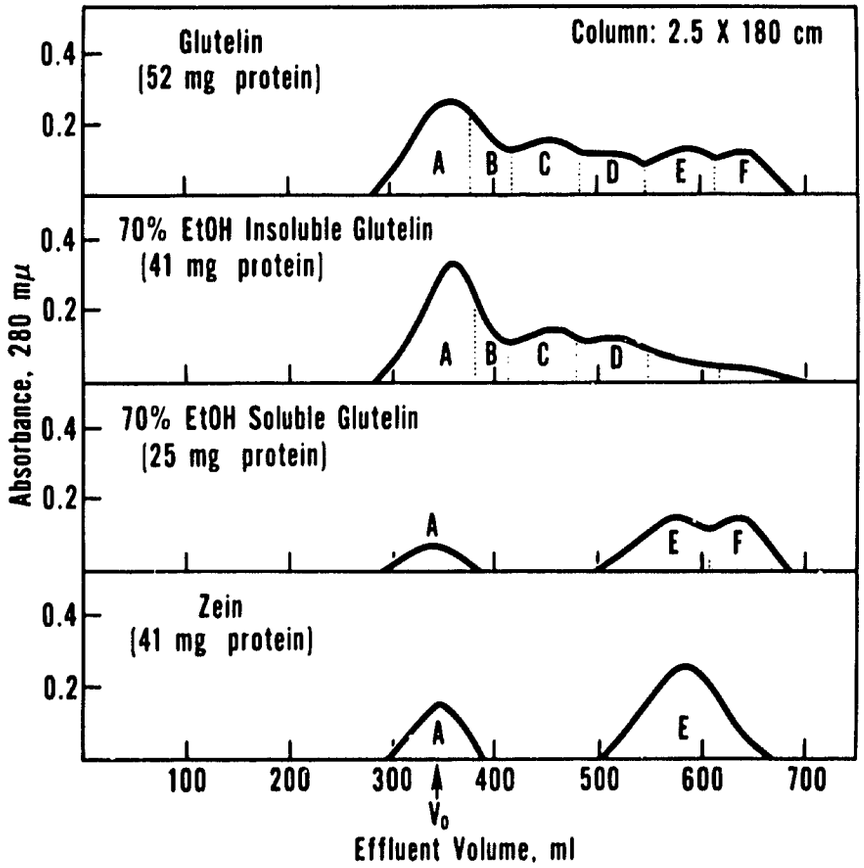


FIGURE 3 Effect of 70% ethanol extraction of alkylated-reduced proteins on Sephadex G-200 chromatography.

TABLE 1 Alcohol-soluble protein subunits in reduced glutelins from normal and opaque-2 maize

Item	Type of protein	Normal	Opaque-2
1	Glutelin in total protein (%)	46.5	55.2
2	Alcohol-soluble protein subunits in reduced glutelin (%)	14.4	7.8
3	Alcohol-soluble glutelin in total protein (%) (1 X 2)	6.7	4.3
4	Zein in total protein (%)	45.8	24.4
5	Zein + alcohol-soluble glutelin subunits, % of total protein (3 + 4)	52.5	28.7

Source: Paulis et al (14).

far the most reliable tool for this purpose. Recent improvements have accelerated its rate of analysis, automatic sample application, and computerized data analysis to a capacity of 96 lysine analyses in 24 hours. However, the instrument and its maintenance are costly.

Alternative methods used by some breeders for analyzing maize lysine include microbiological assay, based on the growth of lysine-requiring bacteria (16), and measuring carbon dioxide liberated by the enzyme lysine decarboxylase (20). Analysis of protein by forming the di- or trinitrophenyl derivatives of lysine, which can be determined colorimetrically, has been widely explored. However, when complete derivatization is sought, solubility of maize proteins becomes a problem. Subramanian and co-workers (18) overcame this problem by using proteolytic enzymes to disperse the protein. Concon (2) developed a procedure based on solubilization of 95% of the maize protein by alkali. These techniques have improved the accuracy and increased the rate of colorimetric analysis of lysine.

However, even simpler methods are desirable. Since we know that the lysine content of maize is related to its complement of different proteins, many investigators have sought to use differences in amounts or properties of the proteins as criteria for protein quality. For example, two groups of workers have reported the successful use of dye binding, based on the Udy procedure, to establish protein quality (5, 9). Since the amount of dye bound is a function of the basic amino acid content of the grain, this procedure permits estimating lysine content.

At the U.S. Department of Agriculture Northern Regional Research Laboratory, we have evolved a simple technique for rapid turbidimetric analysis of zein in extracts of ground maize, and we have shown that a strong correlation exists between zein content and lysine analysis (15). Ground maize (200 mg) is shaken with 20 ml of 70% ethanol containing 0.5% sodium acetate for 60 min. Next, 6 ml of 1.0% sodium chloride solution is added to a 20-ml portion of the supernatant. The turbidity formed due to precipitation of zein is measured in a colorimeter. Differences between extracts of normal and high-lysine maize can be detected visually. In Figure 4, the absorbance due to turbidity at 590 nm g of protein is plotted against lysine content. Lysine content can be approximated from the standard curve by determining the absorbance of the turbid solution. The method has a correlation coefficient of 0.87 and a standard error of ± 0.3 g of lysine/100 g of protein. This simple procedure uses inexpensive reagents and equipment, and is capable of high capacity. It affords a good approximation for initial screening or testing.

BIOLOGICAL EVALUATION OF NUTRITIVE VALUE OF MAIZE PROTEIN

Good amino acid balance is not the only requirement for nutritional value. Maize protein must be digested, and its amino acids must be available

biologically. The subcellular structure of the maize kernel and the solubility of the proteins influence their digestibility. Therefore, additional experiments are needed to evaluate the utilization of protein in grains that differ in protein composition and kernel structure. Enzymatic systems have been developed for tests of *in vitro* digestibility of protein that give good correlation with *in vivo* data. But for a true estimate of nutritive value, tests with laboratory animals are required. Suitable test animals and diets must be investigated and selected if results are to be interpreted properly.

Because of the vitreous nature of dent or flint maize, the grain must be processed for use in food products, either steeped in lime to form *masa* or dry milled by modern, sophisticated equipment. When high-lysine maize becomes available generally, care must be taken that its nutrient content is not diminished by processing and food product preparation. In the past

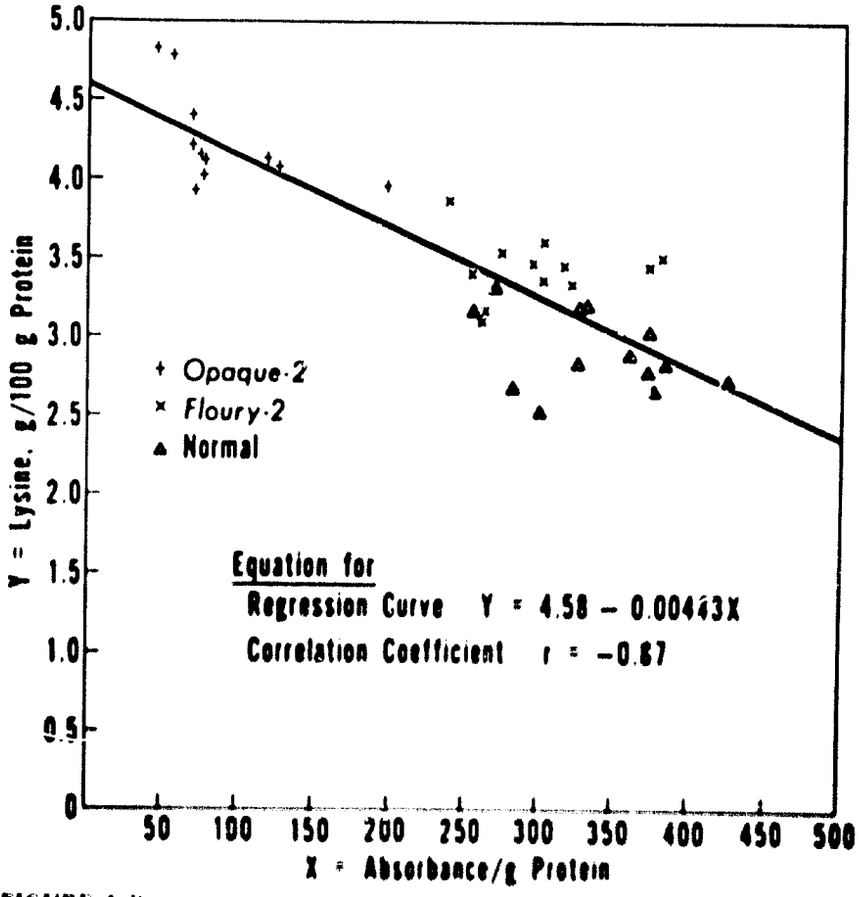


FIGURE 4 Regression curve relating the absorbance at 590 nm/g of protein to grams of lysine per 100 g of protein in maize samples (absorbance measured on 70% ethanol–0.5% sodium acetate extracts made turbid by adding salmon)

little attention has been given to maintaining quality during processing, owing to the poor quality of normal maize protein.

Modern means of dry milling maize to remove germ and hull are used in most parts of the Western Hemisphere, and it is likely that they will become more widely used in other maize-consuming areas, especially as urbanization progresses.

Some of the by-products of the dry milling of maize are now relegated to feed uses. Deoiled maize germ, constituting 10% of the milled products, contains 20% protein that has a favorable amino acid balance (19). But the commonly used expeller process of removing the valued oil from the germ destroys its nutritive benefits. Reaction with carbohydrates during heating converts lysine to a biologically unavailable form.

As shown in Table 2, lysine content, available lysine, and protein efficiency rating (PER) of germ remain high—if germ is gently dried and if oil is extracted at low temperatures with solvents. Available lysine was measured by a chemical procedure. The PER was determined experimentally, based on the growth of rats, using 10% protein diets. Available lysine and animal growth rates are lowered slightly when the germ is dried by heating. However, the high temperatures used to facilitate expeller removal of oil destroy much of the lysine and nutritive value of the germ. In countries where this quality protein would help to diminish protein deficiencies, this sort of processing would be extremely wasteful. Encouragement should be given to processing germ for food use by solvent extraction. This study of maize germ clearly demonstrates the need for biological testing of nutrient availability in cereal products.

CONCLUSION

Research on grain proteins and on chemical and biological means of evaluating protein quality has been stimulated by the discovery of high-lysine maize and the subsequent challenge to make it widely available for use in combating the world protein shortage. Significant advances in these fields are reflected by the following papers.

We wish to thank Ching Khoo, E. T. Baker, and M. J. Wolf of the Northern Regional Research Laboratory for the scanning electron micrographs of sections of maize endosperm (Figure 1), which they prepared.

TABLE 2 Effect of processing on nutritive value of maize germ

Sample treatment for oil removal	Total lysine	Available lysine	PER[a]
Air dried, solvent extracted	[illegible]	[illegible]	[illegible]
Machine dried, solvent extracted	[illegible]	[illegible]	[illegible]
Machine dried, expeller processed	[illegible]	[illegible]	[illegible]

[a] Based on protein efficiency ratio (PER) for casein of 2.5. All data normalized (10% protein) (19).

[*A discussion of this paper can be found on pp. 499–501 of* **Questions and Answers.**]

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CHARACTERISTICS OF PROTEINS IN SINGLE AND DOUBLE ENDOSPERM MUTANTS OF MAIZE

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This report focuses on the characteristics of protein in a series of near-isogenic endosperm mutants from inbred lines of Oh43 and W22 maize. D. V. Glover has noted the production and genetic characteristics of these mutants in previous maize breeding program discussion.

Table 1 shows the amino acid composition of two floury mutants and one starch-modifying mutant in the Oh43 background, and one floury mutant in the W22 background. Columns 2 and 3 compare the amino acid patterns of the near isogenic opaque 2 mutant and its normal counterpart. The relationship shown is very similar to that which we found and reported in 1966 (2) in the inbred W64A and its spontaneous opaque 2 mutant. The lysine in the W64A endosperm was 1.6 g/100 g of protein and 3.7 g in the spontaneous mutant. The tryptophan content was 0.3 g in the W64A normal and 0.7 g in the mutant. These values agree closely with the values shown in Table 1 for the Oh43 normal and its opaque 2 counterpart. Thus it appears that the lysine and tryptophan levels more than doubled when normal endosperm was converted to its isogenic opaque 2 counterpart. Other changes characteristic of the opaque 2 mutant were a substantial drop

TABLE 1 Amino acid composition of defatted maize endosperms[a]

Inbred:	Oh43						W22		
Genotype[b]:	+	o_2	fl_2	o_2fl_2	bt_2	o_2bt_2	+	o_7	o_2o_7
Lysine	1.6	3.5	2.7	2.7	3.3	5.3	2.3	3.8	3.5
Tryptophan	0.3	0.8	0.5	0.8	0.7	1.3	0.4	0.7	0.7
Leucine	16.4	12.1	15.4	12.56	12.3	8.3	15.9	12.5	10.7
Isoleucine	4.3	4.3	4.3	4.07	4.1	4.1	4.3	4.3	4.1
Threonine	4.0	4.4	3.9	3.66	4.4	5.5	4.0	4.4	3.4
Methionine	2.4	2.7	3.7	2.17	3.4	2.4	3.2	3.2	2.2
Cystine	2.1	2.1	1.4	2.23	2.0	2.3	2.4	1.9	2.5
Phenylalanine	6.8	6.0	6.1	5.50	5.6	5.2	6.7	5.2	4.0
Tyrosine	5.9	5.2	5.1	5.0	5.1	4.9	5.8	4.9	3.9
Valine	5.2	5.9	5.2	5.1	5.6	7.0	5.7	6.7	5.4
Histidine	3.0	3.4	2.9	2.8	3.2	3.6	3.6	4.1	4.1
Arginine	3.4	5.1	5.8	4.3	5.0	7.2	3.7	5.2	4.8
Glycine	3.3	4.9	3.2	3.9	4.6	7.4	4.0	5.2	4.3
Alanine	10.1	8.2	8.9	7.6	8.3	7.5	8.5	7.9	5.5
Serine	6.0	5.4	5.3	4.6	5.6	5.6	5.4	5.6	3.9
Aspartic acid	7.5	9.5	7.2	8.7	8.1	10.7	6.7	9.4	7.1
Glutamic acid	30.0	23.6	26.4	24.9	23.6	19.0	27.4	25.5	18.5
Proline	11.3	9.8	9.5	9.4	9.6	8.9	11.5	11.0	10.0
% Protein	11.8	10.1	12.3	10.4	13.4	12.9	8.5	7.3	7.6

[a] Amino acid levels as a percentage of total protein (N × 6.25).
[b] In Tables 1 to 10: o, opaque; fl, floury; bt, brittle; su, sugary; sh, shrunken.

in the level of leucine, with little or no change in the level of isoleucine; an increase in the levels of alanine, glutamic acid, and proline.

In addition to these changes in amino acid levels, there was a drop in the total protein content of the endosperm when the opaque-2 gene was introduced. This drop was also noted in the W64A inbred line.

The effects of the floury-2 gene in the Oh43 background showed in changes in the individual amino acids in the same direction as those observed with the introduction of the opaque-2 gene, but were not so marked. In addition, there was no drop in the protein level of the endosperm.

In earlier studies on opaque-2 and floury-2, we had hoped that the double mutant opaque-2 floury-2 would be superior to both the opaque-2 and floury-2 single mutants with respect to lysine level. However, as shown in Table 1, the opaque-2 floury-2 double mutant was similar to floury-2 in lysine level. Other amino acids were at the opaque-2 or floury-2 level, or were intermediate between these two values.

Kenneth S. McWhirter of the Department of Agricultural Botany, University of Sydney, Sydney, Australia, supplied us with the opaque-7 mutant in the W22 inbred line and the double mutant of opaque-7 and opaque-2. The opaque-7 was a spontaneous mutation in this line, and preliminary data on endosperm amino acid composition and nitrogen distribution have been published (3).

Table 1 shows that the amino acid pattern of the opaque-7 mutant was quite similar to the amino acid pattern of the opaque-2 mutant in the Oh43 background. However, the normal counterpart in the W22 series contained a higher level of lysine and tryptophan than did the Oh43 normal counterpart. This might be expected, since the W22 normal counterpart contained a much lower level of protein in the endosperm (8.5%), and lysine and tryptophan levels are inversely related to protein level. A reduction in protein also was noted in the W22 normal background with the introduction of the opaque-7 gene (8.5 to 7.3%).

The double mutant kernels of opaque-2 and opaque-7 used in McWhirter's studies were obtained as F_3 segregates from the cross W23 L317 opaque-2 W22 opaque-7, and thus were not isogenic. Nevertheless, the amino acid composition shown in Table 1 suggests that no additive effect was obtained with respect to lysine and tryptophan. Apparently, the two floury genes, floury-2 and opaque-7, do not give an additive effect with respect to lysine when combined with the opaque-2 gene. In contrast, double mutants of opaque-2 and other endosperm mutant genes showed an additive effect, as indicated by the studies with brittle-2 in the Oh43 background.

The brittle-2 gene (Table 1, column 6) produced an amino acid pattern very similar to that observed with the opaque-2 gene. In contrast, the brittle-2 gene did not show a reduction in the percentage of protein in the endosperm; on the contrary, there is a definite increase in the percentage of protein (11.8% in the normal, and 13.4% in the brittle-2). The combination of the opaque-2 and brittle-2 genes, however, showed marked changes: lysine increased to 5.3%; tryptophan increased to 1.3%; leucine dropped sharply to 8.3%; the levels of arginine, glycine, and aspartic acid increased; and the levels of alanine, glutamic acid, and proline dropped substantially. The combination also showed an increase in the level of protein in the endosperm, with a level of 12.9%, intermediate between that of the normal and the brittle-2 selection. The genes bt_2, bt_1, su_1, sh_1, sh_2, and sh_4 all give a drastic reduction (60 to 75%) in carbohydrate and protein yield in the endosperm.

EXPERIMENTAL

It is necessary to look at the distribution of proteins in the endosperm to understand the marked changes that occur in the amino acid patterns with the introduction of these mutant genes. For the past 2 years we have been using a fractionation method developed in France by Landry, and Moureaux (1) for this purpose, as outlined in Table 2.

The endosperm is finely ground and defatted, and 1 part of the endosperm is mixed with 10 parts of the solvent. In obtaining the first fraction, 10 parts by weight of 0.5 *M* sodium chloride solution are added to the defatted,

TABLE 2 Landry-Moureaux fractionation sequence D

Fraction	Solvent	Time of agitation (min)	Protein fractions
I	NaCl 0.5*M* (4°C)	60	Albumins
		30	Globulins
		30	
	Water	15	
		15	
II	Isopropanol 70% (20°C)	30	
		30	Zein
		30	
III	Isopropanol 70%	30	
	+ 2-ME 0.6% (v/v) (20°C)	30	Zein-like
IV	Borate buffer pH 10	60	
	+ 2-ME 0.6% (v/v) (20°C)	30	Glutelin-like
		15	
V	Borate buffer pH 10	60	
	+ 2-ME 0.6% (v/v)	30	Glutelin
	+ SDS 0.5% (w/v)	15	Residue

Notes: 2-ME, 2-mercaptoethanol; borate buffer:borate, NaOH, NaCl μ 0.5; SDS, sodium dodecyl sulfate.

finely ground endosperm, and the mixture is stirred for 60 min at 4°C. The mixture is then centrifuged and the extract saved. The residue is treated again with the same volume of saline and stirred for another 30 min. The extraction is repeated a third time for 30 min. Finally, the residue is extracted with the same volume of water for 15 min, and this process is repeated once again for 15 min. The five extracts are then combined to give fraction I. The residue is then treated with 10 volumes of 70% isopropanol at 20°C for three 30 min periods, as outlined in Table 2, to give fraction II. The residue is then reserved for isolation of fraction III, and so on.

The first fraction contains the albumins and globulins, the free amino acids and small peptide fragments, and any other saline soluble compounds. Fraction II contains the prolamin and zein. Fraction III contains zein-like proteins that are soluble in alcohol after the disulfide bonds in the protein have been reduced with 2-mercaptoethanol. The fourth fraction contains proteins that have some of the characteristics of glutelin. The fifth fraction contains the true glutelin, which is a complex, high molecular weight mixture of proteins that can be solubilized only by treatment with a reducing agent and a detergent, sodium dodecylsulfate (SDS) at alkaline pH.

RESULTS: OHIO 43 SERIES

The nitrogen content of each of the five fractions is determined by micro-Kjeldahl, and the residue left after extraction also is analyzed for nitrogen.

Table 3 shows the distribution of nitrogen in the maize endosperms. In

TABLE 3 Nitrogen distribution in maize endosperms[a]

Inbred:	Oh43						W22		
Fraction / Genotype:	+	o_2	fl_2	o_2fl_2	bt_2	o_2bt_2	+	o_7	o_2o_7
I (albumin)	5.8	13.6	9.2	17.0	12.1	22.3	6.9	16.6	17.6
II (zein)	59.0	26.9	49.1	25.0	26.1	2.9	40.6	20.3	8.7
III (zein-like)	5.8	8.4	9.0	15.2	15.4	5.5	15.3	12.0	15.1
IV (glutelin-like)	12.7	14.0	7.6	9.9	8.7	12.2	12.8	18.8	21.3
V (glutelin)	13.8	29.2	22.0	24.8	27.9	48.0	21.0	29.5	33.3
Total N extracted	97.1	92.1	96.7	91.9	90.2	90.9	96.6	97.2	96.0

[a] Percentage of soluble nitrogen.

the normal Oh43 counterpart, the saline fraction made up a very small percentage of the total (5.8%), whereas the zein fraction (fraction II) accounted for almost 60% of the total protein extracted. The zein-like fraction (fraction III) was small (5.8%), the glutelin-like fraction (fraction IV) was intermediate (12.7%), and the true glutelin fraction was only slightly higher (13.8%). The extraction of nitrogen in normal Oh43 was excellent: only 3% of the total nitrogen remained in the residue. Table 3, column 3, shows several changes in the distribution of the protein fractions in the near-isogenic opaque-2 endosperm: the saline fraction more than doubled; the zein fraction (fraction II) dropped from 59 to 26.9%; the zein-like fraction (fraction III) increased slightly (from 5.8 to 8.4%); fraction IV increased slightly; and fraction V more than doubled (13.8 to 29.2%). A similar pattern was observed with the brittle-2 mutant gene, except that there appeared to be a reversal in the relative amounts of fractions III and IV. In contrast, the introduction of the floury-2 gene caused only a moderate rise in the level of saline-soluble protein, a modest drop in the level of zein (fraction II), a reduction in fraction IV, and an increase in fraction V that was intermediate between that of the normal and the opaque-2 conversion.

The combination of floury-2 with the opaque-2 mutant gene produced no major changes in any of the fractions above those observed for opaque-2. In contrast to opaque-2/floury-2, the double mutant opaque-2/brittle-2 showed an increase in fraction I, an almost complete elimination of fraction II, and an additive effect in fraction V.

NITROGEN DISTRIBUTION IN THE W22 SERIES

In the W22 series, the opaque-7 mutant gene in the W22 background brought about changes that resemble those observed with the introduction of the opaque-2 gene into the Oh43 background (Table 3). The combination of the opaque-7 and opaque-2 genes produced a marked reduction in fraction II (zein), from 20 to 8.7% with little change in the other fractions. These changes did not lead to an elevated level of lysine above that of the opaque-2 gene alone, as shown in Table 1.

SDS—DETERGENT ACRYLAMIDE ELECTROPHORESIS PATTERNS

A possible explanation for this anomaly can be found in the SDS–acrylamide gel patterns shown in Figure 1. These patterns show that in all the mutants (except the double mutant of opaque-2 brittle-2) there are two bands which represent the two major protein components of zein. Studies in our laboratory have shown that these two major protein components have molecular weights of approximately 22,000 and 25,000, respectively. In the opaque-2 brittle-2 double mutant, these two bands were com-

pletely absent (Figure 1), and the faster moving material that was observed farther down the tube represented smaller protein fragments with a molecular weight of about 10,000. It seems obvious that true zein is absent in the opaque-2/brittle-2 double mutant, and that the 2.9% of total protein observed in this fraction, fraction II (Table 3), does not represent zein. This finding strongly indicates that the introduction of the opaque-2 and brittle-2 genes into the Oh43 background completely suppresses true zein synthesis. Along with the complete suppression of zein synthesis, there is a spectacular increase in the level of glutelin (fraction V). The marked increase in fractions I and V combined with the disappearance of zein accounts for the unusually well-balanced amino acid pattern observed in the opaque-2/brittle-2 mutant, a pattern that closely resembles the ideal amino acid pattern recommended by the FAO for diets of preschool children.

Table 4 summarizes the nitrogen distributions observed in six near-isogenic endosperm mutants, in which less starch was synthesized, and their double-mutant combinations with opaque-2 in the Oh43 background. In the single mutants, fraction I was variable, but higher in all instances than in the

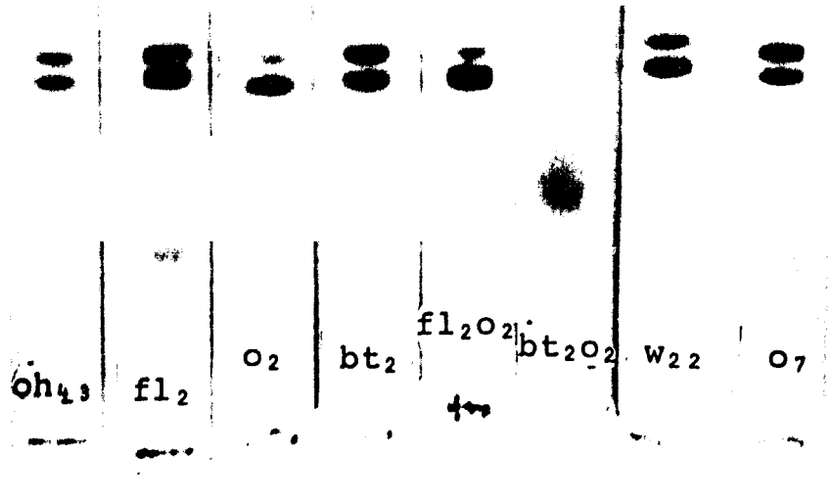

FIGURE 1 Comparison of SDS – acrylamide electrophoresis patterns of zeins (fraction II) isolated by 70% isopropanol from defatted endosperms. Direction of migration is toward bottom of tube.

TABLE 4 Nitrogen distribution in opaque-2 and starch-modifying maize mutants[a]

Fraction	Endosperm													
	+	o_2	su_1	sh_1	sh_2	sh_4	bt_1	bt_2	$su_1 o_2$	$sh_1 o_2$	$sh_2 o_2$	$sh_4 o_2$	$bt_1 o_2$	$bt_2 o_2$
I	5.8	13.6	11.9	8.2	12.3	25.7	8.8	12.1	22.7	39.9	25.3	43.3	23.3	22.3
II	59.0	26.9	27.1	43.7	29.4	30.8	36.0	26.1	3.0	1.8	1.2	6.5	2.7	2.9
III	5.8	8.4	21.9	12.3	9.4	7.7	16.4	15.4	9.0	1.6	1.1	3.9	2.5	5.5
IV	12.7	14.0	9.1	14.4	15.0	8.3	8.3	8.7	14.2	16.4	26.1	8.7	13.1	12.2
V	13.8	29.2	22.8	16.3	23.6	23.6	27.4	27.9	45.3	32.2	35.4	26.8	50.2	48.0
Total N extracted	97.1	92.1	92.8	94.9	89.8	96.1	96.9	90.2	94.2	91.9	89.1	89.2	91.8	90.9
Lysine (% of total protein)	1.6	3.5	1.8	1.9	2.7	3.0	2.3	3.3	3.9	4.8	4.2	4.0	4.8	5.3
Tryptophan (% of total protein)	0.3	0.8	0.3	0.6	0.7	0.8	0.5	0.7	0.8	1.2	1.2	1.2	1.4	1.3

[a] Percentage of soluble nitrogen.

normal counterpart (8.2 to 25.7%, compared with 5.8%). Fraction II (true zein) was lower in the single mutants than in the normal counterpart (26.1 to 43.7%, compared with 59.0%). Fraction III was higher in the single mutants (7.7 to 21.9%, compared with 5.8%), and fraction IV ranged from 8.3 to 15%, compared with 12.7% in the normal counterpart. Fraction V in the single mutants was higher than in the normal counterpart (16.3 to 27.9%, compared with 13.8%). The lysine content of the single mutants was variable, but all values were higher than that observed in the normal counterpart (1.8 to 3.3%, compared with 1.6%). Except in one case (sugary-1), the tryptophan levels were higher in the single mutants than in the normal counterpart.

The double mutants of six endosperm mutant genes with opaque-2 (Table 4) uniformly showed the same additive effect observed earlier with the opaque-2 and brittle-2 combination. Fraction I showed a range of 22.3 to 43.3%, versus 5.8% in the normal; fraction II showed a range of 1.2 to 6.5%, versus 59.0% in the normal; fraction III showed a range of 1.2 to 5.5%, versus 5.8% in the normal; fraction IV showed values both higher and lower than the value of the normal counterpart (8.7 to 26.1%, versus 12.7%); and fraction V showed a uniformly higher level than that found in the normal counterpart (26.8 to 50.2%, versus 13.8%). These changes were reflected in (1) the lysine values, which varied from 3.9 to 5.3% in the double mutants versus 1.6% in the normal counterpart, and (2) tryptophan which varied from 0.8 to 1.4%, versus 0.3% in the normal counterpart.

The data indicate that, with the possible exception of shrunken-4/opaque-2, there was a complete suppression of zein synthesis in the double-mutant combinations. It was obvious from the levels of lysine and tryptophan in these six double-mutant endosperms that the whole kernels should have a high nutritional value.

Studies are now underway to examine the protein fractions in developing endosperms of the opaque-2/brittle-2 double mutant. We expect to find some interesting changes, and hopefully may obtain some clue as to the mechanism involved in the complete suppression of zein synthesis in these double mutants.

AMINO ACID COMPOSITION OF LANDRY–MOUREAUX FRACTIONS

Data have been obtained on the amino acid composition of fractions I to V for the opaque-2, floury-2, brittle-2 and opaque-2/brittle-2 mutations in the Oh43 inbred line, and the opaque-7 mutation in the W22 inbred line. For each of the five fractions, an aliquot of aqueous extract containing 2.5 mg of nitrogen was hydrolyzed in a final volume of 100 ml of 6 *N* HCl for 24 h under reflux. The hydrochloric acid was evaporated *in vacuo* in a rotary evaporator, and the residue dissolved in 10 ml of diluter buffer at pH 2.2.

One milliliter portions were applied to the short and long columns of a Spinco Automatic Amino Acid Analyzer.

Table 5 shows the amino acid composition of fraction I. Fraction I was high in albumins and globulins. The lysine content of fraction I in the normal counterparts of Oh43 and W22 was as high as the content in any of the mutant derivatives. With the exception of brittle-2 and the double mutants of opaque-2 and brittle-2, the levels of lysine were as high as those found in the embryo of maize. Tryptophan analyses have not been made on all the fraction I samples; however, the Oh43 normal contains approximately 0.7% tryptophan and the brittle-2/opaque-2 contains approximately 1.2% tryptophan.

Our findings indicate that the amino acid pattern of fraction I of normal maize endosperm is quite similar to the same fraction in the high lysine mutants. Fraction I is a very nutritious fraction, as evidenced by the excellent levels of lysine, the adequate levels of sulfur amino acids, and the adequate levels of the other essential amino acids. The values for cystine and methionine were variable due to oxidative losses during hydrolysis; however, the high levels of cystine and methionine in the floury-2 were noteworthy, since these are minimal values.

Table 6 shows the amino acid composition of fraction II in the different mutants and their normal counterparts. The recovery of amino acids was satisfactory in all cases, except in the case of the double mutant of

TABLE 5 Amino acid composition of fraction I[a]

Inbred:	Oh43					W22	
Genotype:	+	fl_2	o_2	bt_2	o_2bt_2	+	o_2
Lysine	6.5	5.1	5.8	4.5	5.7	6.1	5.2
Histidine	3.2	2.4	3.5	2.2	2.2	2.5	2.3
Arginine	11.7	6.4	11.8	6.1	7.9	10.4	9.9
Aspartic acid	11.1	9.6	9.2	9.2	7.4	7.9	7.6
Threonine	5.3	4.9	4.2	5.9	5.5	4.2	3.6
Serine	5.8	5.2	4.9	4.7	4.0	4.5	3.9
Glutamic acid	18.5	14.3	15.8	15.7	9.2	10.9	16.8
Proline	6.4	5.1	5.3	7.4	6.3	4.8	4.5
Glycine	8.2	7.5	7.0	6.9	5.8	6.4	5.9
Alanine	8.8	7.7	7.2	8.5	7.7	7.0	5.8
Cystine	1.6	3.9	0.1	4.2	4.0	0.3	0.1
Valine	6.2	6.8	5.3	6.0	4.7	4.7	4.8
Methionine	2.5	3.0	1.5	1.1	0.8	1.7	1.8
Isoleucine	4.6	3.9	4.3	3.5	3.1	3.9	3.4
Leucine	6.4	4.6	6.1	4.4	4.5	5.4	5.2
Tyrosine	4.5	4.2	3.6	4.0	3.6	2.8	3.0
Phenylalanine	4.2	4.5	3.7	3.5	3.1	2.4	3.1
Total recovery	115.3	99.1	97.1	93.6	81.5	85.9	84.9

[a] Amino acid levels as a percentage of total protein (N X 6.25).

opaque-2 and battle-2. Here the recovery was less than half that expected and we have not been able to find hydrolysis conditions that improve it. The material hydrolyzed here should be considered an artifact, since it does not represent true zein. It is possible that nitrogen-containing materials other than protein were present in this fraction, resulting in the lower recovery value.

As would be expected, lysine was very low in fraction II of the mutants and their normal counterparts. The range, excluding the double mutant, was 0.1 to 0.5%. The limited data on tryptophan levels suggest that the samples contained approximately the same range of levels of this amino acid. Arginine was relatively high in floury-2 but low in the other selections. Glutamic acid was consistently high, ranging from 30 to 33%, and proline also was high (10.4 to 11%). Glycine was unusually low (1.5 to 1.9%), and alanine was high, ranging from 10.2 to 11.3%. Leucine also was high, ranging from 19.6 to 21.1%, with a leucine–isoleucine ratio of about 5 to 1. Here again it should be emphasized that no significant differences in the amino acid patterns could be observed between the mutant selections and their normal counterparts.

Table 7 shows the amino acid composition of fractions III. Fractions III and IV were usually not very high; thus, they make only a minor contribution to the final amino acid pattern of the endosperm. Nevertheless, they are interesting. Fraction III is a zein-like fraction and, as a result, the lysine level

TABLE 6 Amino acid composition of fraction II*

Inbred	OH43					W22	
Genotype	x	O_2	o_2	fo_2	o_2fo_2	x	o_2
Lysine	0.2	0.5	0.2	0.1	0.6	0.1	0.1
Histidine	1.6	2.4	1.4	1.0	1.5	1.6	1.7
Arginine	1.9	5.2	1.7	1.6	1.9	1.6	1.6
Aspartic acid	5.9	6.8	5.8	6.1	1.5	6.0	5.8
Threonine	3.4	4.2	3.5	3.6	2.5	3.4	3.6
Serine	5.9	6.2	5.7	6.1	2.5	5.9	5.7
Glutamic acid	32.8	33.5	30.6	31.7	14.5	30.5	30.5
Proline	11.0	11.2	10.5	10.4	7.0	10.9	10.8
Glycine	1.6	1.9	1.8	1.5	5.4	1.5	1.5
Alanine	11.2	11.5	10.4	11.0	5.4	10.4	10.2
Cystine	0.7	0.6	0.2	0.5	0.1	0.1	0.1
Valine	4.1	4.9	5.7	4.5	2.2	4.2	4.6
Methionine	2.2	4.0	1.7	1.9	1.2	1.9	2.5
Isoleucine	4.4	4.9	4.7	4.7	1.2	4.4	4.3
Leucine	20.5	20.2	21.1	19.6	5.1	20.5	19.9
Tyrosine	6.1	6.0	5.8	6.0	5.5	5.5	5.4
Phenylalanine	8.0	8.0	8.5	7.9	2.8	7.7	6.9
Total recovery	121.5	151.6	116.9	118.0	54.5	116.0	114.8

* Amino acid levels as a percentage of total protein (N × 6.25).

TABLE 7 Amino acid composition of fraction III[a]

Inbred:	Oh43					W22	
Genotype:	+	fl_2	o_2	bt_2	o_2bt_2	+	o_2
Lysine	0.6	0.6	0.5	0.4	0.8	0.5	0.4
Histidine	8.0	0.5	5.3	1.9	5.8	4.8	4.2
Arginine	5.2	5.5	2.4	2.2	2.9	4.8	4.5
Aspartic acid	5.1	5.2	5.9	4.9	1.6	2.8	5.7
Threonine	4.5	5.5	4.5	5.7	4.4	4.0	4.5
Serine	5.2	6.0	5.5	6.5	5.1	4.8	5.6
Glutamic acid	25.7	27.0	30.9	31.8	31.2	28.5	31.1
Proline	15.6	12.4	16.2	15.5	15.8	17.1	17.2
Glycine	4.5	2.8	4.2	5.6	6.6	4.7	4.5
Alanine	7.5	9.8	8.4	10.1	6.5	8.2	9.2
Cystine	0.4	0.5	0.6	0.5	0.6	0.2	0.5
Valine	4.7	5.8	5.1	4.0	4.6	5.6	5.0
Methionine	4.5	8.9	6.2	9.2	2.5	5.7	8.0
Isoleucine	2.5	5.2	5.1	5.5	1.1	2.5	2.8
Leucine	15.7	16.5	15.5	17.7	10.5	15.9	16.0
Tyrosine	4.5	4.0	4.4	5.5	6.9	4.6	5.1
Phenylalanine	4.5	6.8	5.8	6.4	6.2	4.2	5.1
Total recovery	112.5	114.5	120.5	125.0	110.7	117.5	126.8

[a] Amino acid levels as a percentage of total protein (N × 6.25).

was still fairly low; however, it was higher than that seen in the true zein fractions, with a range of 0.4 to 0.8%. Only one analysis was made for tryptophan. The brittle-2 mutant had a tryptophan content of 0.1%. Glutamic acid was quite high, with a range of 25.7 to 31.8%. Proline also was high, with a range of 12.4 to 17.2%. Methionine levels were unusual: floury-2 had 8.9%, brittle-2 had 9.2%, and opaque-7 had a level of 8.0%. Since there may have been oxidation losses for this sulfur amino acid, these were minimal values. Cystine values were relatively low (0.2 to 0.6%), but this may have been due to excessive oxidation losses.

Leucine was quite high in fraction III, and isoleucine was relatively low, giving rather unfavorable ratio of leucine to isoleucine (greater than 5 to 1). This was especially noticeable in the case of the double mutant of opaque-2 and brittle-2, where the actual isoleucine level of 1.1% was so low that the leucine–isoleucine ratio was nearly 10 to 1. Proline, as well as glutamic acid, was unusually high in this fraction. Proline ranged from 12.4 to 17.1%, and glutamic acid ranged from 25.7 to 31.8%. An unusually high level of histidine was noted in the normal counterpart of Oh43 (8.0%), but this level was not observed in the normal counterpart of W22. With certain exceptions, the amino acid patterns of the normal counterparts and the high-lysine mutants did not appear to differ markedly in fraction III.

Table 8 shows the amino acid composition of fraction IV. In this glutelin-like fraction, the lysine level was higher, with a range of 1.4 to 2.9%. The

TABLE 8 Amino acid composition of fraction IV[a]

Inbred	Oh43					W22	
Genotype	n	fl_2	wx_2	fl_2	wx_2fl_2	n	wx_2
Lysine	1.8	1.8	2.0	1.7	1.7	1.8	2.2
Histidine	8.2	8.0	7.6	8.4	8.9	9.0	9.0
Arginine	5.0	5.5	5.7	6.0	6.2	5.5	5.4
Aspartic acid	2.7	5.5	6.0	2.6	2.6	2.2	2.5
Threonine	5.5	5.5	5.9	4.8	4.5	5.2	5.7
Serine	5.7	4.4	4.2	4.0	5.0	5.0	5.5
Glutamic acid	25.4	30.0	22.6	25.0	24.0	23.7	26.5
Proline	15.7	14.5	15.9	16.8	17.5	17.4	16.5
Glycine	3.2	3.3	3.0	3.5	3.0	4.1	4.5
Alanine	4.1	5.8	4.4	4.2	4.5	5.9	4.0
Cysteine	0.1	0.1	0.1	0.1	0.1	0.0	0.1
Valine	5.2	5.2	5.8	6.0	6.5	6.0	5.8
Methionine	1.4	1.5	1.5	1.1	1.0	1.1	1.4
Isoleucine	2.4	2.4	2.8	2.5	2.5	2.5	2.4
Leucine	7.9	7.1	8.4	8.6	9.1	8.5	8.5
Tyrosine	2.2	2.7	1.8	1.6	2.1	1.5	1.5
Phenylalanine	2.5	2.5	2.6	2.2	2.5	1.9	1.8
Total recovery	95.5	98.4	97.2	98.1	99.6	95.5	96.5

[a] Amino acid levels as a percentage of total protein (8.4–6.2%).

tryptophan level in W22 and opaque-7 was 0.5%. The histidine was high in fraction IV, ranging from 7.6 to 9.0%. This level usually runs in the range of 3 to 4% in endosperm and in whole seed; thus, this finding is unusual. Aspartic acid was unusually low (2 to 2.4%). Glutamic acid was fairly high, ranging from 21.6 to 30.0%; proline was also unusually high, ranging from 15.7 to 17.5%. The cystine and methionine contents were relatively low. The leucine level was also relatively low, with a ratio of leucine to isoleucine of about 3 to 1. Phenylalanine was uniformly low, ranging from 1.8 to 2.6%. In this fraction, also there were no important differences in amino acid composition between the normal counterpart and the high-lysine mutants.

Table 9 shows the amino acid composition of fraction V. This is the true glutelin fraction, and it was obvious that the lysine content of this fraction was outstanding, ranging from 5.7 to 7.0%. Tryptophan also was high. In the Oh43 series, the normal counterpart contained 1.8% tryptophan, opaque-2, 1.5%; floury-2, 1.7%; opaque-2 floury-2, 1.6%; brittle-2, 1.5%; and opaque-2 brittle-2, 1.8%.

If the plant breeder could in some way modify synthesis in maize endosperms so that only fraction V were produced, he could create cereal grain with an ideal amino acid pattern.

Table 10 shows the ranges of the amino acid levels in the five fractions. Lysine is high in fractions I and V, histidine in fraction IV, leucine and alanine in fraction II, methionine in fraction III, and glutamic acid and

TABLE 9 Amino acid composition of fraction V[a]

Inbred:	4N45					M77	
Genotype:	s	Bt_2	wx_2	bt_2	wx_2bt_2	s	wx_2
Lysine	6.4	5.7	6.7	7.0	6.9	7.0	6.8
Histidine	5.6	2.8	4.8	3.9	3.7	3.0	3.5
Arginine	7.1	6.5	9.6	11.0	7.6	7.0	7.6
Aspartic acid	9.4	11.2	10.7	8.9	10.0	8.3	9.3
Threonine	5.2	5.4	5.7	4.6	5.6	4.2	5.2
Serine	4.8	5.8	5.4	4.9	5.5	5.5	4.8
Glutamic acid	15.2	19.5	15.7	17.1	15.5	15.0	14.4
Proline	5.3	6.2	5.6	6.7	6.4	5.5	5.4
Glycine	5.1	5.6	5.5	5.4	5.8	5.0	4.9
Alanine	7.0	8.0	7.5	6.8	7.2	6.8	6.4
Cysteine	0.2	0.1	0.0	0.8	0.1	0.5	0.0
Valine	7.0	7.4	7.4	7.2	7.7	7.1	6.8
Methionine	2.4	3.1	3.2	2.4	2.4	4.0	2.4
Isoleucine	5.0	5.1	5.5	4.9	5.2	4.9	4.9
Leucine	10.1	10.4	10.4	10.1	10.1	9.4	9.1
Tyrosine	2.4	3.3	3.9	4.9	4.4	2.7	2.9
Phenylalanine	5.6	6.1	5.9	5.6	5.8	5.5	5.2
Total recovery	101.8	114.2	113.5	112.2	109.9	98.3	99.4

[a] Amino acid levels as a percentage of total protein (N × 6.25).

TABLE 10 Ranges in amino acid contents of fractions I to V[a]

Fraction	I	II[b]	III	IV	V
Lysine	4.3–6.3	0.1–0.5	0.4–0.8	1.0–2.9	5.7–7.0
Histidine	2.2–3.3	1.0–2.4	0.5–8.0	7.6–9.6	2.8–4.8
Arginine	6.1–11.8	1.6–5.2	2.2–4.8	5.0–6.2	6.5–11.0
Aspartic acid	7.4–11.1	5.8–6.8	1.6–5.2	2.2–4.0	8.8–11.2
Threonine	3.5–5.3	3.3–4.2	3.5–4.5	1.3–5.2	4.2–5.7
Serine	3.9–5.0	5.7–6.2	4.8–6.3	3.5–4.4	3.5–5.8
Glutamic acid	9.2–18.5	30.5–33.3	25.7–31.8	21.6–28.0	13.0–19.5
Proline	4.5–7.4	10.4–11.2	12.4–17.2	15.7–17.4	5.3–6.7
Glycine	5.8–8.2	1.5–1.9	2.8–6.6	4.1–5.5	4.9–5.8
Alanine	5.8–8.8	10.2–11.5	7.5–10.1	3.8–4.5	6.4–8.0
Cysteine	0.1–4.2	0.1–0.7	0.2–0.6	0.0–0.1	0.0–0.8
Valine	4.7–6.8	3.7–4.9	5.8–5.6	5.2–6.5	6.8–7.7
Methionine	0.8–3.0	1.7–4.0	2.5–3.2	1.0–1.3	2.4–4.0
Isoleucine	3.1–4.6	4.5–4.9	1.1–3.5	2.3–2.8	4.9–5.5
Leucine	4.4–6.4	19.6–21.1	10.5–17.7	7.1–9.1	9.1–10.4
Tyrosine	2.8–4.5	5.4–6.1	4.0–6.9	1.5–2.7	2.7–5.3
Phenylalanine	2.4–4.5	6.9–8.3	4.2–6.8	1.8–2.6	5.2–6.1

[a] Based on data in Tables 5 to 9. Amino acid levels as a percentage of total protein (N × 6.25).
[b] wx_2bt_2 not included.

proline in fractions II, III, and IV. Lysine and glycine are low in fraction II, leucine in fraction I, and phenylalanine and tyrosine in fraction IV.

SUMMARY

The amino acid analyses of endosperms of three floury and six endosperm mutants with modified starch content showed major differences in amino acid patterns compared with the normal isog. ic counterparts. Highest lysine and tryptophan levels were obtained for combining the opaque-2 gene with any one of the following genes: brittle-1, brittle-2, shrunken-1, shrunken-2, shrunken-4, and sugary-1. Protein fractionation data showed that (with the six double mutants just listed) zein synthesis in the endosperm has almost, or completely, been suppressed.

The amino acid patterns of the five Landry–Moureaux fractions differed markedly. Improved endosperm protein quality was related to increases in fractions I and V and decreases in fraction II. The amino acid compositions of these fractions in the normal counterparts were remarkably similar to those in the mutants, suggesting that the mutations had produced no new or unusual proteins, but are responsible for changes in the proportions of the various fractions.

Journal Paper No. 5161, Purdue University Agricultural Experiment Station. This research was supported by the U.S. Agency for International Development under Contract AID/Csd-2809, titled "Inheritance and Improvement of Protein Quality and Content in Maize."

[*A discussion of this paper can be found on pp. 501–503 of* **Questions and Answers.**]

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USE OF SMALL ANIMALS FOR EVALUATION OF PROTEIN QUALITY IN CEREALS

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At their annual planning session in 1972, the Purdue and CIMMYT maize research teams recommended that a group of representative maize, wheat, triticales, and sorghum samples be sent to cooperating universities and tested for protein efficiency response in several species of small laboratory animals. For this work, CIMMYT supplied both the hard and soft endosperm types of opaque-2 maize and representative samples of wheat and triticales. Purdue supplied floury-2 maize and sorghum. The samples were prepared in a standard fashion and distributed among the cooperating laboratories. Identical cereal samples and reference casein were fed to young weanling rats (Purdue), weanling voles (CIMMYT and Pennsylvania

State University), weanling mice (University of Nebraska), and the chick (Washington State University). F. T. Mertz served as coordinator of the project.

Table 1 shows these cooperative samples: ten CIMMYT cereal samples and five Purdue cereal samples were ground in burr mills at CIMMYT and Purdue, respectively. All samples were analyzed in duplicate by the State Chemist Laboratories at Purdue for total nitrogen, moisture, fat, fiber, and ash. Table 2 shows the results of these analyses. Moisture content of the samples ranged from a low of 4.8% in the casein to a high of 8.9% in sample CM-4854. Total protein ranged from a low of 9.3% in Oh43 x B14 (floury-2) to a high of 91.0% in the Animal Nutrition Research Council (ANRC) casein. Fat ranged from a low of 0% in the casein to a high of 4.6% in sample CM-4855. Fiber ranged from 0% in the casein to a high of 2.9% in sample CW-4859. Ash varied from a low of 0.8% in sample CM-4854 to a high of 2.3% in casein, in two triticale samples, and in one sorghum sample.

On the basis of these analyses, (1) the total fat content of the diet was set at 5%, (2) the total mineral plus ash content was set at 5%, (3) no corrections were made for moisture, and (4) fiber content was adjusted to a range of 2.0 to 2.9%. With these parameters fixed, the floury-2 sample established the maximum protein level at which all samples would be fed: a level of 8.8% protein, slightly below the 10% protein level usually used in protein efficiency ratio (PER) determinations. Table 3 shows the composition of the diets used in feeding tests on the rat and the mouse.

TABLE 1 Identification of cooperative cereal samples

Sample	Identification
CM-4854	CIMMYT Veracruz 181, Antigua
	Gpo 2a Veracruz 1, normal maize
CM-4855	CIMMYT Veracruz 181, Antigua
	Gpo 2a Veracruz 1, opaque-2 maize (hard)
CM-4856	CIMMYT Veracruz 181, Antigua
	Gpo 2a Veracruz 1, opaque-2 maize (soft)
CM-4857	CIMMYT Tuxpeño, opaque-2 maize (soft)
CM-4858	CIMMYT Tuxpeño, normal maize (hard)
CM-4859	CIMMYT 7 Cereales, [illegible]
CM-4860	CIMMYT INIA, wheat
CT-4861	CIMMYT PM 132, triticale
CT-4862	CIMMYT PM 2, triticale
CT-4863	CIMMYT PM 15, triticale
Oh43 X B14(+)	Purdue, single cross normal maize
Oh43 X B14(floury-2)	Purdue, near isogenic floury-2 version of Oh43 X B14
IS-8165	Purdue, pigmented sorghum
IS-2519	Purdue, low pigment sorghum
IS-0750421	Purdue, low pigment sorghum
ANRC casein	Purdue, Animal Nutrition Research Council reference casein[a]

[a] Sheffield Chemical Co., Union, N.J.

Table 4 shows the amino acid values obtained at Purdue on the cooperative maize samples and the ANRC casein used in these studies. The values in Table 4 agree reasonably well with the values obtained on identical samples by Villegas at CIMMYT (CIMMYT data are in parentheses). The Purdue values will be discussed here. The floury-2 sample contained only 11% more lysine than its normal counterparts. The hard and soft endosperm of CM-4855 and CM-4856 did not differ significantly in lysine content. Tryptophan levels of the normal selections ranged from 0.5 to 0.7%, the floury-2 selection contained 0.7%, and the opaque-2 selections varied from 0.9 to 1.0%. The casein carried an unusually high level of lysine (8.6%) and a high level of tryptophan (1.4%). Table 4 shows that the range of leucine levels dropped substantially in the opaque-2 selections. Protein in the maize selections varied from 10.1 to 11.0%.

Table 5 shows the amino acid levels in the cooperative sorghum, wheat, and triticale samples. Sorghum sample IS-8165 is a highly pigmented variety, with a lysine only slightly lower than that observed for the other two sorghum selections. The tryptophan values shown for sorghum were obtained with a colorimetric method. The values are uniformly low. Another method, which involves hydrolysis and ion-exchange analysis, was used to show that tryptophan values were above 1% with most sorghums; thus tryptophan is not a limiting amino acid in sorghum protein. Table 5 also shows that lysine values were slightly higher in the triticales samples than in the wheat samples. Tryptophan levels were quite high in wheat and at a satisfactory level in triticales. The leucine was low, and the leucine-

TABLE 2 Approximate analysis of CIMMYT and Purdue cereals

Sample	Total N	Protein[a]	Moisture	Fat	Fiber	Ash
CM-4854	1.62	10.1	8.9	5.7	2.5	0.8
CM-4855	1.62	10.1	8.5	4.6	2.2	1.5
CM-4856	1.50	9.4	8.7	4.2	2.3	1.4
CM-4857	1.55	9.7	8.0	4.4	2.5	1.6
CM-4858	1.54	9.6	7.9	4.2	2.3	1.4
(B43 × B14)(+)	1.52	9.5	8.5	4.1	2.5	1.1
(B43 × B14 (floury-2)	1.49	9.3	8.5	4.2	2.4	1.7
CM-4859	1.95	11.1	5.9	1.5	2.9	1.6
CM-4860	1.98	11.3	6.7	1.4	2.6	1.3
CT-4861	2.56	14.6	6.4	1.6	2.6	1.9
CT-4862	2.60	14.8	7.3	1.9	2.7	2.3
CT-4863	2.74	15.6	5.9	1.6	2.5	2.3
IS-8165	1.57	9.8	8.5	2.7	2.5	1.8
IS-2519	1.87	11.7	6.7	4.3	2.0	1.7
IS-0250421	1.86	11.6	6.4	3.0	2.3	2.3
ANRC casein	14.60	91.0	4.8			2.3

Source: State Chemist Laboratories, Purdue University, Lafayette, Ind.

[a] Maize, sorghum, and casein: N × 6.25; wheat and triticale: N × 5.7.

isoleucine ratio was low and favorable in wheat and triticales. This is in sharp contrast to the leucine–isoleucine ratio in sorghum (approximately 3 to 1). It is believed that the high level of leucine in sorghum is responsible for the high incidence of pellagra in certain parts of India where sorghum supplies the major part of the protein intake. The protein level in these samples varied from a low of 10.3% in sorghum IS-8165 to a high of 15.7% in triticale PM-15. In the feeding tests, however, the protein was adjusted in all cases to 8.8% of the total diet (see Table 3).

RAT FEEDING TESTS

Table 6 summarizes the results obtained at Purdue when the collaborative maize samples were fed to rats for 28 days. In these studies, 10 male weanling rats were used for each cereal. The rats were housed in individual wire mesh cages and supplied with feed and water *ad libitum*. Special feed cups were used, which minimized the scattering of food and permitted accurate recording of the feed consumed. Table 6 shows that there were no significant differences in average weight gained between the three normal maize samples and the floury-2 sample (25.8 to 36.9 g). No significant differences were observed in the average weight gained between the three opaque-2 maize samples and the casein control (82.7 to 87.1 g). Gains in weight of

TABLE 3 Proposed diets containing 8.8% protein

Sample	Sample (%)	Cottonseed oil (%)	Mineral mix[a] (%)	Vitamin mix[b] (%)	Corn starch (%)
CM-4854	87.0	1.8	4.5	1.0	5.9
CM-4855	87.0	1.0	3.7	1.0	7.3
CM-4856	93.5	1.1	3.7	1.0	0.7
CM-4857	80.8	1.0	3.4	1.0	5.8
CM-4858	91.7	1.2	3.7	1.0	2.4
OH43(+)	92.5	1.2	4.0	1.0	1.3
OH43(floury-2)	94.6	1.0	3.4	1.0	0.0
CM-4859	79.3	3.8	3.7	1.0	12.2
CM-4860	77.8	3.9	4.0	1.0	13.3
CT-4861[c]	60.2	4.0	3.9	1.0	30.4
CT-4862[c]	59.4	3.9	3.6	1.0	31.6
CT-4863[c]	56.4	4.1	3.7	1.0	34.3
IS-8165	90.6	2.6	3.4	1.0	3.5
IS-2519[c]	75.2	1.8	3.7	1.0	17.8
IS-0250421[c]	75.8	2.7	3.3	1.0	16.7
ANRC casein[c]	9.7	5.0	4.8	1.0	77.5

[a] Hawk-Oser Salt Mixture Number 3, Nutritional Biochemicals Corp., Cleveland, Ohio.

[b] Vitamin Fortification Mixture for the rat, Catalog No. 40060, General Biochemicals Corp., Chagrin Falls, Ohio.

[c] Add 2% cellulose (Cellu Flour or equivalent) for casein and 0.5% for triticales and sorghum samples.

TABLE 4 Amino acid composition of cooperative maize samples[a]

	CM-4854 normal		Ob43 X B14 normal		CM-4858 normal		Ob43 X B14 floury-2		CM-4855 opaque-2 (hard)		CM-4856 opaque-2 (soft)		CM-4857 opaque-2 (soft)		ANRC casein	
Lysine	2.6	(2.5)	3.0	(2.8)	2.7	(2.5)	2.4	(3.0)	4.5	(4.0)	4.4	(3.7)	4.7	(4.1)	8.6	(8.5)
Tryptophan[b]	0.6	(0.6)	0.7	(0.6)	0.5	(0.7)	0.7	(0.7)	0.9	(1.0)	1.0	(0.9)	1.0	(1.0)	1.4	(1.4)
Isoleucine	3.5	(3.1)	3.6	(3.2)	3.5	(2.9)	2.7	(2.7)	3.5	(2.5)	3.5	(2.1)	3.5	(2.1)	5.2	(4.3)
Leucine	12.7	(13.6)	12.8	(13.9)	13.2	(13.4)	12.3	(11.7)	9.1	(7.8)	8.7	(7.1)	8.6	(7.2)	9.7	(8.9)
Cysteine	1.6	(–)	1.9	(–)	1.6	(–)	1.4	(–)	2.2	(–)	1.9	(–)	2.1	(–)	0.1	(–)
Methionine	2.0	(–)	2.7	(–)	2.1	(–)	2.7	(–)	1.7	(–)	1.8	(–)	1.9	(–)	3.0	(–)
Histidine	3.1	(2.9)	2.9	(2.4)	3.1	(2.7)	2.9	(2.4)	3.7	(2.4)	3.6	(2.6)	3.6	(2.9)	3.0	(2.6)
Arginine	4.8	(4.1)	5.1	(4.5)	4.8	(4.1)	5.5	(5.6)	6.3	(6.0)	6.7	(5.1)	7.3	(5.7)	4.0	(3.5)
Tyrosine	4.3	(3.1)	4.5	(4.0)	4.4	(3.5)	4.4	(4.0)	3.7	(2.5)	3.7	(2.4)	3.9	(2.6)	5.7	(5.6)
Phenylalanine	4.8	(4.4)	5.1	(4.9)	5.0	(4.1)	5.2	(4.7)	5.2	(3.3)	4.1	(2.9)	4.3	(3.5)	5.3	(4.9)
Threonine	3.3	(3.3)	3.4	(3.4)	3.4	(3.4)	3.5	(3.2)	3.6	(3.3)	3.7	(3.3)	3.9	(3.5)	4.2	(4.2)
Valine	4.7	(4.3)	4.7	(4.4)	4.9	(4.7)	5.0	(3.8)	5.4	(4.5)	5.3	(3.6)	5.4	(3.8)	6.7	(6.2)
Aspartic acid	5.8	(7.0)	6.5	(6.9)	6.2	(7.5)	6.9	(6.8)	8.7	(8.7)	8.9	(8.5)	9.9	(9.1)	7.2	(7.2)
Glutamic acid	22.3	(23.4)	22.6	(22.3)	23.1	(23.7)	22.1	(19.7)	19.8	(17.5)	19.2	(15.9)	20.3	(17.0)	27.1	(25.6)
Proline	8.9	(8.7)	8.7	(11.3)	9.0	(8.7)	8.5	(9.9)	8.4	(6.8)	8.1	(6.5)	8.0	(6.6)	11.3	(11.7)
Alanine	7.3	(7.7)	7.5	(7.6)	7.7	(8.4)	7.7	(6.9)	6.0	(5.4)	5.9	(5.2)	6.4	(5.6)	3.1	(2.9)
Serine	4.6	(5.1)	4.8	(5.3)	4.6	(5.4)	4.9	(5.3)	4.3	(4.7)	4.5	(4.1)	4.6	(4.3)	5.7	(5.9)
Glycine	3.5	(3.5)	3.7	(3.7)	3.6	(3.6)	4.0	(3.7)	4.6	(4.4)	4.6	(4.0)	4.9	(4.2)	1.9	(1.7)
% Protein	11.0	(10.5)	10.2	(9.5)	10.5	(10.1)	10.4	(9.6)	10.9	(10.7)	10.7	(11.1)	10.1	(10.2)	88.1	(89.4)

[a] Grams per 100 g of protein (CIMMYT values at parentheses).

[b] Colorimetric method (CIMMYT Bulletin 20). All others, ion exchange. CIMMYT samples not defatted. Purdue sample defatted.

TABLE 5 Amino acid composition of cooperative sorghum, wheat, and triticale samples[a]

	Sorghum IS-8165		Sorghum IS-0250421		Sorghum IS-2319		Wheat 7 Cerros		Wheat INIA		Triticale PM-2		Triticale PM-15		Triticale PM-132		ANRC casein	
Lysine	2.3	(2.2)	2.5	(2.2)	2.5	(2.3)	3.1	(3.1)	3.1	(2.8)	3.4	(3.0)	3.4	(3.4)	3.5	(3.6)	8.6	(8.3)
Tryptophan	(0.3)[b]	( )	(0.6)	( )	(0.5)	( )	1.7	(1.6)	1.8	(1.6)	1.1	(1.0)	1.1	(1.1)	1.2	(1.1)	1.4	(1.4)
Isoleucine	4.2	(3.7)	4.7	(3.8)	4.3	(2.6)	3.8	(2.3)	3.9	(2.7)	3.9	(3.2)	3.7	(2.3)	3.8	(2.8)	5.2	(4.3)
Leucine	13.7	(13.9)	15.0	(15.5)	13.9	(13.8)	7.2	(7.2)	7.2	(7.2)	7.2	(6.7)	6.8	(7.0)	6.8	(7.0)	9.7	(8.9)
Cystine	1.0	( )	1.6	( )	1.0	( )	2.0	( )	2.2	( )	1.8	( )	2.0	( )	1.5	(–)	0.1	(–)
Methionine	1.8	( )	2.1	( )	1.6	( )	1.8	( )	1.9	( )	1.9	( )	1.9	( )	1.6	( )	3.0	(–)
Histidine	2.1	(1.9)	2.5	(2.4)	2.1	(1.9)	2.5	(2.3)	2.6	(2.2)	2.6	(2.0)	2.4	(2.2)	2.5	(2.2)	3.0	(2.6)
Arginine	4.1	(3.8)	4.3	(3.4)	4.1	(4.6)	5.8	(5.3)	5.6	(5.0)	6.1	(4.7)	6.4	(5.9)	6.3	(5.8)	4.0	(3.5)
Tyrosine	4.4	(4.1)	5.1	(4.2)	4.9	(4.0)	3.6	(2.9)	3.7	(2.9)	3.4	(1.8)	3.3	(2.8)	3.2	(2.1)	5.7	(5.6)
Phenylalanine	5.3	(5.4)	6.1	(5.2)	5.5	(4.8)	4.8	(3.7)	4.9	(3.8)	5.0	(3.7)	4.9	(4.7)	4.8	(4.0)	5.3	(4.9)
Threonine	3.2	(3.2)	3.7	(3.7)	3.3	(3.1)	3.1	(3.4)	3.2	(3.0)	3.2	(2.7)	3.0	(3.0)	3.0	(2.7)	4.2	(4.2)
Valine	5.1	(5.4)	5.9	(5.0)	5.5	(4.0)	4.7	(3.2)	4.8	(3.7)	4.9	(4.2)	4.6	(3.2)	4.6	(3.8)	6.7	(6.2)
Aspartic acid	7.3	(7.4)	7.4	(7.6)	7.5	(8.1)	5.5	(6.0)	5.6	(5.7)	6.5	(5.9)	6.3	(7.6)	6.3	(5.9)	7.2	(7.2)
Glutamic acid	25.6	(23.9)	27.4	(25.1)	25.7	(26.3)	36.0	(38.0)	37.0	(37.1)	33.4	(28.9)	32.4	(34.9)	31.9	(28.9)	27.1	(23.6)
Proline	7.9	(8.2)	9.1	(10.3)	8.0	(9.8)	9.9	(9.4)	10.0	(9.4)	9.9	(7.7)	10.0	(9.7)	9.8	(8.8)	11.3	(11.7)
Alanine	9.9	(9.6)	10.3	(11.3)	9.7	(9.9)	3.9	(3.9)	3.9	(3.6)	4.2	(3.6)	4.0	(4.4)	4.0	(3.2)	3.1	(2.9)
Serine	4.4	(4.7)	4.9	(5.6)	4.3	(5.4)	5.0	(5.6)	4.9	(5.3)	4.6	(4.0)	4.5	(5.6)	4.4	(4.8)	5.7	(5.9)
Glycine	3.0	(3.0)	3.6	(3.5)	3.1	(3.2)	4.6	(4.8)	4.6	(4.5)	4.5	(4.0)	4.3	(4.8)	4.2	(4.2)	1.9	(1.7)
% Protein	10.3	(9.6)	12.2	(11.5)	12.7	(11.2)	11.0	(11.1)	11.5	(11.5)	14.9	(16.5)	15.7	(15.0)	14.8	(14.8)	88.1	(89.4)

[a] Grams per 100 g of protein (CIMMYT values in parentheses).
[b] Colorimetric method (CIMMYT Bulletin 20). All others ion exchange. CIMMYT samples not defatted. Purdue samples defatted.

TABLE 6 Protein efficiency ratio values of cooperative maize samples[1]

	CM-4854 normal	Oh43 X B14 normal	CM-4858 normal	Oh43 X B14 floury-2	CM-4855 opaque-2 (hard)	CM-4856 opaque-2 (soft)	CM-4857 opaque-2 (soft)	ANRC casein
Average initial weight (g)	49.6	49.6	49.6	49.5	49.6	49.5	49.6	49.5
Average weight gained (g)	25.8[b]	31.4[b]	32.6[b]	36.9[b]	82.7[a]	87.1[a]	84.4[a]	85.1[a]
Average total feed consumed (g)	183.7[c]	206.1[c]	214.5[c]	227.3[c]	333.5[b,a]	340.2[a]	336.0[a]	290.2[b]
Protein efficiency ratio	1.59[c]	1.71[c]	1.72[c]	1.83[c]	2.83[b]	2.90[b]	2.84[b]	3.34[a]

Source: Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of L. Tanchoco, Department of Biochemistry, Purdue University, Lafayette, Ind.

[1] Duration of experiment: 28 days. Ten male rats in each group. Values with the same superscript are not significantly different from each other at the 1% level.

these last four groups were significantly higher at the 1% level than were the gains in weight of the first four groups. The three normal maize diets and the floury-2 diet showed PER's that were not significantly different at the 1% level (1.59 to 1.83). The three opaque-2 maize diets also showed PER's that were not significantly different at the 1% level (2.83 to 2.90). However, the three opaque-2 maize diets had significantly higher PER values than did the three normal maize diets and the floury-2 maize diet. There was a significant difference in the PER value between the three opaque-2 maize diets and the casein control. The opaque-2 maize diets showed an average PER that was 87% of the PER value (3.34) obtained with ANRC casein.

Table 7 summarizes the weight gains and PER values obtained on the cooperative sorghum, wheat, and triticales samples. This was a separate feeding test for 28 days, using ANRC casein as a control protein. The average total weight gain per animal on sorghum IS-8165 was substantially less than that on sorghum IS-2319. However, the differences in weight gained on the three sorghums were not significant at the 1% level. Average total weight gained on sorghum IS-2319 and the two wheat samples did not differ significantly. The three triticales samples, however, were significantly better than the three sorghum samples; and triticales PM-15 and PM-132 were significantly better than the two wheat samples. Triticale sample PM-2 was not significantly better than the two wheat samples. Weight gain on the control casein was more than twice that observed on the best triticale samples, and was significantly higher than any of the cereal grain samples listed in Table 7.

The PER values show that the PER of the highly pigmented sorghum sample is significantly lower than that of sorghum IS-2319 (0.71 versus 1.28). The PER values of the three triticale samples and the INIA wheat sample were significantly higher than the PER values of the three sorghum samples and the 7 Cerros wheat sample. The two wheat samples did not differ significantly, and there were no significant differences among the three triticales samples. The highest PER value in this cereal group was 2.01 (PM-132), which was significantly lower than the control casein PER value of 3.22. In summary, the average PER value in the triticales was about 62% of the casein control. The wheat samples were about 52% (which is about equal to that of the normal maize shown in Table 6), and the sorghums varied from 25 to 40% of the casein control.

Figure 1 shows the PER range in individual animals on the maize, wheat, triticale, and casein diets. The solid lines represent 28 days and the broken lines 14 days of feeding the diets. The ranges tended to shorten in moving from diets of 14 to 28 days, suggesting that a lower variability among animals was obtained as the animals adjusted to the diet. Nevertheless, opaque-2 maize was distinctly different from the normal and floury-2 maize. Table 8 summarizes the weight gains and PER's of the cooperative maize samples at the end of 14 days. The three opaque-2 maize diets were significantly higher with respect to average weight gained than the three normal diets and the

TABLE 7 Protein efficiency ratio values of cooperative sorghum, wheat, and triticale samples[a]

	Sorghum IS 8165	Sorghum IS 0250421	Sorghum IS 2319	Wheat 7 Cerros	Wheat INIA	Triticale PM 2	Triticale PM 13	Triticale PM 132	ANRC casein
Average initial weight (g)	50.6	50.6	50.6	50.6	50.5	50.5	50.6	50.4	50.6
Average total weight gain 1 (g)	11.6[c]	14.4[c]	19.6[d,e]	27.0[e,f]	26.7[e,f]	32.7[b,e]	38.3[b]	37.8[b]	89.6[a]
Average total feed consumed (g)	141[c,d]	165.2[d]	171.6[d]	193.9[b,e,f]	182.4[c,d]	202.1[b,e]	221.9[b]	213.3[b]	314.2[a]
Protein efficiency ratio	0.71[f]	0.87[e,f]	1.28[d,e]	1.57[c,d]	1.46[b,e]	1.61[b,e]	1.97[b]	2.01[b]	5.22[a]

Source: Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of L. Tanchoco, Department of Biochemistry, Purdue University, Lafayette, Ind.

[a] Duration of experiment: 28 days. Ten males rats in each group. Values with the same superscript are not significantly different from each other at the 1% level.

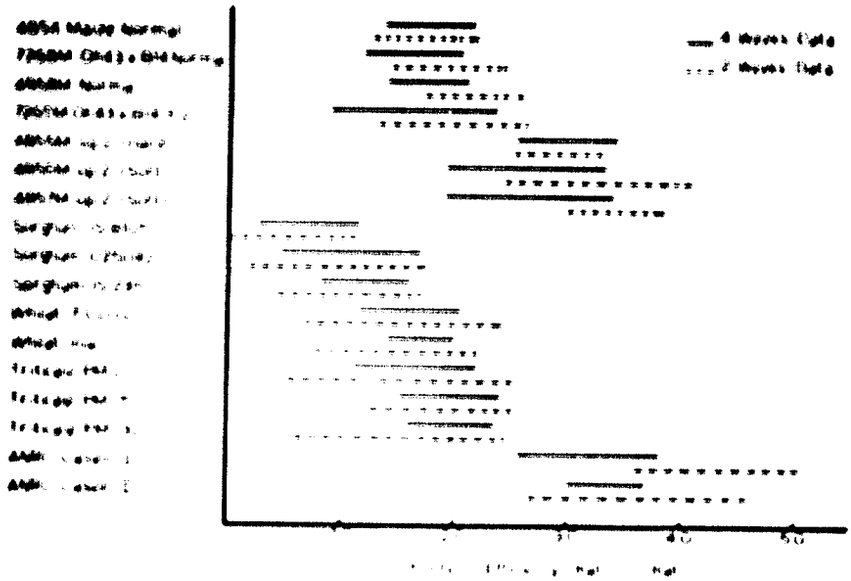

FIGURE 1 Protein efficiency ratio ranges in rats fed cooperative cereal samples. Each range line covers data on 10 rats.

one floury-2 diet. However, at the end of 14 days, the three opaque-2 diets gave significantly lower gains than the casein diet. The same differences were observed in the PER's. The PER value for ANRC casein was unusually high at 14 days, but had dropped 1 point by the end of 28 days. The unusually high PER value for casein observed at 14 days is unexplained.

Table 9 shows the total weights gained and the PER values obtained on the cooperative sorghum, wheat, and triticale samples at the end of 14 days. The average weights gained on the sorghum diets were significantly lower than the average weights gained on the three triticale diets. All the cereal diets showed significantly lower gains than the ANRC casein diet. The PER's of the three sorghum diets were significantly lower than the PER's of the three triticale diets. Again, the sorghum IS 8416's diet was significantly lower in PER than the sorghum IS 2419 diet. All the cereal diets had PER values that were significantly lower than the casein diet. The best of the cereal samples, the triticales samples, had PER values that were 52% of the ANRC casein value.

Table 10 shows the weight gained and PER's obtained with the cooperative maize diets samples at the end of 1 week of feeding. The three normal maize diets and the one floury-2 maize diet showed similar average weight gains. These gains were significantly lower than the average weights gained on the three opaque-2 diets, which in turn were not significantly different from the ANRC casein control. The PER's of the three normal maize diets and the one floury-2 diet did not differ significantly; however,

TABLE 8 Protein efficiency ratio values of cooperative maize samples[1]

	CM-4854 normal	O643 X B14 normal	CM-4858 normal	O643 X B14 floury 2	CM-4855 opaque-2 (hard)	CM-4856 opaque-2 (soft)	CM-4857 opaque-2 (soft)	ANRC casein
Average weight gained (g)	12.4^{c}	16.6^{c}	17.3^{c}	18.5^{c}	33^{b}	37.3^{b}	38.3^{b}	66.9^{a}
Average total feed consumed (g)	83.8^{b}	91.3^{b}	94.4^{b}	94.7^{b}	127.3^{a}	129.2^{a}	133.9^{a}	129.1^{a}
Protein efficiency ratio	1.67^{d}	$2.06^{c,d}$	$2.07^{c,d}$	2.21^{c}	2.96^{b}	3.31^{b}	3.25^{b}	4.38^{a}

Source: Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of L. Tanchoco, Department of Biochemistry, Purdue University, Lafayette, Ind.

[1] Duration of experiment: 14 days. Ten male rats in each group. Same animals as in Table 6. Values with the same superscript are not significantly different from each other at the 1% level.

TABLE 9 Protein efficiency ratio values of cooperative sorghum, wheat, and triticale samples[1]

	Sorghum IS-8165	Sorghum IS-0250421	Sorghum IS-2319	Wheat INIA	Wheat 7 Cerros	Triticale PM 2	Triticale PM 15	Triticale PM 132	ANRC casein
Average total weight gained (g)	3.9^{e}	$7.0^{d,e}$	$8.4^{c,d,e}$	$11.9^{b,c,d}$	$14^{b,d}$	14.5^{b}	16.1^{b}	16.2^{b}	39.2^{a}
Average total feed consumed (g)	$82.5^{c,d}$	77.9^{d}	79.2^{d}	$83.5^{b,c,d}$	$89.6^{b,c,d}$	$90.7^{b,c,d}$	$93.8^{b,c}$	96.2^{b}	122.4^{a}
Protein efficiency ratio	0.52^{e}	$1.0^{d,e}$	$1.19^{c,d}$	$1.60^{b,c,d}$	$1.73^{b,c}$	1.80^{b}	1.93^{b}	1.99^{b}	3.64^{a}

Source: Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of L. Tanchoco, Department of Biochemistry, Purdue University, Lafayette, Ind.

[1] Duration of experiment: 14 days. Ten male rats in each group. Same animals as in Table 7. Values with the same superscript are not significantly different from each other at the 1% level.

their PER values were significantly lower than the PER values obtained on the three opaque-2 diets and the ANRC casein diet. At the end of 1 week, the ANRC casein control had a higher numerical PER value, but the difference between that and the values for the opaque-2 samples was not significant at the 1% level. The average PER value obtained on the three opaque-2 samples was 87% of the ANRC casein control at the end of 7 days, compared with 87% at the end of 28 days.

Table 11 shows the weight gain and PER s of the rats fed on the cooperative sorghum, wheat, and triticale samples at the end of 1 week. In this table, the values with the same superscript are not significantly different at the 5% level. At the end of 1 week, sample IS 8165 provided a weight gain that was significantly lower than the gains obtained on the three triticale and two wheat samples. The net weight gains of the other two sorghum samples did not differ significantly from those of the two wheat samples and two of the triticale samples. All the cereal samples tested provided weight gains that were significantly lower than the ANRC casein control. The PER values of the three triticale samples and the two wheat samples did not differ significantly, but all were significantly higher than the PER values of sorghum IS-8165. After 1 week, however, sorghum IS 8165 was not significantly different in PER from sorghum IS 2319, however, these two sorghums were significantly different in PER at the end of 14 and 28 days of feeding. At the end of 7 days, the triticale samples had PER values of 60% of the casein control, the wheat about 57%, the sorghum 29 to 40%, and the normal maize about 50%. These data suggest that the rat could be used for screening purposes with an experimental period as short as 7 days, provided 10 weanling rats were used per treatment. Under these circumstances, a good differentiation could be obtained between the opaque-2 types in comparison with normal maize, sorghum, wheat, and triticales, with a maximum of about 600 g of each cereal grain required for evaluation.

MOUSE FEEDING TESTS

Protein efficiency ratios were determined on the cooperative grain samples at Nebraska using the suggested levels of protein, casein source, vitamin supplements, mineral supplements, and oil source (Table 3). Five male weanling mice (Swiss Wistar) were used in each group, and feeding tests were conducted for a period of 28 days. The PER values obtained are shown for the cooperative maize samples in Table 12. In comparison with PER values obtained in the rats, most PER values were lower for the grain samples and the casein control. Because of the small group size, the 10% significance level was considered adequate for screening purposes.

The opaque-2 maize sample with hard endosperm did not vary significantly in PER value from the soft opaque-2 maize sample. The PER value of the floury-2 maize (as obtained on mice) was significantly better than the

TABLE 10 Protein efficiency ratio values of cooperative maize samples[1]

	CM-4854 normal	Ob43 X B14 normal	CM-4858 normal	Ob43 X B14 floury-2	CM-4855 opaque-2 (hard)	CM-4856 opaque-2 (soft)	CM-4857 opaque-2 (soft)	ANRC casein
Average weight gained (g)	8.2^{b}	8.6^{b}	8.6^{b}	9.2^{b}	18.0^{a}	18.5^{a}	20.8^{a}	21.1^{a}
Average total feed consumed (g)	42.3^{b}	41.5^{b}	44.6^{b}	43.6^{b}	56.5^{a}	56.7^{a}	58.1^{a}	52.4^{a}
Protein efficiency ratio	2.139^{b}	2.362^{b}	2.163^{b}	2.369^{b}	3.623^{a}	3.680^{a}	4.016^{a}	4.441^{a}

Source: Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of L. Tanchoco, Department of Biochemistry, Purdue University, Lafayette, Ind.

[1] Same as Table 8 except for duration of experiment: 7 days. Same animals as in Table 6. Values with the same superscript are not significantly different from each other at the 1% level.

TABLE 11 Protein efficiency ratio values of cooperative sorghum, wheat, and triticale samples[1]

	Sorghum IS-8165	Sorghum IS-0250421	Sorghum IS-3219	Wheat INIA	Wheat 7 Cerros	Triticale PM-2	Triticale PM-15	Triticale PM-132	ANRC casein
Average total weight gained (g)	1.6^{d}	$4.3^{c,d}$	$4.3^{c,d}$	$6.6^{b,c}$	$7.3^{b,c}$	$7.2^{b,c}$	$7.5^{b,c}$	8.6^{b}	15.2^{a}
Average total feed consumed (g)	41.5^{b}	39.9^{b}	40.1^{b}	39.0^{b}	41.8^{b}	43.4^{b}	42.6^{b}	$43.9^{a,b}$	50.1^{a}
Protein efficiency ratio	0.671^{d}	1.339^{c}	$1.298^{d,c}$	$1.900^{b,c}$	$1.920^{b,c}$	2.035^{b}	1.970^{b}	2.171^{b}	3.345^{a}

Source: Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of L. Tanchoco, Department of Biochemistry, Purdue University, Lafayette, Ind.

[1] Duration of experiment: 7 days. Ten male rats in each group. Same animals as in Table 7. Values with the same superscript are not significantly different from each other at the 5% level.

PER value of its normal counterpart. This was not the case in the rat studies, where the value was higher, but not significantly so.

Table 13 shows the PER values for sorghum, wheat, and triticales. Since all these cereal samples were tested simultaneously, the same ANRC casein control values are shown in both tables. The PER values obtained on the sorghum samples did not show as wide a range as those reported with the rats. However, PER values on these sorghum samples were significantly lower than those obtained on the triticales samples. The PER values on the normal maize samples (except 4854) did not differ significantly from the PER values on the sorghum samples. The mice seemingly responded more favorably to the sorghum samples than did the rats. A greater spread in PER values is obtained between the triticales and wheat samples when tested on the mouse than when tested on the rat. One opaque-2 sample (4857 soft endosperm) was not significantly different in PER value from the three triticale samples. The best opaque-2 maize sample (4855 hard endosperm) had a PER value that was approximately 64% of the casein control, whereas the best triticale sample (CT-PM2) was 92% of the casein control. In summary, the mouse tests were able to distinguish between normal maize and floury-2 maize and between normal maize and opaque-2 maize, but were not able to distinguish between floury-2 maize and opaque-2 maize nor between opaque-2 maize and triticales. These tests distinguished between the control casein and all the cereal grains.

VOLE FEEDING TESTS

Table 14 shows the diet ingredients used in the assay of the cooperative maize samples at CIMMYT and Pennsylvania State University. As shown in Tables 14 and 15, a different salt mixture and casein were used at Pennsylvania State University. CIMMYT used those shown in Tables 1 and 3. Table 15 shows the final diet used in the vole cereal experiments carried out at CIMMYT and Pennsylvania State University. At CIMMYT, 2% corn oil was not added to the casein control (diet 8261 in Table 14).

The results obtained with a 5-day test period in the weanling meadow vole by Villegas and Bauer at CIMMYT with the cooperative maize samples are shown in Table 16. Average weight gain in grams per day varied from a low of 0.22 in 4854 (normal maize) to a high of 0.56 in the ANRC casein control. The PER values varied from a low of 1.30 in 4854 (normal maize) to a high of 2.37 in the ANRC casein.

Because of extreme variations among animals within groups, no significant differences could be demonstrated between maize samples, even at the 10% level. Table 17 shows the values obtained with the cooperative sorghum, wheat, and triticale samples. In these samples, the wheat (7 Cerros) had a PER value that was greater than that of ANRC casein, and only the pigmented sorghum sample had a PER that was substantially below that of the other cereal grains and the casein.

TABLE 12 Protein efficiency ratio values of cooperative maize samples[1]

Mean values	CM-4854 normal	Oh43 X B14 normal	CM-4858 normal	Oh43 X B14 floury-2	CM-4855 opaque-2 (hard)	CM-4856 opaque-2 (soft)	CM-4857 opaque-2 (soft)	ANRC casein
Initial weight (g)	15.36	15.33	15.35	15.36	15.40	15.36	15.37	15.36
Total weight gain (g)	0.08	2.49	2.52	7.33	9.60	6.82	9.02	11.75
Total feed consumed (g)	56.53	75.65	62.58	94.89	108.39	95.18	106.85	84.43
Protein efficiency ratio	0.02[e]	0.37[d]	0.46[d]	0.88[c]	1.01[b,c]	0.81[c]	0.96[b,c]	1.58[a]

Source: Data obtained from the University of Nebraska, Lincoln, Neb., (Department of Food and Nutrition and Department of Agronomy).
[1] Duration of study: 28 days. Five male mice in each group. Values with the same superscript are not significantly different from one another at the 10% level.

TABLE 13 Protein efficiency ratio values of cooperative sorghum, wheat, and triticale samples[1]

Mean values	Sorghum IS-8165	Sorghum IS-0250421	Sorghum IS-2319	Wheat CW-4859	Wheat CW-4860	Triticale CT-4861	Triticale CT-4862	Triticale CT-4863	ANRC casein
Initial weight (g)	15.34	15.34	15.35	15.35	15.57	15.36	15.35	15.35	15.36
Total weight gained (g)	4.49	6.20	5.64	7.35	7.26	11.41	15.37	11.32	11.75
Total feed consumed (g)	104.58	105.81	101.28	101.04	97.48	113.58	119.88	108.01	84.43
Protein efficiency ratio	0.49[d]	0.66[c,d]	0.63[c,d]	0.83[c]	0.85[c]	1.14[b]	1.46[a,b]	1.19[b]	1.58[a]

Source: Data obtained from the University of Nebraska, Lincoln, Neb. (Department of Food and Nutrition and Department of Agronomy).
[1] Duration of experiment: 28 days. Five male mice in each group. Values with the same superscript are not significantly different from one another at the 10% level.

TABLE 14 Diet composition in vole experiment[1]

Lab. ID	Grain (%)	Protein[1] (%)	CHO + O[2] (%)	Fiber[3] (%)	Vitamins[4] (%)	Minerals[5] (%)
CM-4854	70.5	7	65.9	27.1	2	3
CM-4855	71.5	7	92.4	30.6	2	3
CM-4856	71.5	7	62.6	30.4	2	3
CM-4857	71.5	7	63.6	29.4	2	3
CM-4858	76.0	7	63.8	29.2	2	3
CW-4859	65.0	7	64.1	28.9	2	3
CW-4860	61.0	7	64.1	28.1	2	3
CT-4861	47.5	7	66.1	26.9	2	3
CT-4862	48.0	7	65.6	27.4	2	3
CT-4863	44.0	7	65.2	27.8	2	3
Control (8261) (casein)		7	67.0	20.0	2	3

Source: Data supplied by J. S. Shenk, Pennsylvania State University, University Park, Pa.

[1] Protein = Kjeldahl nitrogen X factor.

[2] CHO + O = (protein + fiber) - 95. This value indicates both readily available energy and oil.

[3] Fiber = Alpha cel + % CWC (H. K. Goering) and P. J. Van Soest. 1970. Forage fiber analysis. *Agr. Handbook 379*, Agr. Res. Serv., USDA, Washington, D.C. + cellulose gum.

[4] Vitamin Fortification Mix, General Biochemicals, Chagrin Falls, Ohio.

[5] Salt Mix, Bernhart-Tomarelli, General Biochemicals, Chagrin Falls, Ohio.

The ranges of protein indices shown in Figure 2 provide an explanation for the inability of the animal tests to differentiate between the different cereals tested. Figure 2 shows that 4854 (normal maize) had PER values ranging from 0 to 4.0 in different animals; and for nearly all the cereals

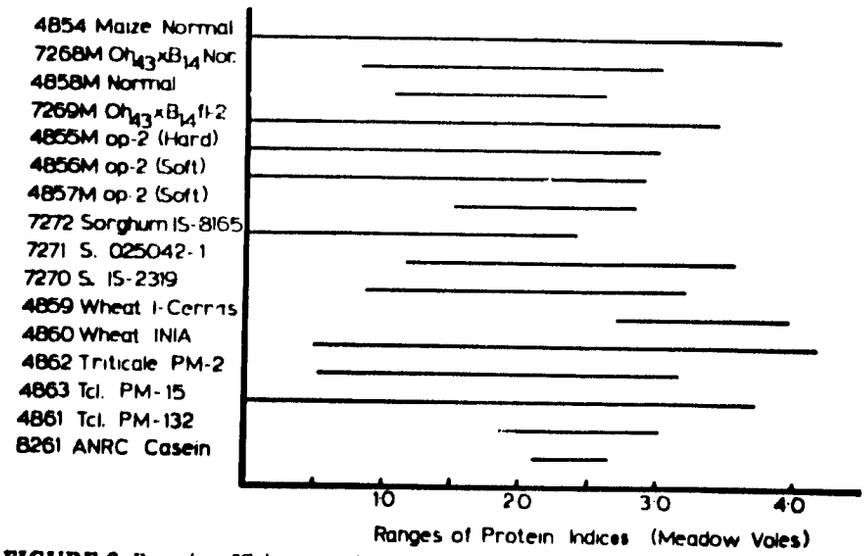

FIGURE 2 Protein efficiency ratio ranges in voles fed cooperative cereal samples at CIMMYT. Each range line covers data on five voles.

TABLE 15 Levels of ingredients in vole experiment

Ingredients	Samples %										
	8261	CM-4854	CM-4855	CM-4856	CM-4857	CM-4858	CW-4859	CW-4860	CT-4861	CT-4862	CT-4863
Meal	8	70.5	71.5	71.5	71.5	73	65	61	47.5	48	44
Vitamin mixture	2	2	2	2	2	2	2	2	2	2	2
Salt mixture	3	3	3	3	3	3	3	3	3	3	3
Alphacel	18	18	18	18	18	18	20	20	20	20	20
Cellulose gum	2	2	2	2	2	2	–	–	–	–	–
Carbohydrate	65	4.5	3.5	3.5	3.5	2	10	14	27.5	27	31
Corn oil	2	–	–	–	–	–	–	–	–	–	–

Source: Data supplied by J. S. Shenk, Pennsylvania State University, University Park, Pa.

Notes:

Meal: The calculated percentage of each cereal in order to provide 7% protein.

Vitamin: Vitamin Fortification Mixture, General Biochemicals, Chagrin Falls, Ohio.

Salt mixture: Bernhart-Tomarelli, General Biochemicals, Chagrin Falls, Ohio.

Cellulose gum: It has to be added just to corn and casein diets.

Carbohydrate: Mixture of 1 part dextrin, 1 part sucrose, 2 parts cornstarch.

Casein: Vitamin-free casein, 14.5% nitrogen, Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 16 Protein indexes of cooperative maize samples[1]

	CM-4854 normal	7268 Oh43 X B14 normal	CM-4858[2] normal	7269 Oh43 X B14 floury-2	CM-4855 Opaque-2 hard	CM-4856 Opaque-2 soft	CM-4857 Opaque-2 soft	8261 ANRC casein
Average initial weight (g)	13.0	12.1	12.2	12.7	12.7	12.9	12.7	12.6
Average weight gained (g/day)	0.22	0.49	0.45	0.36	0.39	0.45	0.49	0.56
Average feed consumed (g/day) (air dry)	2.5	3.7	3.9	3.2	3.6	3.8	3.6	4.2
Protein efficiency ratio	1.30	1.95	2.00	1.76	1.65	1.62	2.27	2.37

Source: Data obtained at CIMMYT, Animal Nutrition Laboratory.

[1] Test animal: weanling meadow voles (16 to 21 days age). Five-day test period; five voles per group. Protein index = grams body weight gain per gram of protein consumed.

[2] Four replications.

TABLE 17 Protein indices of cooperative sorghum, wheat, and triticale samples[1]

	7272 sorghum IS-8165	7271 sorghum IS-0250421	7270 sorghum IS-2319	CW-4859 wheat 7 Cerros	CW-4860 wheat INIA	CT-4862 triticale PM-2	CT-4863 triticale PM-15	CT-4861 triticale PM-132	8261 ANRC casein
Average initial weight (g)	12.4	12.3	12.3	13.0	12.8	12.7	12.3	12.8	12.6
Average weight gained (g/day)	0.25	0.50	0.39	0.90	0.61	0.49	0.77	0.65	0.56
Average feed consumed (g/day) (air dry)	3.4	3.9	3.1	5.0	3.8	3.9	3.7	4.7	4.2
Protein efficiency ratio	1.19	2.12	2.12	3.20	2.69	2.01	2.63	2.37	2.37

Source: Data obtained at CIMMYT, Animal Nutrition Laboratory.

[1] Test animal: weanling meadow voles (16 to 21 days age). Five-day test period; five voles per group. Protein index = grams body weight gain per gram of protein consumed.

tested there were one or more animals having PER values in excess of the highest value obtained on the control casein.

Table 18 summarizes the meadow vole responses (six voles per group) obtained by J. S. Shenk at Pennsylvania State University. Unfortunately, Shenk did not have the sorghum and normal and floury-2 corn samples from Purdue. The data in Table 18 assign a PER value of 2.28 to CM-4857 (opaque 2, soft), which is not significantly different from that of the control casein (2.15). CM-4856 (opaque 2, soft) had a lower PER, but this was not significantly different from the value for the control casein. However, CM-4855 (opaque 2, hard) provided a PER that was substantially lower and significantly different from the control casein (1.41 versus 2.15). The two wheat samples and one triticale sample (4862) did not have PER values differing significantly from the control casein values. However, the other two triticale samples and the two normal maize samples had values that were significantly lower than the control casein values. Based on these data, the voles responded in a much more satisfactory manner to the cereal samples in the experiments at Pennsylvania State University than they did in the experiments at CIMMYT. Nevertheless, in these studies the vole tests were not able to differentiate among opaque-2 maize, wheat, and triticale samples.

CHICK FEEDING TESTS

Since the chick requires a higher level of protein in its diet than does the rat, mouse, or meadow vole, J. McGinnis at Washington State University used a diet with 8% of its protein from a mixture of animal and vegetable proteins of good balance combined with another 6% of protein from the cereal grain to be tested. Day-old male chicks obtained from a commercial source were used in the experiment. The birds were White Rock males, in groups of five chicks each. The chicks were housed in electrically heated, wire-floored battery brooders. The battery heaters were set at about 130°F during the first week of the experiment. At the start of the second week the battery heaters were adjusted to 90°F, and this temperature was kept until the end of the experiment, when the birds were 2 weeks of age. The room temperature was approximately 72°F. Each diet was fed to four replicate groups of five chicks. In all treatments the level of protein in the diet was kept at 14%. Table 19 shows the composition of the diets. For each diet, 6% of the protein was supplied by the test cereal. Glucose monohydrate was added as the adjusting nutrient, so that the diet would contain the proper amounts and proportions of protein from the premix and from the cereal grain being tested. Table 20 is a summary of the body weights of chicks fed different cereal grains. In addition to the cooperative samples supplied by CIMMYT and Purdue, soybean meal, normal yellow maize, triticale, Trailblazer rye, Gaines wheat, high protein wheat, Hiproly barley, and piroline barley also were tested. Each cereal grain was being tested as a supplement to a well-balanced protein base; thus it could be expected that results would

TABLE 18 Protein indices of cooperative maize, wheat, and triticale samples[1]

	CM-4854 normal maize	CM-4858 normal maize	CM-4855 opaque-2 hard	CM-4856 opaque-2 soft	CM-4857 opaque-2 soft	CW-4859 wheat 7-Cerros	CW-4860 wheat INIA	CT-4861 triticale PM-132	4862 triticale PM-2	4863 triticale PM-15	ANRC casein
Average weight gained (g/day)	0.26^{e}	$0.36^{d,e}$	$0.49^{c,d}$	$0.67^{a,b,c}$	0.80^{a}	$0.69^{a,b}$	$0.69^{a,b}$	$0.59^{b,c}$	$0.67^{a,b,c}$	$0.53^{b,c,d}$	$0.58^{b,c}$
Average feed consumed (g/day) (air dry)	$4.57^{c,d,e}$	$4.68^{c,d}$	$4.87^{a,b,c,d}$	$5.12^{a,b,c}$	$5.08^{a,b,c,d}$	$4.98^{a,b,c,d}$	5.57^{a}	$5.41^{a,b}$	$5.05^{a,b,c,d}$	$4.68^{b,c,d}$	3.85^{e}
Protein efficiency ratio	0.87^{f}	$1.08^{e,f}$	$1.41^{d,e}$	$1.89^{a,b,c,d}$	2.28^{a}	$1.98^{a,b,c}$	$1.78^{a,b,c,d}$	$1.59^{c,d}$	$1.90^{a,b,c,d}$	$1.63^{b,c,d}$	2.15^{a}

Source: Data supplied by J. S. Shenk, Department of Agronomy, Pennsylvania State University, University Park, Pa. 16802.

[1] Duration of experiment: 6 days. Six male voles in each group. Values with the same superscript are not significantly different from each other at the 5% level.

TABLE 19 Composition of chick diets

Ingredient	% of diet
Premix	
Meat and bone meal (50% protein)	9.39
Soybean meal (50% protein)	2.68
Dehydrated alfalfa	2.01
Ground yellow corn	16.76
Dicalcium phosphate (Dynaphos)	2.00
Iodized salt	0.25
Trace mineral mix[1]	0.05
Vitamin premix[2]	0.25
DL-methionine	0.20
	33.59
Test material, glucose monohydrate, lysine	64.41

[1] Supplies the following in milligrams per kilogram of feed: Mn, 50; Fe, 50; Cu, 5; Zn, 50; I, 1.5; and Co, 0.5.

[2] Supplies the following per kilogram of feed: Vitamin A, 5,500 IU; vitamin D_3 1,650 ICU; riboflavin, 3.3 mg; choline chloride, 500 mg; calcium panthothenate, 3.3 mg; niacin, 17.5 mg; and ethoxyquin (Santoquin), 125 mg. The vitamin premix was supplemented with B_{12} to provide 0.01 mg/kg of feed.

differ from those obtained with the other tests (in which the cereal grains served as the only source of protein). The body weights indicate that the treatment containing soybean meal was significantly better (tested at the 5% level) than all other treatments. ANRC casein was significantly better than CM-4855, opaque-2 maize (hard), but not significantly better than CM-4856, opaque-2 (soft). CM-4854, normal maize, was significantly less effective as a protein suplement than opaque-2 maize. However, CM-4858 (normal maize), Oh43 × B14 (normal maize), and Oh43 × B14 (floury-2 maize) were as effective as protein supplements as CM-4855, opaque-2 (hard). In series II, with the exception of the treatment containing casein as test material, the test material supplied 5.7% protein to the diet. A level of 0.3% L-lysine was added to all diets, except the treatment containing casein, which was considered to be adequate in lysine (further additions might have created an imbalance of arginine). The addition of lysine to opaque-2 (hard) and opaque-2 (soft) markedly increased the protein supplementary effect of these two cereals and eliminated the growth differences observed in the absence of supplemental lysine. With the added lysine, these two maize samples were significantly superior to ANRC casein as a protein supplement. Addition of lysine to the two normal maize selections gave a positive response, but the response was not as marked as that observed with the opaque-2. Floury-2 maize showed a better response than the normal maizes, giving a supplementary effect not significantly different from that observed with ANRC casein.

When 7 Cerros (CW-4859) wheat was used as a supplementary source of

TABLE 20 Summary of body weights of chicks fed different cereal grains

Source of protein[1]	Average body weight at 2 weeks of age (g)[2]	
	Series I	Series II (lysine supplemented)
Premix	76.0[e,f,g,h]	79.6[i]
Soybean meal	124.7[a]	122.8[a]
ANRC casein	97.9[b]	98.0[d,e,f,g]
Yellow corn, normal	77.7[d,e,f,g]	86.6[g,h,i]
Triticale Trailblazer	71.2[f,g,h]	88.5[f,g,h,i]
Rye	70.4[f,g,h]	77.8[i]
Gaines wheat	74.7[e,f,g,h]	95.1[d,e,f,g,h]
High protein wheat	71.2[f,g,h]	94.2[d,e,f,g,h]
Hyproly barley	85.1[c,d]	101.9[c,d,e]
Piroline barley	78.6[d,e,f]	92.6[e,f,g,h]
CM-4854, normal maize	71.3[f,g,h]	84.5[h,i]
CM-4855, opaque-2 (hard)	81.6[d,e]	114.0[a,b]
CM-4856, opaque-2 (soft)	92.2[b,c]	116.0[a,b]
CM-4858, normal maize	73.5[e,f,g,h]	86.8[g,h,i]
Oh43(+), normal maize	72.7[e,f,g,h]	88.5[f,g,h,i]
Oh43 (fl_2) floury-2	81.2[d,e]	98.5[d,e,f,g]
CW-4859, 7 Cerros	67.4[h]	93.5[e,f,g,h]
CW-4860, INIA	73.6[e,f,g,h]	111.7[a,b,c]
CT-4861, triticale PM-132	75.1[e,f,g,h]	99.8[d,e,f]
CT-4862, triticale PM-2	68.8[g,h]	100.8[c,d,e,f]
CT-4863, triticale PM-15	74.2[e,f,g,h]	97.5[d,e,f,g]
IS-8165, sorghum	69.0[g,h]	98.7[d,e,f,g]
IS-2319, sorghum	68.5[h]	102.0[c,d,e]
S-0250421, sorghum	69.4[g,h]	106.2[b,c,d]

[1] The premix provided 8% protein in all diets with 6% provided by test materials.

[2] Each mean is the average of four replicate groups of five chicks each. Values with the same superscript are not significantly different from each other at the 5% level.

protein, the response was similar to that observed with the three samples of normal maize. This was also true for the other wheat sample, INIA (CW-4860). The three triticale samples also had a protein response similar to that observed with the normal maize samples and the wheat samples. Surprisingly, the sorghum samples also had a supplementary effect about equal to that of the other cereal samples. Only the two opaque-2 samples showed a response superior to that of the other cereal grains. Supplementation of the wheat samples with lysine raised the protein response to that of ANRC casein; and with the INIA sample, the response was not significantly different from that of soybean meal and opaque-2 maize. The triticale samples also showed a significant response on addition of lysine, but these responses were no greater than that observed with sorghum. When supplemented with lysine, both the triticale and sorghum samples gave values equal to that of ANRC casein, but less than that of soybean meal.

Table 21 shows the PER values for the same group of cereal grains tested. The PER value of ANRC casein was not significantly different from that of

soybean meal, and both were significantly superior as supplements to hard endosperm opaque-2 maize. However, soft endosperm opaque-2 maize (CM-4856) had the same supplementary effect as the ANRC casein and the soybean meal. The supplementary effects of the normal maize samples, the floury-2 sample, the two wheat samples, the three triticale samples, and the three sorghum samples were significantly less than those of ANRC casein and soybean meal. However, no single cereal in this group of cereals was significantly different from any other in supplementing action.

The addition of lysine to soybean meal made it significantly superior to ANRC casein. This also was true with the addition of lysine to the hard and soft endosperm opaque-2 maize samples. For these samples the PER values increased, were significantly higher than the ANRC casein values, and were equal to the soybean meal values. Addition of lysine to the other cereal samples provided a significant increase in the PER values, so that none differed significantly from ANRC casein; the INIA wheat sample gave such

TABLE 21 Average protein efficiency ratio of different cereal grains fed to chicks in a modified bioassay[1]

Source of protein[2]	Average PER from 0–2 weeks of age: Series I	Series II (lysine supplemented)
Premix protein	2.20[c,f]	2.27[h]
Soybean meal	2.99[a]	3.24[a]
ANRC casein	2.81[a,b]	2.82[c,d,e,f]
Yellow corn	2.31[d,e]	2.74[d,e,f]
Triticale Trailblazer	2.16[e,f]	2.47[g,h]
Rye	2.11[e,f]	2.29[h]
Games wheat	2.28[d,e]	2.76[d,e,f]
High protein wheat	2.12[e,f]	2.70[d,e,f,g]
Hypoly barley	2.64[b,c]	2.71[d,e,f,g]
Proline barley	2.31[d,e]	2.73[d,e,f]
CM-4854, normal maize	2.10[e,f]	2.57[f,g]
CM-4855, opaque-2 (hard)	2.45[c,d]	3.20[a]
CM-4856, opaque-2 (soft)	2.81[a,b]	3.15[a,b]
CM-4858, normal maize	2.33[d,e]	2.80[d,e,f]
Ob43 × B14(+), normal maize	2.23[d,e,f]	2.87[c,d,e]
Ob43 × B14 (H_1), floury-2	2.46[c,d]	2.94[b,c,d]
CW-4859, 7 Cerros	2.12[e,f]	2.73[d,e,f]
CW-4860, INIA	2.25[d,e]	3.06[a,b,c]
CT-4861, triticale PM-132	2.31[d,e]	2.89[c,d,e]
CT-4862, triticale PM-2	2.18[e,f]	2.85[c,d,e]
CT-4863, triticale PM-15	2.32[d,e]	2.78[d,e,f]
IS-8165, sorghum	2.00[f]	2.72[d,e,f,g]
IS-2319, sorghum	2.02[f]	2.87[c,d,e]
IS-0250421, sorghum	2.09[e,f]	2.88[c,d,e]

[1] Each mean is the average of four replicate groups of five chicks each. Values with the same superscript are not significantly different from each other.

[2] The premix provided 8% of protein in all test diets. The total protein level was 14%.

a good response that it was not significantly different from the soybean meal sample. Interestingly, there was a significant difference in the supplementary effect of the hard endosperm and soft endosperm opaque-2 in the absence of lysine, but the addition of lysine eliminated this difference. Therefore, the chick tests apparently differentiated between the hard and the soft endosperm types when these grains were used as a supplement to an 8% protein base.

SUMMARY

Weight gains and PER's of normal, floury-2, and opaque-2 maize, and of sorghum, wheat, and triticales, have been compared with a reference casein in tests using the rat, mouse, vole, and chick. With 10 male weanling rats per test cereal, significant differences in protein quality were apparent in 7 days, with values comparable to those obtained in 14 or 28 days. A 7-day test with rats would require less than 600 g of each cereal. In the rat tests, no significant differences were observed in either weight gain or PER between hard and soft endosperm opaque-2 maize. The 28-day PER of these two maizes was 85% of the casein control compared with triticales (60%), wheat (50%), normal and floury-2 maize (50%), and sorghum (40%).

With five male weanling mice tested per cereal for 28 days, floury-2, opaque-2, and triticales did not show significant PER differences and showed values that were about 60% of the control casein value. With five or six weanling voles per test cereal for 5 or 6 days, PER values were not significantly different for the opaque-2 maize, wheat, and triticale samples. With four replicated groups of 5-day old chicks tested per cereal (fed as 6% cereal protein in addition to an 8% balanced protein base), PER values were obtained that distinguished diets of hard endosperm opaque-2 from soft endosperm types. However, the hard endosperm types were not different from floury-2, wheat, or triticales.

The chick studies showed the superiority of opaque-2 maize over normal maize, even after lysine supplementation of both. Also, the lysine-supplemented INIA wheat was shown to be remarkably superior to lysine-supplemented 7 Cerros wheat.

Based on these cooperative studies, the male weanling rat tests allowed the best differentiation among the cereals. Because of the chicks' high protein requirement, they were used only to test the supplementary value of these cereal grains when fed in addition to a well balanced protein base.

The research at Purdue University was supported by the U.S. Agency for International Development through Contract AID Csd 2809, titled "Inheritance and Improvement of Protein Quality and Content in Maize."

[*A discussion of this paper can be found on pp. 503–505 of* **Questions and Answers.**]

AN INTEGRAL SYSTEM FOR CHEMICAL SCREENING OF QUALITY PROTEIN MAIZE

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High-quality protein maize is especially significant from a nutritional point of view. Normal maize varieties are usually low in food value, primarily owing to the unbalanced amino acid content and low protein content.

The beneficial effects of incorporating high-quality protein maize into the diets of humans and monogastric animals have prompted maize breeding programs in many countries to improve the nutritional quality of their materials, especially where maize is a staple food.

However, a breeding program for maize, or any crop, usually manages large quantities of germ plasm. If the materials with high-quality protein are to be selected properly, the breeder requires the services of a laboratory.

Incorporating the opaque-2 or floury-2 genes into normal maize with desired agronomic characteristics implies the rapid identification of high-quality lines from segregant materials; such a program requires the joint effort of plant breeders and biochemists.

The maize mutants, opaque-2 and floury-2, whose protein quality is superior to that of normal maize, were identified through chemical evaluation of the amino acid content in the protein only 10 years ago (4, 8). The amino acids limiting the nutritional quality of maize are lysine and tryptophan. The mutant maizes superior in nutritional quality have been designated as *high-lysine* maizes. This designation is correct, but the improved quality is due to the simultaneous increase of both lysine and tryptophan.

When the conversions of normal maize to opaque-2 or floury-2 maize

were initiated, many breeders made a phenotypic evaluation of nontranslucent opaque-2 kernels. This unfortunately excluded any opportunity of obtaining the harder-endosperm-type kernel with high-quality protein that may occur in some opaque-2 populations owing to modifier genes.

The chemical evaluation of diverse genetic material requires the use of simple, rapid, and relatively low-cost analytical methods so that a large number of samples can be analyzed.

Several laboratories have used indirect methods to evaluate protein. This has been recommended, for instance, to determine zein content. Zein is the fraction of the maize endosperm protein lacking lysine and tryptophan. There is a reduction in the prolamine zein in the endosperm in the presence of the opaque-2 gene, and a consequent increase in the other protein fractions that are not deficient in lysine or tryptophan (2, 5, 9).

On the other hand, the dye-binding method has been recommended for evaluating protein quality. This method uses Orange G dye in an acid medium, since the dye will be associated with the basic reactive groups of the protein (lysine, histidine, and arginine) (6, 7).

The CIMMYT Protein Quality Laboratory and the Purdue University Biochemistry Laboratory studied and evaluated different analytical methods commonly used to determine protein, tryptophan, and lysine, in an attempt to present simple and proper techniques to screen and evaluate a large number of samples. After this evaluation, a series of recommendations were made for the protein evaluations of populations and segregant materials produced by the maize breeding programs.

In a general way, the methodology followed by the CIMMYT Protein Quality Laboratory is as follows (11).

METHODS

Sample Preparation for Endosperm Analysis

It is advisable to analyze the endosperm of the sample to readily identify the materials with improved protein quality. The maize endosperm is deficient in lysine and tryptophan, whereas the embryo has a relatively constant and well-balanced amino acid composition, regardless of the genetic background. Also, when analyzing the whole kernel, the pericarp may contribute undesirable pigments that interfere with colorimetric determinations. If whole-kernel analysis is desirable, these factors must be taken into consideration.

In evaluating genetic families,

1. Take a random sample of 10 seeds as representative of each ear.
2. Wash with distilled water if the seeds have been pesticide treated. (If seeds are untreated, eliminate this step.)
3. Soak seeds in distilled water for approximately 30 min. Peel off the

pericarp and remove the germ with tweezers and scalpel. The remaining material of the kernel is considered endosperm tisue. Air dry the endosperm sample overnight.

4. Grind the air-dried sample in a burr mill at the finest setting.

5. Defat ground samples in a Soxhlet continuous extractor with hexane for 6 h.

6. Air dry the samples and grind to a fine powder with a Wig-L-Bug amalgamator.

In certain cases, such as when one maize family with hard endosperm (normal endosperm type) is shown to possess high levels of tryptophan in the protein, it is recommended that a single-kernel analysis be performed on a further subsample. Thus, any segregation within the family can be identified, and grains can be selected that have high levels of tryptophan. The following procedure does not destroy the embryo of the analyzed seed, and thus allows the growing and further analysis of the grain selected as having the highest levels of tryptophan:

1. Take five or six randomly chosen seeds from each family and wash them to eliminate any pesticide, and then air dry them.

2. Drill out a small portion of endosperm in different sites of the kernels with an electric drill using $^1/_{16}$-in. bit.

3. Defat samples in a Soxhlet extractor with hexane.

4. Air dry the sample and grind to a fine powder with a Wig-L-Bug amalgamator.

Protein Determination

The nitrogen content may be estimated by the micro-Kjeldahl procedure (1), and the percentage of protein calculated by using a factor of 6.25.

Reagents

1. Sulfuric acid: sp. gr. 1.84, nitrogen free.

2. Catalyst mixture: 99.0 g of K_2SO_4, 4.1 g of HgO, and 0.8 g of $CuSO_4$.

3. Sodium hydroxide–sodium thiosulfate solution (dissolve 50 g of NaOH and 5 g of $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 100 ml).

4. Boric acid solution (4%).

5. Indicator solution methyl red–bromocresol green (mix 1 part 0.2% methyl red in ethanol with 5 parts 0.2% bromocresol green in ethanol).

6. Hydrochloric acid solution: 0.02 *N*.

Procedure

1. Place a 30- to 40-mg sample in a digestion flask. Add 1.0 g of catalyst powder mixture and 2 ml of concentrated sulfuric acid.

2. Digest 40 min, cool, add a minimum quantity of water to dissolve solids, cool, and place a thin film of Vaseline on the rim of the flask.

3. Transfer the digest to a distillation apparatus, and rinse flask five or six times with 1- to 2-ml portions of distilled water.

4. Place a 125-ml erlenmeyer flask with 6 ml of boric acid solution and 3

drops of litmus indicator solution under a condenser with the tip extending below the surface of the solution.

5. Add 8 ml of sodium hydroxide–sodium thiosulfate solution to a still, and steam distill until about 20 ml of distillate collects.

6. Titrate to gray end point of first appearance of violet.

7. Make blank determination using the same quantity of reagents and the same digestion and distillation periods as for determination.

8. Calculate the percentage of nitrogen:

$$\% \text{ nitrogen} = \frac{(\text{ml HCl in detn.} - \text{ml blank}) \times \text{normality} \times 14.007 \times 100}{\text{mg sample}}$$

$$\% \text{ protein} = \% \text{ N} \times 6.25$$

Tryptophan Determination

Hernández and Bates (3) have observed that the relationship between tryptophan and lysine in the maize endosperm protein is about 1 to 4; thus tryptophan may be used as a single parameter for maize quality evaluation.

For tryptophan estimation, the Opienska–Blauth et al. colorimetric method modified by Hernández and Bates (3) is recommended for its simplicity. With this method it is possible to analyze up to 75 samples with duplicates each day.

Reagents

1. Reagent A: 270 mg of $FeCl_3.6H_2O$ is dissolved in 0.5 ml of distilled water and diluted to 1 liter with glacial acetic acid. (Each bottle of acetic acid must be tested for color development in the presence of tryptophan.)

2. Reagent B: 30 *N* sulfuric acid.

3. Reagent C: a volume-to-volume mixture of reagents A and B is prepared 1 to 2 h before use.

4. Reagent D: papain solution. The enzyme (Papain-Tech. powder from Nutritional Biochemicals Corp., Cleveland, Ohio) (4 mg/ml) is dissolved in 0.1 *N* sodium acetate buffer at pH 7.0. The enzyme solution is prepared daily.

Procedure

1. Weigh 90 to 100 mg of finely ground defatted maize endosperm sample into a glass vial and add 4 ml of papain solution. The tubes are capped and carefully shaken, making sure that the sample is totally wetted. (Blanks must also be carried out with papain solution through this procedure.)

2. Samples are kept overnight in an incubator oven at 65°C.

3. Hydrolysates are removed from the incubator or oven and shaken, then allowed to adjust to room temperature. When this temperature is reached, the supernatant should be clear; if not clear, centrifuge the samples.

4. One milliliter of hydrolysate is pipetted into a test tube containing 4 ml

of reagent C; the mixture is shaken vigorously and the color developed for 15 min at 65°C.

5. After cooling, the solutions are transferred to calibrated tubes and read at 545 mμ on a Bausch and Lomb Spectronic 20.

6. A standard curve is prepared in a range from 0 to 40 g/ml, using DL-tryptophan.

7. The tryptophan content of the sample is calculated from the standard curve and reported on a protein basis.

Lysine Determination

The lysine determination is performed only on those materials with high tryptophan values as selected through the colorimetric procedure, or when the lysine value is required in addition to the tryptophan value.

The recommended colorimetric method was designed by Tsai of Purdue University (10) and modified by Villegas of CIMMYT (11). This method permits analyses of up to 60 duplicate samples each day.

Reagents

1. Papain solution: 4 mg of papain/ml of phosphate buffer, 0.03 *M*, pH 7.4.

2. Carbonate buffer: 0.05 *M*, pH 9.0.

3. Borate buffer: 0.05 *M*, pH 9.0.

4. Copper phosphate suspension: Solution A:2.8 g of $CuOl_2 \cdot 2H_2O$ is dissolved in 100 ml of distilled water. Solution B: 13.6 g of $Na_3PO_4 \cdot 12H_2O$ is dissolved in 200 ml of distilled water. Pour solution A into B with swirling, centrifuge to bring out the precipitate, and discard the supernatant. The pellet is then resuspended three times in 16 ml of borate buffer pH 9, centrifuging after each suspension. After the third washing, resuspend the pellet in 80 ml of borate buffer. The reagent can be used for 1 week.

5. 2-Chloro-3,5-dinitropyridine solution. Prepare, just before using, a solution containing 30 mg of 2-chloro-3, 5-dinitropyridine/ml of methanol.

6. HCl solution: 1.2 *N*.

7. Mixture of amino acids: cystine (20 mg), methionine (20 mg), histidine (30 mg), alanine (30 mg), isoleucine (30 mg), threonine (30 mg), tyrosine (30 mg), glycine (40 mg), phenylalanine (40 mg), valine (40 mg), arginine (50 mg), serine (50 mg), aspartic acid (60 mg), glutamic acid (300 mg), leucine (80 mg), and proline (80 mg). Weigh 100 mg of the amino acid mixture and dissolve in 100 ml of carbonate buffer.

Procedure

1. Weigh 200 mg of finely defatted sample in a vial and add 5 ml of papain solution. Be sure to wet all the sample and shake it at least twice in the first hour of incubation. Carry on blanks with papain solution.

2. Incubate overnight at 65°C. Remove the hydrolysates from the incubation oven and shake; then allow to settle and adjust to room temperature, by

which time the supernatant should be clear; if not, centrifuge. (One aliquot of 1 ml from this hydrolysate can be used for tryptophan determination.)

3. Pipette 1-ml aliquot into a centrifuge tube and add 0.5 ml of carbonate buffer and 0.5 ml of the copper phosphate suspension.

4. Shake the mixture for 5 min and centrifuge.

5. Pipette 1-ml aliquot of the supernatant into a large test tube, and add 0.1 ml of 2-chloro-3,5-dinitropyridine–methanol solution and shake well.

6. Allow the mixture to stand for 2 h at room temperature (shake every 30 min).

7. Add 5 ml of 1.2 *N* HCl and shake well.

8. Add 5 ml of ethylacetate, stopper tubes, mix well, inverting the tubes at least 10 times, and then extract the upper phase, using a syringe adapted with a polyethylene tube. This step must be performed three times.

9. Transfer the aqueous phase to calibrated tubes, and read in the Spectronic 20 at 390 mμ against the blanks.

10. Calculate the lysine content of the sample by comparing with a standard curve and report on a protein basis. Prepare a standard curve in a range of 0 to 200 μg of lysine/ml. Stock solution of lysine: 62.5 mg of lysine-monohydrochloride in 20 ml of carbonate buffer (2,500 μg of lysine/ml). Using carbonate buffer, dilute the stock solution of lysine to 0, 250, 500, 750, and 1,000 μg of lysine/ml. From each solution, take 1 ml and add 4 ml of papain solution (5 mg of papain/ml of phosphate buffer). Pipette 1 ml of each solution into centrifuge tube, add 0.5 ml to the amino acid mixture solution and 0.5 ml of copper phosphate suspension. Continue with step 4 above.

DISCUSSION

On the average, the endosperm analysis shows the following colorimetric values:

1. Normal maize: about 0.45 g of tryptophan and 1.8 g of lysine/100 g of protein (with approximately 9.0% protein in the sample).

2. Opaque-2 maize: about 0.85 g of tryptophan and 3.6 g of lysine/100 g of protein (with approximately 8.0% protein in the sample).

The indicated values can vary according to the genotype or the protein content of the sample. Intermediate values of tryptophan and lysine can be found in the protein materials with the floury-2 gene, or in some samples with hard endosperm.

Using the analytical methods described to evaluate and screen large numbers of maize samples, the laboratory work can be coordinated with that of the breeders. The laboratory reports are essential to the breeders in selecting the desired germ plasm; thus, this coordination can accelerate their programs.

The CIMMYT Protein Quality Laboratory evaluates approximately 7,000 to 8,000 maize samples per year using these techniques. The best materials

selected by chemical screening are reevaluated; a complete amino acid analysis is performed by ion-exchange chromatography, and then the materials are biologically evaluated using laboratory animals. At CIMMYT the meadow vole *(Microtus pennsylvanicus)* has been used for these evaluations. There is still great variability in the results of the biological evaluation. It is necessary to learn more about the meadow vole and how it can be used for screening materials emerging from the breeding programs.

[*A discussion of this paper can be found on p. 505 of* **Questions and Answers.**]

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COST-BENEFIT RELATIONSHIPS IN HIGH-QUALITY PROTEIN MAIZE PRODUCTION

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Opaque-2 maize has been shown to have very significant nutritional advantages over normal maize, in both human and animal nutrition. This high-quality protein maize provides a means for reducing widespread malnutrition. It can supply certain essential amino acids in the diets of low-income families in developing countries. However, superior nutritional quality alone does not assure that opaque-2 maize will find its way into the diet of those who lack high-quality protein. The self-sustained production and consumption of this maize will depend upon several social, technical, and economic requirements.

This report deals with some of the economic issues related to opaque-2 maize production and consumption; its objectives are (1) to analyze the social and private benefits and costs of expanding the production and utilization of opaque-2 maize, (2) to identify differences between social and private benefits and costs, and (3) to suggest government policy measures aimed at reducing such differences, which will assure full realization of the potential net benefits from opaque-2 maize.

After a short definition of social and private benefits and costs, a brief analysis will be made of the potential benefits of opaque-2 maize to South America. This is followed by an examination of the benefits and costs to the consumer, the livestock producer, and the maize producer. A final discussion cites social benefits and costs, and possible government intervention

aimed at obtaining the potential social benefits of opaque-2 maize. Since the economics of utilizing opaque-2 maize in processed foods will be considered separately at this symposium, this report focuses on utilization for direct human consumption and animal feed.

SOCIAL VERSUS PRIVATE BENEFITS AND COSTS

Social benefits and costs refer to those of society as a whole, whereas private benefits and costs refer to those of the individual person or enterprise involved in production, marketing, and consumption of opaque-2 maize. The analyses presented next show that social benefits and costs may differ considerably from private benefits and costs. Private benefits and costs may be such that potential net social benefits will not be realized unless certain corrective measures are introduced.

Potential Benefits of Opaque-2 Maize in South America

Approximately 22% of the maize produced in South America is used for direct human consumption, whereas 62% is used as animal feed (4). Some countries, such as Bolivia (81%), Colombia (72%), and Ecuador (52%), use a higher percentage of the total maize production for direct human consumption. The average per capita consumption of maize in South America in 1970 was estimated at 20.6 kg. Maize thus plays a major role in the human diet in countries such as Bolivia, Venezuela, Paraguay, Colombia, and Brazil, while it is of little importance in Uruguay, Chile, and Argentina.

Table 1 shows the estimated (1970) intake of the two essential amino acids, lysine and tryptophan, from maize, and the potential intake of these amino acids if all the normal maize used for human consumption were changed to opaque-2 maize.

As shown in Table 2, if the per capita maize consumption for 1970 were maintained and all maize were of the opaque-2 type, this maize alone would fulfill the average per capita lysine needs in Bolivia, Venezuela, and Paraguay. Similarly, it would supply more than 50% of total lysine needs in Brazil, Colombia, Ecuador, and Peru.

Opaque-2 maize would supply more than 90% of all tryptophan needs in Bolivia and Venezuela, and would provide more than 50% of total need for both amino acids in Paraguay, Colombia, and Brazil (Table 3). The shortage of essential amino acids in the diets of low-income families in Latin America could be greatly reduced by introducing opaque-2 maize.

The limitations of the preceding analysis should be recognized. Average national consumption data do not reflect intracountry differences, and an adequate average per capita consumption may not reflect a serious level of malnutrition among disadvantaged groups. Although the quantity of maize consumed may be expected to correlate positively with incomes up to a certain level, maize is consumed in relatively large quantities by low-income

TABLE 1 Quantity of maize utilized for human consumption in South America, 1970, the estimated lysine and tryptophan content, and the potential content of a similar quantity of opaque-2 maize

Country	Annual consumption[a] Total (1,000 tons)	Annual consumption[a] kg/capita	Lysine intake[b] (g/capita) Actual (normal maize)	Lysine intake[b] (g/capita) Potential (opaque-2 maize)	Tryptophan intake[b] (g/capita) Actual (normal maize)	Tryptophan intake[b] (g/capita) Potential opaque-2 maize)
Argentina	122	5.0	13.5	26.3	3.2	6.9
Bolivia	213	43.2	116.6	226.8	27.2	59.2
Brazil	2,111	22.6	61.0	118.7	14.2	31.0
Chile	26	2.7	7.3	14.2	1.7	3.7
Colombia	495	23.4	63.2	122.9	14.7	32.1
Ecuador	130	21.4	57.8	112.4	13.5	29.3
Paraguay	92	38.7	104.5	203.2	24.4	53.0
Peru	261	19.3	52.1	101.3	12.2	26.4
Uruguay	5	1.7	4.6	8.9	1.1	2.3
Venezuela	458	41.7	112.6	218.9	26.3	57.1
South America	3,913	20,6	55,6	108.2	13.0	28.2

[a] *Source*: FAO (4).

[b] Estimated on the basis of the following lysine and tryptophan content in grams per kilo of whole grain:

	Lysine	*Tryptophan*
Normal maize	2.70	0.63
Opaque-2 maize	5.25	1.37

TABLE 2 Estimated average lysine intake from normal maize and potential intake from opaque-2 maize compared to needs (mg/day/capita)

Country	Present intake from normal maize: mg/day	Present intake from normal maize: % of need[a]	Potential intake from opaque-2 maize: mg/day	Potential intake from opaque-2 maize: % of need[a]
Argentina	37.5	6.9	73.1	13.4
Bolivia	323.9	59.5	630.0	115.8
Brazil	169.4	31.1	329.7	60.6
Chile	20.3	3.7	39.4	7.2
Colombia	175.6	32.3	341.4	62.8
Ecuador	160.6	29.5	312.2	57.4
Paraguay	290.3	53.4	564.4	103.8
Peru	144.7	26.6	281.4	51.7
Uruguay	12.8	2.4	24.7	4.5
Venezuela	312.8	57.5	608.1	111.8
South America	154.4	28.4	300.6	55.3

Source: Estimated from Table 1.

[a] Estimated on the basis of the need of young adults for 544 mg/day.

TABLE 3 Estimated average tryptophan intake from normal maize and potential intake from opaque-2 maize compared to needs (mg/day/capita)

Country	Present intake from normal maize		Potential intake from opaque-2 maize	
	mg/day	% of need[a]	mg/day	% of need[a]
Argentina	8.9	5.3	19.2	11.4
Bolivia	75.6	45.0	164.4	97.9
Brazil	39.4	23.5	86.1	51.3
Chile	4.7	2.8	10.3	6.1
Colombia	40.8	24.3	89.2	53.1
Ecuador	37.5	22.3	81.4	48.5
Paraguay	67.8	40.4	147.2	87.6
Peru	33.9	20.2	73.3	43.6
Uruguay	3.1	1.8	6.4	3.8
Venezuela	73.1	43.5	158.6	94.4
South America	36.1	21.5	78.3	46.6

Source: Estimated from Table 1.

[a] Estimated on the basis of the need of young adults for 168 mg/day.

families in most Latin American countries. Thus, average consumption data may be less biased in reflecting maize use than for certain other products, such as meats, milk, fruits, and vegetables.

Another factor not reflected by the average data is that related to the differing amino acid needs of children and adults. In this case, however, average data for maize may be less biased than those for certain other products owing to the importance of maize in the diets of small children. Finally, global data, such as those used in the preceding analysis, are frequently collected under adverse conditions and may not be precise descriptions. We should consider the data as approximate magnitudes, rather than as exact figures.

Benefits and Costs to the Consumer

Potential benefits of high-quality protein maize to the consumer may occur in two ways: (1) through improved nutrition, with ensuing improved health and increased earning capacity, and (2) through savings obtained by substituting opaque-2 maize for more expensive foods, such as milk, meats, and beans (maintaining a constant nutritional level).

When discussing consumer benefits, it is important to distinguish between subjective and "real" benefits. Subjective benefits are determined by the individual consumer on the basis of his preference, whereas real benefits are measured by objective standards, such as increased intake of essential amino acids, or reduced disease frequency and/or severity. Furthermore, private benefits and costs might differ from social benefits and costs. Reduced

medical costs, for example, may reduce social costs but have little influence on private costs. Initial discussion will be limited to private benefits and costs, with social benefits and costs discussed later.

In a free market economy, we expect consumer decisions to maximize subjective benefits (given the constraint of income). Lack of knowledge is one factor that may cause a difference between subjective and real benefits. For example, when a group of Colombian homemakers was interviewed, more than half expressed a preference for normal maize over opaque-2 maize at equal prices. This preference was primarily because they did not know how to prepare opaque-2 maize (5). Furthermore, the homemakers did not know of the nutritional advantages of opaque-2 maize. In another study (2), a group evaluated various dishes prepared from normal and opaque-2 maize. Although no statistical analysis was reported, it appears that the people found no significant differences between dishes prepared from normal maize and those prepared from opaque-2 maize. Although the two studies are not directly comparable (consumer choice during purchasing was an important variable omitted from the latter study), the results suggest that an educational campaign explaining methods of preparation would greatly enhance the acceptance of opaque-2 maize. To use our earlier terminology, such a campaign would increase subjective benefits derived from opaque-2 maize relative to those derived from normal maize.

The impact of information concerning the nutritional advantages of opaque-2 maize on acceptance is not clear. Relative nutritional values of traditional foods are fairly well known among small farmers and low-income urban consumers in Colombia (5). However, they do not generally understand the reasons *why* one food has a higher nutritional value than another. Thus, information relating the nutritional value of opaque-2 to that of some well-known foods, such as milk and meats, might speed up the acceptance of opaque-2 maize. This information might have the effect of reducing the difference between subjective and real benefits of opaque-2 maize, whereas technical information on the "higher quality protein" might be less understood and have little impact on acceptability.

As indicated previously, the consumer does not automatically switch from a well-known, but nutritionally inferior, food to a nutritionally superior new food, even though the price of the two foods is equal. There is reason to believe that opaque-2 maize will have to be priced above normal maize to assure its production (this aspect is to be discussed later). Thus the question becomes: Do subjective *benefits* obtained by changing from normal to opaque-2 maize exceed the subjective *costs* associated with the change? How much is the low-income consumer willing to pay for additional lysine and tryptophan? The answer is that we do not know.

While we await more study of the relationship between the subjective value of food and its nutritional value, particularly with respect to opaque-2

maize, we can attempt to estimate certain real benefits of opaque-2 maize to the consumer. We can make these estimates on the basis of the costs of obtaining the same nutrition from alternative sources.

One alternative might be fortification of normal maize with lysine and tryptophan. When Rosenfield (9) added 0.3% L-Lysine and 0.07% DL-tryptophan to maize, he estimated the cost of fortification to be $13.61 (U.S.)/metric ton of maize. The amino acid prices used in the analysis were L-lysine, $2.20 (U.S.)/kg, and DL-tryptophan, $10.00 (U.S.)/kg. The world market price for normal maize is (1972) around $50 (U.S.)/ton. Assuming equal nutritional value of the fortified maize and opaque-2 maize, the opaque-2 maize should command a 27% higher price, based on its higher nutritional value.

Lysine is presently available on the Colombian market at approximately $6.00 (U.S.)/kg, the exact price being determined by the quantity purchased. The Colombian market price for tryptophan was not obtained. Let us assume for this analysis that tryptophan could be imported at $25.00 (U.S.)/kg. The current producer price of normal maize in Colombia is approximately $90.00 (U.S.)/ton. Assuming that the price of opaque-2 maize would have to be 10% above that of normal maize to compensate for yield differences, the additional cost to the consumer would be $9.00 (U.S.)/ton. Thus, opaque-2 maize would supply additional lysine and tryptophan at much less cost than would fortification of normal maize.

It might be more realistic to compare the nutritional and monetary value of opaque-2 maize to that of other available foods, rather than use synthetic amino acids as the "best" alternative. One method would be to establish minimum-cost, nutritionally adequate diets with a linear programming model, including opaque-2 maize among the foods available.

Benefits and Costs for Livestock Producers

Whereas benefits and costs for the consumer are difficult to measure (because of individual preferences and the difficulty of quantifying the benefits associated with improved human nutrition), benefits and costs related to the utilization of opaque-2 maize in livestock production are relatively easy to estimate.

Table 4 and Figures 1 and 2 show the results of an economic analysis of opaque-2 maize for swine in Colombia (7). Analysis was made of relative prices, instead of actual market prices at the time of study, to develop models with applicability for any set of prices. Diets of normal maize and soybean oil meal were compared with opaque-2 maize diets for each of four periods of swine growth: gestation, lactation, growing (20- to 50-kg live weight), and finishing (50 to 90 kg).

Diets compared are shown in Table 5.

The data used for the economic analysis were obtained from nutritional

TABLE 4 Maximum price of opaque-2 maize relative to the price of normal maize and soybean oil meal that can be paid without reducing net returns

Period	Diets compared[a]		a[b]	b[b]	Maximum price of opaque-2 maize in percentage of price of normal maize if price of soybean oil meal is: 150% of the price of normal maize	200% of the price of normal maize
Gestation	CMS	and OM	0.767	0.259	115	125
Lactation	CMS	and OM	0.858	0.176	110	119
Growing	CMS	and OMS	0.665	0.104	87	87
	CMS	and OM	0.208	0.271	12	25
	CM	and OMS	2.581	0.067	278	275
	CM	and OM	1.996	0.000	200	200
Finishing	$CMS_{1,2}$	and OM	0.810	0.158	105	115
	CM_2	and OM	0.988	0.064	108	117
	CM	and OM	1.565	0.000	156	156

[a] See text for explanation of codes.

[b] The coefficients a and b refer to the model $P_o/P_s = a + b(P_o/P_s)$, explained in the text.

experiments by Jensen, Maner, CIAT, and collaborators. The biological results used in the economic evaluation are published in various journals.

Opaque-2 maize may replace normal maize and all or part of the protein supplement. Thus, in addition to the relative nutritional values of normal and opaque-2 maize, the relative benefits and costs of substituting opaque-2

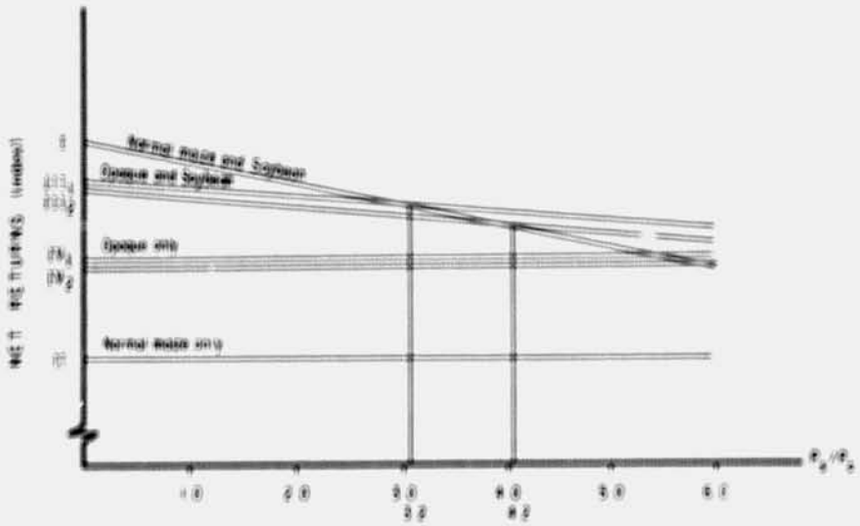

FIGURE 1 Relative net returns from various diets as a function of the price of soybean oil meal (the growing period).

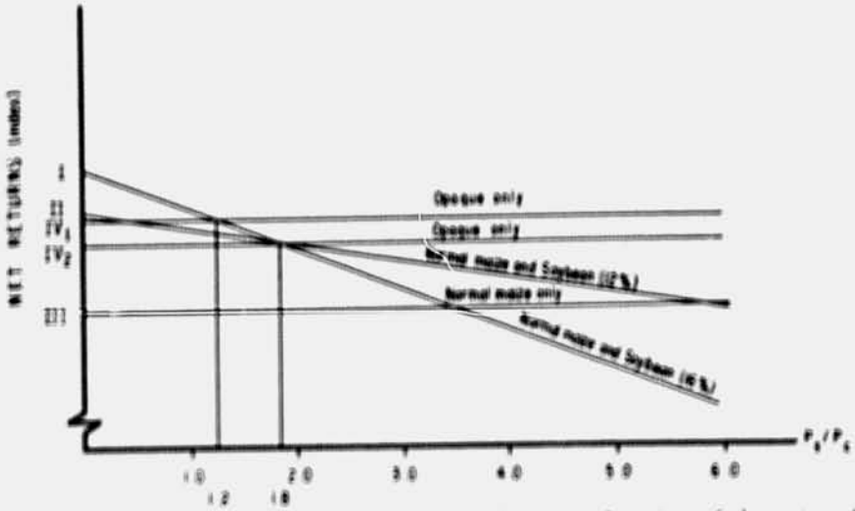

FIGURE 2 Relative net returns from various diets as a function of the price of soybean oil meal (the finishing period)

maize for normal maize depend on the relative prices of the two maizes and the protein supplement.

Table 4 shows the estimated maximum price of opaque-2 maize (relative to the price of normal maize) at which the producer could increase net returns by substituting opaque-2 maize for normal maize in the swine diets. Data are shown for each of the four stages of the swine life cycle. If the estimated maximum price for opaque-2 maize is above market price, the data show that the swine producer who uses a normal maize diet could increase net returns by shifting to opaque-2 maize.

The relationship between the maximum price of opaque-2 maize and the price of normal maize and soybean oil meal is expressed as

TABLE 5 Comparison of diets for swine

Period		Diets compared (O, opaque; N, normal)
Lactation	I	Normal maize (78%) and soybean oil meal (22%) (N/S)
	II	Opaque-2 maize (97%) (O/M)
Lactation	I	Normal maize (79%) and soybean oil meal (17%) (N/S)
	II	Opaque-2 maize (96%) (O/M)
Growing	I	Normal maize (84%) and soybean oil meal (16%) (N/S)
	II	Normal maize (96%) (N/M)
	III	Opaque-2 maize (96%) and soybean oil meal (6%) (O/M/S)
	IV	Opaque-2 maize (96%) (O/M)
Finishing	I	Normal maize (84%) and soybean oil meal (16%) (N/S)
	II	Normal maize (96%) and soybean oil meal (6%) (N/S)
	III	Normal maize (96%) (N/M)
	IV	Opaque-2 maize (96%) (O/M)

$$\frac{P_o}{P_c} = a + b\,\frac{P_s}{P_c}$$

where P_o = price of opaque-2 maize
P_c = price of normal maize
P_s = price of soybean meal

The coefficients a and b may be interpreted as coefficients of an ordinary regression equation. Hence, for each unit change in P_s/P_c, the coefficient b shows the resulting change in P_o/P_c. (A complete description of the mathematical model is given in reference 7.)

The maximum price of opaque-2 maize, relative to the price of normal maize, that the swine producer would be willing to pay can be estimated directly from coefficients a and b for any soybean oil meal price.

The utility of the model can be illustrated by a hypothetical example. Suppose that a swine producer can purchase normal maize at $60.00 (U.S.)/ton, soybean oil meal at $100.00 (U.S.)/ton, and opaque-2 maize at $65.00 (U.S.)/ton. Given these market prices, the producer wants to know whether to use a normal maize–soybean oil meal diet or an opaque-2 diet during the finishing period.

The maximum price he can pay for opaque-2 maize may be calculated directly from the model $P_o/P_c = a + b(P_s/P_c)$ for, using the coefficients shown in Table 4 and the hypothetical prices, $P_o/P_c = 0.8810 + 0.1758\,(100/60) = 1.073$. Hence, the producer can pay up to 7% more for opaque-2 maize. But in our example the market price of opaque-2 is 8% above that of normal maize (65/60). Using these prices, the normal maize–soybean oil meal diet is the producer's best buy.

Table 4 shows the estimated maximum prices of opaque-2 maize when soybean oil meal prices are 150% and 200% of the price of normal maize. If the price of soybean oil meal is 150% of the price of normal maize, it would be economically sound to substitute opaque-2 maize for normal maize during gestation—if the price of opaque-2 maize were not more than 13% above the price of normal maize. The data suggest that opaque-2 maize should replace normal maize and soybean oil meal during lactation only if the price of opaque-2 maize were less than 10% above that of normal maize. If the price of soybean oil meal is twice the price of normal maize, it would be wise to use opaque-2 maize at prices up to 25% above that of normal maize during gestation, and at prices up to 19% above normal maize during lactation.

If a protein supplement is available at a reasonable cost, it does not seem economical to substitute opaque-2 maize for normal maize during the growing period (20 to 50 kg). However, if the protein supplement is available in normal maize diets, feed costs may be reduced considerably by using opaque-2 maize. During the finishing period (50 to 90 kg), it would be economical to use the opaque-2 diet to replace a normal maize–soybean oil meal diet with 16% soybean oil meal, if the price of opaque-2 were less than

5% above the price of normal maize and soybean oil meal were available at a price equal to 150% of the normal maize price. An opaque-2 diet should replace a normal-maize-only diet, unless the price of opaque-2 is more than 36% above the price of normal maize.

The preceding analysis has been aimed at estimating the maximum price of opaque-2 maize that could be paid by the swine producer without reducing net returns, using selected relative prices of soybean oil meal and normal maize.

Another method of analysis would be to establish specific price relationships between opaque-2 and normal maize, and to estimate the relative net returns of the various diets as a function of the price of soybean oil meal. Figures 1 and 2 show the relationship between relative net returns for each of the diets and the price of soybean oil meal for two periods, growing and finishing. Two alternatives are shown with respect to the prices of opaque-2 and normal maize: (1) the price of opaque-2 maize is equal to that of normal maize, and (2) opaque-2 prices are 10% higher. Each line in the figures refers to a diet. The numbers I to IV refer to the diets in Table 5. The lines marked III_1 and IV_1 in Figure 1 and the line marked IV_1 in Figure 2 represent the opaque-2 diets when the price of opaque-2 maize is equal to that of normal maize. The lines marked III_2 and IV_2 in Figure 1 and IV_2 in Figure 2 represent the situation when the price of opaque-2 maize is 10% above that of normal maize.

To maximize net returns, the swine producer should select the diet represented by the top line (given the price of soybean oil meal). He would use diet I (normal maize and soybean oil meal) during the growing period, unless the price of soybean oil meal is more than 320% of the price of normal maize, in which case the normal maize diet should be replaced by a diet of opaque-2 maize and soybean oil meal. If opaque-2 maize is available at the same price as normal maize. If opaque-2 maize is 10% more expensive, the replacement should not be made unless the price of soybean oil meal is more than 120% the price of normal maize.

If opaque-2 maize is available at the same price as normal maize, opaque-2 maize should be used during the finishing period when the price of soybean oil meal is more than 20% above that of normal maize. If the price of opaque-2 maize is 10% above that of normal maize, opaque-2 maize should be used only if soybean oil meal costs more than 180% of the price of normal maize.

Our analysis indicates that it would be economically sound to substitute opaque-2 maize for normal maize during gestation, lactation, and finishing, unless a very inexpensive protein supplement is available or the price of opaque-2 maize is considerably higher than that of normal maize. Use of opaque-2 maize during the growing period does not seem wise unless protein supplement is unavailable or is very expensive.

Data used for the preceding analysis refer to experiments with well-

managed, purebred swine. The validity of the results is not known for non-purebred swine with poorer management. Furthermore, it should be emphasized that the analysis did not attempt to estimate absolute net profits from swine production; rather, it assessed relative net profits associated with diets of opaque-2 and normal maize.

The results found in the analysis are similar to those obtained in Brazil (2). In the Brazilian analysis it was found that, if a protein supplement is available at a reasonable price, opaque-2 maize would not be the most economical feed during the growing period. Comparing an opaque-2 diet to a maize-based commercial concentrate, it was found that the producer could pay up to 18% more for opaque-2 maize than for normal maize during the finishing period, given the existing prices of protein supplements. Compared to a normal maize diet without protein supplement, opaque-2 maize was estimated to be an economically sound diet during the growing period, unless its price was more than 236% of the price of normal maize. Opaque-2 maize was economical during finishing, unless its price was more than 125% of the price of normal maize.

Benefits and Costs to the Producer

The benefits and costs to the producer of opaque-2 maize may be analyzed in terms of three variables: (1) expected cost of production and on-farm storage, (2) expected farm price of opaque-2 maize, and (3) the risk and uncertainty involved in these expectations. As in previous sections of this paper, the discussion will focus on a comparison between opaque-2 and normal maize; thus, it will deal with relative rather than absolute values of costs, price, risk, and uncertainty.

Relative costs. Except for higher seed prices, there seem to be no apparent reasons why the opaque-2 maize cost of production per unit of land should differ from that for normal maize. If, however, the yield capacity of opaque-2 maize is below that of the best normal hybrids, as seems to be the case in Colombia, the cost of production per unit of output will be higher for opaque-2 maize. The cost of on-farm storage of opaque-2 maize also is likely to exceed that of normal maize, because opaque-2 maize appears to be less resistant to insect attacks (5).

For the farmer now growing a local variety with low yield capacity, adopting an improved and well adapted opaque-2 maize is likely to increase yields considerably. For such a farmer, the cost of production per unit of output is likely to decrease. However, costs could be decreased further if a higher yielding normal hybrid were introduced.

Relative price. The price of opaque-2 maize relative to that of normal maize may be expected to be determined by specific supply and demand factors, as well as by government policy measures. Relative production costs and risk and uncertainty are expected to be the most important supply

factors. If opaque-2 maize is expected to yield less than normal maize, or if risk and uncertainty for its use are perceived to be greater, the maize producer will be willing to produce opaque-2 maize only at a price sufficiently superior to compensate for these adverse factors. The most important price-determining factors on the demand side are likely to be relative consumer preferences, prices of available substitute products, consumer purchasing power, and the rate of technical substitution between opaque-2 maize and other products available. The latter factor refers primarily to the demand for opaque-2 maize for livestock feed and processed foods.

If the maize buyer (the individual consumer, livestock producer, or processing firm) prefers opaque-2 maize to normal maize, he may be willing to pay a premium for opaque-2 maize. A mutually satisfactory price may be established and production may become self-sustained. If opaque-2 maize is not preferred and its yields are inferior, it is not likely that a mutually satisfactory price can be established without government intervention.

Risk and uncertainty. Replacing normal maize with opaque-2 maize may increase subjective producer risk and uncertainty for three reasons: (1) opaque-2 maize seems to be less resistant to insect attacks, (2) when initially introduced, opaque-2 maize may not have a well-established market, and (3) the fact that it is a new product tends to increase subjective risks. Although no general monetary cost figures can be placed on these risk and uncertainty factors, it is likely that the farmer will turn his production to a higher-risk product only if expected gains compensate for additional subjective risk and uncertainty.

Based on the preceding factors, we may conclude that self-sustained, large-scale production of opaque-2 maize may be difficult to promote among farmers who have knowledge of a normal hybrid with higher expected yields or lower subjective risk, unless the market price of opaque-2 maize is above that of normal maize. The magnitude of the needed price differential would be determined by yield differences, magnitude of the subjective risk and uncertainty, and the risk-avoiding behavior of the producer. The market will permit the necessary price differential only if the difference in buyer preference is sufficiently large. If such buyer preference does not exist, government intervention probably will be needed to assure self-sustained production and utilization of opaque-2 maize.

Previous discussion has focused on commercial production; however, for the subsistence farmer the potential benefits are those of producer and consumer combined. The subsistence farmer now growing a local variety with low yield potential may increase yields and improve the quality of his diet by introducing opaque-2 maize. In addition to obtaining a better diet, he may be able to produce a marketable surplus. Once this farmer is aware of hybrid seed, his best alternative to opaque-2 maize is not the local variety, but a normal hybrid that may outyield opaque-2 maize. He is then faced with decisions as both commercial producer and consumer. One possible out-

come might be that he would produce sufficient opaque-2 maize for home consumption (thereby obtaining the potential consumer benefits). Or he could produce either opaque-2 or normal maize for sale, depending on relative yields and prices (thereby maximizing producer net returns).

Government intervention. Whereas the benefits and costs to the individuals and firms involved in the production, marketing, and consumption of opaque-2 maize may be relatively easy to estimate, there is no unique measure of the benefits and costs to society. The impact on human nutrition per unit of resources used is one measure that might be used for opaque-2 maize. This measure should include (1) the relative cost of obtaining similar impacts on human nutrition by using other acceptable foods, and (2) the impact on employment, incomes, and income distribution.

If one of a society's goals is improving the protein quality of the diet of low-income families, several alternative strategies may be considered. Besides promoting opaque-2 maize, these plans might include (1) breeding for higher-quality protein in other staple foods, such as cassava and rice, (2) expanding the production and consumption of food presently containing high-quality protein, such as pulses, meats, and milk, (3) fortification, and (4) improving the utilization of the foods presently available through consumer education, income distribution, and the like.

The choice of strategy would then be based on the relative contribution obtained from each for the money spent. The key factors for success, no matter which strategy is chosen, are (1) increasing the availability of low-cost, high-quality protein in a form acceptable to the consumer, and (2) raising the real incomes of the low-income families through increased earnings and reduced food prices.

For present varieties of floury opaque-2 maize, government intervention is likely to be needed to bridge the gap between private and social net benefits. If a flint-type, high-quality protein maize with a high yield capacity were developed, the need for government intervention would probably be reduced greatly (5).

GOVERNMENT POLICIES

Substituting opaque-2 maize for normal maize in South America would appear to have great potential nutritional impact. This impact could be called its social benefits. However, the consumer may be unwilling to substitute opaque-2 maize for normal maize, particularly if the price of opaque-2 maize is above that of normal maize (5). If the market price of opaque-2 maize is not greater than that of normal maize, the producer may still be unwilling to change. These factors are due to private benefit–cost relationships. Thus, government intervention may be needed (1) to assure that the farm price of opaque-2 maize is sufficiently high to pay for its production and (2) to inform the consumer about the nutritional value of opaque-2

maize and provide suggestions on how to use it in cooking. During the introductory phases, government action also may be needed to assume certain of the risks and uncertainties, both in production and marketing.

The most efficient government intervention in most cases would probably be a price-support program that establishes reasonable minimum prices, at the farm and the retail levels, along with a promotional campaign aimed at consumer education. [Results from a recent study in Colombia (5) suggest that the initial promotion of the production and consumption of opaque-2 maize may be severely hampered by the reluctance of the marketing agencies to handle opaque-2 maize. This hesitancy is primarily due to risk and uncertainty with respect to consumer demand. A minimum price at the retail level would sharply reduce this risk and uncertainty.] The cost of such programs would depend primarily on the yield differences between opaque-2 and normal maize, and the willingness and ability of the consumers to pay a premium for opaque-2 maize once they were aware of the differences with respect to nutrition and cooking characteristics. In Colombia, the annual government cost of maintaining a price support program, if all maize used for human consumption were opaque-2 maize, would be $4.3 million (U.S.) (5). This figure assumes that the farm price of opaque-2 maize should be 10% above the price of normal maize to compensate for yield differences, and that the consumer would buy opaque-2 maize only at a price equal to that of normal maize.

Another possible government policy measure should be mentioned. If it should be determined that the potential net social benefits from substituting opaque-2 for normal maize are great, but unrealizable because subjective net private benefits are higher for normal maize (due to relative yields, consumer preferences, and so on) the government may decide to prohibit the production and distribution of normal maize seed. Before such a measure is introduced, however, the government should assure the availability of opaque-2 maize hybrids or varieties suited for all maize producing regions within the country.

The possible implications of such a measure should be carefully studied before it is introduced. If consumers are willing to pay a considerably higher price for normal maize than for opaque-2 maize, the farmer may decide to grow a local variety, producing his own seed. The net result in this case might be a drastic reduction in the total quantity of maize produced. However, if consumers willingly accept opaque-2 maize, the area usually planted to normal hybrids and improved varieties is likely to be planted to opaque-2 maize. The net effect on production would be determined by the yield handicap of opaque-2 maize. This is not a suggestion that the production and distribution of all normal seed be prohibited in countries where sufficient opaque-2 seed is available; however, it is a possible policy measure for consideration.

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THE ECONOMIC AND TECHNICAL FEASIBILITY OF IMPROVING PROTEIN QUALITY OF FOOD THROUGH FORTIFICATION AND SUPPLEMENTATION

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Foods are selected and dishes prepared to conform to individual subjective standards of taste, color, and texture that are considered "good." It is axiomatic that people eat food, not nutrients.

The subjective standards of "good" are determined by the culture of the group and the individual's experiences, and food patterns prevailing in a given culture offer no guarantee that the foods eaten are particularly nutritious. Food habits are deeply ingrained and difficult to change, particularly if the need for change is presented as an abstraction whose benefits lie in the future and cause and effect relationships are difficult to perceive.

However, food habits do change and given sufficient stress these changes may be relatively rapid. For example, food consumption patterns may change rapidly when an impoverished family moves from a rural to an urban setting or when a cheap, easy to grow, high yielding food staple is introduced. Cassava may replace a cereal crop, or breast feeding may be curtailed because the mother must quickly return to work after childbirth.

Rapid changes may be nutritionally deleterious, thus adding to the urgency for improvements to the nutritional quality of new diets. The plant

breeder, the applied nutritionist, and the food scientist whose concern is directed toward the malnourished and impoverished accept the premise that improved nutritional qualities must be built into crops or foods with minimal impact on existing food habits. The discussion of opaque-2 maize has emphasized these points: few people will pay more for a well-balanced protein in maize (an abstraction), and fewer still will accept maize that is nutritionally excellent but considered esthetically unpleasant to the eye or palate. It thus becomes the task of the plant breeder to introduce improved nutrition into crops without increasing cost or affecting acceptability.

The food technologist aiming at diet fortification faces his own set of problems. He must find some point of intervention in the processing chain that leads from the farm to the ultimate consumer, and this intervention must result in a food that is compatible in price and palatability with the foods that are culturally acceptable.

STRATEGIES FOR NUTRITION IMPROVEMENT

Today there is insufficient scientific and technological development and insufficient working experience to permit conclusions regarding the relative merits of breeding and fortification programs as alternative strategies for nutrition improvement. Specific circumstances would determine whether one strategy or the other, a combination of both — or neither — would provide the most feasible approach to solving a given nutritional problem.

Experience to date suggests that, regardless of the approach, meaningful attempts to combat malnutrition require (1) recognition at the national level of a malnutrition problem, (2) governmental awareness of the impact of nutrition on development and active governmental participation in providing solutions. Isolated, uncoordinated efforts dependent on the energies and specific abilities of one individual, or a dedicated group may lead to successful projects involving breeding or fortification. However, such successes may have limited impact unless there is some commitment by government to incorporate the results of these efforts into broader-based programs.

However, governments cannot make realistic commitments unless the feasibility and costs of proposed solutions for nutritional improvement can be estimated. Similarly, some evaluation must be made of the potential effectiveness of the proposed solutions.

Several universities and the international agricultural research centers are heavily involved in developing new crop varieties with support from the major foundations, the U.S. Agency for International Development (USAID), and others.

The USAID Office of Nutrition is supporting several projects in the area of fortification. Fortification efforts are designed to assess feasibility, nutritional impact, and costs, and thus to supply the kind of information that is

useful for nutrition programming on a national scale. The USAID fortification projects seek to improve protein quality in food staples such as wheat, rice, maize, and cassava. These staples are supplemented with amino acids and/or protein concentrates, and with vitamins and minerals. Some of these projects are discussed next.

Wheat Fortification

A pilot study is underway in southern Tunisia to assess the effect of supplementing wheat products with vitamins, iron, and lysine. The study area borders the Sahara Desert and is impoverished [monthly family mean income equals $30 to $40 (U.S.)]. Rainfall is less than 100 mm/year and adequate for only limited agriculture. Infant mortality is estimated at 20% of all live births, with most of the deaths occurring in the first months of life. Infants generally are breast-fed until about 2 years of age; they then eat the usual adult diets.

Wheat is the main dietary staple in these villages and provides more than 60% of the daily caloric intake and over 70% of the daily protein intake. The wheat food products are made from wheat milled in northern Tunisia. All aspects of the milling, distribution, and sale of wheat are under government control.

Twelve villages with a population of about 16,000 (about 3,000 preschool children) have been divided into three study populations. One group receives unfortified wheat; a second group receives wheat products supplemented with vitamins and iron; and the third receives wheat products fortified with lysine, vitamins, and iron.

The various fortification nutrients are premixed in the United States. Two types of premix (with and without lysine) are shipped to Tunis, where the wheat is fortified in appropriate mills at a predetermined rate. Bags of enriched products are identified by color coding. The products are then shipped to warehouses where they are purchased by storekeepers from individual villages. These storekeepers can only purchase products marked with a given color. They are not aware of the nutrition supplement in the bag, only that the color indicates improved quality. All cereal products have been fortified since mid-June 1971.

The composition of the fortification mixture is shown in Table 1. The cost of ingredients is about $8.40 (U.S.)/ton. The cost of lysine accounts for 80% of the total cost. If a metric ton of flour is priced at approximately $200 (U.S.), the ingredient cost for fortifying wheat flour increases this price by about 4.2%. Vitamin and iron fortification accounts for only about 0.5% of that increase.

Hegsted has found that fortification with 0.2% lysine HCl increases utilizable wheat protein by from 3.20 to 5.34%. This is equivalent to an increase of 2.14 g of protein for each 100 g of wheat flour. On this basis the cost of the increased utilizable protein is about $0.34 (U.S.)/kg. This ingre-

dient cost represents only part of the total cost, which must include the cost of (1) added equipment, (2) training technicians, and (3) monitoring the fortified product. Assuming that this increases the cost by 50% a cost of $0.51 (U.S.)/kg for utilizable protein is still a satisfactory figure.

Maize Fortification

A maize fortification project is underway in Guatemala, as discussed by R. Bressani. In contrast to the Tunisian project, where the wheat is centrally processed and then distributed, maize fortification in Guatemala is done at the village level. Two villages are being studied: Santa Maria Cauque (the fortification-supplied village) and Santo Domingo Xenacoj (the control village). Santa Maria Cauque has about 1,500 inhabitants. Living conditions are primitive in both villages and are characterized by a high prevalence of malnutrition, infectious diseases, and growth retardation. Uniquely comprehensive baseline data had been accumulated for Santa Maria Cauque during the previous 8 to 9 years. These data reflect incidence of infection, birth weights, morbidity, and the like, and allow a more precise measuring of the impact of fortification, as follows.

1. Baseline data are available for the fortification-supplied village, thus allowing comparisons between those children born in Santa Maria during 1964–1971 and those born during 1972–1976. Comparisons will be made on a basis of fetal growth, postnatal growth, dietary intake, infant mortality, morbidity, and Shigella infection.

2. Not all families in Santa Maria Cauque receive fortified maize. The same comparisons will therefore be made among children in Santa Maria Cauque whose families receive either (a) fortified or (b) control maize.

3. Children born in the fortification-supplied and control villages during 1972–1976 will be compared for fetal growth, postnatal growth, and infant mortality.

The fortification mix consists of soybean flour (50% protein), lysine, and

TABLE 1 Composition of wheat fortification mixtures used in Tunisian study

Nutrient	Amount in flour (mg/kg)
Vitamin A	57.0 (13,000 I.U.)
Vitamin D	1.3 (2,600 I.U.)
Thiamin	9.63
Riboflavin	5.94
Niacin	67.63
Iron	59.25
L-Lysine	3,000

minerals and vitamins. This mixture is added at the concentration of 8% by weight of dry maize to the *nixtamal*, which increases the protein content of maize to approximately 12%. It also provides the limiting lysine and tryptophan, and the vitamins and iron usually deficient in the diet of the region.

The procedure for maize fortification in the village is simple. Women or children bring the *nixtamal* (lime-cooked, peeled kernels) to the village mill where it is weighed. If the *nixtamal* is to be fortified, the fortification mix is measured out by volume and added to the nixtamal. This mixture is emptied into the hopper of the mechanical mill and is ground. After the grinding, the dough is collected, replaced in the container, and carried home. In the process, the only intervention has been the introduction of minor equipment changes and the addition of supplement.

The fortification mixture is prepared in the United States. The price of the first lot of fortification mixture was $0.57 (U.S.)/kg, transportation not included. At an 8% concentration, the added cost is about $0.04 (U.S.)/kg of maize.

However, soybeans could be grown and processed in Guatemala, and mixed and packaged locally. Using local soybean and imported vitamins, the cost of the fortifying mixture is estimated at $0.22 (U.S.)/kg. At an 8% concentration, the additional cost of maize would be about $0.018 (U.S.)/kg.

Rice Fortification

Following a 1-year pilot study designed to develop methodology, 29 villages in the Chiang Mai area of Thailand were selected for a full field study of rice fortification. These villages have a total population of approximately 13,500, with 1,600 children between 6 months and 6 years of age.

By almost all clinical criteria, the nutritional status of the village is poor. Dietary and clinical surveys indicate that diets are very low in riboflavin, thiamin, and vitamin A. Many cases of anemia, both in children and women, have been reported. Rice provides approximately 80% of the calories consumed.

The villages have been divided into five groups:

1. Raw controls, no treatment.

2. Placebo controls, placebo fortification granules added to rice; day-care centers provided.

3. Rice fortification grains (RFG) containing thiamin, riboflavin, vitamin A, and iron; day-care centers provided.

4. Rice fortification grains containing thiamin, riboflavin, vitamin A, and iron, plus lysine and threonine; day-care centers provided.

5. Rice fortification grain as in group 4; no day-care centers provided.

The fortification mixture used in this study is added in the form of synthetic rice grain (RFG) to the rice as it leaves the mill; it is added at a 1% concentration level. Low-cost RFG dispensers have been built and attached to the village mills used for this study.

The nutrients in the completely fortified RIG include 20% L-lysine HCl, 10% L-threonine, 0.05% thiamin, 0.6% riboflavin, 0.7% vitamin A, and 0.2% iron derived from $FePO_4 \cdot 4H_2O$.

There was a reluctance to add riboflavin because it would affect the color of the RIG. However, riboflavin is greatly needed, and, as of May 1972, has been incorporated into the RIG with no apparent adverse effect on acceptability.

Vitamin A is added as the acetate in an oil solution, and 2,870 international units (IU) are added to each gram of RIG. The vitamin loses activity during processing and storage, and available data indicate that storage of the RIG for 90 days at 30°C results in a combined loss of about 45%. Thus, the RIG, as used, would contain about 1,300 IU of vitamin A/g.

Several independent surveillance measures are used to check whether the RIG is properly added and consumed. This monitoring includes (1) records kept by millers, (2) spot checks of mills, (3) visual checking in houses and day-care centers to spot riboflavin-colored grains, and (4) urine analysis for thiamin.

It is difficult to estimate the cost of a fortification program in Thailand. The ingredient cost for mineral and vitamin fortification is minimal, less than $0.70 (U.S.) metric ton of rice. If another $1.00 (U.S.) is added for miscellaneous costs, the increased rice cost is only about 1%.

Adding lysine and, in particular, threonine increases the cost appreciably. An assumption is made that lysine can be obtained at $2.20 (U.S.)/kg. However, it is difficult to get an accurate estimate of the potential cost of threonine; a projected price of $17.60 (U.S.)/kg seems reasonable at this time. The amino acid cost per metric ton of rice thus would be about $12.00 (U.S.)/ton, raising the cost of rice by another 8%.

A 10% cost increase is high if based only on ingredient cost. But if the demand for amino acids becomes appreciable, these figures become suspect; the engineers of the chemical industry will undoubtedly be unleashed if the demand is evident.

Cassava Fortification

Cassava fortification with soybeans is being evaluated in Brazil. We have approached the question of cassava (*Manihot* spp.) fortification from a more pragmatic viewpoint. The objectives of the projects are (1) to place a fortified cassava flour on the market in the greater Rio de Janeiro area, (2) to check its actual cost, (3) to assess its acceptability, and (4) to determine the requirements for large-scale introduction of fortified cassava.

In the past decade, migration has accelerated from unproductive farmlands to urban centers. Thousands of Brazilians have entered the cities and the commercial marketing system looking for better job opportunities. On marginal incomes, they buy food that is familiar, cheap, and filling — cassava. This has led to increased commercialization of the product. It is sold

in bulk in street markets and increasingly in paper or polyethylene bags in the larger markets. This large-scale commercialization provides the needed intervention point for fortification.

Gramimo, a large, if not the largest, reprocessor and packager of cassava flour has agreed to do the initial test marketing. Preliminary studies indicate that a mixture of cassava and soybean grits is acceptable. We should have a much clearer picture of the feasibility of this approach in late 1978.

COMPARING BREEDING AND FORTIFICATION

It seems apparent that the fortification approach and the "breeding-for-nutrition" effort have similar objectives and problems. It also appears that there are inherent advantages and disadvantages to both approaches. The factors that should be considered in assessing the *suitability* of either approach for improving protein quality can be presented as follows.

Feasibility Considerations

Breeding

1. What is the potential for breeding better protein quality into the major food crops? (For example, protein improvement in cassava may be difficult to accomplish.)
2. Is crop improvement dependent on a long-term research program or is it a simpler matter of incorporating available genetic traits?
3. Are the institutions and trained personnel available for developing a program?

Fortification

1. Is there one food staple that is the major source of calories?
2. What foods can be fortified?
3. Are potential fortificants available locally or must they be imported?
4. Is the food processed centrally or in village mills dispersed throughout the countryside?
5. Are appropriate points of intervention identifiable?
6. Is the food usually ground, for example, maize meal or wheat flour, or is it consumed whole?
7. Can the target group be reached via fortification?
8. What fraction of the fortified food will reach the target group?

Acceptability Criteria

Breeding. Will the new varieties pose problems to the farmer, processor, or consumer? The farmer may find the yields low and insect and disease resistance poor. The processor may discover that the new varieties require modification of existing equipment. The consumer may consider changes in appearance, texture and home processing characteristics undesirable.

Fortification

1. Acceptability problems are minimal if protein quality is improved by amino acid fortification.

2. Acceptability may be more of a problem if protein concentrates are added. Can enough concentrate be added to have a nutritional impact without affecting acceptability? If not, can a suitable technology be developed for incorporating enough concentrate to have an impact?

Inherent Advantages

Breeding

1. Coverage may reach 100% both in rural and urban areas if a wholly acceptable variety is developed. It is conceivable that the food supply of an entire region may be improved with no need for changes in processing or food distribution systems and at no cost to the farmer or consumer.

2. Once successful varieties are produced, the breeding program would require less effort and resources to perpetuate the program.

Fortification. Fortification lends itself to the addition of a wide variety of nutrients—minerals, vitamins, lipids, amino acids, protein concentrates, and so on. The ability to incorporate other nutrients may be most important. Proper utilization of improved protein quality may depend on the proper balance of minerals and vitamins in the diet.

Inherent Disadvantages

Breeding

1. To derive potential benefits from new varieties, subsistence farmers might be required to adopt new agronomic practices, such as irrigation, fertilizer, and pesticides.

2. The farmer may be very reluctant to accept an unknown risk for a possible nebulous benefit.

3. If the new variety is a hybrid, the subsistence farmer may be reluctant to buy seeds each year.

4. New varieties that require large-scale cultivation may exacerbate inequities of income distribution and affect overall crop plantings. Thus, the new varieties may accelerate the departure of the subsistence farmer from the rural areas to the cities and cause a shift in crops planted that may prove detrimental. Witness the increase in wheat production at the expense of legumes in India.

Fortification

1. Potentially limited coverage of rural areas.

2. Expensive in terms of per capita cost of reaching target groups.

3. A fortification program is open-ended. Fortification must be added as long as the food staple is to be improved.

OTHER CONSIDERATIONS

For both breeding and fortification, solving the scientific – technological problem of increasing nutritive value constitutes only the first critical step. For these results to be translated into programs having an impact on the consumer, it will be necessary to ensure that they are indeed "do-able." There must be a realistic way of intervening in the food system. For fortification, this may mean availability of central processing facilities, government intervention by legislation, and a reasonably reliable monitoring system.

Finally, the resultant product must be acceptable to processors and consumers in terms of its processing characteristics, taste, and cost. To date, fortification with amino acids, protein concentrates or simulated rice grains has presented no insurmountable problems of change in processing characteristics. This is because the nutrients are introduced during the milling or grinding process, or immediately after milling, as with synthetic rice grains.

Similarly, the taste characteristics, including flavor, odor, and texture, have not been altered for wheat, maize, and rice, and thus far it appears that the products are acceptable to consumers. For cassava, preliminary taste-panel tests indicate that the use of soybean grits as a fortificant introduces no flavor problem, but a more widespread test under more typical conditions is needed before acceptability can be determined.

In all these commodities, we recognize that the questions of acceptability in terms of taste and processing characteristics will be answered only after widespread dissemination of the products and under actual conditions; this is one of the main objectives of the previously discussed field trials.

The cost factor in assessing acceptability is more complicated. The cost data developed to date are very preliminary and consist largely of estimates of costs of ingredients, mixing, and capital equipment. Logistical and administrative support costs have not been adequately determined, but this is not expected to be a formidable task.

When this is done, however, additional complications remain. How is the cost to be absorbed? Two possibilities appear to be feasible — government subsidy or passing the costs to the consumer in the form of higher prices. Of the two, the latter is the more realistic option. But what will this mean in terms of reaching the groups who most need the added nutrition? If given the choice of purchasing either fortified products at higher prices or unfortified products at lower prices, the low-income consumer may be compelled to choose low price and low nutrition. If the government requires that all this staple food be fortified, this problem would not be encountered. However, the new higher cost might cause increased purchases of other lower-cost nonnutritious food staples.

Cost calculations also will have to consider foreign exchange. If fortificants such as synthetic amino acids, vitamins, and minerals must be imported on a continuing basis, governments may be highly reluctant to add even a modest increment to their foreign exchange burden. In this respect, fortifi-

cation may be more "costly" than indicated by the small percentage of increase in product cost to the consumer.

SUMMARY

The fortification program has advanced to the stage where field trials are underway to determine realistic feasibility. Many answers will be forthcoming in the next year or two. It is likely that feasibility will be established for at least two and possibly three of the commodities, but the degree to which fortification programs are undertaken will depend on several factors. Not the least of these factors will be the relative advantages of utilizing the new varieties produced by breeding. For this reason, food scientists and plant breeders must maintain a continuing dialogue and exchange of information. It also is essential that the products of breeding be similarly examined in terms of "do-ability," nutrition potential, and acceptability in terms of taste, processing characteristics, and cost. Decision makers may choose to implement a proposed program only in terms of subjectively perceived advantages, only to find later that better solutions might have been possible. Responsible advocates of change will present alternatives with evidence of their relative costs and consequences. Such an approach is prerequisite to intelligent decision making.

[*A discussion of this paper can be found on p. 306 of* **Questions and Answers.**]

NATIONAL PRODUCTION PROGRAMS FOR INTRODUCING HIGH-QUALITY PROTEIN MAIZE IN DEVELOPING COUNTRIES

Ernest W. Sprague *CIMMYT, Mexico*

Spurred by the needs of a protein-hungry world, maize research has moved rapidly over the past 10 years. Maize breeders, biochemists, and nutritionists have combined their efforts to develop maize varieties and hybrids that are nutritious, economical, and universally available. Since Mertz, Bates, and Nelson reported the enhanced nutritive quality of opaque-2 maize in 1963, the potential of this high-quality protein food source has been studied rigorously and with increasing success.

Over the past few years, workers have converted many diverse and variable maize populations with inherent supplies of modifiers, and they have selected for a wide range of characteristics in many environments to provide the germ plasm base for high-quality protein varieties and hybrids. Now cooperative international testing of the new materials—at 54 sites in 24 countries in Asia, Africa, and the Americas—indicates that several varieties are fast approaching commercial standards.

This rapidity of progress at the research level suggests that we must now mount an effective campaign for the adoption and production of the new maize varieties on a global basis, thus sharing their benefits with the nutrient-poor people of the world. However, we must plan well. Although there is no blueprint for a system that will match the needs of every country, some general guidelines can be proposed. Successful experiences in some countries and regions can be adapted to other areas. And, hopefully, the unsuccessful projects in some nations can be used to prevent repeat performances.

One factor seems certain: if the world is to realize the potential of high-quality protein in maize, there must be a continuous flow of genetic materials into and among the national maize programs. National efforts must be more sharply defined, and national staffs must be allowed more opportunities to participate at an international level, thus becoming aware of and having access to the best materials and ideas.

The following outline of maize-program requirements illustrates some points of focus for national programs. Organizational and structural limitations are cited, and the final section is devoted to model staffing patterns.

ROLE OF NATIONAL PROGRAMS

The ultimate success of high-quality protein maize depends upon the communications effectiveness of researchers, and their ability to convince national governments of this crop's importance. We scientists, in our traditional, conservative approach, too often have been poor communicators, at least when our efforts have been measured on a mass scale. We tend to be more concerned with relating our findings to other scientists than we are with applying the information at the production level.

Today, we face the challenge of redirecting our thinking and study toward more global needs. We must first convince government planners and policy shapers to make a firm, general commitment in support of agricultural research and production programs. Then, in appropriate countries, we should urge that high priority be given to planning and organizing maize programs.

Historically, however, governments have not placed a high priority on food production until their countries faced famine conditions, or until the cost of importing food reached a level that made self-sustained production the most attractive political and economic alternative. Government commitment is essential; only with this support can an effective national program be organized within the economic means of a country.

RESEARCH AND PRODUCTION PROGRAM LIMITATIONS

National programs usually have several initial limiting factors, including (1) lack of qualified research and production personnel, and (2) lack of an organization structure to effectively generate the technology and extend it to the farm production level.

As outlined later in this report, most countries will require a minimum of 10 to 15 years to develop self-sufficient staffing patterns. Staffing demands often would require that a large portion of a nation's agricultural graduates enter into the production system for a single crop (maize). Governments rarely appreciate the cost and time necessary to staff an agricultural institution. Too often, national planners find it easier to estimate production

targets, rather than plan for an effective research and production program with the staff development necessary to reach production targets.

Other factors to be considered are the following:

1. Budgets for national programs, although extremely important, are seldom the initial limiting factor. Similarly, credit is rarely the first limiting factor to accelerated production, although it often becomes a restraint as production increases.

2. Stable and fair prices for production inputs and for the harvested crop are extremely important. In far too many countries the ratio of the cost of fertilizer to the value of the crop does not provide sufficient economic incentive. Without this profit motive, the best of research and production efforts will have little effect in increasing production.

3. Unavailability of fertilizers and insecticides at the right place and the right time can be a hindrance. Often these supplies are managed by government workers, and their services vary from one country to another. Present systems seldom work well. Many of the inadequacies in planning are due to inaccurate advice from the agricultural sector—sufficient provisions are not anticipated, particularly as production increases rapidly.

4. Understanding of agriculture and the changes that are taking place or could take place is frequently lacking. In my opinion, every country should have a competent production economist who can communicate effectively with the agricultural staffs and with national planners.

NEW APPROACHES

1. Here at CIMMYT we feel strongly that national planners should move away from the traditional systems of agricultural organization built around the academic disciplines. We feel that the older systems should be reorganized into crop-oriented teams. In the past, industrial crops such as rubber, which have been researched by a crop-oriented team, have proved much more successful than food crops, which usually have not been studied by a team of researchers. Maize has been very productive in the United States, perhaps because U.S. seed companies have had their own research and production teams working on the single crop.

2. Means must be found to bring together effective research and extension teams. Extension and production personnel should be crop-oriented, also. For example, they might devote their work to maize during the maize season and, later, to some other crop grown in rotation with maize. It is essential that extension agriculturists be allowed to devote their full efforts to increasing production, rather than diluting their agricultural work with the many community responsibilities traditionally assigned to extension personnel.

In some countries, extension is staffed with the less-qualified workers, or with agricultural graduates having lower academic records. The best-qualified people go into research. Also, the extension staffs usually receive

lower salaries and have fewer promotion opportunities. These discrepancies must be eliminated if extension efforts are to be effective.

The subject-matter-specialist concept does not fit the pattern of most national extension systems. In my opinion, the production-specialist role is essential, and specialists should be assigned to work from experiment station bases, in close liaison with the extension field staff.

3. Pilot projects must be assessed for their potential contributions to the value of a new material, idea, or concept. Some such projects have been successful in the past, but generally they have not been integrated into a truly national program; therefore, pilot projects have had little influence on total national production. Too often they have been expensive and unproductive exercises. *There is no substitute for commitment to national programs.*

4. Maize varieties, not hybrids, should be used in developing countries unless there is a private seed industry capable of producing and selling seed of high quality. Few government-sponsored hybrid seed production programs have proved to be efficient.

Such a situation should not be surprising. Varieties can be developed more rapidly than hybrids. In the same time that lines, single crosses, and hybrids can be increased and ready for sale, further improvements can be made in varieties. Thus, the yield advantage normally assumed with hybrids is often lost.

Hybrid seed programs have a further disadvantage in that as many well-qualified people are required to produce quality hybrid seeds as are needed to develop the hybrids. When few qualified people are available, a nation can ill afford to dissipate its resources with hybrid seed production.

5. In some countries, seed certification, plant quarantine, and variety release policies are nonexistent; in other countries these policies are so strict that they hamper production. In my opinion, production increases should receive the highest priority; seed certification and release policies are not necessary if an appropriate testing program is used. Unfortunately, many developing nations now are being encouraged to an unwise use of time, money, and people to develop and enforce seed control regulations.

PROGRAM STAFFING

Having described some of the limitations, as well as some possible new approaches for national crop programs, we now turn to a description of a model that outlines some of the staff structure and functions for such programs (see Table 1).

One headquarters station and four regional stations will meet the research needs of most nations, with a staff of 20 scientists required to staff the headquarters station and a staff of 6 for each regional station, a total of 44 people. Degree requirements for the staff would be a minimum of 6 Ph.D.'s, 10 M.S.'s, and 28 B.S.'s, or the equivalent. In addition, there should be five

TABLE 1 Model for national crop programs

Research staffing pattern for headquarters			
1 coordinator	Ph.D.	2 research assistants	B.S.
1 breeder	Ph.D.	1 pathologist	Ph.D.
1 associate breeder	M.S.	2 research assistants	B.S.
3 research assistants	B.S.	1 entomologist	Ph.D.
1 research agronomist	Ph.D.	2 research assistants	B.S.
(a team without individual disciplinary programs)			
Production staffing pattern for headquarters			
1 production agronomist	M.S.		
2 research assistants	B.S.		
1 agricultural economist	Ph.D.		
1 research assistant	B.S.		
1 subject-matter specialist	M.S.		
Staffing pattern for regional stations			
1 research agronomist	M.S.		
2 research assistants	B.S.		
1 production agronomist	M.S.		
2 research assistants	B.S.		
1 subject-matter specialist extension	M.S.		

subject-matter specialists with M.S. degrees for extension work, with one specialist posted at the headquarters station and one at each of the four regional stations.

The coordinator is responsible for the coordination of all research and production activities at the headquarters station and the regional stations. He also is an active researcher on the team and may be trained in any of the disciplines. He must understand and implement the concept of a true team. (My concept of a team is a group of people working together and not a group of people who are "cooperating." When people cooperate they usually maintain their own special interests, and their cooperation involves provision of an insecticide or assistance with note taking. The concept here involves a team of people with one captain. The entomologist, for example, need not have special trials for entomology. He could do insecticide evaluation as part of the overall crop production management program. In rare cases he might find a need to test new chemicals.)

A simple, effective program must be worked out that can be executed by available staff, since a program in most countries would have to start with a few, inadequately trained people. As staff numbers increase and capabilities improve, the program enters into more complex research activities.

In the initial stages, when local trained staff are unavailable, a well-trained foreigner, who has the qualifications to function as the coordinator, might be invaluable and could greatly accelerate the progress of the national program.

The program should start by testing quality protein varieties and progeny

that can be supplied by the more advanced international and national programs. It seems likely that varieties could be identified that will be successful in many countries. Of the thousands of progeny that might be tested, it seems certain that a few would be superior for the local ecological conditions. These progeny could be put together as a variety.

To identify varieties and progenies successfully, experiment stations must have efficient testing facilities. A high degree of skill and carefully conducted yield trials would be required to provide the conditions under which the superior materials could express their genetic potential. Variety and progeny testing could be done at all headquarters and regional stations.

The agronomic research would start by assessing management practices, including fertilizer response, rates of insecticide applications, optimum plant population, and planting date, as well as other specifically useful factors. In addition, the production team would start regional farm testing. This would involve testing two or three of the best varieties, at two or three levels of the production variables as determined on the experiment station. The regional farm testing should be done with cooperative farmers on their own land.

Through a regional farm testing program, the farmer could be involved in selecting superior varieties. By participating in the testing program, he could see which levels of fertilizer and which variety might be most successful on his farm. It is highly important that the farmer become interested and self-convinced through personal involvement. Field days at harvest with the help of neighboring farmers could aid to relay information and to show the results of a successful production demonstration program.

The subject-matter specialist could help identify cooperative farmers and provide support for the regional farm testing. He also could organize the extension personnel, helping to prepare large production demonstration plots of at least 0.5 hectare. The production demonstration plots would provide a showcase for the best variety, using optimum economic production practices.

Field days could be held at harvest time at each of the production demonstration plots. If the technological "package" is sound, the farmer growing the demonstration plot will be enthusiastic and might become an excellent "extension" agent. Since the production demonstration plot would be at least 0.5 hectare in size, the center of the field could be saved for seed. The subject-matter specialist and the extension staff should participate in the harvest and explain the reason for saving only isolated seed from the center of the plot. This seed should then be sold to the neighboring farmers.

CONTINUOUS FLOW SYSTEM

The process, or flow system, of variety and progeny testing on the experiment stations, regional farm testing, and production demonstration plots is continuous, with new materials and new farmers. It is critical that all steps be

managed with precision and be successful. In this way, relatively large quantities of new seed will be moved rapidly into the area.

The experiment station will have to increase rather large quantities of genetically pure seed of any variety that goes into the production demonstration plots. Since the variety that will prove to be best is not known in advance, all varieties in the regional farm testing program should be simultaneously increased on the experiment stations.

Figure 1 shows the flow system for materials and technology among headquarters station, four regional stations, five regional farm testing sites operated from each station, and five production demonstration plots in the vicinity of each regional farm trial. The staff patterns outlined previously could manage this volume of work. Production demonstration and seed increase blocks would be planted at the rate of 125 per crop season in the first years of the program. In subsequent years these numbers would probably be insufficient to meet the nations' requirements.

As indicated, extension workers must become better trained and more involved. Several maize production specialists might be trained simultaneously with the development of the research staff, and then posted throughout the maize-producing area of the country. There should be at least one specialist for every administrative division or region of a country. His responsibility would be to organize the extension staff working in his area for successful demonstrations and to explain the advantages of the new high-quality protein maize varieties to the farmers. Along with the subject-matter

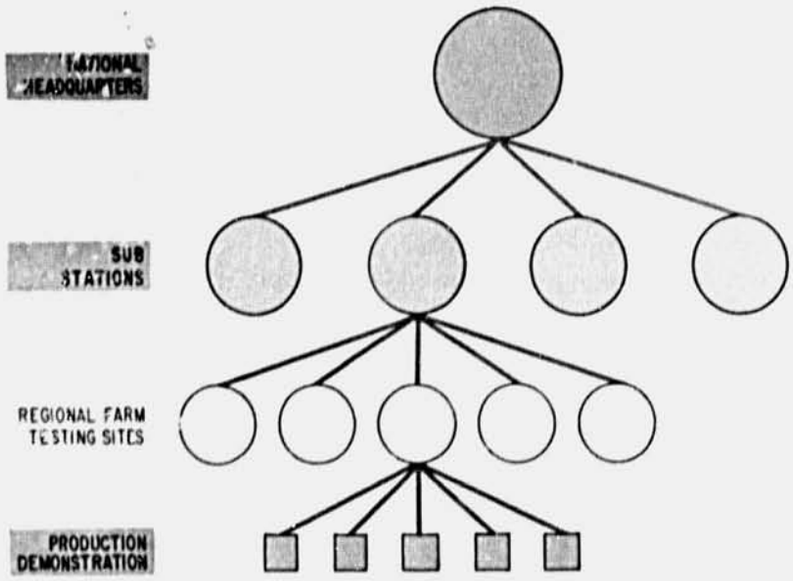

FIGURE 1 Flow system for materials and technology.

specialist, he would organize training programs for the production staff conducted by the research staff at the experiment stations.

Although researchers and extension people might understand the value of the quality protein, it might be difficult to explain this concept to the farmer. The value of quality protein in the family diet cannot be perceived rapidly enough for parents to appreciate it during a short time. Therefore, any variety going into production must be superior in yield to the variety previously grown, and it must be acceptable in appearance.

In some countries, swine trials might convince farmers of the value of new varieties. For example, swine fed normal maize will reach market weight in about 10 months on the average. However, if fed the same amount of high-quality protein maize, the swine would reach market weight much more rapidly. Therefore, swine-feeding programs could be started with farmers wherever production demonstration plots are grown. If the farmer feeds half his pigs with high-quality protein maize and the other half with normal maize, he will see very quickly the feeding value of the quality protein maize. This simple demonstration could be one of the most effective teaching tools available to promote the growing of quality protein varieties.

TRAINING

If a national program is to be successful, a phased staff development program must be organized simultaneously with research and production.

As an example, we can examine the proposed staffing pattern and assume that there are six important maize-growing regions or districts in a country, with a maize production specialist in charge of the maize extension work. Including the research and production team, there would be 55 people participating in accelerating production. All these people, regardless of the degrees they hold, will need 9 months of in-service training if they are to fully understand the possibilities and approaches to an efficient research and production program.

If we assume that a B.S. or equivalent degree could be obtained within the particular country, a phased staff-development program within a 14-year period could include the approximate numbers of people indicated in Table 2.

It can be readily seen that the staff development program is ambitious. In most countries, this sort of staff would be difficult to mobilize on such a schedule.

However, when this training schedule is completed, the program should be able to meet national research and production requirements. Governments should understand that resignations, promotions, and other causes will reduce this initial staff up to 25%. Therefore more people must be trained over a longer period of time.

To summarize, 505 man-months of in-service training and 76 man-years

TABLE 2 Personnel for staff-development program

Year:	1	2	3	4	5
In-service training	10	10	10	10	10
M.S.	2	4	4	4	4
Ph.D.	0	0	0	2	4
Year:	**6**	**7**	**8**	**9**	**10**
In-service training	5				
M.S.	4	4	4	4	4
Ph.D.	4	4	4	4	2
Year:	**11**	**12**	**13**	**14**	
M.S.	4	4	4	2	
Ph.D.	0	0	0	0	

of degree training will be required to meet the staffing needs just described. Approximately $1,000,000 U.S. would be required to finance such a staff development program at present costs.

In addition, a local training program would have to be developed for the extension personnel discussed earlier in this paper. The number will depend on the size of the country, the size of the holdings, and the speed with which a country wishes to increase its production.

Meeting the staffing requirement is only a beginning. Continued opportunities for younger staff improvement would have to be taken into consideration. Also, opportunities for senior-staff study leave would be essential to assure that the national program does not stagnate.

The system described in this paper is not intended as an "absolute" model, but it does provide an outline for a successful program. Although the outline is built around high-quality protein maize, it is not maize specific. The model is equally applicable to other national crop programs.

[*A discussion of this paper can be found on page 506–507 of* **Questions and Answers.**]

Part IX

PROGRESS IN BREEDING FOR QUALITY PROTEIN IN OTHER CEREALS

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This paper's title reminds me of an attempt by a group of distinguished scientists to classify the cultivated sorghums on the basis of botanical characters. They devised 70 classes, and the entries in "class 70" consisted of all those cultivars which could not be fitted into the other 69 classes. This paper is perhaps a little like their "class 70"; general comments are difficult to make that would apply equally to all the cereals under discussion.

I would like to stress the importance of *all* cereals in direct human nutrition, without in any way lessening the emphasis that ought to be placed on their indirect value through livestock feeding. It has been noted that oats have been largely ignored as a human food and are deserving of much more improvement effort as a cereal for the developing world. The Canadian government is financing triticale research work at CIMMYT, primarily for human food.

Many of us are very concerned about those millions of people in the developing world who subsist on the produce of their own patches of land, whose principal input is family labor. This labor input is often seriously weakened by parasites and malnutrition. There can be no Green Revolution for them until they can afford some additional inputs. The only feasible approach would seem to lie in increasing the effectiveness of their labor through better nutrition, and the productivity of their labor through better varieties and agronomy practices. Once they can generate a surplus, they can purchase inputs, and the Green Revolution comes within their reach.

Against this background, breeding for quality protein has to take its place as only one component of the breeding program.

One evident basis for classification among cereal crops is that relating to the genetic control of the amino acid profiles in the proteins. For example, one or more genes have been discovered in barley that produce large changes in the amino acid profile. There may be many behavioral differences between protein genes for barley and those for maize, yet the same kinds of breeding programs are likely to apply to both. Until similar genes have been discovered in the other cereals, protein quality improvement must depend on accumulating genes, each gene having a small favorable effect. Several cycles of recurrent selection may be required to bring about modest changes in the amino acid profile, and substantial changes could require a long time.

Resources are very limited, and it is most important that plant breeders set their priorities correctly in attempting to improve the nutrition of people in the developing world through use of better crop varieties. I am drawing attention again to what has been said in other articles in the hope that the contributors to this session may be able to help clarify our ideas on plant breeding objectives as they relate to particular cereals. Some basic considerations are as follows:

1. When calories are insufficient, protein is used to meet the energy requirements of basic metabolism. Therefore, sufficient carbohydrate is needed to provide enough calories—the concept of net dietary protein calories percentage. A major plant-breeding objective must continue to be yield per unit area.

2. Metabolizable energy for some cereals may be as low as two thirds of the gross energy available in the grain. Thus, the next factor to consider is possible improvement of the metabolizable energy percentage of the grain.

3. The net utilizable protein levels vary considerably among cereal grains. This may be determined largely by the limiting amino acid, normally lysine. There are three aspects to this: (a) breeding for a higher protein content, which must relate to the total production of net utilizable protein per unit area with adequate carbohydrates to provide the calories required; (b) improvement of the amino acid profile of protein, to be thought of as net utilizable protein per unit area and related to the yield of carbohydrate per unit area; and (c) digestibility of the protein, that is, factors, other than limiting amino acids, which influence utilization after protein is produced in the grain, such as the tannins in sorghum.

4. The problem thus becomes one of how plant breeders should arrange the priorities of yield, metabolizable energy percentage, protein percentage, net utilizable protein percentage, and improved amino acid profile in the protein.

5. Finally, are we correct in thinking of cereals alone? In several areas of the world, a diet of cereal and grain legume has been developed. For

example, such diets include maize and beans in Latin America, and millet and sorghums with bambara earth nuts in Africa (the bambara nuts being replaced by peanuts subsequently). We are thinking of a diet that is within the reach of the subsistence farmer. There is good evidence that such crop mixtures give greater production of food per unit of area and labor, and that pest and weed problems can be lessened by their use.

We need to ask ourselves whether improvement of cereals is always the main priority. Grain legumes have not received very much attention so far. Would some of our efforts be better devoted to improving yields of grain legumes, and to dealing with the inhibiting and toxic substances that survive normal cooking processes? In the cereal–legume diet, the limiting amino acid is usually methionine. Lysine is often present in sufficient quantity. For the subsistence farmer's diet, are we right in concentrating on improving lysine in the cereals? Might it not be more important to try to improve the quantity of methionine, either in cereals or in legumes, or in both? Perhaps more plant-breeding work should be directed toward the quality of a diet made up of crop mixtures and developing types more suitable for intercropping systems.

It is possible that we have given too little attention in the past to the cereal–legume diet. The program of the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) will begin this work with both cereals and legumes. The broad problems of priorities in our breeding work will be a major concern.

COMPONENTS OF NUTRITIONAL QUALITY IN GRAIN SORGHUM

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Sorghum is similar to the other cereal grains in many of its nutritional deficiencies, yet it differs in several important respects. The protein quality of sorghum grain is limited by the low lysine content, reflecting the high prolamine content of the endosperm and the relatively small embryo size as a proportion of the mature grain.

Protein availability apparently is limited in some sorghum genotypes by the presence of unidentified polyphenolic compounds located primarily in the testa layer of the grain. These pigmented compounds have not been well characterized chemically and are referred to generically as tannins. We do not fully understand how these tannin compounds influence the nutritional quality of sorghum. Our present hypothesis is that the seed proteins become

complexed or bound with the tannin compounds of the whole grain, and that the complexed proteins are substantially less available for utilization by monogastric animals.

This report presents some of the data and experimental evidence supporting these general observations, and describes briefly our efforts toward improving the nutritional quality of sorghum.

AMINO ACID COMPOSITION

The average protein and amino acid composition of 522 lines from the world sorghum collection is presented in Table 1. The average protein content of these lines is 12.6% and the average lysine content (expressed as a percentage of protein) is 2.1%. A comparison of essential amino acid content in sorghum with experimental rat requirements, as shown in Figure 1, provides a more meaningful assessment of amino acid content from a nutritional point of view. This shows sorghum's deficiency in lysine, and its excessive leucine content. The methionine content of sorghum is low, but considering the cystine content of 1.5%, the overall sulfur amino acid content approaches the experimental rat requirements. The tryptophan con-

TABLE 1 Protein and amino acid composition of sorghum grain[a]

Composition	Mean	Standard deviation
Protein	12.61	1.89
Lysine	2.14	0.35
Histidine	2.01	0.20
Arginine	3.59	0.44
Aspartic acid	7.83	0.77
Threonine	3.26	0.21
Serine	4.52	0.32
Glutamic acid	23.22	1.99
Proline	8.16	0.89
Glycine	3.07	0.27
Alanine	9.89	0.71
Cystine[b]	1.50	–
Valine	5.35	0.31
Methionine	1.80	–
Isoleucine	4.08	0.25
Leucine	14.27	1.21
Tyrosine	4.50	0.32
Phenylalanine	5.19	0.37
Tryptophan[c]	1.31	0.14

[a] Weighted average for 522 lines.
[b] Weighted average for 3 lines.
[c] Weighted average for 9 lines.

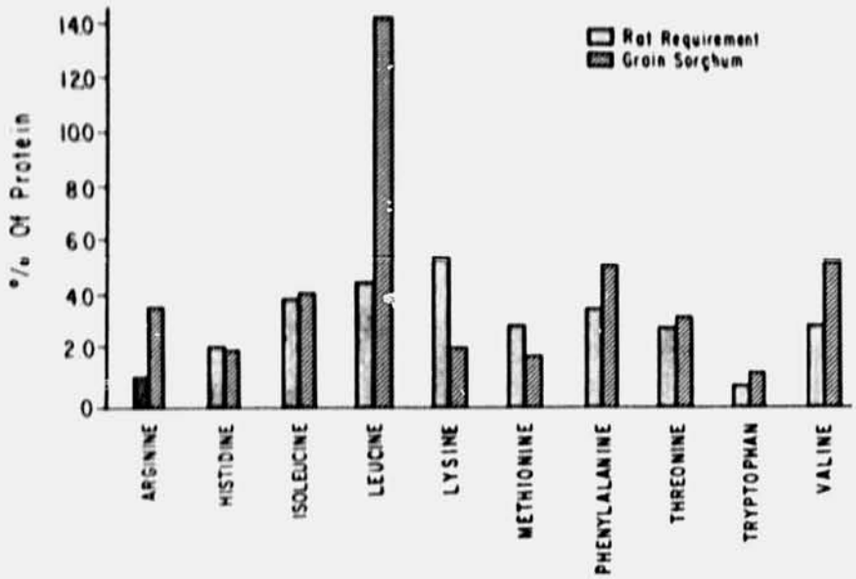

FIGURE 1 Essential amino acids.

tent of sorghum (based on our current evidence) seems to be adequate, in contrast to the low tryptophan content in normal maize.

FRACTIONATION OF WHOLE GRAIN AND ENDOSPERM PROTEINS

Jambunathan and Mertz (3) at Purdue University recently completed the fractionation of sorghum proteins using the procedure of Landry and Moureaux (4). This procedure solubilizes most of the nitrogen of sorghum and yields five different solubility fractions. Results of fractionating whole grain from two low-tannin and two high-tannin sorghum lines are shown in Table 2. The average percentage of nitrogen in each fraction for the low-

TABLE 2 Nitrogen distribution in whole sorghum kernels (lysine as a percentage of protein in parentheses)[a]

	Fraction	Low tannin	High tannin
I	(saline)	16.1 (5.8)	4.9 (3.1)
II	(isopropanol)	14.5 (0.3)	4.2 (0.4)
III	(isopropanol + 2-mercaptoethanol)	18.6 (0.1)	17.7 (0.2)
IV	(borate buffer + 2-mercaptoethanol)	6.3 (2.3)	16.2 (2.9)
V	(borate buffer + 2-mercaptoethanol + sodium dodecyl sulfate)	32.1 (2.8)	52.6 (1.3)
Total nitrogen extracted		87.5	89.9

[a] Percentage of soluble nitrogen.

and high-tannin lines is shown in each column, with the average lysine content shown in parentheses as a percentage of protein for each fraction.

Two conclusions can be drawn from these data. First, the high- and low-tannin lines show distinct differences in the distribution of protein in various fractions, which suggests that the presence of tannins has altered the solubility of sorghum proteins, perhaps by binding or forming complexes with the protein. This idea is supported by the observation that the first fraction in the high-tannin samples is very low in protein content; it would be expected that this fraction would be reasonably high, since fraction I represents the albumins and globulins that are indispensable for the germination and sustenance of the plant.

Second, within the low-tannin sorghums, the *protein* content of fractions II and III is high, but the lysine content of these proteins is very low. For the endosperm fractions presented in Table 3, the differences in protein content between high- and low-tannin sorghum samples are less pronounced, possibly because part of the tannin-containing testa layer was removed with the pericarp during separation of the endosperm from the whole grain. In the endosperm, as expected, the proportion of protein with low lysine content in fractions II and III is higher than in whole-grain fractions, since these are primarily storage proteins.

GROWTH RESPONSE OF RATS RELATIVE TO LYSINE AND TANNIN CONTENT

Since lysine is the first limiting amino acid in sorghum, an experiment was designed to verify the growth response of rats fed sorghum rations supplemented with adequate lysine hydrochloride to fulfill the rat requirement for lysine, plus a 25% excess. The results of this experiment are shown in Figure 2. Two low-tannin lines (IS-2319 and IS-0129) and one high-tannin line (IS-6992) were included in this trial. The rat growth on low-tannin sorghum rations supplemented with lysine, vitamins, and minerals was substantially greater than growth on the ration supplemented only with

TABLE 3 Nitrogen distribution in sorghum endosperm (lysine as a percentage of protein in parentheses)[a]

	Fraction	Low tannin	High tannin
I	Saline	7.3 (5.1)	2.9 (3.0)
II	Isopropanol	16.8 (0.3)	11.3 (0.3)
III	Isopropanol + 2-mercaptoethanol	32.4 (0.2)	27.6 (0.3)
IV	Borate buffer + 2-mercaptoethanol	7.8 (1.2)	10.9 (2.4)
V	Borate buffer + 2-mercaptoethanol + sodium dodecyl sulfate	25.3 (3.6)	40.4 (3.0)
Total nitrogen extracted		89.5	92.9

[a] Percentage of soluble nitrogen.

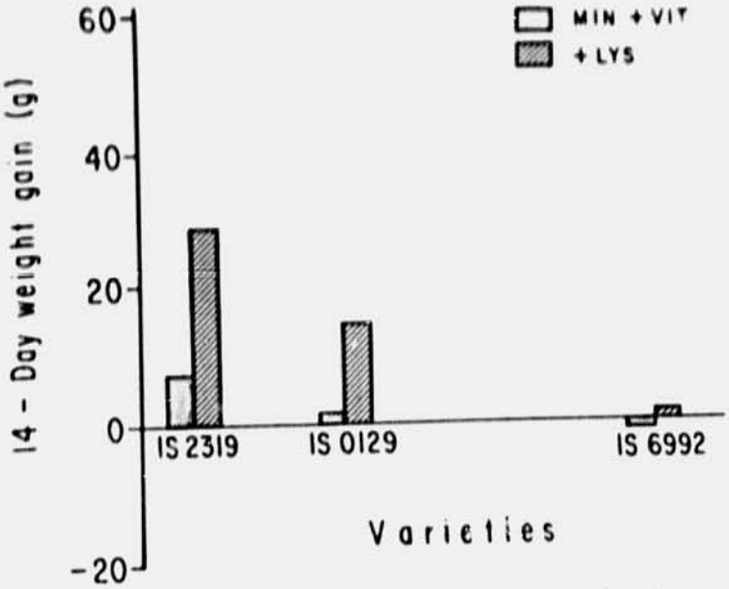

FIGURE 2 Rat weight gains on isonitrogenous samples of supplemented grain sorghum lines.

vitamins and minerals. In contrast, rats fed the high-tannin sorghum (IS-6992) lost weight on the unsupplemented ration and gained only slightly on the ration supplemented with lysine. These results suggest that lysine is the first limiting amino acid in low-tannin sorghum lines, but other factors are first limiting in high-tannin lines.

The results obtained from lysine supplementation experiments conducted at Purdue University are substantiated by data from rat growth trials using unsupplemented sorghum rations. Seventeen low-tannin sorghum lines and ten high-tannin lines, representing the range in variation for protein and lysine content of sorghum lines available from Purdue's screening of the world sorghum collection, were used in this experiment. The results of this experiment, illustrated in Figure 3, demonstrate that rat weight gain is dependent on lysine content in the low-tannin sorghum group, but not in the high-tannin sorghum group. The regression of rat weight gain on lysine content for the high-tannin groups is nearly zero, whereas the regression coefficient for the low-tannin group is 64.79.

We have used the acidified vanillin method of tannin analysis described by Burns (2), which estimates tannin content as *catechin equivalents*. Maxson and Rooney (5) have reported that catechin equivalent values for grain sorghum are correlated with tannin values obtained with other methods. In our experience, the range of catechin equivalents for grain sorghum has varied from 0 to 10. Sorghum lines have been classified as low tannin if the catechin equivalent value is below 1.00.

FREQUENCY OF HIGH-TANNIN SORGHUM GENOTYPES IN THE WORLD COLLECTION

Only limited evidence is available at this time on the frequency of high-tannin sorghum genotypes in the world sorghum collection. Among 472 lines that we have analyzed for amino acid content (Table 4), 300 were classified as low-tannin and 172 as high-tannin lines. The average catechin equivalent values for the low- and high-tannin lines were 0.38 and 3.40, respectively. There were no significant differences between the averages of the two groups in protein or essential amino acid content.

Evaluation of 288 lines collected recently in Cameroon, Africa, has just been completed, and the average tannin and protein content of these lines is shown in Table 5. Approximately 80% of these lines were classified as high-tannin genotypes with an average catechin equivalent value of 4.07. Average protein content was similar for both high- and low-tannin groups, although the two groups were lower in protein content than the overall average of 12.6% reported in Table 1. The lower average protein content probably can be accounted for by the observation that the Cameroon lines represent a collection with unusually large seeds. It can be concluded gener-

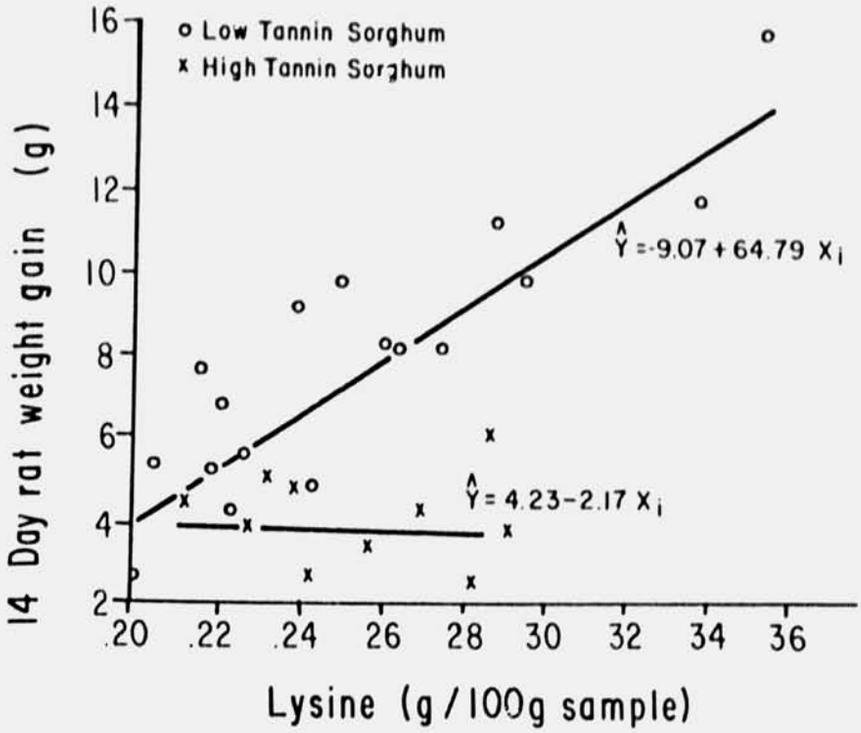

FIGURE 3 Relationships of rat weight gain with lysine content in high-and low-tannin sorghum lines.

TABLE 4 Protein and amino acid composition of high- and low-tannin grain sorghum (amino acid content expressed as a percentage of protein)

Essential amino acids	300 Low tannin ± S.D.	172 High tannin ± S.D.
Lysine	2.11 ± 0.33	2.15 ± 0.35
Histidine	2.02 ± 0.19	1.99 ± 0.21
Arginine	3.56 ± 0.42	3.60 ± 0.43
Threonine	3.26 ± 0.20	3.24 ± 0.18
Cystine[a]	0.94 ± 0.22	0.89 ± 0.22
Valine	5.33 ± 0.29	5.37 ± 0.31
Methionine[a]	1.02 ± 0.53	1.01 ± 0.36
Isoleucine	4.08 ± 0.25	4.07 ± 0.22
Leucine	14.33 ± 1.20	14.17 ± 1.10
Phenylalanine	5.21 ± 0.36	5.12 ± 0.34
Catechin equivalents	0.38 ± 0.24	3.40 ± 2.35
Protein	12.71 ± 1.86	12.42 ± 1.95

[a] Values are low because of loss during hydrolysis

TABLE 5 Frequency of high- and low-tannin sorghum genotypes among 288 lines collected in Cameroon, Africa

	High tannin	Low tannin
Number of lines	229	59
Average catechin equivalent value	4.07	0.49
Average crude protein (%)	10.61	10.93

ally that high-tannin sorghum genotypes comprise a significant proportion of the sorghum germ plasm in some areas of the world.

ALKALI DEHULLING OF HIGH- AND LOW-TANNIN SORGHUM GRAIN

The tannin in sorghum grain is contained primarily in the testa layer beneath the pericarp. This tannin can be successfully removed with an alkali dehulling technique described by Blessin et al. (1). Table 6 shows how dehulling affects tannin content, protein content, rat gain, and feed consumption for the low-tannin Texas hybrid (RS-610) and two high-tannin lines (IS-6992 and IS-8260) from the world sorghum collection. Catechin equivalent values were reduced without substantial change in protein content. Rat weight gain was not significantly improved by dehulling the low-tannin RS-610 hybrid, but was substantially increased by dehulling both high-tannin lines. The higher rat weight gain on dehulled IS-6992 and IS-8260 rations, in comparison with the dehulled RS-610 ration, is due to the higher inherent lysine content of IS-6992 and IS-8260. There was no large difference between hulled and dehulled samples in the amount of feed consumed. This finding indicates that the poor weight gain for whole-grain samples of IS-6992 and IS-8260 was not caused primarily by reduced intake.

TABLE 6 Alkali dehulling of high- and low-tannin grain sorghum

Entry	Catechin equivalent	Protein (%)	14-Day rat weight gain (g)	Feed consumed (g)
RS-610	0.48	9.90	5.26	112
RS-610 (dehulled)	0.10	9.95	6.72	132
IS-6992	1.74	9.4	5.73	99
IS-6992 (dehulled)	0.07	9.8	10.20	101
IS-8260	3.71	8.1	-1.12	95
IS-8260 (dehulled)	0.10	8.4	7.98	97

All our previous tests for differences in response to lysine supplementation between high- and low-tannin sorghum lines have compared lines from the world sorghum collection that differ in tannin content, but that are not isogenic for other characteristics. A more direct test can be made by utilizing the alkali dehulling technique to obtain "low-tannin" control lines. These lines can then be compared with whole grain from the same genetic background but with a high-tannin content. Figure 4 shows the results of an

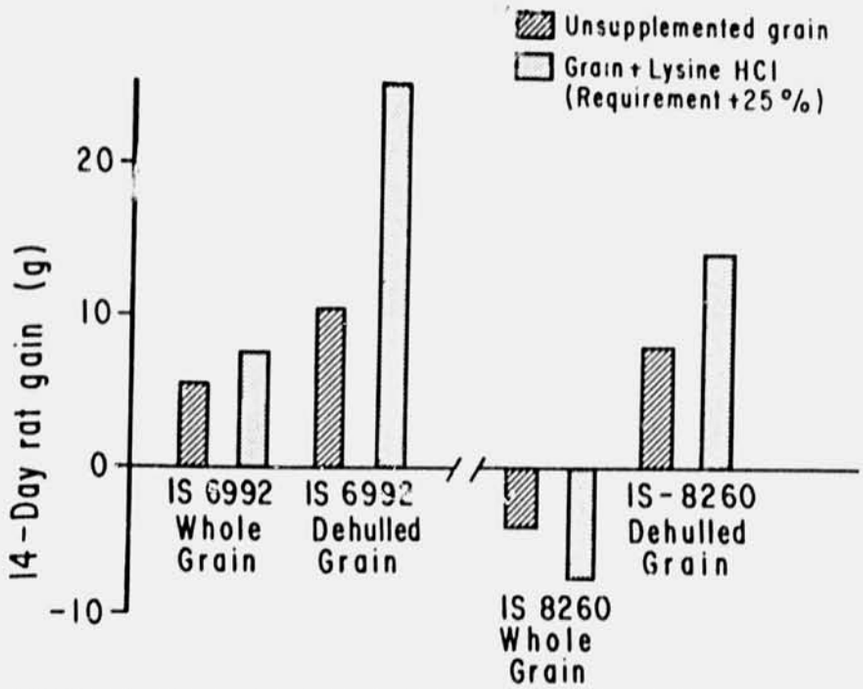

FIGURE 4 Lysine supplementation of whole grain and dehulled high-tannin sorghum lines.

experiment comparing the biological value of whole-grain samples from two high-tannin lines and dehulled counterparts of each line, with and without lysine supplementation. In both cases the rats gained little or no weight on the whole-grain (high-tannin) rations supplemented with lysine, but responded to lysine supplementation of the dehulled (low-tannin) counterpart rations. This evidence, as well as that from other studies discussed here, indicates that low lysine content seems to be the major factor limiting the biological value of low-tannin sorghum grain. However, lysine does not appear to be first limiting for high-tannin sorghum grain.

SUPPLEMENTATION WITH SOYBEAN MEAL

One possible explanation for the lack of rat growth response in lysine-supplemented, high-tannin sorghum rations is that the tannin compounds may produce a toxic effect, which counteracts the expected growth response from lysine supplementation. Data from Schaffert (6) in Figure 5 do not seem to support this explanation, since supplementing the high-tannin rations with only 5% soybean meal produced a very significant increase in rat

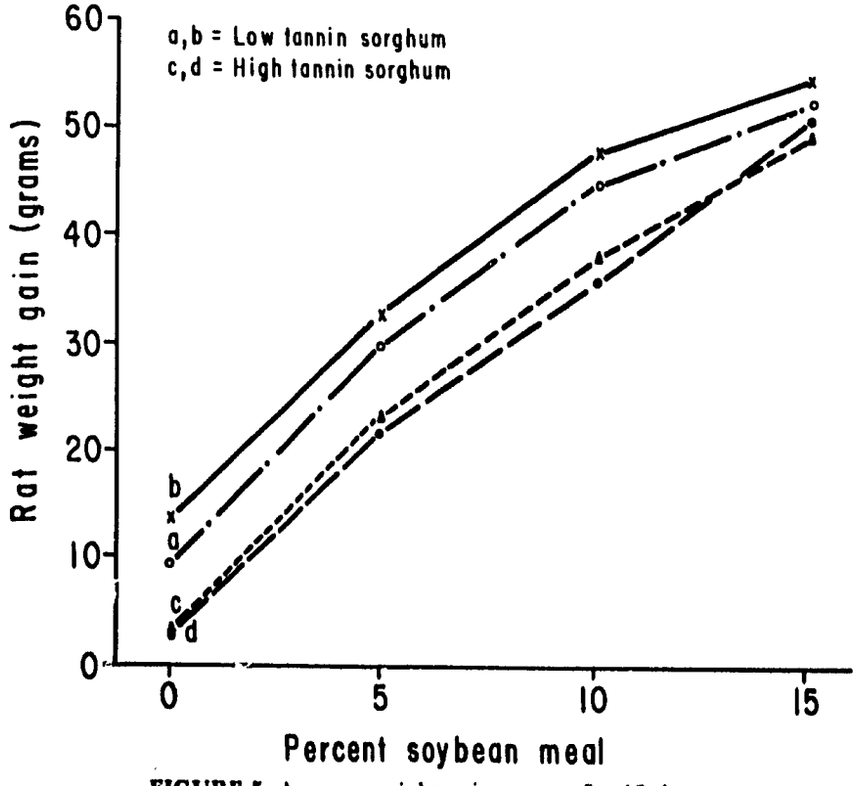

FIGURE 5 Average weight gain per rat for 13 days.

growth. In this experiment, rations containing two low-tannin and two high-tannin lines were supplemented with 0, 5, 10 or 15% soybean meal, and rat growth response was measured in a 13-day trial. It seems unlikely that adding small quantities of soybean meal would dilute any toxic effect to the extent required to account for the substantial gain in rat growth shown in Figure 5. We would expect the low-tannin lines to respond to soybean supplementation because of the soybean's relatively high lysine content. However, it is quite apparent that something else is added with the soybean meal, which can correct a nutritional deficiency or imbalance in the high-tannin rations. The effect of soybean meal supplementation on feed efficiency ratios is illustrated in Figure 6. This again shows the striking improvement in the amount of feed consumed per gram of gain when high-tannin rations are supplemented with 5% or more soybean meal. The most viable explanation for this improvement is that the soybean meal supplies *available* protein, which is deficient in high-tannin lines because the complexed sorghum protein is unavailable to monogastric animals. The evidence obtained by Jambunathan and Mertz (3) on protein fractionation supports this explanation, since the presence of tannin compounds apparently alters the solubility of major protein components in high-tannin sor-

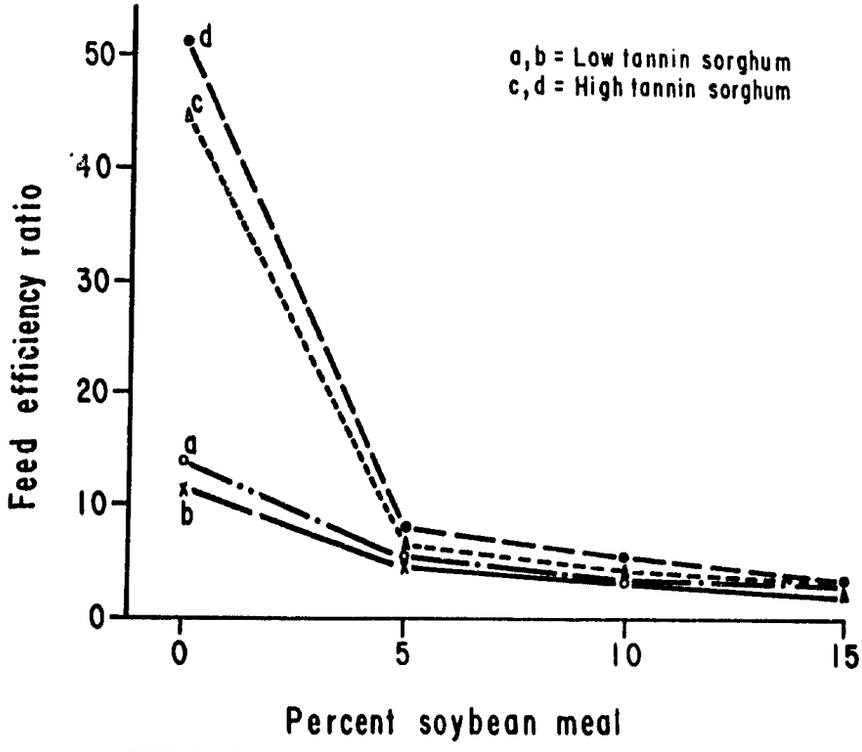

FIGURE 6 Feed efficiency ratios for rats fed for 13 days.

ghum lines. There are other possible explanations that require testing, including the following:

1. Soybean meal may provide some other essential amino acid that is selectively unavailable in high-tannin rations.

2. There may be a deficiency of available inorganic nitrogen in high-tannin sorghum rations needed for biosynthesis of nonessential amino acids and/or other nitrogenous compounds vital for normal growth and development.

3. There may be compounds in soybean meal that can bind more competitively with tannins and thereby replace the protein bound to tannin, thus making the protein more available.

CURRENT STATUS ON IMPROVING NUTRITIONAL QUALITY

If we are correct in our assumption that tannins form complexes with seed proteins, making these proteins less available for monogastric animals, there are several alternatives for improving sorghum nutritional qualities:

1. Use only low-tannin sorghums for consumption by human and other monogastric animals, and find other methods for controlling birds. Resistance to bird damage is a major reason for using high-tannin sorghum germ plasm. Since tannin content is simply inherited and controlled by only one or two major genes, it is easy for the breeder to remove the tannin factors.

2. Use high-tannin sorghum lines for their desirable agronomic characteristics and plan to supplement them with additional protein to meet the nutritional requirement. If this approach is adopted, the possibility must be considered that some of the protein in the sorghum grain is being wasted. We do not believe that this is an economically feasible approach in most areas of the world where sorghum is a major component of the human diet.

3. Use of dehulling procedures or methods of dry milling that could remove the pericarp and testa cells that contain the tannin compounds.

4. Use of additives that could preferentially bind with the tannins and allow the protein to be utilized.

With regard to improving lysine content, there are excellent opportunities for increasing germ size. Grain sorghum germ size ranges from 7.8 to 12% of the whole grain, which is smaller than the germ size in maize. This smaller germ size explains part of the difference in lysine content between normal maize and sorghum; normal maize protein contains approximately 2.5 to 2.8% lysine, whereas normal sorghum protein contains approximately 1.9 to 2.2% lysine. The difference in average oil content between maize (4 to 5%) and sorghum (2 to 3%) also reflects differences in germ size between these species, since most of the oil is contained in the germ. Several sorghum lines have been identified with protein containing 2.5 to 2.6% lysine and with a relatively large germ size. Rapid progress should be possible in selections

from random mating populations derived from selected large-germ lines. This selection should lead to improved nutritional quality.

The prolamine content of sorghum endosperm is high, while the lysine content of the prolamine fraction is very low in sorghum, as in maize. Sorghum is similar to normal maize in many respects. There is every reason to believe that mutants which reduce or block prolamin synthesis also occur in sorghum. Sectioned kernels of most lines in the world collection have now been examined and possible opaque endosperm phenotypes identified. Some of these were grown at Purdue in 1972 and others (light-sensitive lines) will be grown in Puerto Rico. Protein and lysine analysis of these putative opaque lines will reveal whether any low-prolamin lines have been identified. (A report describing these results entitled "High Lysine Mutant Gene *(hl)* That Improves Protein Quality and Biological Vlue of Grain Sorghum" by Rameshwar Singh and J. D. Axtell appears in *Crop Science*, 13:535–539.)

Also, we have initiated chemical mutagen treatments of two colorless pericarp, low tannin, vitreous endosperm sorghum lines for identification of low prolamine mutants. M_2 rows will be grown in Puerto Rico for evaluation in 1973.

It will be necessary to consider each of these parameters (prolamin content, germ size, and tannin content) in improving nutritional quality in sorghum. It is unlikely that any line in the world collection will have all three necessary characteristics. In our estimation, we shall have to identify lines with each component of nutritional quality and then recombine them to obtain genotypes with the desired combination of characteristics for high quality.

This article is Journal Paper No. 5175, Purdue University Agricultural Experiment Station. The work was supported by the U.S. Agency for International Development under Contract AID/Csd-1175, titled "Inheritance and Improvement of Protein Quality and Content in *Sorghum bicolor* (L.) Moench."

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STATUS OF PROTEIN QUALITY IMPROVEMENT IN WHEAT

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In 1966, a research project was initiated at the University of Nebraska with the aim of improving the nutritional quality of wheat through increased protein and improved amino acid balance. Initially, 16,000 entries in the U.S. Department of Agriculture (USDA) World Wheat Collection were screened for lysine and protein differences, and identified materials were crossed to good agronomic wheats. With funds from the U.S. Agency for International Development (USAID), an International Winter Wheat Performance Nursery was developed as part of the Nebraska protein research effort to identify winter genotypes for possible new genes for high protein and high lysine.

GENETIC VARIATION OF PROTEIN AND LYSINE IN WHEAT

Laboratory analyses of 12,500 common wheats from the USDA World Collection of Wheats have indicated protein content ranging from 7 to 22% (Figure 1), with a mean of 12.9%. The genetic component of this variation is estimated at 4 or 5%.

The range in lysine content as a percentage of protein in the same samples was 2.2 to 4.2% (Figure 2). The genetic component of lysine appears to be 0.5%, and it is this portion that should be useful for breeding for improved lysine content.

Protein content and lysine level in wheat have been shown to be negatively correlated. In analyses of 4,100 wheats from the world collection, protein level increases ranged from 10 to 20%, whereas mean lysine as a percentage of protein levels dropped from 3.2 to 2.7% (Figure 3). Only 18% of the variation in lysine was associated with variation in protein content. However, on a sample basis there was a strong positive relationship between protein content and lysine. On a dry weight basis, mean lysine values increased from 0.33 to 0.53%, as the grain protein increased from 10 to 20%.

As stated previously, environmentally induced protein variation is negatively correlated with lysine. However, genetically high protein wheat has

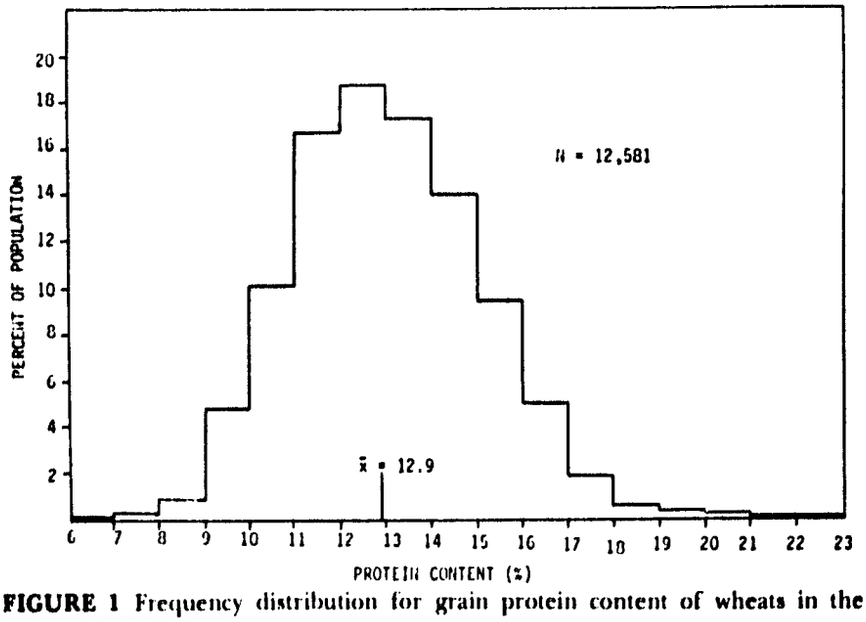

FIGURE 1 Frequency distribution for grain protein content of wheats in the USDA World Collection.

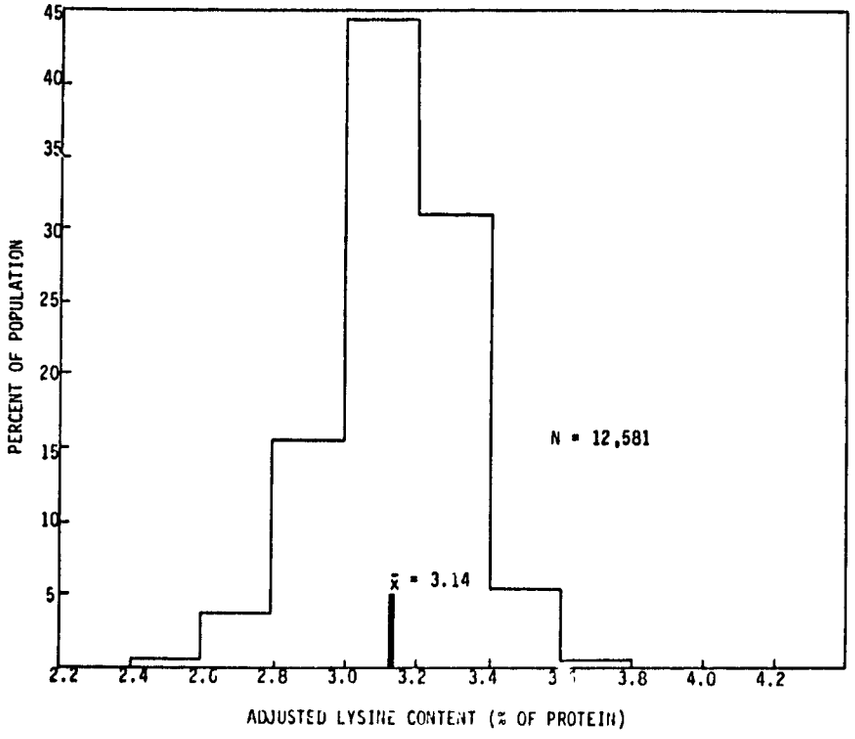

FIGURE 2 Frequency distribution for lysine adjusted to a common protein level of wheats in the USDA World Collection.

been found to be equal to or higher in lysine content as a percentage of protein than are normal wheats grown in the same environment. Interestingly, the negative correlation between protein and lysine appears to become nonsignificant at higher levels of protein.

SOURCES OF HIGH PROTEIN AND HIGH LYSINE CONTENT

Atlas 66, a soft red winter wheat variety, has been used extensively in our breeding program as a source of high protein content. This genetic source originally came from the South American variety Frondoso. Additional genetic sources of higher protein content in wheat are identified in Table 1; NB-542437, SD-69103, CI-7337, CI-6225, and PI-176217 (Nap Hal) show promise. Nap Hal is of particular interest because it also is the most promising genetic source of high lysine content discovered to date in our screening efforts.

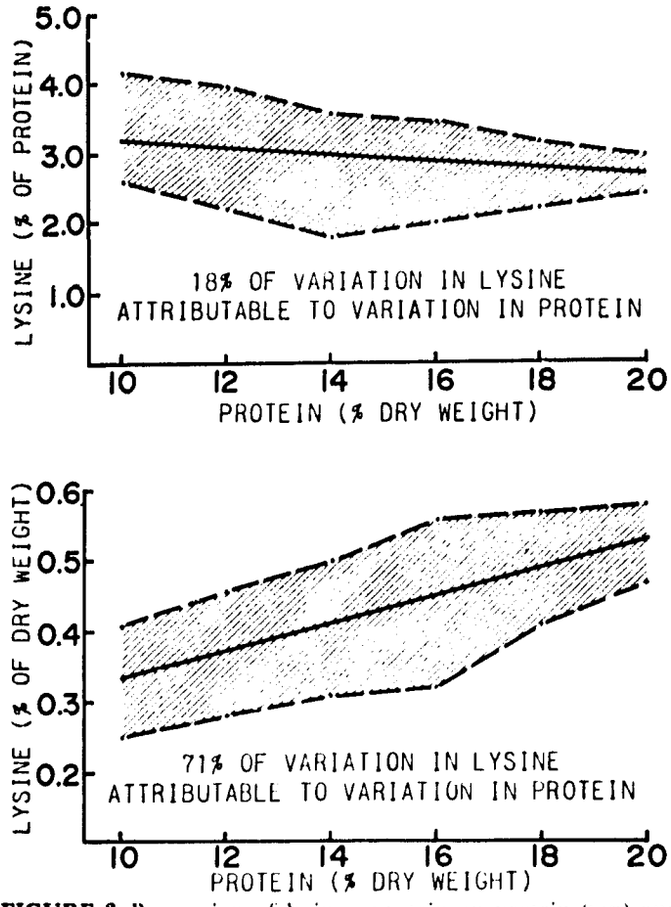

FIGURE 3 Regression of lysine – protein on protein (top) and regression of lysine – dry weight on protein (bottom). Shaded areas indicate dispersal of values about the regression line.

IDENTIFYING THE BEST RECIPIENT GENOTYPES

The need to identify good agronomic winter wheat genotypes with wide adaptation led to the establishment of the International Winter Wheat Performance Nursery (IWWPN) in 1969. Its purposes were to

1. Test the adaptation of winter wheat varieties under a range of latitudes, day lengths, fertility conditions, water management, and disease complexes.
2. Identify superior winter varieties to serve as recipient genotypes for high-protein and high-lysine genes.
3. Test the degree of expression and stability of the high-protein and high-lysine traits in an array of environments.

These purposes are achieved best by continued testing with new varieties

TABLE 1 Useful germ plasm for the nutritional improvement of wheat

Variety	CI or Sel. number	Source	Growth habit[a]	Useful trait
Atlas 50	12534	Beltsville	I	High protein
Atlas 66	12561	Beltsville	I	High protein
Atlas-derived lines	– –	Nebraska	W	High protein
Aniversario	12578	Argentina	S	High protein
Aniversario-derived lines	–	Nebraska	W	High protein
Male fertility restorer	NB 542437	Nebraska	W	High protein
Hume[2] X Nb[4]-Agrus-Tc[7]	SD-69103	South Dakota	W	High protein
Nap Hal	176217	India	S	High protein, high lysine
April bearded	7337	England	S	High protein, high lysine
Hybrid English	6225	England	W	High protein, high lysine
Pearl	3285	Sweden	S	Probable high lysine
22A	5484	U.S.S.R.	S	Probable high lysine
Fultz X Hungarian	11849	U.S.A.	W	Probable high lysine
Fultz-Hungarian X Minturki-Fultz	12756	U.S.A.	W	Probable high lysine
Norin 10-Brevor Sel. 14-derived line	13447	Washington	W	Probable high lysine
Norin 10-Brevor Sel. 14-derived line	13449	Washington	W	Probable high lysine

Source: Proc. First Intern. Winter Wheat Conf. 1972.

[a] I, intense diet; W, winter; S, spring.

from the various winter wheat projects of the world; thus, candidate varieties are solicited for future testing. Three types of candidate varieties are especially desired:

1. New commercial or promising experimental varieties with desirable combinations of high yield potential, acceptable winterhardiness, straw strength, disease and insect resistance, and protein quality and quantity.
2. Lines with improved nutritional value from higher protein quantity and/or quality, even though deficient in some of the traits listed in item 1.
3. Germ plasm lines with especially valuable traits useful in breeding programs.

There was excellent phenotypic expression of the high-protein trait among varieties in the First and Second International Winter Wheat Performance Nurseries. Atlas 66, NB-67730, and Purdue 4930A6-28-2-1, all possessing similar genes for high grain protein, were consistently and significantly higher in protein than other varieties at most nursery sites. Table 2 shows comparisons of Atlas 66 and NB-67730 with Bezostaia, Lancer, and Gaines at three test sites.

The high-protein varieties maintained a protein advantage over the other varieties at sites where nursery mean protein and yield levels were high, as well as where the levels were low.

The IWWPN has identified cultivars having superior yield potential over a wide range of winter wheat growing environments. During 1969–1971, the cultivars Bezostaia, Timwin, and Blueboy were ranked highest. In 1971, NS-611 (Sava), a new entry from Yugoslavia, was ranked first (Table 3).

Cultivars with wide adaptation are used in crosses with lines possessing improved protein and/or lysine content. The IWWPN, which was conducted at 23 sites in 16 countries in 1969 and increased to 44 sites in 27 countries by 1972, provided a unique source of seed samples to determine the stability of genes for high protein and high lysine content over a wide array of environ-

TABLE 2 Phenotypic expression of the high-protein trait (1970)

Variety	Grain protein content		
	Stillwater, Oklahoma, U.S.A. (%)	Martonvasar, Hungary (%)	Cambridge, England (%)
Nursery mean protein	17.8	15.8	12.5
Bezostaia	16.5	14.3	12.3
Lancer	16.2	14.6	12.1
Gaines	16.5	14.1	10.6
Atlas 66	20.6	19.4	13.5
NB-67720	20.9	18.4	14.2
Nursery mean yield (q/ha)	25.4	32.7	31.7

Source: Proc. First Intern. Winter Wheat Conf. 1972.

TABLE 3 Highest-yielding cultivars in International Winter Wheat Performance Nurseries (1969-1971)

Cultivar	Year	No. of sites	Grain yield (q/ha)	Rank
Bezostaia	1969	16	45.2	1
	1970	31	39.5	1
	1971	26	40.4	2
Timwin	1969	16	39.9	5
	1970	31	35.4	2
	1971	26	39.8	4
Blueboy	1969	16	43.5	2
	1970	31	29.0	18[a]
	1971	26	40.3	3
NS-611 (Sava)	1971	26	41.5	1

[a] Seed with low germination.

mental conditions. Samples have been received from every nursery harvested during the past 4 years; only four nursery sites were not analyzed (for technical reasons) during this time.

CURRENT HYBRID POPULATIONS OF SPECIAL INTEREST

Atlas 66 was crossed with Nap Hal to see if the same set of genes was associated with the high-protein trait in both varieties. Also, there was interest in checking the heritability of the higher lysine content of Nap Hal. Protein and lysine data were obtained from the F_2 progeny bulk rows (F_3 and F_4 generations) grown at Yuma, Arizona. The frequency distribution for protein from the F_3 generation appears in Figure 4.

The parent varieties produced grain with similar protein content, which was considerably higher than the protein of Lerma Rojo 64 and Triumph 64 grown in the same experiments. There was distinct transgressive segregation for both high and low protein content among the F_2 progeny rows for both years, which was interpreted as evidence for different protein genes in the parent varieties. Thus, it appears possible to increase the protein content of wheat beyond the Atlas 66 level.

Nap Hal parent rows were significantly higher in lysine content than were the Atlas 66 parent rows, Lerma Rojo 64, and Triumph 64 (Figure 5). The lysine contents were adjusted to 13.5% protein with a regression equation derived from the data in Figure 3 (top). There was evidence of segregation for lysine level in the F_2 progeny rows, with apparent recovery of parental levels of lysine in some rows during both test years.

Nap Hal also was crossed to CI-13449 (a Norin 10–Brevor Sel 14 derived line), thought to be high in lysine. The protein values for the progeny fell

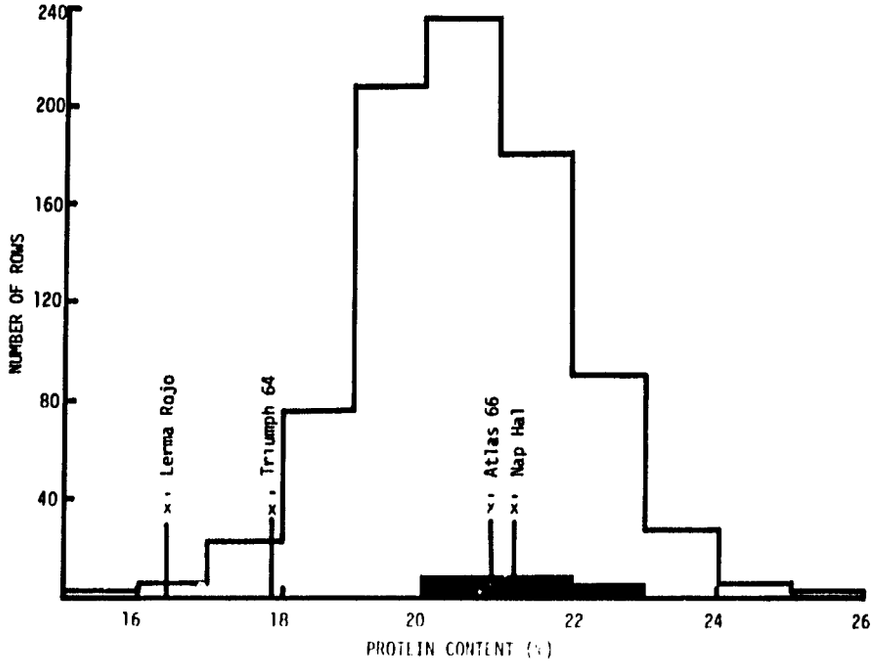

FIGURE 4 Protein frequency distribution for F_2 progeny bulk rows (F_3 generation) from Nap Hal × Atlas 66 cross (1971).

between the parental values, as expected. The lysine values for both parents were similar and higher than the normal varieties, Triumph 64 and Atlas 66. Interestingly, apparent transgressive segregation seemed to produce progeny with lysine values higher than the parents. Improving both protein and lysine content should be possible with the genetic sources identified to date.

BIOLOGICAL EVALUATIONS TO IDENTIFY IMPROVED PROTEIN SOURCES

A small animal laboratory has been established in the Department of Foods and Nutrition in cooperation with the Department of Agronomy at the University of Nebraska with the following objectives:

1. Setting up a mass testing operation for biological evaluation of various wheat materials as sources of protein.
2. Attempting to isolate and define causes of variability among different wheats in relation to protein value.
3. Laying a foundation for establishing general guidelines for predicting the value of wheat materials as sources of protein.

The results of mouse feeding trials, involving high-protein and/or high-lysine wheat varieties as a protein source, will be correlated, using data from human feeding trials with adult and adolescent subjects.

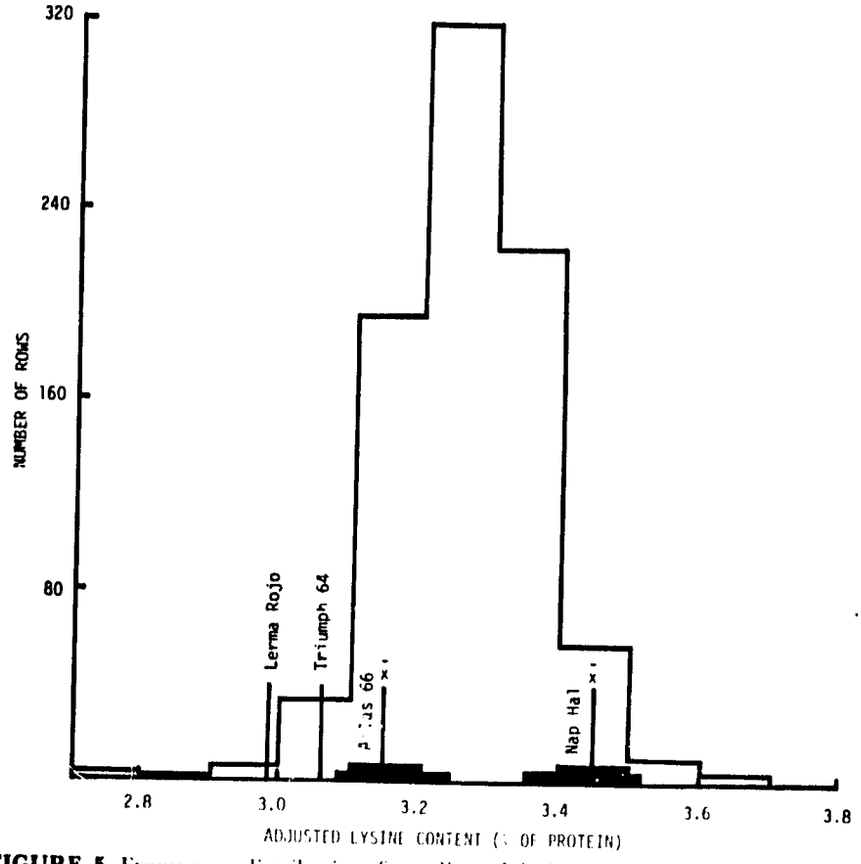

FIGURE 5 Frequency distribution for adjusted lysine among F_2 progeny bulk rows (F_3 generation) from a Nap Hal × Atlas 66 cross (1971).

Correlative studies on high-protein lines have been completed for the pepsin pancreatin digest residue (PPDR) index *in vitro* laboratory method and mouse feeding. The high-protein lines were derived from Atlas 66 and were found to have similar feeding potential.

PLANT PHYSIOLOGY STUDIES

A team approach was followed in research efforts to improve the nutritional value of wheat protein, with research in plant physiology as an integral part of this effort. The nitrate reductase step in the metabolism of nitrogen is thought to be the rate-limiting reaction for the eventual synthesis of protein. Lines and varieties are now being screened and identified for nitrate reductase activity. All high-protein lines to date have been high in nitrate reductase activity. Fortunately, a recent technical breakthrough now permits the quantitative measurement of this rate-limiting enzyme in live leaf tissue. In

addition, accurate identifications now can be made of the factors affecting the nitrate reduction process. Leaf protease levels are thought to be important and are being studied to identify varieties capable of translocating larger quantities of amino nitrogen to the grain for protein synthesis. An *in vivo* method is being used to evaluate nitrogen fertilization practices for maximum and economical increases of yield and grain protein.

[*A discussion of this paper can be found on p. 507 of* **Questions and Answers.**]

RECENT UNIVERSITY OF NEBRASKA PUBLICATIONS

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Kies, C., and H. M. Fox. 1970. Determination of the first limiting amino acid of wheat and tritacle grains for humans. Cereal Chem., 47:615–625.

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Proc. First Intern. Winter Wheat Conf., Ankara, Turkey, June 5–10. 1972. Nebraska Agr. Exp. Sta. MP-28. (See specifically Nutritional research on wheat at the University of Nebraska, V. A. Johnson, p. 110; P. J. Mattern, Analytical procedures and equipment requirements, p. 127; and C. Kies, Nutritional evaluation, p. 134.

Stroike, J. E., and V. A. Johnson. Winter wheat cultivar performance in an international array of environments. Nebraska Res. Bull. 251.

———, J. W. Schmidt, and P. J. Mattern. 1971. Results of the first international winter wheat performance nursery. Nebraska Res. Bull. 245, 84 pp.

———, J. W. Schmidt, and P. J. Mattern. 1972. Results of the second international winter wheat performance nursery. Nebraska Res. Bull. 250.

STATUS OF PROTEIN QUALITY IMPROVEMENT IN OATS

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Walter L. Clark *Hunt-Wesson Foods, Inc., Barrington, Illinois*

Of all the cereal grains, oats have the highest protein content, with overall protein quality second only to rice. Thus, from the standpoint of protein quality, it seems unfortunate that oats rank as only the world's fifth largest cereal crop (30). Wheat, rice, and maize production are each approximately 6 times larger than oat production, and barley 2.5 times as large. Commercial oat varieties grown in the United States range from 15 to 20% protein in the groats (2).

There are at least four possible genetic sources of high protein being explored by oat breeders in the United States and elsewhere. This report highlights these research efforts and discusses some of the more encouraging results in terms of improving oat protein quantity and quality.

OAT BREEDING

Research approaches to improve protein quantity and quality in oats (or combinations of them) (3, 11) include the following:

1. Recombining genes for high protein from various cultivars and common lines of *Avena sativa* and *A. byzantina* (both cultivated common oats).
2. Transferring genes for high protein from *A. sterilis* to *A. sativa* cultivars.

3. Using radiation or chemical mutagens to produce mutations for increased protein.

4. Using other *Avena* species, such as *A. strigosa*, as a source of high-protein genes.

Recently, the Agricultural Research Service of the U.S. Department of Agriculture, Beltsville, Maryland, surveyed the protein content among 3,000 entries in the world oat collection. Analyses were made of whole grain, including hulls. Protein content ranged from 7.8 to 21.9% (3, 11). In 1971, Robbins et al. (24) reported that 289 groat samples of oats contained 12.4 to 24.4% crude protein, with an average of 17.1%. These samples covered a wide range of genetic material (commercial varieties grown in the United States and Canada between 1900 and 1970, plus a few experimental lines). These data suggest that it should be possible to elevate the groat-protein percentages of future oat cultivars above the 15.0 to 18.0% of current cultivars by recombining high-protein genes now present in cultivated lines.

In a recent study at Iowa State University, Frey (11) analyzed groats from 192 random F_6-derived oat lines from a bulk population that was constructed to provide recombinations of genes for lodging resistance. The range of groat-protein percentages was from 14.6 to 32.3% (see Table 1). Commercial cultivars from the same experimental area showed groat-protein percentages from 15.8 to 19.7%.

Frey made a more extensive study (11) of 1,000 F_6 derived lines from another bulk population in which the groat-protein percentages ranged from 11.8 to 23.2%, whereas commercial cultivars averaged 18.4%. These observations of high groat-protein percentages are extremely encouraging, because (1) the high-protein experimental lines were extracted from hybrid-derived populations for which no consideration was given to crossing parents with genes for high protein percentage, and (2) some experimental lines from hybridizations among lines of *A. sativa* had protein percentages well above current commercial cultivars.

In the Robbins et al. study (24), the two groat samples highest in protein content are not considered high-protein oats. Their grain yields were about one third less than check cultivars grown in the same nursery, and thus were abnormally high in protein. In general, there is an inverse relationship between grain yield and protein content in the cereal grains. Likewise, when the fat content in *A. byzantina* oats increases, the protein level decreases (7). This relationship does not necessarily hold for *A. sativa* oats.

Briggle (2) notes that a breeding program is virtually meaningless if selections for high protein content in oat grain are made without considering total grain yield. Pounds of grain protein produced per hectare is a much more realistic measure of progress than either grain yield or protein percentage. On the other hand, protein increase per oat groat is important for the manufacturer and consumer of oat products. An ideal goal would be the development of higher-protein oats with all three factors compromised at

TABLE 1 Frequency distribution for groat-protein percentages of F_6-derived oat lines from a bulk population

Protein percentage class center	Number of lines
33	1
31	2
29	5
27	2
25	4
23	27
21	46
19	71
17	29
15	5
Mean	20

Source: Frey (11).

optimum attainable levels of grain yield, pounds of grain protein, and groat-protein content.

A few of the new varieties of oats and experimental lines now under test average from 1 to 2% higher in protein content than do other varieties compared at similar yield levels. A few test examples of these higher-protein oats are shown in Table 2. The new oat variety Dal, developed by H. L. Shands, Wisconsin Agricultural Experiment Station, and the cultivar OA123-33, developed by Vernon Burrows, Ottawa Experiment Station, are two good varieties showing higher protein obtained at commercial yield

TABLE 2 Groat protein percentages of oat cultivars

Check variety or cultivar	Protein (%)		
	1969	1970	1971
Clintland 64	19.1	17.9	18.2
Gopher	17.4	16.3	17.4
Jaycee	19.6	18.0	18.5
Lodi	18.4	16.6	17.6
Orbit	17.2	16.3	16.8
Canadian OA123-33	-	19.8	-
Illinois 66-2287A		19.2	19.7
Illinois 68-1718	-	-	19.1
Wisconsin X 1289-3, DAL		19.1	19.5
Wisconsin X 1656-1		-	20.0

Note: Amino acid analyses according to Eaker (4).

levels. These recent accomplishments lend a great deal of encouragement to breeders attempting to develop higher-protein oats.

Transfer of genes for high groat protein from *A. sterilis* to commercially acceptable cultivars appears to be relatively easy, since this wild species and cultivated types cross readily (both types have the same number of chromosomes). *Avena sterilis* is a hexaploid oat that grows wild in the Mediterranean area. Approximately 5,000 collections of this species are in the U.S. Department of Agriculture World Oat Collection (3); they were collected originally because of outstanding disease resistance. In *A. sterilis* collections obtained from Israel, groats contained from 15 to 30% protein (2); some samples analyzed exceeded 30% protein.

In 1965, two of the high-protein *A. sterilis* collections were crossed with good agronomic oat varieties. Progenies of these crosses were distributed to oat breeders throughout the United States by the U.S. Department of Agriculture. However, the direct transfer of high protein content proved difficult. In the Beltsville program, selection pressure was applied on *A. sativa* × *A. sterilis* derivatives according to two criteria: (1) high protein content, and (2) agronomic and kernel type (2). When progenies were selected on the basis of protein content only, the high-protein lines resembled the wild parent in seed traits (high hull percentage, awness, pubescence, and slenderness). When progenies were selected for agronomic type, they were either low or moderate in protein content. In general, there was a negative relationship between grain yield and protein content among the *A. sativa* × *A. sterilis* progenies. It was noted, also, that high protein progenies tended to have slender and long kernels. Those selections desirable for milling (with shorter, plump kernels) were lower in protein. These observations allow speculations that a high bran-to-endosperm ratio occurs in *A. sterilis*, perhaps explaining its high protein content. If this is true, it does not represent a genetic source of high protein.

On the other hand, Campbell and Frey (4) found a genetic correlation of 0.07 between groat weight and groat protein content for 10 *A. sterilis* × *A. sativa* crosses, and they were able to select a sizable number of F_2 derived lines having high groat protein and *A. sativa* seed traits. In a second study, Campbell and Frey (5) investigated the groat protein percentage among F_2-derived lines of *A. sterilis* × *A. sativa* and found that the inheritance seemed to be relatively simple (few loci or blocks of loci seemed to account for the segregation patterns). In several crosses, the parental percentages were recovered with as few as 35 to 45 lines tested within crosses.

Since the early work with *A. sterilis* as a source of high protein content involved only a very few individual collections used as parents, this source has by no means been abandoned. Breeders are taking a second look, as demonstrated by Campbell and Frey (4, 5). Perhaps *A. sterilis* collections to be used as potential parents should first be selected for protein content and yield, as well as for kernel configuration.

Frey (11) investigated the protein percentages of mutagen-derived lines of oats, basing his work on findings from mutagen-derived rice studies by Tanaka and Takagi (27). He employed 11 oat cultivars and tested equal numbers of mutagen-derived and check lines in each. For each cultivar, the mutagen-derived population contained lines with protein percentages higher than those of the best check lines.

Data from one of these cultivars show the variation induced (Table 3). For Clintland cultivar, three mutagen-derived lines had groat-protein percentages exceeding those of the best check line. No mutagen-derived line was lower in percentage than the lowest check line. The three superior lines (numbers 27, 28, and 49) from the mutagen-derived population had 21.1, 23.9, and 22.1% groat protein, respectively. Line 49 had a low grain yield, but lines 28 and 29 yielded better than the check mean. A second cultivar, Burnett, a superior line (number 20) from the mutagen-derived population, had 19.9% groat protein and yielded as well as the check mean.

No amino acid analyses have yet been made on the mutagen-derived lines with high groat protein; however, these initial studies do suggest that the grain-protein content of oats can be elevated significantly via mutagen treatment.

Other *Avena* species have been used as a source of high-protein genes, and Burrows was mentioned previously as the developer of the Canadian cultivar OA123-33 (Table 2). This cultivar, just released for commercial development, is based on *A. strigosa*. It has considerable promise, with a groat-protein content of 20% or more and acceptable yield levels.

Another approach that undoubtedly will contribute toward oat quality improvement makes use of the electron microscope and the light microscope (25) to obtain information on the fine structures and time of develop-

TABLE 3 Frequency distributions and means of groat protein percentages for oat lines in mutagen-derived and check populations from Clintland cultivar

Protein percentage class center	Number of lines	
	Mutagen-derived	Check
17	–	6
18	19	22
19	46	43
20	16	13
21	2	1
22	1	–
23	–	–
24	1	–
Mean (protein percentage)	19.2	18.8
Correlation[a]	0.00	-0.09

Source: Frey (3).

[a] Correlations between groat protein percentages and grain yields.

ment of protein bodies in the oat seed (23, 25). Sraon (25) has observed small protein granules appearing in aleurone cell vacuoles 12 days after pollination. It can be presumed that the high-protein strains of oats have more, or larger, protein bodies per seed. If this is true, the protein content of a single seed may be determined with the aid of the electron microscope. Sraon suggests screening the segregating generation by testing individual seeds and saving the embryo portion. This portion, then, can be germinated after detecting the desirable recombinant. The South Dakota Experiment Station plans to initiate a rigorous study of geometric relationships of proteins with other components of the kernel, along with quantitative genetics investigations (26).

Youngs (32) has recently investigated the protein distribution in the oat kernel of seven varieties. He reported that the bran and endosperm contributed the largest amount of protein to the groat. As the total protein of the groat increased, the bran weight and thickness increased; conversely, the weight of the endosperm decreased. Yet the protein concentration of both the bran and endosperm increased. Since hand dissection was used to obtain the four oat fractions, the data should be interpreted with some caution.

The implications of a thicker-bran, higher-protein oat probably are not serious when the entire groat is to be used commercially, such as in some breakfast cereals. There remains the question of possible adverse effects on flavor, color, or texture. However, when oat flour is the prime consideration, Youngs's study (32) suggests that the flour yield is likely to be lower with a high-protein variety. It is encouraging, however, that an increase in endosperm protein accompanies an increase in total groat protein; other food uses may be found for this high-protein flour.

RELATIONSHIP BETWEEN GRAIN YIELDS AND PROTEIN PERCENTAGES

Frey (11) notes that, traditionally, cereal grains have been grown and used as efficient producers of concentrated sources of energy feeds; and the literature repeatedly indicates that grain yields and protein contents in the grains almost always are negatively related. These kinds of findings led to the acceptance of a *universal nitrogen constant* or a ceiling on nitrogen absorption of 318 kg/ha.

Black and Kempthorne (1) have shown this constant to be in error. Furthermore, White and Black (31) showed that plants in pot cultures, could absorb much more nitrogen than the equivalent of 318 kg/ha. These findings suggested that the accepted universal negative relationship between grain yields and grain protein percentages could be attributed to inadequate soil nitrogen in most experiments. The implication is that the reported negative correlations are phenotypically real, but not genotypic in origin.

Two reports from the wheat literature are pertinent to the preceding

findings. Middleton et al. (19) reported that wheat cultivars Atlas 50 and Atlas 66 were equally as productive as traditional cultivars in extensive tests over 3 years and many sites. Yet these two cultivars exhibited from 0.9 to 3.2% more protein in their grain. Johnson et al. (17), using Atlas 66 as a source of genes for high protein, have isolated several "second-cycle" wheat lines that are hard red winter types and combine high grain yields with a 2.5% increase in protein content.

Frey (11) has tested oat cultivars and lines in field experiments where soil nitrogen varied between extremely deficient and adequate levels for plant growth. In these experiments, the correlations between grain yields and grain protein percentages were −0.24 and +0.04, respectively (Table 4). At the Iowa Experiment Station, 80 kg/ha of available nitrogen remained after the crop was mature. The data from oats indicate that nitrogen fertilization rates commonly used for small grains might need to be increased, if the genetic potentials for high yields and high protein percentage in new cultivars are to be exploited. Additional cultural research on oats is needed.

NUTRITIONAL QUALITY OF OAT PROTEIN

Selecting oat varieties for their biological protein value has received little attention, until recently, since most developmental work has concentrated on practical matters such as yield, insect and disease resistance, and ease of harvesting with mechanical reapers. However, some oat types apparently have the unusual property of retaining the biological value of the protein as the selection processes increase the total protein content. Clark and Potter (7) reviewed the composition and nutritional properties of protein in selected oat varieties. They noted that the oat has not been given much research consideration for its protein contribution, an observation supported by the lack of research papers reporting differences in the nutritional quality of proteins of different oat varieties. Most nutritional studies on oat protein have utilized commercially produced rolled oats. Rolled oats are

TABLE 4 Ranges of grain yields and groat-protein percentages and correlations between these two traits for 60 oat lines and cultivars when grown under conditions of deficient and adequate soil nitrogen

Soil-nitrogen condition	Range of protein percentages	Range of grain yields	Correlation
Deficient	15.0-20.0	10-21	-0.24[a]
Adequate	16.0-20.6	11-42	+0.04

Source: Frey (11).
[a] Significant difference at $p = 0.05$.

produced from blends of oats as received by the oat processors from grain market storage facilities, which makes it impossible to state what impact any specific variety might have on the overall nutritional quality of the oat product.

Hischke et al. (12) attempted to correlate the amino acid content of groats of seven oat varieties of known purity, grown at the same location. In rat growth feeding trials, the animals were fed diets containing 60% oats by weight. The lysine content of the oats ranged from 83 to 88% of that of the FAO provisional standard (14), and threonine ranged from 103 to 106% of the FAO figure. The data on methionine and cystine content could be evaluated with caution, since the results were obtained from acid destruction of sulfur-containing amino acids, and no attempt was made to compare such data with the FAO standards. Nevertheless, all seven cultivars had similar protein efficiency ratios (PER) of 2.3 to 2.4 (grams gain in body weight per gram of protein intake). Thus, these animals' PER values did not reflect the differences in amino acid composition that had been found by chemical analysis. This finding further substantiates the conclusions of Weber et al. (29), which are that differences of biological availability of specific amino acids, such as threonine, may be of more importance than the amino acid content of groat protein as found by chemical analysis.

Another basic difficulty in breeding oat varieties for improved protein quality is the relatively large amount of genetic material needed to perform the usual animal-feeding studies (such as tests for PER). Only a few grams of seed are recovered in the original hybridization step, whereas 3 to 4 kg are needed for 28-day feeding trials; thus, it is little wonder that the potential nutritional quality of new varieties has been ignored up to now.

Limited bioassays of oat cultivars, experimental lines, and derivatives of *A. sativa* × *A. sterilis* crosses have been conducted by Elliott and Marcarian (8), using weanling voles as the testing organism. The lesser amount of test material required to feed these animals, plus trial lengths of only 6 days, reduced the total test material requirements substantially. Isonitrogenous diets at 7% protein were fed for 6 days. The low level of protein in the diet presumably put the animals under protein stress, so that protein quality could be assessed. These vole PER's were compared with those for casein. Some oats in these tests had higher PER's than those for casein, but results were variable. Data obtained to date are considered preliminary, and much more research is needed.

The PER for cereal proteins varies with the crop. Wheat, rice, maize, and sorghum have the poorest-quality protein (Table 5) (13, 15, 18, 20). Oats are consistently higher in protein quality, followed by rye and barley. (It should be noted that oats are not degerminated in processing, as are maize and wheat, and thus the nutritional quality of the oat remains intact.)

Frey (11) recently noted that the Mossé (21) investigations show that the PER's of the proteins from different cereals appear to have a direct relationship to the proportion of prolamin, the alcohol-soluble protein fraction

TABLE 5 Protein efficiency ratios (PER) of cereal proteins in diets of rats

Protein source	Protein percentage in diet				
	5.0[a]	7.5[b]	9.5[b]	9.0-10.0[c]	8.0-10.0[d]
Oats		2.3	2.5	1.8	2.2
Rye		2.2	1.8	1.8	
Barley		1.7		1.6	2.0
Corn		1.6		1.4	
Wheat		1.4	1.7	0.9	1.7
Sorghum				0.7	–
Rice	1.7				–

[a] Houston and Kohler (13).
[b] Jones et al. (18).
[c] Howe et al. (15).
[d] Morkuze (20).

contained by the protein (Table 6). Frey (10) reported much earlier that the avenin of groat protein did not increase with an increase in overall protein content, which contrasts with findings for maize zein. Zein proteins and other prolamins are severely deficient in lysine as the major limiting amino acid (21). Frey (10) found that oat cultivars with a protein range from 9.3 to 15.8% all had avenin:protein ratios of 0.18 to 0.19.

Oat flour contains higher quantities of lysine than any other cereal, as reported by Ewart and shown in Table 7 (9, 13, 14). The percentages of methionine have been found to be higher in rice and maize; the threonine percentage is highest in maize. From a quality standpoint, rice protein is slightly above oats. However, if measures of quantity and quality are combined, as shown in Table 8, oats exhibit an overall higher essential amino acid content per 100 g of edible flour when compared with any other cereal grain flour; wheat is next, followed closely by barley and rye. Rice and maize exhibit substantially lesser amounts of these amino acids when compared on this basis.

TABLE 6 Percentages of prolamin fractions in grain proteins of various cereals

Crop	Prolamin	
	Name	Protein (%)
Oats	Avenin	12
Rye	Secalin	40
Barley	Hordein	40
Corn	Zein	59
Wheat	Gliadin	45
Sorghum	Kafarin	60

Source: Mossé (21).

TABLE 7 Percentages of the essential amino acids in flour proteins from cereals

	Percentage in protein of[a]						
Amino acid	Rice[b] (polished)	Oats	Corn	Barley	Rye	Wheat	FAO[c]
Isoleucine	4.6	3.8	3.6	3.6	3.6	3.6	4.2
Leucine	8.0	7.7	11.6	7.2	6.7	6.[illegible]	4.8
Lysine	3.5	4.5	3.5	3.1	3.2	2.0	4.2
Methionine	2.9	1.8	2.0	1.7	1.7	1.8	2.2
Phenylalanine	5.2	5.2	4.9	5.5	4.9	5.1	2.8
Threonine	3.5	3.7	3.9	3.3	3.4	2.7	2.8
Tryptophan	1.3	2.0	0.9	2.0	1.8	1.1	1.4
Tyrosine	4.9	2.6	2.3	2.7	2.1	2.6	2.8
Valine	6.5	5.0	4.9	4.6	4.4	3.7	4.2

[a] Ewart (9).
[b] Houston and Kohler (13).
[c] Howe (14).

TABLE 8 Amino acid content of cereal flours

	Grams/100 edible product					
Amino acid	Oats	Wheat	Barley[a]	Rye[b]	Rice	Corn
Cystine	0.31	0.29	0.28	0.23	0.10	0.10
Isoleucine	0.73	0.58	0.55	0.49	0.36	0.36
Leucine	1.07	0.89	0.89	0.77	0.66	1.01
Lysine	0.52	0.37	0.43	0.47	0.30	0.26
Methionine	0.21	0.20	0.18	0.18	0.14	0.15
Phenylalanine	0.76	0.66	0.66	0.54	0.38	0.35
Threonine	0.47	0.38	0.43	0.42	0.30	0.31
Tryptophan	0.18	0.16	0.16	0.13	0.08	0.05
Tyrosine	0.52	0.50	0.47	0.37	0.35	0.48
Valine	0.86	0.62	0.64	0.59	0.53	0.40

Source: Adapted from Orr and Watt (22).
[a] Grain.
[b] Medium flour.

The amino acid composition of oats is remarkably constant over a wide range of protein content. Recent analyses on lines from *A. sterilis*, the wild oat from Israel, confirm this point (Table 9). In addition, Robbins et al. (24) found only slight correlation between grain protein percentage and lysine percentage in the protein. Of the 289 oat samples surveyed, the average lysine content was 4.2% of total protein. The maximum lysine content was 5.2% and the minimum was 3.2%. This range indicates sufficient variability in the lysine content of oats to expect breeding improvement if at least a major part of that variability is genetic. If most of this variability is caused by environment, improvement through breeding could be difficult.

Average threonine content in the study by Robbins et al. (24) was 3.3% of protein; the maximum was 3.5% and the minimum was 3.0%. The range of

TABLE 9 Percentages of protein and amino acids in grains of *Avena sterilis* lines

Component	Line number (%)		
	1	2	3
Protein[a]	17.0	25.4	21.7
Lysine[b]	4.0	3.9	4.1
Threonine	3.3	3.4	3.4
Cystine	1.8	1.7	1.6
Valine	5.7	5.7	5.9
Leucine	7.8	7.8	7.9
Tyrosine	3.3	3.4	3.4
Phenylalanine	5.5	5.5	5.7

Source: Unpublished data, Quaker Oats Company, 1971.

[a] Percentage of dry weight

[b] Amino acids, percentage of protein

threonine content was limited. The average for methionine content was 2.5%, with a maximum of 3.3% and a minimum of 1.0%. Again, the range was limited.

Thus, correlations between protein and threonine and methionine were highly negative. The oat breeder should pay strict attention to not only protein content and the percentage of lysine, but also to the maintenance of a satisfactory level of the second and third limiting amino acids. In fact, levels of threonine and methionine could be more critical than lysine. The rather low coefficient of variation of the threonine levels indicates that a marked increase in the level of threonine would be difficult to attain through breeding (24). Genetic variability within a population is a prerequisite for progress through selection. Hopefully, *Avena* germ plasm other than that represented in the Robbins et al. study (24) can provide additional opportunities for threonine increases.

Lysine may not be a major concern in chemical analyses, as a direct measure of availability to the ingesting organism. However, chemical analyses of oat protein indicate that threonine is not markedly deficient; but biological data indicate otherwise. For example, Lang et al. (28) have reported that threonine from oat protein was not available to experimental rats, whereas lysine and methionine were available.

Because oat protein has a good biological value relative to other cereals, and because its amino acid composition is quite constant over a wide range of protein percentages, plant breeders now are cooperating in a special experimental design to measure the genetic and environmental variability of protein, oil content, and specific amino acids in oats (4).

An Oat Quality Laboratory was established at the University of Wisconsin in 1970 to assist in this effort, supported jointly by the Agricultural Research

Service, U.S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station. Several thousand grain samples are analyzed for protein content each year at the laboratory. A limited number of samples also are analyzed for amino acid profile, oil content, or other quality criteria. In addition to those services for U.S. oat breeders, the laboratory staff members conduct basic research studies on oat grain quality, and on the physiology of the oat plant, particularly as related to protein synthesis.

SUMMARY

In conclusion, available data on grain protein for oats suggest the following:

1. There appear to be four sources of genes for increasing groat-protein percentage.
2. Oat protein has a high biological value relative to other cereals.
3. The amino acid composition of groat protein is quite constant for all levels of protein percentage. Although some research efforts are devoted to searching for variable amino acid composition in oat protein and to laboratory animal feeding tests with grain from different oat cultivars, the major effort is simply to increase the groat-protein content.

Frey (11) predicts that by 1980 there will be commercially acceptable oat cultivars with 22 to 24% protein content. Hopefully, continued research will lead the way.

We are indebted to L. W. Briggle for information and comments, to V. Burrows, to K. J. Frey for access to and use of his manuscript "Improvement of quality and quantity of cereal grain protein" (11), and to H. L. Shands.

[*A discussion of this paper can be found on p. 508 of* **Questions and Answers.**]

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STATUS OF PROTEIN QUALITY IN TRITICALES

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Triticales are amphiploids of hybrids between wheat species and rye species. Hexaploid triticales originating from crosses between durum wheats and diploid ryes have shown greater promise of developing into a productive crop than the octaploid types obtained from crosses between the bread wheats and rye.

A full-fledged triticale improvement program was begun at CIMMYT in 1967, through Rockefeller Foundation sponsored cooperative arrangement with the University of Manitoba.

In 1972, the Canadian government funded the triticale project through the joint cooperation of the Canadian International Development Agency (CIDA) and the International Research Council (IRC).

EVALUATION OF NUTRITIONAL QUALITY

In past research, the quality evaluations of cereal grains have emphasized physical properties, rather than nutritional-quality characteristics. However, recent years have brought a greater awareness of the need for more plentiful production, as well as more nutritious foods. Discoveries of strains of maize, barley, and other crops having higher levels of essential amino acids have shown the differences in nutritional quality that can occur among strains of crop varieties. Discoveries of growth-inhibiting substances in grain and other plant parts have indicated the need to consider both positive and

negative factors in working with nutritional quality. Increasing the proportion of essential nutrients is not a sufficient answer; these nutrients must be readily available to the biological system, and the effects of the growth-depressant compounds must be minimized.

When CIMMYT began its evaluations of nutritional quality in triticales, the meager amount of information that had been published on the subject was discouraging. In the results of early feeding trials with triticales, some of the findings were decreased feed consumption, lower weight gains, increased liver abcesses, and damaged ruminal epthelium. (It seems probable that ergot could have been responsible for some of the bad results.)

However, one of the most encouraging early reports claimed that, on a pound-for-pound basis, triticales could replace other cereal grains as a poultry feed. Since the early triticales were only about 2% higher in protein content than the cereals they were replacing, the report could not be considered as a strong indication of high protein quality in triticales.

EARLY CIMMYT FINDINGS

Soon after the CIMMYT triticale program began, E. Villegas noted that triticales have a wide range in protein content, and vary considerably in the content of two essential amino acids, lysine and tryptophan. The grain protein content ranges from 10 to above 20%, and the differences in total lysine varied up to 100% among strains grown in the same location.

Among early problems encountered in producing higher-protein triticales were (1) all screening had to be done on a chemical basis, (2) a positive correlation between degree of seed shriveling and protein content, and (3) a negative correlation between protein content and the percentage of lysine in the protein.

Because the concept of breeding for nutritional quality in cereals is relatively new, there are few techniques for screening large numbers of lines for nutritional quality, even in a preliminary or predictive way. Maize breeders are very fortunate in that maize has visible morphological characteristics associated with nutritional-quality characteristics. These characteristics provide a useful screen for better quality before chemical and nutritional evaluations are made. No such associations are, as yet, available for evaluating other cereals.

Fred Elliott of Michigan State University was immensely helpful in working with the screening problems. He noted that protein content and amino acid composition could be measured readily in the chemical laboratory. However, the nutritional availability and the occurrence of various antimetabolites were more difficult to measure, particularly since the nature of these compounds was unknown. Elliott offered to run bioassays on a few of the "best" triticales. He had been experimenting with meadow voles for

several years and had observed that the weanlings had a predictable linear growth response for 7 to 10 days. The vole protein requirements were sufficiently low that satisfactory growth could be achieved with the protein provided by a single grain source. Voles also are very sensitive to growth-depressing factors.

The first results from the bioassays were disappointing, although triticale lines had been chosen that were considered to be the best combination of grain type, yield, protein content, and percentage of lysine. Elliott was undiscouraged, however, and suggested that we send him a larger number of strains for analysis. He later ran bioassays for protein efficiency on 191 strains that had been chosen for his use from a group of almost 300 strains (discards were made of strains that were badly shriveled, very low in protein content, or very low in lysine percentage).

With these strains, he obtained a vole preliminary index (VPI) with average values (from three animals) ranging from 0.07 to 3.5. About 17 values were below 1.0 VPI and 20 of the VPI values were equal to or greater than the values obtained for casein. It was assumed that the low average values were due to low palatability, low values or low availabilities for essential amino acids or other nutrients, or to the presence of antimetabolic compounds; these lines were discarded.

Lines with values equal to or greater than casein were considered desirable and were maintained. Little association was noted between protein efficiency values and the percentage of lysine in the protein or total protein content.

Using the same strains, Villegas investigated the possibility that resorcinols might be one of the antimetabolites in triticales. Resorcinols had been found in rye and were reported to cause growth depression in pigs and rats. Villegas found that the triticale strains had a resorcinol content generally closer to wheat than to rye, and that most of the strains showing low protein efficiency ratios (PER) from vole analysis also had low resorcinol values.

Our program also benefited from the research of nutritionists working with other nonruminant animals. James McGinnis of Washington State University (WSU), working with poultry, had demonstrated that rye, beans, and soybean meal contained growth-inhibiting substances. He offered to run nutritional trials with chicks and laying hens to help analyze the protein quality of our triticale strains.

McGinnis summarized his research on the nutritional quality of triticales, rye, and wheat in a report prepared early in 1972; some of the pertinent conclusions were:

1. Test diets for chicks used in evaluating the proteins of cereal grains must be supplemented with protein or amino acids from other sources, if meaningful results are to be obtained.

2. Among test diets containing 14% protein, 6% of which was derived from a premix and the remainder from the grains to be tested, soybean meal supported significantly better chick growth than any of the cereal grains.

3. No significant differences in chick growth were found among the first five triticale samples from northwestern Mexico, which had been selected on the basis of their performance when fed to voles. The PER values of these five samples were not significantly different from the PER for soybean meal. However, soybean meal supported significantly better growth than the triticale strains.

4. In a trial in which 13 other triticale selections were assayed, some of the triticales gave much better chick growth than did wheat or rye. Some of the triticales' performances were not improved by a penicillin supplement; those unimproved by penicillin were generally higher in PER values than those that showed responses to penicillin supplementation.

5. Significant differences in chick growth were found among 35 different strains of triticales grown in the state of Washington. These differences occurred even when the basal diet was modified to contain a penicillin supplement and lysine, in addition to methionine.

6. One variety of triticale (Trailblazer) grown in Washington was formulated into a practical broiler diet containing meat and bone meal, fish meal, and soybean meal. This diet proved deficient in lysine.

7. When Trailblazer triticale was assayed by the evaluation procedures developed for the other triticales, it was shown to support only slightly more than half the growth rate produced by soybean meal (when both materials supplied 8% of the protein for the diet). Adding lysine and threonine to the Trailblazer diet improved the growth rate significantly.

8. In diets for laying hens, replacing other cereals with rye markedly decreased egg production.

9. In an experiment conducted by Eduardo Rivera (Fomento Avicola de Pacífico S.A.), triticales (strain PM-132) provided egg production comparable to a control diet when replacing all the milo and part of the protein concentrates, such as fish meal, soybean meal, and safflower meal (low levels of fish and soybean meal remained in the diet).

10. In WSU experiments, Trailblazer supported egg production equal to control diets when it replaced all the cereal grain on a pound-for-pound basis. When the protein level was kept constant, however, and other protein concentrates were removed, egg production decreased significantly. Supplementary lysine failed to improve egg production, indicating a deficiency or low availability for some of the amino acids in this strain.

VOLE USE FOR SCREENING

Although the vole appears to have some characteristics which make it useful as a preliminary screen for the availability of nutrients, presence of antimetabolites, and so on, there are several serious questions or objections that have been raised concerning the use of the vole for this purpose:

1. There is little or no association between lysine levels in the protein of triticales and preliminary protein efficiency values obtained from voles.

2. The voles represent a heterogeneous population, providing a wide range of values among animals when fed certain diets.

3. Apparently, the meadow vole can achieve a satisfactory growth response with the levels of lysine that occur in most triticales. We know that lysine is an essential amino acid, which is limiting in cereal grains consumed by chicks, humans, and swine. However, since the lysine content of grain samples can be measured very easily in the laboratory, these data must be obtained to supplement the bioassay data from the voles, if this animal is to be used in screening for nutritional quality.

4. The variability in reaction of different animals is considered by many investigators to be the most serious deterrent to the use of voles. This problem has been encountered with rats and other test animals, also. Experimenters using rats and mice have shown that the best way to reduce variation is to use homozygous strains of animals. For some purposes this is suitable. However, there is a danger in interpreting data from a single homozygous strain to apply to whole populations. There is no more justification in assuming that the values obtained from an inbred line of test animals represent the whole population than the performance of a single inbred line of triticales represents triticales generally.

FUTURE CONSIDERATIONS

The plant breeder is continually faced with the problem of how to measure performance and increase the range of adaptation. He has to live with variability of data. He has learned to accept the fact that, even under the most ideal testing conditions he will obtain variability among locations and years.

Strains of triticales show a wide range in protein content and amino acid content. The evaluations made with voles, chicks, laying hens, and rats have indicated that there are significant differences in the nutritional quality of the protein among triticale strains, regardless of the level of protein occurring in the grain.

Is nutritional quality important enough to put our efforts into its improvement? If so, how can we make progress most rapidly and efficiently? Each year in the triticale program about 50,000 strains are grown in small plots. These strains are screened for plant type, fertility, seed type, disease resistance, tillering, and other agronomic characteristics. Perhaps 1,500 advance to the preliminary yield test each year, and about 300 make it to the regular advanced tests. Until this point in the process, nothing has been done to select for nutritional quality, even in the crudest way.

Using small samples (5 g), Villegas has been able to make chemical analysis of the percentage of protein, percentage of lysine, and resorcinol content on 500 to 1,000 lines per year. With 100-g samples, Elliott and R. Bauer made 200 to 300 bioassays with voles in 1972. Chicks require about 5 kg of seed for

a reliable evaluation, and a laying-hen trial requires about 300 to 500 kg, depending upon how many birds are used and how long the experiment is run.

The greatest possibility for progress appears to lie in screening the observation lines that are sufficiently homozygous to be considered for the preliminary yield test. This constitutes a group of 1,000 to 1,500 lines per year, which can provide up to 500 g of seed above the normal requirements for further field tests and reserve.

COMPARISON OF HIGH-LYSINE GENES AND MUTANTS IN BARLEY AND MAIZE

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Barley and maize are the only cereals that have been shown to have Mendelian, simply inherited major characters for changed amino acid composition of endosperm. Comparing these two very different cereals on the basis of genetic variation in amino acid composition could provide support for a breeding strategy to improve nutritional quality in all cereals, including wheat, triticale, and sorghum.

Genes strongly associated with morphological endosperm character have been discovered for both maize and barley. Although the high-quality-protein maize genotypes seem to have been selected mainly by amino acid analyses among a limited number of mutants displaying modified starch

performance, the five improved barley genotypes were selected by specially devised screening methods (Table 1).

The dye-binding (DBC) method (16) was used to select the first high-lysine barley cultivar, Hiproly (CI-3947), from the world barley collection (7, 22). A recessive gene (lys) located in the seventh chromosome was found in Hiproly (12, 20). The gene increases the lysine grams/16 g N level in endosperm about 30%. The Riso group in Denmark (2, 11) also has shown the efficiency of the DBC method for screening for basic amino acids (lysine). Six high-DBC mutants were found at Riso in mutagen-treated barley material consisting of about 10,000 analyzed offspring lines (3). Two of these mutants (mutants 29 and 86) contain about 15% more lysine grams/16 g N than the parent variety (Carlsberg II) (see Table 1). A recently discovered mutant (mutant 1508) from the Bomi variety is an important breakthrough, showing lysine content increase of about 45% (5.9 g/16 g N) in the preliminary analyses. To our knowledge, this is the highest lysine level reported for a cereal seed.

In barley, and most other cereals, lysine and other essential amino acids (grams/16 g N) are negatively correlated with crude protein and with the amide content of protein; the latter relationship was used by Toft-Viuf (27) in screening for lysine content. Toft-Viuf found another high-lysine barley cultivar (CI-7115) displaying a lysine increase of about 15%. Lysine is relatively constant in CI-7115 and Hiproly, at various levels of nitrogen fertilizer. Among the normal controls, lysine shows a strongly negative correlation with crude protein content owing either to variable genetic material or to environmental effects (21). CI-7115 also is superior to normal lines when DBC values are compared at the same crude protein levels.

The lysine-rich embryo of barley is small (3.5 to 4.5% of the whole seed) compared to the large maize embryo (10 to 20%). The maize embryo makes a much greater contribution to the lysine content of the seed than does the barley embryo. Thus, in cereals, genes that influence endosperm amino acid composition should be compared on an endosperm basis with the embryo removed (17, 19).

INCREASING HIGH-QUALITY PROTEIN PRODUCTION PER HECTARE

Nelson (23) reported that high crude protein percentage in maize seed meal does not appear to be a relevant parameter for selection in plant breeding, because there will be a tendency to accumulate lines displaying a high protein content because of poor starch synthesis. Hiproly (CI-3947) produced very low yields, yielding one third of commercial Swedish varieties. Hiproly seeds were shriveled and displayed lower 1,000-kernel weight and yield capacity compared to a presumably isogenic "sisterline" (CI-4362) with identical plant phenotype. However, by changing the gene background of the lys gene from Hiproly to high-yielding varieties, the poor

TABLE 1 Summary of present high-lysine genes and mutants in barley

Barley	Gene symbol	Lysine[a] (g/16 g N)	Improvement[b] of lysine content in protein (%)	Reference
Hiproly	*lys*	4.0-4.3	20-35	Hagberg and Karlsson (7) Munck et al. (22)
CI-7115 (468)		3.6-4.1	10-20	Toft-Viuef (27), (28)
Mutant 29		4.2	16	Doll (2)
Mutant 86		4.1	14	Doll (2)
Mutant 1508		5.9	45	Ingversen et al. (11)

[a] Normal barley lysine grams/16 g N varies from approximately 2.8 to 4.1 g at crude protein levels from 16 to 8%, respectively.

[b] Approximation referred to the improvement of an "isogenic" line with the same level of crude protein as the high-lysine mutant.

seed type could be modified without losing the protein quality expression of the gene (8).

The yield of the best F_4 line in field trials was about 70% of the standard variety (Ingrid). Of 128 high-lysine lines backcrossed to the commercial variety Mona, 12 lines yielded more than 90% of Mona's plot yields, 6 yielded more than 96%, and 2 yielded more than 100%. The best high-lysine lines are superior to Mona in lysine yield per hectare by about 40%. In the mutation barley material discussed by Doll (2), seed of several lines showed increased content of crude protein percent. However, all these lines were inferior to the parent variety in production of seed and protein. When the seed sizes of the two high-lysine mutants were compared with the mother variety, mutant 29 was slightly lower and mutant 86 was distinctly lower in seed size. Mutant 29 was higher in lysine yield compared to the control, whereas mutant 86 was inferior. The extremely high lysine mutant 1508 yields about 90% of parent variety Bomi.

Seed size problems also are prevalent in opaque-2 breeding. However, there were no negative relationships between lysine and protein levels and yield in crosses reported by Sreeramulu and Bauman (26), indicating good possibilities for obtaining opaque-2 lines with high productivity.

Mutants with depressed yield characteristics but with favorable quality characteristics should be worth considering in breeding programs. Yield improvements for such lines might be difficult to achieve, but the plant breeder appears to have enough evidence to support such an endeavor.

SEED MORPHOLOGY AND APPEARANCE IN RELATION TO AMINO ACID COMPOSITION

The great advantage of the association of a morphological seed character with a change in amino acids lies in the simplified plant-breeding techniques that make chemical screening analyses unnecessary. However, the soft character of opaque-2 endosperm, for example, results in a higher water content and in a greater tendency for the kernel to crack. Problems can occur in harvesting and storage, and consumer acceptability can be affected by the milky appearance of the opaque-2 and floury-2 seeds. To offset such problems, chemical screening in crossbred populations (25, 28) has resulted in obtaining almost normal appearing kernels that retain the protein quality of typically opaque-2 kernels.

The lys gene in barley, in contrast to the opaque-2 gene in maize, is associated with a hard-endosperm character. When the dry endosperm is cut transversely and scanned by electron microscope (Figure 1), the lys barley is shown to hold starch grains and protein bodies firmly attached to the matrix proteins. Normal-lysine-barley scannings (Figure 2) show that small and large starch grains (as well as protein bodies) have fallen off the

FIGURE 1 Scanning electron micrograph from the central endosperm of high-lysine barley (Hiproly) × 1 400. Preparation from a dry, ripe seed.

adherence character to the lys gene in barley. Of 235 F_4 lines emerging from matrix proteins, leaving deep holes (19). The starch protein adherence trait also can be studied in the light microscope by analysis of meal-scrapings scratched with a dissecting needle (17, 19, 20).

A pedigree material (F_2–F_4) from crosses between Hiproly × normal-lysine varieties was screened to study the association of the starch–protein homozygous high-lysine F_2 plants. 17 deviates showed a low degree of starch–protein adherence. The deviates were identical in amino acid composition to 15 related high-lysine controls that displayed a strong adherence trait. However, in the hard endosperm control lines, the negative correlation usually shown between most essential amino acids (grams 16 g N) and crude protein was very low and insignificant. In sharp contrast, the soft 17 deviates in morphology displayed a marked negative correlation between crude protein and essential amino acids in protein. Thus, it seems possible to use a morphological trait to select for changed responses in the relationship between protein and the essential amino acids.

As with opaque-2 maize, it is thus possible with lys barley to change the

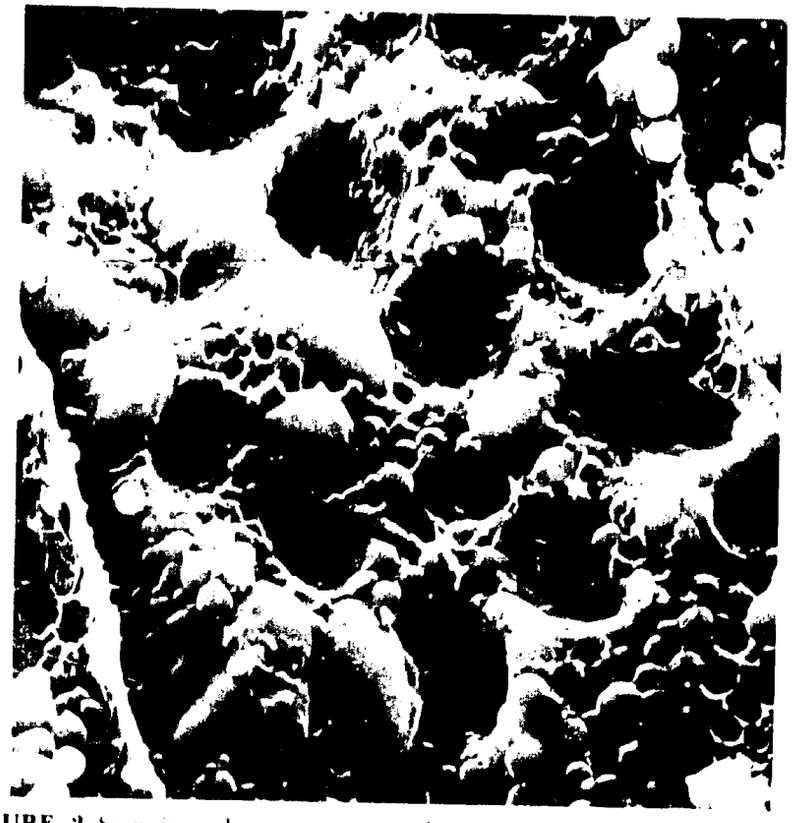

FIGURE 2 Scanning electron micrograph from the central endosperm of normal-lysine barley (CI-4362, "isogenic" to Hiproly in Figure 1): ×1,400. Preparation from a dry, ripe seed

modified gross morphological character of the seed to a normal appearance, retaining the favorable amino acid composition (19).

The ability to induce new opaque-2 mutants with high-lysine content (1) favors the hypothesis that the mutational event affects both a "soft-endosperm gene" and a "protein-regulating gene" that are closely linked. The fact that nonopaque, high-lysine opaque-2 kernels frequently can be obtained by crossing indicates that these effects may be due to modifier genes, and not to crossing over between a "soft-endosperm" gene and a "protein-regulating" gene.

A similar situation is tentatively found in lys barley, but with the kernels displaying a hard endosperm. Serious efforts to modify the structure of recently found mutants in maize (such as brittle-2, which retains the quality protein trait) might be very productive. Further studies of the new barley mutants' morphological construction also will provide new information about the relationship between seed morphology and changes in endosperm amino acid composition.

COMPARING BIOCHEMICAL EFFECTS OF HIGH-LYSINE GENES

A similar pattern of changes in the Osborne extraction fractions (15) was shown for all the mutant genes that improve the amino acid pattern in maize. The more-lysine-rich fractions (albumins, globulins, and glutelins) showed relative increases in the percentage of total endosperm protein, whereas the lysine-poor prolamine showed decreases. This finding also seems valid for barley mutants. Extraction of single lys barley endosperms (nondried, yellow-ripening stage) and subsequent amino acid analyses revealed a doubling of the water-soluble albumin fraction compared to the controls (18). The lysine–arginine ratio also was changed, indicating a reorganization in the relative amount of the water-soluble proteins; this was verified in polyacrylamide electrophoresis of the water extract (18). Figure 3 shows electrophoretic separations of water-soluble proteins from endosperms of the Hiproly and Kristina varieties as compared with three F_2 segregants from the cross between the two varieties. Parents and segregants represented several endosperms of individual plants.

Water extract from Hiproly (dried seeds) was higher in lysine (mol %) compared to normal-lysine cultivars either low or high in protein (21). Ingversen and Koie (9) combined column chromatography with gel electrophoresis and obtained interesting indications that the major increase in lysine content in Hiproly could be due to a drastic increase of one or more extremely lysine rich proteins from the salt-soluble proteins (albumins plus globulins) (10). Similar minor changes also were reported in this fraction of the high-lysine mutants (mutants 29 and 86) as well as the improved cultivar CI-7115 (Table 1).

Contrary to findings for opaque-2 maize, the prolamine fraction of Hiproly is only slightly decreased (20%) when compared with the normal seed fractions. Most Osborne fractions in Hiproly and lys barley, except the prolamins, showed a strongly increased methionine–cystine ratio due to decreased cystine and increased methionine. Thus, the lys gene appears to have produced several side effects in addition to its influence on the water-soluble proteins (19).

The extractability of protein fractions in lys barley seems to be better than that of normal barley due to a less S-S-bridged glutelin network (19). Prolamins in cereals are stored mainly in protein bodies. The development of protein bodies is retarded in prolamin-low opaque-2 and floury-2 maize, whereas protein bodies in lys barley seem to be intact (19). The new, extremely high lysine barley mutant 1508 (Table 1) shows similarity to opaque-2 and floury-2 maize mutants with (1) drastic decreases in the prolamins (from 31% of total crude protein in the control to 10% in the mutant), and (2) radical increases in albumins plus globulins (from 27 to 46%), as reported by Ingversen and Koie (10). Therefore, a strong reduction of protein bodies could be expected in barley mutant 1508.

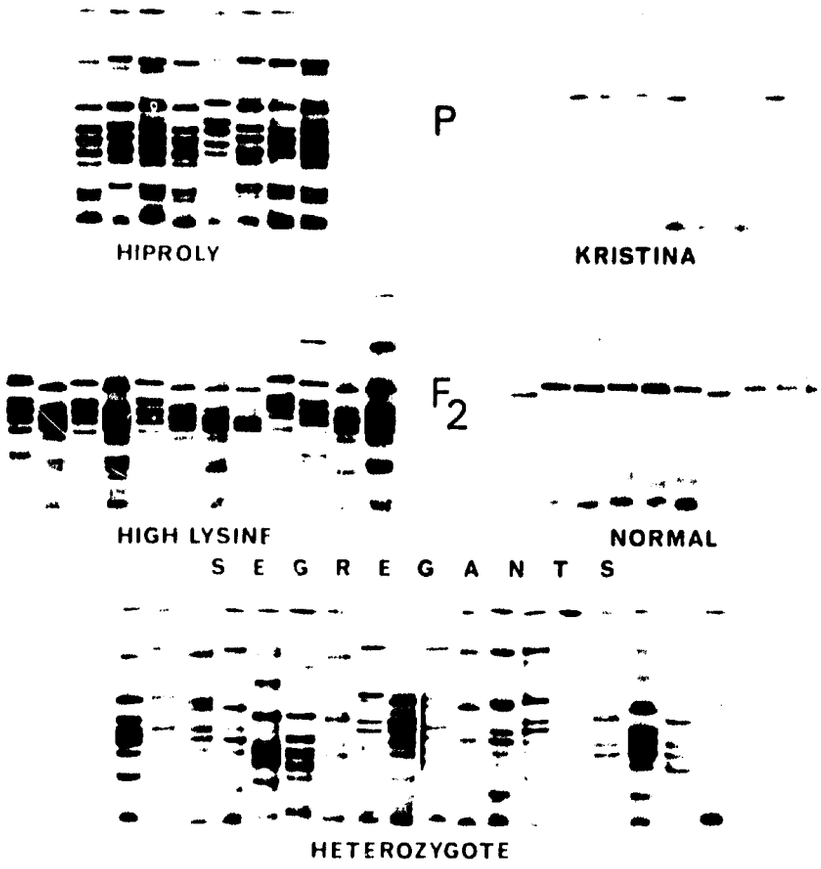

FIGURE 3 Segregation for the lys gene in barley; polyacrylamide electrophoresis of water extracts from single nondried endosperms at yellow-ripening stage. Endosperms from Hiproly and a normal-lysine barley plant (Kristina) are compared with a high-lysine, a normal-lysine, and a heterozygous barley plant in the F_2 from the cross Hiproly (♀) × Kristina (♂). Anodic migration, Tris buffer; Ph 8.6, geles 15%. Extraction with 200 μl of H_2O per endosperm, 10 μl being added to each gel.

From the breeder's point of view, it seems appropriate to conclude that, although crude protein is extremely variable, traits such as lysine grams/16 g N, lysine–arginine and cystine–methionine ratios show very high heritability. In fact, the most striking finding of the above-mentioned barley breeding work in Sweden and Denmark is that even very small improvements, such as 10% in lysine grams/16 g N, can be selected through the DBC method and are found to be environmentally stable between different laboratories and seasons when compared with a standard material.

Several "minor" genes in the gene background can interact with the

"major" ones, generating modified levels of gene expression. This is exemplified by the lys gene in barley (Table 2), where lysine g/16 g N, irrespective of crude protein content, is prevalent at three levels: normal, high lysine, and high lysine modified. The differences are genetically and environmentally stable and can be reproduced (19). Glutamic acid (grams/16 g N) decreases and aspartic acid increases regularly at elevated lysine levels. "Minor" genes should be considered for changes in endosperm proteins, especially in breeding the polyploids (wheat and triticale).

EVALUATION OF NUTRITIONAL QUALITY OF IMPROVED CEREALS IN FEEDING EXPERIMENTS

Proteins from the multicellular aleuron layer of barley are difficult for animals to digest. In wheat and maize there seem to be fewer problems with protein (and amino acid) availability owing to the generally unicellular aleuron layer of these cereals. Figure 4 shows the true digestibility for rats of 17 amino acids from high-lysine (Hiproly) and normal-lysine barley. The important essential amino acids, lysine and methionine, show a very low availability when, for example, compared with glutamic acid and serine. In Hiproly the least-available amino acids show a greater increase in availability when compared to the control than do the most-available amino acids. The increase of the lysine-rich water-soluble proteins in Hiproly, therefore, seems to be reflected in improved lysine availability.

Nitrogen-balance studies (19) were performed on rats (Table 3) to compare the nutritional quality of the high-lysine morphological deviates showing no starch–protein adherence (Figure 2) with that of high-lysine barley adherent controls (Figure 1). A related normal-lysine barley control also was included. Net protein utilization (NPU) was increased by about 70% in the high-lysine lines, compared with 60% in the normal control. The starch–protein adherence trait of the high-lysine lines did not appear to affect true digestibility, biological value, or net protein utilization. Consequently, the morphological character associated with the lys gene in barley

TABLE 2 Modification levels of the expression of the lys gene in barley

Strain	Crude protein (%)	Lysine (g/16 g N)	Glutamic acid (g/16 g N)	Aspartic acid (g/16 g N)
Hiproly	17.7	4.0	21.7	6.3
"Isogenic" CI-4362	17.2	3.0	25.2	4.9
	Segregants Hiproly × *normal* F_4 *plants*			
Normal lysine	13.9	3.6	24.1	6.4
High lysine	12.1	4.5	20.5	8.0
High lysine modified	13.4	3.9	23.0	6.8

Note: Amino acid analyses according to Eaker (4).

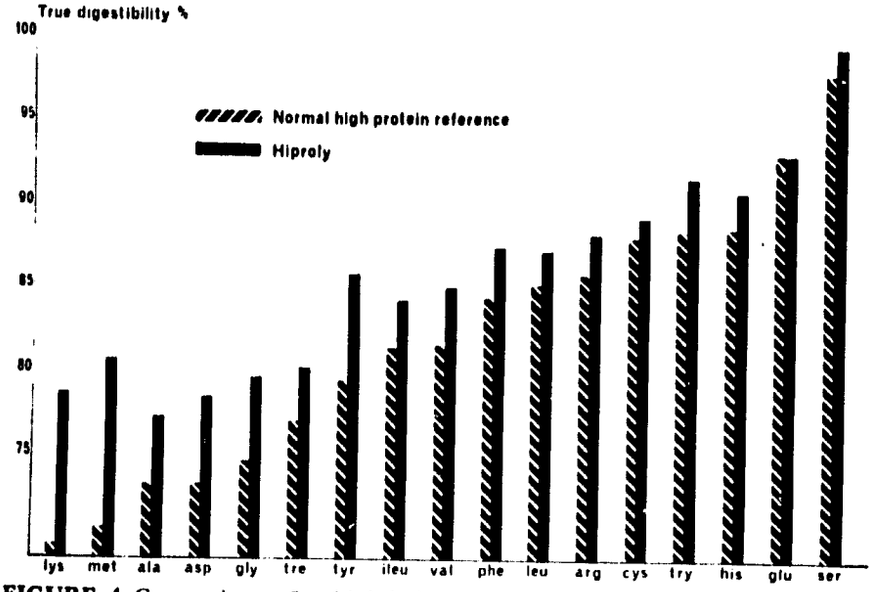

FIGURE 4 Comparison of a high-lysine, high-protein barley (Hiproly) with a normal-lysine, high-protein reference with regard to rat true digestibility (6) of individual amino acids.

seems to have had no observable effect on quality. Rat NPU with lys barley was shown to be equal to that of opaque-2 maize. The adherence character may cause acceptance problems and should be studied further in the Middle East, Far East, and South America, where barley is a major food for about 200 million people.

EVALUATION OF NUTRITIONAL VALUE OF HIGH-LYSINE CEREALS IN PRACTICE

Data discussed in this paper represent only the early stages of an accelerating development in the field of genetic protein engineering in cereals. High-yielding, high-lysine commercial varieties certainly will be released, and their beneficial effects will be established in nutrition laboratories and hospitals. The major question is will the new varieties play an adequate nutritional role in the daily use of feeds and foods? Only thorough scrutiny of how cereals are selected, treated, and used in society can provide an answer. The high-lysine barley breeding program at Svalöf in Sweden is only a part of a general interdisciplinary program to improve the cereals' nutritional value for seed. This work has been in progress since the early 1960s [see compilation by Munck (19)]. Screening studies in the market of compositional and deterioration problems in cereals, feed supplements, and full

TABLE 3 Nutritional quality in feeding tests with rats, displaying different lys lines of barley

Sample	Number of diets	Protein in seed (%)	Lysine (g/16 g N)	Nitrogen true digestibility (%)	Biological value (%)	NPU (%)
Normal control	1	12.6	3.25	84.0	71.2	59.8
High-lysine starch-protein adherent	2	12.9	4.05	85.9	79.2	69.0
High-lysine starch-protein nonadherent	3	12.5	4.07	85.8	78.9	67.7

Note: Nitrogen-balance tests with rats restrictively fed at 9.3% crude protein level in diet. Five animals just weaned were individually fed 9 days. Method according to Eggum and Mercer (5).

feeds have indicated that the introduction of high-lysine barley will provide zero-level economic and nutritional value unless matched appropriately with a screening technique to assess quality, along with a premium-price program. The simple dye-binding (DBC) technique to estimate basic amino acids is ideal for this purpose. A simple, battery-operated field instrument could be constructed.

In developing countries, cultivating high-lysine cereals for food is a much more complicated and diverse problem than introducing high-lysine barley in Swedish feed rations. If the high-lysine cereal is not characterized by some easily recognizable morphological trait, difficulties will arise in its distribution and use. This is especially true among subsistence farmers, where control and extension service are not likely to be available on the scale needed. The new, high-yielding, high-lysine maize varieties are likely to be mixed and intercrossed with normal maize; thus, they would not provide a stable contribution to human nutrition. Morphological markers regulated by the government could be introduced tentatively on the plant or seed of *all* high-lysine varieties released. These characters should be chosen so as not to affect acceptability.

It is evident that several characters of the cereal plant, besides nutritional quality as determined in laboratories and hospitals, are essential for its overall nutritional value to be incorporated in the social structure. Because man is not able to taste or smell essential amino acids in the cereal protein form, indirect, visible, apparent value characters must be attached to the varieties. When the farmer is able to distinguish the variety from others, he might later associate it with improved nutrition and health of his family or with increased animal feeding performance. Still, extension work for the subsistence farmer is an immense undertaking. A "package of locally adjusted simple techniques" must be introduced with benefits that the farmer can personally recognize and exploit. To be able to catalyze nutritional breakthroughs, the plant breeder and his associates must find a *methodology* (19) to evaluate how the production chain selects and transforms the cereal raw material and what this signifies for consumers. The directors for such a program will have to create their own vision of how life should be lived, and how plants and society should be optimized to accommodate their vision. They must then be equally successful in introducing this ideal into the plants and into the social fabric of the community.

H. Doll, J. Ingversen, and B. Koie (Atomenergikommissionens forsogsanlaeg, Riso, DK-4000 Rokslide, Denmark) generously supplied us with unpublished data on their interesting new high-lysine barley mutants.

[*A discussion of this paper can be found on p. 508 of* **Questions and Answers.**]

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Part X

WORKSHOP: CHEMICAL AND BIOLOGICAL ANALYTICAL TECHNIQUES

Workshop discussion centered on (1) chemical analytical techniques, and (2) biological analytical techniques now used in research programs for the breeding of high-quality protein in cereal grains.

CHEMICAL ANALYTICAL TECHNIQUES

Protein Content

Participants agreed that the micro-Kjeldahl method is best for determining the protein content of cereal grain, and can be used in a variety of forms. The simplest version of the procedure is the AOAC method (1), which involves digestion of the sample with sulfuric acid and steam distilling the ammonia at high pH into a boric acid solution. The ammonium borate formed is then titrated with standard hydrochloric acid. A more rapid modification of the micro-Kjeldahl method is direct Nesslerization of the digest and determination of the amount of ammonia colorimetrically (2). The most rapid technique is to analyze the ammonia formed in the digestion step with the Technicon analyzer. This, however, is the most costly method from the standpoint of equipment required.

The rapid-biuret method of Johnson and Craney cannot be used for the determination of protein in opaque-2 maize (3), but could be used for other low-pigmented cereal grains.

Lysine Content

Other amino acids. The automatic amino analyzer is based on the ion-exchange principle and is probably the most accurate method for the determination of lysine, tryptophan, and other amino acids found in cereal proteins. It also is the most costly from the standpoint of the original cost of equipment, upkeep, and manpower. Most analytical chemists consider the amino acid analyzer as the primary standard, since it can determine the level of all the amino acids in proteins with an accuracy of ± 2%. The basic principles of the automatic amino acid analyzer have been described by Moore et al. (4).

The automatic amino acid analyzer is closely followed in accuracy by the gas-chromatographic method. Both the ion-exchange and gas-chromatographic methods require the use of a protein hydrolysate. In addition, the gas-chromatographic method requires the conversion of the individual amino acids into volatile derivatives, adding one more step to the procedure. Methods using the gas-chromatographic column specifically for lysine have been developed by Scheile et al. (5). Gehrke and collaborators (6) have described gas-chromatographic methods for all the amino acids found in cereal proteins.

A third method that is useful for lysine and the other amino acids is the microbiological assay method. Two versions are available: (1) the traditional method, which involves titration of the lactic acid produced by the test microorganism (7), and (2), a more recent method that involves a plate assay in which the diameter of the colony of bacteria is measured. Here the size of the colony depends on the concentration of the amino acid being tested (8).

The estimated cost of a complete amino acid analysis of a cereal grain by the preceding methods would be in the range of $40 to $75 (U.S.) per analysis if done in a commercial laboratory. No cost estimates have been made of a complete analysis in a research laboratory such as at CIMMYT or Purdue.

By using automatic sampling devices on the amino acid analyzer, it is possible to complete a lysine determination in 15 minutes. By programming the equipment for 24 hours, 96 lysine determinations could be made per day. Even with the automated system, however, a large amount of technician time is required for the weighing, grinding, defatting, and hydrolysis of the samples.

Lysine only. The enzymatic lysine decarboxylase method (9) is fast and highly specific for lysine, and can be used in a well-equipped laboratory. In this method, the enzyme specifically releases carbon dioxide from lysine in a protein hydrolysate, and the carbon dioxide is measured quantitatively. For efficiency, the method requires the use of a Technicon autoanalyzer or its equivalent.

Lysine and tryptophan. Several colorimetric methods are now available for the determination of lysine and tryptophan in cereal grains. The tryptophan method now being used at CIMMYT (10) is probably the simplest and fastest

method for a single amino acid. Fortunately, the tryptophan level in maize relates very well to the lysine level and to the quality of the protein. CIMMYT has used this procedure very successfully as an initial screen for protein quality in maize. The principle of the method is to digest a defatted finely ground sample of endosperm with papain overnight, and then to measure the amount of color formed in the supernate with a modified Hopkins–Cole reagent containing ferric chloride and glyoxylic acid.

Three colorimetric methods are available for the determination of lysine. Most widely used is a method originally developed by Tsai et al. (11) at Purdue University and modified by Villegas at CIMMYT (10). In this method, the defatted finely ground endosperm sample is digested with papain to release the lysine. The lysine is then reacted with 2-chloro-3, 5-dinitropyridine and the color measured quantitatively. The other two methods involve extraction of the corn protein with alcohol and alkali: the first uses trinitrobenzenesulfonic acid (TNBS) as a reagent to derivatize the lysine (12); the second uses dinitrobenzenesulfonic acid (DNBS) (13). In either method, the TNBS or DNBS proteins are hydrolyzed for a short period in HCl. The pyridine method is more rapid than the TNBS or the DNBS methods (10).

In the CIMMYT laboratory, Villegas has calculated that the cost for an analysis for tryptophan and micro-Kjeldahl protein is approximately $0.90 (U.S.) per duplicate sample. The cost of determining lysine with the pyridine method is approximately $1.60 (U.S.) per duplicate sample with the necessary nitrogen determination.

Indirect lysine assay. The dye-binding method (14) is a colorimetric method that is less specific for lysine than the preceding methods. In this method an azo dye is added in excess to the finely powdered defatted sample. Then the dye that has not become bound to the basic amino aci is (arginine, histidine, lysine) in the maize protein is measured, and calculations are made for the amount of dye that is bound. The amount of dye bound by the sample per unit of nitrogen correlates well with the level of lysine in that sample. Munck has estimated that (in Sweden) the cost of assaying a single maize sample by dye binding, with the necessary nitrogen determination included, costs about $1.80 (U.S.).

A turbidimetric method for indirectly analyzing for lysine was developed at the USDA laboratory in Peoria and has been described previously by Wall (15). In this method the zein is extracted from the finely ground defatted sample with alcohol. Then it is precipitated and the quantity measured turbidimetrically. Wall found a negative correlation of 0.9 between the zein and the lysine content of the sample. In a commercial laboratory in the United States, a sample analyzed for lysine plus total nitrogen would cost about $6 (U.S.).

In summary, the methods available for determining all the amino acids in a cereal protein are the ion-exchange method using the automatic amino acid analyzer, the gas-chromatographic method, and the microbiological

method. The same three methods can be used for the determination of lysine only. In addition, the following direct methods are available for the determination of lysine: the lysine decarboxylase method, and (on low-pigment cereals) colorimetric methods involving 2-chloro-3, 5-dinitropyridine, trinitrobenzenesulfonic acid, and dinitrobenzenesulfonic acid. Indirect methods are the dye-binding method (for low-pigment cereals) and the turbidimetric zein method for maize. In addition, a rapid colorimetric method can be used for the determination of tryptophan. Since the correlation is very high between tryptophan level and lysine level in maize, it is possible to carry out a screening program using tryptophan analysis alone, followed by lysine determinations on the samples giving the highest tryptophan values. Tryptophan analysis costs much less per sample than does lysine analysis.

BIOLOGICAL ANALYTICAL TECHNIQUES

Workshop participants agreed that the chemical methods for lysine analysis, as described, correlate very well with the biological value of the cereal grains, maize, wheat, barley, and oats. The lysine determinations obtained on sorghum and triticales do not correlate well with the biological value of these materials, because of the presence of factors that combine with the protein or are toxic to the test animal. In the case of sorghum, as Axtell noted, tannins modify the biological response. For example, in two samples of sorghum with the same lysine content, if one sample should be high in tannin, its biological value would measure much lower than would a sample with less tannin content. In the case of triticales, as indicated by Zillinsky, toxins or inhibitors are found in certain selections. These toxins have not been identified. The workshop recommends that additional studies be carried out to identify and isolate the inhibitors or toxins present in certain samples of triticales so that the chemical nature of the toxins can be identified. Hopefully, chemists can then devise simple methods for chemically identifying or detecting these toxins, without the necessity of determining the toxin biologically. Although the exact nature of the protein binding factor(s) in sorghum is not known, chemical methods for determining the level of the tannins or polyphenolic compounds in sorghum are available. Thus it is possible to predict which samples of sorghum will show a low biological value.

Until other methods are available for detecting toxins or other growth-inhibiting agents in cereals the workshop recommends that new selections should be tested (1) for a low protein level (around 10%) for protein efficiency ratio (PER) and (2) for feed efficiency ratio (FER) with the highest possible level of the cereal in the diet, to screen for toxins. Cereal grain also should be tested at the minimum protein requirement level when possible. (It is not possible with cereals containing less than 15% protein.) The weight gains obtained on a particular cereal grain at the minimum protein require-

ment level would be a sensitive indicator of the relative quality of the protein in the cereal grain. As Maner noted, this is the level at which minimum conversion of protein to urea is obtained, concurrently with maximum weight gain.

Participants concluded that it will be necessary to use small animals for the screening of new cereal varieties to detect any toxic materials present. Once toxins have been detected, the material should be brought back to the laboratory, where chemical methods should be devised to quantitatively measure the toxin(s); then a breeding program can be conducted to eliminate them.

The results of the cooperative feeding tests with the rat, mouse, and vole indicate that additional research is needed to improve the efficiency of the mouse and vole as test animals for differentiating between the different cereal grains. When a sufficient amount of sample is available (about 600 g), the rat is considered the best choice for biological evaluation of the protein quality of cereal grains.

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WORKSHOP: METHODS OF INTRODUCING IMPROVED PROTEIN VARIETIES AT THE FARM LEVEL

Participants in the discussion group included representatives from Latin America, Europe, Africa, and Asia, thus providing a wide spectrum of experiences and opinions. It was generally agreed that quality maize had a very real role in the developing world.

The consensus was that many problems presently identified would disappear in the next few years, as varieties of maize with widely differing grain types, higher yield, and disease and insect resistance become available.

The problems of introducing improved protein varieties of maize at the farm level vary in different parts of the world, depending upon (1) the principal use of the maize (mainly as animal feed on farms, as human food on farms, or as free market production) and (2) social, ethnic, and political differences.

PRINCIPAL USES OF MAIZE

Animal Feed

The use of quality protein maize for feeding animals on farms presented the easiest situation, especially for feeding swine. The grain type and color are less important, and the spectacular growth differences are easy to demonstrate.

The provision of small quantities of quality protein maize to farmers with instructions to use it to feed some of their pigs, while maintaining other pigs on normal maize, would be sufficient to demonstrate the feeding value of the quality protein maize. Programs of this type are already underway in Brazil, where there are 68 million pigs, and less than 20% of the farmers provide feeding supplements to their pigs.

Human Food

Quality-protein maize as a human food on farms is somewhat more difficult to introduce. The yield, grain type, and general characteristics of the quality maize must be at least equal to the local maize varieties.

Attempts are being made in Latin America to introduce quality maize along with an improved package of agronomic practices.

Where a few pigs are kept on a farm, they can be used to demonstrate the food value of the quality maize.

Proposals from Latin America and Africa suggest the value of the incorporation of quality maize in school lunch programs to stimulate interest.

Extension, demonstrations, films, and education at the farm level (and for doctors, universities, schools, and for politicians and government employees concerned with farmers) were all mentioned as being of possible value to encourage farmers to grow the improved maize for human consumption on the farm.

Free Market Production

Free market production of quality protein maize requires a competitive product, or one that receives special subsidies to encourage farmers to grow it. Also, there is need for a sufficient market demand to warrant production.

Utilization must be encouraged through the production of acceptable food products, such as baby food, macaroni, soups, drinks, cookies, and school lunches.

Milling and manufacturing qualities must be adapted to industry, or industry must adapt their techniques to the quality product. The cost–profit ratio must be favorable to encourage industrial research.

Several programs are being attempted throughout the world to use quality-protein maize in industry. Further attempts await a hard-endosperm, high-yielding version of the new maize.

A firm commitment by governments to exploit quality maize for both livestock and human consumption is essential if widespread adoption is to be effected.

SOCIAL, ETHNIC, AND POLITICAL DIFFERENCES

Social, ethnic, and political differences among countries will significantly

influence the specific techniques used to introduce quality protein maize into wide-scale use.

Authoritarian governments would be able to effect general use very quickly, if they committed themselves to do so. Some countries do not have monogastric animals, such as pigs, in significant numbers to use as demonstration animals. Methods of food preparation also differ from country to country, thus influencing the type of maize required.

Finally, the workshop group stressed their belief that the new hard-endosperm types now starting to flow from breeding programs could appreciably alter the pattern of acceptance in the next few years.

WORKSHOP: ECONOMIC AND SOCIAL FACTORS IN ACCEPTANCE OF IMPROVED PROTEIN VARIETIES

Group IV discussed economic and social factors in the acceptance of improved protein varieties and aimed its discussion at developing an agenda for research useful to policy makers. The research itself would feature the joint involvement of agronomic scientists, nutritionists, food scientists, and economists.

To keep the discussion within manageable limits, it was assumed that policy makers are concerned with malnutrition and, in particular, with malnutrition arising from shortages of lysine and tryptophan. High-lysine maize was viewed as a potential source of lysine and tryptophan.

In treating high lysine maize, emphasis was given to modified opaque-2 varieties, those combining improved protein with the desirable agronomic and grain characteristics of normal varieties. Given the goal of increasing the availability of lysine and tryptophan, the policy maker has alternative ways of achieving this increase, for example, modified opaque-2 maize, fortification, or grain legumes. It was recognized that he has limited research resources for achieving his goal.

In the course of the discussion it became clear that the issue of increasing the availability of lysine and tryptophan can best be considered in the context of a homogeneous region. It also became clear that it is useful to identify several classes of beneficiaries from increased supplies of lysine and tryptophan. Six such classes were identified: (1) the rural poor (those who directly consume the bulk of their production), (2) the urban poor, (3)

integrated grain–livestock producers, (4) commercial producers of grain (those who sell the bulk of the production from their farms), (5) institutions (for example, school lunch programs and hospitals), and (6) other consumers. Finally, it was recognized that the problem of improving nutrition must be seen in the context of a specific time period.

To portray the relevance of looking at the nutritional problems of specific groups in specified time periods, a potential solution through fortification was considered. This seems to represent a viable alternative for the urban poor in the short run. In the long run, fortification's relative advantage over genetic improvement in crops may decline. For the rural poor, fortification may not be feasible, even in the short run, unless transport and distribution systems are vastly improved.

To make effective choices, the policy maker needs information about the relative costs and benefits from alternative policies—for each of the several classes of potential beneficiaries by region and for given time periods.

The discussion then turned to the kinds of information that policy makers would find useful in assessing the costs associated with improving nutrition through modified opaque-2 maize.

An obvious issue was that of diffusing improved maize among the three classes of producers. It was argued that the slowest to accept the new maize might be the rural poor. To achieve good results, it was agreed that an integrated government program would be necessary. The program would feature improved varieties (improved not only in the sense of having better-quality protein, but also higher yields than local varieties), improved practices, and a dynamic extension program.

Some have suggested using the market for stimulating the diffusion of modified varieties among the rural poor; that is, higher market prices would be offered for high-quality-protein production. Higher prices, however, reduce the benefits flowing to the urban poor. Moreover, maintenance of price differentials requires a simple device for detecting lysine and or tryptophan content in marketed maize.

It was suggested that a way to speed adoption among the rural poor is through the use of high quality protein maize by the integrated livestock–maize producers. The obvious advantages of modified opaque-2 maize as a livestock feed should attract these producers, and the higher yields should attract neighboring farmers. (The Brazilian experience suggests that maize–hog farmers might adopt high quality protein maize more rapidly.)

Beyond information on the costs associated with the diffusion of modified opaque-2 maize and the probable rates of utilization within groups of potential beneficiaries, the policy maker needs information on the likely costs of assembling, storing, processing, and marketing the maize that comes into commercial channels. For example, if the modified opaque-2 and normal maize must be separated for marketing, this handling implies one set of

costs. If, on the other hand, separation is not necessary or if it could be achieved readily, another set of costs is implied.

Similar information is also necessary on the costs and benefits from alternative systems for increasing the availability of lysine and tryptophan.

The committee touched on only some of the critical dimensions of the problem. The ideas implicit in our discussion were that policy makers have alternatives, that choosing among these requires information, and that this information can best be grouped around classes of beneficiaries and the activities associated with diffusing, producing, and utilizing high-quality protein maize.

Aside from the valuable information presented at the conference on nutrition, chemical content, and agronomic characteristics, few of the questions emerging from our discussion have been answered. Effective policy making will require a careful examination of these questions.

Part XI

RESEARCH ON HIGH-QUALITY PROTEIN MAIZE IN SOUTHERN EUROPE

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Maize breeding programs within the European Association for Research on Plant Breeding (EUCARPIA) recently have emphasized efforts to improve the nutritional value of maize. The Southern European Mediterranean Committee of the Maize and Sorghum Section of EUCARPIA has given special attention to the study of the biochemical composition and genetic regulation of maize grain. Some of the main research trends and latest achievements are summarized here.

UTILIZATION OF THE OPAQUE-2 GENE

Extensive interest has been shown in using the opaque-2 gene to improve maize grain quality. Every European country now has backcrossing programs to recover improved lines and to breed high-quality-protein hybrids. Some geneticists are investigating problems arising from the introduction of the opaque-2 gene into the genetic backgrounds of dent and flint lines.

Summaries of the research results obtained in their respective countries have been reported by V. Trifunovic and M. Missovic, Yugoslavia; O. Cosmin, Romania; and I. Kovacs, Hungary. In Spain, F. Sanchez Monge and A. Monteagudo are doing similar research: evaluating and testing several inbreds.

In France, M. Pollacsek and collaborators have developed a program for converting local inbred lines. Their selection program is oriented toward selecting strains that maintain phenotypic flintiness in a homozygote opaque-2 kernel by using a genetic system of complex determinism in which flintiness appears to be due to genic dosage while lysine content stays at high levels. Dominant suppressor genes (Sup^1 o_2) with a 15 to 16 ratio and others of monogenic action also have been detected and studied. The ability of these genes to phenotypically suppress opaqueness has been shown, but lysine content also was shown to have been reduced to normal percentages. Further research is being planned to discover suppressor genes capable of maintaining a high lysine content and restoring flint grain.

In Italy, research is being conducted at three research centers. At the Institute of Genetics of the University of Milan, E. Ottaviano has centered his studies on the variability of the phenotypic effects of the opaque-2 gene within an F_2 population from an opaque-2 single cross. Inferior kernel weight in opaque kernels appears to depend upon a multiplicative effect of opaque-2, and partly on modifier genes affecting o_2o_2 phenotypic expression.

Interaction between a major gene and different modifiers seems to produce high tryptophan content. Protein and oil variability were not shown to be conditioned by the genetic background, although variability was noted.

As a result of this work, opaque-2 genotypes have been selected that have minimum negative effects on grain weight, protein content, and yield. The combining ability of these genotypes has been under evaluation by Salamini and his collaborators for the past few years, and they have carried out an extensive program of backcross conversion of normal genotypes to opaque-2. They now are studying the influence of environmental factors on the expression of the opaque-2 gene within different genetic backgrounds, while selecting opaque-2 strains from opaque-2 synthetics made with materials of a very different origin. This breeding work parallels a program for evaluating the nutritive value of strains conducted in collaboration with A. Piva, University of Piacenza, Italy.

G. Mariani has investigated the influence of environmental factors (plant density and nitrogen fertilizers) on the yield, protein content, and quality of opaque-2 hybrids as compared with normal hybrids. The main results obtained at the breeding center in Bergamo will be presented by Mariani at this conference.

NEW SOURCES OF OPAQUE-2 GENES IN SOUTHERN EUROPEAN GERM PLASM

As part of the cooperative program of the Southern Committee, in collaboration with G. Orio, G. Vandoni, and I. Cabulea, a broad screening to ascertain the occurrence of the opaque-2 gene in Southern European germ

plasm has permitted selection of several sources at the Horticultural Research Center in Minoprio (Como) (see Table 1).

Generally, the floury-2 gene appeared in most Italian, Romanian, and Southern European flint varieties. About 98% of the flint samples pollinated by the floury-2 homozygous stock showed floury-2 in the seed parent, either uniformly or segregating with different frequencies.

It appears that special inhibiting mechanisms do not permit the expression of the fl_2 fl_2 gene in the local panmictic populations, but might operate differently in the presence of special suppressors.

The new sources appear to have great promise. After isolation of the homozygous forms, they will permit utilization of a broader genetic background, thus reducing inbreeding.

NEW SOURCES OF HIGH-QUALITY PROTEIN

To increase knowledge of the biochemical characteristics of European germ plasm, as well as to detect new useful sources of high-quality-protein maize endosperm for further breeding programs, the grain harvested on sibbed plants from about 1,600 samples of different origin was submitted to chemical analysis for fat, protein, lysine, and tryptophan content. Automatic Technicon analyzer equipment provided good reliability and rapid operation.

The results are summarized in Table 2. The data are shown for taxonomic groups, as averages and fiducial limit. It is evident that the variability in Southern European Mediterranean germ plasm is quite high for fats and protein and for lysine and tryptophan content. It also is evident that findings for some groups show an interestingly high percentage of critical chemical components. A preliminary study of correlation values for some characteristics of grain appears to indicate a significant positive relationship between protein and (1) tryptophan and lysine content, and (2) the specific weight of the grain (Table 3).

TABLE 1 Presence of opaque 2 in local maize varieties

		Opaque 2		Opaque 2 modified		Uncertain opaque 2		Total opaque 2 presence
	Cultivars	No.	%	No.	%	No.	%	(%)
Italy	644	20	3.1	7	1.1	10	1.6	5.7
Romania	131	6	4.6	6	4.6	6	4.6	13.8
Yugoslavia	35	5	14.3			1	2.8	17.1
Portugal	28	1	3.6	1	3.6			7.2
Spain	8					2	25.0	25.0
Morocco	32	3	9.4			1	3.1	12.5
France	3	1	33.3					33.3
Total	881	36	4.1	14	1.6	20	2.3	7.9

TABLE 2 Biochemical composition of maize grains in percentage of nondefatted dry grain weight[a]

Racial complex	Sample no.	Lipids (%)			Protein (%)			Tryptophan (%)			Lysine (%)		
		t^{min}	$\bar{x}$	t^{max}	t^{min}	$\bar{x}$	t^{max}	t^{min}	$\bar{x}$	t^{max}	t^{min}	$\bar{x}$	t^{max}
L South European representative collection													
A Popcorn	11	4.12	5.92	7.71	12.40	14.70	17.07	0.12	0.16	0.21	0.12	0.32	0.52
B Small-grain flint	74	4.00	5.70	7.36	10.80	13.40	15.90	0.12	0.16	0.20	0.12	0.27	0.43
C Elliptical flint	20	4.00	5.80	7.10	12.00	13.40	14.90	0.18	0.18	0.18	0.18	0.31	0.43
D Cylindrical flint	90	4.60	5.80	7.60	10.10	13.10	16.10	0.12	0.16	0.19	0.12	0.28	0.44
E Conical flint	22	3.30	5.40	7.40	10.80	13.30	16.00	0.12	0.16	0.20	0.21	0.31	0.42
F Conical derived	27	3.40	5.40	7.30	10.60	12.80	15.00	0.12	0.16	0.20	0.19	0.31	0.43
G Eight-row flint	20	3.90	6.00	8.10	10.60	13.30	16.00	0.12	0.18	0.22	0.24	0.32	0.40
H Eight-row floury	33	3.94	5.30	6.65	10.30	13.70	17.20	0.11	0.16	0.19	0.22	0.30	0.38
I Ancient floury dents	10	3.53	6.30	9.15	11.09	12.70	14.35	0.13	0.16	0.20	0.16	0.24	0.32
L Modern dents	34	3.61	6.00	8.50	10.54	12.80	15.02	0.12	0.16	0.20	0.10	0.26	0.42
M Derived dents	35	4.30	6.40	8.50	11.30	13.10	14.90	0.13	0.18	0.21	0.14	0.23	0.33

TABLE 2 (cont.)

	Racial complex	Sample no.	Lipids (%)			Protein (%)			Tryptophan (%)			Lysine (%)		
			t_{min}	x	t_{max}	t_{min}	x	t_{max}	t_{min}	x	t_{max}	t_{min}	$\bar{x}$	t_{max}
II.	Italian collection													
A	Popcorn	16	3.88	5.71	7.55	12.04	14.47	16.90	0.11	0.16	0.21	0.27	0.35	0.43
B	Small grain flint	154	4.04	5.56	7.08	10.88	12.90	14.92	0.14	0.18	0.23	0.18	0.30	0.42
C	Elliptical flint	82	4.53	5.80	7.07	11.03	12.75	14.47	0.14	0.18	0.21	0.19	0.29	0.40
D	Cylindrical flint	102	3.97	5.51	7.05	10.27	12.48	14.69	0.13	0.17	0.21	0.19	0.30	0.41
E	Conical flint	98	3.72	5.09	6.46	10.17	12.09	14.01	0.13	0.16	0.20	0.21	0.30	0.39
F	Conical dentated	44	3.31	5.20	7.08	10.40	12.12	13.84	0.13	0.16	0.20	0.21	0.29	0.38
G	Light corn flint	38	3.92	5.37	6.83	10.12	12.47	14.81	0.12	0.17	0.21	0.18	0.30	0.42
H	Light corn flours	17	3.23	5.33	7.43	10.48	12.19	13.89	0.12	0.16	0.21	0.18	0.29	0.40
I	Ancient flours dents	9	2.89	5.53	8.18	11.21	12.56	13.90	0.12	0.16	0.21	0.20	0.25	0.30
L	Modern dents	8	3.55	4.83	6.10	10.77	12.77	14.76	0.12	0.16	0.21	0.08	0.27	0.45
M	Derived dents	39	3.76	5.37	6.98	10.89	12.57	14.24	0.12	0.17	0.22	0.16	0.28	0.39
III	Romanian collection													
B	Small grain flint	54	3.88	5.35	6.82	10.29	13.39	16.48	0.12	0.15	0.18	0.13	0.23	0.33
D	Cylindrical flint	62	4.18	5.24	6.32	10.37	13.06	15.75	0.13	0.15	0.17	0.15	0.23	0.31
H	Light corn flours	3	2.31	5.01	7.70	10.92	13.05	15.18	0.13	0.15	0.16	0.26	0.30	0.34
L	Modern dents	48	3.93	4.96	5.98	9.94	12.61	15.28	0.13	0.15	0.17	0.16	0.25	0.33
M	Derived dents	36	3.90	4.96	6.02	9.74	12.43	15.12	0.13	0.15	0.17	0.17	0.24	0.31

[a] Average values ($\bar{x}$) and fiducial limits (t_{min}-t_{max}).

TABLE 3 Correlation coefficients of several factors for samples from Italy and Romania[a]

	Tryptophan	Lysine	Density	Volume
Total protein				
Italy	0.65++	0.27++	0.20++	-0.47++
Romania	0.48++	0.23++	0.08	-0.28++
Tryptophan				
Italy		0.24++	0.11++	-0.20++
Romania		0.18+	0.11	-0.11
Lysine				
Italy			0.09	-0.36++
Romania			0.10+	-0.25++
Density				
Italy				-0.34++
Romania				-0.11

[a] Italy, 607 samples; Romania, 204 samples.

Protein was correlated negatively with both single-grain weight and grain depth. No significant correlations were found between fats and protein and tryptophan, or the other characters mentioned, within the taxonomic groups. However, correlations were found in the flint microsperma (including the Cinquantinis, Quarantinis, and Marano pignoletto varieties).

The data are being analyzed further, however, present findings indicate that it will be possible to use these new sources as donors to develop new programs for increasing protein and fats and for improving the amino acid percentage in the proteins. Breeding utilization certainly will require a heavier effort than simply introducing the opaque 2 gene. Surely, however, utilizing some of these sources will not impose problems such as those stemming from a monogenic system of a single origin.

The materials that are not widely used in current breeding programs will be profitably used where synthetic varieties are preferred, such as in developing countries (where environmental factors, especially drought, are major problems), consequently, a broader genetic base would contribute to better stability of yields.

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EFFECTS OF NITROGEN AND PLANT POPULATION ON GRAIN YIELD, PROTEIN CONTENT, AND QUALITY OF AN OPAQUE-2 MAIZE HYBRID

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The influence of cultural practices on the performance of opaque-2 maize has received scant research attention. This report shows the results of a set of trials performed in Italy. The trials were conducted in two locations over 2 years, at 4.0, 5.6, and 6.9 plants m^2 and increasing rates of nitrogen fertilizer, using a medium season (FAO maturity class 600) opaque-2 hybrid. Tryptophan content was taken as the index of protein quality.

EXPERIMENTAL RESULTS

Tables 1 and 2 show the grain, protein, and tryptophan production per hectare for the two locations (Bergamo, 46° latitude; Rieti, 42° latitude).

The performance of the opaque-2 hybrid was satisfactory. The average grain yield was higher in Rieti than in Bergamo in both years. Statistically significant differences between the 2 years for grain yield and production were reported both at Bergamo and Rieti, whereas differences in protein production occurred only at Bergamo.

In 1970, a significant increase in grain yield was shown at the rates of 100 kg N/ha in Bergamo and 50 kg N/ha in Rieti. The 1971 data results show no differences between nitrogen rates.

Over the 2 years, average grain protein production per hectare increased with increasing nitrogen rates (up to the highest rate in Bergamo and up to

TABLE 1 Bergamo, 1970-1971. Effects of increasing nitrogen rates and plant population on
G grain yield at 15.5% moisture (q/ha)
P protein production (kg/ha)
T tryptophan production (g/ha)

		Nitrogen (kg/ha)												Average of populations and years		
		0			100			200			300					
Year	Plants/m²	G	P	T	G	P	T	G	P	T	G	P	T	G	P	T
1970	4.0	56.4	487	7.38	61.5	553	8.36	61.7	563	8.31	64.6	602	9.14	61.0	551	8.30
	5.6	60.4	525	7.90	66.2	580	9.00	66.1	574	9.26	65.4	593	9.34	64.5	568	8.87
	6.9	60.4	487	7.50	75.3	627	9.74	73.9	654	9.76	70.8	649	9.91	70.1	604	9.23
N rate (avg)		59.1	500	7.59	67.7	587	9.03	67.2	597	9.11	66.9	615	9.46	65.2	574	8.80
1971	4.0	54.3	443	7.66	52.5	458	7.61	53.8	476	8.02	54.8	501	8.44	53.9	469	7.93
	5.6	55.9	438	7.49	54.7	461	7.82	54.6	477	7.92	61.1	539	8.55	56.6	479	7.94
	6.9	62.7	484	8.09	65.1	554	8.85	60.5	528	8.77	59.8	542	8.55	62.0	527	8.56
N rate (avg)		57.7	455	7.75	57.5	491	8.09	56.4	494	8.24	58.6	527	8.51	57.5	492	8.14
1970-1971	4.0	55.3	465	7.52	57.0	505	7.98	57.7	519	8.16	59.7	551	8.79	57.4	510	8.11
	5.6	58.1	481	7.69	60.4	520	8.41	60.3	525	8.59	63.2	566	8.94	60.5	523	8.40
	6.9	61.5	485	7.79	70.2	590	9.29	69.4	591	9.26	65.3	595	9.23	66.0	565	8.89
2-year avg		58.4	477	7.67	62.6	539	8.56	61.8	545	8.67	62.7	571	8.98	61.3	533	8.47

TABLE 2 Rieti, 1970-1971. Effects of increasing nitrogen rates and plant population on
G grain yield at 15.5% moisture (1/ha)
P protein production (kg/ha)
T tryptophan production (kg/ha)

		Nitrogen (kg/ha)												Average of populations and years		
		0			50			100			150					
Year	Plants/m²	G	P	T	G	P	T	G	P	T	G	P	T	G	P	T
1970	4.0	68.1	537	8.99	67.8	567	9.31	68.0	615	9.52	71.3	655	10.46	68.8	593	9.57
	5.6	75.4	542	9.06	81.9	651	10.59	78.7	669	11.45	83.3	704	11.67	79.8	641	10.69
	6.9	83.7	590	10.38	85.9	685	11.23	88.4	747	12.38	91.5	709	11.79	87.4	683	11.19
N rate (avg)		75.7	556	9.48	78.5	634	10.38	78.3	677	11.12	82.0	689	11.31	78.7	639	10.48
1971	4.0	60.3	523	7.30	61.9	535	7.37	61.0	582	7.99	58.0	547	8.12	60.3	547	7.70
	5.6	76.3	637	8.62	71.6	607	8.74	79.6	732	10.27	70.5	620	8.60	74.5	649	9.06
	6.9	83.1	689	9.39	83.1	714	9.22	82.7	738	9.84	83.4	751	10.26	83.1	723	9.68
N rate (avg)		73.2	616	8.44	72.2	619	8.44	74.4	684	9.37	70.6	639	8.99	72.6	640	8.81
1970-1971	4.0	64.2	530	8.14	64.8	551	8.34	64.5	598	8.75	64.6	601	9.29	64.5	570	8.63
	5.6	75.8	589	8.84	76.7	629	9.66	79.1	700	10.86	76.9	662	10.13	77.1	645	9.87
	6.9	83.4	639	9.88	84.5	699	10.22	85.5	742	11.11	87.4	730	11.02	85.2	703	10.43
2-year avg		74.4	586	8.96	75.3	626	9.41	76.3	680	10.24	76.3	664	10.15	75.6	639	9.64

TABLE 3 Bergamo, 1970-1971. Effects of increasing nitrogen rates and plant population on
p protein percentage of the whole grain at 15.5% moisture
t tryptophan ppm of the whole grain at 15.5% moisture
w weight of 1,000 kernels (g)

		Nitrogen (kg/ha)												Average of populations and years		
		0			100			200			300					
Year	Plants/m²	p	t	w	p	t	w	p	t	w	p	t	w	p	t	w
1970	8.0	8.6	151	281	9.0	137	291	9.1	135	291	9.3	142	287	9.0	136	287
	5.6	8.7	131	275	8.8	136	281	8.7	140	279	9.0	143	285	8.8	138	279
	6.9	8.0	124	276	8.4	130	275	8.9	133	287	9.2	141	272	8.6	132	277
N rate (avg)		8.4	129	277	8.7	134	282	8.9	136	286	9.2	142	281	8.8	135	281
1971	8.0	8.2	141	254	8.7	145	253	8.9	149	253	9.1	154	252	8.7	147	253
	5.6	7.9	134	254	8.0	143	241	8.7	145	242	8.8	140	248	8.4	140	241
	6.9	7.7	129	238	8.5	136	234	8.7	145	232	9.0	143	230	8.5	138	233
N rate (avg)		7.9	135	242	8.5	141	243	8.8	146	242	9.0	146	243	8.5	142	243
1970-1971	8.0	8.4	136	267	8.8	141	272	9.0	142	272	9.2	148	269	8.8	141	270
	5.6	8.3	132	254	8.6	139	261	8.7	142	260	8.9	141	265	8.6	139	260
	6.9	7.8	126	257	8.4	133	254	8.8	139	259	9.1	142	251	8.5	135	255
2-year avg		8.1	132	259	8.6	137	262	8.8	141	264	9.1	144	262	8.6	138	262

TABLE 4 Rieti, 1970-1971. Effects of increasing nitrogen rates and plant populations on
p protein percentage of the whole grain at 15.5% moisture
t tryptophan ppm of the whole grain at 15.5% moisture
w weight of 1,000 kernels (g)

Year	Plants/m²	Nitrogen (kg/ha) 0 p	0 t	0 w	50 p	50 t	50 w	100 p	100 t	100 w	150 p	150 t	150 w	Average of populations and years p	t	w
1970	8.0	7.9	133	276	8.4	138	293	9.0	141	272	9.2	147	283	8.6	140	281
	5.6	7.2	128	276	8.0	129	275	8.5	145	260	8.4	141	270	8.0	134	265
	6.3	7.2	128	265	8.0	131	256	8.4	140	259	7.8	134	279	7.8	132	265
N rate (avg)		7.8	126	266	8.1	133	275	8.6	142	264	8.5	141	277	8.2	135	270
1971	8.0	8.7	121	258	8.6	119	253	9.3	131	256	9.4	132	247	9.0	126	253
	5.6	8.3	113	249	8.5	122	251	9.2	129	252	8.8	122	244	8.7	121	249
	6.3	8.3	113	258	8.6	111	249	8.9	119	245	9.0	123	248	8.7	116	250
N rate (avg)		8.4	116	255	8.6	117	251	9.2	126	251	9.1	126	246	8.8	121	251
1970-1971	8.0	8.3	127	267	8.5	128	273	9.2	136	264	9.3	139	265	8.8	133	267
	5.6	7.7	117	252	8.2	125	263	8.8	137	256	8.6	131	257	8.2	127	257
	6.3	7.7	118	261	8.3	123	253	8.6	129	252	8.4	128	265	8.2	124	257
2-year (avg)		7.9	121	260	8.2	125	263	8.9	134	257	8.8	133	261	8.5	128	260

the medium rate in Rieti). Tryptophan production increased in a similar manner.

While protein content steadily increased with increased nitrogen fertilization (Tables 3 and 4), tryptophan in grain remained proportional to the total protein content.

Increasing plant populations resulted in large increases in grain production. Protein and tryptophan production also increased, but at lower rates, owing to lowering of the protein and tryptophan content and lower kernel weight.

Average results over 2 years for each of the two locations are shown in Figures 1 and 2. For the different characters measured, the percentage of variation due to nitrogen rates of fertilization was referred to the mean of the three plant densities at N = 0. The percentage of variation due to plant population was referred to the mean of the lowest population density (4.0 plants/m²) at four rates of nitrogen fertilizer.

The quality of protein of the opaque-2 hybrid as measured by relative tryptophan content was not altered substantially, whereas grain yield in-

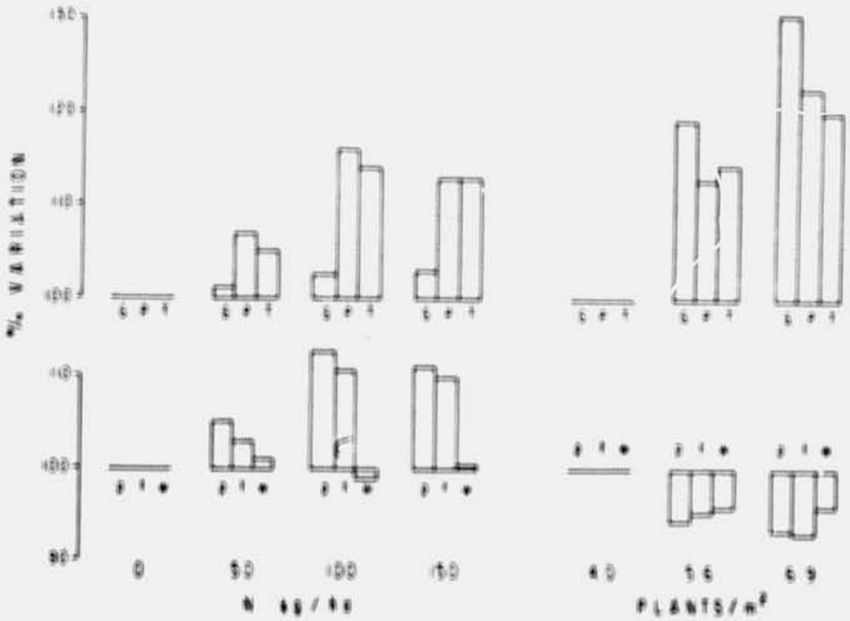

FIGURE 1 Bergamo, 1970 – 1971 averages. Percentage of variations due to nitrogen increasing rates (means of three populations) and to increasing plant population (means of four nitrogen rates)

G	grain yield	p	protein (%)
P	protein production per hectare	t	tryptophan (ppm)
T	tryptophan production per hectare	w	weight of 1,000 kernels

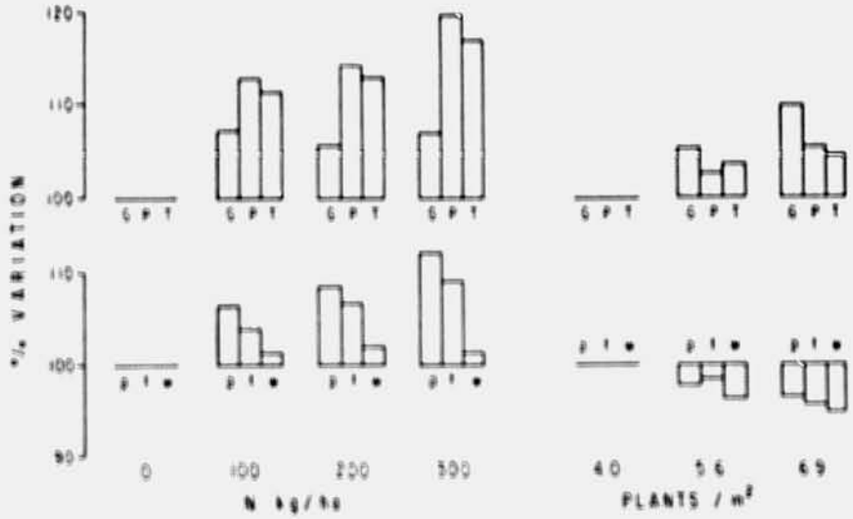

FIGURE 2 Rieti, 1970–1971 averages. Percentage of variations due to nitrogen increasing rates (means of three populations) and to increasing plant population (means of four nitrogen rates).

G	grain yield	p	protein (%)
P	protein production per hectare	t	tryptophan (ppm)
T	tryptophan production per hectare	w	weight of 1,000 kernels

creased owing to nitrogen fertilization or population increase. When nitrogen fertilization did not induce higher grain yields, protein and tryptophan increases in the grain were evident.

STATUS OF RICE PROTEIN IMPROVEMENT

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Rice protein is one of the most nutritious of all cereal proteins. However, the protein content of milled rices is low (7% protein at 14% moisture). This report provides a brief analysis of efforts being made at the International Rice Research Institute to improve rice protein.

PROTEIN CONTENT AND DISTRIBUTION

Second only to starch, protein is the most abundant constituent of rice endosperm (18). In Asian diets, milled or polished rice (*Oryza sativa* L.) provides 40 to 80% of the calories and at least 40% of the protein. The bran (aleurone layers and germ) contains more protein than the starchy endosperm, so the proportion of protein lost in milling is higher than the proportion of weight lost (18). Brown rice usually contains 8% protein and milled rice contains 7% protein (18, 19).

Endosperm protein exists mainly as discrete, 1- to 4-μm particles located between the compound starch granules (Figure 1) (10). The particles are called protein bodies. They are smaller and more numerous in peripheral cells and near the cell walls. Protein bodies have a lamellar structure (25), and their amino acid and protein fraction composition is the same as that of milled rice protein (26).

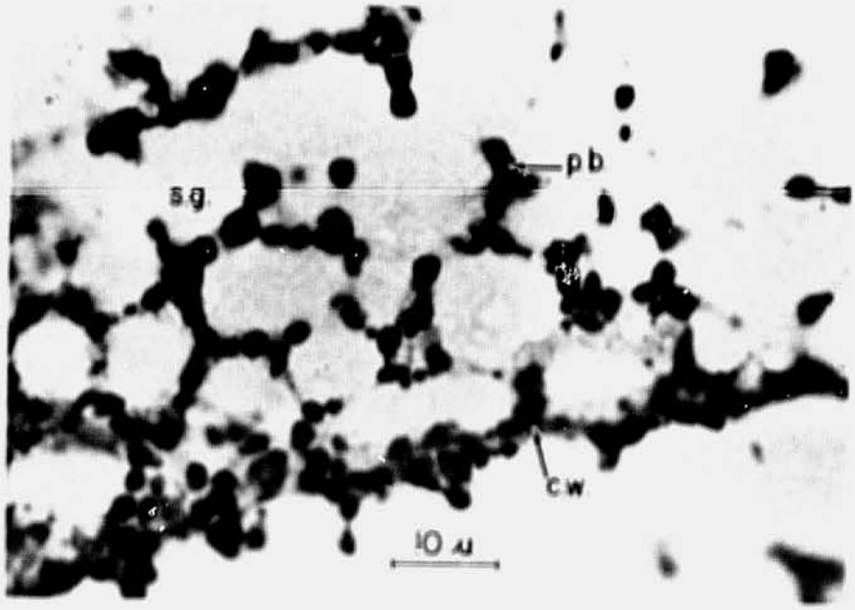

FIGURE 1 Cross section of endosperm of rice variety BPI-76 (11% protein) showing stained protein bodies (p.b.), compound starch granules (s.g.), and cell wall (c.w.) (10). Stain: $HgCl_2$ – bromphenol blue

PROTEIN FRACTIONS, NUTRITIONAL VALUE, AND AMINOGRAM

Milled rice protein is unique among cereal proteins because it is at least 80% glutelin (Table 1) (5, 16). The other fractions, including prolamin, are

TABLE 1 Levels of essential amino acids* and ratio of protein fractions of rice endosperm of IR 8

Property	Protein fraction				Milled rice protein
	Albumin	Globulin	Prolamin	Glutelin	
Isoleucine	4.0	3.0	4.7	5.5	4.1
Leucine	7.9	6.6	11.5	8.2	8.2
Lysine	4.9	2.6	0.5	3.5	3.8
Methionine	2.5	2.5	0.5	2.6	3.4
Methionine + cystine	5.4	2.5	0.8	4.1	5.0
Phenylalanine	3.0	5.5	6.5	5.4	6.0
Phenylalanine + tyrosine	6.9	8.5	15.0	10.5	11.0
Threonine	4.6	4.6	2.9	3.9	4.5
Tryptophan	1.9	1.5	0.9	1.2	1.2
Valine	6.7	6.2	7.0	7.5	7.2
Ratio	5	10	<5	>80	100

Source: Tecson et al. (33).
* In grams/16.8 g N.

present in small amounts. Albumin and globulin are concentrated in the bran, whereas prolamin is evenly distributed in the grain (5).

Compared with other cereal proteins, milled rice protein has an excellent nutritional value due to its high content (about 4%) of lysine, the first limiting essential amino acid of cereal proteins (18, 19). Rices lysine content is similar to that of the endosperm protein of opaque-2 maize (23). Among the protein fractions, albumin has the highest lysine content, followed by glutelin, globulin, and prolamin (Table 1) (33). The high lysine content of rice is due to a low prolamin content (5, 16, 28). Milled rice protein generally has lower lysine content than brown rice protein (18, 19) because of its lower albumin content (5).

BREEDING FOR HIGHER PROTEIN

Breeding Objectives

The International Rice Research Institute (IRRI) began a screening and breeding program in 1966 to increase the protein content of the rice grain. Genetic differences in protein content exist among varieties grown under similar cultural management (2, 17, 20), and it appears reasonable to expect that the protein content of brown rice can be increased 20 to 25%. This corresponds to an improvement of milled rice protein content by 2% (from 7 to 9%).

The IR-8 variety grown by farmers has the same protein content as their traditional varieties, despite its higher yield (14), indicating that protein is not strongly negatively associated with grain yield. If farmers are to accept them, high-protein varieties must yield as well as other varieties. The new varieties must have adequate disease and insect resistance, improved plant type, and acceptable grain quality. Thus, the IRRI will have to produce a series of varieties representing the different grain qualities preferred in tropical Asia, since most national rice-breeding programs lack adequate facilities for protein analysis of breeding lines.

Variability in Protein Content

Protein content (at 14% moisture) may vary as much as 6% within a single variety of rice (17, 20). In IR-8, it varies from 6 to 12% (17). Protein content is generally higher in the grains from the bottom of a panicle (14) and in the grains from early, tall panicles within a hill at high nitrogen fertilizer levels (Figure 2). Protein content is generally lower in grains from plants with close spacing (9) and higher in border hills and in hills next to missing hills (14), at the same application rate of nitrogen fertilizer. Higher application rates of nitrogen fertilizer, particularly during the reproductive stage, tend to increase protein content (14). S-triazine and urea derivatives applied at sub-herbicidal levels during the reproductive stage also increase protein content.

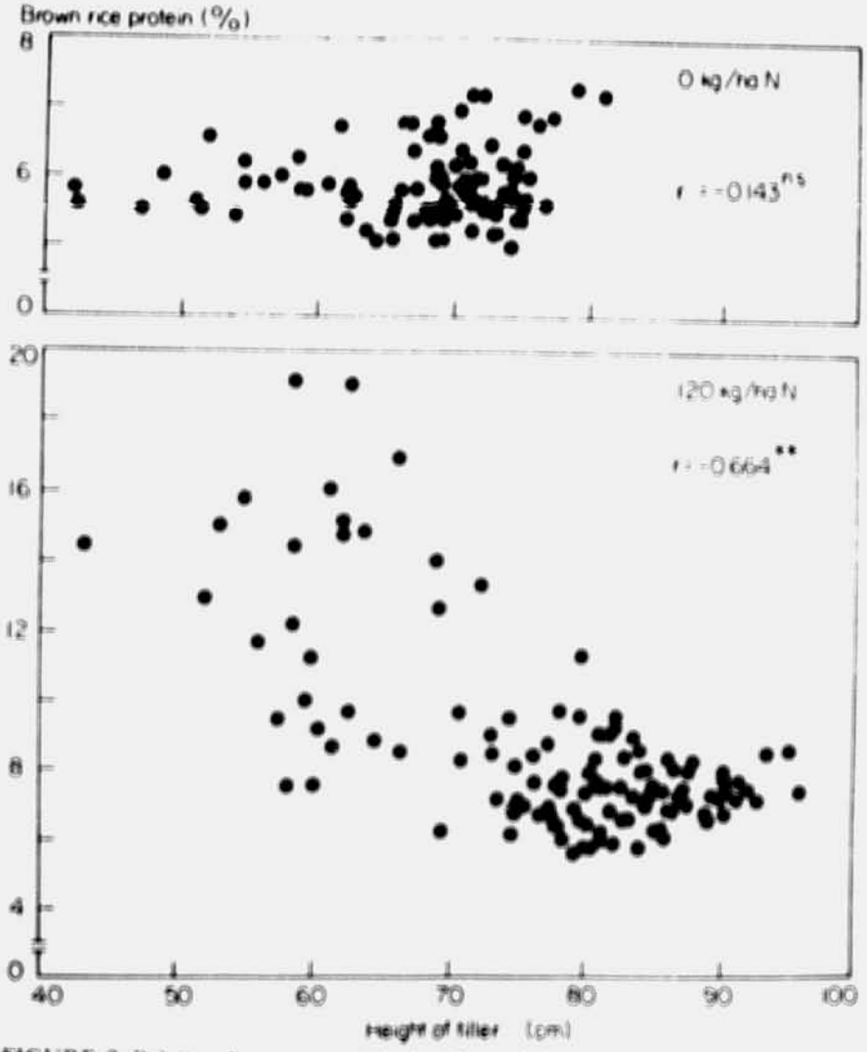

FIGURE 2 Relation between panicle height and brown rice protein content of unfertilized and fertilized (120 kg of N/ha) IR-8 hills, 1970 dry season (14)

independently of nitrogen fertilizer applications (9). Protein content tends to be lower if high solar radiation occurs during grain development (30); thus, protein content is generally lower in the dry season than in the wet season. It has been found extremely difficult to identify varieties with genetically high protein content for use in the breeding program, particularly when the parents are tall, photoperiod sensitive, or otherwise not adapted to the Los Baños environment.

Experimental Techniques

Early field trials showed wide variability in protein content, even in the check variety IR-8. However, later studies showed that protein variability of transplanted flooded rice is minimized by a 20- by 20-cm spacing of two plants per hill and uniform basal nitrogen fertilizer application. Reducing protein variability is essential, because of the low heritability of protein content.

In our work, samples for protein analysis are obtained from the total threshed grains from a hill or from the bulk samples used in determination of plot yields. A 10-grain sample from a hill and a 2- to 5-g sample from a plot are used for analysis. Rapid grinding of dehulled samples is done with a Wig-L-Bug amalgamator for 10-grain samples and a Udy cyclone mill for larger samples. Analysis is done by manual micro-Kjeldahl digestion followed by colorimetric ammonia assay of digested samples with an Auto Analyzer (21). Rice protein is N × 5.95, based on the 16.8% N of rice glutelin (18, 33).

Status of Breeding Program

Several semidwarf breeding lines have been developed at the IRRI that appear to have higher protein content than IR-8 (Table 2) (2). IR-480-5-9

TABLE 2 Relative performance (IR-8 = 100) of several breeding lines and varieties during the 1971[a] and 1972[b] dry seasons

Variety or line		Brown rice protein (N × 5.95)		Rough rice yield	
		1971	1972	1971	1972
IR-8		100	100	100	100
IR-480-5-9	Nahing Mon 5-8/2 × IN-1	123 (5)[c]	120 (8)[c]	95 (5)[c]	85 (8)[c]
IR-160-27-5	Nahing Mon 5-8 × 28-1	118 (5)	106 (2)	96 (5)	96 (2)
IR-667-98-1	IR-8 × (Vaksein × IN-1)	117	107	112	85
IR-1105-15-4	IR-8 × Chow-aung	156 (2)	126 (2)	68 (2)	68 (2)
IR-1105-89-4	IR-8 × Chow-aung	151	125	70	65
BPI-76-1		125 (5)	124	82 (5)	76
IR-1006-26-1	IR-8 × BPI-76-1	115	110	99	82
IR-1006-55-1	IR-8 × BPI-76-1	109	104	90	84
IR-1165-124-1	IR8/2 × BPI-76-1	110	106 (2)	82	85 (2)
IR-1165-155-2	IR8/2 × BPI-76-1	n.d.	104 (5)	n.d.	91 (5)
I.R. (1915)-12	Irradiated IR-8	111	110	91	74

Source: Brandell et al. (2).

[a] IR-8 mean brown rice protein = 6.2 to 8.6%, rough rice yield = 5.1 to 7.5 tons/ha; 60 to 120 kg of N/ha applied basally.

[b] IR-8 mean brown rice protein = 6.5 to 8.5%, rough rice yield = 6.5 to 7.7 tons/ha; 60 to 120 kg of N/ha applied basally.

[c] Number of replicated trials other than 1.

appears to be the most promising of these lines in trials in the 1971 and 1972 dry seasons, and was first identified as having high protein content by IRRI agronomists (2, 9). In repeated experiments the grain yield of the new line has been about the same as IR-8 in the dry season (when bacterial leaf blight infestation is minimal), but it usually has over 20% more protein.

In an experiment conducted by IRRI agronomists in the 1972 dry season, IR-480-5-9 matured at about the same time as IR-22, yielded 8.7 tons/ha, and had 10.8% brown rice protein. IR-22 yielded 8.3 tons/ha with 9.3% protein. IR-480-5-9 has an erect tiller arrangement, matures about 1 week earlier than IR-8, and yields less than IR-8 at low fertility levels. It is susceptible to bacterial leaf blight. It has slightly longer, more slender grain, and seldom shows white belly spots. IR-480-5-9 has an intermediate amylose content and a low gelatinization temperature, which gives it a more acceptable eating quality in the Philippines and Indonesia (17).

All other high-protein semidwarf lines were not consistently higher than IR-8 in protein yield in the 1971 and 1972 dry seasons (Table 2). IR-160-27-3 is not always as high in protein as IR-480-5-9. IR-667-98-1 is a sister line of the higher-protein Korean variety Tongil and has an IR-8 plant type. The better semidwarf lines [from crosses of IR-8 with six high-protein varieties screened from the IRRI world collection (21)], such as IR-1103-15-8 and IR-1103-49-4, usually have at least 20% higher protein content than IR-8, but only 65 to 70% of the IR-8 grain yield (2). The Philippine varieties BPI-76 and BPI-76-1 have produced high-protein-content grain (4, 5), particularly when nitrogen fertilizer is added during flowering. Several semidwarf lines with higher yield than BPI-76-1 resulted from crosses of BPI-76 with IR-8, but are not as high in protein as is BPI-76-1. L4 (1915)-12 has about 10% higher protein than IR-8 and has the IR-8 plant type. These lines and IR-480-5-9 are being crossed among themselves in an attempt to combine divergent sources of high protein content.

Backcrosses and triple crosses were made in 1971 and 1972 to combine high grain yield and high protein content with resistance to disease (particularly bacterial leaf blight) and insect pests. IR-1103 lines and IR-480-5-9 are being used as the high-protein parents. The early generation, high-protein lines from these crosses are being screened for resistance to bacterial leaf blight.

IRRI geneticists are studying the breeding behavior of protein content, using 72 reciprocal crosses involving semidwarf parents.

EFFECTS OF HIGHER PROTEIN ON GRAIN PROPERTIES

Protein Distribution

Protein becomes more evenly distributed in the grain as protein content increases (15). Hence, if 10% (by weight) of the bran is removed from a

sample of brown rice with 8% protein, the milled rice will have 7.3% protein; but milled rice from a sample of brown rice with 10% protein will have 9.4% protein (Figure 3). Histological studies show that the increase in protein content is mainly due to more protein bodies in the endosperm, with a slight increase in their size in some samples (15). Mutants from *gamma* irradiation with high protein content (13.4 to 14.8%) have normal-size protein bodies, however (15). Kjeldahl analysis of brown rice (10- or 20-grain sample) is a more reliable index of protein distribution than is histochemical screening of individual grains (15), which have variable protein content (2, 14).

Grain Quality

High-protein brown rice tends to have higher hardness scores (hard texture) and head (whole-grain) rice yield during milling than does rice with normal protein content (15). It is also more resistant to abrasive milling (5). The thickness of the aleurone layer of brown rice is not necessarily as

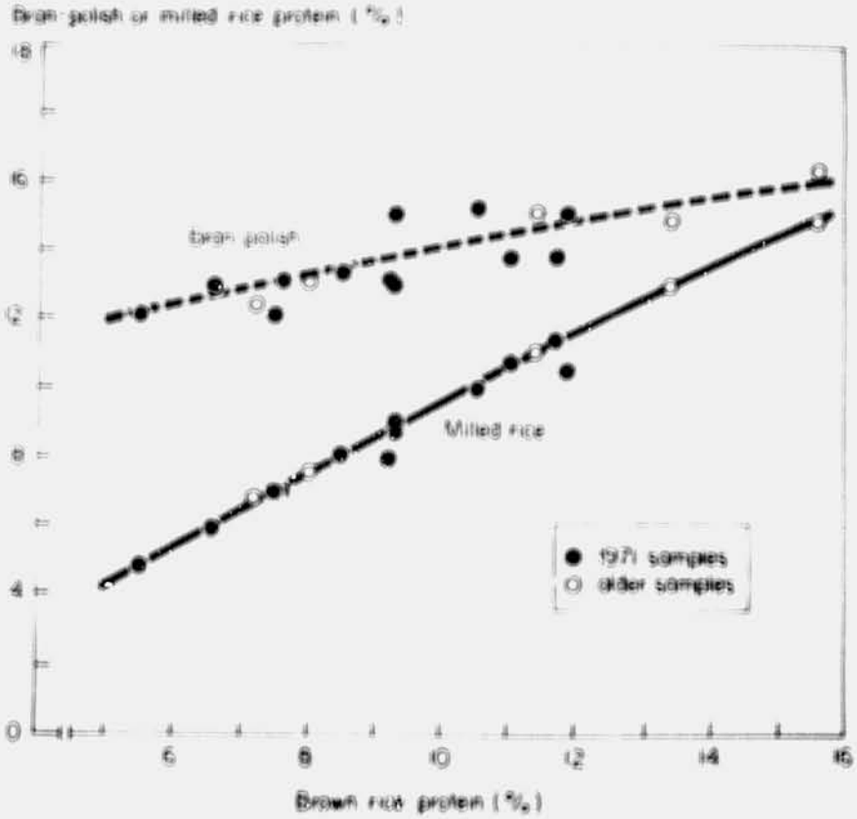

FIGURE 3 Relation between protein content of brown rice and its milling fractions at 10 ± 1% (by weight) bran polish removal (15).

sociated with an increase in protein. Milling removed less than one endosperm cell layer from IR-480-5-9 brown rice.

Although an increase in protein content of 3 or 4% may adversely affect grain color (22), preliminary studies with breeding lines (from the same crosses) differing in protein content indicate that a 2% increase in protein does not necessarily increase the color score of boiled rice (Table 3) (15). IR-480-5-9 milled rice with 9% protein had texture and color scores similar to varieties with 7% protein content. High-protein rice takes longer to cook (22) and tends to be more resistant to disintegration on overcooking than is rice with normal protein content; rice protein, being mainly water insoluble, acts as a physical barrier.

Protein Composition

A large increase in protein content seems to be associated with higher levels of glutelin and prolamin in brown rice (5, 16) and a lower, but significant, increase in albumin (15). The ratio of protein fractions may not vary widely with a 2% increase in protein content, and the lysine content of protein may not decrease by more than 0.3% (4). For example, IR-480-5-9 milled rice with 8.2% protein has 3.8 g lysine/16.8 g N, and another sample with 11.2% protein has 3.5 g lysine/16.8 g N. The lysine content of brown rice above 10% protein is essentially constant, in contrast to the negative relationship between lysine content and protein content when the rice is below 10% protein (Figure 4) (15). Presumably, the effect of increased prolamin content on the lysine level of rice protein is offset by the increase in albumin content.

Nutritive Value

Feeding trials with experimental rats (Table 4) (4) and with human subjects (7) confirmed the nutritional superiority of higher-protein rice due to

TABLE 3 Mean scores by a laboratory taste panel for cooked milled rice of lines from two crosses differing in protein content

Line	Protein (% N × 5.95 at 14% moisture)	Amylose (% dry base)	Mean taste panel score[a]			
			Tenderness	Cohesiveness	Taste	Color
IR 1100-185-3[b]	8.9	26.2	5.2	4.0	2.2	4.0
IR 1100-85-2[b]	10.9	25.4	4.5	3.2	2.2	4.0
IR 1105-64-4[c]	8.6	27.1	4.7	3.7	2.2	4.5
IR 1105-15-8[c]	17.8	25.2	3.5	2.0	1.8	3.7
IR 1105-15-9[c]	12.7	25.0	3.2	2.0	1.5	3.8
LSD (5%)			1.6	1.4	1.3	1.3

Source: International Rice Research Institute (15).

[a] Mean score by a taste panel of six. Numerical scores of 1 to 9 were assigned, 1 representing the least expression of the property in question and 9 the highest expression.

[b] IR-8 × Rikuto Norin 20.

[c] IR-8 × Chow-sung.

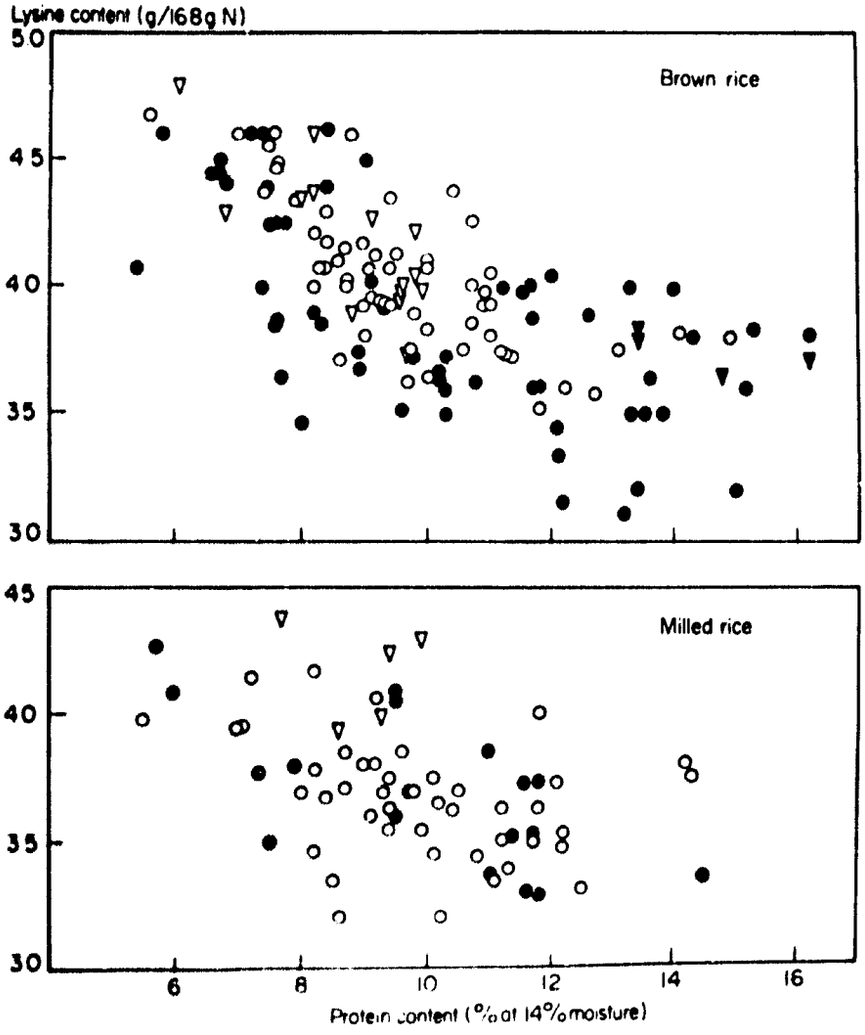

FIGURE 4 Relation between protein content and lysine content of protein of brown and milled rice (15). Open circles, 1971 samples; closed circles, earlier samples; open triangles, higher lysine samples; closed triangles, IRB-100 samples.

the higher levels of all essential amino acids, including lysine, in the milled rice (4, 7). Protein digestibility in rats (11) and humans (7) was not affected adversely by the higher protein content of milled rice. These studies show that a 2% increase in milled rice protein would improve protein intake by at least 10% for diets of growing children and adults in tropical Asia (based on 40% of protein from rice). Weaned children fed mainly rice gruel would increase their protein intake by 25%. In addition, the thiamine content of milled rice also increases with increased protein (15). These increases would reduce the nutritional deficiency among rice-eating peoples.

TABLE 4 Summary of protein quality indexes for four milled rice samples based on weight gain in white rats

Variety	Protein content (%N X 5.95)	Lysine content (g/16.8 g N)	0 to 5% dietary protein: Relative quality (%)[a]	0 to 5% dietary protein: Utilizable protein (%)[b]	Data showing net growth: Relative quality (%)[a]	Data showing net growth: Utilizable protein (%)[b]
Intan	5.7	4.3	80	4.6	47	2.7
IR-8	7.8	3.8	75	5.5	46	3.4
IR-8	9.7	3.7	71	6.9	43	4.2
BPI-76-1	14.3	3.4	57	8.2	42	6.0

Source: Bressani et al. (4).
[a] Based on 75 for casein.
[b] Relative quality X protein content/100.

NITROGEN METABOLISM

In contrast to the results reported for rice by Miller and Mikkelsen (24) and by Schweizer and Ries (31) for other cereals, our studies indicated that seed protein level had no consistent effect on the weight and nitrogen content of 1-month-old rice seedlings grown in soil (14, 15). Field studies at the IRRI in the 1972 dry season showed the rices with a high yield of grain protein translocated more nitrogen from the leaf blades to the developing grains than did rices with average protein yield, regardless of the protein content of the grain (29). Cagampang et al. (6) found that rices with a high yield of grain protein had more free amino acids in the bleeding sap collected from the culm of plants at the mid-milky stage. Since similar volumes of sap were collected in the pairs of lines studied (6), rices with a high yield of grain protein presumably have rates of sap translocation similar to rices with normal protein yield, but more amino acids in the sap.

The developing (mid-milky) grains of IR-480-5-9 had a higher level of soluble protein and free amino nitrogen and a greater capacity for amino acid incorporation than did the grains of IR-8 (29). This finding agrees with our previous results on other rices (8). Thus, the rate of nitrogen translocation from leaf blades to developing grains appears to be directly related to the yield of grain protein, but the rate of protein accumulation in the developing grains appears to be related directly to the percentage of grain protein (29).

SCREENING FOR HIGHER LYSINE

Lysine Variability

High-lysine rices have been reported (32). Although consumption surveys (1) and feeding trials in preschool children (3) show that lysine is not the first

limiting essential amino acid in rice diets, lysine may be the first limiting essential amino acid in rice diets if high-protein rice replaces rice with normal protein content. A higher-lysine rice might benefit weaned infants who are fed mainly rice gruel; however, such an infant diet still would lack the calories and protein for proper infant nutrition (12).

A high-lysine rice can be used to improve the lysine content of promising high-protein rices, such as IR-480-5-9. Preliminary studies indicate that lysine content tends to vary by not more than 0.5 g/16.8 g N of the mean value at any protein level (Figure 4) (15). Samples of 11 wild species of *Oryza* and two interspecific hybrids have aminograms similar to those of cultivated rice (13). Samples of the IRB-100 gamma-irradiation mutants from the Institute of Radiation Breeding, Ohmiya, Japan, which were reported to be high lysine (32), showed average lysine contents varying from 3.6 to 3.9 g/16.8 g N in brown rice (15).

Screening and Identification

To verify the constancy of lysine content in rice, the dye-binding capacity (DBC) was measured for 10,493 varieties in the IRRI world collection. The DBC is a good index of the lysine content of brown rice protein (15). Acilane Orange G dye binds specifically with basic amino acids (27), and only lysine varies with the protein level in rice protein (6, 14, 15). A decrease in the slope of the DBC as a function of protein content occurred above 10% protein, reflecting the change in the relationship between lysine content and protein level at 10% protein (Figure 4).

Thirty-eight varieties had DBC values higher than the mean at their particular protein levels. These were analyzed for lysine content by ion-exchange chromatography with S-β-(pyridylethyl)-L-cysteine as a standard (15). Of these 38 varieties, only two varieties in two crops had lysine contents of 0.5 g/16.8 g N above the mean value (Figure 4). Thus, potentially, lysine content might be raised only 0.5 g/16.8 g N. Lysine analysis is complicated by a variation in lysine content of 0.3 g/16.8 g N within a variety at a particular protein level. In addition, our least significant difference (LSD, 5%) for lysine content was 0.3 g/16.8 g N. The two higher-lysine rices, ARC-10525 and Kolamba-45, had protein bodies of smaller mean size (1.7 to 2.0 μm), in contrast with 2.3 to 2.8 μm for other rices with normal lysine content as measured by light microscopy (15). Variants from these two varieties, with at least 1 g/16.8 g N higher lysine content than the mean value for rice protein, are being screened on a single-plant basis by the DBC technique.

This article was supported by Contract No. PH-43-67-726 from the National Institutes of Health, U.S.A., April 10, 1967, to May 9, 1973, and by a grant from the Rockefeller Foundation, July 1, 1966, to April 9, 1967.

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HETEROSIS OBSERVED IN OPAQUE-2 HYBRIDS FOR YIELD AND YIELD COMPONENTS

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Studies reported here were made on heterosis observed for yield and yield components in different types of opaque-2 hybrids with parents genetically alike in some degree. [The author recently published a summary of his evaluations of similar research with normal maize (1).] Discussion centers on the main results of the measure of heterosis in several opaque single-cross hybrids and an opaque varietal hybrid.

MATERIALS AND METHODS

Open-pollinated varieties, Illinois Syn A o_2, Illinois Syn B o_2, and IHP o_2, were selfed for five generations, and selection was made for the most important traits. From these selections, 28 opaque inbred lines were selected and crossed with two inbred testers: Syn B 6-26 o_2 and IHP 1-1 o_2. Forty plants from each opaque-2 hybrid were examined individually for ear characteristics and maturity at Martonvásár during 1971. Of the 28 inbred lines developed from Syn A, eighteen belonged to Syn A 3, seven to Syn A 4, and three to Syn A 5. Heterosis was expressed as *heterosis index*, the ratio of the observed value of the hybrid to that of the average of its two parents.

RESULTS AND DISCUSSION

Table 1 summarizes the average heterosis index for yield and yield components in the 56 opaque single crosses produced on the testers, Syn B 6-26 and HHP 1-1. Data show that heterosis for various yield components varied in the opaque single cross hybrids studied. The average heterosis index was highest for ear length (144.1%), followed by 119.2% for kernel length, 119.1% for number of kernels per row, and 114.4% for the 1,000 grain weight. Of all the components, the number of rows per ear had the smallest heterosis index (103.4%). The average heterosis index for earliness (96.2%) was not very large; under the conditions studied, the hybrids were only 3.8% (3 to 4 days) earlier than the parental lines.

Table 2 shows the yields of hybrids produced on the two testers. Remarkably, the genetic behavior of the lines tested was essentially the same on both the testers. Yields of Syn A 3-30, Syn A 3-41, Syn A 3-37, and Syn A 3-34 were excellent, practically 1 kg dry grain yield in a 1 m^2 area. Similar grain yields have been reported for the best normal hybrids.

Data in Table 2 also show considerable differences in the value of the hybrids of sublines Syn A 3, Syn A 4, and Syn A 5. The performance of hybrids produced on Syn A 3 sublines is especially interesting. The lowest yield of these hybrids was 85.4% of the mean, and the highest, 118.9% — a difference of 33.5%. The specific combining ability of Syn A 4 sublines had a similar tendency. In this group, the lowest yield was 85.4% of the mean, and the highest, 112.2% — a 26.8% difference. On the basis of these results, the lines tested appear to possess different genetic values, as was the case with the normal inbred lines (2).

The relationship between the performances of hybrids produced on the two testers is shown in Figure 1; the correlation coefficient was $r = 0.7444$, significant at the 0.1% level.

Heterosis for yield and yield components also was studied in one opaque-2 varietal hybrid. This hybrid was produced by crossing Syn A o_2 with Syn B o_2.

TABLE 1 Average heterosis index of the yield components and yield of 56 opaque hybrids produced by crossing with the testers Syn B 6-26 and HHP 1-1

Pedigree	1	2	3	4	5	6	7	8
28 opaque lines X Syn B 6-26 Heterosis index	149.4	99.1	121.7	114.2	118.2	103.4	229.7	95.0
28 opaque lines X HHP 1-1 Heterosis index	138.8	107.6	116.4	124.3	110.6	107.3	217.2	97.5
Mean heterosis index	144.1	103.4	119.1	119.2	114.4	105.4	223.4	96.2

Notes: 1, ear length (cm); 2, number of rows; 3, number of kernels per row; 4, kernel length (mm); 5, 1,000-grain weight (g); 6, shelling percentage; 7, yield per plant (g); 8, days to mid-tasseling.

TABLE 2 Yield of 56 opaque hybrids produced with the testers Syn B 6-26 and HHP 1-1

No.	Lines	Yield per plant (g)			Yield in percentage of the mean
		Syn B 6-26	HHP 1-1	Average	
1	Syn A 3-8	235.0	187.5	211.2	102.0
2	Syn A 3-11	205.0	193.7	201.2	97.2
3	Syn A 3-12	222.5	193.7	208.1	100.5
4	Syn A 3-13	206.2	192.5	199.4	96.3
5	Syn A 3-17	180.0	173.7	176.8	85.4
6	Syn A 3-18	208.7	180.6	194.6	94.0
7	Syn A 3-19	173.7	162.5	168.1	81.2
8	Syn A 3-25	210.0	187.5	198.8	96.0
9	Syn A 3-27	212.5	197.5	205.0	99.0
10	Syn A 3-28	235.0	218.7	226.8	109.5
11	Syn A 3-30	232.4	260.0	246.2	118.9
12	Syn A 3-32	233.7	216.2	225.0	108.6
13	Syn A 3-34	233.7	245.0	239.4	115.6
14	Syn A 3-35	217.5	213.7	215.6	104.1
15	Syn A 3-37	255.0	233.7	244.4	118.0
16	Syn A 3-38	225.6	219.3	222.4	107.4
17	Syn A 3-41	263.7	228.7	246.2	118.9
18	Syn A 3-43	261.2	205.0	233.1	112.6
19	Syn A 4-2	261.2	203.7	232.4	112.2
20	Syn A 4-3	196.2	170.0	183.1	88.4
21	Syn A 4-4	181.2	172.5	176.8	85.4
22	Syn A 4-5	193.7	165.6	179.6	86.7
23	Syn A 4-8	212.5	181.2	196.8	95.0
24	Syn A 4-13	207.5	186.2	196.8	95.0
25	Syn A 4-14	191.2	163.7	177.4	85.6
26	Syn A 5-1	200.0	191.2	195.6	94.4
27	Syn A 5-4	221.2	203.7	212.4	102.6
28	Syn A 5-5	196.2	177.5	186.8	90.2
	Mean	216.9	197.3	207.1	

Table 3 shows yield and yield components of the parental varieties and their hybrid. The table indicates a decrease in heterosis index of all the yield components and grain yield as compared with the heterosis index of opaque-2 single crosses. In comparison with the mean heterosis indices in Table 1, ear length decreased by 32.5%, number of rows by 2.6%, number of kernels per row by 16.6%, kernel length by 9.5%, 1,000 grain weight by 3.6%, and shelling percentage by 4.5%. However, the heterosis index for yield decreased by 144.7%, probably owing to the normal yielding ability of the parental varieties.

Table 3 also shows that the Syn A × Syn B combination yielded nearly 10% more than the average of the parents. A similar situation was shown in the results of the National Yield Trials (3): on seven experimental stations, Syn

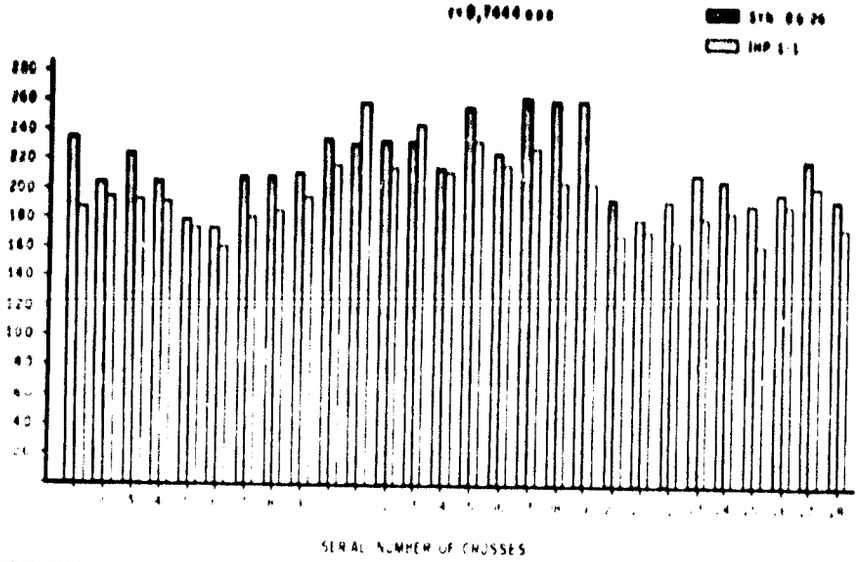

FIGURE 1 Relationship for yield of 56 opaque hybrids produced with the testers Syn B 6-26 and HHP 1-1

A × Syn B produced an average grain yield of 6,860 kg/ha; Syn A yielded 6,010 kg/ha.

SUMMARY AND CONCLUSIONS

Evaluation of heterosis in opaque-2 inbred lines, similar to the normal inbreds, is a part of combination development in our breeding program. As a continuation of the earlier studies, present results can be summarized as follows:

1. Heterosis index of yield and yield components varied in the opaque-2 hybrids studied. The heterosis index of the yield components was largest for ear length: 144.1% in the single crosses and 111.4% in the varietal cross. Number of rows showed the smallest heterosis index: 103.4% for single

TABLE 3 Yield components and yield of opaque varietal hybrid produced with Syn A and Syn B

Pedigree	1	2	3	4	5	6	7	8
Syn A × Syn B	23.4	18.5	42.8	8.5	260.5	83.8	198.7	84
Syn A	20.0	18.9	46.5	8.0	238.5	83.5	181.2	83
Syn B	22.0	17.8	43.0	7.5	231.5	82.6	182.5	86
Heterosis index	111.4	100.8	102.5	109.7	110.8	100.9	109.3	99.4

Notes: 1, ear length (cm); 2, number of rows; 3, number of kernels per row; 4, kernel length (mm); 5, 1,000-grain weight (g); 6, shelling percentage; 7, yield per plant (g); 8, days to mid-tasseling.

crosses and 100.8% for the varietal cross. Of all factors studied, grain yield showed the maximum heterosis index. The average heterosis index of the parental lines was 223.4%, whereas opaque-2 varietal hybrids had an average index of 109.3%.

2. There are considerable differences in hybrid values or specific combining abilities of sublines of jeesu A 3, Syn A 4, and Syn A 5. The best of these were Syn A 3-30, Syn A 3-41, Syn A 3-37, and Syn A 3-34. The other sublines can be used for development of homozygote crosses (o_2 line × o_2 line) and with our recently developed procedure for heterozygote hybrids (normal line × o_2 line × o_2 line).

3. The relationship between the performance of hybrids produced on the two testers shows a correlation coefficient of $r = 0.7144$, significant at the 0.1% level.

4. Syn A × Syn B opaque-2 varietal hybrid on seven experimental stations in the National Yield Trials conducted during 1970 produced an average grain yield of 6,860 kg/ha.

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PROTEIN QUALITY AND NUTRITIONAL VALUE OF HIGH-LYSINE MAIZE (*Short Communication*)

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The higher-quality protein of opaque-2 maize, as compared to normal maize, is attributed to significant changes in the proportions of protein fractions (1 – 6). These changes are accompanied by an increase in the proportions of some essential amino acids: lysine levels increase by 50 to 70%, and tryptophan levels by 40 to 50%. Both arginine and aspartic acid show increases of 15 to 20% above the levels of normal maize.

However, some other amino acids decrease considerably; the leucine content of opaque-2 maize decreases to 7 to 8%, compared with 13 to 14% in normal maize; glutamic acid is 15%, versus 20 to 21%; and tyrosine and phenylalanine are 3%, versus 4 to 5% in protein of normal maize.

FEEDING TRIALS WITH YOUNG PIGS

Breeding studies of opaque-2 hybrids at the Krasnodar Research Institute of Agriculture are being supervised by M. I. Hadjinov. In 1969–1970, the efficiency of opaque-2 maize for feeding pigs was evaluated under commercial production conditions on farms in the Krasnodar area. The evaluation consisted of a single procedure incorporating several designs (7).

Table 1 summarizes the data of the feeding trials for young pigs. Under

TABLE 1 Efficiency of high-lysine maize for pigs (25 to 100 kg) fed diets with different protein levels (summarized data from farms in the Krasnodar area)

Group	Composition of diets	Protein in diet (%)	Average daily weight gain		Feed efficiency		Protein efficiency	
			Grams	% of control	kg feed/kg gain	% of control	g protein/g gain	% of control
1	Opaque-2 maize (77%), sunflower oil meal (8%), pea (7%), alfalfa meal (4%), yeast (1.7%), mineral mixture (2.3%)	15.0	544	105	4.83	94	725	94
2	*Control*: normal maize (77%); the other components are the same as in group 1	15.0	519	100	5.16	100	774	100
3	Opaque-2 maize (85%), sunflower oil meal (3%), pea (3%), alfalfa meal (4%), yeast (2%), mineral mixture (3%)	12.5	522	111	4.99	87	624	87
4	*Control*: normal maize (91%); the other components are the same as in group 3	12.5	469	100	5.75	100	719	100
5	Opaque-2 maize (91%), yeast (2.3%), alfalfa meal (4%), mineral mixture (2.7%)	11.0	522	128	5.42	86	596	86
6	*Control*: normal maize (91%); the other components are the same as in group 5	11.0	408	100	6.28	100	691	100
7	Opaque-2 maize (93%), alfalfa meal (4%), mineral mixture (3%)	10.0	430	193	5.87	66	587	66
8	*Control*: normal maize (93%); the other components are the same as in group 7	10.0	223	100	8.88	100	888	100

Note: All the diets were enriched with vitamin B_{12} (25 mg) and vitamin D_2 (200 IU) per kg of feed.

extreme conditions, the daily weight gain for the pigs fed opaque-2 maize with 10% protein was about double (430 versus 223 g) that of the pigs fed normal maize, feed efficiency (kilograms of feed per kilograms of gain) was 5.87 versus 8.88 kg for normal maize. The trials indicated that opaque-2 could provide a diet producing carcass quality equal to the normal maize diets, but with a saving of 20 to 24% of the protein fed with normal maize.

In physiological experiments with young (33 kg) Landrace pigs, the factors studied were levels of digestibility, nitrogen retention, lysine availability and content of free amino acids in blood plasma (with both restricted food intake and *ad libitum* feeding), and diets containing opaque-2 grain and normal maize grain supplemented with vitamins, mineral microelements, and macroelements. The animals were fed protein-free diets for 7 days after 20 days of maize feeding to determine endogenous nitrogen excreta (feces and urine). The coefficients of apparent digestibility, nitrogen retention, and lysine availability for opaque-2 maize were much higher than those obtained for normal maize for both types of feeding (restricted and *ad libitum*). However, these data did not differ essentially for two types of maize grain, when considering endogenous excreta in their true expression (Table 2).

When pigs are fed the unbalanced protein of normal maize, the essential losses of endogenous nitrogen can be accounted for by the higher metabolic waste of proteins and amino acids for digestion, and by absorption and synthesis of the body's protein. This explanation is supported by tests of the concentration of free amino acids from blood plasma samples taken from fasting pigs; the concentrations for the pigs fed opaque-2 diets did not differ significantly from those obtained from pigs fed normal maize. For example, with the restricted food intake treatment, the plasma lysine level for normal maize was 1.89 mg %, and on the opaque-2 maize it was 2.12 mg %; for *ad libitum* feeding, the plasma lysine level was 0.68 mg % for normal maize and 0.77 mg % for opaque-2 maize.

The total amount of plasma amino acids was 35.66 mg % for the pigs fed opaque-2 maize and 31.25 mg % for normal maize (restricted intake). For *ad libitum* feeding, the total amount of plasma amino acids was 26.83 mg % for opaque-2 maize and 26.46 mg % for normal maize.

EXPERIMENTS WITH RATS

In two other experiments, weanling (48-g) male rats of a Wistar inbred line were fed, individually, a semisynthetic diet similar to normal maize in its amino acid content. Analysis was made of the efficiency of increase or decrease of some amino acids, with the levels of their concentration varied around those levels found in opaque-2 maize (Table 3).

By simultaneously supplementing lysine and tryptophan in the amounts observed in opaque-2 maize, rat growth rates were obtained which were

TABLE 2 Coefficients of digestion, nitrogen retention, and lysine available for pigs fed normal and opaque-2 maize

Item	Unit	Treatment			
		Restricted		*Ad libitum*	
		Opaque-2	Normal	Opaque-2	Normal
Coefficient of N digestion					
Apparent	%	82.1 ± 1.1	77.7 ± 2.5	79.1 ± 1.4	71.8 ± 5.22
True	%	89.4 ± 1.0	87.7 ± 3.1	84.6 ± 1.6	82.4 ± 5.3
Lysine availability					
Apparent	%	83.7 ± 2.3	78.5 ± 1.2	87.9 ± 0.3	77.9 ± 1.4
True	%	89.0 ± 1.4	88.7 ± 0.8	92.5 ± 0.5	90.1 ± 0.6
N retained/N absorbed					
Apparent	%	56.0 ± 1.3	51.3 ± 2.0	61.1 ± 4.9	37.4 ± 1.9
True	%	68.0 ± 1.5	66.4 ± 4.8	71.2 ± 3.5	57.4 ± 2.8
Endogenous N (intestinal)					
Lg/N true digested	g	0.081	0.113	0.065	0.132
in % of opaque-2	%	100.0	139.5	100.0	203.1
Total waste of endogenous N μg					
g N true retained	g	0.162	0.211	0.134	0.361
in % of opaque-2	%	100.0	130.3	100.0	2.694

TABLE 3 Effects of increased or decreased content of some amino acids in opaque-2 protein on growth rate and feed conversion in rats (initial body weight, 48 g; feeding period, 21 days; six rats in each group)

Group	Composition of diets	Experiment 1		Experiment 2	
		Daily weight gain (g)	Feed/gain (g)	Daily weight gain (g)	Feed/gain (g)
1.	Opaque-2 maize (93%), vitamins (2%), mineral mixture (3%), sunflower oil (2%)	1.70	5.10	1.84	5.53
2.	Normal maize (93%), vitamin mixture (2%), sunflower oil (2%), mineral mixture (3%)	0.81	10.5	0.66	10.80
3.	Normal maize (60%), mixture of crystal amino acids (4%), vitamins (2%), mineral mixture (3.5%), sunflower oil (4%), maize starch (26.5%) (amino acid composition is the same as in group 2)	0.78	9.54	0.90	8.90
4.	Group 3 + lysine (0.35%)	–	–	1.15	7.54
5.	Group 3 + tryptophan (0.08%)	–	–	1.12	7.13
6.	Group 3 + lysine (0.35%) + tryptophan (0.08%)	1.36	5.91	1.85	5.08
7.	Group 6 + leucine (0.52%)	2.11	3.97	2.14	4.63
8.	Group 7 + phenylalanine (0.19%) + tyrosine (0.16%)	2.09	4.27	–	–
9.	Group 8 + aspartic acid (0.17%)	2.46	3.94	–	–
10.	Group 9 + glutamic acid (0.5%)	2.10	4.20	–	–
11.	Group 3 + leucine (0.52%)	–	–	1.15	7.46

similar to those observed for the opaque-2 maize (group 6). Addition of these amino acids individually was not very effective (groups 4 and 5). Aspartic acid supplements were effective. High rates of growth were observed when leucine content was reduced to the level at which it is found in opaque-2 maize ($p < 0.001$ for experiment 1 and $p < 0.05$ for experiment 2). Lower concentrations of phenylalanine, tyrosine (group 8), and glutamic acid (group 10) did not affect the growth rate significantly, but did increase some feed–gain ratios.

Notes: For the first experiment Syn A o_2 was taken; for the second, high-lysine hybrid K-81. To maintain the same N content in all the groups with supplementation or reduction of amino acid concentration in the diets, the total amino acid mixture was increased or decreased for the same amount. Mixture of crystal L-amino acids contained (% diet) alanine, 0.3; arginine, 0.22; aspartic acid, 0.27; cystine, 0.09; glutamic acid, 0.72; glycine, 0.15; histidine, 0.1; isoleucine, 0.14; leucine, 0.53; lysine, 0.12; methionine, 0.09; phenylalanine, 0.19; proline, 0.36; serine, 0.19; threonine, 0.14; tryptophan, 0.03; tyrosine, 0.16; valine, 0.2.

The data showed that the nutritional value of opaque-2 maize was associated positively with (1) increased lysine and tryptophan content, (2) greatly decreased leucine content, and (3) increased aspartic acid concentration, resulting in a better total amino acid composition for feeding animals.

The Krasnodar Institute is developing high-protein, high-lysine maize that combines 15 to 17% protein with 0.65 to 0.70% lysine in the grain. These initial lines have protein quality equal to milk protein, a finding that is substantiated by our experiments with rats (Table 4). When the animals were fed a diet of high-protein maize with 0.54% lysine, the growth rate was only a little less than that of animals fed 60 or 100% protein from dry skimmed milk (group 5).

Data from 2-year trials in the State Performance Nursery suggest that high-lysine maizes are being developed rapidly that will compete in grain yields with the high-yielding normal varieties.

According to data obtained from five locations in the Krasnodar area in 1970, the yields of the best high-lysine maize hybrids ranged from 510 kg/ha below the standards of normal maize to 410 kg/ha above. In data from 14 locations in the North Caucasus in 1971, the Ukraine and Moldavia hybrids showed a yield range from 210 kg/ha below the normal maize standards to 450 kg/ha above. The biological trials of these hybrids indicate that their nutritional value is similar to the initial stock of opaque-2 maize.

SUMMARY

1. When feeding pigs opaque-2 maize, it is possible to reduce protein intake up to 20%, as compared with normal maize, and obtain similar growth rate.

TABLE 4 Weight gain and feed/gain in rats fed high-protein opaque-2 maize (the twenty-first day of the experiment)

Group	Composition of diets	Experiment 1				Experiment 2			
		Protein (%)	Lysine (%)	Average daily weight gain (g)	Feed/gain (g)	Protein (%)	Lysine (%)	Average daily weight gain (g)	Feed/gain (g)
1.	High-protein opaque-2 maize	16.5	0.54	3.4	3.13	14.7	0.55	2.69	3.8
2.	High-protein normal maize	17.3	0.30	0.75	8.76	–	–	–	–
3.	Opaque-2 maize	10.3	0.39	1.67	5.10	8.3	0.33	1.87	4.8
4.	Normal maize	9.8	0.25	0.82	10.49	8.4	0.22	0.76	8.4
5.	Normal maize (60%), dry skimmed milk (30%), sunflower oil (5.8%), mineral mixture (3%), vitamins (2%)	–	–	–	–	14.7	0.75	2.92	3.1
6.	Dry skimmed milk (45%), sunflower oil (8%), maize starch (41%), fiber (2%), mineral mixture) (2%), vitamin mixture (2%)	–	–	–	–	15.9	1.00	2.91	2.9

Note: The diets of groups 1 to 4 contained 92% maize grain, 2% vitamin mixture, 3% mineral mixture, and 3% sunflower oil.

2. Apparent digestibility, nitrogen retention, and lysine availability are much higher for opaque-2 maize than for normal maize in feeding trials with pigs.

3. The considerable waste of metabolic nitrogen shown for normal maize is associated with lower digestibility and nitrogen retention.

4. High biological values of this new type of maize can be attributed to higher lysine and tryptophan concentration and to complete change of the total amino acid composition—the significant decreases of leucine, particularly.

5. High-protein maize, with increased lysine content, exhibits a superior nutritional value similar to the protein value of dry skimmed milk.

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Part XII

Questions and Answers

PAPER BY BRESSANI (pp. 38–57)

Q: (1) How many grams of lysine and tryptophan per 100 g of protein were present in the supplemented maize that gave PER values of 2.4?
(2) Was opaque-2 maize–soybean mixture equal to or better than normal maize–soybean–lysine–tryptophan?

R: (1) There were several diets that had a PER value of 2.4. Therefore, it is difficult to know which one you are referring to. However, in all cases the supplementary levels of lysine and tryptophan were 0.35 and 0.05%, respectively, whether from crystalline amino acids or supplementary protein.
(2) The two diets gave the same results. The average nitrogen retention values of four experiments showed equal results from opaque-2 maize–soybean and normal maize–soybean–amino acids.

Q: (1) Should breeders of opaque-2 maize now be concerned with improving total protein, or lysine and tryptophan, or both—particularly as it might benefit young children?
(2) What are the desirable levels of total protein and lysine?

R: (1) Quality protein is, of course, essential for efficient use of protein; therefore, lysine and tryptophan levels higher than in normal maize are important. However, it is also important to increase protein content from about 9 to 14%.
(2) Higher protein will allow children to obtain their protein needs with less bulk. The desirable levels of lysine and tryptophan would be about 300 mg of lysine/g N and about 70 mg of tryptophan/g N.

Q: What is the medical treatment of the children in the diet trials? Could just medicine, plus hygiene treatment, skew your results?

R: When a child comes into the metabolic unit (if he comes in with protein malnutrition signs), he is first given electrolyte solutions, followed by the feeding of vitamins and calories. A few days later he is given protein, with small amounts first, and then increasing levels up to 2 to 3 g/kg/day. Recovery takes 2 to 3 months. During this period of time, the child is cured of infections or parasites. Recovery is followed by a series of medical and biochemical measures. It is essential to standardize the health of children as much as possible, since the extent of protein depletion will interfere with protein evaluation.

Q: What happens to a maize–soybean diet if supplemented only with minerals and vitamins, or only with lysine?

R: Minerals and vitamins will increase protein quality from about 1 to 1.9. With lysine only, I do not know. However, one can predict that tryptophan will become a limiting amino acid.

Q: In your opinion, should corn breeders be concerned with isoleucine, as well as lysine and tryptophan in protein quality improvement? Is it the level of isoleucine alone, or the ratio between isoleucine and leucine, that is important?

R: I do not think breeders should be concerned with isoleucine. First, protein improvement in opaque-2 maize involves a change in protein distribution. There is less zein, which has a high leucine content. Secondly, maize usually is eaten along with other foods that provide isoleucine.

Q: When the maize–soybean diets were fed to children, how many grams of protein were fed per kilogram of body weight?

R: 1.25 g of protein/kg/day plus 100 kcal/kg/day.

PAPER BY MANER (pp. 58–82)

Q: Although endosperm analysis in vitreous grain was found to be almost the same as opaque-2 endosperm, your study indicates that vitre-

ous endosperm was not better than normal. You indicated that perhaps this might be due to availability of amino acids. Nevertheless, if I understood correctly, vitreous whole grain was shown to be better than opaque-2 whole grain. How would you account for this?

R: You did not correctly understand the results. Opaque-2 grain gave better performance than Mexican modified (vitreous) when fed as whole grain to rats. The modified was much superior to normal maize. The endosperm of the modified was much inferior to the endosperm of opaque-2 maize (ICA H-208). The modified endosperm was equal to normal maize whole grain. This would be expected, because the normal maize also contained the germ and more than 9.5% crude protein, whereas the modified endosperm contained only about 6.4% protein. As you know, the germ modifies the protein quality; and protein level is very important in growth and PER. The modified corn (Mexican) was grown in Cali, Colombia, and our analysis showed that it contained slightly less lysine than H-208 opaque-2.

Q: Dr. Maner has found low nutritional values for vitreous opaque-2 high-lysine endosperm, as compared to normal maize grain when fed to rats. Are these results expected if endosperms are processed as usual for human consumption?

R: We would expect similar results, as indicated by Dr. Pradilla.

Q: In your studies, daily gain in body weight was much higher with a diet of maize plus soybean 10% protein, as compared to an opaque-2 10% protein diet. However, opaque-2 plus lysine plus tryptophan plus threonine 10% protein was better than maize plus 10% soybean diet. Would you please comment on the possible reasons?

R: A 10% diet based on normal maize plus soybean meal contains a better balance and level of amino acids than does opaque-2 maize, which is deficient in lysine, tryptophan, and threonine. When these diets based on only opaque-2 maize are supplemented with these amino acids (plus isoleucine to counteract the excess of leucine or to provide a better leucine–isoleucine ratio), then you provide a balance and level of amino acids that gives results equal to or better than a maize–soybean 10% diet.

Q: Have amino acid analyses been run on the vitreous opaque-2 maize endosperm? What differences account for the poor feeding studies with rats? Would this difference be due to kernel structure?

R: The grain of the vitreous maize (Mexican) was grown in Cali, Colombia, and upon analysis contained slightly less lysine than the opaque-2. I do not feel that structure is important for rats, although for voles it may be.

PAPER BY SCHEUCH AND FRANCIS (pp. 102–119)

Q: Is the lysine content of the double-mutant (fl_1/fl_1, o_2/o_2) better than the single-mutant opaque-2? Many double mutants are superior to opaque-2 alone in lysine content.

R: Although we probably have these double and single mutants in highland converted maize varieties, they have not been positively identified and compared. Dr. Cassalett reported that the double mutant is intermediate in quality between opaque-2 and floury-2 single mutant types in lowland backgrounds.

Q: Have you observed any deviation from normal floury endosperm phenotype in the double-mutant opaque-2/floury-2 introduced into Andean maize?

R: We do not have a double-mutant opaque-2/floury-2 in the highland materials in any country in the zone. Present information suggests that the double mutant with floury-1 appears to be identical to the single mutant with opaque-2 alone, in most backgrounds.

Q: Do you think that a qualitative test, such as the staining of the endosperm, might separate samples with high protein quality from those with low protein content? Both materials would have low zein content.

R: That may well be a limiting factor in the use of such a scheme. Many highland materials are low in protein (sometimes from 5 to 7%) owing to the large starch granules. We should probably use other methods with these extreme types, or else concentrate our conversion efforts on varieties with a more reasonable protein level.

Q: In calculating the possible frequency of various genotypes and phenotypes of the floury-1 and opaque-2 combinations, have you taken into account the 3*n* nature of endosperm and possible modifications this will produce in the endosperm character frequencies?

R: All these combinations were calculated, but the precise proportions do not appear in the paper. The exercise is academic in part, owing to the dosage effect of the floury-1, and the probable modifying effects of many of these high-altitude backgrounds. In other words, the precise calculated frequencies would be found rarely in practice.

Q: Since grain color (pericarp or seed-coat color) has been shown to have some effects on nutritional value of the grain in sorghum, is it not conceivable that we might have been overlooking possible effects of certain color maize types on nutritional value? This might be checked further with animal feeding trials with black, red, and other colors.

R: This is possible. There exists a wide range in colors among the races

of maize, both highland and tropical, and these have not been studied as far as I know.

Q: Are you not concerned about the identical phenotypes of the native floury-1 varieties and those converted to opaque-2? How will these be distinguished for commercial purpose and how can a premium be given to the high-quality types?

R: This could be a problem. However, since the great majority of this floury maize in the Andean zone highlands is converted directly, it never moves beyond the farm where it is grown. This minimizes the problem, but we might search for an appropriate marker linked to the opaque-2, which would help in selecting and positively identifying the high-quality types.

PAPER BY DUDLEY ET AL. (pp. 120–135)

Q: Your comment that hard-endosperm opaques are not necessary for the United States—is it based on the fact that you got similar yields with opaque-2 as compared with normals? What about the other parameters, such as losses in harvest, more vulnerability to stored-grain insects, and possible difficulties in utilization owing to soft-endosperm character?

R: The comment was based on similar yield with soft-endosperm types. Since our yield trials were machine harvested, differences in harvest losses were taken into account. Although soft-endosperm types may be acceptable, more rapid acceptance might be obtained with harder-endosperm types.

Q: Heritability values in general were rather high, especially those for grain yield. Could you explain why they were so high?

R: Two factors are probably involved in the heritability values. The experiments were planted at relatively high plant populations (55,000 plants/hectare), which increased genetic variance because of barrenness in some families. Secondly, the experiments were grown in only one location in 1 year. Thus, any genotype–environment interaction would bias the heritability estimates upward.

Q: In your opinion what would be the genetic explanation for higher heritability estimates for yield than those for percentage of lysine? Does this indicate environmental influence or maybe measurement limitations?

R: Higher heritability for yield is largely the result of higher genetic variance for yield with similar error variance. Note the genetic C.V.'s and C.V.'s in tables. I think both environmental influences and measurement limitations are involved.

Q: What selection pressure are you using in your population improvement? Would tandem selection, giving priority first to yield and secondly to percentage of lysine, provide high yield and good protein with less laboratory dependence?

R: Selection intensity was one family selected out of five. A tandem selection program should be effective and practical. However, I have not calculated its predicted effectiveness relative to other systems.

Q: How did you collect samples for lysine and protein analyses?

R: Bulk samples were collected from each plot directly from the stream of shelled corn coming from the sheller and were thoroughly mixed prior to analysis.

Q: What sort of a breeding program or selection scheme do you recommend for converting the opaque-2 lines to vitreous opaque-2 lines?

R: We do not have enough information on genetic control of the modifiers studied to make precise recommendations. However, any system appropriate for a quantitative character with high heritability should work.

Q: Do you have any observations about ear molds on opaque-2 hybrids compared to normals?

R: We do not have detailed experimental data. However, many opaque-2 hybrids appear to be resistant while others are susceptible.

Q: Protein percentage is a function (among other variables) of starch production in the grain, and this is highly regulated by environment. Rather than express selection gains for lysine and protein in terms of percentages, wouldn't it be opportune to think in terms of total value of lysine and protein per kernel and thus, through component multiplication, their value per hectare?

R: Our selection program is based on kilograms of lysine per hectare. Hence, I agree that lysine or protein per hectare is probably of more practical importance than percentage values. However, a certain percentage of lysine is necessary for appropriate growth response from diets. Therefore, both percentage and absolute amounts of nutrients need to be considered.

Q: You indicated low heritability for lysine grams per 16 g N. With barley, our experience is exactly the opposite, as indicated by dye-binding analyses with ion-exchange lysine verifications. What analyses do you use? Do you consider the negative correlation of lysine grams per 16 g N to crude protein?

R: The analyses used are cited in the manuscript. We did not try to con-

sider the negative correlation of lysine grams per 100 g of protein in selecting for lysine per hectare.

Q: I thought that selection for higher protein leads to lower lysine per 100 g of protein. Was your conclusion that, when breeding for more protein, you could obtain more lysine per hectare or per 100 g of protein?

R: The conclusion was that selection for percentage of protein increases the percentage of lysine in the whole kernel.

Q: In your research in selecting for lysine and yield in synthetics, might your results have been different if you could have used animal-feeding trials as a base for determining gains or improvement rather than chemical values? Should this not be the approach if the technique could be worked out, rather than the chemical technique?

R: If animal-feeding response were related to yield in a different manner than is the percentage of lysine in the grain, the results would have differed. I would agree that animal-feeding trials are the ultimate test. However, for intensive selection programs, large numbers of samples per day are required. To date, the cost of animal-feeding trials is much greater than chemical analytical techniques.

PAPER BY JOHNSON (pp. 139–153)

Q: Do you have information on the relative performance of *planta baja* and brachytic-2 types in comparable backgrounds?

R: The *planta baja* appears to be more tolerant of high plant populations and gives relatively higher yields as plant density is increased. At lower stand density, grain yields tend to be about equal.

Q: In normal corn, yield is usually taken at 15.5% grain moisture content, on the average. What figure is used for opaque-2 corn to get an adequate measure of yield (test weight per bushel)?

R: Moisture percentage can be arbitrarily set at any desired level for calculating yields, but there would appear to be no good reason for setting a different level as compared to normal corn.

Q: It seems to me that each cycle yield represents an average yield of crossed plants where heterotic effects are contributing to some degree. This would mean a reduction of yield following random mating crosses if that cycle were to be released as an open-pollinated variety. Do you have any information concerning this possibility?

R: You would assume a possible yield reduction might occur if the matings being made were above average in performance, as compared to

random matings. Otherwise, there would be no reason to expect a drop; and, in fact, the open-pollinated increases of seed derived from these materials have not shown reduced levels thus far in the trials.

Q: Why did you use full-sib selection in population improvement? Did you see a particular advantage to other selection, such as S_1 selection?

R: The decision was in large measure an arbitrary one, but the principal reason was to utilize a system that provided rapid recombination in terms of time. The S_1 procedure requires a longer time to complete a cycle, and therefore would need to provide a much higher gain per cycle to justify its use.

Q: East African, high-altitude materials show a positive response to environment and altitude, whereas materials from low-altitude programs do not. What does your *planta baja* material do?

R: The meaning of "positive" response is not clear, but the *planta baja* selections can be moved farther into other environments than we have found possible with the original populations. This may be at least in part a function of the shorter length of growing cycle required for the *planta baja* types, and their improved resistance to lodging. We really have not attempted to study the point you raised.

Q: Are reciprocal selection progeny being tested over several altitudes for wide adaptation, for example, from 100 to 3,000 m elevation?

R: Although we would like to evaluate progenies in as widely varying environments as possible, there are limits. The tropical materials simply do not mature at 3,000 m elevation, so we are not using such an extreme at this time. There is no differentiation provided among materials at such an extreme environment.

PAPER BY SPERLING (pp. 154–165)

Q: The reaction of Francés Largo in your experiments seems to be atypical when compared to the average of other populations. Can you describe the variety, its origin, relationships, and so on?

R: Francés Largo is an eight-rowed dent from the Dominican Republic that belongs to the race Chandel and is characterized by a small-diameter cob. The normal entry—but not the opaque-2 conversion—was extremely susceptible to stalk rots, thus resulting in low yield.

PAPER BY ORTEGA ET AL. (pp. 178–192)

Q: You gave information about the relative susceptibility to attack by insect of individual families of maize. What is the situation if the stored-

grain insects pests are given free choice of normal, opaque-2, and hard-endosperm opaque-2?

R: Our free-choice tests have indicated that soft-endosperm types (regardless of the presence or absence of the opaque-2) are considerably more vulnerable to stored-grain insects than are materials with harder endosperm.

Q: (1) Moisture content of normal, opaque, and modified grain in storage at 75° F, plus 85% relative humidity, varied over the storage period. Why?
(2) Was consideration given to determining the presence of aflotoxin on the moldy opaque and modified corn ears?

R: (1) All grains, as a whole, tend to reach a moisture stability or equilibrium that varies with the relative humidity of the environment where they are placed.
(2) No, we got information concerning the rate of development of *Aspergillus* spp. only.

Q: You showed slides with good infections of *Fusarium* spp. Did you artificially inoculate the ears? If so, would you briefly describe the procedure of inoculation you used. Also, would you describe the procedure for the artificial inoculation with *Diplodia* spp.?

R: As shown during the presentation, not all the experiments in the different locations were inoculated. At Tlatizapan, we inoculated with a mixture of spores of both *Diplodia* spp. and *Fusarium* spp. by spraying the silks a week after emergence. Diplodia is grown in soaked, sterilized whole oat kernels for about 4 months. *Fusarium* is grown in a mixture of opaque-2 corn and wheat straw, also for about 4 months.

Q: The speaker surveyed interesting disease and insect trends in the opaque-2 maize improvement program. East Africa has been hit very badly by army worm and streak virus attack, which the speaker did not mention. Is any work being done on these two serious maize pests?

R: We are working towards the development of a fall army worm resistant population that originated from Caribbean germ plasm, and results show we are improving its resistance. Streak virus is a disease that does not exist in Mexico. On the other hand, a stunt-resistant population has been developed from Caribbean germ plasm.

Q: The covering of the ear, especially tight over the tip, has been observed to be important in the incidence of various diseases and insects. Was this observed? It would seem to be a worthy breeding objective, and there are great differences in other materials, if not the ones you are working with.

R: We have observed variable results with materials with tight husks, as far as their reaction to ear-rotting organisms in the field. We consider there are additional factors, not only the husks, playing an important role in disease resistance. Our presentation indicated, and your question also suggests, the close relationship found between earworm damage and incidence of ear rots. We also have found that maize cultivars with tight husks (for example, Zapalote Chico, a Tuxpeño called *hoja blanca* from Central Veracruz) and a tight "silk channel" are less damaged by earworms.

Q: Do you think the effect you described, the susceptibility to some pathogens of soft-endosperm opaque-2, can be attributed to physical, nutritional, moisture, or what factors of the endosperm?

R: We have not studied the cause of susceptibility or resistance in some materials, because all the opaque-2 versions do not show susceptibility, as shown during our presentation. It depends on the genetic background. Perhaps, in the case of susceptible genotypes, the combination of all the factors you have mentioned, plus others, is the reason for such a susceptibility.

Q: In your breeding programs for desired resistance, it would appear most desirable to get into a program of developing populations for specific diseases—ultimately developing multiresistance. You would have every known possible source of genetic resistance observed throughout the world as inputs into these populations. The population could be rather specific, with the opaque-2 being included.

R: We are trying to include as many resistance factors as possible in our breeding program. Opaque-2 conversions are carried simultaneously with the development of the corresponding normal populations.

Q: Are there any differences in insect and disease reactions between opaque-2 and floury-2 phenotypes?

R: We have not worked with floury-2.

PAPER BY VASAL (pp. 197–216)

Q: Why not introduce a genetic seed color marker in opaque-2 maizes to clearly distinguish them from normal maize?

R: We can introduce the seed color marker in opaque-2 maizes rather easily. However, farmers and consumers in a particular area have definite preferences for a certain grain color; therefore, introducing color to the opaque-2 seed may pose some problem in its acceptance.

Q: In the diallel analysis of various vitreous types, you refer to P_1, P_2, and so on. Were these stocks homozygous?

R: The parents used in developing the diallel were fairly true breeding for modified phenotype opaque-2 character, but may or may not be completely homozygous.

Q: Have you found the endosperm of modified opaques to be a little softer than that of the true normal types? It seems so to me.

R: Visually there seems to be no difference between the vitreous fraction of modified opaques and the true normal types. The vitreous fraction of modified types may be somewhat softer, but we have made no such determinations as yet.

Q: What effect does location of vitreous fraction have on weevil resistance. Which location would you recommend?

R: The plant protection group at CIMMYT has conducted a test in which they included opaques and modified opaque-2 materials. The entry of the weevils into the modified opaque-2 grain was at random and not essentially restricted to the soft fraction. Therefore, it is not certain which location of the vitreous fraction contributes to weevil resistance.

Q: Your data have suggested an increase in protein content and marked decrease in tryptophan (% g protein). What was the situation with respect to tryptophan content on a sample basis?

R: On a sample basis, there was also a decrease in tryptophan.

Q: Some of your data indicates a lower yield in the modified versions as compared to their opaque-2 counterparts. What is the explanation for this? In relation to this point, would you like to elaborate on your selection scheme for modifiers with reference to sample size, selection intensity, and selection criteria?

R: The material that Dr. Sperling used in his study (especially the modified types) came from the first cycle of selection, and it is likely that many ears that were selected came from progenies that have below-average performance. However, in the data that I presented here, most of the modified types have undergone further selection and most of them were superior to their opaque counterparts. Modified types compared to normal counterparts were somewhat lower in yield, but the differences in most cases were not significant. Furthermore, since gene action controlling modifiers is of partial dominance, such selection schemes as full-sib, half-sib, and S_1 (which exploit additive genetic variance) could be expected to succeed in accumulating the frequency of favorable mod-

ifiers. First, the larger the sample size, the better it is, however, this again depends on the frequency of modifiers in the opaque-2 population. Second, intensity at approximately 20% would be adequate. And third, with regard to selection criteria, one needs to consider all agronomic traits together in families that throw a high frequency of modified phenotype kernels and also have acceptable levels of protein quality and quantity.

PAPER BY BAUMAN (pp. 217–227)

Q: Is opaque-2 corn oil higher or lower in polyunsaturates than oil from normal corn?

R: It would be slightly lower.

Q: Has a comparison been made of the yield of opaque-2 hybrids as a function of available potassium?

R: I believe such a study is being made in Brazil. However, I would not expect a great difference, because the difference in quantity of potassium on an area yield basis is quite small.

Q: Have you obtained 100% true-breeding modified types?

R: Yes, for all practical purposes. However, the degree of modification is influenced by environment. We generally have a greater expression in the Florida winter nursery.

Q: Since you found a positive and significant correlation between protein and P_1 magnesium and zinc, do you think there is a possibility that the accumulation and translocation of these elements are limiting factors in protein or lysine content?

R: I doubt it, but really do not know. This relationship should be investigated further.

Q: Can one incorporate the vitreous opaque-2 type in otherwise agronomically desirable opaque-2 inbreds by backcrossing technique?

R: It may be possible, but an alternating selfing and backcross procedure should be tried.

Q: How can one be sure that the vitreous kernels that approach normal phenotype are actually opaque-2 and not normal?

R: This is quite easily done by crossing the vitreous type as male to a standard opaque-2 type, which will give an opaque-2 phenotype.

PAPER BY ZUBER AND HELM (pp. 241–252)

Q: Your data suggest that h^2 (realized heritability) was often larger than 1. How do you justify this value?

R: We cannot account for this increase in the lysine content of cycle 2 over cycle 1. Possibly, it is due to environmental effect as both cycles were grown in different years. As soon as we complete cycle 3, we intend to grow all three cycles plus the original population under the same environment for an evaluation of selection progress.

Q: What happens to the concentration of protein and lysine in whole-kernel samples of multiple aleurone materials?

R: At this time we do not have sufficient information to answer this question. We plan to compare the amino acid and protein content for single-aleurone layered kernels with multiple layered ones in the same genetic background, as soon as we have suitable homozygous material.

Q: Do you have data on the grams of lysine per 100 g of endosperm protein in the first and second cycles?

R: The number of grams of lysine per 100 g of protein has been included in the paper. For all three populations the second cycle showed an increase over the first cycle in number of grams of lysine per 100 g of protein.

Q: Could you be more explicit concerning the method by which Wolf et al. looked for protein bodies?

R: More detailed information on the microscopic method of examining endosperm tissue for zein bodies may be found in the paper entitled "Mature cereal grain endosperm: rapid glass knife sectioning for examination of proteins," by M. J. Wolf and W. Kwolek (Stain Technol., 45:277–283.)

Q: The breeding scheme for quality protein maize as exposed in a workable procedure appears to simplify some of the complex aspects of breeding techniques worked out by some of the previous speakers. What would then be the practical advantages of this scheme over those of your colleagues working in the same field, so far as time, effort, expenses, and effectiveness are concerned?

R: Recurrent selection for protein quality improvement in normal maize without major endosperm mutants is slow and tedious. More expense is involved owing to the larger number of plants to be pollinated and the larger number of lysine determinations. However, if it is successful, the

problems now associated with some of the endosperm mutants would be eliminated. Thus, the added expense would be justified.

Q: How does your light transmission machine measure transmission in kernels of different thickness?

R: We measured light transmission of kernels having the same relative thickness. We eliminated those kernels of odd shapes and sizes.

Q: Since the double mutant sh_2 su_1 gives high-quality protein as well as translucent endosperm, do you foresee any possibility for its commercial use?

R: Although the double recessive sh_2 su_1 in the inbred line W64A background had a high lysine content and a hard translucent kernel, I have heard that in other genetic backgrounds one might have the soft, opaque type endosperm. It is my opinion that either the modified kernel types found in certain opaque-2 backgrounds or perhaps recurrent selection for improved protein quality among normal types without major endosperm mutants might be more promising.

Q: Is there a likelihood that in your recurrent selection program some major mutant genes favoring lysine-tryptophan content may accumulate?

R: We have noticed some opaque kernels emerging from these populations and are not sure if these are associated with lysine synthesis. We also have found a new mutant in the Midland population, which we call "floury." This mutant is high in lysine, but not allelic to opaque-2. However, we are not sure whether it is allelic to floury-2. We cannot rule out the possibility of other major genes that recognizable phenotypically accounting for the increase in lysine in cycle 2 over cycle 1.

Q: If I remember correctly, some time ago you were screening normal varieties for high lysine and/or protein. You probably achieved some strains with improved protein quality. Would you please comment on your progress in related laboratory work?

R: Several years ago we surveyed a number of exotic strains and open-pollinated varieties for lysine and protein content. The three varieties selected for the recurrent selection study for quality protein improvement presented here were among those with higher lysine values. Although some of the strains surveyed had high lysine content, we had some reservations as to whether the differences in lysine were real since we later found that germ/endosperm ratios may affect lysine content when determined on a whole kernel basis.

Q: What kind of instrument did you use for precisely measuring the

amount of light transmission? Where could this instrument be obtained, and approximately how much does it cost?

R: The quantity of light transmitted through individual maize kernels was measured with an Aminco Photomultiplier Fluorometrophotometer, Model 4-7102. Details on the procedure may be found in the paper entitled "Quantitative measurement of light transmission through corn endosperm," by Paez, Helm, and Zuber. (Cereal Chem. 45:595–599, 1968)

PAPER BY BROWN (pp. 256–264)

Q: Does your information at this stage substantiate the possibility of vitreous hybrids with high lysine being produced without resorting to incorporation of the opaque-2 gene? How would you differentiate these hybrids in production and commerce from normal hybrids with low lysine concentration?

R: We have not identified nonopaque-2 vitreous types of maize that possess lysine levels comparable to the opaque-2 genotypes. The vitreous and semivitreous types I referred to are all homozygous opaque-2, and apparently possess modifiers that influence the phenotype of the endosperm. Unless some easily recognized genetic marker could be incorporated into normal-endosperm, high-lysine hybrids, one would need to rely on chemical analyses to distinguish them.

Q: Once opaque-2 maize is accepted, the control of protein, lysine, and tryptophan (and possibly the whole amino acid spectrum) will have to be maintained both on the farm and at the commercial delivery depots (particularly if a premium is paid for opaque-2 maize). Have you any views on this?

R: This is a good question. In the United States there are no provisions in the grain trade for monitoring the nutritive quality of maize, or any other feed grain for that matter. If the use of opaque-2 maize becomes widespread, and if the producer is to be compensated for quality (nutritive value), some new system will have to be devised. Because of the difficulties of keeping different lots of grain separate in commercial channels, I expect the first significant amounts of opaque-2 maize produced for commercial trade will be grown under contract with feed companies. These companies will undoubtedly depend upon chemical analyses to determine what they are buying in terms of protein, lysine, and so forth.

Q: Do you have any ideas on how one could detect contamination in either seed or farmer production of the modified opaque-2 types?

R: If opaque-2 types are modified to the extent that they are visibly in-

distinguishable from normal maize, detection of contamination and control of purity will have to depend upon chemical tests, unless the use of an endosperm or pericarp marker proves to be feasible.

Q: Do you expect any likely reduction in soybean acreage or of other protein concentrates following the large-scale cultivation of opaque-2 maize in the near future?

R: This depends upon the price of maize and soybeans. In the absence of any major shift in price ratios, I would not expect large-scale production of opaque-2 maize to influence soybean acreage in the foreseeable future. However, if and when soybean production and soybean needs should reach equilibrium on a world basis, opaque-2 maize might well supplant some soybean acreage.

PAPER BY CASSALETT DÁVILA (pp. 265–267)

Q: Wouldn't a policy of price support (compensation for the lower yield of opaque-2 varieties) defeat the very goals that are being sought with the introduction and utilization of opaque-2 varieties in underdeveloped countries? That is, is it not possible that the price of high-lysine maize will be prohibitive to poor people?

R: No. In Colombia the support prices have always been comparable to those of commercial normal maize. The government also has control over the price of processed products so that cost would not increase. The support price is an incentive to production of high-quality protein maize during the initial steps of promotion.

Q: Do you have any special or quick method whereby lysine is determined on commercial opaque-2 maize in Colombia?

R: No. Commercial production is carried out under contract between the farmer and the processing companies. There is some control over quality of the produce. Furthermore, the opaque-2 maize is easily distinguishable phenotypically from the floury types grown in Colombia.

Q: How important has the pollen contamination problem been in commercial opaque-2 maize field production?

R: There have not been major problems. Contracts for production require that the field be under complete isolation in time or in space. There have not been any problems with the small farmers.

Q: What procedure has been used to determine the award of the opaque-2 price differential? Sample size and procedures? Chemical determinations?

R: The award price was considered as a 10% difference in yield between opaque-2 and normal maize. A government organization named IDEMA takes random samples, and identification is done phenotypically.

PAPER BY ROBINSON (pp. 274–280)

Q: Can modified opaque maize be used for the "flakes" type of breakfast cereal?

R: The present varieties of modified opaque maize cannot be used for this type of cereal because they do not provide grits of the necessary size.

Q: Have any efforts been made to prepare and promote breakfast cereal "flakes" made from opaque-2 maize?

R: Not to my knowledge.

Q: What happens to the lysine and amino acid content when *masa* (or ground maize) is washed in water to separate the starch from the chaff to obtain *ogi*?

R: Being unfamiliar with the total process for making *ogi*, I can only surmise that when modified opaque maizes are used there would tend to be some loss of water-soluble protein in the washing operation.

Q: Will you please explain if there is any difference in the total food value (amount of amino acids) of the cooked product (*tortilla*) of normal and opaque-2 maize?

R: The amount of lysine and tryptophan as a percentage of protein do not have a significant change. The total food value in the opaque varieties remains better than in the normals to the degre that they were in the raw state.

PAPER BY WALL AND PAULIS (pp. 281–290)

Q: Dr. Mertz's data seem to indicate that maize having protein with no *zein* and 100% glutelin would be a very satisfactory food. Your method of measuring *zein* content seems extremely simple and reliable for checking vitreous material or normal maize for low levels of *zein*. Did I understand correctly that only grinding and alcohol extraction plus protein determination are necessary for evaluation of lysine in samples?

R: After the *zein* has been extracted from the weighed sample of ground maize, the dispersion is centrifuged and the saline solution is vigorously added to an aliquot of the supernatant. The turbidity that re-

sults from zein precipitation is measured in a colorimeter at 590 nm. This value is corrected to absorbance per gram of protein based on nitrogen analysis of the meal. Estimates for lysine are obtained from plotting the absorbances of extracts versus lysines determined by amino acid analyzer for a series of maize samples of different genotypes. Again, Dr. Mertz and I both presented data indicating that glutelin varies in composition and quality, and this affects the grain lysine content—although the major factor contributing to poor quality of maize protein is its zein content. This variability in glutelin may account for some of the standard error in this method.

Q: A rapid method of determining the zein content is very important for breeders working on opaque-2 maize. Please give us the details of the procedure, if not already published, and the references if it has been published.
(2) What is the minimum sample size permitted in this analysis; that is, is individual kernel analysis possible?
(3) Are the samples defatted before analysis?
(4) Would you recommend the use of this technique for modified, opaque-2 types?

R: (1) The procedure or rapid zein analysis is being prepared for publication and will be submitted shortly to the *Journal of Agricultural and Food Chemistry*.
(2) We are presently using a 200-mg sample/20-ml extractant. We have successfully used as little as 100 mg with a 10-ml extractant. The procedure has been applied to analysis of endosperm tissue with good results. A different standard curve is necessary for endosperm. We believe the method could be used for analysis of an endosperm from a single kernel with appropriate scaling down of quantities of reagents.
(3) The samples do not have to be defatted before analysis, but if whole fat samples are used, analysis must be performed immediately after grinding the grain. Free fatty acids that form in stored, ground maize increase turbidity values. Defatted samples can be used, but a slightly different correlation curve and a slightly higher error are obtained.
(4) We have demonstrated that this procedure successfully differentiates the high-lysine vitreous types from low-lysine dent maizes.

Q: Did you include very low protein normal maize samples in your zein–lysine regression study?

R: Yes, we included both very low and very high protein maize samples in our study. A protein determination based on Kjeldahl nitrogen analysis is used in our data analysis. The lysine content is most directly correlated with the fraction of total proteins that is zein. If maize samples of similar protein content were examined, a satisfactory negative correlation of lysine to turbidity alone could be obtained.

Q: You cite a coefficient of co relation of $r = 0.87$ between lysine content and turbidity brought about by salt precipitations in opaque-2, floury-2, and normal dents. What is the correlation of turbidity and lysine content within the opaque-2 group? the floury-2 group? normal?

R: Within each class of maize, the correlation coefficients between turbidity of extracts due to zein per gram of protein and lysine per 100 g of protein in the grain are not as satisfactory as for the entire range of lysine variability among all genotypes. Within the opaque-2 group the correlation coefficient was -0.59 and within the normal class the r value was -0.36; but within the floury-2 class the correlation coefficient was only 0.05. Thus, within a group having similar lysine analyses, the method fails to distinguish small differences in lysine content. Some of this failure is due to limitations in the analysis of lysine by the analyzer and to the fact that glutelin variation also results in some lysine differences. The major attribute of this method is that it permits rapid differentiation of high, medium, and low levels of lysine content in screening programs.

PAPER BY MISRA ET AL. (pp. 291–305)

Q: What factors do you think could be involved in causing a change in rating of wheats when fed to determine PER and FER values?

R: PER and FER measure different characteristics. In the usual PER method, rations are formulated to contain equal levels of protein. Since grains do not necessarily contain equal levels of protein, this means that different levels of dilutants, such as starch, must be used. The rating or rank order by the PER method is determined primarily by the level of the first limiting amino acid per weight unit of protein in the test material (lysine in the case of wheat materials). An increase in lysine per unit weight of protein would be expected to result in an increase in PER value. However, (1) differences in digestibility (both energy and protein) and the relative presence or absence of materials in the grain influencing protein metabolism, and (2) utilization, or specific amino acid utilization and metabolism, result in alterations from predicted PER rank values based purely on lysine content per weight unit of protein to actually determined PER values in feeding trials. Unless these factors are specifically protein in nature, they are contained in rations differing in amounts and proportions from the original grain.

In the usual FER method, rations are formulated to contain equal levels of grain. Since grains do not necessarily contain equal levels of protein, the rations will vary in protein content, but in the same proportions as found in the original grains. Thus, a higher-protein wheat will result in the formulation of a ration with a higher protein content than a lower-protein wheat. The rating or rank order by the FER method is primarily

determined by interactions between total protein content and the level of the first limiting amino acid on a per unit weight of *grain* basis. How a specific sample will behave is difficult to predict. A high-protein wheat having a relatively low lysine content on a unit weight of *protein* basis may actually provide more lysine per unit weight of *grain* basis than a lower-protein wheat having a relatively high lysine content on a per unit of *protein* basis. There is much that is unknown in the area of amino acid requirements and protein-level interactions. Differences in digestibility (both energy and protein), and the relative presence or absence of materials in the grain directly or indirectly influencing protein or specific amino acid utilization and metabolism, also influence resultant FER values. Materials causing these influences are present in the ations in the case of the FER method in the same level per weight unit of original cereal. Thus, these secondary factors influencing PER and FER values are the same, but are present in different degrees, depending upon the method used. Bioassays are necessary because these factors cannot be predicted accurately (FER less so than PER) from chemical values.

Since factors determining FER and PER rank value differ in type or degree, it is not surprising that relative values by the two methods are not the same. But they should not be, since they constitute answers to quite different question: The really important matter is selecting the appropriate method. In breeding program chiefly concerned with protein quality, the PER method would be appropriate. In a breeding program concerned with the value of the grain as a source of protein (quality and quantity), the FER method is appropriate for screening procedures. Naturally, the nutritionist would like both, plus half a dozen other procedures. But this is obviously not practical.

Q: Why is it that the colorimetric method used for tryptophan in maize is not as efficient in sorghum?

R: These are the two reasons: first, the tannins in highly pigmented sorghum interfere with the digestion of the protein by papain. Second, the color of the pigments carried into solution interferes with the color obtained from the tryptophan with the glyoxylic acid reagent.

Q: Has the modified Landry–Moureaux extraction procedure that is used in your laboratory been used on other cereals like wheat and barley? In the amino acid profile of fractions II, III, and IV, was the percentage of ammonia similar for each mutant?

R: The Landry–Moureaux method has been used successfully in our laboratory with sorghum. We have submitted a paper on this to the *Journal of Agricultural and Food Chemistry*. The ammonia level in our hydrolyzate varies depending on the amide content of the protein and the

degree of deamination of some of the amino acids by the 6 N HCl. The levels were in the range of 2 to 4%, which we consider "normal."

Q: Can you give a cost estimate per sample for the Landry–Moureaux procedure on protein fractions?

R: We do not have any data on this, as we have used it as a basic research tool. The major cost would be labor.

Q: Are some rapid and simple methods available for estimating fraction V, which your data showed was the most desirable?

R: No. Our original alkaline copper fractionation procedure (Mertz, Lloyd, and Bressani, Cereal Chem. 1957) yields a glutelin fraction much more rapidly, but unfortunately the fraction is not identical with fraction V (the copper fraction has less lysine).

Q: How does the weight of "nonzein" double-mutant endosperm compare to the weight of endosperm in "normal" maize?

R: The weight is much lower (25 to 50%) than the normal counterpart, because of reduced starch and protein synthesis.

Q: In your methods for determining lysine, the pericarp is removed. Is the pericarp completely devoid of lysine? What contribution has the pericarp in human nutrition?

R: No. The pericarp contains lysine at a low level (1 to 2 g of lysine/100 g of protein). The pericarp serves only as fiber or roughage in human nutrition because it cannot be digested.

PAPER BY MERTZ ET AL. (pp. 306–329)

Q: By bringing various cereals to a common protein level (8%) for making biological tests with small animals, do you think it would give the absolute picture on the comparative value of the various cereals? If yes, can we use the various cereals as they are for feed preparation?

R: The use of a common protein level of 10%, or lower, tests for protein quality. It does not test for performance of the cereal when fed at a maximum level in the diet. Both methods help to predict the value of the cereal.

Q: In spite of a higher lysine value for floury-2, your data have indicated that growth rate and PER of the rat are no different than when fed a normal maize. Would it be possible that the floury-2 gene may have some other side effect, for example, increasing protein inhibitors?

R: In lysine availability studies on floury-2 carried out by Eggum, and reported here by Dr. O. E. Nelson, no differences were found between floury-2 and normal maize. This would suggest that proteinase inhibitors are not responsible for the relatively poor performance of floury-2 maize.

Q: Is there adequate knowledge on the nutritional requirements of the meadow vole to use it as a test animal? Are amino acids synthesized in the digestive tract of the meadow vole? If so, does this affect their use for this type of analysis?

R: Data on nutritional requirements of the meadow vole certainly are not as extensive as those on the white rat. The vole is used during a narrow range of its life span, just after weaning (16 to 21 days of age) when it is supposed to be functioning as a monogastric animal (no rumen function). Thus, theoretically, the bacteria in the tract should be contributing a large share of the amino acids.

Q: Using different animals, the comparative PER values of various cereals were different in your studies; that is, different cereals were graded differently, depending upon the animal used. Would you like to comment a bit on this aspect?

R: Better conditions must be worked out for the use of the vole and mouse in the assay of cereal grains. In my opinion, the differences in PER observed between the rat and the other two species were due to a lack of sensitivity of the assay procedures using the vole and mouse. The rat classified the cooperative cereal grains as one might predict—in the order of their decreasing lysine contents—except for one sorghum sample known to be high in tannins.

Q: How does the PER of an opaque-2 corn compare with the PER of a high oil opaque-2 corn that contains an increase in the amount of germ protein?

R: If one assumes that the opaque-2 endosperm contains 10% protein with a PER of 80% of casein, and the germ contains 20% protein with a PER of 100% of casein, an opaque-2 maize containing 32% embryo or germ should have a PER of about 90% of casein. This was calculated on the assumption that our opaque-2 maizes with a PER of 85% of casein contain about 15% germ.

Q: Feed intake is obviously very important in the PER calculation. Differences in metabolizable energy contents of diets may influence PER results, as well as differences in protein quality. Would you comment on this?

R: In our cooperative tests with the rat, we adjusted protein, fat, car-

bohydrate, minerals, and fiber to the same levels in all the cereal diets in order to keep metabolizable energy contents of the diets about the same.

Q: In your experience, will the supplementation of normal corn with lysine improve rat growth and PER to levels obtained with opaque-2 maize?

R: In studies carried out by Dr. Bressani with rats, one type of Guatemalan maize had tryptophan as the most limiting amino acid, although in most maize varieties it is lysine. Even with both lysine and tryptophan added to optimum levels, we obtained some evidence to show that opaque-2 maize was still superior. Possibly the leucine/isoleucine ratio or digestibility were involved.

PAPER BY VILLEGAS (pp. 330–336)

Q: Roughly what proportion of the analyses that indicate a high tryptophan level are shown subsequently to be low in lysine?

R: We have not observed any sample with a high content of tryptophan to be low in lysine content. There is a very high relationship between these amino acids in maize endosperm protein.

Q: We have found a direct correlation between the level of lysine and trypsin inhibitor in maize endosperm. Perhaps trypsin inhibitor content also could give an indication of lysine levels in the maize endosperm. Would you give us your comments, please?

R: I consider this correlation very useful for screening materials, if the method for measuring trypsin inhibitor is simple and reliable.

Q: Would you recommend the dye binding method for determination of protein content in a maize screening program?

R: Not to measure protein content, but it can be used to evaluate protein quality.

Q: Is the papain digestion that you described equally effective for hard and soft endosperm opaque-2 types?

R: Yes. We have used papain for all types of endosperm in a large number of samples with no problems.

Q: Which of the following methods would you recommend for screening high-lysine maize in an institution where funds are limited but where precise results are required: the Udy method, paper chromatography, or the spectrometer?

R: I would recommend the Udy method for quality evaluation of the protein, but not for quantity.

PAPER BY FORMAN AND HORNSTEIN (pp. 352–361)

Q: Have you any knowledge of methionine fortification of human diets in field situations, particularly in reference to palatability and acceptability?

R: Not in field trials. However, preliminary evaluation of cassava fortified with soybean protein and methionine ruled out the use of methionine because of flavor problems.

Q: I understood you to say that culturally determined patterns of food consumption bear no relation to nutrition. Isn't it likely that good nutritional patterns have evolved from trial and error experimentation, carried out over generations and featuring interaction between alternative foods and nutritional requirements?

R: Cultural food patterns obviously make for survival, but this does not guarantee good nutrition. An interesting example is widespread vitamin A deficiency in areas where red palm oil is available right at the door.

Q: In your fortification program project at village level, who pays for the basic food or the cost of fortification, the consumer or your agency?

R: In the ongoing field trials, cost of fortification is not passed to the consumer. Concern at this stage is to evaluate acceptability, logistics, and nutritional impact.

Q: For cassava fortification you mentioned cassava and soybean flour mixtures in Brazil and ruled out possibilities of cassava protein improvement by breeding. Is it not true that cassava is capable of protein enhancement by microbial fortification (as reported in the United States), and also by breeding (as reported in Colombia and Nigeria)?

R: Microbial fermentation is a way of enhancing protein in cassava. It is essentially a fortification procedure, with the fortificant prepared in situ. Claims for high protein cassava are apparently based on total nitrogen determinations. Closer examination has shown that about one-half of the nitrogen is nonprotein nitrogen. On a scale of probability, I would guess the probability of finding a truly high protein cassava is low.

PAPER BY SPRAGUE (pp. 362–370)

Q: The most capable students in developing countries appear to be attracted to pure research. What can be done to redirect educational priorities and recognition toward national food programs?

R: Educational institutions have a real responsibility to redirect educational priorities. Unfortunately, although well intentioned, few people in

the educational system have a full understanding of what is required. Ways must be found to bring university staff into international food programs.

Q: Do you think that the level of training (Ph.D., M.S., and so on) is indispensable for the development of these programs?

R: No. People with less academic training could certainly develop this program. It must be remembered, however, that people with the higher academic qualifications are usually those who are promoted into roles of decision making. This allows for a risk—it is possible for people with higher degrees, but less knowledge of the program, to be promoted into decision-making posts. As a result, more incorrect decisions could be made.

PAPER BY MATTERN ET AL. (pp. 387–397)

Q: Some of the protein concentrations shown in your presentation were extremely high in the F_2 or the bulked progeny generations. Are these levels reproducible, and can we hope to reach these levels in a commercial wheat variety?

R: The samples were grown at Yuma, Arizona, under high fertilizer levels, and apparently were under some heat stress during the latter portion of the growing season. It is impossible to predict protein level in wheat in a given crop year, because of the unpredictable environment. However, wheats with a genetic potential for more protein will always be superior to normal types. Under normal growing conditions and yield levels, I would not expect commercial varieties to equal the 25% protein content of the higher materials shown.

Q: How many wheat lines were bioassayed this year, and what could be said about efficiency ratios in these lines?

R: In our first year of operations, we bioassayed approximately 200 samples. Calculations on all these have not been completed. Not all were different lines; some were duplications of lines raised at different locations. Recently, we increased our capacity by purchasing new cages, and, with practice, we can increase our efficiency. Most of our evaluations have been done on an FER basis. One generalization is that statistical differences in wheat grain FER values can be detected. Protein quantity, protein quality, digestibility (and factors that have not been isolated) are involved in these differences. However, one can usually separate those materials which can be predicted to be "good" sources of protein from "medium" and "poor" sources.

PAPER BY SCHRICKEL AND CLARK (pp. 398–411)

Q: How does the quality of protein in oats compare to opaque-2 in human nutrition?

R: Bressani has shown (about 1963 or 1964) that opaque-2 had a higher biological value in child feeding than common oats. No nutritional studies of high-protein oats versus opaque-2 maize have been made.

Q: Iodi has two times the oil content of most cultivars. Have there been any problems with storage or processing of this high-oil variety?

R: Not to my knowledge. Natural antioxidants stabilize the oil in stored Iodi oats. Steaming, plus drying and rolling, serves to stabilize oats because the heat treatment inactivates lipase and oxidative enzymes.

PAPER BY MUNCK ET AL. (pp. 418–431)

Q: What are the phenotypic characteristics of the five or six EMS-induced mutants you reported in barley? Are they smaller and shriveled in any way?

R: Referring to Dr. Doll (Denmark), minor changes occur in plant phenotype. Seeds tend to be slightly smaller. The very high lysine mutant 1508 has slightly smaller seeds and yields about 90% of the high-yielding parent variety, Bomi.

Q: Please provide information on the screening procedure your group uses for identifying high-lysine mutants.

R: The dye-binding (DBC) procedure is used, as referred to in my paper.

Q: What evidence exists that the new EMS-induced variants in barley mendelize?

R: Referring to Dr. Doll (Denmark), the mutants with a 10 to 15% increase in lysine were not giving clear-cut segregation ratios, which might be expected from our research on lys barley, because of the influence of a variable gene background. The high lysine level of two of their lines was confirmed by us in Svälof. The very high lysine mutant 1508 was selected with single-plant analysis in M_3 by Doll. Six plants were found to be high in lysine emerging from the same M_3 line. This should indicate the finding of a genetically stable mutant.

Q: Why is tryptophan not reported in your amino acid evaluations?

R: In comparison to maize, tryptophan in barley is high (1.3 g/16 g N) and sufficient, from the nutritional point of view. There does not seem to be an association of increase in lysine with tryptophan in lys barley, as there is in opaque-2 maize.

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