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Protein Advisory Group of the United Nations System

Nutritional Improvement of Food Legumes by Breeding

Proceedings of a Symposium sponsored by PAG,
held at the Food and Agriculture Organization, Rome, Italy
3-5 July 1972

and

PAG Statement 22:
"Upgrading Human Nutrition Through the
Improvement of Food Legumes"

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Preface and Acknowledgements

Contributors and Participants

WELCOME BY FAO
– Juan Felipe Yriart

OPENING REMARKS OF CHAIRMAN
– Lewis M. Roberts

Keynote Address

BUILDING A PROTEIN REVOLUTION ON GRAIN LEGUMES
– Norman E. Borlaug

PREFACE AND ACKNOWLEDGEMENTS

The PAG has frequently emphasized the need for improvement by agricultural and genetic means of the nutritional value, and particularly the protein component, of staple food crops. Recently, the Group proposed that greater research effort should be devoted to upgrading the food legumes, which are major protein resources in many developing countries. The urgency for improvement of food protein crops is exemplified in India by the fact that the Green Revolution, which stimulated wheat production there in a remarkable way, has not provided similar emphasis to the legumes, whose limited yield potential and low agricultural profitability provide little comparable economic incentive for increased production. As a consequence, their availability as important traditional protein supplements to Indian cereal diets has actually decreased. Accordingly, the PAG Secretariat, with the cooperation of several governments and an international foundation, as well as with full participation of the Secretariats of the Food and Agriculture Organization, the International Atomic Energy Agency, and the Technical Advisory Committee of the Consultative Group on International Agricultural Research, convened in July 1972, at the Food and Agriculture Organization in Rome, an invitational symposium on nutritional improvement of food legumes by breeding.

In the course of this meeting, a group of internationally recognized agronomists, plant breeders, geneticists, nutritionists, biochemists, and food scientists reviewed the latest scientific and technical evidence regarding not only the nutritional and food-use deficiencies of the food legumes, but also aspects of plant physiology, pathology, and production technology which must be tackled if a breakthrough in terms of increased yield, better nutritional quality, and improved consumer acceptance is to be achieved. Specifically, the agenda was designed to identify information needed for achieving the following six objectives for food legume improvement:

1. Higher protein levels and better amino acid profiles.
2. Reduction of toxic constituents.
3. Reduction of flatulence factors.
4. Development of improved processing and consumer acceptance characteristics.
5. Identification of screening methodology useful to plant breeders for measuring relevant chemical, biochemical, nutritional, and toxicological factors.
6. Expansion of research efforts in the areas listed as well as in those related to crop yield and productivity.

In this report of the meeting, the primary papers dealing with each of these topics are followed by a record of the discussion which ensued and recommendations for needed action. In addition, because of time limitations during the meeting, a group of supplementary papers was solicited afterward from various authorities, in order to round out some of the agenda topics.

In a related development, the PAG *ad hoc* Working Group on Guidelines for Plant Breeders met immediately after the Symposium. It took up the recommendations developed there and drafted a PAG Statement (No. 22) titled "Upgrading Human Nutrition Through the Improvement of Food Legumes". This draft statement, later approved by PAG and also published herein, defines desirable objectives and provides a guideline or manual of methods for plant breeders seeking to introduce into their legume-breeding programs recommended procedures for achieving nutritional and food-use improvement in these crops.

The PAG and its Secretariat acknowledge the assistance of the following organizations which co-sponsored the Symposium and the *ad hoc* Working Group:

The Rockefeller Foundation
International Development Research Centre
Overseas Development Administration (U.K.)
Agency for International Development
International Maize and Wheat Improvement Center (CIMMYT)
Campbell Institute for Agricultural Research
Swedish International Development Agency
International Institute of Tropical Agriculture

We are indebted to Dr. Lewis M. Roberts of the Rockefeller Foundation for assistance in planning the Symposium and for serving as Chairman of the meeting.

Members of the PAG *ad hoc* Working Group included Dr. M. Cépède (Chairman – also Vice-Chairman of the PAG), Dr. Ricardo Bressani, Dr. K. J. Carpenter, Dr. J. F. Kelly, Dr. James McGinnis, Dr. Irvin E. Liener, and Dr. K. O. Rachie. United Nations staff participants included Mr. P. Oram, Mr. M. Jalil, Dr. Bruce Nicol, Dr. P. Lunven, Dr. G. D. Kapsiotis (all of FAO), Dr. B. Sigurbjornsson (IAEA), and Dr. M. Milner and Miss E. van Beresteyn (PAG Secretariat). The help of Mrs. Helene Jordan of The Rockefeller University in editing these proceedings is appreciated.

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WELCOME

Juan Felipe Yriart

It is my pleasure to welcome you to the Food and Agriculture Organization on behalf of the Director-General. It is a source of satisfaction for our Organization that so many distinguished leaders in plant research and genetics, as well as in biochemistry and nutrition, are assembled here. We appreciate the initiative taken by the Protein Advisory Group, with whose efforts and work FAO is proud to be associated, in bringing this group together to focus on food-legume research in the vital area of improvement of the nutritional and food qualities of these important food crops. We understand that certain foundations and bilateral agencies have provided sponsorship and assistance to this Symposium, and we express our warm gratitude to them, as well.

In this work, FAO places high priority on the need to improve the quantitative and qualitative characteristics of the protein component in diets of the developing countries. The total protein available in the world today, in relation to global needs, and the problems of quality and distribution of edible protein resources in many developing countries -- particularly in terms of the needs of vulnerable groups -- we call the "protein problem." It is a main concern of FAO, which assigns one of its six main Areas of Emphasis to the consideration of these problems and the promotion of international cooperation toward their solution. Within this framework, as expressed in our close involvement with the work of the Technical Advisory Committee, which counsels the Consultative Group on Agricultural Research, we are convinced that improvement of both greater yield potential and nutritional and food-use qualities of food legumes have been neglected too long. We firmly believe that these protein-rich crops hold great promise in meeting the protein needs of the vulnerable groups, and that expanded research to these ends are of the highest priority. The Technical Advisory Committee and we in FAO eagerly welcome the results of your deliberations and recommendations, and the statement from the PAG on nutritional guidelines for plant breeders that will follow.

We wish you every success in your efforts. Do not hesitate to call on my FAO colleagues and myself for any assistance you may need in your work.

OPENING REMARKS OF THE CHAIRMAN

Lewis M. Roberts

To initiate this Symposium on the Genetic Improvement of Food Legumes by Breeding, I wish to express to each of you my warm personal greetings and to extend to you on behalf of all the sponsoring agencies a most cordial welcome.

When I was invited to serve as the general chairman of this conference, I felt honored and pleased to accept for several reasons. The subject is both important and timely. I very much appreciate the important contributions the United Nations System through its various family members, especially the Protein Advisory Group, is making in helping to overcome protein deficiency. There is a growing awareness that the protein deficit in the world as a whole is a critical and worsening aspect of the total food problem, and is going to be one of the most difficult to overcome.

I would like to express genuine appreciation on behalf of all of us who are here not only to the PAG Secretariat, but also to the sponsoring agencies. These include – in addition to WHO, FAO, UNICEF, and IBRD, which directly support the PAG – the International Development Research Centre of Canada, the United States Agency for International Development, the British Overseas Development Administration, the International Atomic Energy Agency, the Swedish International Development Agency, The Rockefeller Foundation, and supporting companies and institutions.

I should like to say just a few words about the genesis of this meeting. The PAG, at its meeting in December, 1971, approved a recommendation presented in a document prepared by FAO, the International Atomic Energy Agency, and the PAG Secretariat, to establish an *ad hoc* Working Group to develop "Nutritional and Food-Quality Guidelines for Plant Breeders." The recent progress in increased production of wheat, rice, and maize in several developing countries has been spectacular, and has raised hopes that similar advances may soon be possible with other crops. Also recently there has been a significant expansion and acceleration of research efforts to improve the nutritive quality of the major cereal crops. This has been given strong emphasis by the discovery of opaque-2 high-lysine corn. In general, things seem to be moving well on the major cereal front. No so, however, with another group of crops that are of great potential importance in helping to overcome the protein deficiency problem. I refer, of course, to the food-legume crops.

It was decided, therefore, that the initial attention of the *ad hoc* Working Group would focus on the food legumes. It was further decided that the deliberations of the Working Group would be preceded by an informal, but intensive, symposium on "Nutritional Improvement of Food Legumes by Breeding," in

which a small, select group of plant breeders, nutritionists, and biochemists familiar with the problems involved would participate.

The value of this meeting will be judged largely by the quality of the publication that comes out of it. Recently the number of scientists – and also nonscientists – who are interested in helping to increase production of the food legumes has been growing rapidly. Many of these scientists could have made a real contribution to this meeting had it been possible to accommodate them and to pay the expenses of getting them here. We are deeply obligated to produce a publication from this meeting that will be a real stimulus to action and a real help to those now engaged, or who will become engaged, in practical actions that will result in increased production and utilization of the principal food-legume crops.

It is indeed heartening to see the increased interest and attention that is being given to the food-legume crops. Their importance in helping to overcome the protein deficit problem was stressed in a 1967 report of the U.S. President's Science Advisory Committee on the World Food Problem, in FAO's Indicative World Plan for Agricultural Development in 1969, and in a statement issued by the PAG in the spring of 1970, which included the recommendation that "more attention be given in agricultural programmes to the use of crop varieties having potential for higher amounts and quality of protein." These should include not only cereals but also grain legumes. You have probably also seen the "Strategy Statement on Action to Avert the Protein Crisis in the Developing Countries," prepared by a panel of experts from all over the world at a meeting sponsored by the United Nations in New York in early 1971, which strongly emphasized the role of food legumes in helping to solve the protein problem.

In April, 1969, about 16 of the heads of some of the major international assistance agencies met informally at Bellagio, Italy, to discuss how their organizations might work in closer concert to help the human race feed itself better. This group, which came to be known as the Bellagio Group, has met annually since the first meeting, and it has expanded and evolved into a somewhat more formal body that is now named the Consultative Group on International Agricultural Research (CG). There are now about 30 members in the CG, including multinational institutions such as the World Bank, FAO, the Inter-American Development Bank, government foreign assistance agencies, and private foundations. The World Bank is providing a permanent secretariat for the CG at its headquarters in Washington; the secretary is Mr. Harold Graves, who was invited to this meeting but, regrettably, was unable to accept.

The Consultative Group has appointed a Technical Advisory Committee (TAC) of 12 leading scientists in agriculture from different regions of the world. FAO is providing the permanent secretariat for TAC, and the secretary is Dr. Peter Oram, who is with us at this meeting.

At its second meeting on February 3-5, 1970, the Bellagio Group requested that five papers be prepared on areas or problems of special interest to the Group. One of these was on food legumes and what might be done to improve the efforts to increase their production and quality. I was given the assignment of preparing this paper, the main recommendations of which are under active consideration by the TAC.

I have taken a little time to give you this background information because I feel that it has a significant and direct bearing on the character of our meeting. Things are beginning to happen on the food-legume front, and I am confident that now that the initial inertia of neglect of these crops is being overcome, efforts to increase their production and improve their quality will gain momentum.

Undoubtedly, the plant breeders, working with the agronomists, the physiologists, the pathologists, and the entomologists, will be able to develop higher-yielding varieties that will respond well to improved cultural practices and will have a higher level of genetic or natural resistance to diseases and insects. Increasing yields, which are now miserably low, must take first priority. Of almost equal importance, however, is the challenge to improve the nutritive value of these leguminous crops, to overcome the deleterious nutritional factors that are common in the family, and to find ways to improve their cooking, processing, and consumer-acceptance characteristics.

The practical solutions to these nutritive problems in the food legumes, and in other crops as well, will come mainly from the field plots of the plant breeders. But the breeders need a lot of backstopping from the nutritionists and biochemists to arrive at solutions. In other words, an interdisciplinary team approach is required. This is the reason for the mix of plant breeders, nutritionists, and biochemists that has been brought together for this meeting.

Let me suggest that we set for ourselves the following objectives for this meeting: (1) define the status of our present knowledge for each of the topics and subtopics that we will treat; (2) relate in clear and simple terms how the existing knowledge can be put to greater and more effective practical use to solve the nutritional problems of the food legumes; (3) identify gaps in this knowledge; and (4) make recommendations as to how these gaps might best be filled through additional research.

This is not, of course, intended to be a comprehensive list, but I hope you agree with me that these should be among our main goals. May I emphasize that the primary purpose of the presentations listed in the agenda will *not* be to provide comprehensive and historically oriented reviews of the knowledge in the various related fields. I am sure we can safely assume that this highly involved and specialized group already has a good grasp of such background information. Rather, it should be our purpose to provide the group, succinctly and briefly, with the specific information and expert judgment needed to establish the feasibility of our goals, and with an assessment of the procedures, methods, and resources needed to achieve them. I am certain that all of us want very much to see our deliberations and recommendations lead to positive actions and positive results aimed at getting more and better-quality food legumes into the diets of those who need them. The drafting groups will crystalize the important points in our discussions, and I am confident that the publication to come out of this meeting, after having been given the final touches by Dr. Milner and his staff, will have a significant impact in making more effective the present limited efforts to improve the food-legume crops and in expanding and accelerating those efforts.

BUILDING A PROTEIN REVOLUTION ON GRAIN LEGUMES

Norman E. Borlaug

"Green Revolution" refers to the recent rapid rise in yields and production of wheat, rice, and maize in a number of developing countries. Those increases have resulted from the development of high-yielding crop varieties that were capable of responding spectacularly to such improved cultural practices as better land preparation, planting methods, fertilization, weed control, and pest eradication.

The high-yielding Mexican wheat varieties were the catalysts in triggering the Green Revolution. When properly grown by the new technology developed through agronomic research, those varieties often have been able to increase yields from 800 pounds to as much as 4,000 to 5,000 pounds per acre. This spectacular increase has taken place on many millions of acres. As a result, the new seeds and new technology, and the resultant high yields, have made wheat production highly profitable in such countries as India and Pakistan. Therefore, many farmers have begun to shift to wheat part of their land that formerly was grown to grain legumes -- a not unnatural reaction.

Wheat production in India soared from a level of 12.4 million metric tons in 1965 to 26.5 million metric tons in 1972. The increase in per-acre yields of grain accounts for more than 85 per cent of the increase in production; the remainder, however, resulted from an increase in the area sown to wheat, part of which was diverted from winter grain legumes (pulses).

Unfortunately, the pulse yields (chick peas, grains, pigeon peas, mung beans, lentils, peas) remain at a low-yield level, and pulse production is either stagnant or dropping.

Neither new high-yielding varieties of grain legumes (pulses) nor improved technology have been developed, so gradually part of the land that once grew pulses has shifted in winter to wheat and in summer to rice or maize. Of course, the reduction in the production of grain legumes is undesirable nutritionally. Grain legumes are essential in providing the amino-acid balance needed for normal growth development and maintenance of health in those vegetarian diets that depend primarily on cereals and root crops.

What is to be done about this dilemma?

Governments could increase the price of grain legumes and so encourage or speed up the production of pulses. Such action would, however, have a twofold adverse effect. First, the price of grain legumes, which already are the most expensive part of the vegetarian diet, would increase for the consumer. Second, if the price differential were made great enough, some land would be shifted

back from cereals to pulses without, at the same time, increasing per-acre pulse yields. But as these countries also need more cereal grains, such a result also would be undesirable.

My recommendation is to speed up the "slow runners" -- the pulses – by introducing new high-yielding varieties and new technology that will increase pulse yields and thereby result in increased production.

As early as 1965, I anticipated the dilemma of rapid increases in wheat yields and an adverse, indirect, negative effect on grain-legume and oil-seed production. In reports to the governments of both India and Pakistan, I indicated that such a breakthrough on the cereal front would adversely affect grain-legume and oil-seed production – for example, that of safflower, cotton, peanut, and rape – with a concomitant adverse effect on diets. I urged both governments to launch aggressive research programs to increase the yield of these crops. At that time (1966), I wrote the report to The Rockefeller Foundation to which Dr. L.M. Roberts has referred, urging them to stimulate research and production of grain legumes and oil-seed legumes in the semitropics and tropics.

Grain-legume and oil-seed legume crop research and production – despite the prodding from Dr. Roberts, myself, and a few other scientists – remains even today the ugly duckling of agricultural crops. Very little research money is being allocated to the improvement of this diverse group of crops that is so important to human diets in the developing countries.

During May, 1971, the Secretary General of the United Nations named a panel of scientists to examine the world protein-deficiency problem, especially in the developing nations, and to recommend methods of alleviation. These recommendations, which I assisted in drafting, were embodied in a report entitled "Strategy Statement on Action to Avert the Protein Crisis in the Developing Countries," by the United Nations Secretary General's Panel, May, 1971.

We are meeting here today as a working group under the auspices of the Protein Advisory Group (PAG) to decide what action should be taken, if any, to implement one aspect of that report. I refer to the improvement of grain-legume and oil-seed crops from the points of view of genetic varieties, agronomy, plant protection, biochemistry, and nutrition.

Research Priorities

A large number of grain-legume and oil-seed legume crops can be helpful in alleviating the protein crisis of the developing nations. Among the grain legumes are: gram or chick pea *(Cicer arietinum)*; pigeon pea *(Cajanus cajan)*; lentil *(Lens esculenta)*; mung bean *(Phaseolus mungo* and *P. aureus)*; dry beans *(Phaseolus vulgaris)*; lima beans *(Phaseolus lunatus)*; dry pea *(Pisum sativum)*; lathyrus pea *(Lathyrus sativus)*; broad bean *(Vicia faba)*; cow pea *(Vigna sinensis)*; and hyacinth bean *(Lablab niger)*. The most important oil-seed legumes also used for food, either directly or indirectly, are: groundnut or peanut *(Arachis hypogaea)* and soybean *(Glycine max)*.

The number of species shows the complexity of this problem. Some of them are grown during the cooler winter season or at high elevation in the subtropics

or tropics. Others are grown in the rainy season in only the hot, tropical areas. To embark simultaneously on the improvement of all of these species will certainly insure failure. One of the most important responsibilities of this meeting is to provide a guide or priority list of the species on which major research should be undertaken. To establish such a list, we must consider the genetic potential of grain yield for the species, its cultural and climatic requirements, disease and insect problems, nutritional value, and toxic properties, if any.

I believe that the research effort should, for the present, be concentrated on a few species, as follows:

1. Two species for the winter (dry) season in the subtropics.
2. Two species in the heavy rainfall areas of high elevation in subtropical areas or cool, winter, rainfall areas.
3. Two species for the summer seasons of the subtropics.
4. Two or three species for the wet, hot tropics.

Indirect Benefits of Grain Legumes and Oil-Seed Legumes

Although today we are concerned primarily with increasing the production and nutritional value of grain legumes and oil-seed legumes to alleviate the protein deficiency of the developing nations, these crops have several other, indirect, beneficial effects on agriculture. Legumes are symbionts of bacteria that form root nodules. These bacteria can take free nitrogen from the air and fix it in plant-root tissue. Consequently, they increase indirectly the level of soil nitrogen and this, in turn, increases the yields of cereals that may follow legumes in plant rotation. Rotation of grain legumes with cereals also reduces the weed, disease, and insect problems. Grain legumes generally sell at a much higher price per pound than cereals. Therefore, if the yield of grain legumes can be increased, they also will increase farm income.

Need for Interdisciplinary Research

Increased yields and rapidly expanded production can be attained only by an interdisciplinary research approach. Each specie or crop must be improved by:

1. Development of high-yielding varieties responsive to such improved cultural practices as better (or more) fertilizers, weed control, and moisture control. The improved varieties must, insofar as possible, be resistant genetically to the major diseases and insect pests.
2. Development of improved agronomic practices that will permit approaching the maximum genetic yield-potential of the improved varieties.
3. Development of effective measures against disease and insect depredations.
4. Development of biochemical and biological data pertaining to nutritional value and to toxic substances present in the different varieties and segregating lines now being used to develop new crop varieties.

The excellent cooperation between CIMMYT biochemist Dr. Evangelina Villegas and her group (with financial assistance from a UNDP grant) and maize-breeder Dr. Surinder Vasal in the development of "hard-endosperm, high-lysine" maize populations has been monumental in demonstrating the great value of close interdisciplinary research. They have set the pattern for what must be done to improve the genetic yield potential of grain-legume varieties and, at the same time, to improve agronomic type, disease and pest resistance, and nutritional value.

Once new, high-yielding, improved, grain-legume varieties are available, and as soon as good cultural practices and disease and insect protection are developed, they must be hitched to a vigorous, national, crop-production campaign in order to increase production rapidly. Before the production campaign is launched, a realistic policy of grain pricing must be established and guaranteed by government to stimulate the farmer and to assure him a fair return for his investment.

Leads – from CIMMYT's International Wheat Program

I. Varietal Improvement

A. Use a wide genetic base. Collect, study, and use progenitors from throughout the range of species being improved.

B. Make a large number of crosses to build a diverse gene pool. To achieve this rapidly, single crosses, double crosses (between different F_1 generations), and back crosses all should be used.

C. Grow two generations of all breeding materials each year, if possible, to speed varietal development.

D. Grow segregating populations under a wide range of soil, climate, disease, and insect conditions. Whenever possible, exchange genetic materials (and visits) with scientists working on the same crops in other parts of the world.

E. Select for broad adaptation in varietal development.

F. Incorporate resistance to major genetic and/or insect diseases as soon as possible. (See III A)

G. Work closely with biochemists and nutritionists to identify the lines that are outstanding for nutritional value and to discard those that are undesirable from a toxic, palatable, or digestible standpoint.

II. Agronomic Practices

A. Develop cultural practices that will utilize the maximum amount of genetic yield-potential available in a new variety by manipulating:

1. Soil fertility
2. Moisture conservation and utilization
3. Weed control
4. Proper ratio of planting dates

III. Pest Control

A. Develop effective control practices for major insect pests and diseases.

Conclusions

I recommend the funding and initiation of a dynamic, interdisciplinary research and production program on a few carefully chosen species of grain legumes and oil-seed legumes. If the experiences of the CIMMYT wheat and IRRI rice programs are used as a guide or model, if the programs are staffed with imaginative, aggressive, well-motivated scientists, and if bureaucracy can be minimized, I believe that spectacular results can be achieved in increasing the world's plant-protein production.

I am confident that if we act aggressively, great progress can be achieved in raising production of protein grain legumes during the next eight to ten years. The time has come to stop talking about the need to launch programs and to concentrate on achieving a self-evident goal. In summary, let us not slow down. Let us speed up the "slow runner" and build a protein revolution on grain legumes.

I

NUTRITIONAL IMPORTANCE OF LEGUMES FOR HUMANS

Statements

LEGUMES IN HUMAN DIETS AND HOW THEY MIGHT BE IMPROVED

Ricardo Bressani

FACTORS AFFECTING THE NUTRITIONAL VALUE OF BEANS

Werner G. Jaffé

Discussants

Doris Calloway
Irvin E. Liener
B. M. Nicol

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Doris Calloway

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LEGUMES IN HUMAN DIETS AND HOW THEY MIGHT BE IMPROVED

Ricardo Bressani

By now, it is well accepted that supplying adequate amounts of high-quality protein to the increasing population in developing countries is not an easy endeavor.

During the last 20 years, various approaches have been implemented. Among the first was the development of foods with a high-protein content made from nonconventional protein sources. However, because much work is still being done in this area, such foods contribute only moderately in providing the needed protein to small children.

In 1964, the high-protein opaque-2 maize was placed at the disposal of countries that use this cereal grain as their main staple food. After some eight years, its use by humans is still relatively small, and its value in providing better nutrition to rural or undernourished populations has yet to be demonstrated. However, the discovery of opaque-2 maize opened new possibilities in providing people with food of higher nutritive value.

Not long after 1964, a significant increase in cereal-grain production was initiated in various countries -- an increase that has come to be known as the "Green Revolution." Noone can deny the significance of such increase, although recent reports have indicated some negative effects, especially in the production of other food crops in some countries.

During this time, the potential of legume grains as sources of greater amounts of an improved quality of protein was often reported. So far, however, only small and uncoordinated efforts have been made to bring this potential into full reality. Today there are many indications that the time for the agronomic and nutritional improvement of legume grains has finally arrived.

This paper attempts to summarize the large volume of literature devoted to the nutritional importance of beans in human nutrition -- foods that have been chosen as the natural protein supplements to cereal grains since Neolithic times.

Consumption of Legume Grains

Reviews of the subject under discussion have indicated that the Leguminosae include approximately 600 genera, with about 13,000 species. Out of this great number, only about 10 or 12 are of economic importance today, if soybeans and groundnuts are excluded (Aykroyd and Doughty, 1964). Legume-grain crops were domesticated early in history in the major centers where agriculture origin-

ated. Even through legume grains have a world-wide distribution, probably because of their unique capacity to fix nitrogen, some have become more popular than others in various regions. For example, *Pisum*, *Vicia*, and *Lens esculenta* are, in general, more popular in the Middle East. In America, *Phaseolus* is more common, whereas in the Far East and Africa, the Indian species of *Phaseolus*, *Dolichos*, *Vigna*, and *Cajanus* are of greater importance. Preference for one type of legume over another is probably related to the availability of the food in the region; in turn, their availability is determined by environmental conditions that favor higher yields of one over the other specie of legume foods.

Information on the amount of legume food consumed is tabulated in Table I for all countries listed in FAO Food Balance Sheets (1966). The consumption in 63 countries was grouped according to the intake of all legume foods consumed per person per day. As is well known, these figures are the results either of production records or of dietary surveys applied to the whole population; therefore, they may lead to erroneous interpretations. Specific dietary surveys, however, indicate that bean consumption is high in some countries, as shown in Table II. It is obvious that when daily bean intake is high, it provides significant amounts of protein, calories, and other nutrients (Aykroyd and Doughty, 1964; Stanton, et al., 1966; Bressani and Elías, in press).

TABLE I
Legume Intake in 63 Countries of the World

No. of countries	Legume grain intake g/capita/day
33	2-13
10	14-24
15	25-35
1	36-46
4	47-57

(Summarized from FAO Food Balance Sheets, 1966)

In countries where beans constitute a part of the daily diet, consumption starts early in childhood. The results, shown in Table III, were obtained from dietary surveys carried out in Guatemala. They indicate that bean broth is consumed in relatively large amounts by children under two years of age, whereas the bean itself is consumed in only small amounts. As age increases, broth consumption decreases and bean intake increases to values as high as 55 g on the average. In Guatemala and other Latin American countries with similar cultures, beans are valued for the broth that can be obtained by cooking them (Bressani and Elías, in press; Flores, et al., 1966). This broth has been analyzed for some nutrients, as shown in Table IV, where human and cow's milk composition values are also shown. Bean broth is lower in solids and calories, due to lower fat and carbohydrate content, and in other nutrients. However, it is of interest to see that crude protein is slightly higher than human milk values. This crude protein in bean broth should be characterized in terms of amino acids to deter-

TABLE II
Legume Grain Intake from Dietary Surveys

Country	g/person/day
Togoland	13-140
India	14-114
Countries south of Sahara	10-150
Guatemala	50
Nicaragua	85
Venezuela	89
Brazil	48

TABLE III
Consumption of Beans and Bean Broth with Respect to Age in Rural Guatemala

Age	Bean broth g	Cooked beans *(P. vulgaris)* g
1 to 2 years	28	3
2 to 3 years	10	13
3 to 4 years	4	18
4 to 5 years	-	18
Adult	–	50

From Flores, et al., 1966

TABLE IV
Proximate Chemical Composition of Bean Broth, Human Milk, and Cow's Milk (100 g)

Component	Bean broth	Human milk	Cow's milk
Water, g	92.5	87.5	87
Calories	22	67	67
Protein, g	1.9	1.4	3.3
Fat, g	0.1	3.8	3.6
Carbohydrate, g	3.5	6.8	5.4
Ash, g	2.0	0.2	0.7
Calcium, mg	12	32	178
Phosphorus, mg	15	15	80
Iron, mg	0.1	0.1	0.5

mine whether it is of nutritional value, particularly because it is fed mainly to children. As far as is known, such information is not available.

Legume foods are consumed in a variety of forms, but the most common method of preparation is to boil them in water – with or without the addition of salt, onions, or garlic – for three to five hours at atmospheric pressure. They are then consumed or are processed further by mashing them to make a paste, which may either be left as such or passed through a sieve to remove part of the seed coat. This material is then eaten or is processed still further by frying with animal or vegetable fat. The changes in nutritive value that take place in these processes have been evaluated. The results of various processing methods used in Guatemala are shown in Table V (Bressani and Elías, in press). It can be seen that only small changes occur in chemical composition, although the fat content in fried beans is higher, as would be expected. Protein quality tends to decrease from the boiling to the frying stage. The loss in protein quality is probably due to a loss in available lysine, which also decreases.

Other forms of preparation include fermentation, toasting, and germination. Of the three, toasting causes the most significant losses in protein quality.

Protein Content and Quality of Legume Grains

As has been mentioned before, legumes are nutritionally important among vegetable foods because of their relatively high protein content. Therefore, attention is given here only to that particular nutrient.

Protein content of edible species of legume grains, with the exception of the soybean and groundnuts, varies between 18 per cent and 32 per cent. The protein is located in the cotyledons and embryonic axes of the legume grain; only small amounts are present in the seed coat. The cotyledons contain about 27 per cent protein, whereas the embryonic axis and seed coat contain 48 per cent and 5 per cent, respectively. Cotyledons contribute the largest amount of protein, because of their greater weight. Salt-soluble globulins are the predominant class of proteins in seeds of *Phaseolus*, and some of these have been shown to be resistant to hydrolysis by proteolytic enzymes. The presence of such proteins in legume grains may offer an explanation for the low digestibility of legume-grain protein (Seidl, et al., 1969).

TABLE V

Effect of Home Cooking of Bean Preparations in Guatemala

Processing product	Ave. wt. gain, g	PER	Available lysine g/16 g N	Protein %	Fat %	Crude fiber %
Raw beans	0	–	5.83	24.6	1.9	4.6
Cooked beans	34	1.24	6.30	24.9	0.7	2.8
Strained beans	37	1.43	6.35	24.0	0.6	1.6
Fried beans	10	0.87	5.17	17.8	13.3	1.6
Casein	130	2.73	–	–	–	–

In addition to species differences, other factors have been shown to affect the content of protein, as well as other nutrients, including essential amino acids. Table VI summarizes the results of one study carried out with 25 varieties of *Phaseolus vulgaris.* The protein content ranged from 20.1 per cent to 27.9 per cent, and was influenced significantly by both variety and location. Methionine varied from 0.17 per cent to 0.33 per cent, lysine from 1.69 per cent to 2.42 per cent, and tryptophan from 0.14 per cent to 0.22 per cent. This variation was the result of both varietal and location factors, except in the case of methionine, where varietal differences were not significant. In this particular study, it was found that the intervariety coefficients of correlation between pairs of these nutrients were all positive and that a majority of them were highly significant. This implies that genetic factors that cause one nutrient to increase effect an increase in other nutrients, as well (Tandon, et al., 1957).

TABLE VI
Analysis of Variance of Nutrient Content of Bean Varieties Grown in Two Localities

		Mean square			
Source of Variation	D.F.	Nitrogen	Methionine	Lysine	Tryptophan
Varieties	24	0.560**	0.009	0.193*	0.003**
Localities	1	2.869**	0.189**	0.998**	0.041**
Var. x loc.	24	0.090**	0.006**	0.091**	0.001**
Reps. within loc.	4	0.016	0.0002	0.021	0.0002
Exptl. error	96	0.024	0.001	0.019	0.0002

From Tandon, et al., 1957

The essential amino-acid content of legume grains has been studied many times. Representative values indicating the variations reported are shown in Table VII. For comparative purposes, the Table also shows values for beef, because beans have been called "the meat of the poor." This Table shows that legume-grain protein is high in lysine content, a factor of much nutritional significance when beans are considered as supplements to cereal grains. A second factor of interest is that they are low in total sulfur-amino-acid content, which also is important when considered in terms of diets based primarily on such roots as cassava. The next amino acid of special interest is tryptophan, with relatively low value.

Amino-acid content of legume grains depends on species, varieties, localities, and management practices. Of particular significance are the results on the effects of the application of minor-element fertilizers. For example, it has been shown that the uptake of zinc by the pea bean causes increases in methionine. In another study, when *Pisum sativum* was fertilized with sulfur, the methionine content increased from 1.29 to 2.18 g per 100 g of protein (Bressani and Elías, in press).

The nutritive value of legume grains also has been studied extensively. Biological value, representing the amount of absorbed nitrogen retained in the body,

TABLE VII
Range in Essential Amino-Acid Content in Species and Varieties of Legume Grains

Amino acid	Range g/g N	Beef g/g N
Arginine	0.36-0.57	0.40
Histidine	0.08-0.21	0.22
Isoleucine	0.32-0.62	0.33
Leucine	0.20-0.68	0.51
Lysine	0.34-0.71	0.55
Methionine	0.03-0.11	0.15
Cystine	0.01-0.07	0.08
Phenylalanine	0.15-0.49	0.26
Threonine	0.16-0.31	0.28
Tyrosine	0.06-0.24	0.21
Tryptophan	0.01-0.07	0.07
Valine	0.24-0.49	0.35

has been found to be variable and low in most legume grains. Some representative results are shown in Table VIII. Values range from 32 per cent to 78 per cent, and large variations are also observed in varieties of the same species (Jaffé, 1950a; Patwardhan, 1962). It is difficult to explain such high variability, because many factors are involved. However, it is highly probable that the main factor is the relatively low concentration of sulfur-amino acids in legume grains. The beneficial effect of the addition of methionine to beans has been shown many times (Bressani and Elías, in press; Patwardhan, 1962). Table IX summarizes some results. It can be seen that the addition of 0.3 per cent methionine increased the protein-efficiency ratio (PER) in every case. However, it was not equally large among all species. This small effect of methionine addition may in

TABLE VIII
Biologic Value of Some Species and Varieties of Legume Grains

Legume grain	Biologic value %
Cajanus cajan	46-74
Phaseolus vulgaris (black)	62-68
Vigna sinensis	45-72
Cicer arietinum	52-78
Lens esculenta	32-58
Phaseolus aureus	39-66
Phaseolus mungo	60-64
Pisum sativum	48-49

TABLE IX
Effect of Methionine Addition to Various Species and Varieties of Legume Grains

Legume grain	Protein efficiency ratio	
	−Methionine	+Methionine
Phaseolus vulgaris (black)	0.0-0.9	3.5-3.8
Phaseolus vulgaris (red)	0.0	1.7
Phaseolus vulgaris (white)	1.2	2.7
Vigna sinensis (black)	1.0	1.6
Vigna sinensis (beige)	1.0	1.8
Pisum sativum (green)	0.3	2.7
Pisum sativum (yellow)	0.0	1.2
Lens esculenta	0.0	0.9
Cicer arietinum	1.7	2.8

From Jaffé, 1950a

some cases be explained on the basis that methionine is not the most or the only limiting amino acid in some legume species.

The results in Table X were obtained with *Cajanus cajan*. They show that methionine addition has no effect on improving protein quality. The same is true if only tryptophan is added. However, when both amino acids were added, protein quality increased, which indicates, at least in *Cajanus*, both amino acids are about equally limiting (Braham, et al., 1965). The same sort of situation may exist with other legume grains, particularly those that do not respond significantly in protein-quality improvement when supplemented with methionine.

The protein quality of legume grains also can be affected by other factors, such as processing for consumption and storage. Even though these two factors are of importance after harvest, they may have played an important role in the nutritive value reported by various workers. Legume grains contain antinutritional factors, which are destroyed by cooking, and cooking may decrease protein quality, so it is possible that if processing is not carried out under

TABLE X
Amino-Acid Supplementation to *Cajanus cajan*

Amino acid	Amount added %	Ave. wt. gain, g	PER
None	–	48	1.82
DL-Methionine	0.1	35	1.52
DL-Methionine	0.3	30	1.32
DL-Tryptophan	0.1	58	1.81
DL-Methionine + DL-Tryptophan	0.2 + 0.1	118	2.65

From Braham, et al., 1965

equal and standardized conditions, it may contribute to the variability in the biological value reported in the literature. Storage affects cooking quality, because it is common to find hardshell beans in stored beans. Hardshells require longer cooking, which may decrease protein quality. Therefore, it is suggested that optimum cooking conditions should be established for each legume-grain species in order to reduce the variability in nutritive value, as determined biologically. This will be of value for agronomic and nutritional improvement programs. Furthermore, care should be taken to compare bean species and varieties that have been under equal storage conditions.

Role in Relation to Cereal-Based and to Cassava-Based Diets

Among vegetable crops, as already indicated, legume grains contain the highest amount of protein, which in general runs well above twice the level found in cereal grains and significantly more than the levels in root crops. Furthermore, the protein of the legume grains is considered to be a rich source of lysine; sulfur-containing amino acids are the major amino-acid deficiency. On the other hand, cereal-grain proteins are low in lysine but have adequate amounts of the sulfur-amino acids. Therefore, it is evident that legume-grain protein is the natural supplement to cereal-grain proteins. If this is accepted, any agronomic and nutritional improvement in legume foods must take into consideration the nutritional role they play in diets, based on cereal grains and starchy foods. In the specific case of such edible roots as cassava and other starchy foods, such as plantain, legume-grain protein by itself constitutes the only source of protein.

The supplementary effect on cereal grains of legume protein is well documented. On a dry-weight basis, the level of beans ingested usually represents about 10 per cent of the dry weight in diets, at least those eaten in Central America (Flores, et al., 1966). Therefore, in the results presented in Tables XI

TABLE XI
Percent Utilizable Protein from Cereal Fed Alone and from 90% Cereal + 10% Bean Mixtures

Protein source	Protein in diet, %	Utilizable protein %
100% rice	6.9	4.01
90% rice + 10% beans	7.9	4.96
100% maize	8.5	2.41
90% maize + 10% beans	10.3	4.10
100% sorghum	7.7	2.23
90% sorghum + 10% beans	8.6	3.93
100% whole wheat	11.0	4.26
90% wheat + 10% beans	12.0	5.94
100% oats	13.8	8.22
90% oats + 10% beans	14.6	8.73
Casein	10.7	8.02

and XII, the diets fed to rats were prepared with 90 per cent cereal and 10 per cent black beans *(P. vulgaris)*. The resulting performance from these diets was compared with that from diets that contained 100 per cent cereal-grain protein. The effect of beans in increasing the utilizable protein in cereal-legume mixtures also is due to an increase in protein quality. This is shown in Table XI, in which protein content is equalized to about 7.5 per cent among dietary treatments. Even though the presence of 10 per cent beans in diets increased the protein quality of the cereal, the increases were larger for those cereal grains poorer in protein quality, such as maize and sorghum, followed by wheat, rice, and oats.

When protein content of the diet was not adjusted to a fixed level, at least two results are interesting, as shown in Table XII. One is the increased protein content of the diets when they contained 10 per cent bean. This is a significant increase, particularly for children, who require relatively higher protein intake than do adults and who have a smaller stomach capacity. The second point of interest can be seen under the utilizable protein column, which is higher for diets made with cereal and beans. It is also of interest to see that higher increases in utilizable protein are obtained from the cereal-bean mixtures when the cereal is of a low-protein quality – as, for example, maize, sorghum, wheat, rice, and oats, in that order. (The term "utilizable protein" means the product of protein quality and protein content relative to a reference protein, which in the present was casein.)

The evidence presented serves to propose at least two factors that should be considered in bean-quality improvement programs. First is the desirability for higher protein concentration in legume grains, which becomes more significant in terms of child feeding and, second, such protein should be higher in lysine content, as this amino acid is limiting in all cereal proteins tested. Even though

TABLE XII
Protein Quality of Cereal Grain and of Cereal Grain-Bean Diets Fed at Equal Levels of Dietary Protein

Protein source	Ave. wt. gain, g	PER
100% rice	43	2.15
90% rice + 10% beans	56	2.32
100% maize	13	0.87
90% maize + 10% beans	32	1.40
100% sorghum	12	0.88
90% sorghum + 10% beans	30	1.39
100% wheat	19	1.05
90% wheat + 10% beans	41	1.73
100% oats	34	1.60
90% oats + 10% beans	75	2.37
Casein	75	2.71

lysine is not the only limiting amino acid in cereal proteins, increased protein content in beans will carry with it more tryptophan, threonine, and methionine – amino acids that are somewhat deficient in cereal-grain protein.

Even though the presence of 10 per cent beans increases the protein quality of the cereal, such mixtures are still deficient in the same amino acids as the particular cereal under consideration. However, the amounts needed are lower. The information shown in Table XIII will support the above statements. In the present case, maize alone shows a significant response to the addition of lysine and tryptophan; these two amino acids also improve the quality of the mixture of maize and bean under consideration. It should 'so be noted that the addition of methionine to beans, whether or not maize is supplemented with lysine and tryptophan, does not improve the quality of the protein. Similar results have been observed with rice-bean mixtures, in which bean contributes about 10 to 12 per cent of the dry weight of the diet.

TABLE XIII
Amino-Acid Supplementation of Corn-Bean Diets

Dietary treatment	Ave. wt. gain g/28 days	PER
Maize + beans[1]	69	2.11
Maize + Lys + Try + beans[2]	103	2.64
Maize + beans + Met[3]	66	1.93
Maize + Lys + Try[2] + beans + Met[3]	108	2.69
Maize	32	1.21
Maize + Lys + Try[4]	100	2.68

[1] 72.4% maize + 8.1% beans
[2] 0.30% L-Lys HCl + 0.10 DL-Try
[3] 0.30% DL-Met
[4] 0.40% L-Lys HCl + 0.10% DL-Try

The above information has been presented to indicate that any consideration given to improving the nutritive quality of beans must be based on the fact that they are eaten together with other foods, cereal grains in particular. In this respect, it is of interest to analyze the results in Table XIV. In this study, attempts were made to obtain information on what changes should be introduced into beans to obtain cereal-based diets with higher protein quality. The results were obtained by changing those nutritional characteristics in beans that would be reflected in better-quality protein diets.

One can observe, first, that adding beans to a corn diet improves protein content and protein quality, as indicated earlier. In treatment three, the intake of beans was increased to twice the levels commonly found in nutritional surveys; it was assumed that greater production would increase availability, reduce prices, and result in a higher intake. The results show the diet to contain

TABLE XIV
Effect of Various Dietary Treatments on Beans on the Protein Quality of Corn-Bean Diets

Dietary treatment	Protein in diet, %	Protein from maize %	Protein from beans %	Ave. wt. gain, g/28 days	Utilizable protein %
100% maize	8.7	100	0	25	2.93
87% maize + 13% beans	10.6	71	29	48	4.54
74% maize + 26% beans	12.3	51	49	86	6.26
87% maize + 13% beans*	12.3	62	38	72	5.87
87% maize + 13% beans**	12.8	59	41	94	7.09
87% maize + 13% beans***	10.5	71	29	71	5.45

* Bean with a protein content 1.5 times the protein content of common beans.
** Bean with a protein content 2.0 times the protein content of common beans.
*** Beans with a higher lysine content than common beans.

a higher protein content, as would be expected, with values increasing from 10.6 to 12.3. This increase is translated into a higher weight gain, from 48 to 86, and an improvement in protein quality and utilizable protein. In the next treatment, the level of dry-bean intake was maintained at 13 per cent, but it was of a bean variety that contains a protein concentration about 1.5 times that of the usual beans. The effect of such an intervention is to increase the protein in the diet as well as to increase utilizable protein. The same is true when protein content is increased about twice as much as present protein concentrations.

Finally, in treatment six, bean intake and protein content were maintained, as found in nutritional surveys. However, the bean protein simulated in this case represented one with a lysine content higher than normal. The results indicated that this was as adequate a change in bean-protein composition as were the other modifications studied. I believe this to be very important. In other studies we have carried out in Central America, we have found that protein concentration and lysine, tryptophan, and methionine concentrations are closely related. One actually could select for one of these nutrients and come up with an increased concentration of the other nutrients.

For practical purposes, the data shown in this and other tables suggest that much could be accomplished in terms of the protein quality of cereal-based diets if the intake of beans could be increased by increasing availability and reducing prices – provided, of course, that there are no physiological factors limiting the intake. Second, the data suggest that beans containing higher protein or those containing normal protein with higher lysine levels could be nutritionally beneficial to populations who consume cereal-based diets. This applies particularly to preschool populations, where nutrition problems seem to be concentrated. Any alternatives should be as good, and it is up to the agronomist to concentrate on one of the possibilities. Let me emphasize, however, that it is not advisable to

generalize, because the situation recommended is (a) for populations consuming maize; (b) when beans contribute about 13 per cent of the dry weight of the diet; and (c) when the legume food is *Phaseolus vulgaris.*

Some information is also available for cassava-based diets. Results are shown in Table XV. It was assumed that beans contribute 13 per cent of the dry weight of that diet. Under these circumstances, beans with or without higher concentrations of methionine improve growth performance, as indicated by lower weight loss in a 28-day experimental period. The results were expected, because dietary protein is about 4.6 per cent, a level too low for growing weanling rats. The solution would be to increase the intake of beans, and the beans utilized should have a higher methionine content.

TABLE XV
Effect of Small Amounts of Legume Grain on the Nutritive Value of Cassava-Based Diets

Dietary treatment	Protein %	Ave. wt. gain, g
Cassava (100)	1.8	−13
Cassava + beans (87/13)	4.6	− 7
Cassava + beans + Met (87/13/0.3)	4.6	− 6

The results of Dutra, et al. (1971), are shown in Table XVI. In this case, manioc-flour diets with and without methionine were tested in the presence of three levels of beans in the diet. The results are interesting.

First, protein intake is higher as percentage of bean in the diet increases, which is to be expected. Second, best performance is obtained in every case in which methionine is present, which also is to be expected, in view of the well-known sulfur-amino acid deficiency in bean protein. Third, even though protein efficiency appears to decrease as common beans increase in the diet, percentage of utilizable protein is essentially the same.

It must be pointed out that a recommendation for a bean with a high methionine content to complement manioc diets will depend on the actual amount of bean consumed by these particular populations. Although methionine addition fed to humans has resulted in improved quality (Esselbaugh, 1952), the levels needed are smaller than those commonly utilized in rat studies. This is because humans have less need for sulfur-amino acids than do rats (Cox, et al., 1947).

Nutritional Considerations of Legume Grain-Cereal and Legume Grain-Cassava Mixtures

Up to the present time, it has been shown that small amounts of legume protein added to cereal grains improve the amount of utilizable protein in the mixture. It also has been shown that higher intake of beans with a higher protein content and beans with a higher lysine content also could result in better-quality

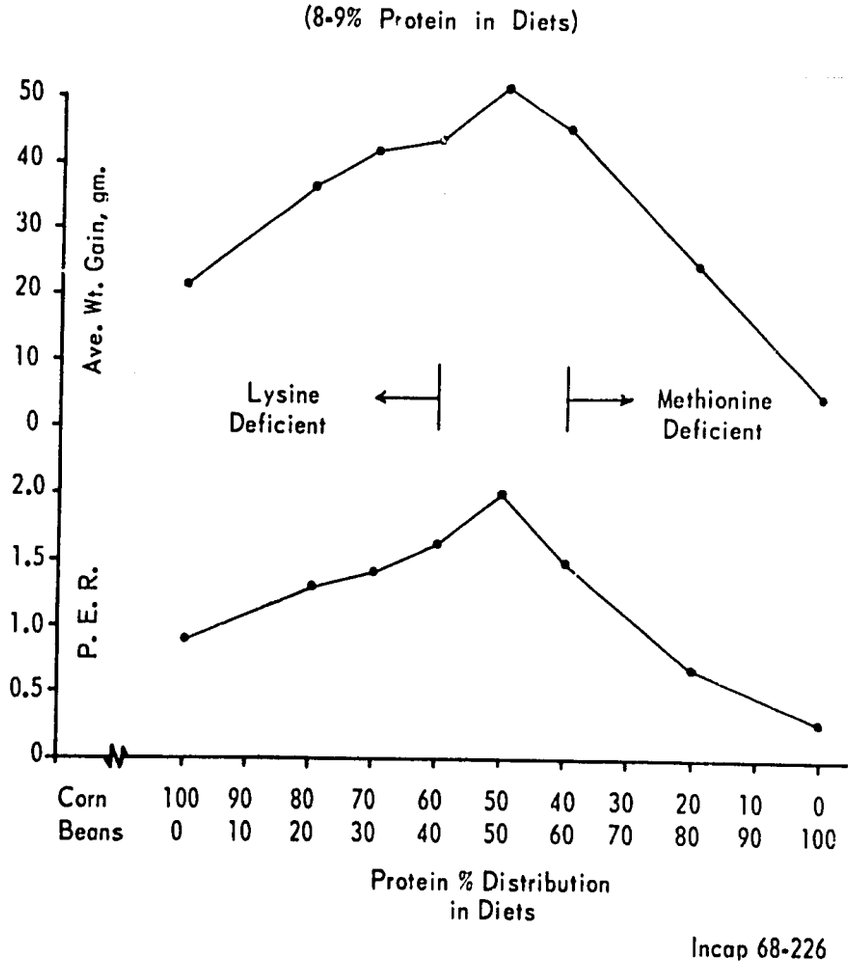

Figure 1. Nutritive value of various combinations of lime-treated corn and cooked black beans.

TABLE XVI
Protein Quality of Manioc Flour with and without Methionine Addition Mixed with Various Levels of Beans

Dietary treatment	Protein % in diet	Ave. wt. gain, g	PER
65% manioc flour + 35% bean flour	8.04	7.7	0.78
65% manioc flour + 0.6% methionine + 35% bean flour	8.85	68.2	2.70
55% manioc flour + 45% bean flour	9.97	14.2	0.96
55% manioc flour + 0.6% methionine + 45% bean flour	10.09	66.8	2.68
45% manioc flour + 55% bean flour	12.17	31.7	1.28
45% manioc flour + 0.6% methionine + 55% bean flour	12.23	71.0	2.27

From Dutra de Oliveira, et al., 1971

diets. Assuming that bean production could be increased easily and that this increased production would stimulate higher intakes, the question is: How much is recommendable in terms of protein quality?

In the results presented in Figure 1, diets for growing rats were prepared. They contained equal amounts of protein, derived in different proportions from maize and *Phaseolus*. The results show that a maximum protein value is obtained when 50 per cent of the protein in the diet is derived from beans and 50 per cent from maize (Bressani, et al., 1962). These figures correspond to 72 g of maize and 28 g of beans. The ratio of maize to beans is 2.6 to 1. Of interest in the Figure is that a mixture of the two components in the ratio shown has a protein quality greater than does each component fed alone.

From the amino-acid content of the two components and comparison to amino-acid patterns, lysine appears to be the main limiting amino acid when maize provides from 50 per cent to 100 per cent of the protein of the diet. On the other hand, methionine becomes the deficient amino acid when greater proportions of protein are provided by beans. Some evidence of this is shown in Table XVII. The addition to maize of small amounts of the three limiting amino acids increased protein utilization from a protein efficiency ratio value of 1.05 to 2.47. The 50/50 mixture was improved by the addition of small amounts of lysine and methionine from 2.10 to 2.42. Whereas the addition of methionine, tryptophan, and leucine improved the quality of bean protein, the effect probably was due mainly to methionine.

Additional results are shown in Table XVIII (Bressani, et al., 1962). The response to methionine is dependent upon the cereal grain and the legume grain used, but is relatively small. This work, carried out by Sirinit, et al. (1965), shows the effect of amino-acid supplementation on optimum protein-quality mixtures of cereal and legume grains. As already indicated, the effect of methionine supplementation is dependent on the cereal grain, as well as on the

TABLE XVII
Amino-Acid Supplementation of 72% Maize and 28% Bean Mixtures

Weight distribution, %		Amino acids added	Ave. wt. gain, g	PER
Maize	Beans			
100	0	None	29	1.05
100	0	Lys-Try-Leu	74	2.47
72	28	None	51	2.10
72	28	Met-Try	59	2.03
72	28	Met-Lys	75	2.42
0	100	None	−3	–
0	100	Met-Try-Leu	23	1.04

TABLE XVIII
Amino-Acid Supplementation of Cereal-Legume Grain Combinations

Weight distribution, %		Amino acid added	Ave. wt. gain, g	PER
Cereal	Legume grain			
Maize (70)	*P. vulgaris*, red (30)	None	47	2.41
		Met	58	2.60
		Met-Lys	42	2.35
Maize (80)	*P. lunatus* (20)	None	52	2.50
		Met	51	2.56
		Met-Lys	46	2.49
Sorghum (70)	*P. vulgaris*, black (30)	None	56	2.54
		Met	58	2.61
		Met-Lys	64	3.05
Sorghum (70)	*P. lunatus* (30)	None	50	2.44
		Met	50	2.47
		Met-Lys	77	3.27
Rice (80)	*P. vulgaris*, red (20)	None	57	2.85
		Met	62	2.98
		Met-Lys-Thr	67	3.20
Rice (80)	*P. lunatus* (20)	None	62	3.08
		Met	63	3.13
		Met-Lys-Thr	73	3.66

From Sirinit, et al., 1965

legume food. For example, the mixtures of maize and sorghum with *Phaseolus vulgaris* are improved when methionine is added, but this is not true with rice. On the other hand, cereals mixed with *Phaseolus lunatus* are not improved in quality when supplemented with methionine.

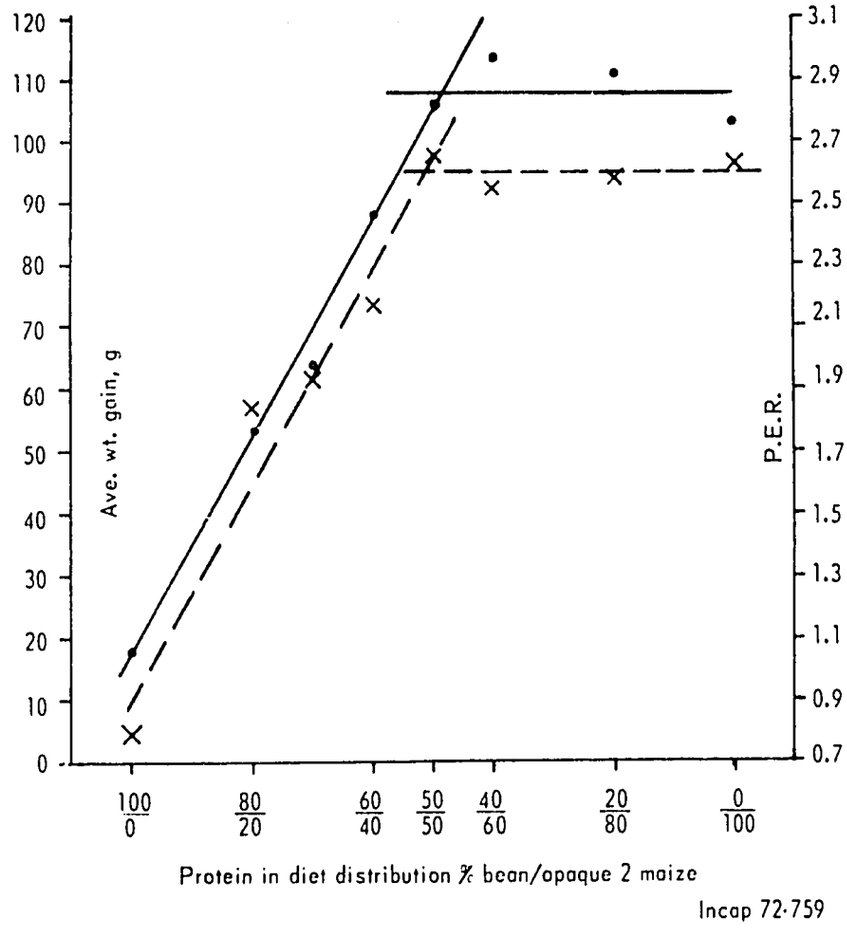

Figure 2. Average weight gain and protein efficiency ratio of bean-opaque-2 maize mixtures.

Similar studies have been carried out with opaque-2 maize and black beans, with the results shown in Figure 2 (Bressani and Elías, 1969). In this example, bean protein is still deficient in methionine; however, opaque-2 maize has essentially the same methionine content as common maize, but has significantly higher levels of lysine and tryptophan. Highest performance occurred when 50 per cent of the dietary protein was derived from each component. However, similar values were observed when maize contributed with greater levels of protein. On the other hand, greater levels of bean protein in the mixture resulted in lower performance, because methionine deficiency increased as bean protein increased in the diet. It should be pointed out that the 50/50 mixture of maize and *Phaseolus vulgaris* gave a PER value of 2.1 and a weight gain of 52 g. In contrast, the 50/50 protein mixture of opaque-2 maize and *Phaseolus vulgaris* gave a PER value of 2.6 with a weight gain in 28 days of 108 g.

From the experimental results shown, the optimum proportions of maize to beans are 78 g of maize and 28 g of beans for a 2.6 to 1 ratio. The results of various nutritional surveys carried out in Central America have permitted the calculation of the actual ratio consumed. These results are shown in Table XIX (Flores, et al., 1966; Flores, 1961). Adult humans show a lower ratio, compared to the highest found in children; the ratio in favor of maize is 14 or 12.1. In both cases, consumption of maize predominates. This permits the conclusion that the diets are low in protein and deficient in lysine.

Mixtures that provide the maximum protein quality have been determined for other cereals and legume grains (Elías, et al., 1969). A summary of such results is presented in Table XX. Rice and *Phaseolus vulgaris* combine into a mixture of high-protein quality when 80 per cent of the dietary protein comes from rice and 20 per cent from beans. Protein quality increases about 16 per cent when the quality of the mixture is compared with the cereal grain alone. Maize gives highest protein-quality values when combined in a 50/50 protein ratio with *Phaseolus* and *Vigna* and in a 60/40 protein ratio with soybean. Increase in quality ranges from 51 per cent to 122 per cent. Wheat combines in a 73/27 protein ratio with *Phaseolus,* which causes an increase in quality of about 49 per cent above wheat alone. These results suggest that the poorer the quality of the cereal the greater the level of legume protein needed to bring about the improvement in quality observed. They also suggest that cereal-based diets could be of higher protein quality if the legume-grain intake was higher than it is at present.

Examination of legume intake in bean-consuming countries shows that, in general, it is relatively low. For example, Table XXI summarizes average intake levels in the Central American countries. The highest intake is 72 g/person/day, which is not enough to give a 2.6 ratio with respect to the intake of cereal consumed (Flores, 1961).

From these and other results, various questions can be raised. Why do people eat only these amounts of beans? Can more beans be consumed? Is consumption limited by their low availability, or is there a physiological limit induced by unrecognized factors, by low protein digestibility, or by a resistance to digestion? If bean consumption is to increase and help solve the world protein deficiency, these questions must be answered.

TABLE XIX
Average Daily Intake of Maize and Beans in Various Areas of Central America

	Maize g/day	Beans g/day	Maize/bean ratio
Guatemala			
Adults	423	58	7.3
Children	281	24	11.7
	295	26	11.3
	277	15	18.5
Honduras			
Adults	398	56	7.1
El Salvador			
Adults	374	60	6.2
Best ratio	72	28	2.6

TABLE XX
Protein Value of Optimum Combinations between Cereals and Leguminous Seeds

Distribution of protein in diet, %			
From cereal	From beans	PER	Increase, %
100 rice	0 beans	2.25	16.4
80 rice	20 beans	2.62	
100 maize	0 beans	0.90	122.2
50 maize	50 beans	2.00	
100 maize	0 cow pea	1.22	50.8
50 maize	50 cow pea	1.84	
100 wheat	0 beans	47*	48.9
73 wheat	27 beans	70*	
100 maize	0 soybean	1.50	90.0
40 maize	60 soybean	2.85	

* NPU

An attempt to answer some of these questions is shown in Table XXII. In this study, which was carried out with young rats, the animals were allowed to eat as they wished from two feeder cups placed in the cage. One contained maize, the other *Phaseolus*. The maize and bean diets were modified (second column) to permit the rat to choose the food that was made more suitable nutritionally.

TABLE XXI
Consumption of Legume Grain in Urban and Rural Areas of the Central American Countries

g/person/day

Country	Urban area	Rural area
Guatemala	45	50
El Salvador	52	59
Honduras	47	41
Nicaragua	50	72
Costa Rica	48	57
Panamá	19	20

TABLE XXII
Effect of Various Dietary Treatments on the Free Intake of Maize and Beans by Young Rats

Foods	Dietary treatment	Intake g/rat/28 days	Intake Ratio	Utilizable protein %
Maize	None	188	3.58	3.11
Beans	None	53		
Maize	Vit-Min-Cal	238	3.87	5.15
Beans	Vit-Min-Cal	61		
Maize	None	257	2.08	6.24
Beans	Vit-Min-Cal	124		
Maize	Vit-Min-Cal	250	2.65	6.19
Beans	None	94		
Maize	Lys-Try	184	3.86	3.81
Beans	Met	47		
Maize	Lys-Try-Vit Min-Cal	272	2.26	6.68
Beans	Met-Vit-Min-Cal	120		
Maize	Lys-Try-Vit Min-Cal	291	2.94	6.10
Beans	Vit-Min-Cal	59		
Maize	Vit-Min-Cal	256	2.28	6.55
Beans	Met-Vit-Min-Cal	112		
Maize	None	199	1.03	9.12
Casein diet (22% prot)	Vit-Min-Cal	194		

Intake from each cup was recorded (third column) and used to calculate the maize-to-bean ratio. The percentage of utilizable protein was calculated from the protein ingested and the protein-efficiency ratio.

The first group was fed on maize and beans without any other dietary treatment. The ratio of maize to beans was 3.58 with a utilizable protein of 3.11 per cent. These results indicate that the animal tried to balance the quality of the protein ingested, because the ratio of 3.6 is closer to the best ratio–2.6–found in other experiments. When both maize and beans were supplemented individually with vitamins, minerals, and additional calories, the animals consumed maize and beans in a ratio of 3.87, similar to that of the previous treatment. Utilizable protein increased, which was to be expected because other needed nutrients were provided. It should also be noted that the intake of maize and beans increased.

The third treatment consisted of supplementing beans with vitamins, minerals, and additional calories, but making no additions to the maize. This treatment stimulated the "free intake" of beans and also of maize, giving a ratio of about 2.1. Utilizable protein was found to be 6.24. The increased intake of beans can be explained on the basis that the animal consumed more to meet its needs for other nutrients. However, it is difficult to explain why cereal intake also increased.

The fourth treatment was the reverse of the third. Maize was supplemented with vitamins, minerals, and additional calories, whereas beans were not. Maize intake was similar to that in the previous study, but bean intake was lower. The ratio of maize to beans was 2.6 and utilizable protein 6.2 per cent.

Factors more directly related to protein quality were tested in treatments 5 to 8. It will be noticed that adding lysine and tryptophan to maize and methionine to beans did not alter significantly the ratio of maize to beans observed in the first group. Utilizable protein increased slightly. When both amino acids and other nutrients are added to the two foods, there is a high intake of both to give a 2.3 ratio with 6.7 per cent of utilizable protein. Treatment 7 improved the protein quality of maize, but not of beans, with other nutrients present in both. This stimulated maize intake, but not that of beans, contrary to what is seen in treatment 8, in which beans, but not maize, were supplemented with methionine to improve protein quality. This resulted in an increase in bean intake, but did not differ greatly from the intakes observed in the third treatment. Finally, the last group was allowed to eat maize and a diet of casein made to simulate beans in protein content. This last group gave the best performance in every measure.

The results of this study were interpreted to mean that, when the rat is allowed to choose its food, it does so by balancing the protein quality ingested. This is stimulated by nutrient supplementation, but not by amino acids alone. Many observations are difficult to explain, and no attempts will be made to do so now. However, the results show clearly that the animals tend to consume more beans when they are made available. It is our opinion, therefore, that efforts should be made to increase production of legume grains to permit cost reductions and thus a greater intake. In the studies shown, availability was not a consideration for intake and, in all cases, the ratio that could be modified tended

toward the best ratio for maize and beans, which is 2.6 (Bressani, et al., 1962).

An important factor in this study is the lower digestibility of beans, as judged by the results of the casein diet. A better control would probably have been meat protein with an amino-acid pattern closer to that of bean protein, even though casein is methionine-deficient, as is bean protein. Casein is also slightly higher in lysine than is bean protein, and definitely higher in tryptophan.

The Problem of Legume-Protein Digestibility

Most people recognize that beans are difficult to digest and may give rise to stomach upsets. Nutritionists are aware that they have a low protein digestibility. At present, it is not known whether these effects are caused by a more rapid discharge from the intestine or by a resistance to protein hydrolysis by the gastrointestinal enzymes. In any case, significant losses of nitrogen occur in feces when beans are consumed.

Some examples from various legume foods are shown in the next tables. Table XXIII summarizes the results of studies on human adults fed Alaska split pea *(Pisum sativum)* (Esselbaugh, et al., 1952). When eggs were eaten, fecal nitrogen represented 14.5 per cent of the nitrogen intake; when peas were fed, fecal nitrogen represented from 20 to 21 per cent of nitrogen intake. Apparently, protein digestibility was somewhat lower for pea protein than for egg.

The same kinds of observations have been made in children. For example, Table XXIV shows results of various studies of children fed *Phaseolus vulgaris* in combination with other foods. Fecal nitrogen values are shown in mg of nitrogen/kg/day, and as percentage of nitrogen intake. It can be seen (last column) that fecal nitrogen increased as milk-protein nitrogen intake decreased. Not all the effect can be attributed to bean protein, because it was given in combination with maize. However, nitrogen losses increased, and beans may have been responsible to some degree.

TABLE XXIII
Fecal Nitrogen and Apparent Protein Digestibility of Human Adults Fed Egg Protein and Split Peas with and without Methionine Addition

Protein source	Nitrogen, gm					App. prot. dig. %
	Intake	Urine	Fecal	Absorbed	Retained	
Egg	5.60	4.90	0.81	4.79	−0.11	85.6
Split pea	5.47	4.62	1.16	4.31	−0.31	78.8
Split pea + Met	5.95	4.21	1.19	4.76	+0.55	80.0

From Esselbaugh, et al., 1952

TABLE XXIV
Fecal Nitrogen from Children and Young Dogs Fed Various Proteins Containing Cooked Beans

Diet	Nitrogen Intake (mg/kg/day)	Nitrogen Fecal (mg/kg/day)	% Fecal N of N intake
	CHILDREN		
Milk	387	70	18.1
25% milk + 75% (maize + beans)	358	98	27.4
10% milk + 90% (maize + beans)	353	134	38.0
100% (maize + beans)	347	107	30.8
	DOGS		
Maize	520	189	36.3
Maize + black beans	635	254	40.0

The results in the lower section of the Table were obtained from young dogs that were fed maize protein both alone and with bean flour. The intake of beans was equivalent to 32 per cent of the total nitrogen intake, and with this relatively small amount, fecal nitrogen increased from 36 per cent to 40 per cent of the nitrogen intake (INCAP, unpublished data).

The low protein digestibility of legume grains has been observed not only among species, but also among varieties of the same species. For example, as shown in Table XXV, Jaffé (1950b) observed that *Cajanus indicus* had a protein digestibility of 59 per cent, in contrast to other varieties that showed values as high as 90 per cent. On the other hand, *Vigna sinensis* showed digestibility coefficients that varied between 86 per cent and 90 per cent. The value for *Lens esculenta* was 93 per cent; for *Pisum sativum*, about 92 per cent.

No experimental results permit us to estimate with precision the variability of protein digestibility of the various bean cultivars, which would allow us to select varieties with higher values. It is possible that grain size has a significant influence, because small seeds have heavier cotyledons.

Recently, Jaffé (Seidl, et al., 1969; Jaffé and Vega, 1968) obtained a protein fraction from black beans that was resistant to in vitro digestion by 10 proteolytic enzymes, even after protein denaturation, and that was inhibitory to trypsin and papain. It is not known if such a protein is present in all legume grains. The classical trypsin inhibitor is thermolabile, so it follows that it cannot be responsible for the low digestibility of the protein observed in some species and varieties of beans.

TABLE XXV
True Protein Digestibility of Legume Grain Species and Varieties

Legume grain	True protein digestibility, %
Phaseolus vulgaris (black)	76.8
Phaseolus vulgaris (white)	84.1
Phaseolus vulgaris (pink)	79.5
Vigna sinensis (black)	90.0
Vigna sinensis (beige)	86.4
Pisum sativum (green)	90.7
Pisum sativum (yellow)	93.9
Cajanus cajan	90.5
Cajanus cajan	59.5
Lens esculenta	92.6
Cicer arietinum	90.5

From Jaffé, 1950b

Physiological Effects

Legume foods are known to contain certain toxic substances classified as: a) tryspin inhibitors; b) hemagglutinins; c) goiterogenic factors; d) cyanogenic glucosides; e) lathyric factors; and f) compounds that cause favism. There are excellent reviews on these subjects, therefore I will not discuss them here. Attention will be given to other, less-known effects of legume foods that could affect their utilization as protein sources for young and adult humans.

Flatulence. One of the best-known problems that results from the consumption of legume foods is increased flatulence. Steggerda, et al. (1966), working with human subjects, indicated that, when beans are eaten, the normal physiology of the gastrointestinal tract is altered so that flatulence production is markedly stimulated.

Rockland (1969) obtained evidence suggesting that the primary cause of flatulence may be the production of gas by gram-positive, anaerobic bacteria present in the gastrointestinal tract after stimulation by unknown factors in dry beans. Furthermore, he suggests that *Clostridium perfringens,* which is the main intestinal anaerobe, is probably the primary source of flatus gases (Rockland, et al., 1969). This hypothesis could be correct, in view of the findings by Kakade and Borchers (1967) that the gas-producing efficiency of rats fed heated navy beans was significantly reduced when antibiotics also were fed. The effects of various antibiotics were also described by Rockland, et al., in 1969, using *C. perfringens* as the gas-forming bacteria. Bornside and Cohn (1965) also observed that the class of bacteria most responsible for the reaction was the gram-positive, anaerobic, spore-forming, clostridia type, which normally inhabits the intestinal tract of animals and humans.

Steggerda, et al.(1966) showed that the gas-producing factor is to be found in carbohydrate fractions of low molecular weight. These include sucrose, stachyose, and raffinose. Rackis, et al. (1970) concluded that these three oligosaccharides are connected with the gas-producing factor when incubated with anaerobes.

The specific factor or factors in beans responsible for flatulence have not been established as yet, even though Murphy (1964) found that the flatulence factor can be extracted from cooked beans with 60 per cent ethyl alcohol. He emphasizes that the factor is low in molecular weight, perhaps even below 10,000. He also reports that the proteins, starch polysaccharides, and lipids of beans are not responsible for gas production.

Sánchez, et al. (1966) indicate that with some cultivars of lima beans, flatulence-stimulation activity does not appear until about one week before the beans are at the green mature state, and continues to increase to a maximum until the dry beans are ready for harvest. More recently, it was shown by Calloway, et al. (1971) that enzymatic treatment of soybeans and alcoholic extraction of whole white beans, as well as other treatments, could decrease to various degrees the flatulence factors in the legume grains studied.

Heat-Sensitive Inhibitors. Not all legume foods are improved by autoclaving, and if the effect of heating is to destroy trypsin inhibitors, it can be concluded either that not all legume foods contain them or that, if present, the concentration or activity is variable among species and even among cultivars. For example, Borchers and Ackerson (1950) determined that of 17 species of legume seeds investigated, eight were improved nutritionally by autoclaving, whereas nine were not. No correlation was observed between the effect of autoclaving on nutritive value and the presence or absence of the trypsin inhibitor in the raw legume seed. Jaffé (1950b; 1968) reported on the digestibility of eight kinds of legume foods fed raw and autoclaved, as well as on the trypsin-inhibitor activity. Autoclaving increased the digestibility of those legume foods with higher initial trypsin-inhibitor activity, which varied from 178 x 10^{-4} units/gm in lentils *(Lens esculenta)* to 4.38 in *Dolichos lablab.* Soybeans and kidney beans also had high trypsin-inhibitor activity values – more than 4 x 10^{-4} units/gm.

Studies at the Institute of Nutrition of Central America and Panama (INCAP) on *Phaseolus vulgaris* revealed that inhibitors are present very early in the formation of the seed. Zimmerman, et al. (1967) reported more activity in the outer layers of the seed than in the inner layers.

Apparently, the function of these inhibitors has not been studied; their significance to the bean plant should receive attention, so that if findings prove they are not useful to the plant, they may be eliminated genetically. On the other hand, because they are high in cystine content, their presence might be useful nutritionally because sulfur-containing amino acids, as already indicated, limit legume protein. However, Kakade, et al. (1969) found in chick studies that the cystine of the navy bean trypsin inhibitor is not released by digestive enzymes.

Hypocholesterolemic Property. Another characteristic resulting from the consumption of legume foods has merited some attention. That is their hypocholesterolemic property. Grande, et al. (1965) compared the effects on serum cholesterol of ingesting the carbohydrate of bread and potatoes with those of

the carbohydrates of legume foods that were isocalorically substituted for sucrose. The diet containing leguminous seeds induced a serum cholesterol level lower by 9 per cent than did that of a sucrose diet. Other reports have confirmed the hypocholesterolemic effects of diets containing a high proportion of leguminous seeds. Mathur, et al. (1961), for example, noted that people of low socioeconomic status in Agra, India, whose staple diet was chick peas *(Cicer arietinum)* had much lower serum cholesterol levels than those who did not consume that diet. In the Netherlands, Groen, et al. (1961) observed that Trappist monks had lower serum cholesterol levels than did Benedictines, and noted that legume foods were an important part of the diet of the former. In studies with 20 volunteers, Luyken and his associates (1962) showed that the introduction of 100 gm of legume foods, mainly brown beans, resulted in a mean serum cholesterol decrease of 12 mg per 100 ml.

Mathur and coworkers (1964) have reported that the cholesterol-depressing action of *Cicer arietinum* in rats fed a diet containing cholesterol and cholic acid is also produced by the fat-free seed and by its lipid extract. It should be said that bean lipids contain relatively high levels of unsaturated fatty acids. Grande, et al. (1965) postulated that the decrease in serum cholesterol concentration was caused by the carbohydrate fraction of the leguminous foods.

Muscular Dystrophy. A further effect from consumption of legume foods, particularly raw, has been the production of a muscular dystrophy in animals. Hogue, et al. (1962) report that when a ration containing raw kidney beans was fed to pregnant ewes, it was effective in producing nutritional muscular dystrophy (NMD) in the lambs. Autoclaving the beans decreased the incidence of NMD; it is suggested that kidney beans contain a heat-labile, vitamin-E antagonist. Autoclaved beans also exhibit negative vitamin-E activity in the prevention of exudative diathesis in chicks. Other legume foods have been reported to have antivitamin-E activity. In 1953, Sanyal reported that *Pisum sativum* contains a factor that decreases the effectiveness of vitamin E in the prevention of fetal resorption in the fat.

Hintz and Hogue (1964) reported that when 50 to 75 per cent raw kidney beans and vitamin E were added to diets of one-day-old chicks, the incidence of nutritional muscular dystrophy was increased to 45 to 100 per cent. This indicated that beans contain an antivitamin-E factor, because the vitamin E-deficient diet produced NMD at the rate of 95 to 100 per cent, whereas 20 to 25 I.U. of vitamin E/kg decreases the incidence to 5 to 6 per cent. Extracting and autoclaving the beans indicated two antagonists to vitamin E. One is alcohol-soluble and heat-stable and the second is not.

Conclusions

It would appear that the most important effort to be made in bean research is to increase the yield, which, it is hoped, would increase bean availability and decrease their cost. Significant advances already have been made in this respect in some countries – for example, El Salvador in Central America. However, the higher-yield effort may be short-lived because of the improper storage conditions

that cause hardshell in the grain, so reducing their cooking quality. In a recent meeting held in El Salvador, farmers continuously complained about the poor storage stability of beans in their country.

Once good storage conditions are achieved, together with greater availability and cost decreases, bean research should concentrate on increasing total protein content. Results indicate that higher levels of lysine, tryptophan, and methionine, in that order, result from beans that contain higher protein levels. We had to screen about 300 varieties of beans in this study, and we found correlations among those three factors. Higher protein content is of benefit when beans are consumed together with cereal-grain and manioc-based diets.

Attempts also should be directed to increasing protein digestibility of some legume-grain species. The factors responsible for protein indigestibility are not known. Trypsin inhibitors could not be totally responsible, because they are destroyed during heating. However, it is possible that available techniques do not permit assays for other heat-stable factors.

In terms of cereal diets, beans with a higher lysine content might prove to be very effective in improving the quality of the diet. However, if digestibility of the protein could be improved, it is probable that the amounts now present are enough to balance the low lysine content in cereal grains.

The flatulence factor is another characteristic of beans that should be eliminated. It is possible that it may be destroyed by certain processing techniques. People who eat beans daily suggest that strained beans produce less gas than do cooked whole beans, and that cooking with certain ingredients also reduces flatulence.

Beans with a high methionine content also should receive attention, particularly for those populations on manioc diets, which contribute little protein.

Higher intakes of bean protein probably could be achieved through the preparation of stable, precooked products, which offer the advantages of improved stability, lower costs of preparation, and possible additional nutrients.

Finally, it should be emphasized that for nutritive-quality screening purposes, it is essential to standardize all procedures to be used after harvest, starting with storage, through processing and drying, to the assay technique. The optimum cooking procedure to inactivate trypsin inhibitors and other heat-sensitive toxic substances probably is not the same for all legume species or varieties, because they contain different concentrations of those compounds. Similarly, some species have a greater tendency than others to hardshell. Standardization and definition of the screening procedures will speed up the nutritional role of legume grains for human populations.

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FACTORS AFFECTING THE NUTRITIONAL VALUE OF BEANS

Werner G. Jaffé

Common beans *(Phaseolus vulgaris)* are among the most important legumes in human nutrition in Latin America, parts of Africa, and India. Unfortunately, they contain a greater number of antinutritional factors than do most other legumes. These factors differ both qualitatively and quantitatively in the enormous number of known bean varieties or cultivars. Consequently, progress in understanding their relative importance has been slow. Nevertheless, the considerable knowledge gained thus far could be applied to work on the genetic improvement of beans.

Raw beans, when fed in an experimental diet, are toxic to animals. When cooked, beans may be ingested without immediate harm. However, to be adequate nutritionally, the diet must be supplemented with methionine, the limiting amino acid in most legumes.

Changes in body weight of young rats fed diets containing 40 per cent raw or cooked red kidney beans, with or without methionine being added, are presented in Table I. As can be seen, only the animals receiving the cooked-bean diet supplemented with methionine were able to grow normally. Rats fed diets prepared with raw beans died within 10-14 days. As shown in the same Table, a similar experiment, in which a white-bean cultivar, the Mountaineer Half Runner bean, was fed gave quite different results. The weight loss of rats consuming the raw-bean diet was much less pronounced than in the previous experiment. Moreover, all of the animals survived the 28-day duration of the experiment. Normal growth was observed again in the experimental group fed the cooked-bean diet supplemented with methionine. Supplementation of the diet with predigested casein had practically no effect on the animals that were fed the raw red beans, but resulted in good growth when raw white beans were fed (Jaffé and Vega, 1968).

The highly significant difference in toxicity between the red and white bean varieties observed in these experiments could be traced to the presence of different types of hemagglutinins. These can be distinguished by means of a simple test in which different blood preparations are used. It has been observed consistently that extracts of bean seeds that are active in agglutinating trypsin-treated blood cells from cows are toxic. This toxicity can be demonstrated by injecting mice intraperitoneally with a crude seed extract (Table II) or by feeding tests. Four distinct types of bean agglutinins can be distinguished in different varieties by this test (Table III). The details of the test and its application to

TABLE I
Performance of Rats Fed Diets Prepared with 40% of Ground Beans and 0.3% of DL-Methionine

Diet	Wt. change/day g
Raw red kidney beans	−2.1*
Raw red kidney beans + digested casein (casitone)	−1.8*
Red kidney beans cooked for 2 h at 85°C	−0.5
Red kidney beans autoclaved at 118°C for 30 min.	+2.8
id. without methionine	+0.4
Raw white beans	+0.1
Raw white beans + digested casein (casitone)	+2.3
White beans cooked for 2 h at 85°C	+1.5
White beans autoclaved at 118°C for 30 min.	+2.4
id. without methionine	+0.3

*All animals died within 10-14 days.

distinguishing between different types of bean agglutinins has been published (Jaffé, et al., 1972).

Prolonged boiling or autoclaving of presoaked bean seeds destroys their agglutinating activity as well as their acute toxic action. We have found that the hemagglutinin activity toward rabbit or human red blood cells is much more heat-sensitive than the toxic activity. However, the agglutinin activity in trypsin-treated cow blood cells persists as long as a toxic action can be detected, as shown in Table II (Jaffé and Brucher, 1972). This leads to the conclusion that the agglutinin test with trypsin-treated cow blood is useful in detecting toxic bean varieties and also for assessing the rate of destruction of the toxic factor by heat.

When whole bean seeds are soaked in water and cooked for human consumption, the toxic factors are usually destroyed. Recently, a mixture of ground beans and ground cereals has been utilized successfully in child nutrition programs. By varying the composition of these mixtures, optimal combinations of proteins from both sources can be reached. Cooking time for these mixtures is shorter than that necessary for the whole seeds. Korte (1972) has recently observed that when these types of mixtures are prepared under field conditions

TABLE II
Hemagglutinating Activity and Toxicity of Crude and Heated Bean Extracts

Variety	Type	Crude extract Hemagglutination		Mortality of injected mice	Heated extract Hemagglutination		Mortality of injected mice
		Rabbit blood	Trypsin act. cow blood		Rabbit blood	Trypsin act. cow blood	
761-MM	A	9	10	6/6	0	9	7/8
San Francisco	B	9	3	0/6	0	0	0/5
Porillo	C	0	12	6/6	0	12	5/5
Alabaster	D	0	0	0/6	0	0	–

The extracts were heated for 2 h to 85°C.

TABLE III
Hemagglutinating Activity of Different Types of Beans on Different Blood Samples

Type of blood	Bean Variety			
	Saxa (A)	Cubagua (B)	Porillo (C)	Mountaineer Half Runner (D)
Rabbit	8	9	0	0
Trypsin-treated cow blood	12	2	12	1
Human AB	6	6	0	0
Swine	11	9	0	0
Pronase-treated hamster blood	12	11	12	10

The highest dilution step of a 10% bean extract producing agglutination within 1 h is indicated.

prevailing in Africa, the toxic hemagglutinin is not always destroyed. This was proved by the agglutinin test and by feeding trials in humans, which resulted in diarrhea and other signs of toxicity.

In other studies, bean plants of toxic type A were crossed with plants of nontoxic type D. Of the total number of F_2 seeds examined, 78 per cent were type A and 22 per cent type D. Type A, therefore, is inherited as a single dominant trait (Jaffé and Brucher, 1972) (Table IV).

Many commercial cultivars belong to the toxic A-type beans, i.e., Saxa, Cubagua, Balinde albenga, Guateina. On the other hand, few commercial cultivars,

TABLE IV
Hemagglutinating Activity in the F_2 – Generation of Crosses of Type-A and -D Bean Plants

No.	Type of cross	Agglutination of rabbit and trypsin-treated cow rbc: Number of seeds +	Number of seeds –
1	A/D	23	13
2	A/D	76	28
3	A/D	22	14
4	D/A	30	6
5	D/A	55	22
6	D/A	241	81
7	D/A	231	77
		678 (73.7%)	241 (26.3%)

Extracts from each seed were tested with rabbit and trypsin-treated cow blood cells blood cells separately. All positive seeds showed activity with both blood types.

mostly white, are of the more desirable D-type (Mountaineer Half Runner, Kaiser Wilhelm, Alabaster). There are considerable quantitative differences in agglutinating strength and toxicity among varieties of any one type of bean. We have encountered varieties of A-type beans that are highly active as agglutinins of trypsin-treated cow blood and are more toxic than other varieties with lower agglutinating action. In a few cases, it was found that a certain cultivar contained both the toxic type-A and the low-toxicity type-B seeds. Of course, there may be other qualitative and quantitative biochemical differences between agglutinins of cultivars with low and high toxicity that have not been detected as yet.

The toxic agglutinins are certainly not the only antinutritional factors in beans. A trypsin inhibitor has been obtained from beans (Wagner and Riehm, 1967). Its concentration varies widely in different bean samples. No correlation seems to exist between trypsin inhibitory activity and digestibility (Table V), as has also been observed in animal experiments (Pusztai, 1967).

A bean globulin, different from the trypsin inhibitor, interfered with the hydrolytic action of seven proteases tested (Seidl, et al., 1969). Its specific action is low, but the bean content of this globulin is much greater than that of the trypsin inhibitor. Its action is not completely abolished by autoclaving, and it could therefore be a factor that is responsible for the poor digestibility of cooked beans – one of the major disadvantages of kidney beans as human food. The poor growth of the rats fed the methionine-supplemented, white-bean diet (Table I) could well be due to this nonspecific protease inhibitor, because these beans are devoid of toxic hemagglutinins and are very poor in trypsin-inhibitor activity. No data on possible variations of this inhibitor in different bean varieties are available. Both in vivo and in vitro tests of digestibility will be required in order to determine the most desirable bean varieties.

The digestibility of starch, as well as proteins, is important, as low starch digestibility is related to poor cooking quality. The in vitro digestibilities by pancreatic amylase of starch from beans with good and bad cooking quality were compared and a relationship between cooking rate and starch digestibility was observed (Hellendoorn, 1969).

An amylase inhibitor is present in most kidney beans (Jaffé and Vega, 1968), but it is destroyed by proper heating and little is known about its physiological importance.

TABLE V
Enzyme Inhibitors and In Vitro Digestibility in Selected Bean Samples

No.	Sample	Trypsin inh. U/g	Chymotrypsin inh. U/g	Digestibility	
				crude	cooked
2035	Red beans	0	0	49	71
2311	Red beans	39	22	56	83
2081	Black beans	65	17	55	80
2041	White beans	0	11	52	91

Among the factors not destroyed by proper heating of beans are phytic acid, which may interfere with iron absorption, and flatulence-producing factors.

The intestinal absorption of iron from beans is poor (Layrisse, et al., 1968). Phytic acid may be at least partially responsible for this, although quantitative data on the relation are still lacking.

The flatulence-producing effect of cooked beans has been the subject of a good number of investigations. Alcohol-soluble flatulence factors have been described (Murphy, 1964), but alcohol-extracted beans still produce considerable amounts of intestinal gas (Hellendoorn, 1969).

In conclusion, it may be stated that in any work related to the genetic improvement of the nutritional value of beans, not only the protein level and amounts of sulfur-containing amino acids should be taken into account, but also the content of toxic hemagglutinins (which can be detected easily in single seeds or even in parts of seeds), and digestibility. Other factors of importance in this context are cooking quality, flatulence factors, and possibly phytic acid.

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DISCUSSANT – Doris Calloway

When we talk about nutritional assets of beans and legumes of all kinds, we tend to forget that they provide many nutrients, in addition to proteins, that are worthy of consideration. They are an additional source of energy, which is required to utilize protein, as well as of vitamins and minerals, such as iron and calcium, that are often needed in the diet. The availability of these nutrients must be considered in the same depth as protein availability.

In countries where protein resources are low, legumes could serve as the main source of protein. There is one example, soybeans, in which the quality of protein is demonstrated to be high for man. In all studies, with one exception (that study was on a protein isolate, rather than whole soy protein), the growth of human infants has been comparable with feedings of protein from soymilk, breast milk, or cow milk. That is, soymilk protein alone maintains adequate growth rates in children (Graham, et al., 1970; Sellers, et al., 1971). In adults, when pure soybean protein has been fed as the sole source in the diet, it has ranked comparably with animal protein (Bricker, et al., 1945). Here, apparently, is one legume that does not need very much improvement (even though some improvement can be shown in biological value if methionine is added).

I believe that most differences in biological quality of legume proteins will be evident only if they are fed at levels that are inadequate to maintain good growth rates in children or nitrogen balance in adults. When enough total protein to meet these criteria is in a diet, many of the differences in the quality of protein sources tend to disappear (Kies and Fox, 1971). I think that is an important consideration: while it may be true that quality is less optimal when levels of protein or energy are limited, these differences may be irrelevant in terms of some ultimate applications.

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DISCUSSANT – Irvin E. Liener

There should be little doubt in our minds that legumes can constitute a valuable source of protein for supplementing the deficiency of lysine in cereal proteins. The remarks I would like to make are an attempt to bring Dr. Bressani's comments more in line with the objectives of this conference. The biochemist has at his disposal a wide variety of techniques for determing chemical parameters which may be directly or indirectly related to the nutritive properties of proteins. These would include, for example, such determinations as amino-acid composition, lysine availability, in vitro digestibility, trypsin-inhibitor activity, and hemagglutinating activity. But the crucial question invariably arises—what do these chemical parameters really tell us about the nutritional properties of the protein? For instance, the amino-acid composition of the protein does not tell us how much of these amino acids are actually available to the animal. The factor which is most likely to affect the availability of amino acids is the digestibility of the protein.

What is really needed, therefore, are techniques for evaluating, in a simple fashion, the digestibility of proteins. Obviously, the best method would be an animal assay, but such assays are of little use to the geneticist or plant breeder who needs a rapid technique for screening, such as some simple in vitro method. It may, perhaps, be desirable to subject the protein to some fractionating procedure, since it is known from the work of Dr. Jaffé in Venezuela that certain plant protein fractions are less digestible than others. Dr. Jaffé has shown that there is a globulin fraction in some legumes that is highly resistant to the action of proteolytic enzymes, even after being subjected to heat treatment. Perhaps we should be concentrating our attention on this particular fraction of protein; this might turn out to be a most useful index of the digestibility of leguminous proteins.

I would like to make a brief comment regarding the effect of cooking or heat treatment on the nutritive value of legumes. The question is frequently raised: Why do we worry about the toxic factors present in legumes if they can in fact be destroyed by cooking? But I would ask in reply: How can we be certain that these factors are always destroyed by cooking under a given set of conditions? We know from our own work that *Phaseolus vulgaris*, for example, requires much more drastic heat treatment for the inactivation of these factors than do some of the other legumes, such as cow peas *(Vigna sinensis)*. If we decide to play it "safe" and apply an excessive amount of heat, then how do we know that we are not causing damage to the protein itself? We all know that too much heat can be just as harmful as not enough heat. In other words, there is an optimum level of heat treatment than can and should be applied for processing plant proteins in order to achieve maximum nutritive value.

These, then, are a few of the remarks I would like to make to indicate some of the problems to which we should address ourselves during the course of this conference.

DISCUSSANT – B. M. Nicol

I would like to say a word or two on world supplies of protein, as we now view them in FAO. The FAO Agricultural Commodity Projections, which have been prepared for 1970 to 1980, show that there is, in fact, no protein lack at the world level or at the *per capita* level. Indeed, supplies of protein available for human consumption at world and per capita levels appear to exceed requirements by about 70 per cent. However, this does not mean that there is no protein *food* problem. Indeed, there is such a problem, which arises from inequalities of distribution between regions, to start with, then inside regions, inside countries, and even inside households, largely because of variations in purchasing power. As a result, a considerable amount of protein-calorie malnutrition is found in virtually all developing countries, because small children cannot eat enough of the traditional bulky diets, which often are based on cereals or roots; they need specially prepared foods that contain concentrated energy and concentrated sources of protein.

The PAG in Paris prepared a statement more or less along these lines, and I made the same statement at a NATO-sponsored symposium on the evaluation of protein that was held at the University of Reading. That statement was reasonably well received: people agreed that distribution, as influenced by purchasing power and the capacity to eat protein foods, is at the root of the so-called protein problem. I prefer to call it a protein *food* problem because it is food, not protein, that is distributed between regions and among members of a household. Unfortunately, I have since heard that I was misquoted on the Reading symposium in Ifé, in Western Nigeria, and credited with saying that there is no longer protein-calorie malnutrition in the world. I trust that I will not be misquoted at this meeting.

One other point I want to make is that the limiting amino-acid pattern of the *total* diet – not of any particular component of the diet – varies from one ecological zone to another. The limiting amino acid is most frequently lysine or sulfur-amino acid, depending on the ecology of the country and the nature of the staple food. Therefore, in breeding it would be advisable to look at the limiting amino-acid pattern of the total diet as eaten, rather than at the limiting amino acid of the staple food. This should apply even in wheat-eating countries, such as the southern coast of the Mediterranean, where enough other food items are eaten so lysine is not always the limiting amino acid. The amino acid of the diet as a whole must not be limited before breeding up any particular amino acid. That, I know, poses something of a problem, because the breeder must become familiar with dietary habits in different ecological zones. However, FAO can provide broad patterns of these specialized diets, which may be of use.

SUMMARY

Doris Calloway

It is almost impossible to summarize this wide-ranging and interesting discussion. Dr. Bressani emphasized that there are many factors to consider in beans, including the percentage, as well as the quality, of the protein that makes up any bean variety. He reminded us that beans are used mainly as a supplementary food, and can be evaluated best in conjunction with complete diets of which beans make up a part. He reported that when maize was supplemented with cooked *Phaseolus vulgaris*, there was no appreciable improvement in nutritive quality of the food mixture with additional methionine, although amino-acid profiles would indicate this to be the first limitation. When protein content of beans is high, because of a difference in variety or in growth conditions, methionine, lysine, and tryptophan are found to be increased simultaneously. As a result, Dr. Bressani said, the quality of bean protein has not been sacrificed in order to increase its quantity. Methionine, lysine, and tryptophan all make important contributions in the supplementation of basic cereal diets. He indicated that about a 50-50 mixture of the cereals and beans is a desirable ratio for the best protein supplementation. This ratio appeared to hold for both maize of relatively low quality and the improved opaque-2 variety.

Dr. Bressani asked us to consider factors that limit the consumption of beans, such as characteristics related to storage, digestibility, and cookability. In this context, it is important to note that cookability and digestibility decline with conventional storage of beans, for when cooking time is increased, lysine quality is diminished and nutrition is affected appreciably.

Dr. Liener asked how digestibility might best be measured in laboratories without access to biological materials. I think none of us responded to that very well. According to Dr. Bressani's evidence, some portion of the bean — the part that suspends most readily in cooking water — is more digestible than the remainder. Dr Liener referred to Dr. Jaffé's bean globulin, a protein that is particularly resistant to digestion. Perhaps breeders should look at specific fractions of beans rather than at total protein digestibility. Dr. Carpenter commented that many products can provide fiber in the crude diets, so that we need not concern ourselves with questions of intestinal bulk in dealing with improvement of beans.

Dr. Nicol pointed out the need to consider the distribution of food protein and energy sources within countries, within households, and even within ecological zones. Even though beans are available in a country and there is enough protein in the world, it does not guarantee that everyone gets equal shares. He also noted that, to evaluate best the nutritional potential of beans, the total behavior of the target population must be considered.

Various speakers reminded us that beans are not all alike. There are many varieties of legumes in the world, many of which have not been well

studied. Perhaps some of these have greater potential than the crops we have found most economical to produce in the past. Even when a bean is grown in a different location, or fertilizer, such as zinc or boron, is added to the soil, a biologically detectable difference in protein quality may result, according to Dr. Bressani. Dr. Boulter noted that biological quality is not always uniform, even though amino-acid profiles, determined biochemically, appear to be the same. Dr. Bressani said that the stage of maturity (green versus mature dry) does not affect the protein quality, but reemphasized that storage of mature dry beans has a major impact on net protein utilization.

Some specific summary points are the following:

Legumes have twice as much protein as cereals and a complementary pattern of amino acids. Ideal supplementation occurs with mixtures in which 50 per cent of the protein is derived from maize and 50 per cent from cooked *Phaseolus vulgaris*, irrespective of whether the maize is ordinary type or opaque-2 (although growth is much better with the latter). Increased protein content in beans is accompanied by higher levels of the key amino-acids, methionine, lysine, and tryptophan.

Digestibility is variable (50 to 90 per cent with different legumes) and needs to be improved. Although some fiber is needed in diets, the key protein sources should have good protein digestibility. Two clues to improvement were noted: the part of beans that suspends readily in cooking broth has higher digestibility than the intact beans, and Jaffé has characterized a bean globulin that is particularly resistant to digestion.

Cookability and digestibility decrease when legumes are stored. Increased cooking time increases fuel requirements and prolonged cooking results in lowered lysine availability and biological value. Nutritional implications of commercial and home-processing requirements must receive attention.

Amino-acid profiles, determined biochemically, do not always correlate well with biological quality of the beans, due to differences in digestibility, availability, toxic factors, etc. Better in vitro methods are needed for measuring these factors, but bioassy will be required until such methods are perfected.

Many varieties of legumes are known, but relatively few have been studied. More information is needed on their composition, agronomic characteristics, and nutritive quality. Effects of environmental conditions on nutritive value and other qualities also are poorly known. Examples were cited of differences within and between varieties in protein content, biological value, and toxic factors.

Legumes provide energy and other essential nutrients (calcium, iron, B vitamins) and the contribution and availability of these must be evaluated. Selection and use of legumes must be considered in the context of the total diet of countries, of populations within countries, and of household members.

RESEARCH NEEDED

Paul Lunven

I would like to classify the research required in two categories: first, nutritional needs; second, plant breeding. It is obvious that much research is needed in the nutritional field, more especially in view of the fact that, as was stressed in the discussion, two different types of beans with the same protein content may have quite different nutritive values. Is that due to digestibility, toxic factors, or other causes? We do not yet know. Therefore, I think that one research field to be emphasized is the digestibility of grain legumes. Dr. Bressani has shown several tables demonstrating discrepancies in the availability of or digestibility of various beans. For example, it was claimed that *Cajanus* has a digestibility varying from 59 to 90 per cent. Such a range should be clarified.

Flatulence factors have also been stressed. Some work has already been done in relation to studies we have carried out here in FAO. In Algeria, for example, where grain legumes form part of protein-rich food for children, the galactans and pentosans are claimed to be responsible, at least partly, for the flatulence effect that has been observed. This area also should receive more attention.

The variability of the screening techniques used in studying the composition of many varieties of legumes requires standardization of either the chemical assay or the bioassay, including the breaking down of protein and the isolation of its different proteinic components. Further research should be carried out on improving and speeding up the bioassay of protein.

Much more research is also needed on heat treatment, which has direct consequences on the nutritive value of legumes. Proper heat treatment should be developed to preserve this and, at the same time, to destroy or counteract all endogenous toxic factors.

Research programs in plant breeding should put more emphasis on the quality of protein. Amino-acid profiles should be studied, and ways and means found to improve the sulfur-amino-acid content of the beans. Also, the point was made that tryptophan, lysine, and sulfur-amino acids increased proportionally with the protein content of grain legumes. Therefore, another area for further research is how to identify and multiply legumes with a high protein content.

I would like to say something here about the high-yielding varieties. These appear to us to be an extremely important aspect of legume consumption in developing countries because small, marginal farmers are not much encouraged by the low yield of the local grain legumes. Thus, the availability of high-yielding varieties would give them economic incentives to grow these beans.

This leads me to another point mentioned by Dr. Bressani with regard to harvest and storage and, in particular, to the quality of the protein in relation to the length of storage time. One of the serious difficulties of grain-legume production in developing countries is the heavy loss caused by insect infestation during the whole process of cultivation, harvesting, and storage. Consequently, the introduction of high-yielding varieties should be accompanied by proper storage

facilities. This will influence price stability favorably and improve availability of supplies throughout the year. This point might be discussed further.

Another discussion concerned the standard in seed quality and size that are claimed to correspond to the protein content of grain legumes. This proved to be an area of disagreement. Perhaps we should clarify whether we need to standardize seeds.

Finally, it was agreed that research was needed on the major, most commonly consumed, species of grain legumes by ecological zones and also on those strains that are not well known but are consumed by many people throughout the world.

My statement can be summarized topically as follows:

Areas of research needed:

In human nutrition:

1. Factors responsible for the poor digestibility and the poor nutrient availability of legumes.
2. Elimination of flatulence factors through improved processing and/or breeding techniques.
3. Need for establishing minimal levels of vitamins and minerals in cereal diets, for legumes to exert fully their potential supplementary role.
4. Developing practical and fast screening techniques such as in vitro assay and bioassay for determination of digestibility and nutritive value. Need for standardization of laboratory techniques (chemical, animal, cooking, etc.) in order to make results fully comparable.
5. Need for a study on the digestibility of protein fractions of legumes.
6. Need for establishing conditions for optimal heat treatment for improving the digestibility and wholesomeness of legumes without lowering their protein efficiency.
7. Need for establishing the limiting amino-acid patterns of *diets* of different ecological zones.

In legume breeding:

1. Need for improving the protein content and amino-acid profile and, in particular, for increasing the sulfur-amino-acid and tryptophan content.
2. Need for high-yielding legume varieties for better incentive to small formers, increased availability, and price stabilization.
3. Need for developing strains with increased vitamin and mineral content.
4. Need for norms on seed quality and size in order to insure optimum yield and nutritive value of the crop.
5. Need for work on the effect of storage on protein quality of grain legumes.

II

STATUS AND POTENTIAL FOR GENETIC IMPROVEMENT OF FOOD LEGUMES

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BASIC RESEARCH NEEDED FOR FURTHER IMPROVEMENT OF PULSE CROPS IN SOUTHEAST ASIA

M. S. Swaminathan

The knowledge gained by concentrated work in the last few years has been summarized in a recent publication of the Indian Agricultural Research Institute (IARI). This report makes it clear that the yield of pulses can be increased substantially from their present low levels. However, even these yields are unsatisfactory when compared with the levels of the present-day, high-yielding varieties of cereals. Also, the realization of these potential yields in pulses may have the paradoxical effect of lowering their competitiveness. For with greater production, pulses may lose the advantage of the higher prices they command at present vis-à-vis the cereals. But, from the point of view of insuring adequate nutrition for the more vulnerable sections of the community, it is essential that prices should be low enough for even the poorest to be able to include adequate quantities of pulses in their diets. Therefore, the yield levels in pulses must be raised to that obtained with cereals.

It has been suggested that pulses have an inherently lower yield-potential than cereals. This may be a reflection of the evolutionary history of the legumes. However that may be, the need for concentrated, basic investigations to understand the causes for the apparently lower yield-ceiling cannot be doubted. These problems are likely to be common for all the pulse crops, so there would appear to be a great advantage in concentrating such basic work at one center. Following are some of the major aspects on which such basic information is needed.

Factors Affecting Photosynthetic Efficiency

In the United States, Canada, and Australia, extensive studies on photosynthesis in relation to crop production have been initiated on sorghum, maize, and soybean. These studies clearly indicate that high-photosynthesis plants are characterized by the enzyme phosphoenolpyruvate (PEP) carboxylase, a low CO_2 compensation point, a high cyclic/noncyclic ratio, a special bundle sheath, and high efficiency of translocation in sorghum and maize (Hatch, et al., 1969). It further appears that photosynthesis may not always be associated with PEP carboxylase. In fact, it has come to be realized that such a large number of characters, which by themselves are dependent upon several enzymes, may not be linked genetically. In some genotypes – by coincidence or, more probably, by selection – most of these characters have combined.

Probably the optimum combinations of all these characters have not yet been incorporated into any one genotype. Therefore, attempts now are being made in several laboratories to establish the relationships of the characters at the level of various taxa. Scientists at the Carnegie Institute in the United States have been able to obtain two species of *Mimulus* that grow under two distinct environments of high altitude and dry conditions. Photosynthesis rates and enzyme composition of these species show characteristics of low and high photosynthesis groups. Interspecific crosses have been successful, and the genetics of this character is being studied. Similarly, two species of *Atriplex*, representing two distinct photosynthesis groups, have been isolated and crossed successfully. The F_1 shows no dominance, but in F_2, genotypes with varying levels of PEP carboxylase were observed (Carnegie Institute Report, 1970). With the exception of these two instances, no serious attempt has been made to understand the genetics of the photosynthesis mechanism in higher plants. Even in this case, emphasis is on carbon-dioxide fixation. By and large, this is true of most laboratories where photosynthesis work in relation to crop production is in progress Similar studies of grain legumes are very essential.

However, the utilization of solar energy is in the production of adenosine triphosphate (ATP). Once produced, ATP need not be used only in the fixation of carbon dioxide; instead, it can be utilized for such other energy-requiring processes as synthesis of protein, fats, etc. Variability in photosynthesis in dark reaction needs extensive investigation. It is all the more important because several enzymes are involved in this process. Therefore, one can expect genetic variation in this very important trait of plants. At the species level, variation has been shown. Recently, Sinha and Khanna (1972) studied photophosphorylation during the growth and development of wheat species and varieties. The existence of variation was obvious. Such studies must be extended to pulse crops.

Another aspect of photosynthetic efficiency is the capacity to utilize effectively high levels of solar energy. Some crops, like maize and sorghums, have the ability to use light intensity of 8,000 f.c. and above. Other crops, such as soybeans, reach the saturation limit at a light intensity of about 2,500 to 3,000 f.c. Little information is available about the behavior of pulse crops, but data on the performance of the same set of genotypes in summer and in the rainy season suggest that the ceiling of light utilization of the presently available varieties of pulses may be as low as in the soybean, if not lower.

A related question is the occurrence of photorespiration in pulses. Obviously, such photorespiration would have a negative effect on the "realized" photosynthesis. Photorespiration has been shown to be present in soybean and field bean, and presumably it also is important in the pulses. It is possible, however, that genotypes may exist that do not exhibit such photorespiration.

Factors Affecting the Harvest Efficiency

The importance of a favorable harvest index, in terms of the partitioning of the photosynthate to the economically useful part of the plant, has come to be

well recognized. In the pulse, little selection would appear to have been exercised in maximizing the harvest index by selection of a suitable plant type. Results of such studies on the cereals can form the starting point, but they cannot be transposed uncritically to pulses. For instance, one of the important considerations in determining the optimum plant type in cereals has been the presence of erect or nearly erect leaves, which allows better penetration of the sunlight in close stands. However, it has been suggested that in some clovers the photosynthetic efficiency, and not the leaf angle, is important. There is, therefore, a great need for basic research on this aspect of pulse crops.

Although high potentiality of photosynthesis is a prerequisite for obtaining higher yields, it obviously cannot be the only factor responsible for high economic yield. So far, the storage capacity of a genotype, usually called the sink capacity, has been determined in terms of such yield components as grain number and grain weight. The significance of these characters and the simplicity with which they can be studied should not be underestimated, for the fact remains that the characters are influenced greatly by the photosynthetic ability of different plant parts and the translocation rates of photosynthates of the plant. Therefore, scientists are turning to the physiology of developing grains and studying the activity of enzymes associated with the synthesis of starch, proteins, and fats. Tsai and Nelson (1969) have shown in maize that very poor activity of starch-synthesizing enzymes exists in the presence of the sugary gene. Tsai and Nelson (1968) also observed that changes in starch content closely followed the activity of starch synthetase. The activity of these enzymes seems to be regulated by inorganic phosphate, potassium, adenosine diphosphate (ADP) and phosphoglyceric acid (PGA). Obviously, then, phosphate and potassium availability could have a profound effect on the enzymes and, in turn, on starch synthesis and grain development.

A simple way to assess the storage capacity of a genotype could be by studying the incorporation of a precursor into the reserve product. By using ^{14}C uptake and its incorporation into starch, scientists have observed that varietal differences exist in tall, medium, and dwarf wheats (Sinha, 1971). Information on these aspects in pulse crops is almost completely lacking, and is needed urgently. Finally, on the basis of these studies, it appears that the future type in crop plants is not to be based on morphological architecture alone, but also on metabolic capacity.

The important aspects of such investigations on variability in photosynthetic efficiency in relation to yield follow.

1. Field conditions:

   a. Differences in the retention of carbon dioxide in plant canopies of different pulse crops and genotypes.

   b. Rates of photosynthesis under field conditions and in relation to canopy structure. The canopy structure can be changed by using different genotypes and by manipulating nutrition and populations.

   c. Rates of photorespiration under field conditions.

d. Respiration of plant canopies in relation to canopy structure, CO_2 concentrations, temperature, and humidity.

2. Laboratory:

a. Variation in photophosphorylation, both cyclic and noncyclic, at the species and varietal level in relation to developmental stages.

b. Response of photophosphorylation components to light intensities.

c. Variation in PEP carboxylase and ribulose, 1-5 diphosphate (RuDP) carboxylase at species and varietal level.

d. Response of carboxylases to light.

e. Genetics of photophosphorylation and carboxylation.

Adaptability to Stress Environments

Pulses are grown predominantly in conditions of moisture stress with limited rainfall. One of the limitations in increasing their production is their intense local adaptation within each region. The recent success in wheat and rice has been due mainly to the wide adaptation of the new varieties. This involves several mechanisms, such as changes in plant type, photo and thermal insensitivity, utilization of nutrients in the early stages of growth, and better harvest index as a result of an efficient mechanism for transfer of photosynthates from leaf to grains. Some of the primitive cultivars are found to possess several adaptative gene blocks, which, if incorporated into these high-yielding varieties, can extend the degree and range of their adaptation. They will also provide the basis for improved disease resistance and nutritional quality normally found in the primitive cultivars. During the past five years, studies by Finlay and Wilkinson (1963) on the biology of adaption in wheat and barley have yielded the information that there is considerable genetic variation among the collections in response to changes in environment. They developed simple statistical techniques to identify such genotypes and to demonstrate that a crossing program between them improves both the yields and range of adaptation.

One of the mechanisms used for millets in India has been the utilization of heterosis, using photo-insensitive male steriles of sorghum and pearl millet. Studies similar to those carried out on wheat and barley, mentioned above, should be done in these crops, particularly for stability of yield under a wide range of moisture stress. This will help to identify genotypes with diverse mechanisms of adaptation to such environment that can be brought together into one genotype.

The paucity of information on the biochemical basis of adaptability has slowed down the improvement program for this character. Indications are available that certain sulfur compounds may be involved in coping with environmental stress. It is necessary to undertake detailed programs of physiological investigations by following the pattern of distribution of sulfur-containing amino acids. This will be done by examining different genotypes of varying adaptability for

these constituents. The studies will be conducted both in the field and under controlled environment. Based on this information, further studies on specific chemical constituents will be undertaken.

1. Field conditions:
   a. Partitioning of dry matter;
   b. Translocation of photosynthates from leaves to seeds;
   c. Seed development;
   d. Effect of growth regulators on the partitioning of dry matter;
   e. Anatomical studies on the translocation channels.

2. Laboratory:
   a. Genetic differences in the translocation of labeled compounds under controlled conditions;
   b. Studies on seed development and capacity to synthesize proteins and carbohydrates;
   c. Study of starch synthesis in relation to seed development;
   d. Effect of temperature on the development of enzymes.

The Indeterminate Flowering Habit in Pulses

Flowering in pulse crops is indeterminate. The processes of vegetative growth on the one hand, and flowering and seed development on the other, go on simultaneously, in contrast to the cereals, in which there appears to be a sharp demarcation between vegetative growth and reproductive development.

The importance of photosynthetic activity at the time of grain development has been clearly brought out in cereals. The position with respect to legumes is not so clear. Apparently, screening should be done for photosynthesis and its efficiency at different developmental stages. The extended growth and flowering periods would mean that photosynthates would be partitioned not only to the developing fruits and seeds, but also to the newer vegetative growth and new inflorescences. The results obtained with cereals may not be wholly applicable to pulses, so it is essential that investigations of this factor be undertaken.

The high rate of flower-shedding in pulses also needs investigation. Recent studies have emphasized that one objective should be to breed pulses for greater seed production per unit of vegetative growth. One method would be to have a greater number of pods. Prevention of the shedding of flowers and young fruits also could make a substantial contribution. Basic investigation on the existence of genotypic variation in respect to this character are needed. An understanding of the reasons for such flower and fruit shedding – whether it is due to limitations of nutrients, hormonal imbalance, light or temperature dependence, or some other environmental influence, are needed urgently.

The indeterminate flowering habit of pulses also results in the fruits maturing in successive flushes. Especially if the mature fruit has a tendency to split open

and expose the seeds, several pickings may be required – a laborious time- and labor-consuming operation. One solution would be to breed for synchrony of fruit maturity. However, the genotypic variability of this character and its genetic control will need to be better understood before the problem can be tackled effectively.

Factors Affecting the Symbiotic Relationship Between Legumes and Rhizobia

A factor unique to the legumes is the symbiotic relationship that exists between them and certain bacteria that are capable of fixing atmospheric nitrogen the legumes can utilize. This property of adding nitrogen to the soil makes legumes an invaluable component of agriculture in such less-developed countries as India. However, optimal efficiency in this process depends on meeting adequately the requirements of the microorganisms. There appears to be considerable specificity in the host-*Rhizobium* relationship. Such specificity may exist at the generic level and also play an important role, as has been shown in soybeans. Among the pulses, such work has not yet been extensive, but there are indications of differences among species of *Phaseolus* in the type and region of nodule formation, which appear to be under genetic control. Again, it has been found that mutants obtained by irradiation differ from the control in their infectability by and symbiotic relationship with given strains of the bacterium. Varietal differences also have been shown to exist in factors toxic to the bacterium located in the seed coat of some pulses.

Another complicating factor is that there need not be a close relationship between efficiency as nitrogen fixers and the competitive ability of bacterial strains or their resistance to bacteriophage. In countries like India, where pulses have been grown from time immemorial, the possibility is that highly competitive bacteria specific to these crops may exist in the soil. Such bacteria may then suppress the improved strains used to inoculate the seed

These and other complexities, such as the adverse effect of nitrogen application on rhizobial infection, has led to the idea that it might be preferable to do away with this symbiotic relationship and feed the legume plant directly with high doses of nitrogen, as is done with cereals. The availability of mutants (natural or induced) that lack the capacity to form nodules (even when the appropriate strain of the bacterium is present) in soybean and some other legumes, such as pea, has made this a feasible proposition.

Attractive as it may look, there are certain difficulties in accepting the proposition fully. First, it is not clear if application of high doses of nitrogen would lead to higher yields of grain; studies on the application of relatively high doses (more than sufficient to compensate for the loss of rhizobial nitrogen) to pulse crops have resulted in excessive vegetative growth, but not in commensurate increases in grain yield. Again, at least in the conditions under which pulses generally are cultivated, it may be difficult to apply very high doses of fertilizers. Second, in developing countries like India, where pulses play a crucial role in nutrition, it appears doubtful that the required quantities of fertilizers would be

available. The wisdom of converting pulses from suppliers of nitrogen to the soil to consumers appears debatable. (Pulses have been estimated to add more nitrogen to the soil each year than do fertilizers in the whole of India.)

Studies on the Biosynthesis of Storage Proteins

There is now considerable information on the biosynthesis and regulation of certain amino acids in microorganisms. The biosynthesis of proteins and amino acids in plants that are a major source of these essentials for human populations have been poorly studied. In bacteria, several mechanisms are known to regulate the biosynthesis of threonine, isoleucine, lysine, and methionine, which have aspartate as an initial precursor and are essential for our nutrition. In bacteria, one can resort to the analysis of mutants on supplemented media to determine biosynthetic pathways. Complex organization in higher plants did not offer any such advantage until recently, when isolated cells were cultured and made to differentiate under controlled conditions. The first paper on the regulation of lysine biosynthesis in plants appeared only three years ago.

From the point of view of fundamental and applied research, it should be essential to know the biosynthesis of amino acids in germinating seeds and in vegetative and reproductive organs, including developing grains. This information is needed in the growing and differentiating cells of different crops. Studies with plants grown under controlled conditions would have a direct bearing on the various aspects of protein synthesis and quality, whereas in vitro studies are important to establish biosynthetic pathways of amino acids.

Conclusion

The results obtained in India on the possibilities for pulse improvement and on the need for an intensive attack on some of the basic problems relating to breaking the ceilings of high yields in these crops (see paper by Swaminathan and Jain, this volume) would suggest that an international research effort involving an interdisciplinary cluster of highly talented scientists could be most effective. Such an effort could become the single most significant effort in the battle against protein malnutrition.

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FOOD LEGUMES IN INDIAN AGRICULTURE

M.S. Swaminathan and H. K. Jain

Food legumes, or pulses, as many of them are commonly known, occupy a unique position in Indian agriculture because they provide the only high-protein component of the average diet. No other country in the world has so much area under these crops as does India. It has been increasingly recognized in recent years that pulses offer perhaps the only, and certainly the most practical, means of solving the protein-malnutrition problem in India at the present stage, as it may take many years to bring about a major advance in the production of high-protein foods of animal origin. Pulses contain 20 to 30 per cent protein on a dry basis, which is nearly three times the value found in the cereals. The per-acre production of protein is higher from pulses, compared to the cereals, and the quantity can be increased greatly if the present varieties are replaced by high-yielding types.

Pulses already constitute an acceptable part of the Indian diet, and the practice of taking them with wheat and rice has been found to have a sound scientific basis. Pulses are generally rich in lysine, an essential amino acid that is often deficient in cere: Greater availability of pulses thus can make a significant contribution to the nation's physical and mental health. In view of this important role of pulses in eradicating protein malnutrition, there has been considerable concern among government leaders, administrators, and scientists that the recently released, high-yielding varieties of cereals may compete with these crops and push them out of cultivation (Swaminathan, 1971). In fact, there already has been some diversion of land from crops such as chick pea to wheat, and it is of paramount importance to insure that intensive production programs, similar to those in cereals, are organized for pulses, too, so that the country's agriculture shows a balanced growth, and the food needs of the people can be met in terms of balanced nutrition. Recent work at the Indian Agricultural Research Institute (IARI) and other centers in the country has shown that, with the development of high-yielding and short-duration varieties of pulses, it should be possible to grow cereals and pulses as complementary, rather than competing, crops.

The pulse crops also derive their importance from their relatively low water requirement; because of this characteristic, they offer a most important means of increasing agricultural production and farmers' incomes in India's dry lands, which constitute more than 75 per cent of the total cropped area in the country. Finally, over the past hundreds of years, pulses have been an important way to

restore the fertility of Indian soils in the absence of adequate manuring. Mehta (1970), who has been one of the leading pulse breeders in India, believes that the pulse crops have been the mainstay of Indian agriculture, inasmuch as they have enabled the land to turn out reasonable quantities of food grains, even when a major part of the area under cultivation has received hardly any manures or chemical fertilizers. Pulse crops are generally included in rotations in most of India, and have helped to keep soils alive and productive through their capacity to fix atmospheric nitrogen.

Area and Production

Pulses occupy nearly 18 per cent of the total area under food grains in India, but their contribution to the total food-grain production is less than 12 per cent (Table 1). Thus, it is clear that, compared to the cereals, pulses give considerably lower yields. This difference has widened in recent years. The production of cereals, more particularly of wheat, has risen significantly during the last five years, but the production of pulses has continued to fluctuate between nine and 13 million tons during the past 20 years, largely as a function of variation in seasonal conditions. During the last four years, the lower trend in production appears to have been maintained, and this is almost entirely because farmers have found it more profitable to grow wheat than pulse crops, such as chick pea.

Factors Responsible for Low Yields of Pulses

Compared to the cereals, the average national yields of pulses have been very low, and the difference continues to widen. An important factor responsible for the low production of pulses in India is that many of the available varieties of these crops take a long time to mature, with the result that their growing season overlaps that of the cereals. Consequently, the per-day productivity of these varieties is low, and the farmer has to choose between the two groups of crops. A factor responsible for the poor yields is that these crops generally have been grown in lands of marginal productivity without the benefit of such inputs as fertilizers and pesticides. One result of this low-level management has been that those genotypes have been selected which have the capacity to do well under adverse conditions and cannot take advantage of more favorable factors of crop growth. This emphasizes the need to build up extensive genetic stocks of pulses from different parts of the world, so that breeding work can be organized to make use of wider gene pools.

Important Pulse Crops and their Improved Varieties

Important Pulse Crops

Although a large number of pulse crops are grown in India, the most important of these are chick pea and pigeon pea, which, taken together, account for

TABLE I
Area and Production of Pulses in India (1969-70)

Total area under food grains (million hectares)	Total area under pulses (million hectares)	Area under pulses as % of the total area under food grains	Total production of food grains (million tons)	Total production of pulses (million tons)	Production of pulses as % of total food grain production
123.57	22.023	17.82%	99.501	11.691	11.75%

nearly half of the total acreage under pulse crops, and for 60 per cent of the total pulse production. The other important pulses are those of the *Phaseolus* group (green gram, black gram and moth), lentil peas, khesari dhal, and cow pea. Some of the less important pulses include species of *Dolichos* and the more-recently introduced soybean. Table II summarizes the more commonly cultivated pulses in terms of their area and production.

Genetic Improvement of Pulses

The work on the genetic improvement of pulses in India can be considered in two distinct phases. The first phase, starting in the early part of the century, ended about 10 years ago, and has been described in a recent publication of the ICAR (Kachroo, 1970). A major characteristic of that phase was that improvement was sought largely through simple procedures of selection from local collections of land races available within the boundaries of the states in which experimental stations were located. This work has been most valuable, as it has resulted in the availability of varieties that are commonly cultivated at present, and has provided a base from which more significant advances in yield can be obtained through intensive plant-breeding programs. The greatest limitation to progress in this work has been the unavailability of restricted genetic variability, with the result that it was not possible to exploit more favorable kinds of gene actions. Also, the selections were made under a low level of management, corresponding to the conditions in the farmers' fields, with the result that the varieties developed in the course of this work are not particularly suited to respond to fertilizers, irrigation, and other inputs. The more important varieties of the different pulse crops that were developed in various parts of the country are summarized in Table III.

The second phase in the improvement of pulses started with the setting up of an all-India coordinated research project, with the participation of a large number of research centers in different parts of the country. This work has already made significant progress and a number of high-yielding varieties have become available, so pulses can be brought increasingly under intensive and commercial cultivation. Some of the important advances are described below.

Wider collection and exploitation of germ plasm. During the last six years,

TABLE II
The Major Pulse Crops of India and Their Relative Areas and Production

Botanical name	English name	Common Indian name	Area	Percentage of total production
Cicer arietinum	chick peas or Bengal gram	chana	41.0	51.0
Cajanus cajan	pigeon pea or red gram	arhar or tur	9.8	11.2
Phaseolus mungo	black gram	urd, mash, or kalai	6.5	3.9
Phaseolus aureus	green gram	mung	5.4	2.5
Phaseolus aconitifolius	–	moth	6.2	2.6
Lens esculenta	lentil	masur	3.3	2.9
Dolichos biflorus	horse gram	kulthi	6.3	3.0
Pisum sativum	peas	matar	5.4	9.8
Lathyrus sativus	chickling vetch	khesari dhal	7.9	7.6
Other pulses	–	–	8.2	5.5
Total			100.0	100.0

efforts have been made to organize extensive collections of germ plasm of the various pulse crops from different parts of the world. Table IV summarizes the genetic stocks that have been collected with the collaboration of the United States Department of Agriculture. These collections have been classified and catalogued, and have been grown at a number of centers all over the country during the past five years. Their seeds have been made available to the pulse breeders in different parts of the country. It should, however, be emphasized that the collections are by no means complete, and represent only a fraction of the world genetic variability. The future success of the pulse-improvement projects in India depend greatly on our ability to enlarge the range of these collections.

TABLE III
Some of the Commonly Cultivated Older Varieties of Pulses

Varieties	Yield in quintals/hectare	Maturity in days
Chick pea		
G.24	18.00	120
T-1	21.00	115
NP 58	18.00	120
G-62-404	16.00	110
RS-10	18.55	125
Chaffa	15.60	108
Pigeon pea		
NP(WR)15	14.50	280
C-11	10.00	200
NO. 148	12.50	188
T.17	14.50	270
CO-1	8.00	180
Green gram		
T.1	8.00	68
T.44	5.50	65
Jalgaon 781	5.40	75
NO. 505	4.70	85
B-1	6.25	70
Black gram		
Mash 1-1	8.00	90
Mash 48	10.00	94
T-27	10.40	130
T-9	9.30	80
Cowpea		
Pusa Phalguni	12.00	80
K-11	12.00	85
JC-10	10.00	85
T-2	15.00	98
CO-1	10.00	110
Lentil		
L 9-12	18.25	110
T.36	17.43	96

TABLE IV
Germ-plasm Collections of Pulses at the Indian Agricultural Research Institute

S. No.	Crop	Indian collection	Exotic collection	Total entries	Countries represented
1.	Gram or chick pea *(Cicer arietinum)*	1803	4817	6620	21
2.	Arhar, or pigeon pea *(Cajanus cajan)*	5028	107	5135	16
3.	Mung, or green gram *(Phaseolus aureus)*	475	1016	1491	18
4.	Urad, or black gram *(Phaseolus mungo)*	310	–	310	1
5.	Lobia, or cow pea *(Vigna sinensis)*	716	706	1422	49
6.	Masoor, or lentil *(Lens esculenta)*	415	528	943	14
7.	Peas *(Pisum sativum)*	418	–	418	1
8.	Khesari, or lakh *(Lathyrus sativus)*	752	87	839	7

New concepts in the improvement of pulses. The pulse-breeding work in India during the past five years has been greatly influenced by the concept of improved plant type, which has proved so very useful in the improvement of wheat and rice. These two have been the crop plants investigated most intensively, but in spite of a long history of research, it has been found necessary in recent years to give them a new form to improve their productivity. Based on this finding, our group at the IARI has reexamined plant types in several other groups of crops, including the pulses (Jain, 1969; 1971). This examination has shown that a change in the plant type can be even more rewarding in the pulse crops, which have received much less attention from the plant breeders than have wheat and rice.

A major reconstruction of the plant type in pulses has required a reduction in their spreading and oushy growth habit so that more plants can be raised per unit of land. Associated with this has been an improvement in their harvest index (weight of grains, i.e., the economic yield/total plant weight, i.e., the biological yield).

A high-harvest index, which obviously is of such great value in crop plants, offers no particular advantage when the plants grow in a wild state. The process

of domestication and subsequent selection of crop plants during the past thousands of years brought some improvement in their harvest index, but the transformation is by no means complete. It can be expected that some of the major advances in crop improvement in the future will come through a genetic reconstruction of plants in the direction of a high-harvest index.

The reconstruction of plant type in terms of improved harvest index is often difficult to achieve without changing the physiological structure, more particularly the photosensitivity. Thus, a major breeding objective in recent years for crops like pulses has been to cut down their excessive vegetative growth, improve the harvest index, and reduce their maturity duration. This last attribute is most important, as it helps to increase the per-day productivity of the pulse crops and makes it possible to fit them into more intensive cropping patterns.

The New Varieties

Based on the concepts outlined above, efforts have been made for the genetic improvement of several of the pulse crops in order to increase their yields and extend their cultivation in both time and space by fitting them into new cropping patterns. These studies, reported by Jeswani and his associates, have been particularly successful with pigeon pea, green gram, black gram, cow pea, lentil, and pea (Jeswani, 1968, 1970 a and b; Jeswani and Ahuja, 1968; Jeswani and Jain, 1969; Jeswani, et al., 1970). Thus, it has been possible to release a number of new varieties of pigeon pea developed during the past five years, and their seed is being multiplied on a large scale by the National Seeds Corporation.

Some of these varieties allow a population of 72,000 plants per hectare, as against the 35,000 allowed by the older types. The maturity duration of these varieties has been reduced to five to six months, which is nearly half that of the older varieties. The reduced duration should make it possible to cultivate pigeon pea in rotation with wheat in the northwestern wheat belt. This has not been possible previously because of the overlapping maturity of the two crops. The per-day productivity of some of the new pigeon-pea strains amounts to nearly 27 kg per hectare, which compares favorably with 17 kg per hectare of the older varieties. Under conditions of suitable management, these varieties have given a yield of 25 quintals per hectare. Three of these varieties, named pusa ageti, sharda, and mukta, were released during 1971. Pusa ageti is the earliest in maturity, taking about 160 days from sowing to harvest. The variety is particularly suitable for growing with high-plant populations. The new variety, sharda, is semi-spreading in its growth habit, and takes about 170 days to mature. The variety is particularly suitable in nonirrigated areas and also for mixed cropping. It has a protein content of 22 per cent. The third variety, mukta, takes about 180 days to mature and has shown wide adaptability. It is relatively resistant to the wilt disease, which is a serious problem, particularly in the northeastern and central parts of the country.

Short-duration varieties marked by a high-harvest index have also been developed in green gram and cow pea. A particularly significant contribution has been

made by one of the new green-gram varieties called pusa baisakhi, which is now widely cultivated in many parts of the country. Ready for harvest within a period of 70 days, pusa baisakhi can be grown as an additional crop during the summer months between the main rabi and kharif seasons. With a yield potential of nearly 10 quintals per hectare, this variety makes a significant contribution to the farmers' income.

Another pulse crop particularly important in the states of Bihar, Madhya Pradesh, and Uttar Pradesh is the khesari dhal; it is known for its adaptability to adverse soil and climatic conditions. This pulse, however, is known to contain a neurotoxic material, and excessive consumption of its grains causes a disease known as lathyrism. After intensive work during the past five years, it has been possible to evolve varieties of khesari dhal that are relatively low in the toxic material. A number of these varieties are being tested at present in the all-India coordinated trials and are expected to be released shortly. Feeding trials are also in progress at the National Institute of Nutrition, Hyderabad.

Chick pea is the most important pulse crop of India and it is generally cultivated in nonirrigated areas or, less commonly, as a second crop under irrigation. As a mixed crop, chick pea is grown as an insurance against the failure of rains. A number of early-maturing varieties with good yield potential have been identified and are being tested in countrywide trials at present. Some of the most important problems in the improvement of chick pea relate to breeding for resistance to the wilt and blight diseases; in the absence of extensive genetic stocks from different parts of the world, it may take several years before resistant varieties become available.

Nutritional improvement. Pulses are known to be characterized by a high protein content, so improvement for yield has received major attention. However, attempts also have been made to increase their protein content and the content of such essential amino acids as methionine and cystine, which tend to be low and so limit the biological value of protein in these crops. As a first step, Kaul and his associates (1971) have undertaken screening of genetic stocks for protein content and quality. Extensive analysis has shown considerable variability, which could be used for upgrading the nutritional value of pulses. The frequency distribution of the samples of chick pea analyzed indicated a range from 1.00 to 3.5 mg methionine/g sample. Considerable variation was also found in the protein content of varieties in the different crops, as can be seen in the data presented in Table V. This variability, and others induced in the course of some mutation studies, left little doubt that pulses can be an even better source of protein than they are at present.

The second phase of plant-breeding work on pulses has resulted in the availability of a number of improved varieties, which combine such desirable characters such as early maturity, high-harvest index, and a good yield potential. The more important of these varieties are listed in Table VI.

TABLE V
Variability for Protein Content in Different Pulse Crops
(1969-1970)

Pulse crop	Variety x location	% Protein Range	Mean
Chick pea	16 x 12	12.4–28.1	19.5
Lentil	10 x 10	20.4-30.5	14.8
Arhar	11 x 5	18.5-26.3	21.5
Green gram	10 x 6	20.8-33.1	25.3
Black gram	15 x 9	21.2-31.1	25.1
Peas	12 x 9	17.2-31.1	24.9
Cow pea	7 x 4	21.2-30.6	24.6

Fuller Exploitation of Genetic Potential

Pulses, more than any other crops, have formed part of an extensive agriculture in India for hundreds of years, and while this fact explains the present low levels of productivity, it also holds the key to substantial increases in production in the future. Recent research at the IARI and other centers in the country has shown conclusively that, with a small investment in improved management, production can be increased greatly from the present levels of 12 million tons. Some of the important factors which should contribute to increased production are discussed below.

Cultural Practices and Chemical Fertilizers

The pulse crops generally have been grown in India without application of any chemical fertilizer. In recent years, extensive agronomic studies by Chowdhury and his colleagues (1971; 1972) have shown that many of the crops respond significantly to the application of phosphatic fertilizers. Improvement in production also can be achieved by new planting patterns, including adjustment of spacing and sowing dates. An impressive demonstration of increase in production after the application of phosphatic fertilizers is provided by the studies of these authors on pigeon-pea crops. The average grain yield of 12.9 quintals per hectare increased to 27.6 quintals per hectare when the crop received 100 kg per hectare of P_2O_5. They concluded that the use of fertilizer at all levels is highly profitable. The net profit per hectare increased from Rs. 964 to Rs. 2341 when the crop received adequate doses of phosphatic fertilizer. Thus, the net increase in profit amounted to 143 per cent, while the increase in cost was 53 per cent. For each rupee invested in fertilizer, a return of Rs. 4.75 was obtained at the high level of fertilizer application.

TABLE VI

Some the Newly Evolved or Tested Varieties of Pulses*

Varieties	Yields in quintals/ hectare	Maturity in days
Chick pea		
C 235	31.5	125
Early S3	29.3	115
BGS-1	25.9	120
Pigeon pea		
S 103	15.00	275
Pusa ageta	27.00	150
Sharda	25.00	160
Mukta	25.00	170
T-21	20.00	154
Green gram		
Pusa baisakhi	10.00	65
Jawahar-45	10.00	80
S-8	12.00	60
PS.7	12.00	60
G.65	10.00	65
Black gram		
Pusa-1	15.00	84
H-10	12.50	95
G-1	15.00	94
Cow pea		
C-152	25.00	88
C-20	18.00	85
Lentil		
Pusa 1-1	20.00	80
Pusa-6	22.00	90

*These varieties have been evolved at a number of research centers, including the agricultural universities. The yields are based on the results of the coordinated trials and agronomic studies at IARI.

Significant increase in yield was also recorded in the case of chick peas, peas, and lentils. However, the response of these three crops was observed only on soils of low fertility, with less than 20 kg per hectare of P_2O_5 available. It was also observed that a starter dose of nitrogen (20 to 25 kg per hectare) was invariably beneficial. The authors also observed a significantly increased yield when fertilizer was applied to green grams.

Whereas chemical fertilizers have been found to be most effective in increasing the yield of pulse crops, significant gains in production have also been observed by Rewari and his colleagues (1968) after rhizobial inoculation. The development of efficient rhizobial strains and their distribution to the farmers offer one of the more practical means of increasing pulse production.

Control of Pests and Diseases

Considerable progress has been made in India during the last five years in the development of short-duration and high-yielding varieties of pulses. The new varieties, however, cannot make a significant contribution to production unless their cultivation is combined with suitable plant protection measures. Few groups of crops suffer greater damage through insect pests and diseases than do the pulses. The development of resistant varieties is already receiving serious attention.

It would, however, be unrealistic to believe that the development of resistant varieties alone would reduce the losses from insect pests and diseases. Plant protection measures must become an integral part of management practices in pulse crops if a major advance in production is to be achieved within a short period. The kind of impact on production of these crops by the use of pesticides and the economic implications of such control measures have been studied by Saxena and his associates (1969; in press). The yield of green gram increased by more than 100 per cent after being treated with pesticides. Highly significant increases in yield also were recorded in chick pea, pigeon pea, and the peas crops.

Strategy for a Short-Term Production Advance

Production programs on pulses must be planned on both short- and long-term bases. It has been estimated that the country will have to produce nearly 30 million tons of pulses by the end of this decade in order to bring about a significant improvement in the present levels of nutrition. A long-term production strategy in pulses will require intensive research in various fields. More immediately, considerable scope exists for doubling the production of pulses in India within a relatively short period. The major components of this production strategy, which is now being adopted, are as follows.

1. Based on the research of the last 10 years, it has been possible to develop a package of practices involving the use of fertilizers, pesticides, and certified seed of improved varieties. This package approach is being introduced in selected areas of pulse production in the country. Its basis is essentially the same as that for the highly successful production programs undertaken during the last five years for cereal grains.

2. Pulses, as explained earlier, have fitted so far into an extensive agriculture. With the availability of short-duration varieties, they are now being brought under intensive cultivation in several parts of the country where conditions for crop growth are favorable. This is being done by fitting pulses into multiple-

cropping patterns, so that farmers with assured irrigation and other facilities can take three to four crops from their land during a one-year period. The government already has adopted multiple cropping as one of its basic approaches for increasing agricultural production in areas of assured irrigation. Pulses are finding an increasing place in such multiple-cropping programs, particularly with the availability of short-duration varieties, as shown by the work of Bains (1968).

3. New cropping patterns. The short-duration varieties of pulse crops have offered other possibilities for increasing production. With the continued rise in the production of wheat during the past five years, and with a major advance expected in the production of rice within the next five years, some of the coarse cereals, like maize and millets, have lost their relative importance. In fact, many farmers are no longer attracted by those crops in view of a continued fall in their prices, with the result that it may be difficult to sustain a program of high-yielding varieties of cereals. They will have to find increasing use as animal feed. The short-duration varieties of pulses can replace some of these cereals profitably. This new development in Indian agriculture should be welcomed, for the additional production of pulses will contribute to improved nutritional standards on the one hand and better maintenance of soil fertility on the other.

The proposed diversion of land from coarse cereal to pulses emphasizes the need for crop planning in India, and should be regarded as a logical consequence of the success of the program of high-yielding varieties of wheat and rice. The cereal crops have accounted for more than 60 per cent of the total cropped area in the country. This is a disproportionately large acreage, and the availability of high-yielding varieties of the more important cereals should be exploited to reduce it considerably. Pulses should find a particularly important place in these new cropping schemes.

Future experimental approaches

Some of the major experimental approaches considered essential for the long-term genetic improvement of pulses are listed below.

1. Genetic upgrading for yield, including gene-action studies heritability and other biometric studies, fertilizer and physiological response, adaptation studies, and studies on stabilization of yield through resistance to diseases and pests.

2. Genetic upgrading for nutritional characteristics.

   a. Genetic and agronomic manipulation for protein, carbohydrate, and fat content.

   b. Screening of germ plasm and mutant strains for nutritional quality, including factors affecting digestibility and toxicity.

   c. Studies on protein quality in terms of amino acids and biological value.

3. Study of genetic and other factors affecting digestibility in pulses.

These studies embrace seed-coat pigments, including polyphenyl residues, fiber contents, hemagglutinins, antitrypsin factors, and favism. The first four have a direct effect on digestibility, while the last is a toxic consideration that also may affect digestibility.

Another study should cover the synthesis in pulses of hydrocyanic and nitrile-containing amino acids, which are responsible for various neurological and gastrointestinal disorders.

4. Key role of germ-plasm collections.

The success of future pulse-improvement programs will depend almost entirely on extensive collections of germ plasm on a world-wide basis. It cannot be emphasized too strongly that the nonavailability of pulse varieties comparable to the high-yielding varieties of wheat and other cereals is almost entirely because it has not been possible so far to make use of more than a fraction of the total genetic variability available in these crops.

Even more important than increasing the yield potential is the question of stabilizing the existing yield potentials. The pulse crops do not always find favor with the farmer, and he is reluctant to invest in an improved production technology because of the wide fluctuations in yields, which are largely the result of susceptibility to diseases and pests. Varieties marked by a high degree of resistance to such diseases as the wilt disease of chick pea and pigeon pea, which accounts for huge losses in unfavorable years, are not available. The newly collected variability also could lead to a major advance in yield through the exploitation of more favorable gene actions, such as those determining heterosis. In some of the pulse crops, such as pigeon pea, heterosis breeding holds considerable promise.

Above all, the new variability should make it possible to shift pulses from the traditional to a more intensive and profit-oriented agriculture. This would require varieties that respond better to improved management, including chemical fertilizers and irrigation. To take one example, the most important pulse crop of India, the chick pea, is at present cultivated largely in dry lands. There is evidence that it should be possible to develop high-yielding varieties of chick pea that can compete successfully with wheat and some of the other cereal crops under irrigation.

It is proposed that irrigation be extended to 50 per cent of India's cropped lands during the next 20 years. Several of the pulse crops, more particularly members of the *Phaseolus* group, are grown during the kharif (winter) season, when conditions for the development of disease are most favorable because of high humidity and excessive rainfall. Long-term strategy requires that these crops be cultivated during the preceding summer months, when the incidence of disease and insect pests is considerably lower. In recent years, it has been possible to identify some genotypes that make possible this adjustment in cropping periods. Additional genetic stocks, particularly those having genes for insensitivity to photo and thermal conditions, must be found to bring about major shifts of this kind. The key to the upgrading the pulse crops lies in exploring, collecting, and exploiting the vast amount of genetic variability available in different parts of the world.

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IMPROVEMENT OF FOOD LEGUMES IN TROPICAL AFRICA

K. O. Rachie

Food legumes have exceptional immediate potential for alleviating human malnutrition in tropical Africa by virtue of several inherent advantages. Universally, they can grow vigorously under a wide range of environments and on poor soils without supplemental nitrogen, which is particularly advantageous for subsistence agriculture in remote areas. The quick growth of some annuals, such as cow peas and dry beans, as well as the extended fruiting habit of long-duration viny species (yam bean, lima bean) and woody perennials (pigeon peas, locust bean) are complementary advantages in complex peasant farming systems. Moreover, they provide the only nonprocessed, storable, and transportable proteinaceous food concentrate for both rural and urban utilization.

Most food legumes have a distinct advantage over other food crops in simplicity of preparation and in multiplicity of edible forms, such as tender green shoots and leaves, unripe whole pods, green peas or beans, and dry seeds. Some food legumes, notably the yam bean and the winged bean, produce edible tubers in addition to the fruit. The excellent nutritional values of most food legumes in terms of proteins, calories, vitamins, and minerals are highly complementary in African diets, which comprise roots and tubers, plantains, cereals, indigenous herbs and greens, native fruits, and minimal animal proteins.

Constraints

Tropical food legumes also have many problems that preclude attainment of reasonable productivity levels and quality product in Africa. These include susceptibility to diseases and pests, narrow range of genetic diversity, inefficient plant types, and low yielding potential. These problems are frequently intensified by prevailing conditions of depleted soils, low capacity of soil to hold moisture, poorly distributed rainfall, high humidities, low insolations, and large numbers of diseases and pests. In addition, some legumes have specific nutrient deficiencies or contain undesirable flavors, flatus factors, and antimetabolites or other toxic substances. However, some otherwise well-adapted, high-yielding, and nutritious species are not utilized because of ignorance or unfamiliarity with their culture and methods of preparation.

The Situation

Tropical Africa contributes 260 millions, or about 19 per cent, of the world's total tropical population. In 1970, this region produced 8.0 million metric tons

of food-legume seeds on 12.6 million hectares, representing 21.8 per cent of production (37 million metric tons) and 22.6 per cent of the area (56 million hectares) devoted to these crops in the world's tropics (FAO, 1971). However, these statistics do not accurately reflect the quantity of grain legumes used directly for human consumption, as they include the industrial oil-seeds – groundnuts (36.6 per cent) and soybeans (6.9 per cent) – which frequently are grown mainly for export, edible oil, livestock feed, and residue used as fertilizer. Moreover, presumably they do not include production estimates on pulses grown in home gardens for direct consumption in various forms. These contribute substantially to local food supplies and total production.

Humid vs. semiarid tropics

Nutrition appears to deteriorate with the increase in annual rainfall, at least in West Africa. In Nigeria, the largest and richest nation in tropical Africa, both per capita calorie and protein availabilities decline from the north to the west and southeast, as shown here for 1963-64 (FAO, 1966).

Major Region	Proteins (g/day)	Calories Per Day		
		Total	Source	
Northern (semiarid)	80	2719	Cereals	= 1,751 (64.4%)
Western (subhumid)	40	1909	Roots and tubers	= 1,018 (53.3%)
Eastern (humid)	33	1774	Roots and tubers	= 1,212 (68.3%)

A somewhat different situation exists in subhumid or humid intermediate elevations such as obtain in Uganda where carbohydrate sources are more than adequate (3,500-4,000 per capita), but where proteins are inadequate, resulting in an estimated five deaths due to malnourishment per 1,000 population – primarily in postweaning children. However, in the highlands, as in the semiarid lowlands, there is a greater reliance on cereals, with consequently better nutrition than in the intermediate and low-elevation humid tropics. A major distinguishing feature of the African humid lowlands is the higher consumption of starchy plantains, roots, and tubers rather than rice and other cereals, as compared with southeastern Asia and other tropical regions.

Among the different sources of marketable food proteins currently available in Nigeria, cow peas are the least expensive on a protein unit basis, being Sh 12-25/kg, compared with Sh 110/kg for rice, Sh 45-90/kg for fish and meats, and Sh 65-130/kg for cassava, or "gari" (Sh = U.S. $0.15). With such high prices for commercial proteinaceous foodstuffs, the ordinary Nigerian householder supplements his diet with indigenous edible herbs, greens, and small fauna (bush rats), which are free for the taking in fields and uncultivated bush near his village. However, this kind of supplementation obviously is more difficult in urban communities.

The Species

Three pulse species beside groundnuts are of major importance in the lowlands and intermediate elevations of tropical Africa. These include cow peas, dry

beans *(Phaseolus vulgaris)*, and pigeon peas in about that order of importance after groundnuts. However, another pulse – chick pea – is an important crop (175 million metric tons in 1970) in the highlands of Ethiopia. Whereas the four pulse species are mainly consumed locally, most of the groundnut crop is exported or "industrialized." Therefore, even in such prominent groundnut-growing regions as Nigeria and Senegal, cow peas are probably even more important in local nutrition, although production is considerably less than groundnuts.

Major grain legumes

Groundnuts. This crop *(Arachis hypogaea)* is extensively grown throughout West Africa and from the Sudan to South Africa on the east (4.2 million metric tons on 4.3 million hectares in 1970, omitting north and south Africa). Nigeria, Senegal, and Sudan are the major producing countries, with 2.2 million metric tons on 2.4 million hectares or 15.3 and 16.4 per cent of area and production, respectively, of the world's tropical groundnut crop. However, this represents a decline in the production recorded for 1961-65, probably as the result of a weakening in the European market. This crop is largely confined to the medium and coarse-textured soils of lowland savannahs and intermediate elevations in tropical Africa. Major centers for improvement include the Institut Recherches Agronomie Tropical (IRAT) at Bambey, Senegal, with cooperating centers in other growing regions of French Africa: the Institute of Agricultural Research at Zaria, Nigeria; the Agricultural Research Station at Serere, Uganda; and ARS-Lilongwe, Malawi (partially supported by the Overseas Development Administration of Britain and the FAO).

Cow peas. The distribution of this crop *(Vigna unguiculata* Walp.) is similar to that of groundnuts – that is, along the southern fringes of the Sahara from the west coast to East Africa and southwards. It performs best on well-drained soils in the semiarid to subhumid intermediate to lowland savannahs. This is the most important pulse crop in tropical Africa, and in 1970 the major growers – Nigeria, Niger, Upper Volta, and Uganda – together produced 1.03 million metric tons on 2.8 million hectares, or 91 and 87 per cent of the world area and production, respectively. Cow peas grow vigorously, and many varieties are quick-maturing (60 to 80 days). Four major plant types can be identified, including climbing, prostrate, semiupright, and erect. Yields can be reasonably high, but the crop is susceptible to a large number of pests and diseases, particularly in West Africa (Nigeria), where the species originated.

The plant is useful in many forms: Seedlings, tender green leaves, immature pods, and unripe green seeds are cooked as vegetables, whereas the mature, dry seeds are used in many preparations as a pulse. The seeds are highly palatable, very nutritious, and remarkably free of antimetabolites and other toxic principles. Recent work done by Boulter (1972) at Durham University (UK) suggests that protein contents may run higher than suspected (22.9 to 34.6 per cent in 21 cultivars tested); and that biological values (63.2 per cent) and net protein utilization (53.3 per cent) of unsupplemented cow-pea seeds may run 30 and 37 per cent, respectively, above the same values obtained for *Phaseolus* beans in rat feeding trials. This, in part, may be attributed to higher contents of sulfur-bearing

amino acids. Methionine ranged from 1.42 to 1.57 per cent and cystine was 1.02 per cent of crude protein in these experiments. Cow peas also showed lower levels of such toxic principles as protein enzyme inhibitors than did dry beans. However, Boulter has also shown that further supplementation of cow-pea meal with methionine can raise the protein values to 95 per cent that of egg albumin.

Yields of dry seeds are not high under farming conditions in Africa (3.7 quintal per hectare), but, under favorable conditions, productivity levels of 1,500 to 2,000 kg/hectare are realized within 60 to 70 days from planting or 20 to 35 kg/hectare/day of concentrated edible product. Good management and pest control pay off handsomely. For example, the 1971 first-rains varietal trials at IITA averaged 1,660 kg/hectare, and broadly-adapted, high-yielding strains like Jebba Pea A and Kwara ex-Kwoi-C yielded 2,226, and 2,219 kg/hectare, respectively. Varieties that performed well in both seasons in spite of an untimely early drought in the second season included Pale Green and New Era, with mean yields for both seasons of 1,591 kg/hectare and 1,585 kg/hectare, respectively (IITA, 1972).

Centers of improvement in French-speaking Africa include Bambey, Senegal, and other IRAT stations in West Africa; in Nigeria, Moor Plantation (MANR), University of Ibadan, and the International Institute of Agricultural Research, all located at Ibadan; University of Ife (Western Region) and the Institute of Agricultural Research located at Kano in the north; in Uganda at Makerere University; and in South Africa at Potchefstroom.

Dry beans. Haricot, kidney, french, and navy or dry beans all belong to the same species *(Phaseolus vulgaris* L.) and either are synonyms or cross freely with one another. Africa produced 908 thousand tons on 1.38 million hectares with mean yields of 6.6 Q/ha in 1970; but the major producers were areas of intermediate elevation and moderate climate, such as Uganda, with 260 thousand tons on 430 thousand hectares and Burundi with 200 thousand tons on 210 thousand hectares.

Dry beans are quick to mature — about 60 to 75 days — but do not make as vigorous, aggressive growth as do most other grain legumes. In the low humid tropics, particularly, they are susceptible to several pests and diseases. In addition, they do not tolerate water stress for any length of time. The seeds are high in antimetabolites and the flatus factor, which tend to limit intake to some extent. Although this crop has been extensively worked on in the Western Hemisphere, it has been largely neglected in Africa. Major improvement efforts have been established in Uganda at the Kawanda Experiment Station (Ministry of Agriculture) and at Makerere University, with assistance from ODA.

Pigeon peas. The only production recorded for Africa is in Uganda and Malawi, but pigeon peas (*Cajanus cajan* Mill sp.) are grown extensively throughout the semiarid to subhumid tropics as a perennial garden crop or mixed with other species. Almost every rural household grows a few bushes in its garden for local consumption. Because it is grown infrequently in pure stands on a field scale and the produce is almost entirely consumed locally, it is suggested that available statistics on this crop are grossly underestimated.

The pigeon pea is much slower to become established than are most other

grain legumes and does not compete well with weeds or other crops in the early stages. It is more widely adapted in the tropics than are many other legumes, and both long-lived perennials and short-duration annual forms are available. The leaves are smaller and narrower, and the foliage is more open. It appears less susceptible to diseases and pests than cow peas – particularly in the more humid areas.

Green beans are relished as a vegetable, particularly in the Caribbean and South America; but the major use for the dried peas is in India and Africa. The nutritive quality of the grain is excellent, and yields can be high in short-duration types – up to 2,000 kg/hectare in East Africa and over 5,000 kg/hectare in India have been recorded (RPIP, 1968). It is one of the few partially cross-pollinated species (10 to 30 per cent) among the grain legumes – an important advantage in the population-improvement breeding system. There is little interest in improving this valuable crop, although a modest breeding effort recently has been mounted at Makerere University in Uganda, with assistance from The Rockefeller Foundation. However, the newly established International Crop Research Institute for the Semi-Arid Tropics, located near Hyderabad, will mount a major program on *Cajanus*.

Soybeans. This crop *(Glycine max)* has been and is being tried widely throughout Africa, but is grown only to a limited extent in East Africa – primarily Ethopia, Tanzania, and Uganda – and in Nigeria, where a production of 12 thousand tons on 14 thousand hectares was recorded in 1970. The total production for the continent, including South Africa, was 29 thousand tons on 43 thousand hectares in the same year. Most of the production in Nigeria was grown for export, but there is increasing interest in local processing and "home" consumption, with recent advances on simple methods of deactivating off-flavors and antimetabolites.

Investigations in both Uganda and Nigeria show that this crop can be highly successful and that technological advances achieved elsewhere – even in temperate regions – can be applied to the tropics. For example, several varieties from the cornbelt in the United States perform amazingly well in southern Nigeria. Soybeans tend to shatter readily in dry weather and some varieties lodge, but generally they have fewer pest and disease problems at this stage of development and perform better on poorly drained soils than do many other legumes. Bud and pod borers and some other insects are much less devastating to soybeans than to cow peas – perhaps partly as a result of profuse pubescence on the pods, stems, and leaves. Nematodes and bacterial pustule *(Xanthomonas phaseoli* var. *sojense)* were the most important disease problems of this crop at IITA in 1971. However, there appears to be good resistance to bacterial pustule among the genetic stocks grown in our breeding nurseries. Screening for nematode resistance has not yet been started.

Productivity levels in soybeans can be exceptional in well-managed plantings. It is essential to inoculate with the appropriate strain of Rhizobium, and at Ibadan there is also good response to modest applications of fertilizer. During both the first and second seasons (April and August plantings) at IITA, dry-grain yields exceeding 2,900 kg/hectare were attained in varieties maturing out within

90 days in the first season and 72 days in the second season. Highest mean yields in both seasons were obtained from the following (IITA, 1972): CNS = 2,573 kg/hectare; Bossier = 2,553 kg/hectare; Clark 63 = 2,538 kg/hectare; Amsoy = 2,499 kg/hectare.

Perhaps the major deterrent to growing soybeans is utilization. The crop is unfamiliar and not generally relished for direct consumption. Therefore, at the outset, efforts at introduction have tended to emphasize the industrial and export potentials. However, recent progress in a practical method of deactivating lipoxidase in the seed testa has made it possible to prepare bland, pleasing dishes without the "beany" or "painty" off-flavors. Preliminary experiments at the University of Ibadan have demonstrated that some highly acceptable native dishes can be prepared with heat-treated soybeans.

Chick peas. Cicer arietinum is really a subtemperate crop of the Middle East, but it is also grown successfully at intermediate to high elevations in the tropics. It is a fine-leaved, bushy plant with good yields of highly palatable and nutritious grain. Practically all the production of chick peas in tropical Africa is confined to Ethopia; there is little interest in it elsewhere.

Minor Species with Potential

There is a great need for food legumes adapted for special conditions – particularly the more humid tropics. Several promising species that show low levels of susceptibility to pests and diseases have been observed cursorily at Ibadan. These include the yam bean *(Sphenostylis stenocarpa)*, the "cow pea of the humid forests," which is cultivated and also grows wild east of Benin and in southeastern Nigeria; the winged bean *(Psophocarpus tetragonolobus)*, which is not cultivated in West Africa but is well adapted, free of pests, and exceptionally high in both protein (35 to 37 per cent) and oil (15 to 17 per cent); the lima bean *(Phaseolus lunatus)*, which is remarkably free of problems in southern Nigeria and is more widely grown in the subhumid to humid regions of West Africa than previously suspected; and the jack bean *(Canavalia ensiformis)*, with exceptionally large seeds, vigorous early growth, and high yielding potential. The locust bean *(Parkia* spp.) is a tree native to West Africa, and is used chiefly as a flavoring in soups and other dishes, but it is not cultivated generally or tended as a crop.

Strategy for Improvement

The decision to mount an intensive improvement program on a specific grain legume must be based on a favorable judgment of the potential for that species. Ideally, it would be highly desirable to be assured of the following:

1. The crop is already used extensively and is a preferred food or else would be used if the economic production potential were developed.

2. The species is highly productive and has the potential for sustained improvement in yielding ability.

3. Pest and disease problems are minimal, or else possibilities exist for developing stable host-plant resistance. If developing resistance is impossible or impractical, other control measures should be inexpensive, convenient, and safe to apply.

4. Opportunities exist for improving the quality of the product in terms of nutritional value, acceptability, palatability, and for industrial purposes.

The plant improver would much prefer to work with those species that (a) are convenient to grow and evaluate in different environments; (b) have a broad genetic base; and (c) are readily manipulated by breeding. These characteristics for tropical grain legumes may be described in more specific terms as follows:

1. A broad range of genetic diversity with a high degree of compatibility between extreme types.

2. Convenience in controlled crossing to facilitate making large numbers of hand crosses. In legumes, large flowers with uncoiled styles are much easier to emasculate and manipulate.

3. Short or medium duration to facilitate a rapid rate of generating information and to permit a rapid turnover in generations in breeding programs.

4. A reasonable level of natural outcrossing and availability of simple, inherited genetic male sterility mechanisms to sustain massive gene flow in population-improvement systems.

5. Easily-grown, "rugged" plants that germinate quickly and have rapid early development.

Cross-pollination in grain legumes is effected by insects, as contrasted with pollination by wind in the cereals. This imposes certain constraints on population-improvement systems that are dependent on massive gene flow, particularly when pest-control regimes are necessary. Some legume flowers (cow peas) contain nectar glands, which help attract bees and other pollen-carrying insects. It is possible that these glands attract undesirable insects as well.

Potential for Improvement

An arbitrary assessment of the different species can be made in respect to their potential for improvement, using the criteria described in the previous section. On that basis, the food legumes most deserving of improvement are, in descending order: (1) cow peas, (2) pigeon peas, (3) groundnuts, (4) *Phaseolus* beans, and (5) soybeans. However, this evaluation does not take into consideration the distribution of populations, the need for industrial and export crops, or peculiar regional conditions. In respect to the last, suitable food legumes have not yet been identified in the very humid and rain-forest tropics or in isolated and other areas where pests and diseases are not well controlled on any of the above species. The needs for proteinaceous foods are often greatest under such conditions, and it may be more expedient to concentrate efforts on less-familiar, but better-adapted and pest-free species like yam, lima, and jack beans for those conditions.

The Improvement Process

Meaningful progress in crop-improvement programs can be achieved only by multidisciplinary teams of scientists working together on common problems. At the HTA, a team of six senior scientists (Ph. D. level), including an agronomist, a biochemist, a breeder, an entomologist, a pathologist, and a physiologist, were assigned full time to grain-legume improvement by the end of 1972. Future additions to this team are expected to include a cytogeneticist, a second breeder, and a microbiologist. The microbiologist will investigate the rhizobial nitrogen-fixing systems in tropical legumes.

The improvement process can be divided into three major aspects, each of which involves two or more scientists (subprograms) and staffs at any one time. A comprehensive effort at the international center of excellence level would include the following objectives:

1. Plant Improvement

   a. Assemble and maintain a broad range of germ plasm to establish a "World Collection." Collect systematically in centers of diversity to obtain all available genetic diversity.

   b. Evaluate and screen the collection for important agronomic and botanical characters, including growth habit, yield potential, genotype/environment interactions, disease and pest resistance, and quality of product (primarily seeds).

   c. Concentrate desired characters, if quantitatively inherited or broadly based, by proved breeding methods — particularly by using "recurrent selection."

   d. Recombine desired characters into improved stocks, utilizing either varietal or population-improvement methods. Population improvement is considerably more efficient if a high rate of controlled hand or "natural" intercrossing can be effected.

   e. Select superior segregates or populations and test over a wide range of environments. Superior varieties or composites are increased and released.

2. Plant Protection

   a. Evaluate thoroughly methods of control through cultural practices, biological agents, and chemical means.

   b. Screen "total genetic diversity" for resistance or tolerance and include in breeding objectives (see also 1-b, above).

   c. Study the etiology and life habits of pests and diseases occurring in the regions and areas of interest.

3. Plant Growth and Development

   a. Evaluate total genetic diversity for desirable plant architecture and

physiological efficiency – for example, screening for nonphotorespiration.

b. Examine the nonmanageable constraints to attain higher levels of productivity, such as bud and pod abortion, and extension of flowering period. Examine the role of moisture and carbon dioxide in plant growth, insolation and light quality on plant development, interactions of the plant with environment, and other stress factors.

c. Study in depth and under a broad range of environments the influence of management factors on the growth and productivity of different genotypes and on new strains as developed. These factors include land cultivation, time of planting, plant nutrient levels, populations, spatial arrangements, irrigation, and other controllable factors.

d. Investigate the rhizobial nitrogen-fixing system in different species. This would include consideration of genetic factors in both the host plant and symbiont – primarily a concern of microbiology.

Inherent in all these investigations are training and outreach activities, which are essential at both the national and the international levels. International organizations such as IITA are obliged to treat all requests on an equal basis in the following ways: training young scientists and production specialists; providing information, germ plasm, and other assistance; and acting as a catalyst for stimulating interest and activity in national programs served by that institution. Specific outreach activities include organizing cooperative varietal trials and experiments, maintaining and supplying germ plasm, distributing information through reports and periodicals, and organizing professional workshops and conferences.

Conclusions and Summary

The Green Revolution has been extraordinarily effective in meeting the caloric needs in cereal-growing regions in late-developing countries. On the other hand, there has been little increase, if any, in the production of more concentrated protein foods of either plant or animal origin. It is therefore suggested that improvement of food legumes would be the most practical and efficient means of solving this deficiency.

The leguminous species with the greatest potential for improvement in the tropical regions of Africa include the following:

1. Cow peas – for lowland, semiarid savannahs to subhumid forest and savannah ecoclines.

2. Groundnuts – for lowland, semiarid savannahs for both industrial and food purposes.

3. Soybeans – for the subhumid forest and savannah ecoclines and intermediate elevations. In the longer term, soybeans may become increasingly

important as an inexpensive and nutritious protein source in processed foods for urban communities.

4. Pigeon peas – broadly adapted from humid to semiarid conditions and at low to intermediate elevations. Fit well into longer-term rotations.

5. *Phaseolus* beans – best adapted for intermediate elevations and favorable moisture conditions.

As yet, no species have been identified as good for adaptation to the very humid tropics and to high pest and/or disease pressures. However, several diverse genera may be promising candidates: yam, lima, jack, and winged beans. For intermediate to higher elevations, chick peas and broad beans should be considered.

Improving tropical food legumes will require the same magnitude of inputs as now are dedicated to cereals and industrial crops. However, much of the basic procedural technology is well understood, and rapid advances can be expected. Thus, within two decades, it should be possible to reach the same level of advancement with such unimproved pulses as cow peas and pigeon peas as that which required half a century of massive effort for the major cereals. Of course, the present development base is much higher for the oilseed legumes - soybeans and groundnuts - than for the pulses.

Rapid improvement of food legumes will be made by competent and dedicated teams of scientists representing the broad array of disciplines involved in the process and well supported with staff, equipment, and other facilities. Given this magnitude of support, real progress will be made toward balancing the food resources of developing regions in the tropics. In this context, the seventies and eighties could well become the "era of the food legumes," just as the fifties and sixties belonged to the cereals.

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FOOD LEGUMES IN LATIN AMERICA

Mario Jaïl

In spite of the ever-increasing importance of export crops in Latin America, the rural economy is still based largely on the cultivation of subsistence crops (maize, wheat), roots and tubers (potatoes, cassava), and plantain, which occupy by far the largest part of the area used for subsistence farming. It is therefore obvious that food legumes play only a very modest role in Latin American agriculture, largely because of the small amount of food gained for effort expended, as compared with that obtained from starchy crops for the same effort.

There is adequate potential in the region for a substantial increase in food-legume production for human consumption. However, to break away from the traditional system of monoculture, which prevails in many countries, to a diversified cropping, and to intensify the production of food legumes will call for considerable effort on the part of breeders and agronomists in such work as plant breeding, improvement of soil fertility, seed production, mechanization and improved equipment, improving crop rotation, developing more efficient disease- and pest-control methods, and reducing the losses of harvested and stored products.

The need for the expansion of food-legume cultivation in the region is great. The influence of food legumes on land productivity and soil fertility in cultivated land through crop rotation is undisputed today. Their primary importance, however, is that they are one of the cheapest sources of protein for human consumption. As the price of animal protein is expected to rise, it seems reasonable to provide solutions for replacing it in part with food legumes.

Cultivated Area and Production of the Most Important Food Legumes

In recent years, the insignificant increase in production of food legumes, as compared with the population needs, has widened the protein gap. Table I gives the region's area, production, and yield figures for all the major food-legume species, and reveals that their production has remained static. Table II shows an interesting increase in the production trend of soybeans but a decrease in the production of groundnuts; both are food legumes used mainly in the manufacture of edible oil.

Dry bean *(Phaseolus vulgaris)* is the most common food legume in Latin

TABLE I

Regional Production of Food Legumes in Latin America (Mainly for Direct Human Consumption)

	Dry beans	%	Dry broad beans	%	Chick peas	%	Dry peas	%	Lentils	%	Pigeon peas	%	Lupins	%	Other food Legumes	%
1966																
Area	6,415	89.3	296	4.1	176	2.5	151	2.1	38	0.5	44	0.6	4	0.1	57	0.8
Production	3,712	86.9	175	4.1	147	3.4	120	2.8	29	0.7	32	0.6	4	0.1	28	0.7
Yield	5.8		5.9		8.4		7.9		7.6		7.3		10.0		4.9	
1967																
Area	6,469	88.9	324	4.5	196	2.7	139	1.9	41	0.6	44	0.6	4	0.1	61	0.8
Production	4,052	88.4	191	4.2	148	3.2	105	2.3	24	0.5	31	0.7	4	0.1	30	0.7
Yield	6.3		5.9		7.6		7.6		5.9		7.0		10.0		4.9	
1968																
Area	6,350	88.3	307	4.3	231	3.2	149	2.1	42	0.6	44	0.6	5	0.1	61	0.8
Production	3,797	87.8	167	3.9	177	4.1	97	2.2	19	0.4	33	0.8	6	0.1	31	0.7
Yield	6.0		5.4		7.7		6.5		4.5		7.5		12.0		5.1	
1969																
Area	6,707	89.1	310	4.1	201	2.7	149	2.0	47	0.6	45	0.6	5	0.1	61	0.8
Production	4,056	88.2	184	4.0	149	3.2	110	2.4	31	0.7	33	0.7	6	0.1	31	0.7
Yield	6.0		5.9		7.4		7.4		6.6		7.3		12.0		5.1	
1970																
Area	6,923	89.2	323	4.2	199	2.6	150	1.9	51	0.7	45	0.6	5	0.1	61	0.8
Production	4,294	88.3	198	4.1	164	3.4	106	2.2	28	0.6	35	0.7	6	0.1	31	0.6
Yield	6.2		6.1		8.2		7.1		5.5		7.8		12.0		5.1	

Source: FAO Production Yearbook, 1970.

Area – in thousand hectares
Production – in thousand metric tons
Yield – 100 kg/hectare
% – percentage of total area and production

TABLE II

Regional Production of Groundnut and Soybeans in Latin America (Mainly for Edible Oil Purposes)

	1948-52	%	1961-65	%	1966	%	1967	%	1968	%	1969	%	1970	%
Groundnut (in shell)														
Area	371	89	952	70	1,171	66	1,211	62	1,081	54	1,072	48	1,065	43
Production	349	86	1,169	71	1,519	66	1,312	58	1,227	55	1,204	44	1,376	42
Yield	9.4		12.3		13.0		10.8		11.4		11.2		12.9	
Soybeans														
Area	46	11	408	30	610	34	747	38	934	46	1,151	52	1,438	57
Production	55	14	471	29	779	34	941	42	1,009	45	1,512	56	1,937	58
Yield	12.0		11.5		12.8		12.6		10.8		13.1		13.5	
Total														
Area	417	100	1,360	100	1,781	100	1,958	100	2,015	100	2,223	100	2,503	100
Production	404	100	1,640	100	2,298	100	2,253	100	2,236	100	2,716	100	3,313	100

Area – in thousand hectares
Production – in thousand metric tons
Yield – 100 kg/hectare
% – percentage of total area and production

Source: FAO Production Yearbook, 1970

America, accounting for more than 80 per cent of production. Next in importance are dry broad bean *(Vicia faba)*, chick pea *(Cicer arietinum)*, and dry peas *(Pisum sativum* and *P. arvense)*. Other important food legumes are lentil *(Lens esculenta)*, pigeon pea *(Cajanus* spp.) and lupin (*Lupinus* spp.).

Past and Current Work on Genetic Improvement

The information given herewith is more for the purpose of illustrating the lines of work than to give an exhaustive account of the work carried out.

The genetic improvement of food legumes has been limited to beans *(Phaseolus vulgaris)*, which, together with corn, constitutes the basis of the tropical Latin American daily diet.

Bean diseases are the major limiting factors to yield. Breeding work has been carried out recently, aimed at obtaining resistant selections to rust *(Uromyces phaseolus)*, anthracnose *(Colletotrichum lindemuthianum)*, angular leaf spot *(Isariopsis griseola)*, Cercospora leaf spot, bacterial and viral diseases in Colombia, Mexico, Brazil, Peru, and Central America. Resistant or tolerant bean selections to *Xanthomonas phaseoli*, mosaic, *Isariopsis*, and rust have been obtained in Central America.

In 1963, the Instituto Interamericano de Ciencias Agricolas (IICA) set up a Basic Food Crops Unit at the Tropical Centre (IICA-CTEI),* and since that time priority has been given to research on beans. The Regional Office for the northern part of IICA (IICA-ZN)† took charge of the coordination and technical support of the Central American Project for Production and Improvement of Beans and Other Food Legumes in 1965.

Genetic research, especially on the heritability of yield components and grain quality, coupled with cytogenetic studies, mainly on induction of mutations, have enabled the program to speed up bean improvement. From 1963 to date it has produced 14 improved bean lines from introductions or crossings. Several of them have outyielded all other entries in various regional trials in Central America and in national tests in Brazil and Venezuela. Their experimental yields often are seven to eight times larger than the average commercial bean yield of 500 kg/ha in Central America. Between 1967 and 1970, the program released four of them as improved varieties.

Every year, from 1966 to 1970, the PCCMCA‡ bean-yield trials included an average of 90 varieties or lines in a "nursery" group, 14 in a "black bean" group, and 13 in a "red bean" group. Some of the entries came from national institutions and some from the program. They were planted not only in all the countries of the Central American isthmus but also in other Latin American countries, such as Colombia and Venezuela. From the results obtained, PCCMCA

*CTEI – Centro Tropical de Enseñanza e Investigacion

†ZN – Oficina de IICA, Zona Norte

‡PCCMCA – Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios.

varieties have been recommended in the following countries and numbers: Guatemala, two; El Salvador, four; Nicaragua, five; and Costa Rica, six. One of them, developed by the program, was specifically recommended for the lowlands of Guatemala.

High-yielding, white-seeded, bean cultivars were scarce in the PCCMCA trials. By treating a black-seeded PCCMCA variety with a chemical mutagen (ethyl methane sulfonate), the program developed two white-seed mutants. In a field trial this year in IICA-CTEI, one mutant (NEP-2) yielded 2268 kg/ha, i.e., 22 per cent more than the best white-seeded PCCMCA cultivar present in the trial.

Except for the change in seed color, the mutants possess all the other important features of the parent variety, such as erect plant type and resistance to common bean mosaic virus. Moreover, their seed-protein content is higher by about three percentage points. Breeder seed of NEP-2 is being increased for further testing and eventual release of this mutant as an improved variety.

Brazil, Colombia, Venezuela, and Peru are also working in the improvement of other food legumes (chick peas, dry peas, lentils, pigeon peas, and cow peas). The main line of work consists of the introduction and evaluation of varieties, particularly for disease resistance.

Because of the wide adaptability of soybean as a crop and its high content of protein and oil, both of which are in short supply in Latin America, an almost regional interest in soybean has been developed in recent years. Varieties from Asia and the United States have been transferred successfully to several countries, notably Colombia, Brazil, and Venezuela, but considerable varietal development, testing, and production research appear to be needed in other countries.

Research on food-legume improvement in Latin America has followed, first, the pattern of collection of native varieties in germ-plasm banks. Such collections have concentrated mainly on *Phaseolus vulgaris*, and less on other food-legume species.

The basic exploration work, done by Bukasov in the late twenties, gave a comprehensive view of the variations. In more recent times, several local collections have been established in Central America, Colombia, Peru, and Brazil. Two of them are of special interest. One recent collection of primitive types, led by Miranda Colin in Mexico and kept at Chapingo, showed interesting ecological relationships between beans and insects. The other was the intensive exploration work done from 1965 to 1967 in Central America and Mexico by H.S. Gentry of the United States Department of Agriculture. This collection has been evaluated for certain characteristics, such as disease resistance, with optimum results. Apparently the Mexican material collected there by Gentry has genes resistant to the most important bean diseases.

According to an FAO partial survey in Latin America, germ-plasm collections on *Phaseolus vulgaris* are kept in the countries given below.

Country	Number of Entries
Brazil	645
Chile	700

Country (Cont'd)	Number of Entries (Cont'd)
Colombia	4000
Costa Rica	8000
Jamaica	60
Mexico	610
Peru	288
Venezuela	1487

Research Needs on Nutritional Improvement of Food Legumes

Knowledge, as well as research work, on the nutritional quality of food legumes is very limited in most developing countries of the world. Generally, the protein content of food legumes, which, in existing varieties, varies from below 20 per cent to above 30 per cent, needs to be increased. With a few exceptions, legume proteins are deficient in sulfur-containing amino acids. Methionine and cystine contents, therefore, need improvement. Digestibility of most legume proteins (and carbohydrates) is poor. Up to 25 per cent or more of the protein contained in some is indigestible and not absorbed from the intestinal tract. Generally, the cooking qualities should be improved to allow cooking in 1 to 1-1/2 hours after adequate soaking. Other factors, such as antimetabolites (antitryptic and hemagglutinins, and others) should be reduced or eliminated completely. Flatus production and general acceptability, including taste, need active investigation.

Like any other character, protein and other properties are also influenced by the genotype, the environment, and their interactions. Analysis of germ-plasm collections have revealed considerable variability for protein quality and quantity. However, before this variability can be exploited profitably, it is necessary to know whether the character is stable under varying environmental, agronomic, and biological conditions. Furthermore, improvement in protein quality and quantity and in other desirable characteristics should occur simultaneously with increased yield.

The Important Role of Germ-Plasm Collections in the Improvement of Food Legumes

In Latin America, the improvement of the nutritional value of food legumes may come mainly from a collection/evaluation approach, rather than from any other method. This is because of the huge natural variability, still untapped in many ways, and, with the exception of a few countries, the lack of permanent programs of selection. One important factor to consider is that selection aimed at a specific purpose, e.g., to obtain specific disease resistance, is conditioned very often by narrow ecological limits, and the improved varieties have a limited area of adaptation. It is, therefore, the introduction/testing approach in which the future work may give quicker and less expensive results.

Disease and insect resistance, as indicated before, is found in the native populations of beans and may be incorporated by crossing. An important economic factor is that selection has been aimed at the bush beans. Climbing types may fit better in the native agricultural pattern, but because bush beans are predominant in developed agriculture, little attention has been paid in Latin America to climbing varieties.

Also, very little attention has been paid (except in Colombia) to the highland types of the common bean and to the other three species of beans. The tepari is disappearing rapidly; the scarlet bean is becoming a relict; and virtually no selection is being carried out on the lima bean.

Among the introduced species, pigeon pea is by far the most promising. Selection work is carried out in Puerto Rico, and small collections are maintained in a few other places (El Salvador). They are also of some importance in Brazil. The cow pea has not received much attention. However, in the lowlands of Brazil, Peru, and Venezuela, varieties — probably of American origin — are cultivated in those areas too hot and wet for beans. More germ plasm of pigeon pea and cow pea should be brought in from outside and tested, because these two crops are more adaptable than common beans.

Latin America badly needs well-established gene banks, both for its autochtonous materials and for introductions. These gene banks, of a permanent nature for conservation and rejuvenation, should be associated with a network of evaluation stations, where the materials may be screened for yield, resistance, etc., and followed by a seed-production scheme to supply the farmers with the selected varieties. At the same time, the seed banks may become the source of material for advanced purposes in breeding.

Recommended Approach for Regional Cooperation

Research work considered essential for the genetic improvement of food legumes would require a closely-knit team effort. Responsibility for in-depth research and information service could be assumed by such existing international/regional institutes as IICA and CIAT. Consideration should also be given to making use of national institutes — strengthening them as appropriate — through assistance from UNDP and other aid-giving agencies. Solutions of many of the problems mentioned will take time. In the meantime, measures to increase output of food legumes are essential.

A strategy that will give farmers the possibility of taking maximum advantage of existing knowledge to increase areas planted to food legumes and productivity should be concentrated for a short-term basis on:

Organizing, on a multicountry basis, cooperative programs (uniform nurseries and standardized variety trials) to introduce and test improved varieties of the main food legumes in order to secure an adequate stock in the countries. This is being done successfully by IICA-ZN with *Phaseolus* in Central America.

The benefits to be derived from such cooperative programs are:

Determination of the comparative adaptation and yield of the principal regional varieties and of those bred in other countries with similar agro-climatic conditions.

Use of the nursery as a vehicle for the introduction of promising commercial varieties and outstanding advanced generation lines from a breeding program of one country into other countries, thus reducing duplication of effort.

Development of basic information on adaptation and yield, including disease and pest resistance, of different varieties under a wide range of physical and biological environments, thus enabling quick release of suitable varieties for production on a larger scale than at present.

Establishment of a "scientific" bond between scientists and programs in the participating countries.

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UNIVERSITY OF ILLINOIS INTERNATIONAL SOYBEAN PROGRAM

Earl R. Leng

The Program for International Research, Improvement and Development of Soybeans (PIRIDS) was established in 1969 at the University of Illinois. It operates as part of the Office of International Agricultural Programs in the College of Agriculture. I have been Program Director of PIRIDS since its inception.

General Organization and Funding

Initial funding of PIRIDS was from a Rockefeller Foundation grant. The University of Illinois, through its Office of International Programs and Studies and the Office of International Agricultural Programs, contributed heavily to the operation of PIRIDS between July 1, 1969, and June 30, 1972. A contract from the U.S. Agency for International Development was awarded, effective September 1, 1971, and part of the program's operation now is supported by this contract.

Locations of Operations

PIRIDS has established contacts and research linkages with virtually every country in the world where soybeans are being investigated as a field crop, excepting the Republic of China and the Eastern European communist countries. Even in the latter, research linkages have been developed in Romania and preliminary discussions have been held with soybean workers from the U.S.S.R.

Cooperative field trials, to study varietal performance, have been carried out or are under way (June, 1972) in the following countries:

Latin America: Colombia (3 locations); Ecuador (2 locations); Brazil (3 locations); Belize.

Africa: Sierra Leone; Nigeria (in cooperation with IITA); Ethiopia.

Asia: India (3 locations); Ceylon; Thailand (2 locations); Vietnam; Indonesia (2 locations); Pakistan.

Middle East: Jordan.

Agronomic-cultural practice trials and studies of response to *Rhizobium* inoculation have been conducted, on a cooperative basis, in Colombia, Ecuador,

India, and Indonesia, and additional trials now are under way in Belize and Sierra Leone.

Study of simple processing methods for direct food utilization of soybeans are based at Urbana, Illinois. A strong program, closely linked with the principal operation, is under way at G.B. Pant University in India, under the direction of a University of Illinois professor affiliated with PIRIDS. Preliminary demonstrations of the basic process have been conducted in Colombia, Brazil, India, Thailand, Indonesia, and the Philippines. Contacts established in Brazil, Colombia, and Thailand will enable much more detailed work and demonstrations to be undertaken in the very near future.

Principal Results to Date

The work of PIRIDS to date has clearly shown that:

1. Soybean yields in equatorial latitudes can exceed 3,000 kg/ha, using varieties presently available, provided that proper cultural practices are used and favorable growing conditions prevail.

2. It is possible to develop effective cooperative relations and research linkages with soybean research personnel in many LDCs in tropical and subtropical regions.

3. The major present limitations on soybean yields, when the crop is grown according to local practices, are generally related to:

   a. Insufficient productive potential of "local" varieties.

   b. Low soil fertility, particularly unfavorable pH and low supply of P.

   c. Very low plant populations.

   d. Absence of effective *Rhizobium* inoculation.

   e. Damage by insects, plant diseases, and weeds; these are often aggravated by the generally weak condition of the crop and the low level of cultural practice, including weed control.

   f. Growing of soybeans as a "catch crop" in unfavorable seasons, particularly when moisture supply is insufficient.

4. Because some "local" varieties make reasonable vegetative development, even under unfavorable growing conditions, and some "modern" varieties yield very well when grown under favorable conditions, it should be possible to breed high-yielding "combined" types with broad adaptation to tropical and subtropical conditions.

5. The cultural practices necessary for high yields can be adapted to conditions of the LDCs in satisfactory ways and at relatively low cost to the cultivater.

6. Indigenous manufacture of effective *Rhizobium* inoculant should be feasible in many LDCs. Even where this material cannot be produced effectively, the cost of imported inoculant is low.

7. Techniques developed at the University of Illinois make it feasible to produce attractive, highly nutritious food products from whole soybeans at low cost.

Detailed Studies

1. Studies on a neurotoxin from *Lathyrus sativus* (work of Professor Betty Haskell and Tara Mahta).

a. We have devised a new synthesis of the beta-N-oxalyl-L-alpha, beta-diaminopropionic acid, neurotoxin from *Lathyrus sativus*, from monoethyl monochloro-oxalate and L-alpha, beta-diaminopropionic acid.

b. We have confirmed that the product of the revised synthesis is identical to the natural product by a comparison of infrared spectra, mass spectra, and gas-liquid chromatography tracings of two different chemical derivatives, and by comparison of biological activity in yeast assays.

c. We have confirmed that the natural toxin is a beta-N-oxalyl derivative (and not an alpha-N-oxalyl derivative) carrying both natural and synthetic toxins through a sequence of reactions whose anticipated product is dinitrophenylserine: treatment with fluorodinitrobenzene, hydrolysis of the amide bond, conversion of the free amino group to a diazonium salt, and hydrolysis of the diazonium salt. The product of this degradation scheme for either synthetic or natural toxin has a nuclear magnetic spectrum identical to that of dinitrophenylserine.

d. To confirm that the natural toxin is a derivative of L-alpha, beta-diaminopropionic acid (and not of D-alpha, beta-diaminopropionic acid), we have treated both the toxin and the alpha, beta-diaminopropionic acid isolated from the toxin after hydrolysis with tryptophanase, an enzyme which acts only on L-isomers of amino acids. Either the natural toxin (or the alpha, beta-diaminopropionic acid isolated from the toxin) is readily converted to pyruvic acid by a highly purified apotryptophanase preparation.

e. We have investigated the specificity of the neurotoxin as an amino-acid antagonist in nutritional experiments with microorganisms. Resting-cell experiments demonstrate that the neurotoxin behaves as a potent antagonist of the transport of amino acid across the microbial cell wall.

2. Methods Currently Used for Screening Soybean Populations for Chemical Component (Hymowitz and Walker, 1970; Walker, et al., 1969).

a. *P, K, Ca, Mg, B, Cu, Fe, Mn, Zn, and Na*

Sample Preparation

Seed or leaf samples are dried at 55°C for 3 days and ground in a Wiley mill or Stur-Dee Mitey Mill. A 0.5 g sample is ashed at 200°C for one hour, then at 500°C for 15 hours. The ash is taken up in 5 ml of an internal standard.

Instrument

Jarrell-Ash Mark IV Ebert Direct Reading Stigmatic Spectograph.

b. *Total Oil – Nondestructive Analysis*

Sample Preparation

Seeds are dried at 55°C for five to seven days in a forced draft incubator to a moisture content of about 3 per cent before 1 g of whole seed is analyzed for oil content by the method of Collins, et al. (1967). (See also Hymowitz, et al., 1970).

Instrument

Varian PA-7 Nuclear Magnetic Resonance (NMR) Process Analyzer.

c. *Fatty Acid Content*

Sample Preparation

A 1 g sample of seed is ground to a fine powder for 6 minutes in a Spex ball mill or for 15 seconds in a Stur-Dee Mitey Mill. The method described by Singh, et al. (1968) and Singh and Hadley (1968) is used for extraction of the oil and making of methyl ester.

Instrument

Aerograph Hy-Fl 600°C gas chromatograph with a 232.5 cm x 0.32 cm copper column packed with 15 per cent diethylene glycol succinate (DEGS) on 80-100 mesh chromosorb (A)W. The column is maintained at 205°C with helium carrier gas flowing at a rate of 30 ml/min to the hydrogen flame detector.

d. *Protein Content – Dye-Binding Method*

Sample Preparation

The soybeans are ground in a Bauer Mill or for 15 seconds in a Stur-Dee Mitey Mill. Only those particles that rest on a 40-mesh sieve after passing through a 30-mesh sieve are used for the dye-binding method.

A 100 mg sample is weighed and transferred into a 60 ml polyethylene wide-mouth bottle. A plastic bead 0.9 cm in diameter is placed in the bottle with 25 ml of acid orange 12 reagent solution (Hymowitz, et al., 1969; Singh and Hymowitz, 1971; American Oil Chemists Soc., 1967). The bottle is placed horizontally for 105 min on a reciprocal shaker that produces approximately 150 strokes per min. After shaking, the solution is filtered through a fiber-glass filter placed on a No. 4/0 Husch porcelain funnel into a test tube up to the 15 ml level. The filtrate is clear. The calibration curve is based on Kjeldahl values.

Instrument

Vdy Analyzer Co. colorimeter equipped with a flow-through cuvette that has a path length of about 0.3 mm.

e. *Soybean Trypsin Inhibitor A_2 – Qualitative*

Sample Preparation

An oily sample of finely ground soybean meal is well mixed in a mortar with 3 ml of a buffer solution containing 2 ml of buffer (0.092 M trishydroxymethylaminomethane and 0.023 M $CaCl_2$ 2 H_2O brought to pH 8.1 with HCl) and 1 ml of 0.4 g of sucrose. The mixture is decanted into a test tube and centrifuged at 2,000 x g for 10 min in a clinical, unrefrigerated centrifuge. After centrifugation, the supernatent is decanted into a storage vial and stored at 0° to 2°C until electrophonesis (Hymowitz and Hadley, 1972; Yen, et al., 1971). A 30-μ liter volume of the final supernatent is sufficient to show the presence of trypsin inhibitor band(s) when electrophonesis was performed by the procedure of Singh, et al.

Instrument

Canalco Electrophonesis Constant Rate Source Model 300B and a Canalco Quick Qal Destaener.

f. *Sugar Content*

Sample Preparation

Whole mature seeds are placed in a forced draft incubator for 5 days at 48°C. The moisture content at the end of the 5 days is approximately 3 per cent. Seeds are ground in a Bauer Mill, 4 g of meal is weighed, wrapped in 15-cm Whatman filter paper.

Oil is extracted from the meal in a Soxhlet extraction apparatus with petroleum ether for 24 hr. Afterward, sugars are extracted from the defatted meal by repeated washings with 80 per cent ethanol. Protein in the extract is precipitated by a solution of lead acetate, and excess lead precipitated with sodium bicarbonate. The pH of the solution is 6.4 to 6.8. The sugar solution is brought to volume in a 50-ml volumetric flash. A 10-ml sample is centrifuged for 30 min at 2,000 x g and stored in a 10-ml vial at −15°C.

A sugar-trimethylsilyl derivative is made, using 2 ml of trimethylsilylimidazole in dry pyridine at 15 mg per ml. The derivative is injected directly into the gas chromatograph (see Hymowitz, et al., 1972; Hymowitz and Shu, 1969).

Instrument

A Beckman GC-45 gas chromatograph with dual "U"-shaped columns, dual hydrogen flame conization detectors, a-c electrometer linear temperature programer, two column injection inlets, and equipped with a 1-mv Beckman linear potentiometric recorder is used.

Dual stainless-steel columns 1.98-m long are packed with 2 per cent OV-17 on Diatoport S (80 to 100 mesh). Stainless-steel ferules are used in the columns. Fixed temperatures are: inlet 300°C; detector line 335°C; and detector 350°C. Gas flow rates are: air at 275 cc per min; hydrogen at 45 cc per min; helium carrier at 90 cc per min; and helium makeup at 30

cc per min. The linear programer is set at an initial temperature of 150°C and final temperature of 330°C with a rise time of 16 min.

g. *Range of Chemical Components in Soybean Seed*

Chemical Component	*Range*
Protein %	52.2-35.1
0.1%	23.4-11.4
Palmitic %	17.4- 9.3
Stearic %	7.0- 2.2
Oleic %	29.6-15.2
Linoleic %	59.6-33.8
Linolenic %	15.0- 4.3
Sugar % (U.S. northern varieties)	16.5- 6.2
Sucrose %	67.5-41.3
Raffinose %	15.8- 5.2
Stachyose %	35.2-12.1
Trypsin inhibitors	Rf 0.79 allele Tl^1
Electrophonetic banding pattern	Rf 0.75 allele Tl^2

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GENETIC IMPROVEMENT OF *PHASEOLUS VULGARIS*

A. M. Evans

The genus *Phaseolus* has contributed crop plants to agriculture in both the old and the new worlds. *Phaseolus* beans are among the oldest established crop plants, and radio-carbon dates show that *P. vulgaris* was domesticated in Central America about 7,000 years ago (Kaplan, 1965). It was introduced into Europe in the sixteenth century, and since then it has become a common leguminous crop throughout most of the world. In most tropical countries it is a valuable pulse crop, whereas in temperate regions, although dry beans are grown, varieties have been developed for fresh pod consumption and for processing as frozen vegetables.

Genetic Diversity

Studies of the genetic variability within the species have been carried out in Cambridge over a number of years. (McWalter, 1964; Rathgen, 1965; Smartt, 1969; Froussios, 1970). The aim has been to give information on the potential for the genetic improvement of this crop. A collection of some 4,000 entries has been assembled (1972). These include varieties from the center of diversity of this species in Central and South America, wild populations from Mexico, land races from Uganda, and varieties from temperate North America and Europe.

Intraspecific Differentiation in Cultivars

Under greenhouse conditions, Froussios (1970) described four growth forms. Under field conditions at Cambridge, using a greater range of material, Pegington (unpublished, 1972) has recognized five races (Table I). These races vary in habit, leaf size, internode length and internode number. The survey of their fruiting characters shows variation in pod length and texture, in seed numbers per pod, and in seed size (Table II).

The pattern of evolution appears to be as follows:

CENTRAL AMERICA

Race 1 a

Small-seeded, indeterminate climbers

small-seeded, indeterminate semiclimbers → *Race 2*

small-seeded, indeterminate bush types → *Race 3*

small-seeded, determinate multinoded types → *Race 4*

SOUTH AMERICA

Race 1 b

Large-seeded, indeterminate climbers

↓

large-seeded, determinate bush types

↓

Race 5

TABLE I
Variation in Vegetative Characters in *P. vulgaris* Varieties

Race	Habit of plant	Internode length	Number of internodes	Leaf size
1	Indeterminate climber	long	17-35	1(a) small 1(b) large
2	Indeterminate semiclimber	long	14-30	small
3	Indeterminate bush	short	13-25	small
4	Determinate climber	short	12-15	small
5	Determinate bush	long	3-5	large

TABLE II
Variation in Fruiting Characters in *P. vulgaris* Cultivars

Race	Pod length (mm)	Seed number per pod	Seed size (mg)		Pod texture
1	75 to > 150	3-7	1 a small 1 b large	200-800	parchmented leathery, and fleshy
2	< 75 to 112	5-7	small	200-400	parchmented and leathery
3	< 75 to 112	5-7	small	150-300	parchmented
4	< 75 to 112	6-8	small	150-250	leathery
5	75 to > 150	3-4	large	400-1000	leathery and fleshy

TABLE III
Characteristics of the Eight Parental Varieties

Varietal name	Country of origin	Growth habit	Pod type	Mean seed size (mg)
Panameno (PM)	Colombia	Determinate bush	Leathery	693
Tipo Comun (TC)	Colombia	Determinate bush	Leathery	582
Cuarentano 54-P (CT)	Costa Rica	Determinate bush	Leathery	411
Masterpiece (MP)	Britain	Determinate bush	Leathery	519
Glencarse (GC)	Britain	Determinate bush	Fleshy	414
Tenderwhite (TW)	U.S.A.	Determinate bush	Fleshy	389
Guatemala 14-2 (GM)	Guatemala	Indeterminate bush	Parchmented	198
Venezuela 41 (VZ)	Venezuela	Indeterminate bush	Parchmented	172

It is clear that enormous variation occurs in *P. vulgaris* cultivars and this can be exploited by breeding. There is also variation in varietal response to photoperiod, and the selection of varieties for north-temperate use has primarily involved selection for tolerance of long days.

Exploitation of Variability by Breeding

The breeding program at Cambridge is a joint enterprise with the University of Makerere, Uganda. Hence, day-neutral types were chosen as parental varieties for breeding. These types contain a morphologically predetermined pattern of development, irrespective of day length. In considering the most desirable

growth habit, little appeared to be gained in using the climbing types, so the choice was based on the bush types, which are more suited to the agronomic circumstances of mechanical harvesting. The decision to use both the determinate and indeterminate bush types was made because of the possible use of both these types in Uganda (Leakey, 1971).

Eight bush varieties of *P. vulgaris* were selected from the collection to include representatives of different geographical areas. Details of the growth habits, pod types, seed sizes, and country of origin are given in Table III. The eight parental varieties were crossed in a diallel fashion, and 56 F_1 families, including reciprocals, were produced. A total of 1,894 pollinations were made and 1,356 pods were produced, giving a 72.1 per cent success overall. The F_1 hybrids were allowed to self-pollinate to produce 56 F_2 progenies. Parents, F_1, and F_2 progenies were grown in the field, using a 5^3 lattice design, as described by Phipps, et al. (1944). Ten seeds were sown per single-row plot with a 25-cm intrarow and a 60-cm interrow spacing; three replicates were used. All the component characters of yield were scored, but only total seed yield per plant in parents, F_1, and F_2 progenies will be considered here (Tables IV, V a and V b).

In the parents, the mean yields ranged from 42.5 to 47.9 g per plant, whereas the range was from 23.7 to 74.8 g in the F_1 progenies. There was evidence of considerable heterosis. This was defined as a significant positive deviation, over all crosses, above their top parent or a significant negative deviation over all crosses from their bottom parent. The degree of heterosis was expressed as a percentage of the corresponding parent mean. There was significant positive heterosis at the 1 per cent level of significance in the F_1 progenies, but much of this was lost in the F_2 (Table VI). On omitting the progenies that included parents GM and VZ, there was a decrease in heterosis in the F_1 but more was maintained in the F_2 (Evans, 1970).

On considering only the six determinate bush varieties, the highest yields in the F_1 and F_2 were from single-plant progenies of CT, GC, and TC.

TABLE IV

Adjusted Means of F_1-Yield per Plant (g)

	PM	TC	CT	MP	GC	TW	GM	VZ	Mean
PM	42.5	57.7	62.4	53.8	58.2	62.1	66.5	61.7	58.1
TC	55.4	45.2	52.6	59.8	62.5	62.5	65.0	66.8	58.7
CT	55.9	57.4	45.3	59.5	64.3	64.8	71.0	64.5	60.3
MP	57.5	56.8	52.5	47.9	63.8	61.4	61.2	70.1	58.9
GC	66.3	54.3	74.8	58.9	46.8	42.4	71.3	63.7	59.8
TW	50.3	49.7	55.2	42.3	39.6	23.7	58.0	56.0	46.9
GM	58.4	70.5	62.5	69.2	66.3	65.2	37.8	51.3	60.2
VZ	60.3	63.1	62.9	61.9	61.7	59.8	42.9	40.7	56.7
Mean	55.8	56.8	58.5	56.7	57.9	55.2	59.2	59.4	57.4

TABLE Va
Adjusted Means of F_2 -Yield per Plant (g)

	PM	TC	CT	MP	GC	TW	GM	VZ	Mean
PM	42.5	52.5	56.4	53.3	52.3	45.6	45.8	43.7	49.0
TC	48.0	45.2	51.0	45.8	60.3	55.6	46.9	46.8	50.0
CT	47.5	48.7	45.3	49.9	56.4	51.1	40.8	30.7	46.3
MP	55.2	51.8	49.1	47.9	50.7	44.8	32.8	37.6	46.2
GC	53.0	57.1	60.0	55.4	46.8	42.8	43.7	45.0	50.5
TW	52.1	53.7	49.8	41.0	41.9	23.7	41.9	41.7	43.2
GM	40.5	48.0	42.6	37.9	43.3	50.7	37.8	36.6	42.2
VZ	46.0	37.9	41.3	40.6	46.9	37.8	48.1	40.7	42.4
Mean	48.1	49.4	49.4	46.5	49.8	44.0	42.2	40.4	46.2

Overall S.E. = 0.67 L.S.D. (at 5%) = 1.79

TABLE Vb
Analysis of Variance of 5^3 Lattice Trial of Parents, F_1 and F_2 Progenies

	D.F.	Mean Square	F
Replicates	2	4774.9018	+++
Blocks	72	69.8348	++
Populations	124	298.0567	+++
Blocks + error	248	51.0151	
Error	176	43.3161	

(For the estimation of F, the blocks and Error M.S. has been used)

TABLE VI
Degree of Heterosis in the F_1 and F_2 Progenies

	Degree of heterosis (F_1)	S.E.	Degree of heterosis (F_2)	S.E.
All progenies	+31.74	2.251 +++	+ 3.47	1.967 n.s.
Omitting GM & VZ	+23.27	2.997 +++	+ 10.19	2.057 +++

F_3 Progeny Rows

The mean yields from 5 m drilled F_3 progeny rows (Table VII), show that the highest-yielding F_3 progenies were obtained from the CT and TC parents (393 g), whereas the GC progenies, which had performed well in the F_1 and F_2 generations when grown as spaced plants, were very poor under the F_3 competitive conditions.

TABLE VII
F_3 Yield per 5m Rows

	PM	TC	CT	MP	GC	TW	Mean
PM	310.3	328.9	399.5	320.2	289.9	214.0	310.5
TC	389.7	380.9	399.5	345.6	331.6	239.2	347.7
CT	398.1	386.3	397.8	362.2	337.0	263.7	357.3
MP	320.5	391.3	390.3	327.7	275.2	171.1	312.3
GC	296.8	318.5	292.6	245.7	179.3	158.6	248.6
TW	212.0	210.5	253.0	219.9	153.1	108.3	192.6
Mean	321.3	336.7	355.4	303.5	261.2	192.5	294.9

S.E. = 18.22 L.S.D. = 51.36

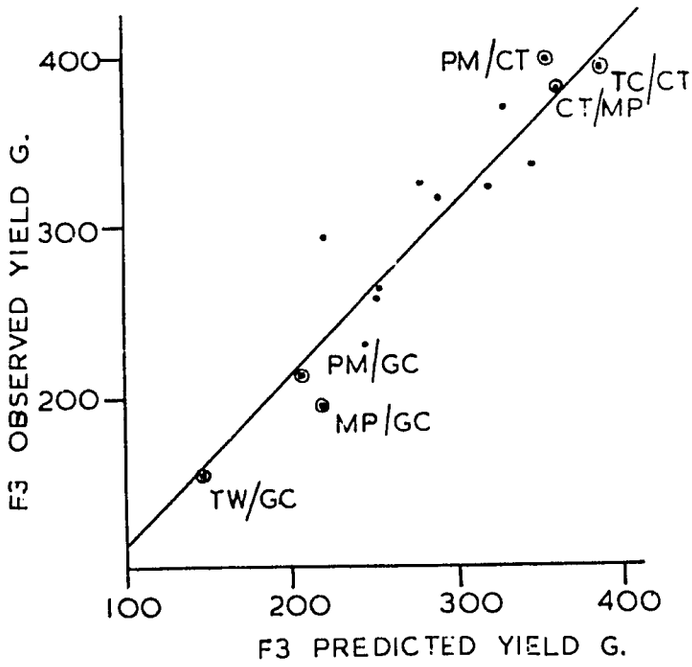

Figure 1. Predicted and observed values for the three highest- and three lowest-yielding progenies in F_3.

A striking feature of the F_3 yield data was that the observed values showed a highly significant correlation ($\gamma = 0.959^{**}$) with the expected values calculated from the means of parental yields (Figure 1). Most of the genetic variation is thus additive, and there is likely to be response to selection for high yield in future generations. The mean yields of the F_3 progenies was 295 g (1967 kg/ha) and of the CT/TC progeny was 393 g (2620 kg/ha).

So far, the program has shown that there is a genetic potential for yield improvement in this material. An F_3 composite bulk population was also grown in 1971 at both Cambridge and Makerere Universities, and we plan to compare the performance of the bulks at these contrasting sites. It is hoped that this will assist in breeding for stability and adaptability in the two environments and will contribute to both breeding programs.

Crude Protein Estimation

A limited amount of information is available on the crude-protein percentage determined from seed samples from spaced plants of the six determinate bush varieties:

Variety	% protein	Yield/plant (g)	Yield of protein/plant (g)
PM	26.9	42.5	11.43
TC	25.0	45.2	11.30
CT	24.4	45.3	11.55
MP	22.8	47.9	10.92
GC	23.5	46.8	10.99
TW	25.1	43.7	10.96
Pooled S.E.	1.08	0.67	

The main trends are that the highest-yielding variety (MP) has the lowest per cent protein and the variety with the highest per cent protein (PM) has the lowest yield. The highest yield of protein per plant is given by a variety with yield and protein near to the average (CT).

Breeding for Yield and % Protein in Grain Legumes

A few workers have studied the relationship between yield and protein content in grain legumes and in nearly all cases have reported a negative correlation.

Crop	*Correlation coefficient (r)*	*Authority*
Soybeans	– 0.33	Johnson, et al., 1955
	– 0.08	
Phaseolus beans	– 0.635	Tandon, et al., 1957
Phaseolus beans	– 0.23	Rutger, 1970
	– 0.36	
Peas	Low + correlation (n.s.)	Füredi, 1970
Phaseolus beans	– 0.446	Leleji, et al., 1972

Although percentage protein is inversely related to yield, Leleji, et al., (1972) have shown that enough variation occurred in F_2 and F_3 progenies from high and low crude-protein lines to allow selection of genotypes that combined high yields with above-average crude protein content. However, they postulated that many plants produce seed high in percent crude protein because they have a limited seed production or sink capacity. Therefore, it may be necessary to construct plant types with a larger sink capacity before high seed yield and high crude protein can be combined in one genotype.

At present, and until a greater range of variation has been explored or has been generated in hybrid progenies, the plant-breeder's strategy must be to select first for high seed yield and, within his high-yielding lines, to select for above-average crude protein content, thus insuring the highest protein yield per acre.

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THE MAKERERE PROGRAM

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Mankind still struggles to bring the population-food supply equation into balance. The lack of balance of the vital nutritive elements, especially protein, seems to be particularly critical in developing countries and certainly in Uganda. Although the national protein production is respectable, the distribution is poor and the protein quality in terms of essential amino acids -- especially methionine and lysine -- compounds the complex problem. Oland and Stabursvik (1970) have shown that the diet of most Ugandans contains enough total protein but lacks these essential amino acids. At the moment, about 70 per cent of the protein in the human diet world-wide comes from vegetable sources and 30 per cent from animal sources (Roberts, 1970). The high-protein foods utilized in Uganda are mainly of animal origin, but all of them are too expensive for regular dietary consumption and tend to be regarded as luxuries.

As an approach to meeting the protein needs of the poor in Uganda in particular and eastern Africa as a whole, the Makerere grain-legume improvement program was started in 1965. The major objectives in the program were: (1) improvement of protein, by which is meant high-protein content and better amino-acid composition (more methionine); (2) achievement of high yield potential; (3) identification of disease and pest resistance and also control; and (4) establishment of production practices. The crops involved were soybean (*Glycine max* (L.)Merr., cow peas (*Vigna unguiculata* L.), beans (*Phaseolus vulgaris* L. Savi), pigeon peas (*Cajanus cajan* L.), and, more recently, groundnuts (*Arachis hypogea* L.) and peas (*Pisum sativum* L.). A substantial amount of germ plasm of each crop has been assembled and tested at the University Farm.

This paper gives a brief summary of the work so far completed and that in progress on each of the crops except peas, and suggests future research trends on these crops at Makerere University.

The Soybean Program

Improvement work prior to 1970.

Greenway (1945) has reported that soybeans first were introduced to Uganda around the turn of this century. However, the crop failed to establish itself as an important food crop, and it was not until the outbreak of World War II that an

export market for seed stimulated production. That production declined with the end of the war. The early history of soybeans in East Africa has been described by Rubaihayo (1969), and Rubaihayo and Leakey (1970). In 1965, research activity in soybeans was revived once more. Genotype collections were made and evaluated at both Kawanda Research Station and on the University Farm at Kabanyolo.

Information on the periods from sowing to first flowering and to maturity, branching habit, mean seed weight, and per cent protein on 104 introduced varieties and selections from Uganda land-races has been published by Rubaihayo and Leakey (1970). This work revealed that, for the maturity classification system used for cultivars in the United States, based on the response of genotypes to the changing photoperiod from north to south, does not hold when the same cultivars are grown at Kabanyolo, which is on the equator and at an elevation of approximately 1,219 meters above sea level. Leakey and Rubaihayo (1970) discussed this further and put forward a hypothesis concerning soybean adaptation at the equator that implicates temperature rather more than photoperiod. Confirmation for the hypothesis appears to be forthcoming from A.P. Hughes (personal communication), who is currently undertaking controlled environment studies on soybeans at Reading University. His work has shown that night temperature, in particular, influences the length of the juvenile period and the time to maturity. It would seem likely that this response of a genotype to night temperature, as well as to photoperiod, will determine its suitability for any given location (and elevation) in East Africa.

Rubaihayo and Leakey (1970) established that most of the material from the United States and Japan matured in 85 to 100 days, whereas the local selections took rather longer between 100 and 130 days. Lines introduced from low elevation areas in Tanzania required much more than 130 days to complete their growth and were deemed unsuitable for Uganda, as their growth period would exceed the length of each of the biennial rainy seasons in most years.

Rubaihayo (1969) also carried out a number of agronomic investigations and studied such factors as spacing, fertilizer response, planting date, the effect of weed competition, and the response of the crop to weed-control measures, both mechanical and chemical. Although the number and scope of these experiments was limited, valuable data were gathered to allow the formulation of a "management package." Several seed yields in excess of 2,530 kg/ha were recorded, and these performances led to increased optimism for the crop.

Soybean Improvement Work Now In Progress At Makerere

Since September, 1970, Radley (1972) has continued to evaluate the genotype collection, which now is comprised of 172 entries. In each season, the entire collection is planted out and a range of observations is made on them. The aims of the exercise are: (1) to make selections from the assembled pool of varieties for direct entry into the regional-variety testing program organized by the Uganda Government Department of Agriculture; (2) to choose parents for

the hybridization program that was initiated in late 1970; and (3) to study the genotype by season interaction. Some indication of the extent of the diversity within the collection may be gathered from Table I (Radley, 1971), in which extreme values for the characters measured are shown for the first-rains season of 1971. It is proposed to continue this exercise until the end of 1972, when an evaluation of five seasons will have been completed.

In 1971, a whole range of crosses was made by Hawtin, who is undertaking inheritance studies of the crop. The six characters being scored are: number of days to flowering and maturity; plant height and main-stem node number; the number of pods; seed size; seed yield; and the ratio of seed weight to total plant dry-matter at harvest.

The influence of the population-density factor on the inheritance of the above characters is being evaluated in a study involving three of the crosses, viz., Bukalasa 4 x Tokachinagaha, Bukalasa 4 x Clark 63, and Bukalasa 4 x Willett 1. In the program as a whole, the policy is to cross lines, which might be described as possessing local adaptation (insofar as they develop between 17 and 22 nodes on the main stem during the course of growth and mature in 110 to 130 days at a plant height of 50.8 cm to 89 cm), with the introduced American and Japanese

TABLE I
Extreme Values in Various Plant Characters of Soybeans, Obtained in the Genotype Evaluation Exercise, First-Rains Season, 1971

Plant character	Lowest value recorded		Highest value recorded	
Days to flowering	25	(Hill)	55	(Hernon 237)
Days to harvest	88	(Manitoba Brown)	133	(Hernon 237)
Mean plant height (cm)	12.7	(Ani)	109.2	(3H55I-7/19/3)
Mean node number (main stem)	6	(Norin 2)	24	(Hernon)
Mean branch number	0	(Harbinsoy)	8	(Hernon)
Mean pod number/plant	9	(Norin 2)	100	(Yellow Keeble)
Mean number locules/pod	1.47	(Akazaya)	2.65	(Blackhawk)
Mean number seeds/pod	1.33	(Akazaya)	2.51	(Blackhawk)
% seed aborts	1.5	(Shirohachikoku)	32.6	(Avoyelles)
100-seed weight (gm)	8.73	(Norredo)	25.21	(Sag)
Height from ground of lowest-borne pods (ins)	ground level		7 inches	(Hernon 237)
Seed yield (kg/ha)	4.33	(Norin 2)	3178	(3H55I-4/9/3)
% protein	31.8	(Belgian Congo)	43.7	(Norredo)
% oil	15.0	(Norredo)	23.3	(Dalmar 43)

cultivars, which at Kabanyolo mature in 85 to 105 days with only 8 to 12 nodes on the main stem, but which are characterized by a very high Crop Index.

Both to augment the breeding program and to aid crop management generally, a number of agronomy/physiology studies also are being undertaken. Two of these studies concern mature plant size and its relationship to plant density and maturity period and to the length of the rainy seasons in Uganda. These studies are as follows:

1. Genotype x density x spatial arrangement experiments, in which the genotypes are chosen to reflect the range that exists in the genotype collection with respect to mature plant size.

2. Genotype x planting date x density experiments, in which the genotypes selected are those which contrast markedly in maturity period. These experiments were started in September, 1970, and are still being carried out.

A selection of data from experiment 1 is shown in Tables II and III (Hawtin and Radley, unpublished data). These data show clearly the interaction between genotype and plant spacing and also serve to demonstrate the very large differences that exist between genotypes with respect to the efficiency with which assimilate is channeled into the economic part of the plant, namely the seed. Thus, although Bukalasa 4, with its longer growing period, accumulated rather more dry matter than did Clark 63, the much higher Crop Index of the latter was responsible for its exceeding Bukalasa 4 in terms of seed yield.

Other agronomy investigations that have been concluded are a nitrogen x potash x Rhizobium inoculation trial and a weeding study, in which hand and chemical means of control were compared. The fertilizer-response work has been discontinued, as this aspect is now being handled by the Uganda Government Department of Agriculture, but it is intended that the weed control studies be continued at Makerere.

The most recent development in the soybean research program involves the use of experimental mutagenesis by Bulungu as a means of creating genetic variability. The objectives of this program are: (1) to establish the optimal conditions under which seed treatments should be done and to establish the

TABLE II

Main Effects of Density (Meaned for Three Spatial Arrangements) on the Seed-Yield Performance (kg/ha) of Four Contrasting Genotypes, Second-Rains Season, 1971

Plant Density	Bukalasa 4	Willett 1	Clark 63	Tokachinagaha
67,200 per ha	1912	2047.3	2429	1345
134,400 per ha	1886	2242.0	3052	1951
201,400 per ha	1859	2037.0	3124	1999

TABLE III
Selected Growth and Development Parameters for the Four Soybean Genotypes Featured in the Genotype X Density X Spatial Arrangement Experiment, Second-Rains Season, 1971*

Parameters	Bukalasa 4	Willett 1	Clark 63	Tokachinagaha
Plant height at maturity (cm)	93.0	74.4	45.0	22.1
Main-stem node no.	17.9	16.0	13.6	9.4
Total dry matter/plant (gms) maximum recorded during the season	30.7	28.3	24.8	14.5
Fraction of total dry matter channeled into seed	29%	32%	48%	45%
Number of days from sowing to harvest	122	116	102	87
Height from ground level of the lowest-borne pods (cm)	26.4	11.7	2.8	2.5

*Figures represent the mean of nine spacing treatments and three replications.

most appropriate conditions for handling treated seed; and (2) to screen treated material for potentially useful macromutations in M_2 plants. The mutagens being used are the chemical mutagen ethyl methane sulphonate (EMS) and ionizing radiation. The variety being used in the initial studies is Bukalasa 4. Although this is a reasonably good variety, it has the following shortcomings: a tendency for pods to shatter; a long growing period of approximately 125 days; the basal branches tend to sever from the main stem; the mean internode length may be considered too great; it is susceptible to bacterial pustule disease.

Future Research Work at Makerere

In the immediate future, almost all of the areas of research mentioned above will be continued. In the longer term, it is likely that the breeding component of the program will make increasing demands on the resources available. The emphasis in the breeding program will be toward the production of nonshattering, nonlodging, high-yielding plant types with a high combined yield of oil and protein.

Much of the work carried out heretofore has been biological in nature and oriented towards raising yields. For the future of the crop to be assured, how-

ever, work with a technology bias directed toward reducing costs of production is going to be equally necessary. In particular, low-cost aids for seeding the crop, harvesting, threshing, and seed cleaning are much required. It is in recognition of this need that the Department of Agricultural Engineering initiated programs recently in the development of both a seeder and a thresher.

The Pigeon-Pea Program

The local pigeon-pea varieties vary from 2 to 4 meters in height and generally require eight months to produce their first crop of mature seeds. It is generally planted in rows 1.5 meters apart and is frequently mixed with such other crops as sorghum, finger millet, and groundnuts. The average yield in the 1963/64 census was estimated to be 168 kg/ha (Dunbar, 1969). Pigeon pea, however, is potentially a very productive crop, and yields up to 5,000 kg/ha have been reported from India (Anon., 1967). The short-duration cultivars are believed to be potentially higher yielding than long-duration ones (Rachie and Wurster, 1971). Unfortunately, no active breeding work has been done in the past on pigeon pea in East Africa.

A program was therefore initiated in 1968 at Makerere to produce high-yielding, short-duration cultivars of pigeon pea adaptable to Ugandan conditions. The additional benefits of short-duration cultivars consist of: the possibility of taking two crops in a year under bimodal rainfall conditions in the tropics; using pigeon pea in a double-cropping system; and avoiding damage from insects pests, as the short duration would limit build-up of insects.

The improvement program started with assembling a world collection of 5,000 varieties of pigeon peas and screening them at Makerere during 1968 and 1969. A set of 500 varieties was selected to represent most of the genetic variability. This "representative nursery" has been grown in Uganda, Nigeria, and Colombia, and results are being assembled to study their behavior in widely separated locations. It is hoped that, with the above studies, it will be possible to prepare a catalogue of genetic stocks in the near future.

The initial progress of this program has been reported by Khan and Rachie (1972). A number of single-plant selections were made from the elite lines and also from crosses between the selected elite lines. The progeny of these elite lines were bulked and tested in preliminary yield trials. The most promising lines were tested further. The plant spacing in trials was 80 cm between rows and 20 cm between plants. In the last three years, more than 100 lines have been tested in these trials. As a result, a number of promising breeding lines have been identified. Data on eight of the lines that have been tested in several trials are presented in Table IV. In their yield potential, these varieties range from 1,304 to 2,237 kg/ha and take 125 to 140 days to mature. Their heights range between 0.89 and 1.67 meters. The average number of locules and seeds per pod range between 4.175 and 4.425, and 3.975 and 4.575, respectively, and are essentially the same in all the varieties.

The measure of adaptability was made by the regression approach suggested by Finlay and Wilkinson (1963). This consists of using the regression coefficient

TABLE IV
Yield Potential and Other Parameters of Some of the Advanced Breeding Lines of Pigeon Peas at Makerere*

Uganda collection number	Source	Average days to first flowering	Average days to maturity	Average plant height (cm)	Average no. of main branches	Average no. of locules in pods	Average no. of seeds per pod	Yield poten-tial (kg/ha)	b**
1 (CIVEL)	Uganda	84	127	107	5.50	4.425	4.300	1959	0.57
16	Philippines	88	132	135	7.25	4.200	4.100	1304	0.86
948	India	85	125	115	9.25	4.200	4.070	1700	0.86
959	India	84	128	120	7.00	4.200	4.200	1997	0.99
2288	India	87	128	139	4.75	4.175	3.975	1683	0.77
1447		89	129	121	4.75	4.75	4.575	1193	0.79
3035	India	71	130	167	7.25			2237	
5417		75	140	89				1923	1.00

*Except in last two lines, data are based on 1971 second-rains results in varieties UC 1 to UC 1447. In varieties UC 3035 and 5417, such data are based on 1970 first-rains results.

**Regression of variety means over trial means.

b of variety mean over trial mean as a measure of adaptability. Uganda selection UC 1 had the lowest regression coefficient value (0.57) and was therefore considered to be the most stable. The exotic varieties ranged in their regression coefficient values between 0.77 and 1.00.

The degree of out-crossing at Makerere has been found to be 8 to 22 per cent, depending upon the season. This emphasizes the need for pollination control, so elite lines must be grown in isolation for bulking purposes.

Using the data obtained from yield trials, heritability estimates and correlation coefficients have been calculated. The details of the data have been reported by Khan and Rachie (1972). Heritabilities of days to first flower and days to maturity were high (0.7 to 0.9 and 0.74, respectively). On the other hand, plant height and plant width exhibited low heritabilities. The heritability of the grain yield, however, varied widely (0.43 to 0.87), depending on the season and the genotypes included in the trial. Grain yield was positively significant when correlated with plant width, length of main branch, pods per main branch, pods per plant, threshing factor, and grain/straw ratio. The correlation between yield and days to flower was negative and significant ($P < 0.001$). Grain yield also was found to have negative and significant correlation with the incidence of *Cercospora* leaf spot.

Future Program

With our increasing familiarity with the pigeon-pea material, we have realized that considerable basic studies in various fields are necessary if the breeding program is to be carried further. Therefore, a subprogram has been started to carry out basic studies in allied fields.

It is felt that we have reached the limits of the breeding program itself, as far as the applicability of straight selection is concerned. An extensive hybridization program has been started between local and exotic types and also between exotic types x exotic types. To utilize the high outcrossing percentage, two composites have been formed. At present, these composites have been left for random mating for three to four generations. Thereafter, they will be used as base populations for trying some of the breeding methods normally employed in cross-fertilized crops. The allied studies consist of general genetic studies, a search for male sterility, the growth analysis of important architectural types, and studies in flower abscission. A program to study the sources of resistance to *Cercospora* leaf spot and its genetics also has been started.

The Cow-Pea Program

The Uganda Census of Agriculture, 1966, Vol. III, shows (Table V) that cow pea is one of the most important pulse crops of Uganda, ranking third in importance only after beans and groundnuts.

Because of the importance of cow peas, the Faculty of Agriculture initiated,

TABLE V
Hectares* of Important Grain Legumes in Uganda

Crop	Grown Pure	Mixed	Total
Beans	143,000	619,000	762,000
Groundnuts	166,000	214,000	380,000
Cow peas	73,000	118,000	191,000
Pigeon peas	31,000	98,000	129,000

*Excluding Toro and Karamoja Districts. (Source: Uganda Census of Agriculture, 1966, Vol. 3)

in 1965, work on the improvement of that pulse crop. When the program was started, very little empirical data existed on agronomic practices, the varieties grown, and the control of pests and diseases of this crop.

Cow peas are grown more in the drier parts of Uganda. Traditionally, they may be grown in pure stand or mixed in varying proportions with other crops, such as finger millet, sorghum, maize, sim-sim, and cassava.

Experiments to determine optimum plant populations have been carried out. Mehta (1970) worked with populations that ranged from about 26,000 to 430,000 plants per hectare and, using three varieties, obtained average yields of 1,500 to 1,700 kg/ha at the lower plant populations of between 26,000 to 48,000 plants per hectare. With higher populations, yields were lower.

Little response to fertilizer applications has been recorded. Using the variety Emma, Mehta (1970) obtained a yield increment of 21 per cent with single superphosphate (a phosphate fertilizer that contains 16 to 21 per cent available P_2O_5). Calcium ammonium nitrate and potassium sulphate did not cause significant increases in yield. Also, S.O. Keya (1972), unpublished), working with the varieties Iran Grey and Emma, obtained no responses in grain yield to nitrogen or phosphorus application.

Ordinary farmers practice virtually no crop-protection measures on cow peas apart from weeding, although cow peas in Uganda are generally infested with pests and diseases. The most important insect pests of cow peas in the Lake Crescent area are pod borers, *Maruca testulalis* Geyer; the pod-fly larva, *Melanogromyza chalcosoma* Spencer; and storage weevils. Control of the pod borer increased yields from 1,500 to 2,100 kg/ha (Mehta, 1970). Koehler and Mehta (1969) have shown that infestation by *M. chalcosoma* reduces germination, retards radicle development, and increases the proportion of abnormal seedlings, but there have been no experiments aimed at controlling this pest.

Infestation of threshed dry cow peas by weevils during storage is often severe, but data are not available to show the extent of infestation under different storage conditions or the most effective way of controlling weevil damage.

There are several diseases of cow peas in the Lake Crescent area, of which the most important are the stem, pod, and leaf diseases caused by *Synchytrium dolichi*, *Assochyta phaseolorum*, *Dactuliophora tarrii*, and sometimes *Colleto-*

trichum lindemuthianum. Several fungicides have been tried and many of them, especially Dithane M45, control these diseases and so increase the yield from 2,100 to 2,700 kg/ha (Mukiibi, 1969).

Current work on the interaction of weeds, pests, and diseases (Mukiibi, unpublished) indicates that weeds alone depress pod formation and grain yield by up to 100 per cent and is the most important single negative factor. Yields were reduced rather more by insect pests than by disease. There was no correlation between insect infestation and fungal diseases, but the absence of weeds led to severe attack by *Assochyta* and *Dactuliophora* leaf spots.

Mehta (1970) made 43 collections from various parts of Uganda. He also made introductions from other countries. Table VI shows some of these collections with the highest yield potential.

The variety V.U. 12 (Emma) appears to be the most promising, but this may be because it was tested in the same area (Kabanyolo) from which it was collected. By growing 34 cultivars collected from Uganda and other parts of the world, Mehta (1970) was able to select four promising varieties -- Iran Grey, Pale Green, Lalita-2, and Emma -- on the basis of yield and pest and disease tolerance. The first two varieties were collections from South Africa and the latter from Uganda. Mehta (1969) reported work on the hybridization program. This work was carried on up to the F_3 generation and unfortunately was abandoned when he left for Nigeria.

Table VII shows the current work being done by Oland and Stabursvik on important pulse crops as part of a crop-improvement program with respect to protein quality. The quality of the cow-pea protein, based on essential amino-acid composition with 1.3 per cent methionine, is slightly better than that of the other grain legumes.

Although the cow pea is one of the most important pulse crops in Uganda, research on agronomic aspects and the selection and breeding of improved varieties is still inadequate and, in some cases, lacking. Rather more has been done on aspects of crop protection, but the importance of soil-borne and virus diseases has not yet been examined sufficiently.

TABLE VI
Yields Obtained in Variety Trials of Cow Peas

Variety	District of Origin	Estimated Yield (kg/ha)
V.U. 12 (Emma)	West Buganda	2890
Serere Local	Serere	1870
V.U. 11	Lango	1560
V.U. 8	Acholi	1460
V.U. 13	Lango	1170

TABLE VII
Percent Composition of Essential Amino Acids in Some Pulse Crops in Uganda

AAs	Beans	Soybeans	Cow Peas	Pigeon Peas	Ground-nuts
Lysine	6.8	6.3	6.7	6.8	3.0
Threonine	4.7	4.1	4.1	3.8	2.7
Valine	5.4	4.7	5.2	4.8	4.3
Isoleucine	4.5	4.3	4.3	4.3	3.5
Leucine	7.8	7.1	7.4	6.8	6.4
Tryptophan	1.0	1.2	1.0	0.8	1.2
Methionine	1.0	1.2	1.3	1.1	1.1
Cysteine	1.2	1.5	0.7	0.5	1.3
Phenylalanine	5.5	4.9	5.7	9.0	5.6
Tyrosine	3.2	3.6	3.1	2.5	3.8

The Bean Program

Yield is a paramount objective in the bean program. The plant type especially sought is of upright habit; high yield potential; resistance to such diseases as mosaic, anthracnose, and blight, which most varieties lack. The main emphasis of work since 1967 has been on the use of hybridization between large-seeded, Colombian-bred Diacol Nima and its derivatives, produced from crosses in the Kawanda program, and stringless vegetable varieties carrying the ARE gene (Leakey and Simbwa-Bunya, 1972; Bannerot, 1965; Mastenbroek, 1960). The last-mentioned was bred at Versailles by Bannerot. This program has given rise to a range of new, high-yielding lines of both dry-seed and green-pod types.

In addition to the above objectives, canning acceptability — which, in effect, means the "correct" or traditional shape of a Michigan pea bean combined with rapid soakability of the dry grain — is a major criterion in haricot-bean improvement. Although we attach great importance to protein content and quality as measured by the methionine content of the protein, we are not in a position to elevate this to a major place in selection of segregating populations because we lack a suitable screening method. Steps are being taken to rectify this lack, and it is expected that the *Pediococcus cerevisae* rapid-plant assay of Bolinder (1970) will provide the future basis of protein selection at Makerere. This has much to recommend it over the widely used UDY, or dye-binding, analysis for grain-legume work, because the basic amino acids for which this latter method is specific are not relatively deficient in any of the pulses. A total-sulfur rapid assay would be very useful, but we have not yet adopted one.

When planted in East Africa, most well-known and widely grown beans suffer severely from bean rust caused by *Uromyces phaseoli*, and on this account cannot be produced on a commercial scale. A survey of a world collection of

beans in Uganda in recent years (Leakey, Atkins, and Magara, 1972) has shown widespread susceptibility to prevalent races of rust, but about 20 accessions with small seeds and reasonable adaptation have indicated resistance to some of those races. Increasing attention is being given to breeding and selecting for tolerance to *Xanthomonas phaseoli*, to which many otherwise promising new introductions and lines selected from recent crosses are proving susceptible (Leakey, 1972). No strong resistance is available, but a number of lines, such as ICA Guali, from Colombia, and Jules and Tara, bred in Nebraska (Coyne and Schuster, 1969; 1970), are being studied as potential parents.

Agronomic and variety trial experiments have been carried out. Results in Table VIII give an indication of the potential yields that can be obtained. Yields generally vary from season to season between 1,300 kg/ha and 2,400 kg/ha.

The Groundnut Program

Groundnuts (*Arachis hypogea* L.) are grown extensively in Uganda (Table V). As a crop, they have been established in the country for well over a century (Jameson, 1970). In the 1920s and 1930s, the limited range of types grown in the country was supplemented with foreign introductions by the Uganda Government Agriculture Department. These, together with some of the local types, were kept at the Government Research Station at Serere and were subjected to selection work and agronomic studies. To augment this material, a comprehensive collection was made of all the main varieties grown in Uganda, and further foreign introductions were made from 1960 to 1962 by Tiley (1962).

From 1963 to 1966, Tribe (1963) carried out the first and only hybridization work in Uganda. His main aim was to incorporate resistance to the damaging

TABLE VIII
Results of a Spacing and Fertilizer Trial with a Standard Bean Variety (Banja) and a Promising Variety (Diacol Nima)

		Kg of mixed fertilizer/ha			
Plant/acre	Variety	0	628	1255	2510
37,400	Banja	879	1333	1534	1417
	Diacol Nima	1397	1588	1565	1930
72,900	Banja	1296	1836	2094	2016
	Diacol Nima	1653	2202	2473	2551
108,500	Banja	1177	2223	2594	2287
	Diacol Nima	1745	2594	3183	2857
145,900	Banja	1459	2253	2890	2569
	Diacol Nima	1893	2723	3210	3260
271,200	Banja	1457	2392	2834	2790
	Diacol Nima	2118	3091	3268	3399

virus disease, rosette, into potentially high-yielding types. He selected from the collection those varieties that were resistant, such varieties, as a rule, being poor yielders. These varieties were crossed with rosette-susceptible types, which nevertheless were potentially high yielders, provided that they were grown under rosette-free conditions. The resultant generations were grown out and promising selections were back-crossed for yield. Unfortunately, there has not been a breeding program in Uganda since then. It is hoped that one will be initiated in the near future.

Currently, groundnut improvement work is confined mainly to selection and testing of promising varieties in the government collection at Serere, based on district, variety, and trial-yield data.

A physiological study that involves the correlation of the growth characters within the crop with seed yield also is being carried out at Makerere University by J.M. Ashley. It is hoped that such information will provide a useful bank of data for future breeding work at the single-plant selection level. In this study, one variety with each of the three distinct growth habits – Spanish, Valencia, and Virginia – was chosen, and under field conditions each was treated in various ways which altered the phenotype. The changes in growth character were correlated positively or negatively with yield. Findings then were tested with a wider range of genotypes to see if general conclusions could be made within each of the three groups. Protein and amino-acid studies of groundnuts so far completed indicate that no significant change is brought about by any of the treatments given, when compared with control conditions.

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BREEDING FOR NUTRITIONAL IMPROVEMENT

J.P. Meiners and S.C. Litzenberger

Food legumes (grain legumes, pulses) were among the earliest crops to be cultivated by man, and have constituted for thousands of years an important source of protein for him and his domestic animals. Today as many as 20 species of food legumes are regularly consumed to a greater or lesser extent for human nutrition. Of these, some nine species — soybeans, peanuts, beans, peas, broad beans, lentils, chick peas, pigeon peas, and cow peas — can be considered sufficiently important in human diets to warrant substantial efforts on genetic improvement, because they are being grown and used in various places as a staple food commodity. These nine legume species are listed in Table I, together with certain cereal and root/tuber crops, for comparison on world production figures, average yields, protein content, nutritional value, and other information pertinent to their value as providers of protein for human nutrition. As can be readily noted from the information in Table I, the food legumes listed are not equally productive for protein per hectare, nor are they equal in quality.

Research on the genetic improvement of good legumes for nutritionally related factors is just in the beginning stage. However, some results of research in the field have been published and several excellent research programs are in progress. However, most programs have been superficial, with little continued concentration of effort. Moreover, until very recently, initiation of breeding programs has been limited because of the lack of suitable methods for screening germ plasm and breeding material for improved nutrition (Kamra, 1971). To date, nearly all of the breeding work for nutritional factors has involved higher protein and better amino-acid balance.

Breeding for Higher Protein

Probably the first of the food legumes to be investigated for protein content was the soybean, even though it has the highest protein content of any food crop. In fact, the interest of soybean breeders in protein content goes back more than two decades and experimental lines have been routinely analyzed for protein as well as for oil content. Although the protein content has contributed as much as or more than oil to the rapid growth of the soybean industry in the United States, until recently it has been difficult to obtain encouragement for any emphasis on increased protein in soybeans. Consequently, research has pro-

TABLE I

Relative Efficiency of Selected Food Crops in Worldwide Protein Production

Crop	Selected Country or Region	Total World Production	Average Yields	Average Protein Content	Nutritional Value	Protein Content Nutri'l Coefficient	Actual Avg. Yield Protein Nutrition	First Limiting Amino Acids
		(1000 MT)	(kilos/ha)	(%)	(% of egg protein)	(%)	(kilos/ha)	
Grain Legumes								
Groundnut (in shell)								Cystine, Methionine
	India-Pakistan	4,582	640	–	–	–	96.3	
	Africa	4,219	810	–	–	–	122.0	
	Mainland China	2,200	1,160	–	–	–	174.5	
	Latin America	1,246	1,150	–	–	–	173.1	
	North America	1,153	1,980	–	–	–	297.9	
	Far East	1,287	1,030	–	–	–	155.1	
	Near East	292	780	–	–	–	117.4	
	World Total	15,034	850	25.6	69	17.7	128.0	
	%	14.81						
Soybean								Cystine, Methionine
	North America	30,269	1,810	–	–	–	427.2	
	Latin America	903	1,050	–	–	–	247.8	
	Mainland China	10,670	800	–	–	–	188.8	
	Far East [1]	1,161	700	–	–	–	165.2	
	Europe	50	930	–	–	–	219.5	
	Africa	23	770	–	–	–	171.7	
	USSR	528	620	–	–	–	146.3	
	World Total	43,613	1,300	38.0	62	23.6		
	%	43.0						

TABLE I (Cont'd)

Dry Beans *(Phaseolus vulgaris)*							
Latin America	4,003	590	–	–	–	61.4	Cystine, Methionine
Pakistan – India [2]	1,969	259	23.9	32	7.6	19.7	
Mainland China	1,390	700	–	–	–	72.8	
North America	833	1,530	–	–	–	159.1	
Africa	805	600	–	–	–	62.4	
Europe	800	230	–	–	–	23.9	
Near East	195	1,100	–	–	–	114.4	
Far East	650	840	–	–	–	87.4	
Total	10,708	470	22.1	47	10.4	48.9	
%	10.6						
Dry Peas *(Pisum sativum)*							
USSR	4,818	1,540	–	–	–	174.0	Cystine, Methionine
Mainland China	3,100	910	–	–	–	102.8	
India – Pakistan	950	920	–	–	–	104.0	
Europe	529	1,170	–	–	–	132.2	
Africa	352	730	–	–	–	82.5	
North America	197	1,630	–	–	–	184.2	
Latin America	101	690	–	–	–	78.0	
Near East	7	1,750	–	–	–	197.8	
Total	10,115	1,150	22.5	50	11.3	130.0	
%	10.0						
Chick Peas *(Cicer arietinum)*							
India – Pakistan	6,500	689	–	–	–	73.7	Cystine, Methionine
Africa	316	630	–	–	–	67.4	
Europe	215	590	–	–	–	63.1	
Near East	206	910	–	–	–	97.4	
Latin America	150	720	–	–	–	77.0	
Far East	58	580	–	–	–	62.1	
Total	7,445	690	20.1	53	10.7	73.8	
%	7.3						

TABLE I (Cont'd)

Broad Beans *(Vicia faba)*							Cystine, Methionine
Mainland China	3,000	980	–	–	–	95.0	
Europe	767	1,130	–	–	–	108.5	
Africa	341	830	–	–	–	79.7	
Near East	328	1,500	–	–	–	144.0	
Latin America	189	590	–	–	–	56.6	
Far East	11	1,220	–	–	–	117.1	
Total	4,636	990	23.4	41	9.6	95.0	
%	4.6						
Pigeon Peas *(Cajanus cajan)*							Methionine, Cystine
India – Pakistan	1,745	653	–	–	–	53.5	
Africa	38	380	–	–	–	31.2	
Latin America	31	700	–	–	–	57.4	
Far East[1]	15	188	–	–	–	15.4	
Total	1,829	630	20.9	39	8.2	51.7	
%	1.8						
Cow Peas *(Vigna sinensis)*							Methionine, Cystine
Africa	1,004	370	–	–	–	49.2	
Far East	26	620	–	–	–	82.6	
North America	25	630	–	–	–	83.8	
Near East	16	1,230	–	–	–	163.6	
Europe	9	820	–	–	–	109.1	
Oceania	3	530	–	–	–	70.5	
Total	1,083	390	23.4	57	13.3	51.9	
%	1.1						

TABLE I (Cont'd)

Lentils *(Lens culinaris)*							
India – Pakistan	458	510	–	–	–	50.5	Methionine, Cystine
Near East	223	770	–	–	–	76.2	
Africa	128	590	–	–	–	58.4	
Europe	60	670	–	–	–	66.3	
North America	31	1,240	–	–	–	122.8	
Latin America	22	590	–	–	–	58.4	
USSR	74	1,320	–	–	–	130.7	
Total	996	620	24.2	41	9.9	61.4	
%	1.0						
World Total	101,347						
%	100.0						
Cereal Grains							
Wheat	332,500	1,460	12.2	68	8.3	121.2	Lysine
Rice (Paddy)	284,200	2,150	7.5	76	5.7	122.6	Lysine
Maize	251,100	2,370	9.5	55	5.2	123.2	Lysine, Tryptophan
Sorghum and Millet	85,100	770	10.1[3]	41[3]	4.1	31.6	Lysine
Barley	130,700	1,740	11.0	78	8.6	149.6	Lysine
Oats	54,200	1,680	13.0	79	10.3	173.0	Lysine
Sunflower	9,944	1,270	12.6	93	11.7	148.6	Lysine
Root-Tubers							
Potatoes	315,500	13,800	2.0	53	1.1	151.8	Cystine, Methionine
Sweet Potatoes (Yams)	134,400	8,400	1.3[4]	82[4]	1.1[4]	92.4[4]	Cystine, Methionine
Cassava	85,625	8,700	1.6	85	1.4	121.8	Cystine, Methionine
Plantain	12,724	15,000	1.1	71	0.8	120.0	Cystine, Methionine

[1] Includes India and Pakistan
[2] Primarily mung beans
[3] Analyses are for sorghum; for pearl millet respective value would be 9.7 and 6% with a protein content coefficient of 6.5%.
[4] Analyses are for sweet potato; for yam respective values would be 2.4 and 75% with a protein content coefficient of 1.8%.
Sources: 1969 Production Yearbook, Vol. 23, FAO, and 1970 Amino Acid Content of Foods and Biological Data on Protein, No. 24, FAO.

gressed slowly in improving soybean protein content.

In soybeans, the problem of negative correlation of protein content and yield has long plagued cereal breeders. In addition, there is a negative correlation between oil and protein content (Hartwig, 1969). Recently, breeders have succeeded in selecting against these negative associations, and two varieties – Protana and Bonus, with approximately 43 per cent protein – have been released. Compared to this is the world-wide average protein content of soybeans, with approximately 38 per cent. Lines of soybeans with 46 to 48 per cent exist, but both yield and oil content are reduced (Hartwig, 1969). Hartwig feels that cultivars with 10 to 15 per cent higher protein are a distinct possibility (ibid).

Breeding for higher protein in other food legumes has not advanced much beyond the stage of screening to determine how much variability exists. Considerable variability has been reported, but extremes may be due to adverse environmental conditions and thus may be meaningless in terms of genetic improvement. The screening by several workers of collections of germ plasm of beans has indicated that significant variability in protein content may be the result of either environment or genetic factors.

Rutger (1971) screened 343 bean lines and found that protein varied from 19 to 31 per cent, with a mean of 24.6 per cent. Protein content was positively correlated with late maturity and negatively correlated with seed weight. Yield and protein content were not significantly correlated, indicating the possibility of breeding high-yielding, high-protein bean cultivars. Dr. Rutger found significant variation in protein content as a result of location and year, indicating the necessity of growing the seed of the lines in a single location and year to decrease variability in environmental effects.

Silbernagel (1971) screened large numbers of lines introduced from many countries, and identified selections from these lines ranging from 16 to 33 per cent protein. He also found that protein percentage is influenced by environ- . .ental factors, with considerable variation between locations in one crop and between crop years. Moreover, seed quality was found to be negatively correlated with protein percentage. However, some lines are consistently high in protein, and crosses have been made between high- and low-protein lines to study the inheritance of protein content. Dr. Silbernagel has several lines in the F_3 generation, but has lost his funding for this bean improvement work and has not been able to pursue it further.

In the case of mung beans, the research just started at the Missouri Agricultural Experiment Station under an AID contract is of importance (Yohe, et al., 1971; 1972). Protein analysis of 313 strains in 1970 and 126 strains in 1971, most of which were USDA plant introductions from many countries, indicated a range in protein of from 19.1 to 28.3 per cent, with a mean of 24 per cent in 1970, and a range of 22.1 to 31.2 per cent with a mean of 26.5 per cent in 1971. Although a statistical analysis was not performed, none of the 10 highest protein strains was among the 32 highest-yielding strains in 1971, although the latter ranged from 19.1 to 27.4 per cent protein. As in the case of beans, yield, seed weight, and protein content appeared to be negatively correlated.

Recently, several workers in Hungary have published results of research on

breeding peas *(Pisum sativum)* for increased protein content. Kurnik, et al. (1970) concluded that pea varieties commonly cultivated are heterozygous for protein content, which can be raised 2 to 3 per cent merely by selection. Furedi (1970) crossed varieties of peas differing in protein content and morphobiological characters. He obtained significantly lower percentage of protein in the F_1 with a return in the F_2 and F_3 to protein levels comparable to those of the parents. In general, protein content of the progeny was intermediate between the two parents and was inherited quantitatively. Using irradiation and chemical mutagens, Dudits (1970) obtained a mutant with 27.1 per cent protein, which was 4.6 per cent higher than the original variety, but yielded 16 per cent less. One mutant, however, was higher in both protein content and yield.

Working with a number of food legumes in India, Krober, et al. (1970), of the AID/USDA-sponsored Regional Pulse Improvement Project, reported significant varietal differences in five of seven crops -- chick pea, pigeon pea, mung bean, pea, and cow pea. Urd bean and lentil varieties were not significantly different in protein content. The investigators reported significant differences caused by location in all of the crops.

This sample of work on total protein content indicates that the level can be improved by breeding and selection of most crops. However, it is necessary to control variation carefully because of environmental conditions and to screen germ-plasm collections systematically in order to locate high-protein lines.

Breeding for Amino-Acid Balance

In breeding food legumes for improved protein quality or better amino-acid balance, research has progressed only to the point of examining the range of variability and determining if the level of amino-acid content is genetically controlled.

Again, the early work in this area involved soybeans. In the 1950s, Krober (1956) found substantial variation in methionine content in 14 soybean varieties tested and concluded that it was possible to breed soybean varieties high in methionine. However, as far as is known, breeders have not followed up on this lead. One reason may be the limited range of methionine content in lines examined thus far (Bernard and Cremeens, 1969). Another reason may be lack of a suitable method for screening large populations of breeding material for methionine content. In fact, the lack of such methods undoubtedly has discouraged breeding for higher methionine and cystine content in all of the food legumes, as well as in other crops (Kamra, 1961).

By modifying a microbiological assay for methionine, Kelly and coworkers (1971) at the Campbell Institute for Agricultural Research overcame the lack of a suitable method for screening breeding material, and have made considerable progress in initiating a breeding program in beans for methionine content. Using the microbiological assay method, Kelly (1971) has screened 3,600 bean cultivars, lines, and single-plant selections and has found that the level of methionine is determined genetically and that sufficient variation exists to permit improve-

ment through hybridization and selection. Kelly feels that, through breeding and selection, strains of beans with a content of critical amino acids twice that of varieties now being grown can be attained. This would bring the amino acids in bean protein close to that of egg protein.

Of critical concern is to know the genetic relationship between methionine content in the bean lines and their yielding ability, as, in the final analysis, yield remains most critical in the development of improved strains of any one crop.

Other research projects on improving the amino-acid content of beans are under way – notably those of Dr. Wood and coworkers at Colorado State University (1969) and Dr. Ronald Echandi of the University of Costa Rica, who is utilizing a modification of Dr. Kelly's microbiological method as an assay procedure.

More must be known about the variation in methionine content of other food legumes and the nature of its heritability. The work with mung beans at the Missouri Agricultural Experiment Station, as mentioned above (Yohe, et al., 1971), indicates that, in 313 strains studied, methionine content ranged from 0.55 per cent to 1.78 per cent of protein, with a mean of 1.20 per cent. Unfortunately, the strains high in methionine have tended to be low in protein.

Recently, a project in India at Punjab Agricultural University, financed through PL 480 funds of the USDA, has been activated to determine genetic variation of protein and amino-acid content in several of the good legumes. Another project has been proposed at another Indian university, and represents a small additional effort on a large problem.

Breeding for Other Nutritionally Related Characters

Although there is considerable interest in the breeding of food legumes for lower levels of flatus-producing factors, toxins, trypsin inhibitors, and other antimetabolites, progress in this area also has been hampered by the lack of techniques. An exception to this is the work at the Indian Agricultural Research Institute (IARI) on breeding for lower neurotoxin in khesari *(Lathyrus sativus)* (Swaminathan, 1969). Mutants obtained with irradiation and chemicals (EMS) appear to breed true for low neurotoxin and to be controlled by several genes. This work is continuing to be supported by the USDA under a PL 480 grant to IARI. Germ-plasm collections of khesari are being screened to determine the extent of natural variation of the toxin.

Other toxins of importance in food legumes include that causing favism in broad beans. However, except for that of the lathyrism-producing toxin, no published results have been encountered on breeding to lower either toxins or flatulence in food legumes. We understand that breeding programs on flatulence are being initiated in beans at Michigan State University and in soybeans at the University of Illinois.

The ultimate proof of improved nutritional quality of a commodity is improved growth or development of the human or other animal that consumes it. Although chemical or microbiological tests are used to indicate quantity or

quality, laboratory animals also must be used finally as indicators of nutritional improvement resulting from a plant breeding program. We have failed to find in the literature a record of any breeding program, or of any screening of germ plasm, to enhance the nutritional value of food legumes that has utilized laboratory animals or humans to measure the biological value of both parent material and its progeny. Once breeding programs have reached the stage at which there is some stabilization in populations superior in nutrient value, as measured by protein content, amino-acid balance, or other characteristics, they must be assayed with test animals or humans, as currently is being done in improving protein content and quality in cereals.

Sources of Variability in Food Legumes

Throughout this paper, we have emphasized the necessity of locating genetic variability for a given attribute as the first step in the breeding program. Let us examine, therefore, the current status of germ-plasm collections of the food legumes.

There are numerous collections of seeds of food legumes. These may be large or small and broad or narrow in geographical base. Large collections of species grown commercially in the United States exist at the Regional Plant Introduction centers of the U.S. Department of Agriculture or elsewhere. These include 8,000 accessions of beans, 4,000 of peas, 4,500 of peanuts, 4,600 of soybeans, and 600 of cow peas.

Of the food legumes not grown to any appreciable extent in the U.S., the most comprehensive collections are those assembled and distributed by the USDA/AID Regional Pulse Improvement Project (USDA, 1966-1970). During its brief existence in India and Iran, some 20,000 collections of pulses were assembled. Some 15,000 of these now are part of the Plant Introduction germ-plasm collections of the Agricultural Research Service, U.S. Department of Agriculture. These collections include the following crops and number of accessions: *Cajanus cajan* (pigeon pea) 4,000; *Pisum sativum* (English pea) 400; *Cicer arietinum* (chick pea) 4,000; *Phaseolus vulgaris* (common bean) 1,500; *Vigna sinensis* (cow pea) 1,200; *Lens culinaris* (lentil) 2,100; *Phaseolus aureus* (mung bean); and *P. mungo* (urd bean) 2,000.

Unfortunately, at the present time the USDA does not have adequate funds to provide for a continuing program of seed collection, increase, cataloguing, classification, etc., for these large collections. Some most welcome help of a temporary nature has been supplied by The Rockefeller Foundation, AID, and the FAO, but a more adequate, long-range, funding program to maintain these as working collections should be provided.

Considering the germ plasm of food legumes available in the world, the present collections are undoubtedly incomplete (Creech and Reitz, 1971). With the advent of improved varieties of food legumes, many of the natural cultivars may disappear, with the resultant loss of desirable characteristics. Every effort should be made in the near future to enlarge these collections, particularly from geo-

graphical centers of origin. These collections also should include wild relatives of the crop species.

A second source of genetic variability is that of induced mutations. However, it should be resorted to only when natural variability does not exist. More than 99 per cent of mutations are economically undesirable, so the need for working with extremely large populations is a serious limitation to mutation breeding (PSAC, 1967).

It is important to combine nutrient quality with other desirable, genetically controlled characteristics, so the use of male sterility for composite crosses and, where male sterility is not available, the use of gametocides should be considered.

Discussion

Programs to increase supplies of food legumes cannot depend on more land being devoted to their cultivation, but rather on achieving higher yields per area cultivated. Without increased yields and/or comparative favorable prices, legumes cannot compete with other less nutritious, but more profitable, crops. Thus, the first priority in a breeding program is increased yield. Along with, and a part of, increased yield is improved adaptation, resistance to diseases and insects, and other environmental hazards, plus responsiveness to fertilizer and other management input factors. Ample evidence from developed countries and limited evidence from developing countries indicates that breeding for improved grain yield and resistance to diseases is likely to result in substantial increase in the supply of food legumes.

Coincidental with the effort to increase yield by breeding, but secondary to it, must be the effort to improve the quality of food legumes through breeding for higher protein, better balance of amino acids, and lower toxins, flatus factors, and antimetabolites. Limited experience in developed and developing countries on a few of the crops indicates that some improvement is possible.

Because the breeding effort is one of exploiting genetic variability not now generally identified, it is highly important that the collections of germ plasm are as complete as possible. Although present collections are only now beginning to be evaluated, high priority should be given to plant exploration to secure the full range of genetic variability. In addition, efforts should continue to exploit variability obtained through induced genetic mutations and to make use of the latest techniques to combine desirable characters.

An ecologically appropriate environment must be an integral part of the improvement program for each species. Malnutrition is primarily a problem of the tropics, so most improvement programs will have to be located in those environments. From the U.S. point of view, possibly Puerto Rico is the best location for screening species adapted to the more humid tropics.

Finally, as with all crops, it is essential that breeding programs keep the genetic base as broad as possible. To do otherwise is to invite catastrophe.

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ON THE QUEST FOR QUALITY IN THE FIELD BEAN

M. W. Adams

From the standpoint of human needs, the most urgent problems in dry beans resolve to the necessity of markedly improving their yielding ability across broad geographic regions and improving the biological value of their seed proteins.

Beans have to be made to yield well, else other crops will be grown in their place. We have to develop types possessing zonal adaptation, because to put the quality factors we desire into the hundreds of locally adapted varieties of Central and South America would be a Herculean task and a sheer waste of technical resources.

With respect to quality, we now acknowledge such desiderata as the reduction of toxic substances, elimination of flatus, ease of cooking, flavor, retention of wholeness and firmness when processed, and amino-acid balance in the storage proteins of the seeds.

The comments that follow will be directed to this last aspect of quality, with particular regard to breeding for both quality and yield.

The breeding strategy which I think should be adopted to achieve both yield and quality can be stated in three short sentences:

1. Relax selection for high protein per se.
2. Aim for 20-22 per cent protein.
3. In this range of protein, concentrate on selection for yield and for the sulfur-amino acids.

In the remainder of this discussion, I will offer a rationale in support of this strategy.

Of first importance is the report by Leleji, et al. (1972) of negative correlations in F_2- and F_3-generation plants between yield and percentage of crude protein ($r = -.45$). Rutger (1970) had previously found negative, but nonsignificant, correlations between yield and protein in beans. The highest total protein per plant was produced by plants that were average or below in percentage of crude protein. (Seed yield and protein yield were correlated with a r value of +.96). Leleji, et al., speculate that an inadequate sink size in the lower-yielding plants becomes overloaded with protein as compared to carbohydrate, giving a high protein percentage and generating the negative correlation.

The negative relationship of yield and protein becomes even more significant when viewed against a second negative association, that of percentage of crude

protein with grams of sulfur-containing amino caids per 100 grams of protein (Figure 1). The higher the protein, the lower the cystine and methionine. The highest levels of cystine and methionine occur in beans at about 20 to 22 per cent of protein.

The best quality (most cystine) lines out of some 200 plant introduction lines bioassayed at Michigan State University by R. J. Evans and Doris Bauer had 1 gram of cystine per 100 grams protein at a protein level ranging from 19.6 to 20.6 per cent. In contrast, the highest percent protein line at 30 per cent had a cystine level of .6 grams. One hundred grams of the best quality bean would be equal to 110 grams of the highest protein bean. This relationship between cystine-methionine and percent protein also was found in 49 strains indigenous to Central America, seed of which was provided by IICA at Turrialba, Costa Rica, and in eight standard types from the United States. In the latter set, the correlation between cystine and methionine was positive and significant at r = +.83 (Figure 2).

What is suggested by the negative relationship of protein to cystine and methionine is that the additional protein stored, above about 21 per cent, is of a progressively lower quality with respect to sulfur-amino acids. The plant breeder

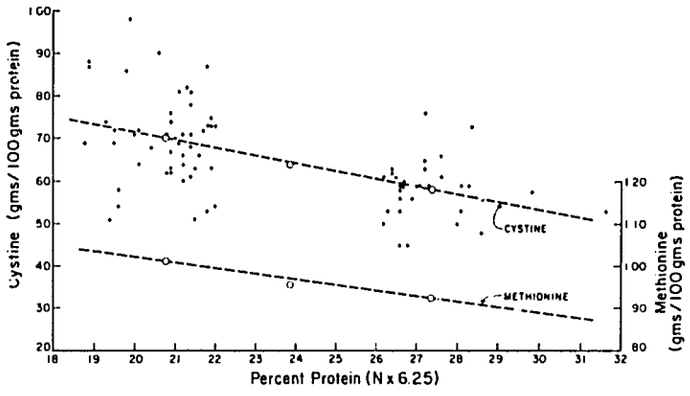

Figure 1. Relationship trends of cystine and methionine with crude protein in 198 plant introduction lines of *Phaseolus vulgaris*. Data points are shown only for cystine values below the 22% protein level and above the 26% protein level. The dotted lines in each case connect the means of the two swarms indicated by open circles. Not shown are the cystine values of lines which fell in the 22%-26% range. For methionine, no individual values are shown, only mean values of the lower, middle, and upper swarms of points.

^{r}CYSTINE-NITROGEN	=	—	.73*
^{r}METHIONINE-NITROGEN	=	—	.84**
^{r}(CYSTINE + METHIONINE) - NITROGEN	=	—	.84**
^{r}CYSTINE—METHIONINE	=	+	.83**

Figure 2. Simple correlations of sulfur-amino acids with nitrogen from analyses of eight commercial bean types of the United States.

CYSTINE SELECTIONS	PERCENT PROTEIN
LOWEST 12%	24.78
HIGHEST 12%	22.09
METHIONINE SELECTIONS	
LOWEST 12%	25.13
HIGHEST 12%	21.56

Figure 3. Percent protein (N x 6.25) in lines selected for cystine and methionine by truncation from lowest 12% and highest 12% of a population of 198 PI entires.

might advisedly select in populations of 20 to 22 per cent protein for high sulfur-amino acids, as well as high grain yield, which should be easier of attainment, in view of the relationship of yield to low protein (Figure 3).

The distributions of 198 lines of dry beans for total cystine and methionine (as measured by bacterial bioassay) is presented in Figure 4. Of these, 49 averaged .70 gms cystine/100 gms protein at an average percentage protein of 20.8. At this level, and with no further addition of a cystine-containing protein as the protein percentage rises to 27 per cent, the expected concentration of cystine would be .54 gms/100 gms protein. Actually, in the 33 lines averaging just over 27 per cent protein, the cystine concentration was found to be .59 gms/100 gms protein. It is inferred that the additional protein stored above the 21 per cent level is a very low cystine-containing protein. To a large extent, this seems to be true also for methionine. If so, the additional protein is depressing the biological value, and because it costs the plant more energy to produce the "empty protein" than carbohydrate, it is also depressant to yield.

So, on this preliminary evidence, the optimal breeder strategy for achieving both quality and yield in beans is to work with populations at about 20 to 22 per cent protein, putting together genes for greater sink capacity with the factors for quality.

The Gene Basis For Cystine-Methionine in Beans

What is known on this subject, of value to the breeder, is scanty indeed. Leleji (1971), on the basis of data from one cross, very tentatively suggested partial

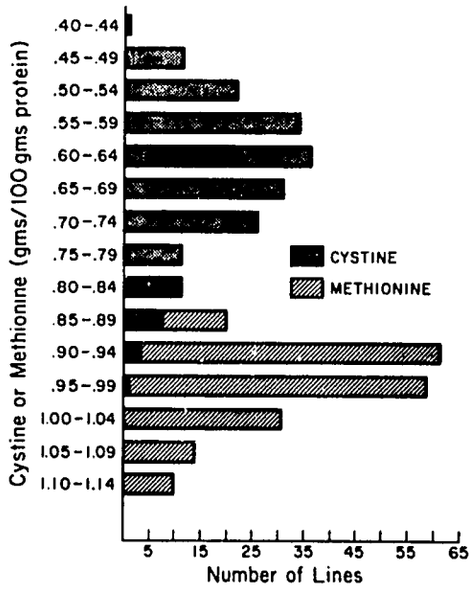

Figure 4. Distribution for cystine and methionine in 198 plant introduction lines of *Phaseolus vulgaris*. (Data of Evans and Bauer, Mich. State Univ., 1971)

dominance of genes for low available methionine over genes for high. He also demonstrated joint determination of methionine levels by the maternal genotype and by the genotype of the seed in which the amino acid was measured.

At this preliminary stage of genetic analysis, I think breeders might be more interested in the evidence, if any, relating to whether variation in levels of methionine-cystine is due to genes controlling the synthesis of these amino-acids per se, or depends more upon regulator genes that control the ratios of various storage proteins in the seed, some containing greater amounts of the sulfur-amino acids than others.

Conclusive evidence on this point is not available for beans at this writing, although Wood, et al. (1969) have indicated they expect to obtain such evidence soon. Boulter (1972) has already shown cystine-methionine differences in two major storage proteins of *Vicia faba*, which presumably implies differential genetic regulation as a means of increasing or decreasing the cystine-methionine level. Boulter has also produced evidence that the limitation on methionine in *V. faba* seed protein is not caused by a failure of synthesis of methionine, and hence its unavailability for incorporation into protein, by showing that exogenous supplementary methionine supplied to an in vitro protein-synthesizing system caused no change in the proportion of methionine in the proteins formed in the system.

This strongly inclines one to the view that, in order to effect a change of methionine level in the whole seed, the breeder must shift the ratio of high methionine-containing proteins to low methionine-containing proteins.

Csonka and Jones noted in 1933 that phaseolin, the chief protein of soybeans and other legume seeds, was deficient in cystine. They also asserted that differences in nutritional value among varieties was explainable on the basis of the various seed proteins and their proportions.

Earlier, Waterman, et al. (1923) had identified two globulin proteins – alpha and beta – in the navy bean. Nutritionally, the alpha-globulin was most important, as it was richer in lysine and the sulfur-amino acids. In 1938, Jones, et al., stated that alpha-globulin was seven to eight times richer in cystine than beta-globulin. Evans and Bandemer (1966) found that the alpha-globulin of navy beans was richer than the beta in cystine, methionine, and tryptophan. Wood, et al., in 1969 proposed that varieties high in methionine could be developed by combining into a single genotype the regulator genes that control the presence of specific proteins already high in methionine.

Kelly (1971), performing methionine bioassay on an extensive array of foreign plant introductions and domestic bean varieties, produced compelling evidence for genetic differences between lines. Using the navy bean variety Sanilac as a standard and giving it a relative value of 100 for methionine level (1 mg available methionine per gram protein), Kelly found some lines showing values in excess of 200 per cent. There is no direct evidence in Kelly's data on the genetic control of methionine level in beans. However, he identified seven classes or levels ranging from a low of 30 per cent of standard Sanilac to 200 per cent and above, each class differing by some 25 to 40 percentage points from the next lower one. Since he found an L.S.D. at the 1 per cent level of significance of 30 percentage points, each class probably indicates a true genetic step-differential.

If this is so, then varying levels of methionine must depend upon something more complex than a single regulator gene switching synthesis from a methionine-rich protein to a methionine-poor protein.

If one postulates three proteins in beans differing in methionine content, each protein at one of two levels, eight combinations are possible. For illustrative purposes, we have arranged the following comparison, relating the three-protein scheme to the seven methionine classes of Kelly:

Protein ——→	"A"	"B"	"C"	Level	Level	Kelly's
Methionine level →	3X	2X	1X	of	of	methionine
N-level ——→	1X	1X	1X	N	methionine	classes
Combination	1	1	1	3X	6X	30 and lower
or	1	1	2	4X	7X	30-54
level of each	1	2	1	4X	8X	55-79
factor	2	1	1	4X	9X }	80-119
	1	2	2	5X	9X }	
	2	1	2	5X	10X	120-149
	2	2	1	5X	11X	150-200
	2	2	2	6X	12X	200 and higher

In this scheme, combinations 4 and 5 give the same intermediate level of methionine, which reduces the number of classes from 8 to 7, in fortuitous harmony with the seven classes identified by Kelly. This array also gives a positive correlation between available methionine and percent nitrogen, which is consistent with the association found by Kelly ($r = 0.73$).

Can a regulator-gene model be postulated such that a negative association would appear for cystine-methionine versus percent protein? An affirmative answer can be given, provided two conditions prevail:

if, of two proteins, one has substantially more sulfur-amino acids than the other; and

if, for each unit increase in the first protein, there is a greater-than-unit decrease in the second protein. These conditions are not unreasonable of fulfillment.

Thus, the evidence and arguments adduced up to this time clearly favor the view that graded levels of methionine-cystine do occur and can be explained by regulator-gene differences affecting the balance of various storage proteins in the seeds.

Conclusions

The correlations between yield and protein, although tentative, favor the view that higher-yielding genotypes will be associated with lower levels of protein. Additionally, there are data to indicate that the cystine-methionine content of

bean protein is improved at the lower levels of protein. An obvious course of action for the bean breeder, therefore, is to (1) relax selection for high levels of protein; (2) build populations centering on 20 to 22 per cent protein; and (3) carry out yield and quality selections at this intermediate level.

The expectation is that higher levels of yield and quality could be achieved with less difficulty than by any alternative strategy.

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COW PEAS IN NIGERIA

F. A. Bliss

Cow Pea as a Source of Protein

The most extensive production of cow pea, *Vigna sinensis* L., is in West Africa. In Nigeria, it is the principle source of dietary protein (Oyenuga, 1967). The dried seeds are prepared in a variety of ways and eaten either singularly or in combination with other foods. Most highly-regarded cultivars grown in Nigeria have large seeds with a rough testa that is either white or brown. These traits are often associated with a short soaking time for testa removal and desirable cooking traits and they are necessary for rapid, wide acceptance of new cultivars.

Yields are generally low because there are few improved cultivars and the growing plants are often severely attacked by numerous diseases and insects. There is, however, considerable potential for greater production. The currently available cultivars have been shown to respond to added fertilizers, particularly phosphorus, and timely applications of recommended pesticides often increase yields considerably. Early maturing genotypes that show little or no response to day-length and have a determinate, upright growth habit on which the pods are borne high off the ground are available in germ-plasm collections. Combining these traits with locally acceptable, high-quality seed types will contribute greatly by allowing plants to be grown more densely and providing broader ranges of adaptability, ease of cultivation, management, and harvest. Some progress has been made in identifying and incorporating biological resistance to certain viruses (Bliss and Robertson, 1971) and considerable variability exists for resistance or tolerance to some other important diseases (Onesirosan and Barker, 1971).

Protein and Amino-Acid Composition

The mature seeds of cow pea have been reported to contain approximately 24 per cent protein (Amirshahi and Tavakoli, 1970) and 1.0 g methionine/100 g protein (Evans and Bandemer, 1967). Methionine is usually considered to be the primary limiting amino acid, but isoleucine and tryptophan have also been found to be low (Evans and Bandemer, 1967). The synthesis of storage protein appears to progress rapidly in the cotyledons as the seeds develop. A globulin fraction is predominant and an albumin fraction comprises a smaller portion in the mature seeds. Storage-protein components have been separated on acrylamide gels where

qualitative differences in banding patterns have been observed (T. C. Hall and F. A. Bliss, unpublished data).

Field-grown seeds of the cultivars and breeding lines that were used at the outset of the breeding program at the University of Ife, Ile-Ife, Nigeria, were analyzed for seed protein using the Kjeldahl procedure. A Beckman automatic amino-acid analyzer was used. Fifty-gram samples of each lot were ground in a laboratory Wiley mill and dried under vacuum at 50°C for three cays. One hundred mg aliquots were hydrolyzed with 50 ml of 6N hydrochloric acid. The hydrolysis flasks were flushed with nitrogen and evacuated before hydrolysis at 110°C for 22 hours. After hydrolysis, the hydrochloric acid was removed by vacuum distillation, the residue dissolved in pH 2, O.2N citrate buffer, filtered and made to 25 cc volume. One ml of the resulting solution was placed on the analyzer columns and analyzed for basic, neutral, and acidic amino acids. The plant materials were chosen without prior knowledge of protein quantity and quality and represent a wide range of seed types, maturity, day-length response, plant types, and adaptability to several different environments. Most were used because of such desirable traits as disease resistance, plant type, seed quality, and local adaptability. They appear to give a good estimate of the ranges of values one can expect from a nearly random sample of cultivars (Table I). Analyses of other accessions have shown several to be both higher and lower in their protein and methionine content.

Variability Among Existing Strains

The phenotypic expression of seed protein and amino-acid content of beans have been found to vary depending on the genotype and several environmental factors (Tandon, et al., 1957; Rutger, 1970; Leleji, 1971). Few estimates of heritability of protein quantity and quality traits in pulses have been reported, particularly for cow pea. We were interested in identifying promising parental lines in the material available and in determining the extent to which genetic and nongenetic factors may influence protein synthesis and storage. We also wished to determine whether the analyses made on seeds produced in the biotron and greenhouse in Madison, Wisconsin, would characterize adequately the same genotypes when grown in the field in Nigeria. Data were also collected on seed yield and yield components of the cultivars studied.

The grain yield/plant, per cent protein (Kjeldahl N) and available methionine, estimated using the procedure of Kelly, et al. (1970), of eleven cultivars grown at three locations during long (13-hr) and short (11-½ hr) days are presented in Table II. The significance of location, day-length, genotype, and the various interactions as measured by appropriate F tests are presented in Table III. Day-length had little effect, except that two cultivars were day-length sensitive and flowered only during short days. Genotype was important in determining differences in per cent protein and methionine content, but genotype x location interactions were also significant, indicating that caution must be used in selecting for these traits under nonfield conditions. The primary use of the biotron and greenhouse should be in the early stages of a program when genotypes

TABLE I

Percent Protein and Amino-Acid Content of Dried Seeds of Cow-Pea Cultivars Grown at Ile-Ife, Nigeria
(aa content in g aa/g protein)

Cultivar	% Protein	Lys	His	Arg	Met	Gly	Ala	Val	Leu	Ile	Ser	Thr	Phe	Pro	Tyr	Asp	Glu
								Duplicate Determinations									
Westbred	28.2	5.91	2.97	6.27	2.07	5.42	5.99	6.73	9.56	5.32	7.05	5.10	7.47	5.07	4.60	12.76	24.60
Westbred	27.6	5.86	2.93	7.61	1.11	5.37	6.12	6.43	9.17	5.16	6.00	3.57	7.88	6.39	4.21	10.68	22.36
Oyo Brown	26.5	6.00	2.92	6.38	2.31	5.66	7.09	7.47	10.83	6.04	7.77	4.99	9.12	9.25	4.67	16.67	25.97
Oyo Brown	27.3	5.01	2.19	4.89	1.85	4.68	5.91	5.92	8.95	4.58	6.49	4.72	6.83	7.17	4.38	14.61	22.52
Oyo White	27.7	5.24	2.34	5.48	2.20	5.45	7.00	6.84	10.15	5.30	6.82	4.24	8.68	5.81	4.68	14.69	22.30
Oyo White	28.1	5.26	2.42	5.28	1.91	5.52	6.31	7.13	9.68	5.02	7.19	5.15	7.73	6.29	4.40	15.99	26.61
Blue eye	25.7	5.30	2.66	5.81	.79	5.39	6.02	6.39	9.79	5.20	7.32	4.82	8.39	6.99	3.36	16.85	24.57
Blue eye	25.5	5.46	2.50	5.43	1.78	5.57	6.33	9.91	9.91	5.28	6.91	4.95	8.00	5.27	4.21	16.72	25.33
								Single Determinations									
Lagreen	28.3	5.37	2.55	6.16	2.10	5.90	7.06	8.10	10.36	5.88	5.62	3.26	8.17	7.09	2.78	15.78	23.96
Topset	30.2	5.57	2.76	6.73	1.85	5.26	5.87	7.26	9.59	5.43	5.04	4.87	6.40	4.91	3.98	16.32	25.98
Bechuana	27.4	5.00	2.40	4.57	1.62	6.27	6.50	7.99	10.07	6.25	7.60	6.41	8.82	5.84	5.17	17.33	28.00
II-V-5-6	27.1	4.44	2.05	4.10	1.61	5.57	5.36	6.18	8.85	4.79	6.94	5.18	5.75	5.03	3.34	15.71	26.37
Dixielee Sel.	22.9	5.52	2.75	5.90	1.65	5.27	6.53	7.04	9.95	5.73	5.96	3.78	8.53	5.55	4.44	13.38	20.94
New Era	26.6	5.78	2.87	5.76	2.07	6.06	6.36	7.71	10.12	5.62	7.12	5.28	7.63	6.34	4.79	15.76	26.51
Akure Loc. Brown	27.1	5.07	2.55	5.17	1.66	5.41	6.19	7.31	10.04	5.64	4.10	4.79	7.90	5.10	4.23	14.36	25.74
Climax	29.1	4.08	2.41	4.73	2.10	5.27	5.77	6.91	9.30	5.25	5.69	4.31	7.31	4.45	4.20	13.38	21.43
Producer	29.1	5.12	2.62	5.99	1.45	5.27	6.03	6.43	9.51	5.15	6.78	4.52	8.01	7.45	4.12	15.04	23.77
Miss. Silver	23.9	5.82	2.76	5.82	2.03	6.33	6.43	7.41	10.39	5.80	7.41	5.78	8.11	6.69	4.45	16.26	27.08
Blackeye 1239	28.4	5.85	2.84	5.84	1.89	5.18	5.55	6.70	8.98	5.27	6.40	4.57	7.02	4.35	3.97	14.03	23.48
Kafinsoli Brown	28.2	5.28	2.71	6.05	1.88	5.41	6.38	7.09	9.94	5.62	7.39	5.15	8.36	6.67	4.13	15.89	25.36
Bunch Purple Hull	27.9	4.78	2.32	4.98	2.03	5.56	6.07	7.20	9.77	5.45	6.78	5.15	7.17	5.50	4.19	15.77	26.02
Knuckle Purple Hill	28.7	5.00	2.71	4.99	.78	4.69	5.55	7.16	10.06	5.70	6.09	4.25	9.22	5.00	4.45	15.71	22.33
Mean	27.4	5.32	2.58	5.68	1.76	5.46	6.18	6.97	9.78	5.41	6.57	4.76	7.85	6.00	4.21	15.03	24.53

TABLE II

Yield (g Seed Plant), % Protein and Amino Acid Content of Dry Seed of Cow-Pea Cultivars Grown at Different Day Lengths in Different Locations

	Ife				Biotron				Greenhouse			
			Methoinine				Methionine				Methionine	
	Yield	% protein	mg/g[1]	%[2]	Yield	% protein	mg/g	%	Yield	% protein	mg/g	%
					Short day							
Topset	31.2	28.6	2.47	.86	10.3	26.8	2.28	.85	9.6	22.8	2.02	.89
Lagreen	29.2	26.2	2.26	.86	17.2	23.4	2.10	.90	9.6	22.6	1.72	.77
Climax	17.7	26.2	2.33	.89	20.6	25.0	2.16	.86	9.8	22.6	1.97	.88
II-V-5-6	39.4	26.4	2.07	.78	15.0	23.4	2.05	.88	4.5	15.0	.79	.52
Miss. Silver	26.2	24.4	2.05	.84	15.0	22.3	1.40	.63	8.4	19.4	1.03	.54
Bechuana	29.0	26.3	2.31	.88	10.9	24.4	2.14	.87	8.9	21.0	1.49	.71
Dixielee Sel.	29.9	26.0	2.25	.86	15.6	24.0	2.13	.89	12.1	21.8	1.64	.75
Westbred	28.4	25.6	2.08	.83	9.4	24.3	2.13	.87	5.0	22.2	2.00	.90
New Era	28.4	27.1	1.98	.73	13.3	24.7	2.05	.83	7.2	17.1	.96	.55
Producer	40.3	27.3	2.18	.80	24.3	24.5	2.14	.88	11.8	23.8	1.31	.56
Local Brown	27.0	25.9	2.44	.94	7.1	23.3	2.17	.94	3.9	21.4	1.48	.70
$\bar{x}$	29.7	26.3	2.22	.84	14.4	24.2	2.07	.85	8.3	20.8	1.49	.70
					Long Day							
Topset	17.0	29.6	2.78	.94	3.5	29.4	3.00	1.00	3.00	25.8	3.02	1.18
Lagreen	38.1	26.9	2.78	1.04	11.1	26.9	2.48	.92	5.70	23.4	2.21	.94
Climax	31.0	26.1	2.87	1.10	3.4	26.7	3.03	1.13	4.95	21.1	2.52	1.20
II-V-5-6	37.4	26.9	2.42	.90	11.0	27.3	2.60	.95	3.05	20.4	1.53	.75
Miss. Silver	23.6	25.4	2.30	.91	14.8	25.2	2.03	.81	4.25	21.8	1.59	.73
Bechuana[3]												
Dixielee Sel.	22.5	27.9	2.69	.97	13.3	26.6	2.61	.98	8.45	22.3	2.09	.91
Westbred	23.0	26.0	2.69	1.04	9.4	26.8	2.51	.94	3.60	26.5	2.24	.84
New Era	35.3	27.1	2.57	.95	11.4	27.3	2.33	.85	3.15	21.4	1.36	.63
Producer	32.4	27.9	2.57	.92	15.3	27.9	2.84	1.02	5.40	26.1	1.86	.71
Local Brown[3]												
$\bar{x}$	28.9	27.1	2.63	.97	10.3	27.2	2.60	.96	4.61	23.3	2.04	.88

[1] mg of met/g bean flour
[2] g met/100g protein
[3] photoperiod-sensitive, does not flower during long days

TABLE III
F Values for Tests of Significance to Determine the Effects of Various Factors on Yield, % Protein, and Methionine Content of Cow-Pea Seeds

Factor	Yield	% protein	Methionine mg/g flour	Methionine g/100g protein
Location	124.08**	17.40	26.00*	1.40
Daylength	8.18	8.60	6.05	0
Loc x Dl	2.58	14.22**	.89	2.12
Genotype	2.76	2.64**	8.05**	6.04**
Geno x Loc	1.14	4.92**	3.04*	3.29*
Geno x Dl	0.72	1.50	1.21	1.57
Geno x Loc x Dl	1.09	2.27*	1.06	.78

** Significant at .01; * significant at .05.

capable of synthesizing large amounts of protein and methionine are being sought and in studying the mechanisms regulating synthesis. More plants can be grown under the uniform biotron conditions throughout the year, and these facilities can be used to supplement the field program. However, further evaluations for yield, protein, and amino-acid content must be made on plants grown in the field under conditions similar to those in which they will be grown ultimately.

The cultivars studied here represent only a small portion of the genetic variability available. Analyses of additional accessions from the "Nigerian grain-legume gene bank" have identified plants that produce seeds having in excess of 30 per cent protein and up to 2.5 mg methionine/g flour. Considerable variability for tryptophan also exists. A number of *Vigna* accessions have been reported to produce over 30 per cent protein, with the highest being 36 per cent.*

Thus, it appears that there is sufficient genetic variability to expect considerable gain from selection in segregating populations.

Combining Improved Protein and Grain Type

The cross of Producer x Late Kabba has been studied extensively regarding per cent protein and, to a lesser extent, available methionine. Late Kabba is a typical local cultivar that is short-day sensitive and rather indeterminate, with numerous prostrate side shoots on which the pods are borne. The seeds have a rough testa, which is either white or white with brown flecks. Producer was

*Report from the Alabama Experiment Station. Minutes of the meeting of the S-9 Technical Committee "New Plants." Auburn, Alabama, July, 1972.

introduced into Nigeria, where we found it to b one of the higher-yielding cultivars. It is largely day-neutral, has large, light-brown seeds with smooth testa and is usually high in total seed protein, but only intermediate in available methionine. We were interested in selecting plants that combined large seeds having a rough brown or white testa with a higher protein content than that of Late Kabba.

Seed-coat texture and color were found to be simply inherited, with ratios of 25 brown: eight cream and 26 smooth: seven rough seeds being observed in the F_2 population. The results of selection for increased per cent protein in F_2 and F_3 populations are presented in Table IV. The two families of greatest interest after one cycle of field selection are 4/1605 and 43/1662, whose mean protein values exceeded both parents. The former appears to be uniform for cream seed color, rough testa, and medium seed size, whereas 43/1662 is segregating for these three seed traits. Further field selection is underway in these and other families where high protein phenotypes were observed. Methionine content of the F_2 individuals ranged from .92 to 1.98 mg/g flour compared to estimated values of 1.55 and 1.74 for Late Kabba and Producer, respectively. There did not appear to be an obvious relationship between high or low methionine content and per cent protein. Further analysis of the F_3 families for methionine is underway.

Summary

Cow pea is an important source of dietary protein in many tropical and subtropical areas, particularly in West Africa. The potential for raising yields through breeding improved cultivars that respond to better cultural practices is considerable. Methionine, and perhaps isoleucine and tryptophan, have been reported to be limiting amino acids. Significant variability in seed protein and methionine content exists among the accessions held in various collections. Percent protein and methionine content appear to be moderately heritable, and progress has been made by selecting for a combination of high protein content and grain types suitable for Nigeria.

Acknowledgments

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TABLE IV

Distribution of Parents and Individuals Within the F_2 and F_3 Populations of the Cross Producer (high protein) x Late Kabba (Desirable Seed Type), Based on % Protein in Mature Seeds

Population of family		Parent value (% protein)	% Protein 20.0-	20.1-22.0	22.1-24.0	24.1-26.0	26.1-28.0	28.1-30.0	30.1+	Mean	n	s^2
					Greenhouse, Madison							
F_2 Producer			2	0	4	9	8	6	4	26.5	33	
								5	1	28.5	6	
					Field, Nigeria							
F_3 families	70/1676	19.0				8	2			25.7	10	.57
	58/1667	22.3			1	2	5		2	26.8	10	5.14
	34/1655	23.5				1	7			26.6	8	1.30
	37/1657	24.6		1	2	5	2			24.8	10	2.37
	78/1685	24.9			1	7	2			25.4	10	.82
	42/1660	25.0			3	4	2	1		25.2	10	3.41
	21/1617	25.0			1	4	5			26.1	10	1.37
	4/1605	25.1					3	6	1	28.7	10	.95
	52/1665	25.3			5	4	1			24.0	10	2.74
	35/1656	25.5			3	6	1			24.6	10	1.48
	75/1675	25.7				3	5	3		27.2	11	3.02
	43/1662	26.2					5	4	1	28.6	10	1.17
	79/1686	26.7		1	1	2	4	1		26.2	9	5.73
	8/1607	27.0				2	2	1		26.4	5	1.47
	39/1659	30.6				4	5	1		26.2	10	2.08
Producer Late Kabba		28.5			1	1	6	7	1	27.6	16	2.54
					3	3		1		25.0	7	3.31

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DISCUSSANT

John F. Kelly

Rather than comment specifically on the papers that have been presented, I will give a short review of the work we are doing. I think this report is a logical extension of what already has been reported. We have found genetic variation in methionine and total nitrogen levels of *Phaseolus vulgaris* from three different sources, and have begun making crosses to determine their inheritance and to transfer the character to commercial types. Most of our work is based on a rapid adaptation of Ford's *Streptococcus zymogenes* assay for available methionine which we'll be discussing later (Kelly, Firman, and Adams, 1971; USDA ARS 74-56).

The first sources of variability we investigated were the USDA world collection and some beans from other collections. Table 1 shows some of the early results from our searches for variability. We then started screening the world collection and, since about 1968, we have looked at some 3,500 individual lines and a total of some 10,000 samples, including single seeds. Subsequently, we have found crude protein levels in *Phaseolus vulgaris* ranging from 17 to almost 35 per c[illegible] available methionine, 0.8 to about 3 mg/g total methionine, 2 to 4 mg per g[illegible] of bean. The ratio of available to total methionine ranged from .20 to .81.

TABLE 1
Variation in Crude Protein and Methionine Levels Among Six Bean Introductions

Line	% Crude protein	Available methionine (mg/g)	Total methionine mg/g
P.I. 172032	–	–	1.5
P.I. 282693	3.1	2.4	3.9
P.I. 207227	–	0.1	–
P.I. 194181	–	1.9	–
Haiti #6	1.7	0.9	2.1
P.I. 180758F7	3.2	1.4	–

Table II summarizes in another form the potential in *Phaseolus vulgaris*. It is apparent that there is a wide variation among the beans, depending on their place of origin.

Mexico, Scandinavia, Central America, Western Africa, and Central Africa have low methionine levels. The Caribbean samples had high levels, as did a few samples from China. We found high methionine among these assorted lots, which caused us to look within these lots for further variation, and we found a wide range of available methionine. This selection method alone, simply looking within existing material on hand, could be a significant means of improving the methionine levels in currently used breeding lines and mixtures - just a matter of roguing out the low and selecting out the high ones.

TABLE II
Frequency Distribution for Available Methionine in Bean Lines Broken Down by Geographic Origin

Place of Origin	Available Methionine (% of standard) 0-50	51-100	101-150	151-200	>200	N	Mean
Canada USA	37	181	133	29	9	389	97
Mexico and Central America	167	724	237	13	2	1143	80
Caribbean	1	10	24	4	0	39	126
South America	34	168	102	15	1	320	91
W. Europe	10	86	65	9	0	170	97
E. Europe	26	155	147	18	3	349	99
Middle East	29	362	357	47	3	798	102
Africa	7	99	35	4	0	145	85
Central Asia	5	68	37	10	0	120	95
China	0	3	13	4	2	22	142
Pacific	0	12	15	2	0	29	106
Total	316	1868	1164	155	20	3524	92

The third source of variability (Table III) we exploited was commercial cultivars - largely types that are grown for their immature seeds. Primarily, they are green-bean types from American seed producers. The varieties are very likely pure lines in almost all cases, except perhaps for some of the old land varieties. We have reported this in *Journal of the American Society for Horticultural Science* (1971, 96:561-563). If we take samples and put them in a replicated trial, the differences hold up; indeed, in almost all tests involving lines with a variation of, let us say, 33 per cent over or under our standard, we can be confident that the lines will behave predictably. As I have pointed out, crude protein levels in *P. vulgaris* varied considerably. The levels of both crude protein and total nitrogen proved to be markedly affected by environment. In addition, we find variation on individual plants from pod to pod, and from seed to seed

TABLE III
Variation in Available Methionine Levels Among Six Bean Cultivars

Cultivar	Available methionine (mg/g)
Bush Blue Lake (23618)	2.0
Bush Blue Lake 290	1.7
Bush Blue Lake (23620)	2.3
White Kidney	1.6
Sparton Arrow	0.7
French's Horticultural	0.6

within single pods; much more variability than we have found with methionine. A similar degree of variation is found in some of the cow peas we have examined, but our sampling was small.

More recently, we have begun work on the soybean. Here, we have found a range of about 31 per cent to 52 per cent protein. This, too, is largely affected by environment. However, there is a tendency for those that are high in protein to remain high under different environments. We are also looking for methionine variation in the soybean. So far, we have not found as large a variation, nor have we been able to duplicate our results as well; we're having analytical problems. The USDA has found small differences in methionine that originally were considered to be insignificant. However, the mean for the soybean is about 1.2 per cent methionine and ranges to 1.6 per cent – perhaps a very significant variation.

DISCUSSANT

P. Oram

I have been asked to take as my subject the activities of the newly formed Consultative Group on International Agricultural Research in relation to the food legumes. The Consultative Group has a Technical Advisory Committee, whose objective is to identify major gaps in research and, within those gaps, to indicate major priorities. (A gap, of course, doesn't necessarily mean a priority.) Once the priorities are identified, the TAC suggests the sort of approach that might best be adopted to solving the problem and, in particular, attempts to define fields of research where a strong interdisciplinary team might make a significant impact on a major problem of development. A project of this nature probably requires international support for its establishment, on the assumption that most developing countries could not support such large-scale research on their own.

One of the first concerns of the Technical Advisory Committee to the Consultative Group was the food legumes, partly because of the well-recognized need related to their contribution to protein nutrition, partly because a number of its members were at the Bellagio Group meetings, where the problems of their low yield and disease susceptibility were emphasized. Dr. Robert's report was its starting point, and his recommendations were basically (and I am simplifying) to separate the main food legumes on an ecological-zone basis and to have the work carried out by appropriate international institutions within the zones to which those crops were most suited ecologically and, therefore, well accepted by the people. He examined the possibility of a central "food legume research institute" and concluded that, although this might give some economies of scale, so many species of grain legumes were involved and so many problems were location-specific that they could not be tackled adequately at one center. He did identify six species for priority: soybeans, groundnuts, chick peas, pigeon peas, *Phaseolus*, and cow peas.

The TAC discussed his report and also a report by Dr. Swaminathan, which argued the case for a central facility to undertake work on problems common to a number of the major food legumes, of which a number can be identified, and where it seemed that a particularly strong and specialized team, using sophisticated equipment, might achieve a significant breakthrough in yield. This has not yet been achieved with most leguminous species widely grown in developing countries, although, as we have heard today, there are indications that substan-

tial improvements in productivity can be demonstrated experimentally by conventional breeding methods, by improved agronomic-cultural practices, and by pest, disease, and weed control.

Nevertheless, some disappointment has been felt at the progress that had been achieved by conventional methods - and it is true that, on the whole, yields of food legumes in developing countries remain remarkably low. This is one of the reasons that has led people to argue the need for a special facility that would undertake the more difficult tasks and try to raise the yield ceiling more dramatically; in other words, try to provide new horizons.

The TAC therefore decided to set us a subcommittee, composed of Dr. Hopper, the president of the Canadian IDRC, Dr. Swaminathan, and Dr. Pereira, head of the East Malling Research Station, who has worked in Africa for a number of years as head of the Research Council of the Central African Federation.

This subcommittee is looking at some of the issues facing the TAC, but before I say anything more about those, I would like to mention that both the Consultative Group and the Technical Advisory Committee came into a dynamic situation in which there were a number of ongoing proposals for research at existing institutions: for example, at IITA in Nigeria, CIAT in Columbia, and IICA in Costa Rica, along the lines recommended by Dr. Roberts.* I will describe these briefly before defining some of the remaining gaps.

IITA is working on cow peas, pigeon peas, and, to some extent, on soybeans and lima beans. CIAT is working mainly on field beans. It did have a proposal to work on soybeans but, according to their latest budget proposal, the soybean program is in suspension for the time being. A reason for this may be that there was considerable feeling at the TAC meeting that the more basic aspects of the work on soybeans might be centered in the United States, which has a major research capability for this crop, and that only duplication would result if too much work, other than that of an applied nature, were done elsewhere.

In addition to this work, which is mainly concentrated on the humid tropics, there appears to be a gap – and an important one – with respect to the drier areas of the tropics. Here the TAC has recommended the establishment of a new Institute for Crop Research in the Semi-Arid Tropics (ICRISAT), the constitution of which has been signed in India, which will work on sorghum, millet, chick peas, and pigeon peas, and which will have global responsibility for certain specialized work on breeding and agronomy, as well as on the physiology and biochemistry of those crops. It was debated whether this institute should also work on groundnuts, which fit into the systems of the semiarid tropics, but it was decided, for the time being, not to overload it. (The problem of overload has also been one of the main factors that argued against the establishment of a central facility for all food legumes.)

New proposals under scrutiny by the TAC include suggestions for what one might call "outreach" work related to ICRISAT in Africa and possibly also in

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*IITA, International Institute for Tropical Agriculture; CIAT, Centro Internacional de Agriculture Tropical; IICA, Instituto Interamericano de Ciencias Agricolas de la OEA.

Brazil, which has offered facilities. There are comparable conditions in Africa, northeastern Latin America, and parts of Southeast Asia, where semiarid conditions exist and where no major work is being conducted on food legumes at the moment. The problem is that in Africa, as Dr. Rachie has said, cow peas are the main legume. Chick peas and pigeon peas are not as important, so results from ICRISAT may not be too widely applicable to Africa under the present circumstances. Possibly, special programs should be set up -- programs linked to the main ICRISAT institute, but which could cover the specific needs of Africa in respect to the food legumes, even if these differ somewhat from the species being worked on in India, e.g., cow peas. A team in the field, with Hugh Doggett as team leader, is looking into this problem at the moment.

There is also a gap, in my opinion, in Southeast Asia. Dr. Swaminathan's paper, excellent though it was, related mostly to South Asia. Conditions are very different in Southeast Asia, not only in the species grown, but in the way in which they are consumed. In Indonesia, for example, there is a large acreage of soybeans and groundnuts, which are used not for oil but for direct human consumption. In the Far East, also, soybeans are used in the diet quite differently from their use in the United States, for example. Research on these end uses is not being covered adequately at the moment. Mung beans are another important crop in that area, particularly for fitting into multiple-cropping systems. FAO has submitted a proposal to the United Nations Development Program for a possible linkage program to ICRISAT for work on various food legumes and other rain-fed crops in Southeast Asia, but I am not sure if this adequately covers the more humid areas of the monsoon climate.

Other than this, the main gap -- geographically, ecologically, and related to species -- lies in the Mediterranean Zone with its cool, rainy winters and warm, dry summers. The principal species grown there are lentils, broad beans *(Vicia faba)*, and various species of *Lathyrus*. (These are also important, but to a lesser extent, in South Asia and in some parts of Latin America.) How can this gap be filled? There is a proposal to have a review mission take the field with TAC funding to see what needs to be done in the Near East and North Africa, not only for legumes, but for other crops, as well.

I think, on the whole, that a large amount of work is going on at the applied and adaptive level. The problem is no longer being ignored. The total program -- supported by international funds and including areas where new requests are likely to be supported -- comes to something like $1 million to $1-1/2 million per annum, if one includes the IICA proposal for work on field beans in Latin America, the CIAT work, work at IITA, and the proposals coming up from Africa and from ICRISAT. There is a weakness on the economic side. We don't know nearly enough about the factors affecting demand for and consumption of food legumes, marketing problems, or various related aspects, such as consumer preference, price responses, storage, and so on. Perhaps these considerations need more attention at existing institutes.

Two other supporting services exist, which I think will be helpful to work on food legumes in general, and which are being funded by the Consultative Group. The first is a Food and Agriculture Organization project to develop a current

research information system (CARIS), which aims to cover all the developing countries eventually, but which, at the moment, is in the pilot-study stage and is being initiated in 14 West African countries. These countries form the West African Rice Development Association. But the system will not be confined to rice. It will cover the research situation on all crops and other aspects of agricultural production in those countries. If this is successful, and money is available, it will be expanded and will provide an up-to-date directory of ongoing research and the research facilities in all of the developing countries.

The second proposal is to establish a network of Plant Genetic Resources Centers with a coordinating unit and a governing board, which would include both developed and developing countries, as well as international research centers, and would provide for regional centers, which would assist in the collection, exploration, and evaluation of plant genetic resources. Again, food legumes are being given high priority in this proposal, which was drawn up by a group of 12 leading geneticists at Beltsville, Maryland, in March, 1971.

In conclusion, I would like to summarize the questions facing the TAC and, in addition, those that face this meeting, in looking at the needs for research – particularly for research on breeding as related to the food legumes. The first question is: "How can we prevent overlapping?" or "How much duplication is permissible?" To what extent should duplication be avoided, and to what extent is duplication – or replication, if you like – desirable? (The avoidance of duplication sometimes can be overstressed.) But undoubtedly there is duplication at present not only in some of the proposals from international centers, with competing claims – for example, for world collections of germ plasm of some of the food legumes – but also in the symposia and the studies that are now being proposed. In addition to this PAG symposium, the TAC also has been considering holding a symposium. So has the National Research Council in the United States. Might it be possible that we are overproducing symposia?

The question of whether there should be a central facility of some magnitude, comparable to CIMMYT (the Wheat and Maize Institute in Mexico), which would work on the more fundamental problems of the good legumes, is still unresolved. What could one such facility do that the individual international centers couldn't do? Where would it save money, staff, equipment, and so on? Where would it provide economies of scale and economies of force? How could it cover the missing links? How many species can be handled effectively by any one institute, whether it is working on adaptive or basic problems?

You might consider what developed countries could do to cooperate in supporting the over-all program. Could they fill the gaps on more fundamental problems, which might otherwise require that a central institute be established, thus saving a very large cost in new facilities?

My last point relates to the possible balance of need as related to resource allocations between short-term breeding and adaptive research, e.g., on diseases, and the long-term research that might have to be done at some central institute(s). Can this meeting suggest any short- or long-term priorities?

DISCUSSANT

J. H. Hulse

A number of speakers have referred to the unique feature of the food legumes – their ability to fix atmospheric nitrogen. It is well known, of course, that the nitrogenous enzyme system present in the root nodules is responsible for the biological adaptation of atmospheric nitrogen to ammonia. Until about 1962, when Hardy, of DuPont, described his method of assay for nitrogenase, it was difficult to determine the activities of the enzyme, either during the life of the plant or among different cultivars. He described a method by which he found that acetylene is reduced by nitrogenase to ethylene and that one could, by gas chromatography, determine the amount of ethylene produced and, therefore, the activity of the nitrogenase. (I would like to point out parenthetically that, since Hardy's publication, some 200 other papers have discussed nitrogenase activity, but fewer than 1 per cent of them have originated in the developing countries.)

Quite recently, Canadian scientists developed a simple colorometric method for ethylene that makes the nitrogenase determination method portable. They now are in the final processes of patenting the system, which can determine nitrogenase activity in the field during the life of the plant.

It may be that the paucity of papers from the developing world can be attributed to the cost of the previous system and its requirement for a somewhat elaborate laboratory in which to carry it out. The new Canadian system can use either a portable color meter or a color-grade standard, and is sensitive to 2 x 10^{-7} moles of ethylene, which is sufficient to measure nitrogenase activity in plants about three weeks after germination.

Early work indicates that the duration of nitrogenase activity varies among plants, certainly in field peas. The simplified method may enable plant breeders to make a more serious study and look for cultivars that either fix nitrogen earlier in the plant's life or continue fixation during pod-filling. Nitrogen fixation ends in many legumes about the time the pods start to fill. I would suggest that the areas of nitrogenase activity and the rate and duration of nitrogen fixation in various legumes are worthy of more study.

Reference

Hardy, R.W.S. 1973. Applications of the acetylene reduction assay for nitrogenase. *Soil Biol. Biochem.* (in press).

SUMMARY

Donald Boulter

The speakers brought out a certain difference of opinion on one or two points. For example, I sense that some people felt that already there is sufficient variation in the existing legumes and that we should start with what we already know and move forward. On the other hand, there was a feeling that, in some cases, we had to go out and assemble additional varieties and get world collections together. That was one area of disagreement. Possibly another was the number of different species of legumes that we should actually try to tackle.

Having said that, I would like to pass on to our Chairman's remarks. He reminded the biochemists (of whom I've said I'm one) and the nutritionists that the plant breeder is dealing with a complex problem, and every time we introduce a new dimension, it produces a tremendous amount of additional work for him. If he is going to tackle the problem within a feasible time span, we obviously must limit the number of those new dimensions. This was brought out again by another speaker, who also pointed out that the farmer – normally a subsistence farmer – is going to be concerned about yield. Therefore, yield, adaptation, and resistance to disease are going to be major considerations in all of the programs discussed. It was suggested that we must not scatter our shots too broadly, that there are many legumes, and that progress in legume improvement must be a team effort – a sentiment echoed by many speakers.

Dr. Swaminathan's paper made the point that the situation in India is unique, in that the primary high-protein food is legumes. He also pointed out some reasons why considerable work on legumes should continue. They are high in lysine; they can adapt to dry areas; and often they are responsible for restoration of soil fertility. He pointed out, as well, the danger that, in India, production of pulses has not increased greatly because the cereal program is so successful. The major problem with the pulses is their low yield and this, he said, was attributable to a veriety of reasons. For instance, they often have been poorly managed or grown in marginal lands; there has been insufficient genetic variation in the breeding programs that have been employed for the different legume crops.

He broke the breeding program down into two phases. The first phase embraces simple selection procedures, which are being carried out with a restricted germ plasm. The second phase is the breeding program, now being carried out in collaboration with the USDA. A considerable collection of legumes has been assembled and already various improved varieties have been produced. They are breeding for an improved plant type (a need that has come up time and again in these sessions) and also for an increased harvest index or biological yield. Dr. Swaminathan also mentioned the need for control of pests and diseases, the establishment of proper rhizobial strains, and he felt that, given the package approach and new cropping patterns, it should be possible to double the produc-

tion of pulses readily. He referred to various experimental approaches for high yield, nutritional values, digestability, the shortening of the maturation, and even, in some cases, a shift of the season in which the legume was grown.

Dr. Jalil spoke about the situation in Latin America, where the major food legume is *Phaseolus vulgaris,* followed by *Vicia faba* and chick pea. Most of the genetic improvement work has concentrated on beans, and here the bean diseases are the limiting factor. He feels there is a great potential for increasing yields, but stressed that the problem is difficult of solution. The major program in Latin America will be the collection and evaluation of major varieties. Dr. Jalil, too, stressed that team effort is an absolute essential -- that there should be regional training programs, and regional laboratories for evaluation. He also mentioned that the soybean is doing extremely well in Latin America; that there had been cooperation with Dr. Leng of the University of Illinois; and that, although groundnuts had decreased in their productivity, there is a considerable increase in demand for soybeans, which are being used mainly for oil.

Dr. Rachie spoke to us about legumes in Africa south of the Sahara. He emphasized the tremendous potential for the legume in that area -- in both the dry and the humid regions. He said that they grow vigorously on ever poor soil, and that the dry seeds are possibly the only storable, transportable protein with concentrated vitamin content. However, he also pointed out the tremendous constraints on increased productivity, mentioning, among other problems, plant diseases, the narrow range of genetic diversity, and inefficient plant types -- the same difficulties that face other parts of the world. He also discussed the wide climatic variations from the northern, semiarid regions to the more humid southern regions. In the north, the protein calorie intake averages roughly 2,900 calories per day and 80 grams of protein. In the humid regions, intake is something like 1,744 calories per day and 33 grams of protein -- which clearly is inadequate. This is because in the north the staple is cereals; in the south the staples are roots and tubers. Although groundnuts are grown extensively in the north, they are used mainly for oil or for cattle feed, so cow peas are the first legumes in these areas followed by *Phaseolus,* pigeon peas, and chick peas. Soybeans are grown, but acceptability is low. This, of course, is one of the great advantages of the cow pea: it is readily grown, readily eaten, and West Africans like it. However, Dr. Jalil did point out that soybeans may well have a great potential as urbanization develops in these areas.

It is doubtful that cow peas and soybeans can adequately meet the needs in the tropics. Yam bean, grown east of Benin from the Congo to Angola, is much less susceptible to pests and plant diseases. However, he mentioned the possibility of introducing pigeon pea, wing bean, lima bean, and jack bean. He pointed out that, in trying to choose a legume on which to work, major considerations must be given to the local acceptability of the crop, the range of genetic diversity, and the level of outcrossing. He also stressed the importance of team effort.

Dr. Kelly, from the Campbell Institute, has been screening the world collection of *Phaseolus vulgaris* for variation in crude protein and methionine. The results appear to be encouraging - something between 17 and 32 per cent crude protein, 0.8 to 3 per cent methionine. He showed that there were differences

according to the geographic area from which the *Phaseolus* was obtained originally.

In addition, Dr. Kelly has done some work on soybeans, where there is a variation of 31 to 52 per cent, although the variation in methionine in that species is not so great — 1.2 to 1.6 per cent. He pointed out that the actual protein levels in soybeans were affected by environmental interactions. That problem was also mentioned as a possible subject for further discussion.

Dr. Leng stressed that virtually all the main legume species have enough variability, and that it is vital to have a time framework of ten years in which to introduce new varieties. He also brought out the need for restructuring, and he gave us an illuminating example of the soybean, which was, in fact, restructured in about four years. He suggests that we must go for high yield, and feels that legumes satisfactory in this respect are pigeon peas, cow peas, and soybeans. He suggested that we should start with what we have, and the success of the corn-belt soybeans in Nigeria was adduced as evidence.

Dr. Oram discussed the work of the Consultative Group. He explained that its objectives were to identify major gaps and major priorities, and then to see what could be done. They used food legumes as a point of departure, because it is a well-recognized need, and they took Dr. Roberts' excellent report as a basis of their discussion. It was pointed out that this was, in fact, an ecological approach, and that one of the main questions was to try to decide if they would have a central institute that would deal with a large number of legumes, or if there should be several institutes in which one or two legumes would be studied. The final decision was in favor of the latter.

Dr. Oram discussed a series of gaps which they felt existed, for instance, in the semiarid tropics, and informed us of a new institute (ICRISAT) whose charter was signed in India during these sessions. The institute will be concerned with sorghum, millet, chick peas, and pigeon peas. He discussed ways in which gaps in Southeast Asia and in the Mediterranean Zone could be closed. In this context, he mentioned overlapping, which he didn't think was necessarily a bad thing, but which, on the other hand, obviously should be controlled. For instance, he posited an institute working on some of the more fundamental aspects of legumes, on the missing links, so to speak, and on how many species it is actually possible to work on — a point brought up by Dr. Borlaug earlier. He also discussed the role of the developed countries in these programs, suggested that we should have short- and long-term priorities and indicated, to some extent, what these might be.

Dr. Evans talked about the Cambridge work on *Phaseolus vulgaris*, which is conducted in conjunction with the Makerere program in Uganda. The program is testing for genetic diversity in this species, as well as for yield, adaptability, and protein content. The advantages of such a joint program are obvious, because the studies can embrace Cambridge in one climatic region and Uganda in quite another.

Dr. Rubaihayo also spoke about the Makerere program. As did a previous speaker, he emphasized that there is sufficient protein per capita in Uganda, but there is still a protein food problem because of distribution. Animal products

would be too expensive, so a breeding program in legumes is now in progress. Its aims are the improvement in protein quality, in this case methionine content, and also protein quantity; to give high yields; identify and eliminate pests; develop resistant varieties; and introduce good management practices. They have, in fact, collected considerable germ plasms of soybeans, cow peas, beans, pigeon peas, groundnuts, and peas. There have been some promising results in increases in the amount of productivity per hectare, and some of the programs are not being pursued as actively as others because of various technical reasons.

Dr. Wayne Adams works on *Phaseolus*. He raised one or two points on whether primitive varieties, which grow in low-fertility conditions, have led to the selection of varieties that would be unable to respond to fertilizer and mineral treatment. He pointed out that his group has established that there is great potential for response even among such plants – certainly an encouraging sign. He also talked about the ideal plant type, and on the whole he felt that he would be able to achieve this. He also raised some interesting questions for the plant breeder. He said we must establish some priorities; for example, we should relax the search for high protein and concentrate on yield and adequate biological value. In effect, he pinpointed for us that we cannot have everything, and therefore must make some hard decisions.

RESEARCH NEEDED

Dr. Rachie

This afternoon's meeting has, I believe, helped to place this conference in perspective by giving weight to the plant-breeding side of the problems. Several people have contributed to the various points I am going to make, so I will not attempt to indicate them all by name.

I think the entire conference has agreed that a matter of primary importance in the improvement of the grain legumes is to increase productivity. Under this heading, we must increase yield per se to make the grain legume attractive commercially to grow and, of course, to bring good-quality protein within the reach of all economic classes. It appears that everyone agrees that we need to restructure these plants and to increase the harvest-yield index.

In stabilizing yields, it will be particularly important to gain some measure of control over insects and diseases through the genetics of the plant itself and by other means, as well. We must also bear in mind that, in the areas where these crops are grown, they must be adapted to a wide range of conditions, including moisture limitations, acidity, and other soil factors. There also is a place for developing high-yielding, short-duration types, as has been mentioned by several people. However, when one gets into the rhizobial nitrogen-fixing system, one tends to limit oneself to very short-duration types. I wonder if this isn't, partly, what Dr. Adams was talking about in relation to his protein levels. For example, take a cow-pea plant that matures in 60 days, bearing in mind that the Rhizobium probably doesn't become very meaningful until after at least two or three weeks and flowering begins in, say, 35 days at the earliest. Now we are talking about a 60- to 65-day crop, and have very real problems in getting enough nitrogen into the sinks to provide for adequate sources of that nutrient.

Most of us agree that we should not sacrifice grain quality in the effort to improve yield. There may be some merit, however, in Dr. Adams' point of view that, in the short term, we may have to look at lower-protein types in order to gain yield breakthroughs.

One thing of which I am deeply convinced is the need for acceptability when we deal with human consumers. In this area we cannot compromise. So I would add this to our quality problem.

I am not quite as discouraged as is Dr. Adams about improving the deficient amino acids. I think that perhaps, with the many species we have to work on, there are real possibilities for making meaningful progress on, say, the sulfur-bearing amino acids. This is an area in which we must intensify our efforts. Also, we cannot compromise on antimetabolites and must strive to decrease them in new and improved strains.

The basic key to improving grain legumes will be to assemble and evaluate germ-plasm collections. I would like to suggest that, as we look around the

world, Africa has been neglected as much as any region, and that the early mounting of the collecting program must be considered as a matter of the highest priority. A large number of species ostensibly originated there, so there is a very wide range of genetic diversity. I would also suggest that the IITA has some excellent facilities in a $20-million plant set up near the modern city of Ibadan to serve as a host for such a project if it were to be mounted in that area. This is just a suggestion. Many other places could host such a project. Once collected, these materials must be evaluated and re-sorted, duplicates must be eliminated, and the various important agronomic, biochemical, and other characters assessed. We are starting to do this with the world collection of cow peas, but it is required for other species, as well. Obviously, we don't have anything like the total range of genetic diversity in *Vigna*.

Hand in hand with the work of genetic improvement must go that of management, and particularly important are investigations on fertility levels, on the rhizobial system, on populations and spacial arrangements, and on other kinds of management problems. Of course, this is a dynamic program. It must go hand in glove with the development of new and improved varieties.

I also think it essential that a few species be selected and studied intensively. I believe we have agreed that the *Phaseolus* beans, cow peas, and soybeans are primary species. We are not convinced that these will fit all possible needs, but we may have a great lacuna of knowledge and materials in the humid tropics. Sometime, somewhere, we should look at species for these areas. In this same context, some participants have mentioned the need for a global project on soybeans, and I would propose that the conference recommend that such a decision be made as soon as possible.

Dr. Oram mentioned the possible need for basic investigations at a separate center. I would question this, for I think that basic investigations are needed in several regions and under several wings in areas of wide crossings in physiological aspects. This would include more efficient plants; the limitation of sinks, often caused by excessive dropping of pods; photorespiration; and other physiological considerations.

We have also mentioned the basic work in rhizobial systems, and several people have discussed the use of populations. These are excellent tools in terms of both improvement and distribution of genetic materials, but much needs to be done on these in terms of (1) developing the mechanism that would include male sterility and insect carriers of pollen, and (2) studying the problems basic to developing and maintaining them.

A further area that was mentioned by Dr. Oram concerned economy. This should include economic problems involved in short-term and long-term cropping and in how various legumes would fit into various farming systems. I would not like to consider all the points that Dr. Oram discussed, because many of these will depend, first, on basic policies at a high level.

Obviously, research needs are very extensive, and can be summarized as follows:

Improving Productivity

1. Yields per se: Grain legumes must be economically attractive to producers. Most breeders feel it would be necessary to restructure the legume plant both morphologically and physiologically, and to increase the harvest yield index (Swaminathan).

2. Improve the stability of yields: Diseases and pests must be brought under control; involve the development of elite, widely-adapted varieties and populations with good response to high levels of management under variable conditions of moisture, soil characteristics, insolation, temperatures, and other factors.

3. Develop special types, specifically suitable for multi and mixed cropping. Among legumes, these might include short duration, climbing, spreading, or slowly developing plant types.

Retention and/or Improvement of Grain Quality

1. Increase of total crude protein is an important objective, but should not deter the drive for higher yields.

2. Increasing the proportions of deficient amino acids – particularly the sulfur-bearing amino acids and tryptophan – are legitimate, immediate objectives. Effective, quick, screening methods for specific amino acids are needed urgently.

3. Reduction of various antimetabolites and flatulence factors are of primary importance in some species, notably *Phaseolus* beans and soybeans.

4. Final assessment of nutrient qualities must be made on living organisms or with highly correlated in vitro techniques.

5. Organoleptic characteristics desired by the majority of consumers must be retained (and, if possible, improved).

Systematic Collection and Evaluation of Grain Legumes

1. The important species should be collected in major regions of diversity and use as soon as possible, to prevent irreparable losses of invaluable genetic materials through replacement by improved strains.

2. Evaluate collections for their important agronomic, botanical, and biochemical characters when grown under different environments and conditions.

Improvement of Management Conditions

1. The response of existing elite and newly developed varieties to fertility levels under different environments must be determined on a continuing basis.

2. Tillage methods, planting methods, and population dynamics require similar studies.

3. The utilization of different and improved genotypes in various cropping systems are essential. The feedback to breeders in developing special-purpose types will be invaluable.

4. Improvement of rhizobial nodulation as related to management factors on genotypes will be essential to improvement in this area.

Essential Knowledge of Growth Processes

1. In breeding, it will be important to keep abreast of new developments in wide crossing, inducing haploids, polyploidy, induction of mutations, and "vegetative" hybridization.

2. Population-improvement methods utilizing male sterility (genic or chemically induced), and more efficient insect vectors require intensive immediate study to improve the rate and efficiency of the improvement process.

3. Physiological investigations should involve studies of more efficient plant structures and physiological processes, the problem of fruit abortion, nonphotorespiration, metabolic pathways, and hormonic relationship.

4. The rhizobial system must be examined for identifying more efficient host plants and symbionts, and the management factors developed to optimize the total symbiotic response.

Production Economics

The economic factors involved in growing both improved and local cultivars of grain legumes under different conditions and locales require careful study. These studies will be particularly relevant to developing more efficient cropping systems and new plant types designed for those situations.

III

NUTRITIONALLY RELATED FACTORS IN LEGUMES REQUIRING GENETIC IMPROVEMENT

A. Protein and Amino Acid Profiles

Statements

INCREASING PROTEIN QUANTITY AND QUALITY
– J. F. Kelly

PROTEIN QUANTITY AND QUALITY IN HIGH-YIELDING BEANS
– M. H. Dickson and L. R. Hackler

AMINO ACID PROFILES OF *CAJANUS CAJAN* PROTEIN
– W. Vernon Royes

BREEDING FOR PROTEIN QUANTITY AND QUALITY IN FIELD BEAN *(VICIA FABA)* AND BARLEY *(HORDEUM VULGARE)*
– L. Munck, E. Holmberg, K. E. Karlsson, B. Löfqvist, and J. Sjödin

THE AMINO ACID COMPOSITION OF *VIGNA UNGUICULATA* (COW PEA) MEAL IN RELATION TO NUTRITION
– D. Boulter, I. M. Evans, A. Thompson, and A. Yarwood

THE JOINT FAO/IAEA PROGRAM ON THE USE OF NUCLEAR TECHNIQUES TO IMPROVE SEED PROTEIN
– Bjorn Sigurbjornsson and Robert Luse

BIOLOGICAL VALUE OF LEGUME PROTEINS AS INFLUENCED BY GENETIC VARIATION
– Satinder Bajaj

Discussant

K. J. Carpenter

Summary

K. J. Carpenter

INCREASING PROTEIN QUANTITY AND QUALITY

John F. Kelly

The nutritional contribution of legumes might be improved through increased yields, increased protein concentration, or improved protein quality. Depending on the figures one uses, a good case can be made for any one of the three. Ideally, we would make the greatest improvement if we were to increase crop yield, protein quantity, *and* protein quality. As a minimum, we should aim to increase any of the three without decreasing the other two. Each of these three factors is probably quantitatively inherited (certainly for yield), and increased protein levels are frequently, but not always, associated with decreased seed yields, so it is unlikely that maximum yields, protein levels, and protein quality can all be achieved in a short-term breeding program.

Protein levels of legumes are influenced significantly by environment. Perhaps the best long-range study of protein-environment interaction has resulted from the U.S. Regional Soybean Laboratory Uniform Soybean Tests (Anon., 1965c). In several locations there is little year-to-year variation within a variety. However, in a given year, the location-to-location variation for a variety is likely to be greater than the variation in protein levels among cultivars in a given test. Thus, in one 1970 test, Kent soybeans contained 36.3 per cent protein at Stoneville, Mississippi, and 43.5 per cent at Warsaw, Virginia. Similarly, Hill and Md 64-429 contained 35.5 per cent at Keiser, Arkansas, and 42.7 per cent at Stuttgart, Arkansas. The varietal mean ranges for the two tests were only 37.5-41.2 per cent, respectively. These locational differences are not generally maintained over several seasons.

Despite the environmental influence, a wide range of genetic differences in protein levels has been observed in soybeans (31 to 52 per cent [Anon., 1965a]) and common beans (17 to 32 per cent). Conscious efforts at breeding soybeans for higher protein levels have resulted in release of commercial varieties with up to 43 per cent protein. Because of the general tendency for high protein to be accompanied by low oil, and the demand for high-oil soybeans, rapid progress has not been made in achieving maximum protein levels.

The importance of protein level in a legume is illustrated by the data in Table I. The Pimero lima bean exhibited the highest PER of all *Phaseolus* beans we assayed, but because of its low level of protein more than twice the consumption of beans was required to give twice the weight gain of the Sanilac standard.

The importance of amino-acid balance in nutrition has received less than its

TABLE I
Composition and Biological Value of Lima Bean

	% of Sanilac standard
Protein	60
Total methionine/g bean	54
Available methionine/g bean	41
Total sulfur/g bean	74
Available methionine/g N	42
PER (3 weeks)	163
Gain/g consumed (3 wks)	95
Grams consumed (3 wks)	220

deserved attention by crop breeders. The discovery of high lysine in opaque-2 corn did much to change this. However, the search for better amino-acid balance in seed crops goes back many years before that important discovery, and small varietal differences in essential amino-acid levels have been reported for several crops (King, 1964; Lantz, et al., 1958; Russel, et al., 1946; Tandon, et al., 1957). Until recently, these differences were considered by some to be unimportant. For example, at the U.S. Regional Soybean Laboratory, Krober screened most of the USDA world collection of soybeans for methionine and found a range of methionine levels from 1.1 to 1.6 per cent of total protein, most samples being in the 1.2 to 1.4 range. Like most legumes, and indeed like most plant proteins, soybean protein is first limiting in the sulfur-bearing amino acids (Table II). Expressed as a percentage of total protein, the soybean protein contains only 56 per cent as much methionine and cystine as the FAO hen's egg (Anon., 1965b; Table III). In terms of A/E (amino acid/total essential amino acid), it contains 71 per cent of the hen's-egg level. By comparison, the respective percentages for beef are 68 and 79. Thus, a 33 per cent increase from 1.2 per cent to 1.6 per cent methionine would result in soybean protein with a chemical score similar to that of beef. Such a scoring system does not take availability and digestibility into consideration. Somewhat greater increases would be needed for the common bean.

In addition to the variations in amino-acid balance, which can be largely attributed to varietal differences, a wide range of values has been reported from various sources. These probably are attributable in part to genetic differences (Anon., 1970; Orr and Watt, 1968; Table IV).

Probably without exception – it is the exceptions that we are searching for – the first amino-acid levels requiring improvement in legumes are methionine and (or) cystine. It must be determined if these amino acids can be increased together or if it is more practical to concentrate on one or the other, taking into consideration the importance of maintaining the proper balance between them (Shannon, et al., 1972). What of the second and third limiting amino acids? In the common bean, cow peas, and mung beans, a 50 per cent increase in sulfur-bearing amino acids would probably result in tryptophan becoming the limiting amino acid. In lima beans, even a small increase in methionine or cystine would

TABLE II
Essential Amino Acids (Percentage of FAO Hen's Egg) in 11 Leguminous Crops

	Trypt	Thre	Isol	Leuc	Lys	Meth	Meth plus Cys	Phenyl	Phenyl plus Tyr	Val
Common beans	58	85	86	98	116	33	37	95	94	83
Broadbeans	58	64	95	99	88	13	21	72	69	68
Cow peas	6[illegible]	77	73	85	102	50	52	90	82	77
Lentils	53	70	80	80	95	23	28	76	71	75
Lima beans	59	93	88	94	104	52	57	102	85	86
Mung beans	46	61	84	103	107	35	31	83	64	81
Peas	67	76	85	94	115	39	46	87	91	77
Cow peas (green)	67	74	75	79	102	45	-	96	–	75
Lima beans (green)	81	88	93	92	98	35	40	89	86	89
Peas (green)	52	72	69	71	74	25	34	66	63	56
Soybeans (mature)	86	78	81	87	98	43	56	85	80	72

TABLE III

Amino-Acid Balance in Selected Proteins (FAO Hen's Egg = 100)

	Beef		Common Bean		Soybean	
	mgm/g N	A/E	mgm/g N	A/E	mgm/g N	A/E
Isoleucine	79	91	86	99	81	100
Leucine	93	107	97	112	87	109
Lysine	135	157	115	134	98	122
Total aromatic	74	86	92	108	80	101
Threonine	87	100	85	99	78	95
Total S-bearing	68	79	36	42	56	71
Tryptophan	73	84	58	68	86	106
Valine	76	89	83	96	72	90
Total essential	87	-	86	-	80	–

TABLE IV

Reported Range in Levels of Limiting Amino Acids (mg/g N) of Legumes

Amino Acid	Common bean	Soybean	Peanut	Cow pea	Chick pea
Methionine	28-131	53-114	33-100	50-119	34-106
Cystine	21-108	51-114	11-106	48-106	50-94
Tryptophan	32-101	75-88	45-90	66-70	25-94
Valine	213-388	250-375	142-307	250-325	213-356
Threonine	192-356	200-285	116-207	178-300	219-263

make tryptophan first-limiting. In soybeans, valine becomes limiting with about a 50 per cent increase in sulfur-bearing amino acids, and in green peas either tryptophan or valine become limiting with a similar increase. In several of the legumes, threonine is the third limiting amino acid. Whether any emphasis should be put on the second and third limiting amino acids must depend upon the amount of increase that can be effected with methionine and cystine and upon the amino-acid composition of the other protein sources that are normally consumed with the legume. It also may be valid further to improve other amino acids (e.g., lysine) already present in abundance, based upon this latter consideration.

An important question to be answered when changes are observed in one or more amino acids is how other amino-acid levels are affected. The genes that affect amino-acid patterns probably obtain their effect through their influence on the structure of a complete protein. Thus, the increased level of lysine in opaque-2 corn is accompanied by changes in other amino acids as a result of a reduction in the ratio of zein to glutelin (Mertz, et al., 1964). Similar differences

in amino-acid composition have been reported for the various protein fractions obtained by ultracentrifugation of soybean protein (Catsimpoolas, et al., 1967; Roberts and Briggs, 1965). The two most abundant fractions, 7 S and 11 S, differ significantly in their amino-acid levels. Cystine, methionine, threonine, tryptophan, and glycine are lower in 7 S, whereas arginine and phenylalanine are higher. There is evidence that soybean cultivars differ in their contents of proteins (Larson, 1967). Among common bean varieties we have found large differences in the levels of albumins (8.5-18.6 per cent) and globulin (9.8-14.0 per cent). The level of methionine is generally higher in the globulin than the albumin fraction. Many fundamental studies of legume protein fractions have formed a base for further work needed in relation to amino-acid balance.

Our initial efforts can be aimed at attaining a high chemical score based upon amino-acid content, but ultimately we must aim for maximum biological value for human beings. Rather than spending years on research and breeding to improve amino-acid balance, only to find that nutritional value has not been increased proportionately, the breeder must work closely with nutritionists, physiologists, food technologists, and biochemists, who, at the same time, are trying to determine the factors that give maximum biological value acceptability, digestibility, availability, and utilization.

If legume proteins are to replace or substitute for animal proteins, the animal proteins should be the standard for comparison and the goal should be to meet that standard. Otherwise the legume proteins should be promoted on their own merits and those deficiencies (compared to the established standard) should be made up by balancing the diet with other proteins or through supplementation.

It has never been easy to characterize the ideal protein. The task of establishing guidelines for legume-protein improvement is likely to be even more difficult, but we should be able to establish at least some minimum standards based on our current knowledge, with the intention of revising them as we learn more.

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PROTEIN QUANTITY AND QUALITY IN HIGH-YIELDING BEANS

M. H. Dickson and L. R. Hackler

Genetic improvement of protein quality and quantity in beans *(Phaseolus vulgaris* L.) and other legumes is now assuming greater importance, due in part to the "Green Revolution," which has taken care of the immediate need for more food.

Beans are an acceptable and widely used source of vegetable protein, but both higher protein content and improved amino-acid balance is desirable. Recently, at various locations throughout the United States, as well as in other countries, programs have been initiated to study and solve these problems. The first operation in all cases has been to identify sources of high-protein beans and also beans with higher-than-average levels of desired amino acids. At the New York State Agricultural Experiment Station in Geneva we have looked at several of these factors since 1967.

Results and Discussion

Efficiency of nitrogen utilization

Leleji (1971) compared PI 179444 and PI 229815, low- and high-seed protein lines respectively, for their protein content in the different plant parts at various stages of development. Table 1 shows that the leaves of the young plant of the high-protein line were higher in N than were those in the low-protein line, but in the mature plant the leaves of the low-protein line were almost twice as high in N as in the high-protein line. Apparently, in this pair of lines, PI 179444 is much less effective than PI 229815 in transferring nitrogen out of the plant parts and into the seed. These results are similar to these found in wheat (Johnson, et al., 1968), where varieties high in protein translocate a higher percent of their nitrogen from the vegetative portions to the grains than do varieties low in protein.

A factor that may influence protein concentration is the yield. Generally, PI 179444 gave a higher yield than PI 229815. If nitrogen distribution is influenced by sink size (seed yield), the latter line with the smaller sink should have more N in the vegetative parts. This was not supported by the analyses made after seed began to form (Table 1). Although the percentage of nitrogen in the roots of PI 229815 was higher, suggesting that uptake is a factor contributing to efficiency in N utilization, it appears likely that translocation is as important. A 3.42 per

TABLE I

Distribution of Nitrogen in the Various Tissues of Dry Bean Lines

(Data are expressed as percent nitrogen on dry-weight basis)

Plant Tissues	Weeks after nitrogen application									
	2nd		4th		6th		8th		10th	
	229815	179444	229815	179444	229815	179444	229815	179444	229815	179444
Roots	3.93	3.06	3.39	3.10	2.77	2.56	2.04	1.85	1.94	1.73
Leaves	4.66	4.45	3.85	4.09	2.02	2.29	1.86	2.60	1.38	2.15
Stems	2.58	2.71	2.54	3.19	1.12	1.18	.85	1.05	.81	.70
Hulls					2.63	3.32	1.08	1.16	.77	.43
Seeds							3.98	3.27	3.84	3.10

cent increase in percent protein occurred with PI 179444 when N was increased from a low level (3 g/plant) to a high level (16 g/plant), while the corresponding increase in PI 229815 was only 1.17 per cent (Table II). This lack of a more noticeable increase probably reflects a more effective uptake and translocation of N from the vegetative parts to the seeds. Thus, we might conclude that beans such as PI 229815 are more suitable for low N soils, whereas PI 179444, with its response to high N levels, is more adapted to high N soils.

Sources of high-protein lines and inheritance

Rutger (1970) observed a wide range of protein content of different varieties of beans (19-31 per cent). The environment also has a considerable effect on protein content (Singh, 1969; Silbernagel, 1970; Stuber, et al., 1962).

In our studies (Leleji, et al., 1972) of protein inheritance, F_1 seeds of the cross PI 169760 x PI 229815 (low- and high-protein parents) showed highly reciprocal differences in protein level. In contrast, there were no reciprocal differences in F_2 seed (Table III). This indicated that the maternal genotype controls the percentage of seed protein, but that the absence of reciprocal differ-

TABLE II
Efficiency in N Utilization as Reflected in Seed Yield, Percent Protein, and Protein Yield

Genotype	N applied (g/plant)	Seed yield[1] (g/plant)	Percent protein	Total protein (g/plant)
PI 179444	3	18.0	16.2	2.93
	6	22.6	18.6	4.20
	16	31.9	19.7	6.27
PI 229815	3	11.2	25.9	2.91
	6	15.9	27.0	4.29
	16	21.6	27.1	5.85
LSD (19:1)		8.3	1.0	
(99:1)		11.4	1.4	

[1] Mean of 3 plants

ence in F_2 seeds indicates that maternal cytoplasm does not influence the crude protein of progenies. This conclusion is the same as Singh (1969) reported in soybeans.

The F_2 and backcross progenies indicated that high-protein content is recessive, with broad-sense heritability estimates of 49.3 per cent and narrow-sense heritability of 20.1 per cent (Table IV). Other populations in the same study showed broad-sense heritability of 30-50 per cent and indicated that two to four major genes were involved. There were no clear examples of transgressive segregation, although a few F_2 plants exceeded the parental extremes.

Rutger (1970) and Tandon, et al. (1957) have shown bean-seed yield to be negatively correlated with percent protein, and in our trials (Leleji, et al., 1972),

TABLE III
Frequency Distribution of Percent Crude Protein of Seeds of PI 196760, PI 229815, their F_1 and F_2 Seeds, and Reciprocals

	No. plants/class of % protein											
Pedigree	17.5	19.5	21.5	23.5	25.5	27.5	29.5	31.5	N	$\bar{x}$	S.D.	C.V.
PI 196760	4	1	5	4					14	20.4	2.40	11.8
PI 229815					7	5	1	1	14	27.1	1.33	4.9
PI 196760 x 229815 F_1	3	6	5						14	19.8	1.28	6.4
PI 229815 x 196760 F_1					4	8	1	1	14	27.3	1.73	6.4
PI 196760 x 229815 F_2	1	4	2	2					9	21.2	2.17	10.2
PI 229815 x 196760 F_2	1	4	2	5	1				13	21.7	2.40	11.1

TABLE IV
Frequency Distribution of Percent Crude Protein in PI 169760, PI 229815, and their F_1, F_2, and Backcross Progeny

	No. plants/class of % protein											
Pedigree	17.5	19.5	21.5	23.5	25.5	27.5	29.5	31.5+	N	$\bar{x}$	S.D.	C.V.
PI 169760	14	8	1						23	18.6	1.41	7.6
PI 229815				6	8	5	2	1	22	26.8	2.09	7.8
F_1	3	6	7	5					21	21.4	1.72	8.0
BC_1*	5	8	8	6					22	21.1	2.03	9.6
F_2	7	28	29	32	10	5	1		112	22.5	2.48	11.0
BC_2**		1	4	6		1	1		13	23.4	2.64	11.2
Mid-parent										22.7		

* Backcross to PI 169760 ** Backcross to PI 229815
Broad-sense heritability = 49.3%; narrow-sense heritability = 20.1%

the same observation was apparent, r = .446 (Figure 1). However, bean-seed yield and gross-protein yield were positively correlated, r = .964 (Figure 2). Similar results have been observed for cereals (Grant and McCalla, 1949; Stuber, et al., 1962) and soybeans (Johnson, et al., 1968). Although percent protein and seed yield were negatively correlated, there was enough variation to indicate selection for high yield; medium-high protein should be attainable and would probably be the most productive course to take. Stuber (1962), in wheat, likewise found it was possible to combine high yield with high protein.

Generally, high-protein lines contain a higher percentage of nonprotein N, but this need not be, based on per-unit of protein nitrogen (Johnson, et al., 1968). There were enough exceptions in our small population of 21 lines to offer good

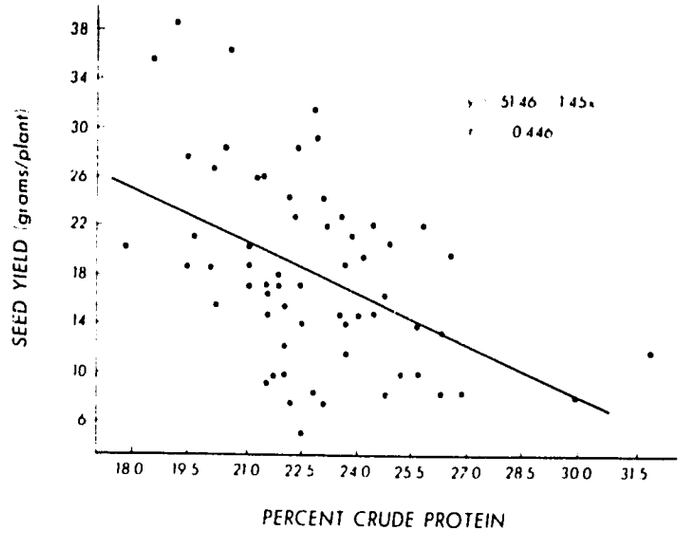

Figure 1. Correlation of seed yield and percent crude protein of F_2 bean plants from the cross PI 169917 x PI 229815.

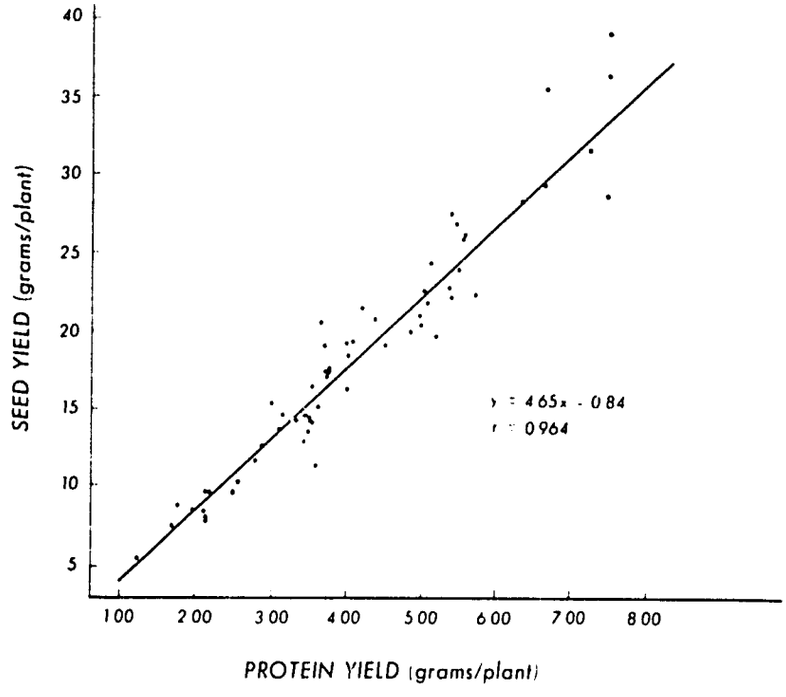

Figure 2. Correlation of seed yield and protein yield of F_2 bean plants from the cross PI 169917 x PI 229815.

opportunity for selection for high-protein and high-amino N (Hackler and Dickson, 1972).

Protein quality

Although beans are high in protein, the level of the sulfur-amino acids (AA) is lower than desirable if beans are to supply a balanced source of protein in conjunction with a cereal.

Kelly (1971) analyzed over 20,000 bean lines and established that there was as much as a sevenfold range in available methionine, with the high lines being more than twice as high as Sanilac, his standard. Of these, the Asgrow Bush Blue Lake lines were among the highest.

In crosses of a low available-methionine line (195-4R68) x Bush Blue Lake 240, a high available-methionine cultivar, there were significant reciprocal differences in the F_1 seed (Leleji, et al., 1972). There was no difference between selfed and F_1 seed when 195-4R68 was the female, but highly significant differences between F_1 and selfed seeds of BBL 240, indicating available methionine is influenced by both maternal and filial genotypes. The effect of the seed genotype became more apparent from the segregation pattern of the F_2 seed. In the F_1 and F_2, the mean available methionine content was between the mid- and low-parent, indicating partial dominance of low- over high-available methionine.

In another unpublished study of AA inheritance involving a 10 x 10 diallel, we found a considerable number, but not all of the AA crosses showed reciprocal differences in the AA content of the seed from the F_1 plants.

In an analysis of 15 sulfur-amino acids in 21 varieties and Plant Introductions (Hackler and Dickson, 1972), there were surprisingly high correlations between many AAs, indicating an excellent chance of selection for more than one at a time; aspartic acid and glutamic acid and methionine were more independent than were the other AAs. Table V shows the correlations obtained for seven of the eight essential AAs; only valine and methionine, methionine and isoleucine, methionine and threonine, and isoleucine and lysine did not show significant correlations with each other. In the same study of 21 varieties, each AA showed wide ranges in concentration, in some cases exceeding 100 per cent difference between lines. Figure 3 shows the scatter diagram of levels of methionine and lysine in 21 lines, and shows the opportunity for selecting for high levels of both AAs at the same time. Levels of N and AA were independent in almost every case.

Conclusions

Breeding for high percent protein in beans of high AA quality will not be simple and will involve good cooperation between breeder and chemist. There are several stages at which selection will have to be made: selection for efficiency in utilization of available nitrogen, whether applied artificially or obtained via root nodules; selection for plant efficiency in transferring the protein precursors from the plant parts to the seed; selection for yield in combination with medium-high levels of protein; and selection for high percentage of plant nitrogen as amino N. At the same time, selection for high methionine would be

TABLE V
Correlations between Essential Amino Acids in Bean Seeds

	Methionine	Isoleucine	Leucine	Phenylalanine	Lysine	Threonine
Valine	.24	.87**	.66**	.66**	.48*	.67**
Methionine		.17	.59**	.63**	.78**	.30
Isoleucine			.68**	.61**	.37	.44*
Leucine				.90**	.64**	.56**
Phenylalanine					.73**	.53*
Lysine						.60**

* and ** = Significant at 5% and 1% levels, respectively

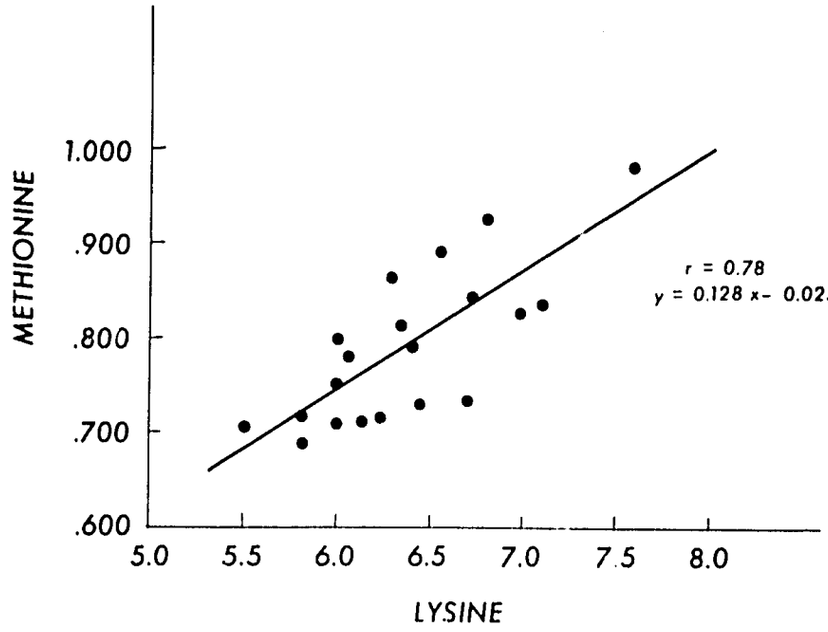

Figure 3. Correlation of methionine and lysine in g/16g N for 21 bean lines.

desirable, and it also would be advisable to consider lysine, tryptophan, and threonine. Any new techniques permitting simpler and speedier chemical analyses will greatly expedite progress; this is especially required for such a program, because it appears that in most cases the inheritance of gross protein and individual AA is polygenic and generally recessive. However, with the aid of modern technology, these improvements in the bean are possible. This should improve the diet of those people whose chief source of protein may be beans.

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AMINO-ACID PROFILES OF *CAJANUS CAJAN* PROTEIN

W. Vernon Royes

Cajanus cajan is nutritionally important to the Caribbean. Genetically it may be regarded as a wild species, in that it is far from the limit of its productive capacity in all respects. Considerable variation for most characters also exists. These are the major reasons for its inclusion in the breeding programs of the University of the West Indies.

Considerable progress has been made over the last twelve years in a number of agro-economic characters. Very recently, protein quantity and quality have made their appearance in the list of priorities. The material presented here was obtained while mastering the techniques of analyzing this plant protein. There is every reason to believe that further refinement is possible.

Materials and Methods

Ten plants, in two groups of five each, of variety GI 26/2 were grown on a soil with a generally low nutrient status. This variety, developed at the University of the West Indies, has been selfed for the last ten generations and genetic variation is expected to be at a minimum. Two pounds (900 grams) of ammonium sulphate was applied to each of the five plants in one group; the control group was not treated.

Mature, but green, pods were harvested from all plants. The seeds were removed from their pods and dried intact in an air oven at 105° for 48 hours. The samples were then pulverized in a stainless-steel "Micromill" (Chemical Rubber Co.). The testas were not removed.

Samples for hydrolysis (c.200 mg) were weighed into ampules and hydrolyzed for 24 hours with 5-ml portions of constantly boiling HCl (triple-glass distilled). The ampules were nitrogen-flushed before sealing. One sample was lost.

After hydrolysis, the considerable deposit of "humin" was removed by filtration through a glass sinter (No. 3) and the clarified solution and washings were taken to dryness on a water bath (60°C) *in vacuo*.

For amino-acid analysis, the dried hydrolysates were made up to 10 ml with 0.01 N HCl, and 0.1 ml aliquots were applied to a 60 x 0.6-cm amino-acid analyzer (Locarte, London) column. The column was eluted over a period of seven hours, using a system modified from that of Piez and Morris (1960), with norleucine as internal standard.

The charts were integrated manually and the results computed, using a FORTRAN II program developed for the I.B.M. 1620 computer.

Results are expressed as percentage amino acid of total amino acids recovered. Tryptophan was not estimated.

Results

The results of the current analyses and those quoted in *Amino Acid Content of Foods and Biological Data on Proteins* by the Nutrition Division of the FAO, are given in Table I.

There are no statistically significant differences in amino-acid content or total protein between the two treatments, except in the case of methionine.

Methionine constitutes 0.86 per cent of the protein in the samples from plants that had received no ammonium sulphate, but only 0.05 per cent for those to which ammonium sulphate had been applied. The difference was statistically significant at the 5 per cent level of probability. Haque and Walmsley (1972, in press) have shown that the nitrogen-sulfur ratio is a far better indicator of the state of sulfur nutrition than are sulfur values by themselves. The amount of nutrient applied in this case is very high. The tendency for legumes to display a greater luxury consumption of nitrogen than of sulfur may have resulted in a disturbed nitrogen and sulfur metabolism. This led to the present result, which was the opposite of that expected.

Because of the low level of occurrence of methionine and the ease with which it is oxidized, technical difficulties arise in estimating its presence in protein. In addition, these two factors lead to high coefficients of variation, and this, together with the low level of statistical significance, strongly suggests that much more reliable estimates are required before complex metabolic explanations can be found.

There are, however, highly significant differences — better than the 1 per cent level of probability — between the current profiles and those quoted by FAO. The values for valine and isoleucine are both 30 per cent higher in the current samples, and those for phenylalanine, lysine, and histidine are 30 per cent, 50 per cent, and 17 per cent higher, respectively, in the samples quoted by FAO.

I have been unable to determine the method of sampling used by the worker quoted by FAO, and the differences may be the result of the material having been sampled at different stages of maturity or to the presence or absence of the testa in the sample. However, in my opinion, at least a major portion of the differences are due to varietal differences.

It should be noted that the protein levels are similar. Critical comparison would, however, be unwise, because much stricter attention would have to be paid to the moisture content of samples. In addition, the method does not take into account tryptophan, which is destroyed, or amide nitrogen, because total protein is computed from the summation of amino-acid residues.

Discussion

Of the differences observed, that of lysine is most encouraging. However, these results do little more than point out the poor state of knowledge. We need

TABLE I

Amino Acid Profiles of *Cajanus cajan* Protein

Amino Acid	Current Data					FAO Quoted Data		
	$Na(NH_4)_2SO_4$ Mean of 5	$2\ lbs(NH_4)_2SO_4$ Mean of 4	No.	Overall Mean	Coeff. of Var.	No.	Mean	Coeff. of Var.
Proline	3.34	3.83	8	3.5	21.0	7	4.5	8.7
Aspartic acid	10.62	10.55	9	10.6	6.8	7	10.9	10.1
Glutamic acid	21.80	21.67	8	21.8	5.4	7	21.3	4.8
Threonine	3.92	4.15	9	4.0	13.9	7	3.3	4.3
Serine	4.40	4.27	9	4.3	10.0	7	4.7	8.6
Glycine	4.26	4.12	9	4.2	6.0	7	3.7	3.8
Alanine	4.80	4.90	9	4.8	8.4	7	4.8	10.3
Valine	5.38	5.42	9	5.4	9.2	7	4.1	5.2
Isoleucine	4.62	4.67	9	4.6	5.0	7	3.5	4.0
Leucine	8.18	7.87	9	8.0	8.1	7	7.2	3.8
Tyrosine	2.32	2.10	8	2.2	6.5	7	2.3	4.0
Phenylalanine	7.26	7.17	9	7.2	13.1	7	9.4	6.0
Lysine	6.08	5.35	9	5.8	9.5	7	8.7	5.0
Histidine	3.68	3.40	9	3.6	7.7	7	4.2	5.7
Arginine	5.62	5.50	8	5.6	8.0	7	5.5	4.7
Cystine	0.40	0.89	4	0.6	46.1	7	1.1	18.0
Methionine	0.86	0.50	9	0.7	27.1	7	0.6	17.6
as Protein % dry wt.	17.94	18.60	9	18.2	6.6	7	20.9	–

to know the relative worth of testa and cotyledon to assess the value of split peas from which the testa is removed. We must determine the changes that occur in the quality and quantity of protein as the seed matures to determine the relative nutritional merit of the green pea and the less acceptable dry pea.

The results also suggest that no gross differences may be expected in amino-acid profiles or total protein over the normal range of nutrient status found in economic crop production. However, this should be determined with greater certainty.

The range of variation that exists in the germ plasm and the relevant heritabilities should be estimated before far-reaching decisions are taken on structure and methods of a breeding program. It should also be noted that the advisability of seeking higher total protein and improved digestibility at the expense of improved amino-acid constitution may vary widely from region to region as the level of protein supplied by animal protein varies.

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BREEDING FOR PROTEIN QUANTITY AND QUALITY IN FIELD BEAN *(VICIA FABA)* AND BARLEY *(HORDEUM VULGARE)*

L. Munck, E. Holmberg, K. E. Karlsson, B. Löfqvist, and J. Sjödin

Programs for nutritional improvement of protein in barley and field bean were started in 1961 and 1967, respectively, in Svalof and Lund. Both crops are used mainly as animal feeds in Sweden, unlike the Middle Eastern and Mediterranean countries, where they are utilized as human foods. In the Swedish barley program, a broad spectrum of analyses was used in inventories to estimate how barley was grown, treated, and utilized, stressing the nutritional aspects (Munck, 1972). Screening methods were developed to estimate the nutritional quality through feeding tests with mice. A screening method for basic amino acids and lysine through dye-binding (DBC) with acilane-orange dye was introduced (Mossberg, 1969). From this experience, a tentative developmental program for barley nutritional quality was proposed. It considered measures to prevent deterioration from moist harvest weather, and emphasized the genetic improvement of the barley plant with respect to protein quantity and quality (Munck, 1972).

Field bean is a marginal crop in Sweden and displays a long time of maturation in the temperate regions. Earliness of high yields is regarded as the key factor of the breeding program. Screening methods were applied to study the effect of selection in a genetically variable material. As a model, the possibility of producing protein isolates from untreated Leguminosae seeds was investigated, comparing field bean with soybean and chick pea (Löfqvist and Munck, 1969).

Improving the Crude Protein Content in the Seed

For malting purpose, barley breeding in Sweden has been traditionally directed toward breeding for low-protein content (N x 6.25). The present range of protein content in commercial varieties is 9 to 12 per cent. Environmental influences seem to be more pronounced than variation due to variety. Our present experience indicates that stable increases of protein content to the level of 14 to 15 per cent might be obtained without affecting yield. When selecting for still higher protein levels, an accumulation of lines with low starch synthesis and small seeds will dominate. These lines also display a low protein production and therefore are worthless. Evidently, yield must always be considered, especially in breeding programs for improved protein quality.

In the field bean, selection for low and high crude-protein content was performed during three consecutive years on plant-levels in three different populations. All the populations responded to the selection and the data obtained in

1971 from one of these are demonstrated in Figure 1. Seed size and other yield parameters were not significantly influenced either by the high- or the low-protein selection.

Improving the Amino-Acid Balance

In barley, an increase of crude-protein content – due either to variety or to fertilizer – most often results in a decreased concentration of essential amino acids, such as lysine, in percentage of protein. This is well known and is found in all cereals except oats (Munck, 1972). However, calculated on a whole-seed base, essential amino acids (%) do increase; from a nutritional point of view, this indicates an improvement.

In the field bean, we found a pronounced effect of selection for high- and low-protein content in the seed (Figure 1). However, if we consider the contribution of amino acids to the difference in nitrogen between "high" and "low" lines, arginine displays a very high increase, whereas the important sulfur-amino acids are almost at "nil" level of contribution (Table I A). In barley, similarly, ammonia, glutamic acid, and proline are highly increased, whereas lysine, threonine, and the sulfur-amino acids show a low level in their N contribution to the total N increase. It is obvious, especially in field beans, that the plant breeder must consider if it is wise to select for increased crude protein. The meager effect of increasing protein content, as reflected in the sulfur-amino acid levels, was verified by Adams (1972, these Proceedings) in *Phaseolus*. These considerations should be extended to other legumes. The unbalanced amino-acid patterns obtained with increased protein content in barley and field bean are the result of increased production of storage proteins with specialized amino-acid composition.

In barley, the DBC method as related to Kjeldahl protein was used in the World Collection as a screening method for cultivars high in lysine. A high-lysine gene (lys) comparable with opaque-2 and floury-2 genes in maize was found in the World Collection (Table I B). The new barley gene also displayed increased content of other essential amino acids (Munck, et al., 1970). In Figure 2, it can be seen that the recessive lys gene segregates with respect to (1) DBC (mmol/16gN) and (2) lysine (g/16gN) content irrespective of crude protein content. The lys gene thus represents an example of a deviate from the "natural law" of the negative correlation between essential amino acids (g/16gN) and crude protein (%).

In legumes, an increased content of lysine might be advantageous, considering their use in mixed diets with cereals. We therefore tried to use the DBC method for selection in two years. However, it was not possible to obtain a selection response with regard to DBC (mmol/16gN). This may be due to a variation of high-molecular protein in the analyzed material overlapping a possible variation in basic amino-acid content (g/16gN), as suggested in Figure 3. It should be realized that the acilane-orange dye at pH 2.5 precipitates proteins in the meal suspension, and the subsequent decoloration is measured in a short-path, cuvette

TABLE I

A. The Amino-Acid Contribution (%) to the Varietal N Increase in Normal Barley and in the Broad Bean

Amino acids	Amino-acid contribution to N increase %	
	Normal Barley	Broad Bean
lys	2.4	4.4
his	3.2	3.4
NH_3	21.6	7.4
arg	6.5	40.2
cys	0.7	0.3
met	0.8	0.0
glu	17.7	7.4
pro	12.2	3.1
N recovered as amino acids %	93.0	92.0
Protein (N x 6.25) increase g/100g	6.4	9.0

B. Amino-Acid Composition (g/16gN) of Normal Barley, High-Lysine Barley, and Broad Bean

Amino acids	Normal Barley CI 4362*	High-lysine Barley (Hiproly)	Broad Bean
lys	3.0	4.0	6.3
his	1.9	2.1	2.7
NH_3	3.2	2.8	1.8
arg	4.3	4.8	10.8
cys	1.9	1.7	2.2
met	1.8	2.0	0.7
glu	25.2	21.7	17.9
pro	12.8	10.8	4.7
protein %	17.2	17.7	33.8

*Regard as isogenic with Hiproly, with the lys gene as major difference.

colorimeter. Low-molecular peptides, etc., that contain basic groups thus are not precipitated. As shown in Figure 3, the percentage of high-molecular (TCA-precipitable N) is very well correlated with DBC (mmol/16gN) in three field bean varieties at widely different stages of development. It must then be stressed that the DBC measurement could reflect *both* a rise in high-molecular protein with increasing maturation *and* a variation in basic amino acids in this protein. The DBC method could consequently be used as a screening tool for high content of basic amino acids, provided that the stage of maturation at harvest is well defined and equal for all samples under comparison.

Concerning the sulfur-amino acids, no convenient screening method is yet available. However, we are trying to develop such a method, based on a micro-

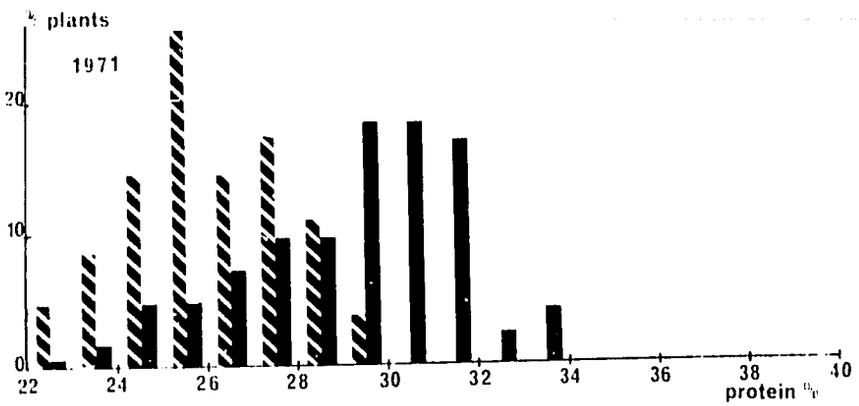

Figure 1. The effect of three years' selection for high (black columns) and low (striped columns) crude-protein (N x 6.25) content in field beans.

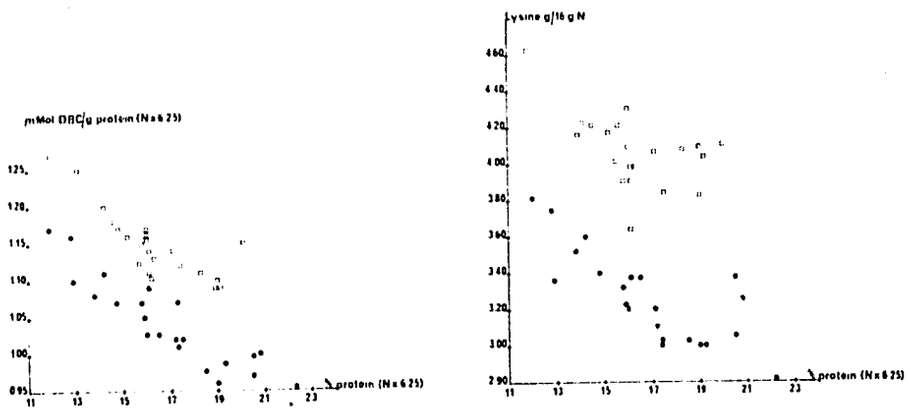

Figure 2. Segregation for (A), dye-binding (mmol acilane-orange/gN x 6.25) and for (B), lysine (g/16gN) in F_2 plants from a cross between Hiproly x normal barley. Dots = normal lysine segregants; open squares = high-lysine segregants.

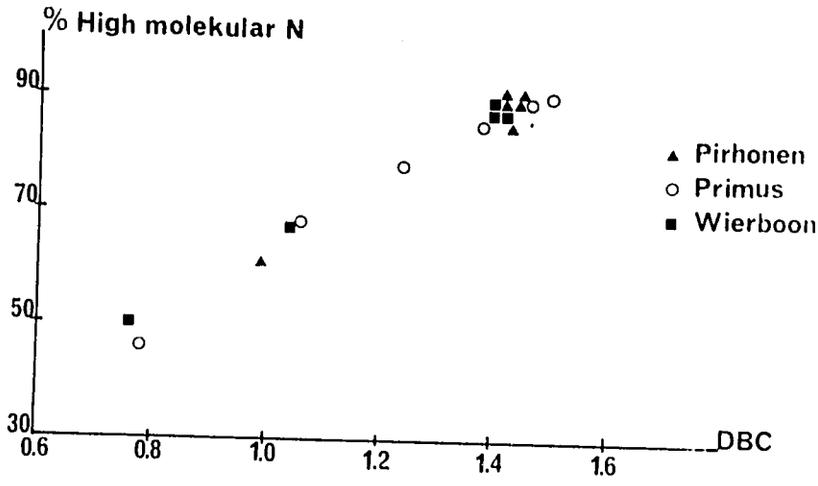

Figure 3. Correlation between percentage high-molecular, TCA-precipitable N and dye-binding (mmol acilane-orange/gN x 6.25) in broad beans.

colorimetric titration procedure. The method is based on the possibility of establishing a correlation between organic sulfur and sulfur-amino acids (methionine and cystine) in the legume samples. The capacity of the equipment is in the order of 10 analyses per hour. The seed meal is pyrolysed in hydrogen atmosphere at 550°C. The pyrolysis products are then reduced to hydrogen sulfide over a platinum catalyst at 1150°C. The generated sulfide is then titrated in a colorimetric cell, using silver ions as titrant. In order to avoid interference from hydrogen cyanide and volatilized inorganic sulfur, certain pretreatments of the samples had to be undertaken. So far, we have obtained good correlation between ion-exchange chromatography analysis and the pyrolysis method, and a preliminary selection was made in spring, 1972. Analysis of this selection is under way at the moment and will give indications concerning the applicability and validity of the method as well as the possibility of selecting for sulfur-amino acids in field beans.

Nutritional Quality and Toxic Substances

The increase in lysine in barley was caused by the doubling of albumins rich in essential amino acids (Munck, 1972). This was reflected in improved nitrogen retention and NPU in mice and rats. The availability of lysine in cereals is especially poor, as shown in experiments with rats and swine. Results indicate that the increased albumin level caused by the lys gene is available in barley

TABLE II

Nutritive Quality of Broad Bean, Chick Pea, and Soybean Protein Concentrates and Unextracted Residue Fed to Mice. Methionine Added to 2.5 g/16g N

Diets	Dietary crude protein %	Growth 14 days (g)	Consumed protein (g)	PER
Protein concentrates				
Broad bean	13.8	9.7	6.7	1.46
Soybean	11.6	6.5	4.8	1.36
Chick pea	11.8	10.3	5.4	1.92
Unextracted residue				
Broad bean	9.6	6.7	4.1	1.61
Soybean	10.8	1.7	3.4	0.47
Chick pea	9.3	3.9	3.7	1.07

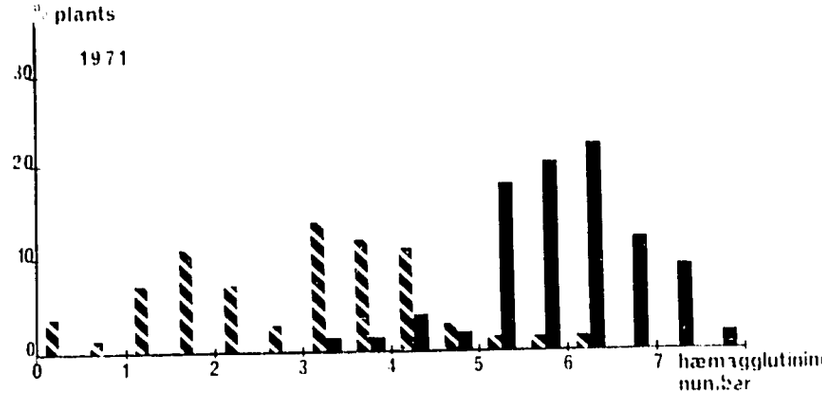

Figure 4. Selection for high (black columns) and low (striped columns) hemagglutinin content in field beans.

(Munck, 1972). Net protein utilization for rats was similar for lys barley and opaque-2 maize (Eggum, 1972, personal communication).

The protein-evaluation problem is much more complex in legumes than in cereals, both because of considerations of amino-acid content and availability and the effects caused by widely differing toxicants. This can be exemplified by the disease favism in man, resulting from the ingestion of *Vicia faba*. Only certain genotypes are sensitive, and the disease is not reproduceable in laboratory animals. Consequently, this toxic factor was not considered in our field-bean breeding program.

By means of protein isolation in more or less pure form, other deleterious substances could be eliminated. The response with laboratory animals (mice) fed whole-seed meal, protein isolate, and residual fraction (obtained after protein solubilization) could be used to trace toxic and availability effects in legumes and to localize them to different fractions. Thus, mouse growth is equally high when the animals are fed the three kinds of field-bean diets mentioned above, with methionine supplementation. The unextracted protein fraction in chick pea displays a low performance when compared with the whole seeds and the protein isolate. This is in contrast to the soybean, in which the isolate is superior to both whole soybean and unextracted soybean protein, both with methionine supplementation. This demonstrates an improved nutritional quality obtained through protein fractionation, shown in Table II for the three kinds of legumes (values from whole-seed meal not reported here).

Even if, in mice studies, the nutritional quality of whole broad bean is as good as that of its protein concentrate, we have been looking at the variation in hemagglutinins and antitrypsins. We have been successful in selecting for high and low hemagglutinin content (Figure 4). We have yet to ascertain if the considerable differences obtained will be reflected in the physiology of animals fed extreme "high" and "low" diets. Our results with tryptic inhibitors are more difficult to interpret. A variation in morphology of the seeds seems to interfere with our extraction technique, and this leads to values too low for plants with hard seeds.

From the comparison between barley and broad bean, it could be concluded that further selection in legumes for high content of sulfur-amino acids (g/16gN) at a high level of crude protein in the seed should be profitable, just as high lysine (lys) barley, which was obtained by selection, deviates from the negatively correlated lysine g/16gN-crude protein in normal barley.

Acknowledgement

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THE AMINO-ACID COMPOSITION OF *VIGNA UNGUICULATA* (COW PEA) MEAL IN RELATION TO NUTRITION

D. Boulter, I.M. Evans, A. Thompson, and A. Yarwood

Cow peas are an important part of the diet of many West Africans and a considerable literature on them exists. Rachie (these Proceedings) has outlined the reasons why *Vigna unguiculata* (cow pea) has been chosen as one of the main legumes of the legume grain-improvement program of the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Although it is realized that the first priority in the cow-pea grain improvement program is to increase yields, presumably by controlling pests and diseases, nevertheless, it is also felt that an attempt should be made to increase the concentration of protein and/or its percentage of sulfur-amino acids, since it would appear that these amino acids are limiting from a nutritional viewpoint (Harvey, 1970; Sellship, 1962).

Increasing the protein content of cow peas would give more utilizable protein per area cultivated, and increasing the sulfur-amino acid content would improve the percentage of protein biologically available per given weight of meal eaten. On this basis, a cooperative program of work has been instituted by the Botany Department, University of Durham, and the International Institute of Tropical Agriculture at Ibadan. This paper sets out the development of our ideas and gives some preliminary results. While these refer to cow pea in West African diets, they are of general interest for legume grain-improvement programs with other legumes for other regions.

Before we could attempt to determine the nutritional status of the cow pea with a view to its improvement, it was necessary to satisfy ourselves that anything found out about cow peas was relevant to the situation in West Africa, because cow peas are not eaten alone but as a "minor" part of the diet. Thus, the fact that cow peas might be deficient in sulfur-amino acids would be unimportant if they were part of a diet that was not so deficient overall.

The staples used in Nigerian diets are: (1) sorghum and/or millets; (2) yams; (3) cassava; (4) sorghum or millets and yams; (5) yams and cassava. All of these meals have much lower concentrations of protein than does cow pea, especially yams and cassava. The root crops, yams and cassava, also are deficient in sulfur-amino acids. The sorghums and millets are marginally satisfactory for methionine but are low in cysteine and deficient in lysine. Although there have been no amino-acid composition determinations on diets themselves, and diets vary from area to area (Nicol, 1959; Johnson, et al., 1969; Hauck, 1961; Collis, et al.,

1962), it is likely that areas in which cassava, yams, or mixtures of these, and areas with a sorghum or millet and yam staple, will be deficient overall in sulfur-amino acids. Where sorghum and millet alone are the staples, the first limiting amino acid may be lysine rather than the sulfur-amino acids. At present, most areas use up to 5 per cent legumes (usually mainly cow peas) in their daily diets. Legumes contain a much higher concentration of protein than do the above staples, and their protein is more than adequate in its lysine content and in the other essential amino acids, except for the sulfur-amino acids and tryptophan. Thus, although legumes represent only a small part of the diet, they contribute significantly to the protein requirements; increasing their contribution probably represents the easiest way to increase the protein concentration in a diet. However, this alone would be of limited value unless the percentage of sulfur-amino acids also was increased, as in most diets these amino acids are already limiting. In those areas where lysine is likely to be critical, it may be necessary to increase contents of both sulfur-amino acids and especially to avoid increasing the content of one while, at the same time, decreasing that of the other.

There are three different approaches, then, to diet improvement in this respect:

1. Supplementation with sulfur-amino acids.
2. Changing the proportions of the diet components and, if necessary, adding new ones.
3. Improving the amino-acid profile of one component via a breeding program.

Data in the literature are limited, so it is not possible to decide unequivocally which of these three approaches is the best and, in any case, different solutions may apply to different regions. It is probable, therefore, that all three approaches should be followed. However, largely for socioeconomic reasons, it was agreed to exclude the first approach, as this requires (a) production of the necessary amino acids or their purchase; (b) their distribution; (c) satisfactory and acceptable addition of them to the diet.

The second approach requires the establishment of typical daily food intakes and the determination of their amino-acid composition, as well as that of their component parts, and then changing, as necessary, their relative proportions in the light of local economics and social practices.

The third approach is being pursued by the International Institute of Tropical Agriculture. For its success, it requires the establishment of screening methods for total protein and sulfur-amino acids that are satisfactory for use in breeding programs for improved grain legumes. We have already referred to the reasons for the initial choice of cow pea, rather than other legumes.

Cow-pea varieties with improved protein-amino-acid profiles would also be important in feeding postweaned infants, where the amount of food comfortably eaten is not large. They could also play an important role in feeding the growing urban population, especially because that population is cut off from incidental protein supplement by leaves and small animals of the "bush."

Results

1. *Nutrition*

The amino-acid composition of cow pea var. "Prima" meal is given in Table I.

The quality of cow-pea protein was assessed using the Thomas-Mitchell procedure for Biological Value. Unsupplemented cooked cow-pea meal was assayed, as well as cow-pea meal supplemented with (a) L-cystine up to the level of L-cystine found in a standard egg albumen; (b) L-cystein and L-methionine up to the levels found in the egg albumen; (c) L-methionine up to the combined levels of L-cystine and L-methionine found in the egg albumen. The results are given in Table II.

TABLE I
Amino Acid Composition of Cow-Pea Globulin and Meal (g Amino Acid/16gN)

	Globulin[1]	Sealed tube Prima meal[1]	Reflux method Prima meal[2]
Asp	12.48	9.65	10.98
Thr	3.56	3.47	3.65
Ser	5.92	4.89	4.79
Glu	19.03	15.60	16.06
Pro	4.33	3.28	3.80
Gly	3.24	4.01	3.98
Ala	4.19	3.81	3.99
Val	6.36	5.09	4.80
Met	1.57	1.40	1.12
Ile	5.37	4.46	4.17
Leu	9.23	7.47	7.30
Tyr	3.91	2.86	3.35
Phe	7.93	5.05	8.04
His	3.01	3.06	3.04
Lys	6.61	6.27	6.58
Arg	7.24	6.36	6.69
½ Cys[3]	0.40	1.01	0.88
Try	0.79[4]	0.85[4]	0.85[4]
g/16g N_2	105.17	88.59	91.07

[1] Duplicate analyses were performed at three different times: 20, 48, and 72 h. Values for Asp, Glu, Pro, Gly, Ala, Met, and Tyr, are average values of all determinations. Ser and Thr values were extrapolated to zero time assuming first-order kinetics (Hirs, et al., 1954). Values for Lys, His, Arg, Val, Ile, Leu, and Phe are the maximum values obtained after 72 h.

[2] Values on the average of three analyses. Refluxing was carried out under N_2 for 24 h, as described by Cavins, et al. (1972).

[3] Determined as cysteic acid after performic acid oxidation.

[4] Average values. Figures for tryptophan using the methods of Horn and Jones (1945), Villegas and Mertz (1971), and Spies and Chambers (1949) respectively were: 0.61; 0.79; 0.98 (globulins), and 0.70; blank; 1.00 (meal). The method of Villegas and Mertz could not be used with meal.

TABLE II
Mean % True Digestibility, Biological Value, and Net Protein Utilization of the Five Test Diets*

	True digestibility % ± S.E.	Biol. value % ± S.E.	Net protein utilization % ± S.E.
8% Alb.	97.87 ± 0.72	101.72 ± 2.54	99.52 ± 1.85
Cow-pea meal (unsupplemented)	87.04 ± 0.70	58.17 ± 2.31	50.60 ± 1.83
Meal + cystine (a)	90.48 ± 2.88	80.26 ± 1.87	72.47 ± 0.94
Meal + cystine and methionine (b)	86.80 ± 0.47	94.61 ± 1.26	82.12 ± 1.07
Meal + methionine (c)	85.03 ± 1.16	95.84 ± 1.45	81.46 ± 0.87

*Feeding trials conducted by A. Thompson and G. Simpson at the Department of Agricultural Biochemistry, University of Newcastle upon Tyne.

2. *Screening Methods*

(a) Total Protein: Using ground cow-pea meal, the UDY dye-binding method for estimating total protein has been shown to be reproducible to ±1 per cent, the error averaged over 21 varieties (Table III). In order to obtain reproducible results, it was essential to use the same weights of meal (320 mg) and the same volumes of dye reagent (40 ml).

The average error in the estimation of protein by micro-Kjeldahl method was ±2.5 per cent and Figure 1 gives a calibration line for the percentage of protein (based on Kjeldahl nitrogen) versus unbound dye concentration. The method was accurate to ±12 per cent of the protein concentration; differences less than this are, therefore, not significant.

(b) Sulfur-Amino Acids: Seventeen varieties of cow-pea meals, both untreated and extracted with 70 per cent (v/v) ethanol, were examined for their total sulfur contents and for the amounts of sulfur-amino acids. The material was dried for 2½ hours in a vacuum oven at 60° prior to analysis. Total sulfur was determined by wet digestion with perchloric-nitric acid mixture, and subsequent turbidimetric analysis of barium sulphate (Mottershead, 1971). Methionine was determined on 22-hour hydrolysates of meals with 6N HCl at 105°C, by means of LOCARTE autoanalyser; cysteine plus cystine were determined by performic acid oxidation (Schram, et al., 1954), and subsequent analysis of cysteic acid on a LOCARTE autoanalyser. The method was checked with various compounds, and the percentage recovery of total sulphur and the comparative data, are given in Table IV. Table V shows the data for unextracted meals, and Table VI for alcohol-extracted meals.

The results show that among the examined varieties of cow-pea meals, there is only a small variation in the contents of total sulfur and sulfur of sulfur-amino acids. The remaining four varieties were examined for total sulfur content and

TABLE III
Nitrogen Content of 21 Varieties of Cow Pea

Variety	% N	% Protein (N x 6.25)	Concentration of unbound dye
57 New Era	5.17 ± 0.01	32.3 ± 0.04	0.481 ± 0.003
70 No. 58-185	5.37 ± 0.06	33.6 ± 0.35	0.479 ± 0.006
76 Prima	4.76 ± 0.07	29.7 ± 0.42	0.489 ± 0.003
95 Renostar	4.23 ± 0.06	26.4 ± 0.37	0.549 ± 0.006
176 C5723-11	4.49 ± 0.18	28.0 ± 1.12	0.522 ± 0.008
179 Louisiana Purchase	4.29 ± 0.02	26.8 ± 0.14	0.537 ± 0.009
201 Cal Blackeye	4.59 ± 0.03	28.7 ± 0.19	0.517 ± 0.008
335 Jebba Pea A	4.87 ± 0.05	30.4 ± 0.31	0.444 ± 0.005
494 C5714-14	4.95 ± 0.01	30.9 ± 0.69	0.537 ± 0.003
74 Kwara Ex-Kwoi "C"	4.0 ± 0.14	25.0 ± 0.87	0.505 ± 0
91 51C-421-2	4.13 ± 0.20	25.8 ± 0.12	0.512 ± 0.005
530 Ibadan-2	3.67 ± 0.01	22.9 ± 0.06	0.556 ± 0.003
946 Kano 2461	4.52 ± 0.03	28.3 ± 0.19	0.447 ± 0.01
1101 Kano 6032	4.12 ± 0.01	25.8 ± 0.06	0.529 ± 0.005
30 No. 112	5.36 ± 0.04	33.5 ± 0.25	0.434 ± 0.0002
31 Emma	4.96 ± 0.26	31.0 ± 1.62	0.483 ± 0.008
32 Iran Grey	4.76 ± 0.59	29.8 ± 3.69	0.483 ± 0.002
37 Pale Green	4.99 ± 0.25	31.2 ± 1.56	0.495 ± 0.004
1418 No. 189230	4.06 ± 0.08	26.5 ± 0.50	0.527 ± 0.01
1499 Q. Anne	5.53 ± 0.44	34.6 ± 2.75	0.400 ± 0.003
1503 Cardinal	5.43 ± 0.11	33.9 ± 0.69	0.466 ± 0.008

TABLE IVa
Recoveries of Sulfur from Various Compounds

Type of Sample	% Recovery of total sulfur
K_2SO_4	100.5 ± 0.5
L-Cystine	103 ± 0
Lysozyme (from chicken white)	95 ± 1
L-Methionine	100 ± 1

TABLE IVb
Relationship between Total Sulfur (% S_t) and Sulfur-Amino Acids (% S.a.a.)

Type of Sample	% S_t	% S.a.a.
Lysozyme (from chicken white)	2.06 ± 0.04	2.04
Albumen (from egg)	1.41 ± 0.01	1.36
Casein – fat free	0.69 ± 0.028	0.69

TABLE V
Relationship between Total Sulfur (% S_t) and Sulfur-Amino Acids (% S.a.a.) for 17 Varieties of Cow-pea Meals*

Variety of Cow pea	% S_t	% S.a.a.
IV u Prima (our main stock)	0.216 ± 0.01	0.164
76 Prima	0.190 ± 0.018	0.153
91 51C-421-2	0.202 ± 0.006	0.151
95 Renostar	0.208 ± 0.011	0.154
179 Louisiana Purchase	0.224 ± 0.016	0.150
335 Tebba Pea A	0.233 ± 0.015	0.164
494 C5714-14	0.201 ± 0.005	0.138
530 Ibadan-2	0.204 ± 0.004	0.134
1101 Kano 6032	0.204 ± 0.012	0.145
946 Kano 2461	0.247	0.151
201 Cal Blackeye	0.224	0.150
74 Kwora ex-Kwoi "C"	0.237	0.161
70 No. 58-185	0.173	0.161
37 Pale Green	0.205	0 152
57 New Era	0.196	0.144
30 No. 112	0.245	0.174
176 C5723-11	0.232	0.171

% S_t = .2141 + .7966 (% S.a.a. – .1533

*Up to four separate digestions were performed, shortage in available material being a limiting factor.

the data (Table VII) confirm this observation. The proportion of total sulfur contained in sulfur-amino acids is high, between 61 and 93 per cent for unextracted meals of different varieties.

The linear relationship between total sulfur and sulfur-amino acids is significant at the 5 per cent confidence level, although not at the 1 per cent, when analysis of variants is applied to the data. The correlation between these two parameters for the meals extracted with 70 per cent ethanol appears to be stronger, but because of the smaller sample size it was less accurately established. The relationship between the amounts of protein-sulfur and protein-nitrogen in cow-pea meals is given in Table VIII. Nitrogen/sulfur ratios are reasonably constant and the average value is 28.7, with the lowest and highest values deviating from it by 11 per cent and 25 per cent respectively.

Discussion

Literature figures for the amino-acid composition of cow-pea meal are often incomplete and vary considerably one from another. However, apart from the sulfur-amino acids, tryptophan, leucine, and isoleucine values of Khan and Bar-

TABLE VI
Relationship between Total Sulfur (% S) and Sulfur-Amino Acids (% S.a.a.) for 9 Varieties of Cow-Pea Meals Extracted with Boiling 70% (v/v) Ethanol. Values Calculated on the Basis of the Weight of the Meals Prior to Extraction.

Cow-pea variety	% S_t (Protein S)	% S.a.a.
Prima V.u.	0.160 0.161	0.164
494 C.5714-14	0.149 0.153	0.138
95 Renostar	0.149 0.145	0.154
179 Louisiana Pur hase	0.142 0.141	0.150
76 Prima	0.156	0.153
335 Tebba Pea A	0.151	0.164
91 51C-421-2	0.149 0.151	0.151
1101 Kano 6032	0.157	0.145
530 Ibadan-2	0.123	0.134

The regression line = % S_t = .1491 + .5733 (% S.a.a. .1507)

ker (1957), we are in substantial agreement with their figures and also with those of Busson, Carbiener, and Berperet (1959).

Lack of agreement in reported amino-acid composition values for a variety arise because of: (1) different origins of source materials; (2) varying conditions of hydrolysis; and (3) varying methods of analysis.

With regard to (1), it is important that the plant material has been grown under standard conditions, and that only normal seeds have been selected for analysis. With regard to (2), the oxygen-free, sealed-tube metho ' oore and Stein, 1963) is to be preferred; although providing a large volume of acid to protein, the reflux method under nitrogen gives essentially the same results (see

TABLE VII
Sulfur Content of Some Cow-pea Meals

Cow-pea variety (extracted with 50% ethanol)	% S_t
1418 No. 189230	0.158
1499 Q. Anne	0.177 ± 0.017
1503 Cardinal	0.212 ± 0.009
32 Iran Grey	0.206 ± 0.003

TABLE VIII
Relation Between the Amounts of Protein Sulfur and Protein Nitrogen in Cow-pea Meals

Cow-pea variety	% S (protein)	% N (protein)	Nitrogen/ sulfur
Prima V.u.	0.160 0.161	4.35 4.11	27.2 25.5
494 C.5714-14	0.149 0.153	4.26 3.98	28.6 26.0
95 Renostar	0.149 0.145	3.89 4.09	26.1 28.2
179 Louisiana Purchase	0.142 0.141	3.77 3.73	26.5 26.5
76 Prima	0.156	4.90	31.4
335 Tebba Pea A	0.151	5.16	34.2
91 51C-421-2	0.149 0.151	4.60 4.47	30.9 29.6
1101 Kano 6032	0.157	4.05	25.8
530 Ibadan-2	0.123	4.42	35.9

Table I, for example). With regard to (3), ion-exchange (Moore and Stein, 1963) or gas-liquid chromatography (Gehrke, et al., 1971) are recommended. Methionine can be determined accurately by either of these methods; cystine should be determined as cysteic acid (Spackman, et al., 1958) and tryptophan by at least two different methods.

As compared with the FAO reference protein (FAO/WHO, 1965), the limiting amino acids of cow-pea meals are methionine, cysteine, and tryptophan. Considering the sulfur-amino acids together (see below), there is a need to increase both these and tryptophan by 43 per cent in comparison with the FAO standard.

The feeding experiments show that cooked cow pea meal is a reasonable source of protein (B.V. of the order of 60 per cent), but that it is deficient in cysteine, hence the increase in B.V. on supplementation with this amino acid. It is also clear that most of the added methionine was used to supply a deficiency of cysteine; methionine can be metabolized to cysteine in the rat, but the reverse reaction does not occur to any great extent. From the amino-acid composition data, it appears likely that methionine is also required and that some of the exogenously added methionine in the feeding experiments went to supply this need. Unequivocal evidence of this will rest on a further feeding experiment, i.e., if cysteine is added to the diet until there is no response and then a further response is elicited by added methionine.

It is also clear that any other limiting amino acid is of minor importance, since B.V. of 95 per cent were obtained by sulfur-amino acid supplementation alone and that, in this variety, heat-stable antimetabolites are not important. It is known that man has essential amino-acid requirements similar to that of rats, but these findings cannot be extrapolated quantitatively to man.

Before attempting to develop suitable screening procedures for increased protein concentration and percentage sulfur-amino acids in cow peas, it is well to consider whether attempts to achieve this appear likely, theoretically. Can we, in fact, change the amounts and quality of proteins in this direction without making seeds inviable? Boulter, Evans, and Derbyshire (1972) have discussed this in a recent paper, concluded that it should be possible and, further, that successful varieties with more sulfur-amino acids are likely to have changed proportions of the storage proteins, which constitute the major part of the seed proteins

Screening methods are needed to (1) screen the world collection of varieties, races, etc.; (2) determine heritability of the nutritional quality; (3) be used in the grain-improvement breeding program. Initially, we are concentrating on methods for (1). Screening methods must be reasonably accurate, fast, and use small amounts (mgs) of material, if they are to be of use in breeding programs.

With cow pea, existing methods for screening total nitrogen as a measure of protein are satisfactory, and a preliminary screening using the UDY Protein Analyzer could be used to select high-protein varieties, which subsequently could be subjected to automated micro-Kjeldahl analysis (Varley, 1966).

Figure 1 shows that not all the varietal values fall on the calibration line, and the divergence of ±12 per cent is greater than the overall experimental error of the two techniques. This is due to one or the other of the following: (1) inhomogeneity of the sample of material used; (2) variation in the amount of starch in the samples; (3) difference in the amounts of the basic amino acids – histidine, lysine, and arginine. The percentage protein content of the 21 varieties ranged between 25 per cent and 34 per cent (see Table III). High dye-binding values in the initial UDY screen could be due to a high content of protein with a constant basic amino-acid composition, or to a somewhat lower protein content with higher-than-average basic amino-acid composition. Varieties with the latter composition would be eliminated during the second screen, using the automated Kjeldahl method.

For sulfur-amino acids, existing methods, i.e., ion-exchange chromatography, GLC, and microbiological methods, are too time-consuming.

Although for the small range of varieties examined (Table IV), total sulfur cannot be regarded necessarily as being a good measure of sulfur-amino acids, the preliminary results are encouraging, and we are presently extending the range of the varieties investigated. If the correlation is sustained, it will be necessary to see if total sulfur correlates not only with the sulfur-amino acids, but also with PER. This will require the development of fast biological screens, using smaller amounts of material than for the rat-feeding experiments.

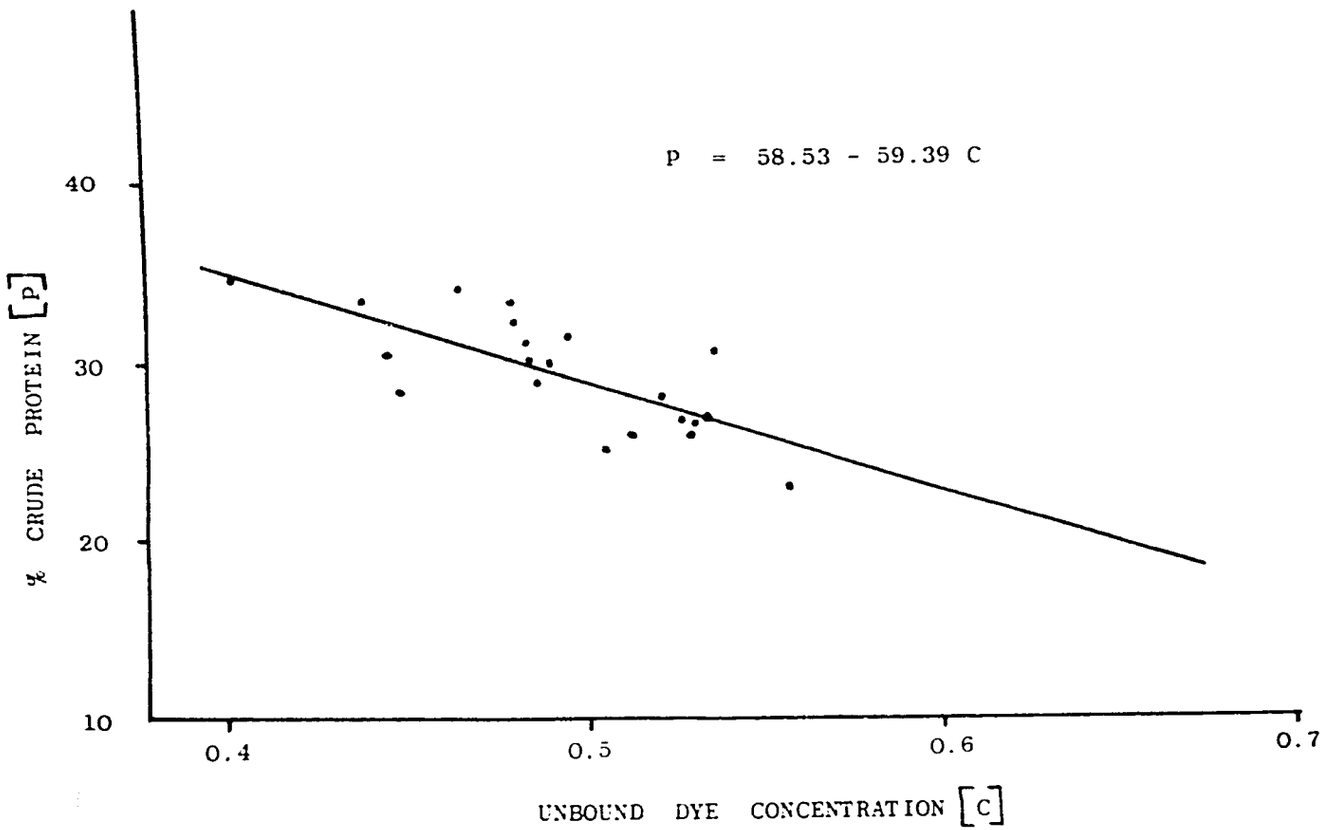

Fig. 1. Correlation between dye-binding values and micro-Kjedahl. P = 58.53-59.39 C; r = -0.722 for 19 degrees of freedom.

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THE JOINT FAO/IAEA PROGRAM ON THE USE OF NUCLEAR TECHNIQUES TO IMPROVE SEED PROTEIN

Björn Sigurbjörnsson and Robert Luse

In June, 1968, an FAO/IAEA Panel of Experts was convened at Röstanga in Sweden to consider new approaches to breeding for plant-protein improvement. The experts (geneticists, plant breeders, analytical chemists, biochemists, physicists, and nutritionists) prepared a set of recommendations, which have served as guidelines for subsequent program activities by FAO and IAEA, on the use of nuclear techniques for seed-protein improvement (Panel Proceedings, 1969). A general conclusion of this Panel was that plant breeding can provide the needed acceleration in the production of high-protein and high-lysine crops, provided rapid mass-screening techniques for protein and for certain limiting amino acids are developed. Although the Panel was primarily concerned with improvement of cereal-seed protein, it also paid attention to legumes, especially the need to free many legume species of toxic substances. As a result of the Panel recommendations, a Coordinated Research Program was initiated in 1969 with research contract-holders working on rice, wheat, and soybeans in various Asian and Latin American countries.

In 1970, an FAO/IAEA Symposium on Improving Plant Protein by Nuclear Techniques was held in Vienna, attended by scientists from 33 countries. Most of the papers dealt with the use of radiation or radioimmetic substances to induce changes in the protein content of seeds, and with new and improved techniques of seed-protein analysis and mass screening (Panel Proceedings, 1970).

In 1971, the government of the Federal Republic of Germany concluded an agreement with the IAEA to sponsor a five-year program of research on the use of nuclear techniques to improve seed protein. Nearly one million dollars was granted to support the program, which at present has research contracts with 16 institutes in developing countries in Asia, Latin America, and Africa. In addition, the grant supports developmental research in plant breeding and nutrition at institutes in Germany, as well as services and research carried out in the IAEA laboratory near Vienna. This direct technical participation by scientists in the donor country is an important feature of the program, because it directs the resources and skills of these institutes toward finding solutions to problems of the developing countries.

In addition, the program provides for coordination of research activities

through visits by consultants and advisers, through publication of results, and through annual coordination meetings.

The first full-scale coordination meeting within the expanded joint FAO/IAEA/GSF program was held at the headquarters of the host institute, the Gesellschaft für Strahlen- und Umweltforschung (GSF), at Neuherberg near Munich, Germany, 26-30 June, 1972 (see Appendix to this paper). It was attended by all research contract holders, in addition to a number of scientists from developed countries who cooperate under the program by holding research agreements with the IAEA or who had been invited to advise on special aspects of the program. The scientists reported highly encouraging results in the improvement of protein quantity and quality in various cereals, as listed below.

Hans Doll and co-workers from the Danish Atomic Energy Commission, Risö, reported screening 6,000 lines of mutagen-treated barley and detecting six high-lysine mutants. The lysine content is from 15 to 45 per cent higher than that of the mother varieties, with a maximum yield depression of 25 per cent.

M.S. Haq, of the Atomic Energy Centre in Dacca, Bangladesh, reported five rice mutants with protein content higher than the parents, and with comparable yield. These mutants were initially screened on the basis of earliness.

Y.E. Chu from Taiwan found that irradiation of *japonica* rice seeds results in a reduction of average protein content in M_2 generations. Standard deviation is increased, as compared to the irradiated control. Up to a limit, the higher the dose, the higher the deviation. This material is now being grown in the M_3 and later generations, where more quantitative results may be expected.

C. Harn, of the Radiation Research Institute, Seoul, Korea, reported that mutant rice lines(M_3 – M_5) had protein contents from 70 to 170 per cent that of the mother line. The protein content of the high-protein mutants increased with N-fertilization much more than in the mother line. Most importantly, the grain yields of high-protein mutants were not statistically lower than those of the mother variety.

S. Tanaka, of the Institute of Radiation Breeding in Ohmiya, Japan, reported the protein content of 718 rice mutants derived from both a nonglutinous and a glutinous variety, 754 native varieties collected from many remote areas throughout Japan, and 265 commercial varieties grown in 817 locations. The variation of protein content was the largest in mutants (4 to 16 per cent), relatively large in native varieties (5 to 12 per cent), and the smallest in commercial varieties (5.5 to 10 per cent). This suggested the potential of breeding high-protein rice varieties. Because Japanese rice varieties have been bred without the aim of protein improvement, it was thought that mutants can play an important role in this field. As several high-protein M_3 lines, which segregated for no visible mutants, have been obtained, it is expected that it will be possible to breed high-protein rice varieties with the superior agronomic traits of the original variety.

In general, it may be said that cereal breeders using induced mutations and careful selection and screening techniques have found high-protein mutants with a frequency of roughly one in a thousand lines. Protein contents have been found that are higher than in native – even "exotic" – varieties, so it is felt that

the use of induced mutations has proved of great value in providing breeding materials for raising the protein content of cereals.

In contrast to the cereal breeders at the coordination meeting, who typically summarized five or more years of work in mutation breeding, the legume breeders were just starting mutation-breeding programs. Preliminary reports were made by the following contractors:

P.R. Rubaihayo, of Makerere University, Kampala, reviewed the breeding work done in Uganda on a number of grain legumes. A substantial amount of germ plasm has been assembled and tested. Mutation breeding of soybean and *Phaseolus* bean has begun, with the objectives to improve protein content and quality, yield potential, and disease resistance.

A project to improve the yield and grain quality of legumes commonly eated in the Caribbean was outlined by C.A. Panton of the University of the West Indies, Kingston, Jamaica. Soybeans have been treated with gamma rays and are now in the M_2 plant generation; later, the project will expand to include *Cajanus cajan* and *Phaseolus vulgaris*.

Vicia faba, or field bean, occupies a prominent position in the diet of the Egyptian population, being a low-cost source of good protein. A.M. Abo-Hegazi, of the Atomic Energy Establishment, Cairo, told of his project to improve yield, protein content, and methionine content in this crop by using gamma radiation to induce mutations.

Because soybean may provide much-needed protein to the hill-tribe people in northern Thailand, improvement of the crop in regard to earliness and plant type through mutation breeding is the aim of the research contract awarded to S. Smutkupt, Chiengmai University.

Beans and chick peas are traditional food sources in Chile, so the application of mutation breeding to improve their protein quality is an important aspect in the project placed at the Catholic University of Chile in Santiago. A. Bravo and co-workers now have second-generation material in the field.

From research work that has continued over a much longer time, W. Gottschalk, University of Bonn, has found that two fasciated mutants of peas and a fasciated recombinant seem to be very promising for use in pea breeding because of their high protein production. The increase of seed-protein content per plant of these genotypes was 20 to 70 per cent during three subsequent generations. Moreover, one of the fasciated mutants shows a 33 per cent increase in sugar content.

The Research Coordination Meeting participants emphasized the need for faster screening methods for both protein content and protein quality. Reports on the development of such techniques were given by a number of contractors and cooperators, as follows.

W. Schön of the University of Göttingen has tested a number of methods to estimate protein content (Kjeldahl, Dumas, ninhydrin, biuret), and has devised a method suitable for half-seeds. Rapid methods for estimating the limiting amino-acid, lysine, are now under study.

Optimal conditions for the enzymatic hydrolysis of grain protein are being developed by A. Fritz, Bavarian Institute of Agronomy and Plant Breeding,

Freising, so that large numbers of samples may be prepared for analysis of such amino acids as lysine by specific colorimetric tests.

R.A. Luse, of the FAO/IAEA Joint Division, reported on the application of a nuclear technique — that of induced electron-emission spectrometry — to the analysis of seed protein. Elemental analysis for N (total protein), S (methionine, cystine cysteine), and C (total carbohydrate) may be done rather quickly by this technique with a minimum of sample preparation.

Other nuclear techniques for protein estimation were discussed by H.G. Niemann and H. Glubrecht from the Institute for Radiation Botany, Hanover. High-energy gamma activation to analyze seed nitrogen is promising. Also under study is the estimation of certain amino acids in the form of iron complexes, using Mössbauer spectroscopy.

It was pointed out that methods presently available permit fairly rapid analysis (100 to 300 samples per day), and that in many breeding programs this satisfies the demand. However, as larger programs are developed, or when screening of much larger populations in early generations is justified, a need remains for methods capable of screening thousands of samples per day. This problem will continue to occupy the cooperators.

The need to determine nutritional value in mutant-derived varieties was also stressed at the meeting. B.O. Eggum, of the Agricultural Research Laboratory, Copenhagen, reviewed the subject of biological availability of amino acids in grain protein and indicated new areas of investigation that should be studied. A number of speakers commented on the need to study the morphological and physiological status of protein bodies within the seed, as this may provide insight on the problem of protein nonavailability.

The meeting participants drafted a set of recommendations dealing with the objectives and strategy of plant breeding for seed-protein improvement, and also with the methodology and future developments for analysis of protein content and quality. These recommendations will form a part of the proceedings from the research coordination meeting, to be published by the IAEA early in 1973 (Panel Proceedings, 1972).

APPENDIX

Draft recommendations, FAO/IAEA/GSF Research Coordination meeting on seed protein improvement, Munich, 26-30 June 1972

Nutritional Sub-group

1. The final assessment of an improved genotype is on its ability to increase the performance of animals, including man. Because of the high cost of animal performance trials, these can be conducted in only a small number of genotypes. We consider it essential that all improved protein genotypes proposed for commercial use be first subjected to animal performance tests. Efforts should be made to develop more efficient testing procedures.

2. High-protein genotypes have been produced which failed to give the

pected response in animal performance. Therefore, we would like to encourage research to elucidate the norms underlying such lack of performance, with the hope that this knowledge will provide tools to aid plant breeders at earlier solution stages.

3. Similarly, a better understanding of the basic processes of the biosynthesis of plant storage proteins could lead to more efficient screening techniques, and research in this area should be encouraged in appropriate laboratories.

4. Suggested methods for evaluating the protein quality:

a) Complete amino-acid analysis.

b) In vitro digestion (PPD – pepsin pancreatin digest) (PDR – pepsin digest residue), in combination with amino-acid analyses of the liberated amino acids.

c) Evaluation by the use of Tetra Hymena Pyriformis W – but this method needs to be refined.

d) Animal experiments: mice, rats, and pigs, and the criteria PER, TD, BV, and NPU can be determined. Blood urea measurements are also suggested to be of great value. Refer to the paper entitled "Improving plant protein by nuclear techniques," presented by Mauron at the 1970 Vienna meeting (page 303).

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BIOLOGICAL VALUE OF LEGUME PROTEINS AS INFLUENCED BY GENETIC VARIATION

Satinder Bajaj

Protein deficiency, which is prevalent in many parts of the world today, has challenged scientists for years. Geneticists have bred plants to alter specific characters, such as color, size, shape, disease resistance, and yield. Biologists studying plants and animals have shown that specific proteins are characteristic of certain families (Boulter and Thruman, 1968, Dalby and Lillevik, 1969). The nutritional implications of this type of work are obvious: it is possible that quantitative, as well as qualitative, variation occurs with genetic variation, and can be enhanced further by suitable crosses. In order to address efforts directly to this problem and search for sources of protein for human consumption, several important factors need attention:

1. The food should have a high content of protein. If a protein increase is made, the food should contribute sufficient protein to the over-all diet.

2. It should not contain any toxic factors, especially if they are difficult to remove by treatment or preparation.

3. It should be easy to grow, and the yield must be sufficient to make its production economical.

4. If possible, it should be easy to store and transport. This becomes especially important in tropical countries, where there is very little refrigeration.

Considering these requirements, cereals and legumes appear ideally suited to provide an increasing portion of nutritious food for the world's population. Although cereals are consumed in larger quantity as staple foods, legumes contain more protein (20 to 30 per cent vs. 10 to 20 per cent in cereals).

In those countries in which populations depend heavily upon vegetable sources of protein, the cereal diets usually are supplemented with legumes, which contribute sufficient lysine (Patwardhan, 1956). The limiting amino acid of cereal-legume diets is methionine.

Although many studies have been conducted on the influence of genes on protein quantity, very little work has been done to show their effect on protein quality. The quality of protein in a food depends on several factors, including its digestibility, the availability of amino acids, the amino-acid composition, and the presence of toxic factors. Systematic studies to improve the quality of protein of seeds requires information on: (1) the quantity and quality of protein present, and (2) the types of protein and their relationship to nutritional quality.

Protein Quantity

The procedure usually accepted for determining the amount of protein in foods is based on nitrogen content. The nitrogen values can be converted into figures for protein by means of factors that do not distinguish among amino acids, proteins, and other nitrogen-containing compounds. The protein in some types of foods (meat, dairy products, and eggs) is recognized as being of high biological value. The biological value quoted in handbooks and food-composition tables are averages of values found in different varieties of the same food, thereby indicating that genes influence the quantity of protein in foods. That fact is generally overlooked by nutritionists and plant geneticists. The need to rectify this situation became clear when it was shown that some varieties of maize contain more lysine and tryptophan than others (Mertz, et al., 1964).

Protein Quality

The quality of protein in a food depends on its constituent protein fractions. Two factors are of importance in this respect – the biological value of different protein fractions, and their concentration in the food. If one of these protein fractions has a higher biological value than the other, it must either (1) have a better combination of essential amino acids, especially those that are limiting for the food, or (2) the amino acids present must be more readily available.

That certain proteins are better endowed with limiting amino acids than are others has been demonstrated in the alcohol-insoluble protein-glutelin of maize (Dimler, 1966). Varieties of maize that contain more lysine and tryptophan have a high glutelin content, and a biological value similar to that of casein (Mertz et al., 1965).

In earlier work (Bajaj, 1969; Bajaj, et al., 1971 a, b), I emphasized the value of legume proteins for human nutrition, and was able to demonstrate the importance of water-soluble proteins as indicators of protein quality and superior nutritional value. The albumin fraction was shown to contain two to three times the lysine and sulfur-amino acids present in other protein fractions. Furthermore, equations developed from the correlation between albumin and protein quality were used to predict biological value with accuracy and speed. Because current interest in legume proteins is directed toward a search for quick, reliable assays for protein quality, the data are presented herein.

Experiments

Peas *(Pisum sativum)* were grown at the Horticulture Center of Michigan State University in 1966. The strains used in the breeding program were derived from crosses involving the following varieties: Early Perfection, Miragreen, Miracle, Jade, and Green Seed Perfection: foreign plant introductions 167250, 169604, 173059, and 175232; and breeding lines 56-118 and 56-184. Rainfall was adequate for growth. The peas were dried on the vine, harvested by hand, and stored at 4°C and in low humidity (25-50 RH) until used. Alaska peas, a commercial variety, grown and harvested under standard conditions of commercial seed production, were used as a standard of reference.

The peas were ground in a Wiley Mill until the powder passed through a

20-mesh screen. All samples were analyzed for nitrogen by the Kjeldahl method (Association of Official Agricultural Chemists, 1970). The nitrogen value was multiplied by a factor of 6.25 to facilitate comparison of the nitrogen fraction of the peas with casein (Hegsted, 1964), the reference standard in biological assays.

For the biological assays, either pea meal or casein was added to the basic diet at the expense of sucrose (Bajaj, et al., 1971a).

For one week prior to the experiment, weanling male Sprague Dawley rats were fed on a grain ration (Bajaj, et al., 1971a). Five rats were assigned to each group in such a way that the group weights did not vary by more than 3g. Because 28 lines of peas could not be assayed simultaneously, three separate experiments were performed. Casein was repeated in every experiment, and certain lines of peas were assayed two or three times. Food and water were provided ad lib. Food intakes and weight gain were measured every week for three weeks.

One extra group of five rats in each assay was killed at the start of the experiment (body-composition controls). The carcass (minus the gastrointestinal contents) of each of these rats was autoclaved, homogenized, and a sample was analyzed for nitrogen. The remaining rats were killed at the end of the three-week experimental period, and the carcasses were treated in the same manner. Nitrogen retention was calculated from the body-nitrogen content of the test animals, minus that in the body-composition controls.

Protein quality was calculated as protein efficiency ratio (PER):

$$\text{PER} = \text{weight gain (g)/g protein eaten,}$$

and nitrogen incorporation efficiency (NIE):

$$\text{NIE} = \frac{\text{nitrogen retained in the carcass} \times 100}{\text{nitrogen intake}}$$

The extraction of the pea proteins and subsequent fractionation was a modification of the method of Raacke (1957). The extraction procedure and subsequent amino-acid analysis of the albumin and globulin fractions have been described in a previous publication (Bajaj, et al., 1971b).

The microbiological method (Ford, 1960) for evaluating protein quality was applied to different fractions isolated from peas. *Streptococcus zymogenes* was used as the test organism, because it has the same requirement for exogenous amino acids as does the growing rat, and is able to utilize intact proteins.

Results and Discussion

Based on nitrogen values (N x 6.25), the protein content of vine-dried peas varied from 21 to 28 per cent (Table I). However, the total protein content of different strains of peas, as measured by their nitrogen content, may be a poor index of their protein quality. This was evident when it was observed that the protein in certain strains of peas gave PER values that were as much as five times that given by another strain with the same nitrogen content (Table I). Nitrogen content may not be an accurate estimate of true protein, as different strains of peas may contain different quantities of nonprotein nitrogen (NPN). The NPN

TABLE I
Protein Quality of Different Pea Strains As Determined by Rat Bioassay

Protein source[1]	Percentage protein in the supplement (Nx6.25)	NPU	PER	Percentage protein in carcass	Experimental weight gain of rats (g in 3 wks)
Casein	87.5	52.3	2.7	18.8	75.6
66-1	24.4	32.5	1.4	22.6[2]	33.8
66-2	24.4	25.9	1.2	20.2	31.6
66-3	25.9	16.1	0.7	21.2[2]	23.4
67-4	25.5	39.5	2.1	18.7	39.2
66-5	25.8	9.6	0.4	21.0[2]	7.7
66-6	25.4	30.5	1.4	21.8[2]	33.8
66-7	27.2	36.8	1.8	20.4	42.6
66-8	27.2	26.3	1.1	22.7[2]	33.0
66-9	27.2	22.2	1.0	22.2[2]	21.6
66-10	28.1	32.6	1.6	20.2	20.4
66-11	28.2	30.9	1.4	21.1[2]	34.6
66-12	22.9	33.6	1.5	21.6[2]	37.4
66-13	28.5	28.1	1.4	18.8	28.8
66-14	26.9	33.6	1.7	19.8	35.8
66-15	26.7	37.5	1.8	20.2	34.8
66-16	28.3	27.1	1.3	20.3	27.2
66-17	27.7	34.4	1.6	20.4	32.0
66-19	25.2	31.5	1.5	20.1	24.8
66-20	23.5	33.7	1.6	20.2	27.8
66-22	21.6	35.4	1.8	19.3	60.8[3]
66-23	24.8	37.7	1.8	20.0	35.8
66-24	26.3	41.2	2.0	20.1	35.2
66-26	25.1	35.7	1.7	20.2	35.2
66-27	23.6	26.6	1.2	20.8	22.0
66-28	23.7	23.4	1.1	20.2	24.6
67-28	21.2	32.3	1.6	19.5	20.8
66-31	26.7	44.4	2.2	20.2	37.1
66-33	26.9	33.6	1.8	18.6	36.6
66-34	28.5	34.3	1.8	18.5	38.2
66-38	28.2	25.5	1.2	19.9	13.0
66-52	23.5	26.2	1.3	20.0	43.2[3]
Alaska	22.9	13.6	0.6	21.5[2]	3.0

[1] The numbers refer to the different varieties of peas. The first two figures indicate the year when the peas were grown.
[2] Significantly different from casein ($P < 0.05$).
[3] The weight gains are higher in this experiment because the animals were fed experimental diets for a period of 5 weeks instead of the 3 weeks in all other experiments.

may contain peptides and amino acids that are nutritional equivalents of protein, as well as other nitrogen-containing compounds that cannot be utilized by monogastric animals.

The highest PER value obtained with a strain of peas was nearly 80 per cent of the mean value for casein. This is not due to abnormally low values for casein, as is evident from the agreement of our PER values for casein with reported values (Morrison, 1964). The explanation for the similarity in PER values is that, although the rats fed on the best pea rations grew at only half the rate of those

receiving casein, they also consumed considerably less food. The generally lower food intakes and weight gains of the pea-fed rats did not reflect consistant reductions in appetite (in relation to body size), because the appetite quotients calculated according to Carpenter (1953) were essentially the same for all groups (Table I).

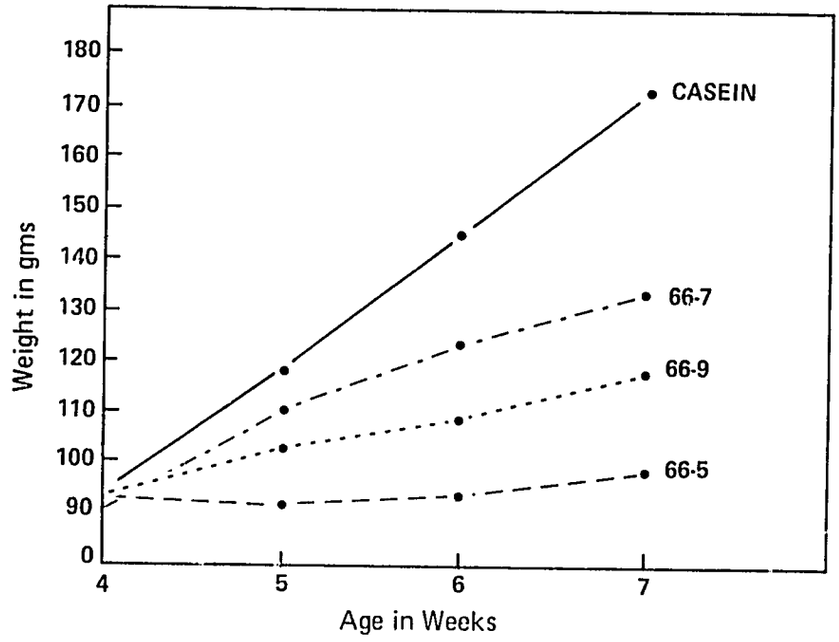

Figure 1. Growth curves of rats fed pea and casein diets.

NIE (Stucki and Harper, 1962) is a measure of the efficiency with which the animal converts dietary nitrogen to carcass protein. As such, its magnitude is determined by the animal's weight gain and carcass composition. The ratio of weight gain to food intake is determined by PER, so the important factor in differentiating NIE from PER is the percentage of protein in the carcass. That percentage in rats given certain lines of peas was significantly higher ($P < 0.05$) than that in rats given diets containing casein. This difference suggests that the rats given pea diets did not have as much fat in their carcasses as did those fed on casein. Both NIE and PER appeared to be equally effective measurements for evaluating protein quality of peas. This is evident from the high correlation ($r = 0.90$) between them. Thus, peas can be ranked equally effective on the basis of PER or NIE, but not on the basis of weight gain alone (Table I). There appears to be no significant return from the extra work of carcass-nitrogen analysis, which is required for the determination of NIE but not of PER.

From rat growth curves (Figure 1), the different pea lines can be designated as supporting (1) fairly good growth, (2) mediocre growth, or (3) bare maintainance of initial weight. Rats given poor-quality diets usually showed an initial loss of weight, which was regained later.

The PER of the 11 lines of peas selected for fractionation experiments ranged from 0.46 to 2.20g weight gain per g protein eaten, and the albumin content ranged from 0.53 to 1.06g N per 100g pea meal (Table II). Expressing the albumin in terms of nitrogen eliminates the need to choose a factor for converting N to protein. The moisture content in all of these strains of peas ranged from 6 to 7 per cent, and for this reason all values are expressed on the basis of air-dried samples.

In a preliminary experiment, the albumin content was determined for the nine strains of peas used in that bioassay. There was a high correlation between the PER values, as determined by the bioassay and the albumin content of peas. When a quadratic curve was fitted to these data, 99 per cent of the variation in PER could be explained by differences in albumin content (r = 0.998). This high

TABLE II
Albumin Content of Pea Strains and Their Protein Efficiency Ratio

Strain no.	Albumin N* (% pea meal)	PER
66-5	0.53	0.46
Alaska	0.53	0.63
66-3	0.57	0.74
66-38	0.61	1.30
66-28	0.62	1.16
66-14	0.63	1.45
66-9	0.63	1.00
66-16	0.63	1.34
66-1	0.64	1.44
66-19	0.65	1.57
67-12	0.66	1.56
67-28	0.67	1.65
66-4	0.67	1.82
66-26	0.68	1.76
66-7	0.71	1.80
66-34	0.71	1.86
66-17	0.72	1.70
66-15	0.72	1.90
66-31	0.78	2.20
66-23	0.82	1.88
66.24	1.06	2.05

*g per 100 g air-dried meal.

correlation suggested that the following equation of a quadratic curve could be used for the prediction of PER from the albumin content (X), as determined by the procedure used in this study:

$$PER = 23.2X - 12.8X^2 - 8.2 \qquad (1).$$

To check the validity of the equation, an additional group of pea strains was analyzed for albumin. These had been assayed for PER values. The latter values were compared with those calculated by inserting the albumin content into

equation (1). They differed from the PER values secured by rat bioassay by 0.0 to 0.18. The correlation between PER and albumin was still very good (r = 0.97). This prompted a pooling of data from the two experiments, which resulted in the following equation:

$$PER = 24.7X - 13.6X^2 - 8.8 \qquad (2).$$

The correlation of the PER data from the 21 strains of peas, with values secured from calculations using equation (2), was 0.949.

At the lower levels of albumin, a unit change in albumin content produces a large change in PER. These data do not permit an indication of the relation of PER values and albumin concentration beyond the ranges in the samples studied. When peas with higher albumin concentrations become available, the upper regions of the curve should be explored.

The correlation coefficients for PER values and total N, extractable N, or the globulin fraction were: 0.42, 0.02, and 0.32, respectively. All these values are so low as to make them of dubious value in predicting the biological value in pea samples. In contrast to these low values is the high correlation coefficient for PER and the albumin content of the peas. Furthermore, the isolation of pea albumin is simpler and subject to less interference than is the isolation of other fractions.

On the basis of bioassays, the S-containing amino acids and lysine have been suggested as limiting the biological value of pea proteins (Campbell, 1963). The relatively high proportion of S-amino acids and lysine found in the albumin fraction of peas (Table III) may be an important factor in explaining the excellent correlation between the concentration of that protein in peas and the corresponding biological value, as measured by PER. A much higher content of methionine plus cystine occurs in the albumin than in the globulin fractions isolated from the strains of peas investigated. Although the difference in lysine proportion is not so striking, its level in the albumin fraction is higher than in the globulin fraction. A larger quantity of lysine and sulfur-containing amino acids has been reported in the albumin fractions of other legumes (Mathur, et al., 1968) and maize salt-extractable proteins (Misra, et al., 1972).

The distribution of values in Table III, when compared to the FAO (1957) pattern of essential amino-acid requirements, indicates that the albumin fraction contains all the essential amino acids, except one, at levels that are more than adequate to satisfy the FAO requirements. The exception is the sulfur-amino acids, but even here the albumin fraction contains twice as much as the globulin (Table III).

The evidence that the biological value of albumin is superior to that of globulin was obtained from microbiological assays. This method was used because only small amounts of proteins could be isolated from the available pea samples. Relative values for different samples were based on bacterial growth response produced by casein. On this basis, the albumin fraction produced growth that was equal to 117 to 128 per cent that of casein, with no significant differences among the nine samples of albumin assayed (Table IV). Corresponding values for the globulin fractions isolated from the same nine strains of peas

TABLE III
Amino-Acid Distribution in Hydrolysates of the Albumin and Globulin Fractions of Proteins Extracted from Peas

Amino Acid	Isolated Pea Proteins* Albumin†	Globulin‡
Lysine	10.6 ± 0.4§	7.9 ± 1.2
Histidine	2.9 ± 0.1	2.3 ± 0.1
Arginine	5.7 ± 0.3	8.8 ± 0.6
Threonine	5.2 ± 0.1	3.2 ± 0.2
Cystine	0.8 ± 0.1	0.3 ± 0.04
Valine	5.5 ± 0.2	5.5 ± 0.2
Methionine	1.1 ± 0.1	0.5 ± 0.1
Isoleucine	6.1 ± 0.3	5.9 ± 0.2
Leucine	5.9 ± 0.3	8.7 ± 0.2
Phenylalanine	5.7 ± 0.4	3.1 ± 0.1
Aspartic Acid	11.2 ± 0.7	9.9 ± 0.2
Serine	3.8 ± 0.2	3.6 ± 0.2
Glutamic Acid	15.9 ± 1.4	23.9 ± 0.6
Proline	4.8 ± 0.2	4.3 ± 0.1
Glycine	5.0 ± 0.1	3.6 ± 0.1
Alanine	5.2 ± 0.2	3.7 ± 0.1
Tyrosine	5.2 ± 0.3	3.1 ± 0.1

*g per 100 g of amino acid hydrolysate.
†Average of analyses carried out on four samples of albumin isolated from the following pea strains: 66-10, 66-24, 66-15, 66-4. Each sample was analyzed in duplicate.
‡Average of separate analyses carried out on 2 samples of globulin isolated from pea strains 66-15 and 66-24. Due to inadequate sample size, 3 strains of peas had to be combined to provide the starting material for the isolation of another globulin fraction. This was also analyzed for amino acids. Each of these three analyses was done in duplicate.
§Mean ± S.E.

ranged from 64 to 100 per cent (average = 85.6 per cent). These values were significantly lower than those for albumin. The large variation among the globulin values probably reflects the difficulty in securing fractions of uniform composition. The globulin fraction may contain its two major proteins, legumin and vicilin, in variable proportions. These two proteins differ markedly in biological value, as estimated by the microbiological procedure; legumin is far superior to vicilin (Bajaj, 1969).

Summary and Conclusions

From the evidence presented, it can be inferred that:

1. Nitrogen is a poor index of the protein quality of the seeds.

TABLE IV
Microbiological Assay Values* of Protein Fractions Isolated from Pea Meals

Sample	Protein fraction		
	Albumin	Globulin	Residue
66-3	118 ± 10	85 ± 9	48
66-5	119 ± 8	64 ± 2	56
66-7	117 ± 8	71 ± 6	29
66-9	121 ± 7	90 ± 5	28
66-23	126 ± 9	94 ± 6	29
66-24	128 ± 9	76 ± 3	28
66-26	120 ± 3	93 ± 6	32
66-28	120 ± 5	100 ± 6	48
Alaska	128 ± 11	97 ± 4	52

*All values are expressed as the percentage of response produced by the sample when compared with the response secured with casein.

2. Variation in protein quality and quantity exists in different varieties of peas, even if they contain the same percentage of nitrogen.

3. Ninety-four per cent of the variation in PER of the peas studied can be explained on the basis of differences in the albumin content.

4. The high correlation between the albumin content and PER is expressed by the equation:

$$PER = 24.7X \quad 13.6X^2 \quad 8.8.$$

The above equation can be used to predict the PER of peas after the albumin content is estimated chemically.

6. The biological quality of the albumin fraction is better than that of the others.

7. The albumin fraction contains a higher amount of the essential amino acids, particularly lysine and sulfur-containing amino acids.

This information indicates that albumins in legumes may be important as indicators of nutritional quality, and could be used in routine assays for protein biological value, such as are required by plant scientists.

The albumins in legumes form 8 to 40 per cent of the total protein, the remainder being in the globulin form. The two globulins, i.e., legumin and vicilin, vary in nutritional quality. Legumin has a better protein quality than does vicilin, a superiority that has been ascribed to its amino-acid composition, particularly sulfur-containing amino acids.

Although the albumin fraction contains a variety of enzymes and proteins, which make it difficult to manipulate genetically, the factors that influence its production need more attention. Factors such as cooking and processing, which alter the quantity of albumin, and their relation to the biological quality of protein — with emphasis on the albumin content — need to be studied in various legumes. The effect of sprouting and its influence on the albumin and the

quality of protein is of great interest, especially in those legumes that are consumed in this form.

Acknowledgements

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DISCUSSANT

K.J. Carpenter

As nutritionists, we must try to explain the nutritional problems to breeders, and they must reciprocate with problems of their own. Dr. Bressani has reported the results of feeding experiments on diets in which a considerable proportion of the protein came from cereals, and half, or less than half, from beans. Supplementation of these diets with methionine gave a much smaller response than that obtained when cooked beans form the sole source of protein in an experimental diet. Figure 1 explains this difference. Beans, by themselves, give a large response to methione because it is the only amino acid that is seriously short. However, both lysine and threonine are present in all cereals only at low levels, in relation to requirements.

As bean protein is mixed with increasing proportions of cereal protein, the levels of lysine and threonine in the mixture decline until these two amino acids are almost, or quite, as deficient as is methionine. Under these conditions of increasing proportions of cereal proteins, the expected, possible response to extra methionine falls and then disappears (see Figure 1).

Maize differs from the other cereals only in having an additional deficiency of tryptophan, and these theoretical calculations are in complete agreement with Dr. Bressani's findings for the diets of Guatemalan children, who receive about two-thirds cereal protein and one-third bean protein. We are, of course, most interested in the children, because they are at the highest risk from protein deficiency. As Dr. Calloway has said, if the amounts of protein are increased, the relative deficiencies of individual amino acids are much less important.

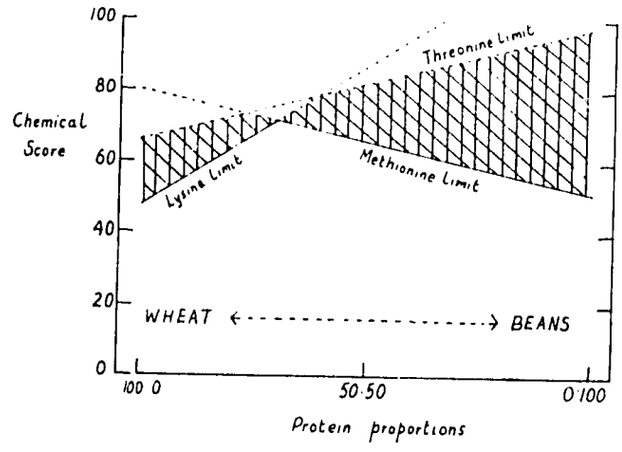


Figure 1. The calculated chemical score of whole wheat and *Phaseolus* bean proteins in different proportions. The score for each limiting amino acid is obtained by expressing its concentration as a percentage of that in a completely balanced protein. The shaded area on the left represents the theoretical improvement possible from increasing the lysine level and that on the right from increasing the methionine level (reproduced from Carpenter, K.J. 1970. *Proc. Nutr. Soc.* 29:3).

SUMMARY

K.J. Carpenter

Protein and Amino-Acid Profiles

The percentage of protein in crop samples of any one species of legumes has been found to vary greatly. Much of this has been due to environment; for example, the same varieties have shown values ranging from 24 to 33 per cent protein. But there are also genetic differences, so selection can lead to consistently higher nitrogen levels. Radiation techniques to induce mutations have not been much used with grain legumes, so far, but we have heard from Dr. Sigurdbjornsson how experiments are now being organized to do this on a very large scale, indeed.

With regard to amino-acid patterns, these have proved relatively constant, and certainly nitrogen fertiliaztion does not seem to improve protein quality; it isn't poor soil status that gives poor protein quality. Sulfur-amino acids have always proved to be the limiting factor. It has been pointed out that there are some differences in sulfur-amino acid levels and although, in the past, they have been thought to be almost negligible, they have been confirmed, and it is thought that now they should be pursued. However, it has also been pointed out that in whole diets, where not just beans but "beans and cereals" are the staple protein sources, methionine is not always the first limiting factor and that one gets at best only a small response to methionine. This may not be true everywhere, but it is in many places. As a result, the amino-acid situation is not entirely straightforward, and we must be careful that changing one amino acid does not have an unfavorable effect on others. In other words, a lot remains to be found out.

It has been emphasized that, although changes in protein content and in amino-acid pattern may be beneficial, even protein nutritionists do not suggest that such changes should have priorities over the yield and the general acceptability of the material.

Toxic factors

Dr. Liener pointed out experimental results in which there was no correlation – even with heated soybean meals – between the sulfur-amino acid content of particular samples and the response of rats in PER experiments. This reinforces the reasons for thinking that possible toxins and depressant factors must be considered, but we must consider them in the context of cooked materials, rather than raw. It was pointed out that trypsin-inhibitor activity can be assayed fairly simply. Nevertheless, there is probably something more to this, because cooked material still seems to show some inhibitory activity in rats, although it is not detected in the ordinary assay methods. Hemagglutinin can cause most of the growth depressions obtained with raw *Phaseolus* beans, and they survive dry heat, so it is important not to dismiss these completely for all forms of heated materials. It is already realized that there are other extremely important, com-

plex, toxic problems with particular types of beans: goiterogens, estrogens, cyanides, etc., and that more things are still to be discovered. It is also clear that the results obtained in assays with animals may depend on the species being used – the chick or the rat, for example.

It seems, therefore, that further research is needed, first, in developing fast, simple, biological assays and investigating the reported discrepancies between the results in animal assays and the results of chemical predictions. Sometimes, on further investigation, some apparent discrepancies disappear.

Second, there is an obvious need for a continuing study of toxins, with particular relevance to cooked material and, third, a continuing study of amino-acid methodology. The problem is twofold: to discover the facts and to try to get the facts into perspective for the plant breeders.

III B. Antitryptic and Other Toxic Factors

ANTITRYPTIC AND OTHER ANTINUTRITIONAL FACTORS IN LEGUMES

– **Irvin Liener**

Discussant

James McGinnis

ANTITRYPTIC AND OTHER ANTINUTRITIONAL FACTORS IN LEGUMES

Irvin Liener

For reasons that scientists have yet to fathom, Nature has seen fit to endow many plants with the capacity to synthesize a wide variety of chemical substances that are known to exert a deleterious effect when ingested by man or animals. Included among such plants are the many varieties of legumes, which constitute an important part of the diet of large segments of the world's population. If such toxic substances do, in fact, exist in plant foodstuffs commonly consumed by man, why are their effects not manifested more frequently in the population? It would appear that, through trial and error, man has not only learned to avoid those foods which produce immediate ill effects; he has also devised ways and means of eliminating such effects from other foods. For example, cooking and other common means of preparation have, in many instances, proved to be effective in destroying many of the toxic constituents in legumes. Nevertheless, circumstances may arise whereby complete detoxification may not always take place, as, for example, in the inadequate commercial processing of soybean products.

Abnormal patterns of food consumption may however, prove harmful, as exemplified by the periodic eruption of lathyrism in certain parts of India. This illness is associated with the consumption of certain varieties of *Lathyrus* during times of famine, when cereal grains are in short supply. Then we have the well-known examples of favism, which affects certain individuals who consume the bean *Vicia faba*. These few examples serve to illustrate what might be expected to become more commonplace as the shortage of protein foods becomes more acute and people are forced to become more indiscriminate in their choice of life-sustaining plant foods.

Protease Inhibitors in Soybeans and Other Legumes

Historical Background

Substances which have the ability to inhibit the proteolytic activity of certain enzymes are found throughout the plant kingdom, particularly among the legumes. These protease inhibitors have attracted the attention of nutritionists because of the possible role they might play in determining the nutritive value of plant proteins. It was not long after soybeans were introduced into the United States that Osborne and Mendel (1917) made the significant observation that soybeans had to be heated in order to support the normal growth of rats. Kunitz

(1945) subsequently isolated from raw soybeans a protein that had the unique property of combining with trypsin to form an inactive complex.

It was logical to assume that the trypsin inhibitor was the substance responsible for the poor nutritive value of unheated soybeans. The hypothesis that its effect could be readily explained on the basis of its ability to inhibit intestinal proteolysis was appealing. In addition, the fact that methionine exerted a marked improvement on the nutritive value of raw soybeans (Table I) was taken to indicate that the trypsin inhibitor somehow interfered with the availability or utilization of methionine from the raw bean.

Mode of Action in the Animal Organism

It now appears that the true explanation for the growth-inhibitory property of the trypsin inhibitor is not simple. Although more than 25 years have elapsed since Kunitz first isolated a trypsin inhibitor – years which have witnessed literally hundreds of papers on the subject (see review by Liener and Kakade, 1969) – there is still a decided lack of certainty regarding the significance of the trypsin inhibitor and its mode of action in the intact animal. There seems to be little doubt that hypertrophy of the pancreas represents one of the primary physiological effects produced by feeding raw soybeans (Booth, et al., 1960) or the isolated inhibitor (Nesheim, et al., 1962). Booth, et al. (1960) are of the opinion that pancreatic hypertrophy leads to an excesssive loss of endogenous protein secreted by the pancreas. Since this protein, consisting largely of pancreatic enzymes, is rich in cystine, the resulting effect is a net loss of sulfur-containing amino acids from the body. That would explain why the need for methionine, which is limiting in soybean protein anyway, is rendered even more acute in diets containing raw soybeans. Recent evidence indicates that trypsin or chymotrypsin in the intestine suppress pancreatic enzyme secretion by feed-back inhibition, and that trypsin inhibitors evoke increased enzyme secretion by counteracting the suppression produced by trypsin (Green and Lyman, 1972; Niess, et al., 1972).

Trypsin inhibitors also have been found in a large number of other legumes, including the peanut, navy bean, lima bean, etc.; in fact, all legumes that have been looked at to date have been found to contain trypsin inhibitors to varying degrees. The exact nutritional significance of these inhibitors is not clear, because there does not appear to be any clear-cut correlation between the trypsin-inhibitor content of various legumes and the beneficial effect which heat has on their nutritional value (Borchers and Ackerson, 1950). The presence of other growth inhibitors, which will be discussed later, no doubt tends to obscure whatever detrimental effect the trypsin inhibitors per se may have on growth.

Factors Affecting the Trypsin Inhibitor Content

Heat treatment. The extent to which the trypsin inhibitor in legumes is destroyed by heat is a function of the temperature, duration of heating, particle size, and moisture conditions – variables that are controlled closely in the com-

mercial processing of soybean-oil meal in order to obtain a product with maximum nutritive value. In general, autoclaving in an atmosphere of steam at 15 lb/sq^2 in for 15 to 10 min serves to inactivate the trypsin inhibitor of soybeans almost completely. If the beans first are soaked overnight, steaming at atmospheric pressure serves to inactivate the trypsin inhibitor. The extrusion cooking process, developed for producing a full-fat soybean flour for village use in underdeveloped countries, yields a product that is free of trypsin inhibitors and is reported to be nutritionally equivalent to good-quality, toasted soybean flour (Albrecht, et al., 1967).

The trypsin-inhibitor activity inherently present in soymilk can be eliminated effectively by proper heat treatment of the liquid product or by spray-drying (Hackler, et al., 1965). It is of significance to note that properly processed soybean milk has a nutritive value that is almost equivalent to that of cow's milk (Shurpalikar, et al., 1961).

Comparatively few studies have dealt with the effect of heat treatment on the trypsin-inhibitor content of other legumes. Although the trypsin-inhibitor content of groundnuts may be destroyed effectively by heat treatment, little or no improvement in nutritive value is apparent (Borchers and Ackerson, 1950; Anantharaman and Carpenter, 1969). When an improvement in nutritive value does accompany the inactivation of trypsin inhibitors, the destruction of other growth inhibitors in some legumes (see below) may becloud the issue.

Germination. Although germination is known to result in an improvement in the nutritive value of soybeans and a number of legumes, that effect appears to be unrelated to any changes in trypsin-inhibitor activity in the germinated seeds (Liener and Kakade, 1969). The reason for the beneficial effect of germination on the growth-promoting property of legumes remains unknown.

Fermentation. Some investigators have reported that the nutritive value of such fermented soybean preparations as tempeh and natto is somewhat enhanced as a result of the fermentation process (Smith, 1963). These findings are consistent with the observation that there is no pancreatic enlargement in rats fed diets containing tempeh (Smith, et al., 1964), indicating that the trypsin inhibitor had presumably been destroyed by the heat treatment involved in tempeh preparation (the beans are boiled for 30 min prior to the fermentation).

TABLE I
The Effect of Heat and Methionine on the Nutritive Value of Soybeans for Rats

Diet	Protein Efficiency
Ray soybean meal	1.33
Autoclaved soybean meal	2.62
Raw soybean meal + 0.6% methionine	2.42

From Liener, et al., 1949

Soybean isolates. More recently, considerable attention has been given to the nutritional properties of soybean isolates and the textured meat analogues fabricated therefrom. Although the nutritive quality of these textured food products has been reported to be essentially equivalent to casein or beef, the protein efficiency of the original protein isolate was very low and could be improved by heat treatment (Bressani, et al., 1967). These results would indicate the possible presence of residual growth inhibitors in the protein isolate -- inhibitors that were inactivated during the process of converting the isolate into fiber. We have had occasion to examine a number of these textured products for antitryptic activity, and, as the data in Table II show, the soybean isolate and fiber are surprisingly rich in trypsin-inhibitor activity. The processed meat analogues have a reduced, but measurable, level of antitryptic activity.

Varietal Differences

Most plant-breeding studies involving legumes, notably soybeans, have been directed to changes in oil and protein content and amino-acid composition, with little attention being paid to trypsin-inhibitor content. In a study supported by the U.S. Department of Agriculture, our group at Minnesota undertook an examination of a large number of different commercial and experimental strains of soybeans for their trypsin-inhibitor content (Kakade, et al., 1972). An attempt was then made to establish what relationship, if any, might exist between these activities and the nutritive properties of the protein as measured by rat assays. Figure 1 shows a scatter diagram of the trypsin-inhibitor activity in relation to PER for 26 different varieties or strains of soybeans representative of the more than 100 samples that were screened initially. The absence of any correlation between trypsin-inhibitor activity and PER is immediately apparent. Quite unexpectedly, however, a highly negative significant correlation was observed between the weights of the pancreas and PER (Figure 2).

TABLE II
Trypsin Inhibitor Activities of Soybean Flour, Isolate, Fiber, and Finished Textured Products

Product	Antitrypsin Activity TIU/gm dry solids* $\times 10^{-3}$	% of soyflour
Soyflour (unheated)	86.4	100
Soybean isolate	25.5	30
Soybean fiber	12.3	14
Chicken analogue	6.9	8
Ham analogue	10.2	12
Beef analogue	6.5	7

*Trypsin-inhibitor activity determined by the method of Kakade, et al. (1969).
TIU = trypsin-inhibitor units, as defined in this reference. (From Liener, 1971)

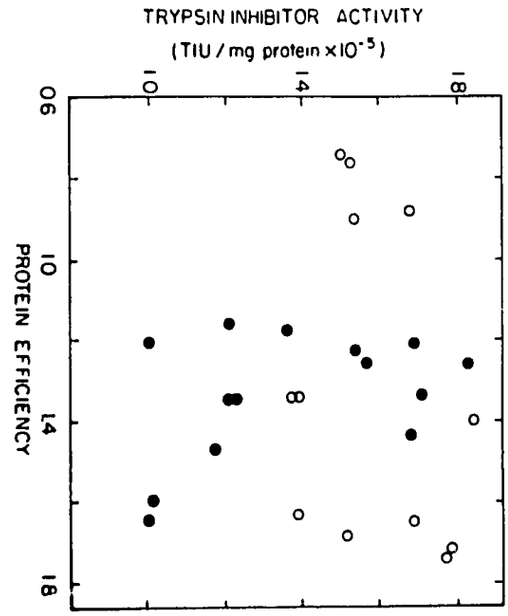


Figure 1. Scatter diagram relating trypsin-inhibitor activity to protein-efficiency ratios of various soybean samples. (Taken from Kakade, et al., 1972)

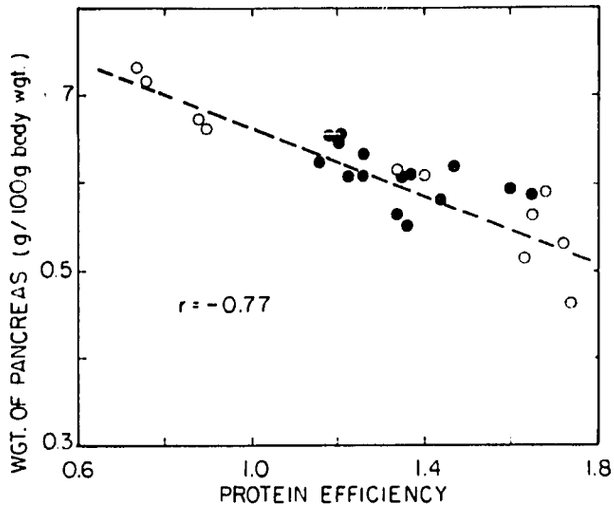


Figure 2. Relationship of weights of pancreas to protein-efficiency ratios of various soybean samples. r = coefficient of correlation. (Taken from Kakade, et al., 1972)

A possible explanation for the discrepancy noted here may be that the measurement of antitryptic activity as measured in vitro does not reflect the true inhibitor content of the soybean. For example, the trypsin-inhibitors might be bound in such a fashion that they cannot be extracted by the aqueous solvents generally employed for their assay. This might explain why the water-insoluble residue of soybeans, although low in antitryptic activity, nevertheless inhibits growth and causes pancreatic hypertrophy (Saxena, et al. 1963). One cannot, of course, exclude the possibility that there may be present in raw soybeans a factor totally unrelated to the trypsin inhibitor, but nevertheless capable of causing pancreatic hypertrophy and an inhibition of growth.

Another factor that complicates this whole picture is that there are different kinds of trypsin inhibitors in soybeans, and it is possible that these also may differ in their phsiological effects. This possibility is suggested by a recent report (Yen, et al., 1971) that one variety of soybean, with a trypsin-inhibitor component that is electrophoretically different from that observed in regular commercial varieties, is nutritionally superior to two commercial varieties with which it was compared. Thus, any screening program of soybeans and legumes for trypsin-inhibitor activity should take into account not only the levels of antitryptic activity but also molecular differences in the inhibitors themselves.

Possible Significance in Human Nutrition

Essentially all of the work dealing with the physiological effects of the trypsin inhibitor has been done with experimental animals. As with most nutritional studies, we are tempted to extrapolate the results obtained with animals to human subjects. But, in the case of the soybean inhibitors, are we really justified in doing this? It is of significance to note that virtually all of the in vitro studies with trypsin inhibitors have involved the use of *bovine* trypsin, because of its ready commercial availability as a crystalline preparation. More recently, however, it has been demonstrated that *human* trypsin is essentially uninhibited by the soybean inhibitor (Feeney, et al., 1969); Travis and Roberts, 1969).

Furthermore, it may be pointed out that ovomucoid, the egg-white trypsin inhibitor (as demonstrated with bovine trypsin), likewise is incapable of inhibiting human trypsin, which perhaps may explain why ovomucoid has virtually no effect on the nitrogen balance of human subjects (Scudamore, et al., 1949). From these various lines of evidence, therefore, one is tempted to conclude that, despite the considerable body of evidence which implicates the trypsin inhibitor as a factor contributing to the poor nutritive value of improperly processed legumes in animals, they may have little, if any, relevance to human nutrition.

Other Antinutritional Factors in Legumes

Phytohemagglutinins

Another substance that appears to be distributed universally among the legumes is a protein with the unique property of being able to agglutinate red blood cells – the so-called phytohemagglutinins (Jaffé, 1969). A number of years ago, we were able to demonstrate its ability to inhibit the growth of rats (Liener,

1953). It may be estimated from the data in Table III that the soybean hemagglutinin can account for approximately 25 per cent of the growth inhibition that raw soybeans produce in rats.

We next turned our attention to the possible nutritional significance of the phytohemagglutinins present in other legumes, particularly those which enjoy popular consumption in some of the less-developed countries. For this purpose, we obtained samples of a black bean from Guatemala, the Bengal gram and red gram from India, the mung bean from the Philippines, and a domestic variety of the kidney bean. The effect of heat on the nutritive value of these beans is shown in Table IV, where it is apparent that the growth-promoting properties of only the black and kidney beans were improved by heat. As shown in Table V, these same two beans were also the only ones which displayed a significant level of hemagglutinating activity.

TABLE III
Growth-Inhibitory Effect of the Soybean Hemagglutinin (SBH)

Protein component of diet	Weight gain gm/2 weeks	% Growth inhibition
25% heated soybean meal	60	0
25% raw soybean meal	28	43
25% heated soybean meal + 0.8% SBH	45	26

From Liener, 1953

TABLE IV
Effect of Heat on Nutritive Value of Some Legumes

Source of Protein	Gain in weight gm/day Raw*	Heated
Phaseolus vulgaris		
Black bean	−1.94 (4-5)	+1.61
Kidney bean	−1.04 (11-13)	+1.48
Cicer arietinum		
Bengal gram	+1.25	+1.16
Cajanus cajan		
Red gram	+1.33	+1.74
Phaseolus aureus		
Mung bean	+1.05	+1.07

*100% mortality observed during period (in days) shown in the parentheses. (From Honovar, et al., 1962)

TABLE V
Hemagglutinating and Antitryptic Activities of Crude Extracts* of Raw Legumes

Legume	Hemagglutinating activity HU/ml	Antitryptic activity TIU/ml
Phaseolus vulgaris		
Black bean	2450	2050
Kidney bean	3560	1552
Cicer arietinum	0	220
Cajanus cajan	0	418
Phaseolus aureus	0	260

*A 10% suspension of the finely ground meal in 1% NaCl, clarified by centrifugation. (From Honovar, et al., 1962)

The phytohemagglutinins from these two varieties of *Phaseolus vulgaris* were isolated in pure form and fed at various levels to rats in a basal ration containing 10 per cent casein. From the data presented in Table VI, it is evident that an

TABLE VI
Effect of Purified Hemagglutinin Fractions from the Black Bean and Kidney Bean on the Growth of Rats

Source of hemagglutinin	Purified hemagglutinin in diet (%)	Average gain in weight (gm/day)	Mortality* (days)
Black bean	0.0	+2.51	
	0.5	+1.04	
	0.5**	+2.37	
	0.75	+0.20	
	1.2	-0.91	15-19
	2.3	-1.61	12-17
	4.6	-1.72	5-7
Kidney bean	0.0	+2.31	
	0.5	-0.60	13-16
	0.5[b]	+2.29	
	1.0	-0.87	11-13
	1.5	-1.22	4-7

*100% mortality observed during period recorded here. Blank space indicates that no deaths were observed.

**Solution of hemagglutinin boiled for 30 min and dried coagulum fed at the level indicated. Hemagglutinating activity was completely destroyed by this treatment.

From Honovar, et al., 1962.

inhibition of growth was obtained at levels as low as 0.5 per cent of the diet, the kidney-bean hemagglutinin being much more effective in this respect than the black-bean hemagglutinin. In fact, at a level of 0.5 per cent, the kidney-bean hemagglutinin caused 100 per cent mortality after about two weeks, whereas 1.2 per cent of the black-bean hemagglutinin was necessary to produce a similar mortality rate. These data also show that the toxicity of these phytohemagglutinins may be destroyed by moist-heat treatment. We have found it necessary to soak whole beans overnight before autoclaving in order to effect complete destruction of the phytohemagglutinins.

Although studies involving the direct feeding of purified phytohemagglutinins have been limited, the presence of the substance in such a large number of legumes suggests that they may be of general nutritional significance and that their possible toxic effects should not be discounted. There have been occasional reports that inadequately cooked beans (*Phaseolus*) have produced toxic effects in humans (Faschingbauer and Kofler, 1929). Recommendations for the use of bean flour for baked goods (Anon., 1948; Marcos and Boctor, 1959) that may be subjected to dry heat, rather than cooking, therefore should be viewed with caution.

Goiterogens

Goiterogenic substances are found most commonly in plants of the cabbage family, which include such common edible plants as cabbage, turnips, cauliflower, kale, Brussels sprouts, rapeseed, mustard seed, etc. Among the legumes, however, only the soybeans and peanuts have been reported to produce goiterogenic effects in animals (Van Etten, 1969). Unheated soybeans, for example, cause a marked enlargement of the thyroid gland of the rat and chick, an effect that can be counteracted by administering iodide, or partially eliminated by heat.

An example of the therapeutic effectiveness of iodide in overcoming the goiterogenic effect of soymilk is shown in Figure 3. Several workers (Hydowitz, 1960; Van Wyk, et al., 1959) have reported a number of cases of goiter in human infants fed soybean milk. Apparently, the heat treatment employed for sterilizing these particular soybean preparations was not sufficient to destroy the goiterogenic agent. Iodine supplementation, however, abbreviated this goiter condition in infants (Van Wyk, et al., 1959). The particular agent responsible for the goiterogenicity of improperly processed soybean protein remains unknown.

Rats fed groundnuts also develop enlarged thyroids, but in this instance the goiterogenic principle has been identified as a phenolic glycoside that resides in the skin (Sreenivasan, et al., 1957). It has been suggested that the phenolic metabolites formed from this glycoside are iodinated preferentially, and thereby deprive the thyroid of available iodine. Thus, the goiterogenic effect of groundnuts is effectively counteracted by iodine supplementation, but not by heat treatment.

Cyanogenetic Glycosides

It has been known for a long time that a wide variety of plants are potentially toxic because they contain glycosides from which HCN may be released by

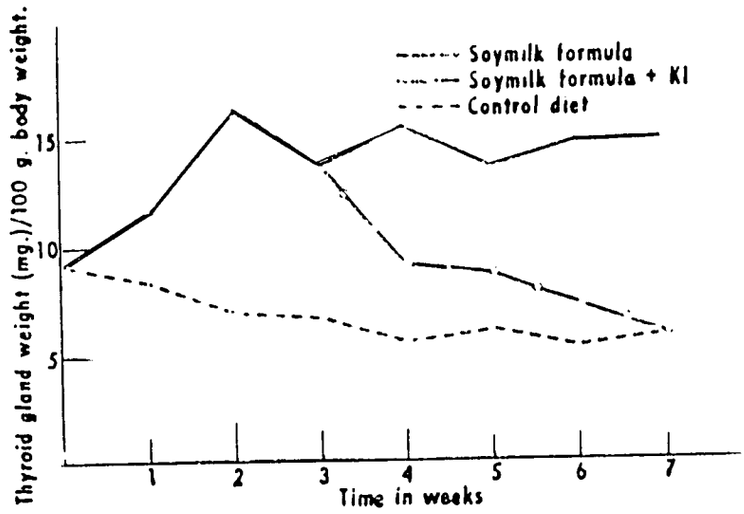


Figure 3. Effect of a soymilk diet, with and without iodine, on the thyroid gland of the rat. (Taken from Anderson, 1961)

hydrolysis (Montgomery, 1969). Legumes predominate in terms of their cyanide-producing potential. In the years immediately after the turn of the twentieth century, and again during World War I, lima beans imported into Europe from tropical countries (Java, Puerto Rico, and Burma) were responsible for serious outbreaks of cyanide poisoning, and cases of human intoxication from the consumption of certain varieties of lima beans are not uncommon to lay in some of the tropical countries. It is reassuring to note that most of the lima beans consumed in the United States and Europe at the present time are well below the toxic levels implicated in fatal cases of poisoning.

Cyanide in the form of HCN is released from a glycoside (*phaseolunatin*, in the case of lima beans) through the action of an enzyme present in the plant tissue (see Figure 4). Hydrolysis occurs rapidly when the ground bean meal is cooked in water, and most of the liberated HCN is lost by volatilization. Further cooking also leads to the eventual destruction of the enzyme. Yet many cases of human intoxication have occurred even with cooked lima beans. It has been reported (Gabel and Kruger, 1920) that when lima beans, which had been cooked to destroy the enzymes responsible for cyanide formation, were fed to human subjects, cyanide could be detected in the urine. This has led to the supposition that perhaps enzymes secreted in the intestinal tract or by the microflora of the colon may be responsible for releasing HCN after ingestion of the cooked beans.

Antivitamin Factors

The inclusion of unheated soybean meal (or the protein isolated therefrom)

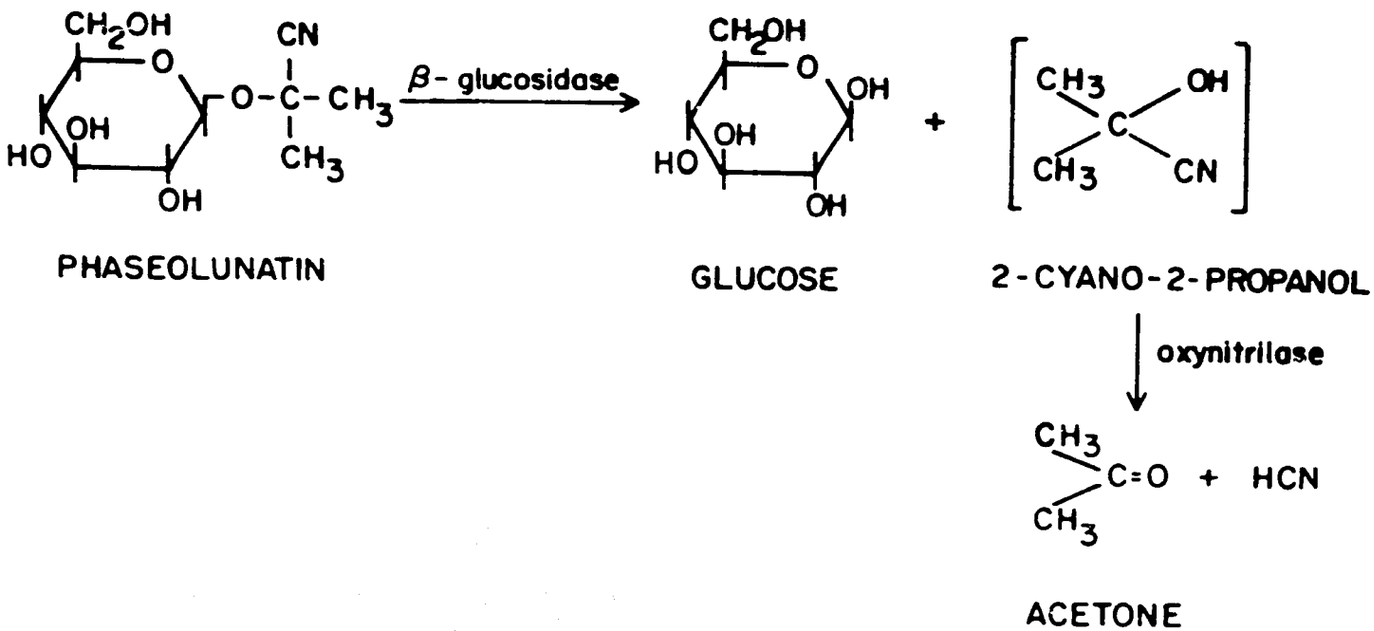


Figure 4. Enzymatic release of HCN from phaseolunatin, the cyanogenic glycoside of lima beans (*Phaseolus lunatus*).

in the diet of chicks may cause rickets, unless higher-than-normal levels of vitamin D_3 are added to the diet (Carlson, et al., 1964). The rachitogenic effect could be eliminated by autoclaving the soybean meal, but supplementation with calcium or phosphorus was ineffective. Along the same lines, raw kidney beans are believed to contain an antagonist of vitamin E, as evidenced by liver necrosis in rats, and muscular dystrophy and low levels of plasma tocopherol in chicks (Hintz and Hogue, 1964; Desai, 1966). The antivitamin-E effect of raw kidney beans can be eliminated partially by heat treatment. The identity of the antivitamin-D factor of soybeans and the antivitamin-E factor of kidney beans is unknown.

Metal-Binding Constituents

The inclusion of isolated soybean protein in animal diets has been noted to lead to a decrease in the availability of certain trace minerals, such as zinc, manganese, copper, and iron (Odell and Savage, 1960). In fact, an anemia caused by iron deficiency may be induced in monkeys on a soybean-protein diet, an effect which may be eliminated by heat treatment or chelating agents such as ethylenediaminetetraacetate (EDTA) (Fitch, et al., 1964). Peas (*P. sativum*) have also been shown to contain a factor that interferes with the availability of zinc for chicks (Kienholz, et al., 1962). Autoclaving the peas eliminated the requirement for supplemental zinc. Because zinc supplementation was only one-third as effective as autoclaving, the presence of an additional heat-labile growth inhibitor in peas was postulated.

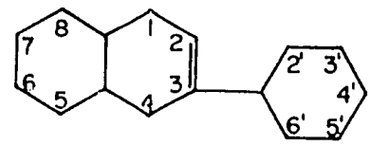
The exact mechanism whereby certain plant proteins exhibit this ability to interfere with the availability of metals is not known, although it may be related to the observation that a soy protein-phytic acid complex has a special affinity for metal ions (Odell and Savage, 1960).

Estrogenic Factors

Factors capable of eliciting an estrogenic response in animals have been shown to occur naturally in certain legumes (Liener, 1966). Chemically, these have been identified as derivatives of isoflavone, combined with a sugar residue (Figure 5), the latter being attached to one of the hydroxyl groups. Genistein and daidzein have been isolated from the soybean and shown to have estrogenic activity when tested on female rats and mice. It is doubtful, however, that the normal consumption of legumes would provide sufficient amounts of these substances to elicit a physiological response in humans

Toxic Amino Acids

Mimosine. Although the legume *Leucaena glauca* has been used primarily as a forage crop for livestock, particularly in Hawaii, certain segments of the population in Indonesia are known to consume portions of this plant as part of their diet. A loss of hair frequently has been observed among those individuals who have eaten the leaves, pods, and seeds in the form of a soup (Van Veen, 1966). The toxic effects of *L. glauca* have been attributed to its content of mimosine (Figure 6), which is structurally related to tyrosine, and, when administered to rats, acts as a metabolic antagonist of tyrosine.

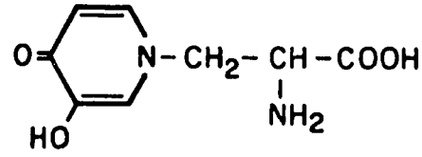


ISOFLAVONE

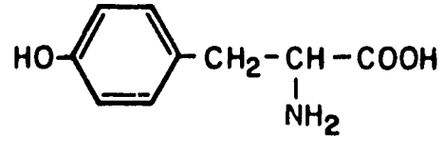
Genistein	4',5,7-trihydroxyisoflavone
Daidzein	4',7-dihydroxyisoflavone
Isogenistein	2',5,7-trihydroxyisoflavone
Tatoin	8-methyl-4',5-dihydroxyisoflavone
Methylgenistein	8-methyl-4',5,7-trihydoxyisoflavone
Methylisogenistein	8-methyl-2',5,7-trihydroxyisoflavone

Figure 5. Structure of naturally occurring estrogens.

FOUND IN LEUCAENA GLAUCA



Mimosine



Tyrosine

Figure 6. Comparison of the structure of mimosine with that of tyrosine.

Djenkolic acid. In certain parts of Sumatra, the djenkol bean *(Pithecolobrium lobatum)* is a popular food (Van Veen, 1966). The bean is not particularly toxic, but it sometimes leads to kidney failure, which is accompanied by the appearance of blood and white, needle-like clusters in the urine. The clusters are a sulfur-containing amino acid known as djenkolic acid (Figure 7), which is present in the bean in the free state to the extent of 1 to 4 per cent. Despite its structural similarity to cystine, it apparently does not act as an antagonist of cystine, but simply escapes metabolic degradation and, because of its insolubility, crystallizes out in the kidney tubules and urine.

Unidentified Growth Inhibitors

Despite the vast amount of research that has been done on the soybean, the possibility still exists that there may be present in raw soybeans some still-unidentified growth inhibitors. Schingoethe, et al. (1970), for example, recently have reported the isolation from raw soybean meal of a growth-inhibitor fraction that was free of trypsin-inhibitor activity. Although this factor has not been completely purified and characterized, it appears to have a low molecular weight and is composed, at the present state of purification, of at least eight positively charged peptides.

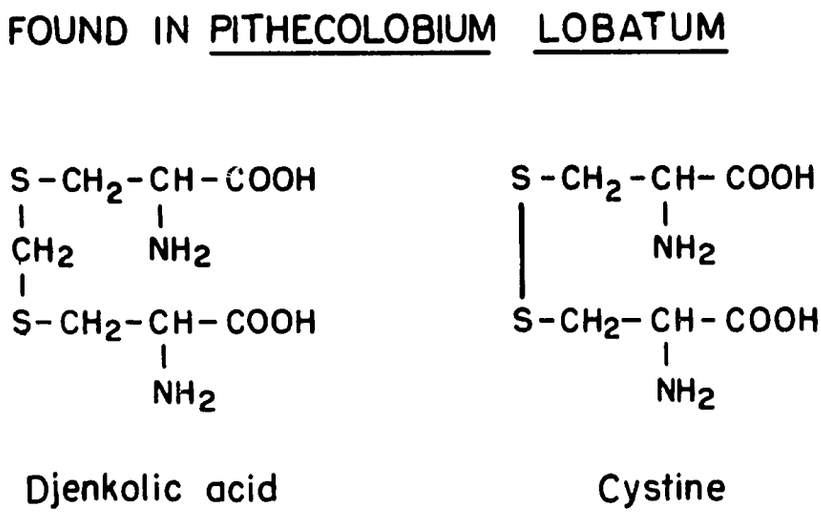


Figure 7. Comparison of the structure of djenkolic acid with that of cystine.

Lathyrogens

Lathyrism is a disease associated with the consumption of certain species of peas belonging to the genus *Lathyrus*. This subject has been reviewed recently by Rao, et al. (1969). The disease is particularly prevalent in India, especially during periods of famine that result from droughts when the crop fields become blighted and, as an alternate crop, a type of pea referred to as chickling vetch or *L. sativus* is cultivated. Those who eat this plant frequently suffer from an affliction of the central nervous sytem that causes weakness and paralysis of the leg muscles and, in extreme cases, death (neurolathyrism).

Attempts to identify the causative agent of human lathyrism have been complicated by the fact that another species of *Lathyrus* – *L. odoratus*, or sweet pea – produces in rats a type of lathyrism (osteolathyrism) characterized by skeletal deformities. In contrast to this, rats thrive quite well on *L. sativus* and do not display the nervous disorder associated with man's consumption of this species. Historically, the lathyrogen of the sweet pea was the first to be isolated, and was identified as β-(N-β-glutamyl)-aminopropionitrile, although β-aminopropionitrile is equally as active as an osteolathyrogen (Figure 8).

Several groups of workers in India have succeeded in isolating from *L. sativus* a compound that may very well be the causative principle of human neurolathyrism. This compound was identified as β-N-oxalyl-α,β-diaminopropionic acid (Figure 8), and its injection in young chicks, rats, and monkeys produced severe neurotoxic symptoms. Any conclusion that this compound is, in fact, the causative agent of human lathyrism must await the outcome of experiments involving the oral ingestion of the neurotoxin by suitable experimental animals.

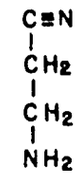
Assuming that oxalyl-diaminopropionic acid is the causative factor of human lathyrism, it may come as a surprise to learn that all of the misery associated in the past with the consumption of *L. sativus* could have been avoided by a relatively simple detoxification procedure, involving steeping the dehusked seeds overnight, then steaming them for 30 min or roasting them at 150° for 20 min. Amino-acid analysis of the protein of *L. sativus* indicates that it is rich in lysine, and is therefore potentially useful as a lysine supplement.

Breeding species and varieties of *Lathyrus* that are genetically deficient in the neurotoxin is also a possibility. In extensive surveys of different species of *Lathyrus* (Bell, 1964) and different varieties of *L. sativus* (Jeswani, et al., 1970), a number of samples were found to have little or no oxalyl-diaminopropionic acid. Unfortunately, many of these samples also contained other compounds that could be lathyrogenic, among them α,γ-diaminobutyric acid and β-cyanoalanine (Figure 9), which also are known to produce neurotoxic effects when injected into animals. It is obvious that any serious breeding program involving *Lathyrus* must take into account the possible role of these compounds, as well, in the pathogenesis of lathyrism.

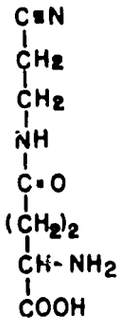
Favism

Favism is a disease characterized by hemolytic anemia. It affects certain

OSTEOLATHYROGENS



β-aminopropionitrile
(BAPN)

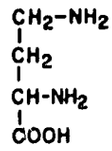


β-(N-γ-glutamyl)-aminopropionitrile

Found in
L. odoratus
L. pusillus
L. hirsutus

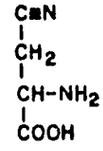
Figure 8. Structures of compounds that cause osteolathyrism, found in certain species of *Lathyrus*.

NEUROLATHYROGENS



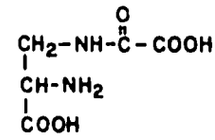
α,γ-diaminobutyric acid

Found in
L. latifolius
L. sylvestris



β-cyano-L-alanine

Found in
V. sativa



β-N-oxalyl-α,β-diaminopropionic acid

Found in
L. sativus
L. cicera
L. clymenum

Figure 9. Structures of compounds that act as neurotoxins, found in certain species of *Lathyrus* and *Vicia*.

FOUND IN VICIA FABA

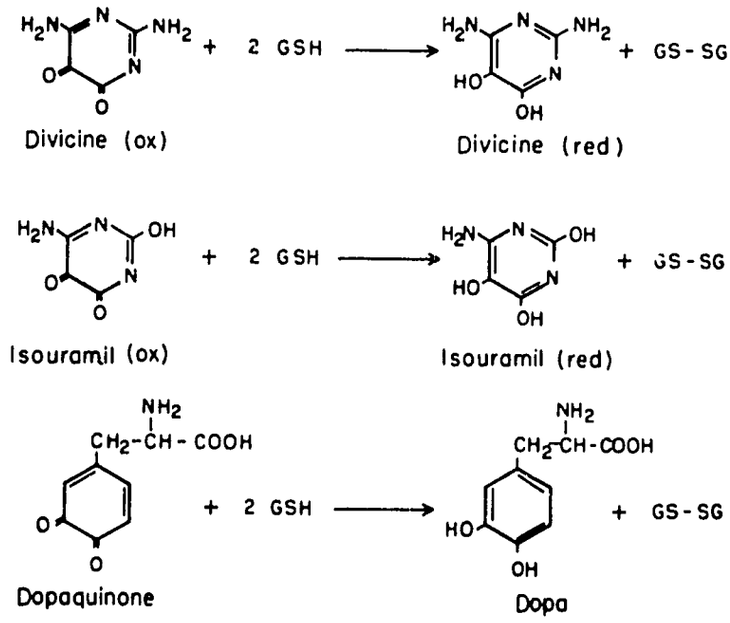


Figure 10. Compounds found in *Vicia faba* that catalyze the oxidation of glutathionine (2GSH → GS-SG). The oxidative destruction of glutathionine may be the underlying biochemical event responsible for favism.

individuals after they have ingested fresh, raw, or cooked broad beans (*Vicia faba*). The subject of favism has been reviewed recently by Donoso, et al. (1960) and Mager, et al. (1969). The disease is confined largely to the inhabitants of countries surrounding the Mediterranean basin, although individuals of the same ethnic background residing in other countries frequently suffer from favism. Individual susceptibility to this disease is believed to be of genetic origin, and an attack is frequently precipitated simply by exposure to the pollen of the blossoms of this legume.

One of the difficulties in establishing the pathogenesis of favism is that it has not been possible to reproduce the disease in experimental animals. In fact, the broad bean can serve as a satisfactory source of protein in feedstuffs, although it does tend to be somewhat deficient in the sulfur-containing amino acids (Eppendorfer, 1971; Nitsan, 1971).

The red blood cells of individuals who are prone to favism exhibit a number of biochemical abnormalities, the most significant of which are reduced levels of glutathione and glucose-6-phosphate dehydrogenase activity. Reduced levels of both of these factors are believed to destabilize the integrity of the cell membrane, so that exposure to some component of the broad bean then precipitates the hemolytic crisis.

Recent evidence would suggest that the substances responsible for favism are pyrimidines that occur naturally as β-glycosides (Mager, et al., 1965). These substances, known as divicine and isouramil, have the structures shown in Figure 10, and are the aglycone moieties of vicine and convicine, respectively. In vitro experiments have demonstrated that divicine and isouramil rapidly decreased the glutathione content of red blood cells deficient in glucose-6-phosphate dehydrogenase, and could thus account for the hemolytic effect exerted by broad beans. In addition, broad beans are rich in DOPA quinone, which also has been shown to catalyze the oxidation of reduced glutathione (Buetler, 1970).

It is a curious fact that divicine was first discovered in the common vetch, *Vicia sativa*, and the garden pea, *Pisum sativum*, in 1891 by Schulze. Divicine is also toxic when injected into experimental animals and, because of its occurrence in *Vicia sativa*, was at one time thought to be the causative factor of lathyrism (Anderson, et al., 1925). Divicine is not confined to the broad bean, and this makes it difficult to accept the idea that the compound can be responsible for a disease which, to our knowledge, can be produced only by the ingestion of this particular bean.

Conclusions

It should be apparent that, although there are numerous examples of so-called toxic constituents in legumes, they nevertheless have provided a valuable source of protein to man over the centuries. This can be attributed, in part, to the fact that man has learned how to detoxify them by suitable preparative measures.

The varied nature of our diet also minimizes the contribution of a toxicant from any one foodstuff. Nevertheless, there is the ever-present possibility that the prolonged consumption of a particular legume that may be improperly processed could bring to the surface toxic effects that otherwise would not be apparent. As the shortage of protein becomes more acute, it is not unlikely that much of the population of the world will be faced, in the future, with a more limited selection of protein foods, most of which will be of plant origin and, hence, potential carriers of toxic constituents. The food scientists and plant breeders should at least be cognizant of such a possibility, and be prepared to apply their knowledge and skill to meeting this challenge.

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DISCUSSANT

James Mc Ginnis

We have found that when *Phaseolus* beans are cooked adequately and fed to chicks, the best growth is about two-thirds that we would expect with a good, normal diet. Even when we corrected the bean diet, as far as we could, with amino-acid supplements, minerals, and vitamins, the growth performance was poor. We determined metabolizable energy — the energy the chick can derive from bean diets — and found it to be unexpectedly low. We could not explain why. Every other chemical analysis indicated that we should have higher values. But we must take the answer the animals give us.

About three years ago, we again became involved in examining the nutritional values of *Phaseolus*. We looked at different varieties and found, as was to be expected, that when fed raw, they were all poor in value. We got highly significant responses to cooking; all of the varieties tested responded to methionine. But, again, the value was low. Another aspect was a serious diarrhea we found in the animals that were fed high levels of beans. We then remembered the work that had been reported on raw soybeans, in which penicillin was part of the mixture, and tried an antibiotic supplementation of diets composed primarily of raw or cooked *Phaseolus*. Much to our surprise, we found a far greater growth response to the antibiotic mixture than we did with the amino-acid supplementation. In that situation, in which two-thirds of the protein was from *Phaseolus* and the other third from a mixture, amino acid obviously was not the most limiting deficiency. Rather, it was something that antibiotics could overcome.

My interpretation is that we are dealing with certain heat-stable factors in beans that depress growth in some way. This depression is counteracted or overcome by antibiotics. I think it is an indirect action on the intestinal microflora. I do not believe it is a nutrient defect, unless we are seeing the same kind of thing that has been observed in germ-free animals: intestinal microflora that compete effectively with the host animal for nutrients. For example, one scientist who has studied amino-acid requirements of germ-free rats versus "contaminated," or normal, animals has found that the methionine requirement for the normal growth of germ-free rats is only about 40 per cent of that needed for the normal animals. So here, again, we may be supporting or stimulating a much more competitive microflora. This may be related to the types of organisms that are involved in flatulence.

But we must look at the total nutritional effects of these bean products. Dr. Bressani discussed a product made from casein and carbohydrate that attempted to simulate bean protein. The test animals consumed a far greater quantity of that mixture, based on a free choice with corn, than of any of the other beans offered to them. We have made the same finding. (Of course, we are told that chicks do not have taste buds, but our observations certainly indicate that they have a tremendous ability to discriminate and select — on what basis, I do not know.) This kind of finding could play a most important role in the total nutritional value of bean products. After all, a bean is 20 per cent to 25 per cent protein.

We also have found recently that perhaps we can overcome the antinutritional effects in beans — and in cereal grains, as well — by a mild type of acid hydrolysis. This will improve the value of beans as much as will the antibiotic. The finding suggests that we are looking at a component in beans and other materials that is either not utilized by the test animal and so is directly harmful, or is not utilized and serves to stimulate some intestinal organisms in an adverse way. Young animals are affected more seriously than are the adults, as we have found by feeding some of the products to laying hens, which tolerate them very well.

As we look at the total problem of inhibitory effects, we must be aware of different results with different kinds of animals. Here we should make what progress we can, from a breeding standpoint, as rapidly as possible. Then we should attempt approaches to improve plants in ways other than by breeding.

III C. Flatulence

Statements

GAS-FORMING PROPERTY OF FOOD LEGUMES
– D. H. Calloway

INVESTIGATIONS OF FLATULENCE PROPERTIES OF DIFFERENT FOOD PRODUCTS
– James McGinnis

THE POSSIBLE ELIMINATION OF LEGUME FLATULENCE BY GENETIC SELECTION
– Edwin L. Murphy

Discussant

Earl R. Leng

GAS-FORMING PROPERTY OF FOOD LEGUMES

D.H. Calloway

Legumes are notorious inducers of flatulence. The first evidence of flatulence in average, healthy adults is a sharp rise in hydrogen concentration of the breath about five to seven hours after eating large amounts of beans (Figure 1) (Calloway, 1968). Shortly thereafter, gas begins to be passed from the anus. Both the breath-hydrogen concentration and the rate of gas egestion diminish gradually over the next two hours or so. Both parameters are idiosyncratic; some persons have large increases in breath hydrogen without passing gas after a dose of beans that causes marked flatulence in others (Calloway and Burroughs, 1969). In a given person, the proportion of intestinally produced gas that is excreted via the lungs and via the anus varies to a limited extent from day to day. However, while it is true that hydrogen concentration may be elevated in the breath without other evidence of gas formation (bloating, borborygmus, passage of gas), the converse situation (in which increased amounts of gas would be expelled from the anus without a rise in breath hydrogen) has never been recorded in tests of flatulence due to foods.

The dominant components of flatus passed from the anus at peak production rates are carbon dioxide and hydrogen (Figure 1). Some persons pass large amounts of methane, as well, but others do not (Calloway, 1966). Large volumes of flatus usually contain relatively small amounts of nitrogen and oxygen; these atmospheric gases are major constituents of flatus when passage rate is low or when flatulence is caused by swallowing air – a quite different phenomenon from food-induced gaseousness.

Radiographic evidence indicates that the rise in breath hydrogen occurs when food residues are passing from the lower ileum into the ascending colon (Murphy, 1964a). There the abundant microflora ferment undigested food carbohydrates, forming carbon dioxide and hydrogen as the primary end-products (Calloway, et al., 1966)*. Methane will be formed farther along in the colon if the individual's gut is colonized by methane-producing species. Carbon dioxide is added to the luminal gases from bicarbonate-rich intestinal secretions, food carbonates, and endogenous metabolism.

*Other minor volatile compounds can be formed (e.g., hydrogen sulfide, ammonia) and a number of nongaseous substances (e.g., lactic acid, histamine, vitamins). Increased bacterial growth and mild laxation also follow consumption of foods high in poorly digested carbohydrates. This may have beneficial effects in terms of health (Burkitt, 1969; Painter 1972), but the accompanying increase in fecal-nitrogen loss lowers the apparent digestibility of the food protein.

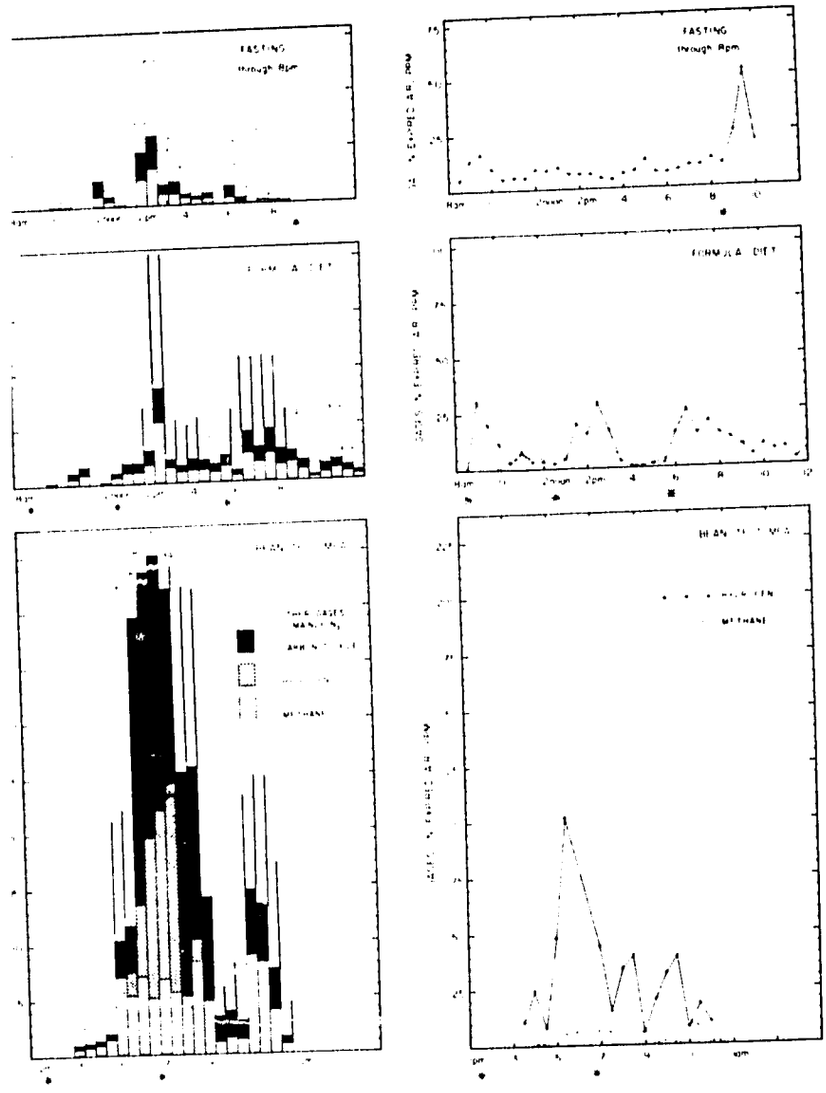

Figure 1. Diagrams on the left represent the volume of flatus gas collected each 30 minutes by rectal catheter from one subject under three conditions: *top*, fasting for 24 hours with formula meal at 8 pm (*); *center*, bland formula meals at regular intervals (*); *bottom*, test meal consisting of 100 gm dry weight of white beans and formula meal five hours later (*). Curves on the right show concentration in ppm of hydrogen and methane measured simultaneously in the breath. (From Calloway and Murphy, 1968)

Gases formed in the gut diffuse out of the lumen into the blood, and gases present in the blood and tissues diffuse into the lumen, depending on their partial pressures on either side of the intestinal membrane. Diffusion rate is a function of molecular weight, and is affected by the thickness of the membrane through which the gas diffuses and by the differing solubilities of the gases in the aqueous and lipoid layers. When production rates are high and the partial pressures of the bacterially produced luminal gases rise, diffusion into the blood is favored and the gases are transported to the lungs, where partial pressures again favor their outward diffusion. Because hydrogen and methane are present in negligible amounts in normal atmosphere, they will tend always to appear in the breath when they are formed in the gut. And, as there is no known nonbacterial metabolic pathway for production of these gases, their presence in the breath is a good indicator of bacterial fermentation in the gut. Because it is ubiquitous, intestinal carbon-dioxide production cannot be traced in this way, of course.

There are a number of ways in which foods might affect the amount of gas formed in the intestine and the route of its disposal. If the food contains substances that man cannot digest but that his bacteria can, the food provides a rich substrate for bacterial gas formation. This situation exists in normal individuals with the alpha-galactosides present in legumes and with milk sugar in lactase-deficient persons. Foods contain substances that directly affect gastrointestinal motility and blood flow (e.g., pressor amines); that have surface-active properties (e.g., saponins); that interfere with respiration (e.g., nitrates); and so forth. Bacterial action on the food may form other pharmaco-active substances, such as hydrogen sulfide, a carbonic anhydrase inhibitor. There is even the possibility that foods may affect the nature of the intestinal flora, as in the postulated cases of the *Lactobacillus bifidus* factor in breast milk and the antibiotic activity of the mold-fermented soybean food, tempeh.

In view of this complexity, it is not surprising that the flatulence factor(s) (FF) in foods have not been characterized fully. FF has been removed from white beans by exhaustive extraction with 60 per cent aqueous ethanol; the extract contains oligosaccharides (stachyose, raffinose, verbascose) and small peptides (Murphy, 1964b).

Administration of as little as 2 gm of stachyose causes a measurable increase in breath hydrogen (Calloway and Murphy, 1968). Pulmonary excretion of hydrogen after a 5-gm dose of raffinose was almost as great as after a 100-gm feed of beans, but flatus egestion was not increased appreciably above control values when the sugar was eaten (Calloway and Murphy, 1968). Higher dosages of raffinose (8-17 gm) result in typical flatus egestion (our unpublished findings). These dosages exceed the amount of stachyose and raffinose found in common food legumes, but may be in the range of total alpha-galactosides present. Complete quantitive analyses of these oligosaccharides have not been reported to date.

Some food-processing methods reduce FF effectively (Calloway, et al., 1971). Precipitated soybean curd (tofu) has essentially no FF, and isolated bean proteins also are free of FF. When soybean grits are fermented with *Rhizopus oligosporus* to produce tempeh, flatulence is reduced markedly. Experimental

treatment with an alpha-D-galactopyranosidase enzyme reduces FF in soybean flour. Ethanolic extraction of whole white beans results in a product with lessened flavor but with reduced FF. A number of other treatments, all involving repeated aqueous extractions at various pH's, marginally reduce FF, but have adverse effects on flavor and color.

Until the food substances responsible for induction of excessive formation of gas in the intestinal tract are characterized completely, FF must continue to be quantified by biological assay. Various assay techniques have been proposed, but only certain human tests are fully satisfactory.

Microbiological Methods

In the interests of simplicity, speed, and economy, several workers have attempted to assay FF by measuring the amount of gas produced by bacteria acting on foods or single nutrient substances in vitro. Bacteria used were mixed cultures derived from human ileostomy and colostomy residues (Calloway, et al., 1966), and dog intestine (Steggerda, 1968; Rackis, et al., 1970); or pure cultures of *E. coli* (Steggerda, 1968) and *Cl. perfrigens* (Rockland, et al., 1969). In all cases, gas was evolved from bean substrates with anaerobic culture methods. All mixed cultures showed the capacity to ferment stachyose, but neither of the organisms tested in pure culture did so. The most obvious weakness of this method is lack of specificity: in all instances, as much or more gas was produced when the substrate contained common monosaccharides or disaccharides as when bean fractions were added. Also, to the extent that flatulence results from a combination of bacterial fermentation and other factors affecting the gut, bacteriological methods cannot provide complete answers.

Gas Present in the Bowel

Gas content of rat viscera has been measured at various times after bean and control feeds by a method based on the different specific gravities of gas-containing and gas-free tissues (Hedin and Adachi, 1962). The extirpated intestines were brought to volume in constantly boiling HCl and weighed before and after digestion and readjustment of volume. Because carbonates present in the gut react with the acid to produce gas, this method overestimates gas volume. The measured volume would include food-carbonate contributions and would reflect potential effects of dietary treatments on bicarbonate secretion. Composition of luminal gas was determined in these studies by analysis of gas aspirated from the gut *in situ*, and would not have included carbon dioxide evolved from carbonates. Thus, volume of component gases (other than CO_2) would be overestimated by applying the observed composition to the observed volume.

Because much more gas leaves the gut than is retained (Calloway, 1968), this method attempts to measure the "tip of the iceberg." The rat intestine contained about 1 ml of gas four to six hours after a purified control diet was fed, and 2 to 2.5 ml when beans were fed. The coefficient of variation is in the order of 15 per cent, but dietary effects have been detected reliably by this method

when it is in skilled hands (Hedin and Adachi, 1962; Hellendoorn, 1969). Unfortunately, for present purposes, larger volumes of gas were recorded in rats fed a diet that does not cause flatulence in man (purified amino-acid diet) than in those fed beans (Hedin, 1962). Gas volumes also were increased inconsistently (Hedin, 1962), unchanged, or reduced (Kakade and Borchers, 1967) after antibiotic treatments.

Volume and composition of gas present in loops of duodenum, jejunum, ileum, and colon of anesthetized dogs were measured after three hours of fermentation in vivo; gas evolved with bean homogenate increased progressively caudad and was eliminated or reduced markedly by various antibiotic treatments (Richards and Steggerda, 1966). Because it requires special operative skill and is very expensive of animal life, this method is better reserved for fundamental research than for routine screening of products under development. The procedure also involves unavoidable interference with respiration, blood flow, and so forth, and cannot be regarded as physiological.

Hydrogen Excretion in the Rat

Production of gases of exclusively bacterial origin, hydrogen and methane, can be measured by housing an animal in a closed system of known volume with adequate arrangements for life support, i.e., removal of carbon dioxide, sufficient water, and oxygen supply. Such a scheme has been devised and tested with a view toward using hydrogen production to assay the gas-forming potential of foods (Gumbmann and Williams, 1971). Maximum production rate of hydrogen occurred four or five hours after beans were fed, but for convenience the investigators chose to measure hydrogen content of the chamber 18 hours after the test began, when steady-state conditions were thought to exist. The coefficient of variation ranged from 24 to 80 per cent in a series of experiments (average, 53 per cent) in which 3.2 to 7.7 ml of hydrogen per rat was recorded. Factors identified as contributing to variation were pre-test diet and duration of legume-feeding. There would also have been substantial and probably variable contributions of hydrogen (and methane) from feces that collect in the cage during the test (our unpublished findings). In addition to the enormous problem of standardizing a procedure subject to variation of this magnitude, the method has a scientific weakness. Carbon dioxide is a major component of flatus, particularly with large volumes of legume-induced gas; it is not and cannot be measured with this technique because endogenous production is many orders of magnitude larger. Carbon dioxide and hydrogen are not always found in the same proportions in flatus. Therefore, this method, which measures only one component, cannot be expected to correlate well with tests that measure all constituents of flatus.

Gas Content of Stools

Gas content of stools from healthy persons can be sufficient to lower the average specific gravity from 1.09 to as little as 0.98 (Levitt and Duane, 1972).

The gas present was found to be mainly methane, with very little hydrogen. Nearly half of a typical adult population fail to show evidence of significant methane formation (Calloway and Murphy, 1968), and methane does not figure importantly in the typical occurrence of flatulence five to seven hours after a meal (Calloway, 1966). However, analysis of the constituent gases and measurement of fecal specific gravity (by means of a pyknometer) might be useful in judging other effects of dietary treatment on lower-colonic fermentation in individuals whose tract is colonized by methane-forming organisms. The method has not been tested in this way.

Hydrogen Content of Expired Air

Breath-hydrogen concentration can be used to predict that a food may be gas-forming due to the presence of unabsorbable carbohydrates, but not that the food is certain to cause noticeable flatulence. Although it cannot predict the probable volume of flatus, total gas production cannot be estimated properly without knowledge of pulmonary excretion.

Minor gases are difficult to measure at concentrations found in the expired air (2 to nearly 200 parts per million). Breath samples must be collected and stored in containers impervious to them, usually multilaminated, metallized plastic bags (Calloway and Murphy, 1968). Hydrogen concentration in this range may be measured by a helium ionization detector after chromatographic separation with a molecular sieve column (Calloway and Murphy, 1968). If only less-sophisticated instrumentation is available, a rebreathing technique may be used with suitable precautions to raise the hydrogen concentration to detectable levels (Levitt, 1969).

For valid assessment of treatments, measurements must be made in several people or several times in a few people, and in a proved nonflatulent control. In addition, a standard bean-feeding must be included as a periodic check. The test is costly, in that subjects must be studied for several hours under comfortable conditions with stipulated exercise and freedom from emotional stress, and the original investment in equipment is considerable.

Volume of Egested Flatus

Two techniques are known for measuring the gas content of the human gut. The first, a plethysmographic method, requires bulky and complicated apparatus and gives widely different values in different investigations (Calloway, 1968). The other method involves flushing out the luminal gas by infusing an inert carrier gas, argon (Levitt, 1971). This method requires intubation of the subject and a high flow rate, 30 ml per min, of infusion gas, which entails discomfort ranging from mild distention to severe cramps and vomiting (Levitt, 1968). Responses observed under these conditions would be distorted by the procedure: at high flow rates, gases that might otherwise be removed by pulmonary ventilation would be swept from the gut; intestinal motility is known to be altered, which would change the rate at which food residues move along the

alimentary canal and alter the substrate-microflora relationships; and some alteration of intestinal secretion and blood flow might be anticipated. Except among sadists, the technique would not be acceptable in any case for the repeated investigations required for product development.

It is quite easy to collect gas from the anus, and many investigators have done so. Most have used intrarectal catheters of various types attached to a collection vessel by means of tubing. Catheters are not entirely satisfactory, because they cause mild discomfort to the subject and occasionally become plugged with fecal matter, which prevents gas collection. An alternative, and equally effective, technique is to affix to the shaved, alcohol-cleansed, and dried buttocks an adhesive colostomy patch with an attached plastic tube. To permit some physical activity, it is preferrable to collect the gas in an impervious bag (v.s.) The volume of gas collected can be measured manometrically, and its composition determined by conventional chromatographic methods (Calloway and Murphy, 1968). Gas can also be collected by a fluid-displacement technique in an ordinary gas apparatus, provided the fluid is properly selected with respect to solubility of gases (Kirk, 1949).

Gas volume and composition have been measured with even simpler equipment (Murphy, 1964b). The rectal collection tubing is attached first to a drying trap (anhydrous calcium sulfate), then to a U-tube filled with sodium hydroxide-impregnated asbestos (which reacts stoichiometrically with carbon dioxide), and then vented to the atmosphere. Composition and/or carbon-dioxide content of rectal gas is measured in samples taken at frequent intervals from the tubing proximal to the body; the total amount of carbon dioxide passed is determined by weighing the U-tube at the same frequent intervals. From this information, the total volume passed can be computed with accuracy, if sufficient measurements are taken.

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INVESTIGATIONS OF FLATULENCE PROPERTIES OF DIFFERENT FOOD PRODUCTS

James McGinnis

The results of studies conducted at Washington State University, using in vitro techniques and pure cultures of *Clostridium perfringens,* show clearly that factors other than carbohydrate content may have a marked influence on the gas production by this organism. Studies of this type were undertaken because of the observed growth-depressing effects of high levels of dried beans on chicks and because of the surprisingly large response to antibiotic supplements of chicks fed such a diet.

A decision was made early in these studies to provide a supply of readily fermentable carbohydrate in the form of glucose so that we might study the influence of factors other than carbohydrate -- or at least factors other than glucose – on gas production. One medium, described by Boyd and associates many years ago, has been used by many investigators and was used in some of these studies; a second medium was devised in our laboratory. It was found that addition of a bean homogenate to either of these media would stimulate gas production, and it was also observed that many different products had this property. Some typical results obtained in our studies are summarized in Tables I and II. It can be seen that the ash component of beans was as active as the bean homogenate in stimulating gas production when added to the W.S.U. medium, but was much less effective when added to the Boyd medium.

Many other insoluble materials have also been found to stimulate gas production by this organism when added to the W.S.U. basal medium. The results obtained with the Boyd medium indicate that it is probably deficient in more than one factor necessary for maximum gas production by *Clostridium perfringens.* The addition of yeast extract to this basal medium stimulated gas production, and a combination of yeast extract and ash from either bean or soybean meal was more effective than was either material added alone.

Discussions on the results we have obtained indicate that microbiologists have known for years that the presence of solid particles in a liquid medium will stimulate the growth of *Clostridium perfringens.*

TABLE I
Influence of Medium and of Adding Beans or Bean Ash on Gas Production by *Clostridium perfringens*

Supplement mg/tube	Gas Production			
	Boyd Medium		WSU Medium (C-2)	
	3.75 hrs	6.75 hrs	3.75 hrs	6.75 hrs
None	0	0	5.0	35.3
25 beans	0	0	16.3	69.7
50 beans	0	6	17.3	73.3
25 bean ash	0	7	22.3	>75
50 bean ash	0	5	16.0	>75
25 beans + 25 bean ash	0	12.3	36.3	>75
50 beans + 50 bean ash	0	14.0	39.0	>75

Washington State Univ., 1971

TABLE II
Effect of Different Supplements to the Boyd Medium on Gas Production by *Clostridium perfringens*

Supplement mg/tube	mm gas production after incubating	
	2 hrs	3 hrs
None	0	3.0
50 beans	++	6.7
50 bean ash	++	6.7
50 soybean meal ash	2.0	14.0
15 yeast extract	4.5	48.1
Yeast extract + beans	18.0	75
Yeast extract + bean ash	25.0	75
Yeast extract + soybean meal ash	25.3	75

Washington State Univ., 1971

THE POSSIBLE ELIMINATION OF LEGUME FLATULENCE BY GENETIC SELECTION

Edwin L. Murphy

Traditionally, man has turned to genetic selection to solve his many problems encountered in raising legumes: better yields; nematode and blight resistance; color and size of seed; etc. In any proposed program for plant breeding to eliminate or reduce legume flatulence, the geneticist has been frustrated by the lack of published flatulence data and a reliable assay method rapid enough to handle the hundreds of samples generated. One scientist at a U.S. plant-introduction station wanted 3,000 bean samples assayed for flatulence. This would be a five-year project at our present rate of testing with human subjects.

When flatulence is measured in human test subjects under controlled conditions, differences in degree of gas response are noted among members of the legume family (Murphy, 1964; Calloway, et al., 1971). Dry navy, kidney, and pinto beans (Table I), produce more flatulence than do dry peas, peanuts, mung beans, or soybeans. Some of the difference in reported data on flatulence is probably due to differences in the variety of legume tested rather than to techniques of measurement used or to subject response.

In our laboratory, difference in measurable flatulence response in human subjects had been obtained with related members of the *Phaseolus lunatis* group (Table II). When fresh frozen mature lima beans, Fordhook variety, are fed, the result is similar to a bland control meal. The dry seed of the Fordhook variety, which is not normally sold as a dry bean, also did not produce a significant degree of gas formation. By comparison, the Ventura variety of dry lima bean increased the elimination of intestinal gas tenfold in this study.

When the genetic parent, Fordhook concentrate, of the bland Fordhook variety was fed to several test subjects, the flatulence response was equivalent to the *Phaseolus vulgaris* group. So the flatulence factor appears to have been lost in the development of the commercially frozen bean.

We obtained a sample of a reportedly gasless member of *Phaseolus vulgaris*, Pike's Jacobs Cattle Bean, which was the result of a cross of a reportedly gasless, heirloom "Jacobs Cattle" bean (*Phaseolus vulgaris*) with the Black Mexican variety. When fed to human test subjects, the hybrid raised the level of formation of intestinal gas to about four times that of the bland control meal, compared to the expected tenfold response common to the *Phaseolus vulgaris* group.

The significance of these limited flatulence measurements on members of the dry bean family is that there appears to be a genetic transference of the degree

TABLE I
Flatus from Different Legumes

	Ratio*
Phaseolus vulgaris	
California small white	11.1
Pinto	10.6
Kidney	11.4
Phaseolus lunatus	
Lima, Ventura	4.6
Lima, Fordhook	1.3
Phaseolus mungo	
Mung	5.5
Glycine max	
Soya, Lee, or Yellow	3.8
Arachis hypogaea	
Peanut	1.2
Pisum sativum	
Pea, dry	5.3
Pea, green	2.6
Bland test meal	1.0

*Ratio of flatus from test meal of 100 g (dry weight) for a three-hour period measured from four to seven hours after ingestion as compared to a bland test meal.

TABLE II
Flatus from Lima Beans

Varieties	Human subjects		
	DC	RD	BM
	(Total volume (cc) for 3-hr peak period)		
Fordhook			
Formula	50	0	19
Green mature	41	0	14
Dry	93	0	25
Ventura			
Dry	471	45	166

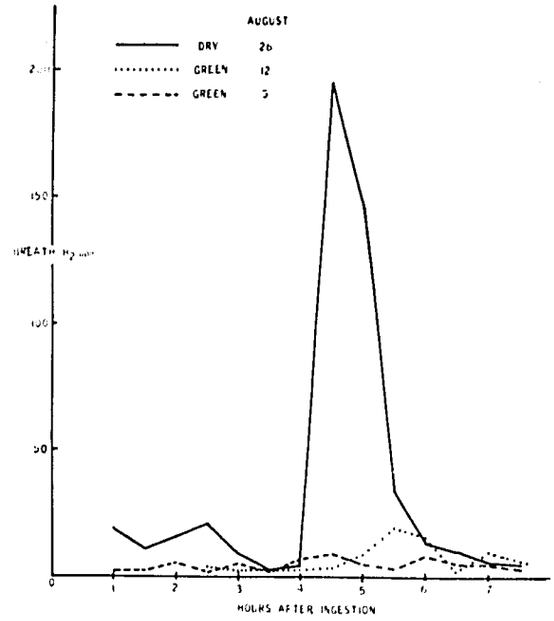

Figure 1. Breath hydrogen after ingestion of beans of different maturity.

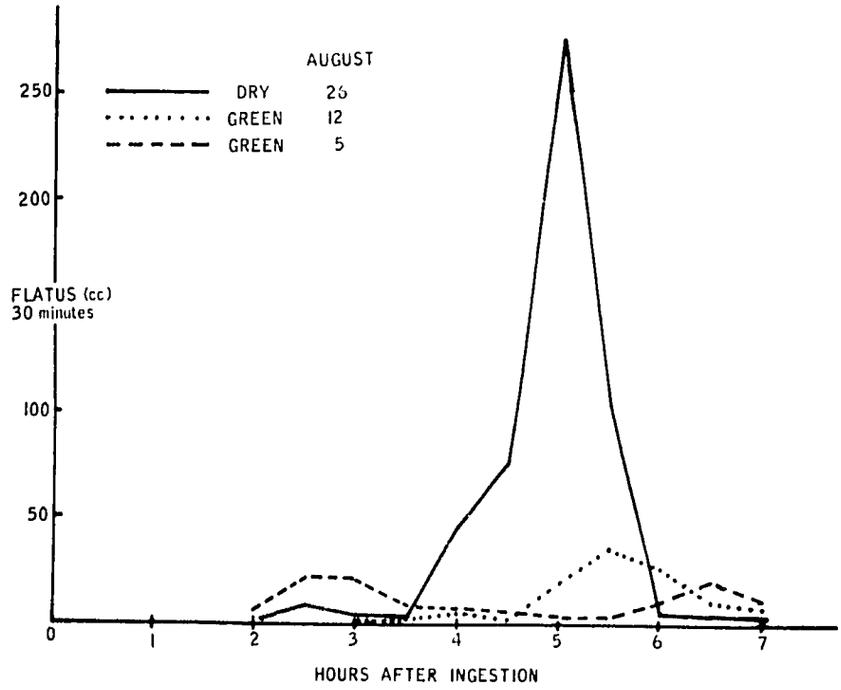

Figure 2. Flatus after ingestion of beans of different maturity.

of flatulence response. This suggests a possibility of reducing this undesirable legume trait by genetic selection.

In any genetic study of flatulence, the degree of plant development must be taken into consideration. In general, the immature green seed induces no flatulence in test subjects compared to the usual degree experienced with the mature dry seed. In the Ventura variety of lima bean, the flatulence principle is not present three weeks before green maturity or maximum seed weight, but begins to appear about two weeks prior to this stage (Figures 1 and 2). This is indicated by the appearance of a slight rise in breath and flatus gases associated with the three-hour measurement period during which bean-induced flatulence is experienced in this test subject.

The fresh-frozen pea is marketed at a stage of development at which the flatulence principle is already present. When test subjects are fed equivalent test meals of fresh-frozen green peas, wrinkled-skin variety, and dry peas, smooth-skin variety, the increase in flatus formation for the fresh-frozen green pea is almost one-half that of the dry variety (Table I).

The flatulence principle evidently does not disappear rapidly from the cotyledons of the germinated bean. When the California small white variety of *Phaseolus vulgaris* was germinated to a point of radicles 10-11 mm long with developing root laterals and fed to a test subject, the flatulence response was equivalent to an equal weight of the ungerminated dry seed. Similar results were obtained when soybean and mung-bean sprouts were fed test subjects (Calloway, et al., 1971).

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DISCUSSANT

Earl R. Leng

When Dr. Hymowitz in our group at the University of Illinois first came to us, he began to work with Dr. Steggerda, who, as Dr. Calloway has mentioned, was involved with this problem for a long time, specifically with soybeans. Dr. Hymowitz's work grew out of our interest in direct food uses for soybeans. Since Dr. Steggerda's death, the flatus work at the University of Illinois has not been continued, so I will attempt to report only on the range or variation that Dr. Hymowitz has found working with the northern collection of soybean varieties.

As many of you may know, the germ-plasm collection is divided into two parts: one is maintained at Urbana, Illinois; the other at Stoneville, Mississippi. Dr. Hymowitz has followed an analytic method very much like that Dr. Calloway has outlined. Essentially, after oil extraction and protein precipitation, a sugar residue remains. This is analyzed chromatographically. Dr. Hymowitz has found ranges in total sugar varying from around 6 per cent to more than 16 per cent in the varieties used. More interestingly, a wide range exists in the distribution of different types of sugars. The total sucrose fraction varies from 41 per cent to 67 per cent; some varieties have only about two-fifths, others as much as two-thirds, of the sucrose fraction.

Raffinose, which may or may not be implicated, as Dr. Calloway has said, varies from 5 per cent to more than 15 per cent of the total sugars; stachyose from 12 per cent to more than 35 per cent. These figures clearly suggest an enormous range of variability within the northern soybean varietal collection. In the frequency of the distribution of the different sugars, there are no such things as the non-raffinose or the non-stachyose type, but from very low to high ranges are present.

Obviously, the correlation of these is needed in dietary use by human subjects, but we are not in a position to do this. It merely leads to the suggestion that, if soybeans are going to become a more important item of the human diet, correlations should be made. It also suggests that similar situations probably obtain in other types. For instance, it must be the case with the Fordhook derivative as opposed to the Ventura or other types. I would be most interested to hear any other evidence on this point.

III D. Processing and Food Acceptance Problems

Statements

UTILIZATION PROBLEMS IN FOOD LEGUMES
H.A. B. Parpia

TESTA PIGMENTS IN *CAJANUS CAJAN* CANNING QUALITY AND FLAVOR
W. Vernon Royes

BREEDING FOOD LEGUMES FOR IMPROVED PROCESSING AND CONSUMER ACCEPTANCE PROPERTIES
M. W. Adams and C. L. Bedford

Discussant
G. D. Kapsiotis

Summary and Research Needed
Irvin Liener

UTILIZATION PROBLEMS IN FOOD LEGUMES

H.A.B. Parpia

Food grains constitute nearly 80 per cent of the diet of a large majority of the people who live in technologically less advanced countries of the world. Quantitavely, cereals occupy the first place as the source of calories and protein, and grain legumes are the next (Aykroyd and Doughty, 1964). Qualitatively, however, grain legumes are more important, as they are the main sources for certain important amino acids, such as lysine and threonine. Cereal legume diets continue to be deficient in sulfur-amino acids, such as methionine and cystine. Animal proteins are out of reach for a large majority of the low-income groups of populations. Therefore, a sound program of breeding is necessary to upgrade the quality of vegetable proteins, particularly the legume proteins, in order to raise the nutritional standards of these people.

The position with regard to the production of legumes is not happy at present. As a result of the emphasis laid on improving the crop yield of cereals, legume production has not only remained stagnant, but actually has suffered a set-back in terms of per capita availability. Recently, however, more attention has been paid to increase the per hectare yield of grain legumes, to improve the economy of their cultivation, and to develop varieties for different agro-climatic conditions. If, simultaneously, due stress can be given to them, they can make a much more significant contribution to overcoming protein-calorie malnutrition in most parts of the world.

The objective of this paper is to discuss the place of grain legumes in the diet of the low-income groups of populations in developing countries, with particular reference to India, and to indicate the important areas where greater coordination is required in research and development work between breeding programs and postharvest technology.

Production and Consumption of Grain Legumes

India is the largest producer of grain legumes in the world (Table 1), and efforts have been made in recent years to develop high-yielding varieties suitable for different agricultural tracts in the country. The "relay cropping" recently developed by the Indian Agricultural Research Institue, New Delhi, follows the cultivation sequence of wheat-legumes-maize-oilseed crops. This system of cropping yields 90 quintals of cereals, 60 quintals of grain legumes, 15 quintals of

TABLE I
Estimated Production of Pulses in Different Countries in 1970*

	Million metric tons
World total	44.4
Europe	3.4
North + Central America	2.9
South America	3.0
Asia	15.3
Africa	4.5
Oceania	0.1
India	11.9
Guatemala	0.07
Mexico	1.5
Philippines	0.05
Thailand	0.13
Uganda	0.37
Ethiopia	0.50

*Production Year Book, Vol. 24, FAO, Rome, 1970

protein-rich legume fodder, and 8 quintals of oilseeds under the conditions studied. Preliminary efforts in improving the protein and amino-acid contents of these crops appear to be quite promising (Bains, 1968).

The consumption of legumes in some selected parts of the world is given in Table II. The importance of legumes in the diets in different parts of the world varies, depending on dietary patterns, availability, and local traditions (Patwardhan,1962).

The per-capita consumption of legumes varies in different states within India (Patwardhan, 1962), as in the world elsewhere. The consumption in the state of Tamil Nadu is as low as 16g per capita, whereas in Madhya Pradesh it is as high as 55g (Gopalan, et al., 1971; Table III).

Surveys indicate that there does not appear to be protein deficiency in diets based on cereals when their legume content is 50 to 80 g and the calorie intake is sufficient (Parpia, 1971). Some typical diets containing cereals and legumes that can meet the protein requirements adequately are given in Table IV.

The currently available 12.24 million tons of grain legumes in India can provide 64.5 g of grain legumes per capita per day, which corresponds to about 14.2 g of protein from this source (Parpia, 1971; Directorate of Economics and Statistics, 1968). This would indicate that there should be no protein deficiency in the diet, but uniform and equitable distribution is not possible in practice, because of widely varying consumption patterns and uneven income levels. If grain legumes are to meet the protein requirements of a majority of the popula-

TABLE II
Consumption of Legumes in Certain Developing Countries*

Country	Consumption (g/capita/day)	Remarks
Philippines	14	
Western Java	6	
Mexico	42	
Venezuela	80	Rural workers
	89	Urban workers
Guatemala	44	Poor Ladinos
	57	Other Ladinos
Guatemala	51	Poor Indians
	58	Other Indians
Togoland countries (13)	13-140	
South of Sahara	10-150	
India	14-114	Consumption unit
New Guinea	3	
	27	Includes peanuts
Japan	5.5-7.8	Exclusive of 44 to 65 g of soybean and its products
Haiti	30	

*Patwardhan, V.N. 1962. *Amer. J. Clin. Nutr.* 11:2

TABLE III
Consumption Pattern of Legumes in India*

State	Consumption (g/capita/day)
Madhya Pradesh	55
Uttar Pradesh	55
Gujarat	43
Rajasthan	43
Bihar	42
Punjab	35
Maharashtra	33
W. Bengal	30
Andhra Pradesh	29
Mysore	26
Kerala	24
Tamil Nadu	16
Jammu & Kashmir	8
All India	34

TABLE IV
Low-cost, Balanced Diets (in Grams) Containing Liberal Amounts of Pulses for Preschool Children and Expectant and Nursing Mothers

	Preschool children				Expectant mothers		Nursing mothers	
	2-3 years		4-6 years					
Foods	V*	NV*	V	NV	V	NV	V	NV
Cereals	150	150	200	200	400	400	450	450
Pulses	60	50	70	60	90	70	100	80
Green, leafy vegetables	50	50	75	75	150	150	150	150
Other vegetables, roots, and tubers	30	30	50	50	75	75	75	75
Fruits	50	50	50	50	50	50	50	50
Milk	300	200	250	200	300	200	300	200
Fats and oils	20	20	25	25	40	40	40	40
Sugar (refined and raw)	30	30	40	40	30	30	30	30
Meat and fish	–	20	–	20	–	20	–	20
Eggs	–	–	–	–	–	1	–	1

*V = vegetarian; NV = nonvegetarian

tion, their production will have to be increased by at least 25 per cent and linked with a planned rise in income levels and a sound program of education.

Storage and Handling

Food grains, including pulses, undergo substantial quantitative and qualitative losses as a result of rodent and insect infestation and infection by fungi during postharvest handling, storage, and distribution. Such losses are particularly heavy in India, where over 70 per cent of the food grains produced are stored and consumed in villages under very inadequate conditions for controlling infestation and infection. Grain legumes stored in overground structures are attacked by the pulse beetle (*Bruchus chinensis*), which causes heavy destruction of the grain and makes it unhygienic (Parpia, 1971) because of the presence of excreta; the insect damage further creates favorable conditions for microbial infection. The pulse beetle passes through eight overlapping generations in one year. The progeny of 50 eggs can reduce the weight of the grains (gram) by about 49 per cent in just six months (Singh, 1964). Grains infested by these pests are contaminated with metabolites, such as uric acid, show increased fat acidity, and become unfit for human consumption in a short time (Venkat Rao, 1960). Recent studies have shown that insect infestation significantly decreases the protein-efficiency ratio (PER) of split chick pea and pigeon pea (Table V). The milling yields of legumes are also reduced as a result of insect infestation (Table VI). Thus, the losses are both quantitative and qualitative.

Several techniques have been developed and adopted for controlling insect infestation. The resident infestation in grain legumes can be destroyed by fumigating with a mixture of ethylene dibromide and methyl bromide. This also controls fungi. Cross-infestation by insects can be prevented by treating the jute bags with special pesticidal emulsions. A single treatment by a combination of fumigation and pest-proofing of bags can preserve the food grains in sound condition for about six months (Parpia, 1971).

Like other food grains, legumes stored in humid conditions are also attacked by molds. The survey carried out by the Central Food Technological Research Institute in the coastal areas of Mysore State has shown the presence of *Aspergillus* sp. and *Penicillium citrinum* and their toxic metabolites in food grains (Annual Report, 1970a; Parpia and Sreenivasamurthy, 1970). Considering the high potency of aflatoxins as a hepatotoxic agent, the health hazard can be serious (Amla, et al., 1971). The growth of mold on food grains can be controlled by postharvest fumigation with (1) a mixture of ammonia and phosphine; (2) a mixture of methyl bromide, ethylene dibromide, and chloropicrin; (3) ethyl formate; and (4) chloropicrin alone. The last two are meant essentially for grains in small quantities, as their vapor pressure is low. These treatments are sporicidal in action and so minimize the hazard of the development of fungal toxins during storage.

Cooperation of breeders to develop grain-legume varieties with increased resistance to postharvest infestation may alleviate storage problems and reduce the need of chemical treatments for control of insect infestation and microbial infection.

TABLE V
The Protein Efficiency Ratio of Chick Pea and Pigeon Pea Infested by Insects (10 Female Rats Per Group; 10% Protein in Diet. Experimental Period: 4 Weeks)

Protein source	PER
I. Chick-pea dhal (control)	2.21
Chick-pea dhal (infested)	1.83
II. Pigeon pea (control)	2.04
Pigeon pea (infested)	1.66

TABLE VI
Effect of Insect Infestation on the Yield of Dhal from Bengal Gram

	% Kernel Damage	% Dhal yield
Chick pea (uninfested)	2	82
Chick pea (infested by insects)	15	65

Milling

About 80 per cent of the grain legumes produced in India are dehusked and milled to produce splits (dhal) before consumption. Traditional methods of dehusking and splitting are defective and give low yields, because of powdering and breakage. These operations have been modernized, and suitable equipment has been designed and fabricated by CFTRI. The milling yields of dhal can be increased 10 to 15 per cent by adopting this new technology (Table VII). On a national scale, adoption of the new technology can add nearly 1.5 million tons of grain legumes to present food supplies (Parpia, 1971), and can improve the quality of milled legume by minimizing the scouring of the outer surface of the grain, which has a higher percentage of protein than does endosperm.

Recent studies have shown that milling quality of grain legumes in different parts of India varies widely as a result of varietal and agro-climatic characteristics (Table VIII). The presence of gums determines the extent to which the husk adheres to the endosperm. Bold-grain varieties of pulses can be milled more easily than can small and medium varieties, and require less drastic pre-milling treatments. The development of loose-husked varieties should be given priority in order to improve milling out-turns, improve the quality of dhals, and reduce the processing costs.

Table VII also shows that although substantial improvement has been made in milling out-turns through modernization of technology, there is still some difference between the results so far achieved and the yield theoretically possible. This

TABLE VII
Per Cent Average Yields of Dhal from Legumes by Different Processes*

Legume	Home scale process	Conventional commercial methods	Improved CFTRI process	Maximum theoretical yield
Chick pea *(Cicer arietinum)*	75	75	82	89
Pigeon pea *(Cajanus cajan)*	68	75	83	89
Black gram *(Phaseolus mungo)*	69	71	82	88
Green gram *(Phaseolus radiatus)*	64	65	82	89

*Kurien, P.P. Data from CFTRI, Mysore

TABLE VIII
Yield of Dhal from Different Commercial Strains of Red Gram Grown in India*

Strain	% Average yield	
	Traditional method	Improved method
Lathur white	75	84
Hyderabad red-white mix	72	81
Bihar red-white mix	70	81
U.P. gray	68	80
Mysore red	64	79

*Kurien, P.P., et al. CFTRI, unpublished data, 1970

gap could perhaps be bridged to a significant degree by collaboration between breeders and postharvest technologists.

Toxic Factors in Legumes and Their Elimination

Many legumes contain deleterious, biologically active factors such as trypsin inhibitors, hemagglutinins, cyanogenic glucosides, saponins, and goiterogens. Toxic compounds which cause such diseases as lathyrism and favism are also present in some legumes (Liener, 1962).

Trypsin inhibitors present in many legumes are generally inactivated by cooking. Hemagglutinins agglutinate the red blood cells of various species of animals. Incorporation of purified hemagglutinins from different grain legumes in synthetic diets has been found to inhibit the growth of albino rats. The nutritive value of the legume proteins can be improved by destroying the activity of these compounds by heat treatment (Parpia, 1971).

In order to destroy the toxic factors in black beans (*Phaseolus vulgaris*) and field beans (*Dolichos lablab*), they first must be soaked in water before being subjected to heat treatment. The toxic compounds in the *Lathyrus* species can be eliminated by soaking the seeds in warm water, which is then discarded. Investigations carried out recently at the Indian Agricultural Research Institute, New Delhi, have shown that some strains of *Lathyrus sativus* contain only traces of the toxin and hence are safe for human consumption (Swaminathan, et al., 1970). By cultivating strains with low content or developing new varieties in place of the common ones that are highly toxic, it may be possible to eradicate lathyrism in India within the next five to six years. Favism is a serious problem in several African countries where the *Vicia faba* is consumed in large quantities. By selective breeing, it may be possible to develop varieties of these legumes with a low toxin content. Similarly, it should be possible to develop varieties of several other legumes with low levels of trypsin and growth inhibitors.

Legumes as Daily Food

After cooking or puffing, legumes are widely consumed as a part of the daily diet in many traditional food preparations of India. Compared to soybeans, grain legumes are easier to cook under home and rural conditions and are more acceptable in all parts of the country.

Cooked Legumes and Dhal

Whole-grain legumes are soaked in water overnight and the soaking water drained off. They are boiled in water for 30 to 40 minutes, and consumed after salt and spices are added.

Dhal or Dehusked, Split Legumes

These are cooked by boiling in water for 40 to 50 minutes, or 20 to 25 minutes in a pressure cooker, where available in certain urban areas. Salt and spices are added to taste and the cooked, mashed dhal is consumed along with cereal preparations or as soup.

Puffed Legumes

Puffed chick pea and dried peas are widely consumed throughout India. Studies with albino rats have shown that puffing does not affect adversely the nutritive value of the protein of the chick pea (Acharya, et al.., 1942).

Traditional Savory Preparations

Some of the common savory preparations containing split legumes are idli,

papad, and adai. Idli is a common breakfast dish prepared in many parts of India by steaming fermented mixed dough of ground rice and black-gram dhal. An instant idli mix developed at the Central Food Technological Research Institute has proved very popular and is being manufactured by several commercial organizations in Indian cities (Swaminathan, et al., 1963). Papads or papadams are paste goods prepared from black gram, green gram, and other varieties of dhals and produced both on a home and commercial scale throughout India. Extensive studies have been undertaken for rationalizing* and mechanizing the processing operations (Parpia, 1971).

In a country like India, which consists of several agro-climatic regions, the cooking and organoleptic qualities of grain legumes vary widely. In order to insure optimal consumer acceptance and to encourage greater use of grain legumes, it is urgently necessary for plant breeders, in cooperation with the technologists, to develop strains with better taste, flavor, and processing characteristics.

Breeding for Nutrition and Safety

As shown in Table IX, legume proteins are rich sources of lysine and threonine, but are deficient in methionine and tryptophan. As I have pointed out, the protein efficiency ratio of most raw legumes is low, due to the adverse effects of trypsin inhibitors, growth inhibitors, and hemagglutinins present in them. When heat processed optimally, the inhibitors are inactivated and a marked increase in the PER is observed (Venkat Rao, et al., 1964; Table X). When cooked legumes are incorporated in cereal diets at about the 15 per cent level, together with vitamins and minerals, so as to provide about 2.5 per cent extra proteins, the

TABLE IX
Chemical Composition and Amino-Acid Make-up in Some Indian Grain Legumes*

	Protein %	Amino Acids (g/16gN)				
		Lysine	Cystine	Methionine	Threonine	Tryptophan
Bengal gram	17.1	6.4	0.8	1.7	4.8	0.6
Black gram	24.0+	6.0	0.7	1.1	4.3	0.5
Cow pea	24.6	6.2	0.7	1.0	3.2	0.6
Field bean	24.9++	8.1	1.0	0.7	3.3	0.5
Green gram	24.0+	7.0	0.6	1.0	3.5	0.4
Peas	19.7++	11.4	1.2	1.3	4.9	0.7
Red gram	22.3+	6.4	0.8	0.9	3.4	0.2

*Venkat Rao, S. 1964
+Estimated in dehusked, split grains (dhal)
++Dried material

TABLE X
Effect of Heat Processing on the Nutritive Value of Legume Proteins (Protein Level in Diet, 10% Experimental Period: 4 weeks)

Protein Source	PER
Pigeon pea, raw *(Cajanus cajan)*	0.68
Pigeon pea, autoclaved	1.43
Soybean, raw	0.7
Soybean, autoclaved	2.1
Horse gram, raw *(Dolichos biflorus)*	0.7
Horse gram, autoclaved	1.7
Black bean, raw *(Phaseolus vulgaris)*	loss of body wt.
Black bean, autoclaved	1.35

dietary deficiencies and growth rates of rats are markedly improved (Daniel, et al., 1965; Table XI).

Experiments carried out in recent years have shown that the deficiency of methionine and tryptophan in legume proteins can be corrected by supplementing with synthetic amino acids (Table XII). For example, the PER of red gram

TABLE XI
Supplementary Value of Legumes to Rice and Wheat Diet*

	% Protein content**		Wt. gain/week (g)	
	(a)	(b)	(a)	(b)
I. Rice diet (RD)	8.5	8.5	5.7	13.7
RD + Bengal gram (2.5% extra proteins)	11.0	11.0	8.0	19.1
RD + red gram (2.5% extra proteins)	11.0	11.0	7.9	19.3
RD + soybean (2.5% extra proteins)	11.1	11.0	8.0	21.6
RD + skim milk powder (2.5% extra proteins)	11.1	11.1	22.2	23.5
II. Wheat diet (WD)	11.3	11.0	9.7	17.6
WD + Bengal gram (2.2% extra proteins)	13.4	13.3	13.7	21.7
WD + soybean (2.2% extra proteins)	13.6	13.5	16.3	22.5
WD + red gram (2.2% extra proteins)	13.3	13.0	15.3	21.8
WD + skim-milk powder (2.2% extra proteins)	13.6	13.5	23.9	24.0

*Daniel, et al., 1965
**(a) Without vitamins and minerals
(b) With addition of vitamins and minerals

TABLE XII
Amino-Acid Supplementation of Legume Proteins*

Foodstuff	% Protein level	Experiment duration (days)	Weight gain (g)
Kidney bean, black *(Phaseolus vulgaris)*	10	21	0
+ 0.3% methionine	10	21	65.1
Cow pea *(Vigna sinensis)*	10	21	6.3
+ 0.3% methionine	10	21	31.5
Lentil *(Lens esculenta)*	10	21	2.1
+ 0.3% methionine	10	21	10.5
Chick pea *(Cicer arietinum)*	10	21	27.3
+ 0.3% methionine	10	21	60.9
Pigeon pea *(Cajanus cajan)*	10	21	4.2
+ 0.3% methionine	10	21	6.3
Lima bean *(Phaseolus lunatus)*	10	10	4.0
+ 0.1% methionine	10	10	30.0
Snap bean *(Phaseolus vulgaris)*	10	10	1.0
+ 0.1% methionine	10	10	16.0
Pea *(Pisum sativum)*	10	10	3.0
+ 0.1% methionine	10	10	23.0
Black bean *(Phaseolus vulgaris)*	10	28	45.0
+ 0.2% methionine	10	28	124.0
Lathyrus pea *(Lathyrus sativus)*	12	21	6.0
+ 0.6% methionine	12	21	30.0
Green gram *(Phaseolus radiatus)*	12	21	14.4
+ 0.6% methionine	12	21	22.7
Black gram *(Phaseolus mungo)*	12	21	20.0
+ 0.6% methionine	12	21	38.2

*Venkat Rao, S. 1964

increases from 1.4 to 2.6 when supplemented with methionine and tryptophan (Annual Report, 1970b). In the case of black gram, supplementation with methionine alone was sufficient to increase the PER from 1.7 to 2.7 (unpublished data from CFTRI, Mysore).

Studies on children conducted at CFTRI have shown that consumption of large quantities of legumes (130g per day) leads to increased production of flatus. Bengal gram was found to produce the highest amount of flatus, whereas green gram produced the least. The serum and liver cholesterol levels of rats fed with the polysaccharide fractions prepared from different legumes were lower than in rats fed on corn starch. The cholesterol-lowering effect was also observed when the polysaccharide fractions were included in an atherogenic diet (unpublished data from CFTRI).

Concerted breeding programs are necessary for evolving strains with better amino-acid makeup, particularly including methionine and tryptophan, and possessing other desirable properties, such as reduction of flatus and blood cholesterol among the consumers.

Utilization of Grain Legumes in Weaning and Supplementary Foods

The worst sufferers from protein-calorie malnutrition are the vulnerable groups of population consisting of preschool children, pregnant women, and lactating mothers in low-income groups. The availability of milk and other animal products to meet their requirements is very low, and such products are out of their reach. Much work has been done in different parts of the world to upgrade the quality of vegetable proteins through mutual and amino-aid supplementation, improved processing, and utilization (Parpia, 1971) to overcome the widespread problem of protein-calorie malnutrition.

Parched or Toasted Split Chick Pea

The preparation of parched chick pea is an extensive cottage industry in India. Toasted, split, chick-pea flour has been used as an ingredient in certain processed protein foods developed in India and West Asian countries. Feeding experiments with albino rats have shown that toasted chick-pea flour with vitamins and minerals added is an effective supplement to low-protein diets that are based on tapioca and maize (Table XIII), which are the principal staple foods in these areas (Tasker, et al., 1962).

Realizing the importance of grain legumes as traditional food items, as well as for their nutritive qualities, the Central Food Technological Research Institute has developed a protein concentrate called Indian Multipurpose Food Supplement from Bengal gram and edible peanut flour. This product is a very adequate supplement to cereal diets in India and can be added to traditional recipes without involving any change in dietary habits. There are five commercial units for producing Multipurpose Food in different parts of the country.

Precooked, Dehydrated, Dried, Weaning Food

A process has been developed for the preparation of a precooked, roller-dried food based on a blend of wheat flour, dehusked chick pea, green gram dhal, and peanut flour fortified with vitamins and minerals. The food contains 27 per cent protein and has a PER of 2.2 as compared with 3.0 for milk. Incorporation of dehusked sesame flour at a 15 per cent level has raised the PER of this product to 2.5. Another product of this type, developed through collaborative efforts with a cooperative industrial organization, is being marketed successfully.

Uncooked mixtures composed of grain legumes, cereals, and peanut flour and known as Bal-Ahar, have also been produced and used extensively in famine- and distress-affected areas to prevent dietary deficiencies among vulnerable groups of the population (Parpia, 1971).

Vegetable-protein mixtures, that is Laubina 104 (parboiled wheat, 60 parts; parched chick pea, 28 parts; and skim milk, 10 parts) and Laubina 105 (parboiled wheat, 70 parts; and chick pea, 28 parts) have been developed in West Asian countries, based on the local food patterns. These foods are acceptable to preschool children and are effective in the treatment of protein-calorie malnutrition. The consumption of the foods vastly improves nitrogen retention in normal, as well as malnourished, children (Asfour, et al., 1965).

TABLE XIII
Supplementary Value of Chick Pea and Other Protein Foods to Maize-Tapioca (M-T) Diet*

Diets	% Protein content of diet on moisture-free basis	Average weekly gain in body weight of rats (g)
M-T + 27.6% peanut flour	19.40	17.5
M-T + 29.1% of 3:1 blend of peanut flour and skim-milk powder	19.40	18.5
M-T + 53.3% chick-pea flour	19.80	17.2
M-T + 51.1% of 3:1 blend of chick-pea flour and skim-milk powder	19.80	17.8
M-T + 35.3% Indian Multipurpose Food Supplement (Formula C)	19.80	18.3
M-T + 38.2% of protein food containing coconut meal	19.60	1[illegible].0
M-T + 40.6% skim-milk powder	19.40	19.1
Maize-tapioca	5.08	0.6

*Tasker, P.K. 1962

Blends of Wheat Flour and Toasted Chick-Pea Flour

The protein-efficiency ratio and over-all nutritive value of blends of wheat and Bengal gram flours (Bal-Ahar type of product) in various proportions, namely 80:20 (Blend I), 70:30 (Blend II), and 40:60 (Blend III), have been determined in albino rats. The protein contents of the blends were 14.2 per cent, 16.1 per cent, and 20.7 per cent, respectively, on a dry-weight basis.

The PERs of the three blends were 2.59, 2.49, and 2.34, respectively. They were further improved by supplementation with lysine, methionine, and threonine (Daniel, et al., 1969).

The mean weekly gains in body weight of rats fed on Blends I, II, and III, fortified with vitamins and minerals, were 18.3 g, 15.8 g, and 16.9 g, respectively, over a period of four weeks. Further improvements were noticed when the limiting amino acids were also supplied.

The results indicate that a blend of 80 parts of wheat and 20 parts of Bengal gram containing about 14.0 per cent protein might be adequate to meet the protein needs of growing children when the blends are supplemented with vitamins and minerals.

If the basic quality of the raw material, particularly of grain legumes, that goes into production of such foods, can be improved in amino-acid make-up, vitamins, and the elimination of deleterious factors and flatus production, it should be possible not only to upgrade the quality of various weaning and infant foods, but also to reduce their costs and make their commercial manufacture more economical.

Summary

Quantitatively, grain legumes are second in importance only to cereals for overcoming protein-calorie malnutrition in developing countries of the world; but their qualitative importance, as compared to most cereals, is even greater as sources of lysine and threonine for the low-income groups.

Production of grain legumes and their utilization have suffered a set-back over the last few years because they were not given the required attention in breeding for better yields per hectare and in raising their nutritive value, as was done with cereals. It is now being realized that the situation must change without further delay and high priority must be given to improvement of grain legumes. The time is opportune to include in the breeding program a number of factors that call for research and development to overcome dietary deficiencies, and to make legumes safer for human consumption. It is clear that there is need (1) to improve the amino-acid balance to make them better supplements to cereal diets; (2) to reduce and possibly eliminate toxic and deleterious factors that affect nutrition adversely; (3) to improve flavor and acceptability; (4) to introduce certain improvements in the processing characteristics to raise the milling out-turn; and (5) to improve storage qualities to minimize the damage due to infestation and infection. If the yield per hectare could be raised and combined with all the above factors — or at least some of them — it should be possible to increase the production and consumption of grain legumes by making them more economical for the cultivater and bringing them within the reach of a larger number of consumers. In that event, the consumers might use sufficient quantities to overcome nutritional deficiencies in their diets. Close cooperation among breeders, postharvest technologists, and nutritionists can help to achieve the objective more effectively.

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TESTA PIGMENTS IN *CAJANUS CAJAN:* CANNING QUALITY AND FLAVOR

W. Vernon Royes

A wide range of testa color, seed shape, and size exist in *Cajanus cajan.* A number of varieties were obtained from the Indian subcontinent. These have varied in testa color from a very dark purple, through a dark and light brown, to heavily speckled beige types. Nearly all West Indian material has a beige testa, some being more or less lightly speckled around the hilum.

There were four pigments in all the types I examined spectroscopically before 1965: two dark pigments had absorbance maxima at approximately 600 and 630 nm, and two light pigments had maxima at 350 and 490 nm. The pigments, not atypically, are unstable in solution and present separation and analytical problems. The differences between types were in the relative amount of each pigment present.

When *Cajanus cajan* is cooked or canned with the testa in place, those that appear most lightly pigmented when dry become an olive green with more or less brown coloration, even when they are harvested in a green state. The more heavily pigmented types with larger proportions of the dark pigments become dark brown to purple on cooking, even when cooked as peas and rice, but the flavor, if one is intrepid enough to eat them, is very good. West Indians, highly educated or not, are of the concerted opinion that these varieties are extremely unattractive when cooked, and it is interesting to note that they are mostly used on the Indian subcontinent and elsewhere as split peas from which the testa is separated.

Sanchez-Nieva, et al. (1961), have studied the factors affecting the quality of canned pigeon peas. Many improvements have been achieved, especially in the turbidity and color of the brine, but the color remained an olive green or brown. G.M. Sammy (1971) assessed the canning potential of the highly inbred variety G.I.26/2. By varying his conditions, he was able to obtain a product of higher quality with the uniform material than with the mixtures normally available, but the color remained unchanged.

S.C. Harland has mentioned that in a market in Grenada, West Indies, in 1926 he found four seeds of *Cajanus cajan* with white testas.

In 1965, I was given about two pounds of seed in Jamaica. It had been bulked by the Agricultural Development Corporation, and the seed coat of this type was a translucent pearl white. Even the seed scar was colorless. Upon spectroscopic

analysis, it was shown to contain only the lightest of the pigments, and that at only 25 per cent the level of the previously known lowest concentration.

I grew a third of an acre of this type, and hand-reaped it carefully for canning by International Food in Port of Spain. They hand-shelled the peas and canned them in the usual manner. The brine in the tins was clear and the peas were a bright green color, but they did not taste anything like pigeon peas.

There was very little flavor, and the taste was that of unseasoned split peas. This fiasco shows that palatability and consumer acceptance cannot be disregarded, no matter how difficult it may be to test it accurately and objectively. It may be possible that naturally occurring or artificially produced mutants may appear that will contain leuco-forms of the present pigments and still retain the flavor, but this work may have to await the elucidation of the structure of the pigments.

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BREEDING FOOD LEGUMES FOR IMPROVED PROCESSING AND CONSUMER ACCEPTANCE PROPERTIES

M.W. Adams and C.L. Bedford

Edible legumes reach the consumer in various forms:

1) green vegetables, such as snap beans, limas, garden peas, or pigeon peas, in the fresh or frozen state;
2) matured dry grains, such as dry beans, peas, limas, lentils, or cow peas, in prepackaged form or in bulk, obtained from the village store or market place;
3) precooked whole grains in tin cans, with plain water, brine, pork, tomato sauce, or molasses added;
4) roasted, flour, soups, etc.

It is essential, first, to identify those properties of legume grains that appear to be important to the consumer and to the processor. We will be concerned with the properties of seeds that are related to the use-forms as noted in 2) and 3) above.

Usage as Dry Seeds

Edible legume seeds come in many sizes, shapes, and colors. Consumers have acquired specific preferences for various combinations of size, shape, and color of seeds and, in some countries, these preferences are formalized in the commercial class designation. Examples in dry beans *(Phaseolus vulgaris)* include (a) the navy bean, an ovoid white bean weighing 16-20 gms per 100 seeds; (b) the kidney bean, a large, elongated, ovoid seed, dark red, reddish-brown, light red, or white in color, weighing from 50-60 gms per 100 seeds; (c) the pinto bean, a medium-sized, flattened seed with dark reddish-brown, striped mottling overlying a light tan self color, weighing 35-45 gms per 100 seeds; and many other classes similarly distinguishable.

Visual Seed Characteristics

The legume breeder is cognizant of visual preferences and does not often violate the class restrictions when introducing new varieties. Although these readily identifiable seed characteristics determine the acceptance class, it is generally recognized that intrinsic differences in cooking quality and flavor are

associated with the size, shape, and color patterns of the seeds. The breeder, therefore, at a first approximation, is reasonably assured of having achieved or maintained an acceptable level of cooking quality and flavor in the new variety, provided he selects carefully for the appropriate class characteristics. Fortunately, these properties are highly heritable and are generally easily recovered in early segregating generations of crosses.

Mechanical Damage

An additional property of the dry seed that is of importance to both the consumer and the processor is its susceptibility to mechanical damage, expressed as seed-coat breakage and splitting of cotyledons as a result of abusive handling in harvesting and cleaning operations.

As a general rule, the larger and more irregularly shaped seeds are more sensitive to mechanical abuse than are the smaller, more nearly rounded seeds. Moisture content of seeds at harvest and handling stages is probably the most critical factor. Even within the recognized commercial classes, however, differences exact in degrees of tolerance to abuse and can be selected for by the breeder.

Preliminary screening for resistance to mechanical abuse can take place as early as the F_3 generation. A sample of at least 50 seeds is recommended, with the seeds previously brought to an equilibrium moisture level that is known by experience or preliminary trial to be conducive to damage. The test procedure depends upon imposing an impact of sufficient force to damage perhaps about 50 per cent of the seeds of the standard varieties to some significant degree. A constant impact may be achieved most simply by dropping the seeds against a hard surface from some arbitrarily chosen height. When various types of beans at about 12.5 per cent moisture level are dropped 10 meters onto a slanting steel surface, they usually will incur sufficient damage to permit detection of genetic differences.

A simple, power-driven, rotary batting device may be used for samples that weigh 100 grams or more, or, when only a sample of 50 to 100 seeds can be spared, a more precise impact tester is recommended, such as that developed at Michigan State University in the Department of Agricultural Engineering.

Soaking Time and Cookability

Rapid uptake of water is a desirable attribute of legume grains used for food. The hilum and micropylar areas usually admit water readily, but seed coats differ strikingly in this regard. Both the age of the seed and the genotype of the maternal parent are important regulatory factors. A well-hydrated seed coat admits water freely to the cotyledons, resulting in softening of the whole seed. Therefore, breeding lines can be tested for soakability either by measuring the weight-gain in soak water per given period of time, or by pressure-testing a soaked sample with a shear press.

In cookability testing, hot, soft water should be used on seeds graded for

uniformity in size, age, and initial moisture content. A device such as the experimental cooker that was built at the USDA Western Regional Research Laboratory for testing the cooking time in beans appears suitable for any of the legumes. The test can be conducted on single seeds or on any number up to 100 at a time; the results with the larger samples are expressed as number of minutes to achieve the stage of 50 per cent cooked. Each run may require up to 60 minutes for completion, so the system is not efficient for use in early generations, where results on hundreds or thousands of test lines might be desired. This particular system of evaluating cookability should be reserved for use where only advanced breeding lines or selected strains need to be tested.

Evaluation as a Processed Food

For convenience, palatability, and preservation or enhancement of nutritive value, the food legumes are often supplied to the consumer as canned products. Acceptability to processor and consumer depends upon several quality factors, most of which have not been defined explicitly enough for quantitative measurement.

Before taking up these factors, we will outline the canning procedures appropriate for the navy bean, this being one of the legume grains commonly processed.

1) Raw product — clean, free of foreign material, free of seed-coat defects. Seed should be fully mature and of uniform size.

2) Moisture content — should fall in the range of 12 to 18 per cent, although beans hydrate and cook more readily at lower moisture levels. Seeds to be canned should have been kept at a moisture level of 12-14 per cent and at a temperature of 15°C or below for a period of six to eight weeks to insure that the canning test, when conducted, truly represents varietal differences.

3) Soaking — this step serves to insure uniform expansion in the can during the thermal process, and insures product tenderness. Legume seeds are usually hydrated to a moisture level of 53 to 57 per cent. Soak time is related to temperature: 8-16 *hours* at 15°-20°C, or 20-40 *minutes* at 82°-100°C. The shorter times are preferred to prevent growth of bacteria.

4) Water — a long, soft-water soak will leave the bean more tender or even mushy. Hard water tends to toughen the skins and firm the texture of the cotyledons. Water containing 25-50 ppm calcium is considered optimum. If too-hard water cannot be softened chemically or by ionic exchange resins, the firming effect can be counteracted by the addition of 0.1 to 0.2 per cent sodium polyphosphate to the soak.

5) Blanching — after the beans have been soaked at temperatures of 15°-70°C they are usually blanched in 80°-95° C water for three to eight minutes to eliminate the air and to equalize moisture. Legumes hydrated at higher temperatures need not be blanched if adequate soaking time has been used to obtain the 53-57 per cent moisture level. Overblanching causes skins to split.

6) Filling — with fully presoaked beans at a moisture level of 53-57 per cent,

about 225 grams are required to fill a one-pound can (450 gms). The can is then filled to the brim with hot brine, sauce, or diluted molasses, and sealed, ready for the retort cooker.

7) Cooking -- it is important that there be no delay between can closing and processing. The sterilization process necessary depends to a considerable extent upon the sauce formulation. Brines require less processing time than do sauces that contain tomato pulp and starch. The proper processing time should be obtained from a laboratory connected with the canning industry. The process may be 10-30 per cent longer than that necessary for sterilization in order to produce canned beans with the desired degree of tenderness. The cans should be water cooled immediately after processing.

8) Equalization - the beans will continue to absorb water for several days after processing, so an equalization period of at least two weeks should be allowed before subjecting test beans to the grading process.

Processing legume seeds by canning is clearly not a simple, inexpensive matter as it now is conducted. Much labor, time, special equipment, and several hundred grams of seed of each line are required for the processing alone. The bases for selection of improved breeding lines require additional expenditures to conduct the various quality tests on the canned product. As now conducted, therefore, the *full* procedure generally should not be employed in the early, segregating generations of crosses, but only after the populations have been reduced in number of lines to an elite group of otherwise acceptable lines - perhaps 50 to 100 - by selection and by elimination of other characteristics. When the number of lines has been reduced further, and if sufficient seed of each line is available, it is recommended that the most promising lines be submitted to a commercial processor for evaluation by an independent, nonpartisan judge.

Quality Evaluations of Processed Product

On what grounds, now that the legume seeds have been processed, shall the quality evaluations be made? We note, first, the more subjective of these evaluations.

1) Wholeness - the tendency of the legume seed to remain whole throughout the processing operations -- not to break apart, burst, or disintegrate. In the navy bean, it is common for transverse breaks to appear across the hilum region, but the bean retains its shape and contents.

2) Consistency - the fluid is slightly viscous and clear, not cloudy or grainy; it separates or drains readily from the beans. The starch-protein matrix of the cotyledons does not rupture to release starch granules into the external fluid.

3) Freedom from defects - no extraneous material, loose skins, or mashed beans.

4) Flavor - must be scored by a taste panel. Storage molds, container odors, and volatile chemicals stored with or near legumes may impart undesirable flavors to the canned product. There are also genetic differences in flavor components that can be detected by skilled tasters.

5) Color – pigments in the seed coat that escape detection in the dry seed may impart an off-color to the cooked product. Iron and copper in the white-seeded legumes are inducers of a bluish tinge that consumers find unacceptable. In some red kidney beans, a dark-colored, water-soluble pigment of the seed coat exudes into the fluid, producing a slightly bitter taste and an off-color fluid.

The factors 1), 2), 3), and 5), above, are scored by visual inspection of a canned sample. The food technologists have not yet given us the criteria or tools by which to measure these factors clearly and objectively, although color may be measured by color-difference meters. However, the situation is somewhat better regarding the factor of texture.

6) Texture – the textural parameters of beans are firmness, gumminess, and adhesiveness. Firmness is measured by the force required to penetrate a substance, and is perceived on first bite. Gumminess is related to the energy required to disintegrate a substance, and is perceived during chewing. Adhesiveness is the force required to remove the material from the mouth, and is also perceived during chewing. These parameters can be evaluated with reasonable accuracy by a sensory panel and also can be measured quantitatively by machines, such as the Kramer Shear Press, the Instrom Universal test machine, and the Ottawa Texture Measuring System, with necessary force-recording systems and test cells. Initial studies have shown that consistent results can be obtained when the canned beans are washed with cold tap water to remove sauce and the measurements are made at a selected, constant temperature. The objective results have shown a high degree of co relation with the sensory evaluations ($r = .950$ or better) for texture. Further studies with objective testing methods will be required to establish values that can be used routinely to indicate bean texture.

There are no comprehensive studies on the source and nature of variation in the quality factors referred to above. From limited studies of special factors, and on the basis of experience, the assumption has emerged that at least a portion of the variability observed among lines depends on genetic differences. But it is quite clear that the length of processing, the temperature of the soak, the hardness of the water, and the character of the added fluid all play an important role. In addition, the initial moisture level of the seed is known to affect the rate of water uptake during soaking. Therefore, the breeder must expect to standardize the growing and handling conditions for all lines to be tested, and to follow a standard regimen during processing and in evaluation.

In practice, in order to assure acceptance of the new selection by growers, the breeder generally will find it necessary to compromise between the best level of quality possible in a particular program and the levels of disease resistance and agronomic performance that must be maintained. There seems no escape from this. The operational stratagem followed by most breeders, we suspect, involves holding his population at a given, satisfactory level of disease resistance and agronomic performance by recurrent backcrossing, while selecting strongly for quality components. Contrariwise, breeders hold to a given level of quality by

periodic (cyclic) crossing to a known, acceptable standard while selecting for improved disease or agronomic performance.

This strategy provides no obvious answer to the problem of improving quality when no satisfactory, high-quality, recurrent parent is available. One solution to this problem lies, first, in identifying the component nature of "quality" – flavor, firmness, texture, etc.; second, in searching the germ-plasm resources for lines of special flavor, tenderness, texture, or whatever; and, third, synthesizing from these sources a new combination of quality components by composite crossing or by stepwise insertion of one component after another into a common genetic background that is already suitable on other grounds.

Almost nothing is known about the relative ease or difficulty of working with these components in breeding legumes. Genetic analysis of subjectively determined quality characteristics usually is not satisfactory. Such progress as has been made up to now has been largely by the fortuitous recognition of lines with acceptable or superior quality and the incorporation of a significant proportion of their germ plasm into all new strains via backcrossing.

We need clear identification of quality components, simple, inexpensive and quantitative tests for measuring them, studies on the genetic and environmental causes of variation in quality components, and, finally, studies on the problem of component interaction as these quality factors are brought together in various combinations.

DISCUSSANT

G.D. Kapsiotis

Like other speakers, I have my own bias, which is to processed infant foods in which legumes constitute a substantial part of the formula. One problem that gave us trouble in developing such products is the color of the legume seed-coat in the flours. So far, we have not used fava beans; chiefly, we use lentils and, of course, soybeans. If the seed coats are not entirely removed in milling, the product may be colored with small specks (the residual seed hilum), which are particularly noticeable in the cooked product (Kurien, 1971). The development of strains with light husks or with loose seed coats might make our work, and that of the miller, much easier. Also, the consumer undoubtedly would find the product more attractive.

Second, I would mention some of our experience, which indicates that certain protein fractions, such as globulins, might be difficult to digest. We used alpha amylase to treat the mixtures to which I refer. As a result, I can give you some figures from the work we conducted with the TNO Food and Nutrition Research Institute in the Netherlands. After the heat and enzyme treatments, the digestibility of these mixtures rose from 79 per cent to about 98.5 per cent. This might suggest that the hard-to-digest protein fractions of the legumes, which sometimes comprise 55 per cent of the food mixture, benefit from the combined enzyme and heat treatment. Heat treatment alone did not provide such an increase in digestibility.

The other area on which I would like to comment is the low acceptance of insect-infested legumes, which Dr. Parpia mentioned. But low acceptance also can be found in new varieties of legumes. Probably the best approach for the plant geneticist is to be certain that, during genetic manipulation, the organoleptic characteristics of the most commonly used legumes are not altered, so that negative consumer reactions may be avoided.

Of course, eliminating bitter or other objectionable tastes should be of concern to the geneticist; however, I suggest that if the elimination of such tastes can be achieved easily by simple processing, the breeding objective might be given secondary priority.

I would like to make a small correction in Dr. Parpia's paper. In passing, he mentioned that *Laubina* is a product developed and used in the countries of the Near East. However, for the last ten years, *Laubina* has been — and still is — a laboratory curiosity that has never found its way into commercial channels.

Reference

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SUMMARY AND RESEARCH NEEDED

Irvin Liener

I will start with Dr. Calloway's presentation on flatulence. She described the sequence of events which are involved in the pathogenesis of flatulence, pointing out, first of all, that we are concerned here with the production of gases, namely CO_2 and hydrogen, by the action of bacteria on a particular substrate or substrates. Apparently the flatulence factor(s) can be eliminated effectively by fractionation; for instance, the protein isolate of beans is not flatus-producing, and one can extract the flatulence factors at various pH's. They also can be extracted by aqueous ethanol. But these, of course, are not the best procedures, because they are so complex as to be impractical.

Thinking that the causative factors of flatulence might be the oligosaccharides, Dr. Calloway carried out a series of experiments with stachyose and raffinose. It appears that extremely high levels of these particular oligosaccharides are necessary to produce the same types of effects as are produced by the beans themselves, so apparently they are not the only flatulence factors involved. Dr. Calloway pointed out the possibility that a galactosidase deficiency might be responsible for the formation of gas that is associated with flatulence-producing factors. In terms of determining which beans produce the most flatulence, it appears that *Phaseolus* heads the list, followed by soybeans and mung beans, in that order. Peanuts seem to be devoid of this activity.

Dr. Calloway also presented data on lima beans, showing the varietal differences that exist regarding flatulence production. From her data it was apparent that humans have individual susceptibilities. She described a simple type of apparatus used in the study of flatulence in human subjects, making clear that she had little faith in the microbiological method; apparently it is not sufficiently specific. Rats are not satisfactory either, because one cannot detect CO_2 in their flatus by present methods.

In his comments, Dr. Leng referred to the work of Dr. Hymowitz, at the University of Illinois, who studied the sugar content of various soybeans, pointing out that sugar content ranges from 6 to 16 per cent. Of this sugar, 41 to 60 per cent is in the form of sucrose, 5 to 15 per cent in the form of raffinose, and 12 to 35 per cent in the form of stachyose. Such data might prove valuable if research points to these oligosaccharides as being important causative factors of flatulence.

Dr. McGinnis then described experiments in which he tested the production of gas by *Clostridium perfringens* on various types of substrates: the Boyd

medium and a medium that was developed in his laboratory. His results made it evident that gas production can be caused by rather simple materials – for example, bean ash itself is an effective stimulant to the production of gas by *Clostridium*. Apparently, this is caused by the effect of surface particles. Thus, when considering the use of a microbiological technique for evaluating flatulence caused by legumes, it is important to realize the various factors that might be involved in gas production and to take these into account when developing appropriate investigative procedures.

Dr. Rao presented a paper prepared by Dr. Parpia that relates to the experience of Indian workers in legumes. The paper emphasized the effects of milling, and indicated that the type of milling used to remove the husks from the legumes can play an important part in the yield. For instance, by using a particular technique that was developed at the Central Food Technology Institute, it was possible to obtain a yield of 82 per cent, which is much higher than the yield from some of the home methods. In addition, Dr. Parpia's paper pointed out varietal differences in yield. Apparently, soybeans are not readily acceptable in India because of cooking difficulties. Pressure-cooking could solve that problem, but a question of economics intervenes. Could, indeed, the average Indian afford a pressure cooker to cook soybeans effectively?

The various legumes in India are prepared by a variety of cooking methods, which usually begin by soaking them overnight and then boiling them. There also is a puffing process, which begins by soaking the beans and subsequently toasting them in hot sand. In a commercial process, another type of puffed legume is produced, presumably equivalent to our puffed cereals. This has enjoyed some popularity.

Among the most serious legume problems in India are rodent and insect infestation, which cause a significant reduction in the yield of stored legumes. One storage problem that may not be obvious is its effect on both the quantity and the quality of legume protein. Apparently, rodent and insect infestation greatly reduces legume PER. This may be due to deleterious metabolites developed during storage as the insects penetrate the legumes. Therefore, one important need for India is to seek varieties of beans that are pest-resistant. This is not an easy objective, but it should be investigated.

Dr. Parpia's paper then summarized research needs in India, most of which have been discussed here: amino-acid balance, toxic factors, milling properties (that is, producing varieties that can be milled more readily and so increase the yield), and developing pest-resistant varieties.

Dr. Royes related some of the experiences he has had with color variations in pigeon peas. Apparently the color of the pigeon pea is due to four different pigments; the color one gets depends on the relative proportions of these pigments in any one variety. When these beans are dried, the pigments become intensified and the bean darkens. As a result, they are less acceptable to the consumer. Pigeon peas can be canned to produce a rather satisfactory product. Dr. Royes managed to obtain a variety of pigeon pea that was relatively white. When these were canned, their appearance was acceptable, but their taste was

not. They tasted more like split peas. So we can't always correlate color with flavor; apparetly they are unrelated variables.

Dr. Kapsiotis called attention to the use of legumes in infant foods, emphasizing the important deleterious role of seed coats. If the coats are not eliminated, the product is colored and undesirable; he suggested the development of varieties of legumes with loose coats that could be removed readily. He mentioned that treatment with alpha amylase increased the digestibility of proteins from 75 to 90 per cent. He also emphasized that we should not tamper with the inherent organoleptic properties of the legumes, because in doing so we might decrease their acceptability. He stressed that elimination of the bitter principles by processing should be given preference over attempting to breed out undesirable taste factors.

Dr. Munck described his experiences with the broad bean. The objective was to try to isolate proteins from the broad bean, extracting the bean with alkali, and then precipitating the purified protein at an acid pH. They compared the amino-acid content of the isolated protein with that of the original bean and found very little difference. He also described an experiment in which two varieties of broad bean were studied for protein solubility as a function of the days of development of the plant. There were definite differences and he suggested that perhaps one should exploit these differences in a breeding program.

Dr. Kelly gave us the viewpoint of the processors' requirements Their first consideration is cost; it must be low and the yield must be high. A second criterion is uniformity in the product, which is vital for a commercial manufacturer. Texture and flavor are also important; the product must be acceptable. He then pointed out that government grades must be met -- another criterion that must be kept in mind.

I would like to spend a minute or two here in mentioning a point that I failed to include in my own talk -- the nutritional significance of protease inhibitors in human nutrition. Most of the work that has been done with trypsin inhibitors has related to the action of these inhibitors -- such as those from soybeans -- on bovine trypsin. But more recently, experiments have been undertaken to see what effect trypsin inhibitors have on human trypsin, a consideration of direct concern to us. Surprisingly, human trypsin is *not* inhibited by the soybean inhibitor. In addition, ovomucoid, which is the inhibitor in egg white, does not inhibit human trypsin, and we know from tests with human subjects that ovomucoid has no deleterious effects. From these various lines of evidence, I am forced to conclude that the trypsin inhibitors are really of little significance in human nutrition.

I would also call to your attention the presence of the trypsin inhibitor in some of the soybean protein isolates that are being used nowadays for textured vegetable proteins or meat analogues. It is true that trypsin inhibitors can be found in these isolates, and that the trypsin-inhibitor activity persists even after the isolates have been converted to fibers and the fibers made into various types of food. For this reason, some people say that perhaps we should avoid those meat analogues. But in view of the evidence just presented, I think that we need have no worries about such residual trypsin inhibitors.

IV

ANALYTICAL AND MASS SCREENING TECHNIQUES (CHEMICAL, BIOCHEMICAL, TOXICOLOGICAL, NUTRITIONAL)

A. Methods Available and Others Needed

Statements

USEFUL PROCEDURES AND NEEDED IMPROVEMENTS
K. J. Carpenter and J. A. Woolfe

QUANTITATIVE RELATIONSHIPS BETWEEN TOTAL SULFUR AND METHIONINE AND CYSTINE IN LEGUMES
Wayne M. Porter, John Axtell, and W. F. Keim

ENZYMATIC DETERMINATION OF INSOLUBLE, INDIGESTIBLE RESIDUE OF BEANS
E. W. Hellendoorn

CHARACTERIZING BEAN SEED PROTEINS AS A BASIS OF SCREENING GERM PLASM
Donald R. Wood and C. Vernon Cole

USEFUL PROCEDURES AND NEEDED IMPROVEMENTS

K.J. Carpenter and J.A. Woolfe

Sample Preparation

A single bean cannot be a satisfactory sample of a particular growth of a particular variety. Exact sampling procedures are a matter for discussion, but a minimum of 10 beans is needed for grinding to a fine powder.

Moisture Content

It should be made clear in all publications if analyses for nutrients are expressed on a "wet weight" or a "dry weight" basis. In practice, the variability in moisture content is not high. On the other hand, the determination easily can be added in as the first stage in determination of protein content by re-weighing the samples taken after 16 h in a drying oven (105°C).

If some groups continue to express their analyses on a wet-weight basis, those who *do* determine moisture content could express their values for protein, etc., adjusted to a standard 10 per cent moisture content. This would remove any large, systematic difference between the two types of analytical values.

Protein Content

The following procedure has worked satisfactorily with large numbers of samples. Approximately 150 mg of finely ground bean meal is weighed into a paper cup (made from a cigarette paper) on a torsion balance. Each sample is weighed out in duplicate. A block of 50 cups is then dried at 105°C overnight. Each cup is re-weighed, after cooling, to give the dry weight of the sample and then dropped into numbered Kjeldahl flask and digested with 5 ml of concentrated N-free sulfuric acid and 3 ml "100-volume" hydrogen peroxide. After the contents are clear, boiling is continued for 30 minutes.

After cooling, samples are diluted to 100 ml (up to a graduated mark on the stem of the flask) with water, and mixed thoroughly. Batches of digests are then put through a Technicon auto-analyzer for nitrogen, using sodium phenate and hypochlorite as color reagents. On each "tray" of 40 sample containers for the analyzer we include a set of 5 standards of known nitrogen content. These "phenacetin" standards have been put through the full digestion procedure. A typical day's run would be 4 to 6 trays, and would also include one analysis (in

duplicate) of a standard bean meal. The results are only acceptable if (1) the phenacetin standards have given a linear response, with little variation from tray to tray; (2) the "standard bean meal" has given a mean value within 0.5 "percentage units" of the over-all mean value from earlier runs; and (3) that the replicates are within 1.0 percentage units of each other for each individual bean sample.

This can be a very satisfactory procedure with a low coefficient of variation (see below), but from time to time gross aberrations can occur, and it is vital that there is a checking system that picks these out. These could be due to blockages in the analyzer system, contamination of sample cups, or to operator errors (e.g., in writing down weights).

It is doubtful if dye-binding methods should be used for selection in legumes, because they measure reactive groups, and the values obtained are necessarily a function of both protein quality *and* quantity. The position would be different if there were no rapid method for large-scale measurement of protein quantity alone.

Sulfur-Containing Acids

Total Sulfur

If this measure were to prove a useful indirect measure of methionine + cystine, it would be a great convenience. A workable auto-analyzer procedure has been published (B.E. Mottershead, *Laboratory Practice*, 20: 483, 1971). Information is needed as to whether, with a wide variety of samples, total S/N is closely correlated with methionine/N and/or (methionine + cystine)/N. (It is important that S/N should be the criterion, because we already have N values that correlate with amino acids as percentage of sample.)

Methionine

The only published procedure which, even from the claims of its proponents, appears to meet the need for routine screening of a large number of samples in a general analytical laboratory is that of Ussary and Gehrke (1969). This adapts the McCarthy and Sullivan color reaction with sodium nitroprusside on papain digests, for use in automated equipment. Interference from histidine and tryptophan is overcome by adding an excess of glycine, and both reproducibility and recovery of added methionine were reported to be satisfactory for a series of soybean samples. Since then, more difficulty has been encountered in obtaining acceptable reproducibility (Gehrke, private communication).

It is now a matter of priority (if necessary in coordinated experiments) to check the values obtained for a wide variety of legumes against the corresponding values obtained by a reference procedure (e.g., ion-exchange chromatography on oxidized hydrolysates). If the agreement is good, those needing large numbers of methionine values will then feel justified in asking for funds to purchase the special equipment required.

Until now, microbiological assay has provided the only means of relatively large-scale assay for methionine, and one has had to make the best of it. The advantages are that it requires no expensive, specialized equipment. The disadvantages are that precision is relatively low and that, for people otherwise engaged in purely chemical work, a new type of procedure has to be mastered and, in practice, assays can fail inexplicably or give aberrant results.

Kelly (1971) has concentrated on the simplification of the assay procedure of Ford (1962). The problem is to find a rational compromise between speed and precision. If the difference between legume varieties in methionine g/16g N is small, precision must be good in order to discriminate even between extremes in the range of material available.

Ion-exchange chromatography is the present ideal, but materials require a preliminary oxidation so that methionine units are converted to the more stable sulphone form. It is difficult to visualize the results from such analyses being adequate for a plant-breeding program. Gas chromatography has been proposed, but its use in such a program has not yet been proved, and there seem to be difficulties in the extension of procedures suitable for pure proteins (e.g., Inglis and Edman, 1970) to such complex materials as legumes.

It is to be expected that, when the same variety has been grown under conditions that lead to the crop's varying in protein content, there will be a similar variation in the content of methionine (as per cent of sample). It remains to be determined how far methionine g/16g N remains stable.

Cystine

There is controversy over what proportion of a child's requirements for sulfur-amino acids can be supplied by cystine. It is commonly accepted that in mixed diets it may be the total supply of methionine + cystine that is limiting. If this is so, we really need to have cystine, as well as methionine, values for beans. If methionine is being determined as the sulphone by ion-exchange chromatography, cystine will also have been converted to cysteic acid, and its value can probably be read off the same chromatogram by manipulating the buffer conditions. If other procedures are being used for methionine, quite separate analyses would be required for cystine. Microbiological assay is possible, but has given more problems than with other amino acids. A color reaction that could be automated would be of great value.

Trypsin Inhibitors

The procedure using the synthetic substrate BAPA, as used for soybeans by Kakade, Simons, and Liener (1969), is simple and satisfactory. For material of low-fat content, the initial fat-extraction step is unnecessary.

It is doubtful whether assays of uncooked legumes are relevant to the selection of material intended to be grown as human food and to be cooked before use. There is no evidence that significant amounts of trypsin-inhibitor activity survive any of the usual cooking procedures. Routine screening for this character is not recommended, therefore.

Hemagglutinins

It is easy to prepare crude aqueous extracts of legumes and to determine whether they cause agglutination with a particular sample of blood. It is, however, a very different matter to generalize from one blood to all bloods. In any case, as with trypsin inhibitors, the activity is destroyed by cooking. Routine screening is not recommended.

Flatulence Factors

These survive ordinary cooking and may be the crucial factors that limit the adoption of a diet containing increased proportions of soya products and *Phaseolus* beans (e.g., Steggerda, et al., 1966; Calloway, 1966). There is, therefore, a need to find means of assaying the relative flatulence activity of different varieties of legumes.

There have been suggestions that the trouble arises from the stachyose and raffinose in the beans that are not digested rapidly in the small intestine (unlike sucrose or starch), but can be fermented in the large intestine (unlike cellulose and most structural carbohydrates). If this is confirmed, we would need simple methods of analyzing for the combined level of these two polysaccharides in test materials.

Methods have also been proposed for in vitro assay of gas production, using bacterial cultures from human intestines (Rockland, et al., 1969). These will need to be calibrated against the results of in vivo tests. Although heroic experiments have been performed with human volunteers, there is an obvious need for satisfactory animal assays. Rats have been used (Kakade and Borchers, 1967), although they appear to tolerate high levels of cooked *Phaseolus* beans without any difficulty. The dog appears to be at least as sensitive as man (Richards, et al., 1966; 1967).

There is an urgent need for work to discover whether people in certain areas, such as East Africa and Central America, where there is a regular and considerable consumption of legumes, have become adapted to ingestion of flatulence factors. If they have not done so, the establishment of meaningful, rapid assays for these factors deserves the highest priority (Dr. Calloway has already spoken on this subject).

Feeding Tests With Laboratory Animals

It has been demonstrated with many animals (rats, chicks, laying hens, pigs, etc.) that raw *Phaseolus vulgaris* beans have a depressing effect on performance, and such experiments do not require further repetition. At this Conference we are concerned with how legumes (cooked for human consumption) can be evaluated as a human food (and, in particular, a toddlers' food) by feeding them to animals. However, only a limited number of samples can be tested, both because testing is slow and because kilogram quantities are needed in most procedures.

The commonest procedure has been to measure protein efficiency ratio (PER)

with rats. The test material included in the diet is at a level contributing 10 per cent protein to the diet; it is the only source of protein. (Thus, a legume with a 25 per cent protein content is included as 40 per cent of the diet.) Growth and feed consumption of young rats are then measured for a period varying from 10 days to 6 weeks. For a legume of low methionine content, the PER (g gain/g protein eaten) is always low as compared to the result obtained with a protein source of well-balanced, amino-acid composition and no toxic properties.

If the methionine content of the material is being determined separately in any case, and one can only afford one feeding test for any particular sample, it seems preferable to carry out a PER determination with added methionine. In these conditions, cooked and dried *Phaseolus* beans have given values equal to casein (Kakade and Evans, 1965; Woolfe and Carpenter, unpublished results). Such a result confirms that no amino acid other than methionine is seriously deficient in the protein, and that the cooked beans contain nothing seriously growth-depressant or appetite-depressant for the rat. However, these results seem too good, unless, contrary to expectations, children receiving 40 per cent cooked beans (+ methionine) in their diet could also do as well as on a casein diet. It appears that dogs would be unable to do so.

We are forced back to the need for direct investigation of the tolerance of legumes by children before we can judge which animal can be used as a model if the experiments are designed to test for toxic or appetite-depressant factors in cooked legumes.

Tests of protein quality seem less urgent, but when they are carried out are they to be for methionine, amino acids other than methionine, or, alternatively, for ability to supplement cereal protein? The potential value of material as a protein source is, of course, predictable from its amino-acid analysis. The extra information that a feeding trial for protein quality can give is an idea of how far the actual quality is below the potential (because of unavailability or imbalance), but not every experimental design will provide such information. If the experiments are with young rats, nothing seems to be gained by extending the experimental period beyond 10 days, but both a positive control (e.g., casein) treatment and a negative control (i.e., N-free) are important.

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QUANTITATIVE RELATIONSHIPS BETWEEN TOTAL SULFUR AND METHIONINE AND CYSTINE IN LEGUMES

Wayne M. Porter, John Axtell, and W.F. Keim

Ion-exchange, resin-elution chromatography is a precise method for determining the amino-acid content of foodstuffs, but it is very expensive and time consuming, especially for sulfur-containing amino acids. Miller and Naismith (1958) found a highly significant correlation between the total sulfur content of a diet and its net dietary-protein value. Miller and Donoso (1963) used the sulfur-nitrogen ratio to predict the net dietary-protein value. These findings indicated that a determination of total sulfur content of legumes might be used as a screening technique for the sulfur-containing amino acids, methionine and cystine.

In investigations of genetic control of protein quantity and quality in grain legumes by Sandhu (1969) and Porter (1972), both used an analysis of total sulfur content (Blanchar, et al., 1965) as an indicator of nutritive quality. Sandhu found a coefficient of determination of 0.89 for the regression of sulfur as per cent of dry matter on the sulfur-containing amino acids as per cent of protein in six lines of chick pea, *Cicer arietinum* L.

Porter, using five lines of dry bean *(Phaseolus vulgaris)*, one line of black-eye pea *(Vigna sinensis)*, and one line of mung bean *(Phaseolus aureus)*, found a coefficient of determination of 0.87 (highly significant) for the regression of total sulfur as per cent crude protein on the sulfur-containing amino acids as per cent of protein. Significant regressions were also obtained for values of PER on both the sulfur-containing amino acids as per cent of protein ($r^2 = 0.71$) and total sulfur as per cent of crude protein ($r^2 = 0.60$). The regressions of PER on methionine or cystine alone as per cent of protein were not significant.

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ENZYMATIC DETERMINATION OF INSOLUBLE, INDIGESTIBLE RESIDUE OF BEANS

E.W. Hellendoorn

Indigestible Carbohydrate Content of Beans

Dry beans *(Phaseolus vulgaris)* contain on average about 11 per cent water, 22 per cent crude protein, 4 per cent ash, and 1.5 per cent fat, the total carbohydrate content "by difference" thus being about 60 per cent (Watt and Merrill, 1963).

The starch content amounts to about 48 per cent, the percentage of starch found depending on the method of determination. However, not all this starch can be made available easily as glucose by enzymatic hydrolysis. The digestibility depends on the condition of the beans and on cooking time (Hellendoorn, 1969). The starch of raw beans, which had been soaked overnight, is about 20 per cent digestible. The digestibility of bean starch rapidly increases with cooking time. After one hour of normal cooking, the digestibility rises to about 85 per cent and, after one hour of pressure cooking, the digestibility of beans with good cooking properties may go up to about 95 per cent. However, the digestibility of starch of bad cooking beans never reaches such a high value, not even after pressure cooking. A small percentage of the beans will not cook to softness at all. Accordingly, some relationship exists between the cookability of beans and their digestibility.

Taking 85 per cent as an average for bean-starch digestibility, a calculated total quantity of about 20 per cent of the dry bean content appears to be indigestible. This figure is in accordance with the values calculated by McCance and Widdowson (1960) for unavailable carbohydrates of beans; one of their earlier papers mentioned a variation of from 17 to 30 per cent (McCance, et al., 1936).

The crude fiber content often is taken as a measure for the percentage of indigestible carbohydrates in food products. The crude fiber content of dry beans as given in food-composition tables is from 2 to 4 per cent, showing that a great discrepancy exists between crude-fiber content and calculated quantity of unavailable carbohydrates.

Crude-fiber content is determined by chemical analysis, either by the Weende method, i.e., an alternating acid and alkali extraction, or by the Scharrer-Kürschner method, which involves an oxidative treatment. Moreover, van Soest (1966) gives a crude-fiber determination technique that uses detergents. None of

these purely chemical determinations of fiber content has anything to do with the physiology of digestion in the human digestive tract.

Direct enzymatic determinations of unavailable carbohydrates have been given (Williams and Olmsted, 1935, Weinstock and Benham, 1951). We preferred to determine the insoluble residue after a pepsin-hydrochloric acid treatment of the food product during the night; this was followed by a short pancreatin treatment at pH 7.0, using a detergent. By this method, however, such water-soluble, but indigestible, bean components as pectin and galacto-oligosaccharides are not determined. The insoluble residue contains, besides indigestible carbohydrates (cellulose, hemicellulose, and lignin), some indigestible nitrogenous non-protein material. Conditions of the combined pepsin-pancreatin treatment are chosen to give optimal digestion of carbohydrates and protein. Consequently, the values determined for unavailable carbohydrates and protein are minimal.

Unavailable carbohydrates of beans may give rise to minor digestive disorders. Carbohydrates that are not enzymatically digested and absorbed in the small intestine are fermented by bacterial enzymes in the large intestine; only traces are found in the stool. Lower fatty acids and gases are formed in the intestine as products of fermentation, causing an intensified motility of the gut and flatulence (Hellendoorn, 1969).

Procedure

A known quantity of the beans to be analyzed is cooked, and finely divided in a blender to pass a 20-mesh sieve. Next, 50 ml of this suspension (with a dry matter content of 1 to 2 g, containing no more than 1 g of starch) is put in a 250-ml glass container; 100 mg pepsin (Merck USP, 1:10,000) and 50 ml 0.2 N hydrochloric acid are added, and the content of the container is well mixed, after which the sample is incubated 18 h (preferably during the night) at 40°C in the presence of some thymol.

After the pepsin digestion, the mixture is neutralized with 4 N sodium hydroxide solution, adding 50 ml buffer solution pH 6.8 (made by mixing equal volumes of 1/15 molar KH_2PO_4 and 1/15 molar $Na_2HPO_4 \cdot 2H_2O$ solutions), 100 mg pancreatin 4NF (Merck), and 300 mg sodium laurylsulfate. For pancreatin digestion, the sample is incubated one hour at 40°C.

After digestion, the mixture is made slightly acid (pH 4 to 5) with 4 N hydrochloric acid, a weight quantity (about 1 g) of Hyflo Supercel is added, and the suspension is centrifuged. The supernatant liquid is decanted through a 1-G3 crucible. The sediment is whirled up with water and centrifuged again. Washing the residue by centrifugation and decanting the supernatant liquid through the glass filter crucible is done three times with water and three times with acetone. The residue that remains after the last centrifugation is placed carefully in the crucible; attempts are made not to break it up into small pieces. The glass crucible with the combined sediment and filter residue is then dried at 105°C to constant weight (drying time from 4 to 6 h).

The weight of the dried residue is expressed as a percentage of dry matter of

the sample that shows the quantity of insoluble, indigestible carbohydrates in the sample.

In the same way, the nitrogen content (by micro-Kjeldahl determination) of the residue can be expressed as a percentage of the nitrogen content of the sample, which shows the quantity of indigestible crude protein in the sample. Subtraction of this value from 100 yields the digestibility coefficient of protein.

Results

By way of example, the results are given of the analysis of some types of pulses (Table I). The brown beans investigated were in the dry, raw state. After soaking the beans overnight and pressure cooking (one hour at 110°C), the mixture of beans with cooking water was analyzed. Three commercial canned products also were analyzed after the brine solution was decanted.

The figures show that the indigestible residue determined by the enzymatic method is far greater than is expressed by the figures obtained through the crude-fiber determination.

However, the percentage of the indigestible residue is lower than the unavailable carbohydrate content "by difference." Two causes for these differences have already been mentioned: (a) the pepsin-pancreatin digestion was carried out to

TABLE I
Main Constituents of Four Types of Pulses

Constituents	Brown beans (pressure cooked, total)	White beans (canned, decanted product)	Dun peas (marrow-fat) (canned, decanted product)	Peas (canned, decanted product)
Dry matter	80.5	29.9	26.2	24.9
		Percentage of Dry Matter		
Protein (N x 6.25)	23.0	24.3	22.5	21.9
Fat	1.8	--	-	–
Ash	4.0	–	--	–
Starch (enzymatic as glucose)	52.6	44.0	45.4	42.0
Crude fiber	(2.8)	(2.4)	(2.9)	(2.1)
Indigestible residue	15.0	15.7	19.6	13.2
Digestibility coefficient of protein	(95.7)			
Total	96.4			

give minimal residues; (b) the soluble indigestible food components are not included in the determination. The second point also applies to the total composition of the brown beans in question, as a few percentages escape analysis.

Summary

A simple enzymatic method is proposed for the determination of insoluble, indigestible residues of food products, which replaces chemical crude-fiber determinations. The percentage of true indigestible residue of pulses appears to be far greater than their crude-fiber content.

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CHARACTERIZING BEAN-SEED PROTEINS AS A BASIS OF SCREENING GERM PLASM

Donald R. Wood and C. Vernon Cole

The discovery of the effect of the opaque-2 mutant on the amino-acid composition of endosperm proteins of maize (Mertz, et al., 1964) has raised hopes that significant improvement can be made in the nutritional value of cereal and seed proteins. Screening of other crops has been done on the assumption that the maize achievement can be expected for other crops. It now seems to be true, however, that the possibility of finding an opaque-2-like mutant in other crops is low. If a new screening strategy is needed, we should look at the component seed proteins with the realization that improvement in protein nutritional value depends on combining them in new ways.

Materials and Methods

Cultivars of beans were grown to maturity in a constant-temperature room in 4-liter polyethylene containers with four plants per container. Aerated nutrient solutions were modified for bean culture. Sulfur was supplied from a stock solution with a specific activity of 0.333 m Ci ^{35}S/mM SO_4-S.

Mature seeds were ground in a blender mill to pass through a 60-mesh screen. The ground meal was extracted in 5 per cent sodium chloride (1 g/20 ml) in 50-ml centrifuge tubes (Powrie, 1961). Polyvinylpyrrolidone (PVP) was used in the extraction process to complex the phenolic compounds. The mixture was agitated on a wrist-action shaker for 30 minutes at room temperature. The extracts were centrifuged at 5,000 rpm for 5 minutes. The clear liquid was decanted from the tube through a fine nylon mesh. Each sample was extracted three times and combined and dialyzed at 2°C against phosphate buffer at pH 7.9. Extracts were concentrated by dialysis against Aquacide.*

Discontinuous vertical-gel electrophoresis was run on an E-C apparatus. The pH of the spacer buffer was modified from 6.7 to 7.3 (Williams and Reisfeld, 1964), to adapt the system to the separation of bean-seed proteins. Gels were fixed and stained with 0.25 per cent commassie blue in 7 per cent acetic acid and 25 per cent methanol for 15 minutes (Reed and Bieleski, 1968). After

*Mention of trade names is for the convenience of the reader and does not imply approval to the exclusion of other products that may also be suitable.

clearing, the gels were photographed and densitometry tracings were made on a Densicord 542 densitometer, using a 545 nm filter.

Unstained gels were sectioned into 2.55 mm sections and put into liquid scintillation vials and counted in Brays scintillator medium for radioactive sulfur. A fluorescence related to bean protein was eliminated by adding 1 ml of water to the vials and storing them in darkness for two hours before counting.

Protein quantity was determined by micro-buret (Itzhaki and Gill, 1964) and absorption in the ultra-violet using the 260/280 ratio (DeMoss and Bard, 1957).

Gel filtration was carried out by packing fully swelled G200 Sephadex in a 1.5 x 90 cm column. Exclusion volume was determined with blue dextran. The column was calibrated for molecular-weight determination by the method of Andrews (1964) with the following proteins: ribonuclease (Worthington), soybean trypsin inhibitor II-s (Sigma), bovine serum albumin (Sigma), ovotransferrin (Sigma), and bovine γ globulin (Sigma). The column was eluted with phosphate buffer at pH 7.9.

Results and Discussion

Experimental studies conducted in our laboratory illustrate some of the possibilities and problems to be resolved if meaningful breeding programs are to be planned to improve the nutritional quality of seed legumes. An experiment using experimental variables of cultivar and source of sulfur was conducted in the greenhouse. The data obtained, shown in Table I, were interpreted to mean that the source of sulfur influenced nitrogen and sulfur components of bean seeds. However, the ratio of methionine to protein in these data was stable. Under the conditions of this experiment the relative proportions of seed proteins were not shifted markedly by sulfur treatment, so that the relative proportions of the amino acid was a function of the cultivar grown. Additional evidence for this interpretation was provided by the data in Table II, which showed that the tested cultivars differed significantly in the proportion of methionine per unit of protein. In this case, the protein-N was precipitated by 0.8 M trichloroacetic acid (TCA) and the nonprotein-N was soluble in TCA. A reliable, easy, inexpensive analysis for seed protein that would not be influenced by nonprotein nitrogen sources would be useful in genetic studies.

One way of exploring the range of nutritive values of beans as a human food is to look at the component seed proteins that are characteristic of the species. Separation by polyacrylamide gel electrophoresis, as shown in Figure 1, reveals a diversity of protein bands from one variety to another. As pointed out by Favret (1967), the presence or absence of a particular band may be shown to be under genetic control. A survey of a broad range of germ plasm should reveal the alleles of the loci that determine the presence of seed proteins associated with each possible band. It is possible that some bands will occur in every bean genotype and that other bands will occur in rare genotypes. It might be expected that each variety or genotype would exhibit a unique permutation of the possible seed proteins and thus show a different pattern upon separation by gel electrophoresis.

Table I
Content of Bean Seeds Grown to Maturity on Various Sulfur Sources

Sulfur source	Percent				mg Methionine / g protein
	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Methionine	
Sodium sulfate	4.02	.511	3.38	.408	0.90
Sodium sulfite	3.78	.410	3.29	.397	0.90
Sodium thio-sulfate	3.94	.466	3.33	.396	0.88
L-cysteine-hydrochloride	4.60	.627	3.79	.475	0.89
DL-methionine	4.17	.564	3.42	.406	0.89

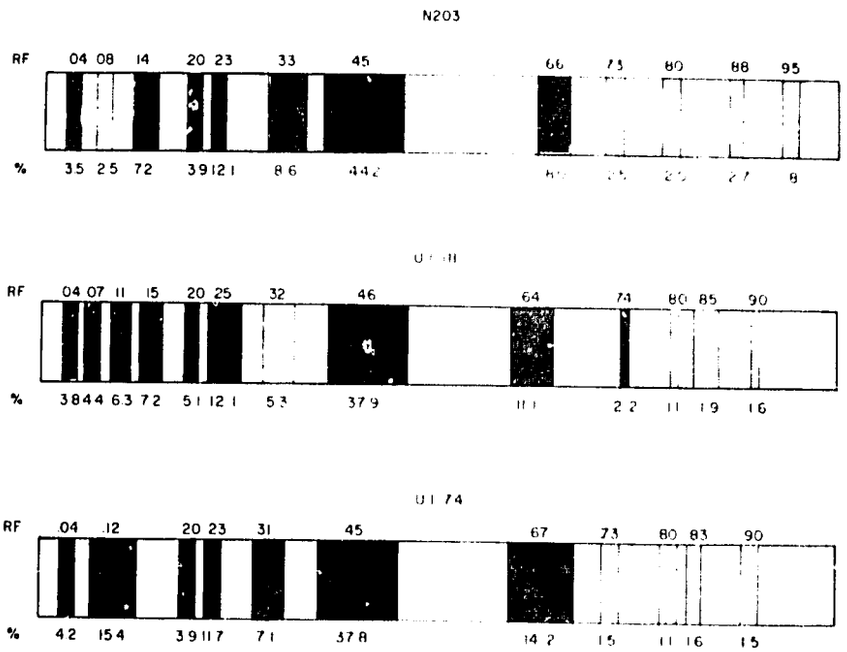

Figure 1. Patterns of bean-seed proteins of three varieties separated by polyacrylamide gel electrophoresis.

Gel filtration provided a useful supplement to the gel electrophoresis techniques. Separation of seed proteins on Sephadex G200 yielded fractions of different molecular weights, as shown in Figure 2. When these fractions were further separated by polyacrylamide gel electrophoresis, bands not evident in previous separations were revealed (Figure 3). Individual bands may be characterized for a number of factors related to human nutrition. Such factors should include an assay of the amino-acid content of each protein, its digestibility during feeding trials, its proteolytic and/or anti-proteolytic activity, if conjugated with other moieties, and whether it functions as a hemagglutinin or has other harmful effects. Whether the seed proteins are components of aleurone grains also should be known, as this fact may facilitate utilization research.

We were able to demonstrate, using the negative staining procedure of Uriel and Berges (1968), that the band with an RF of 0.9 was a trypsin inhibitor. Confirmation of this fact and the possibility of more than one trypsin inhibitor comes from circumstantial evidence related to the known high cystine content of bean trypsin inhibitor. Vogel and Wood (1971) were able to show that aleurone grains near the surface of cotyledons were high in protein-bound sulfhydryl

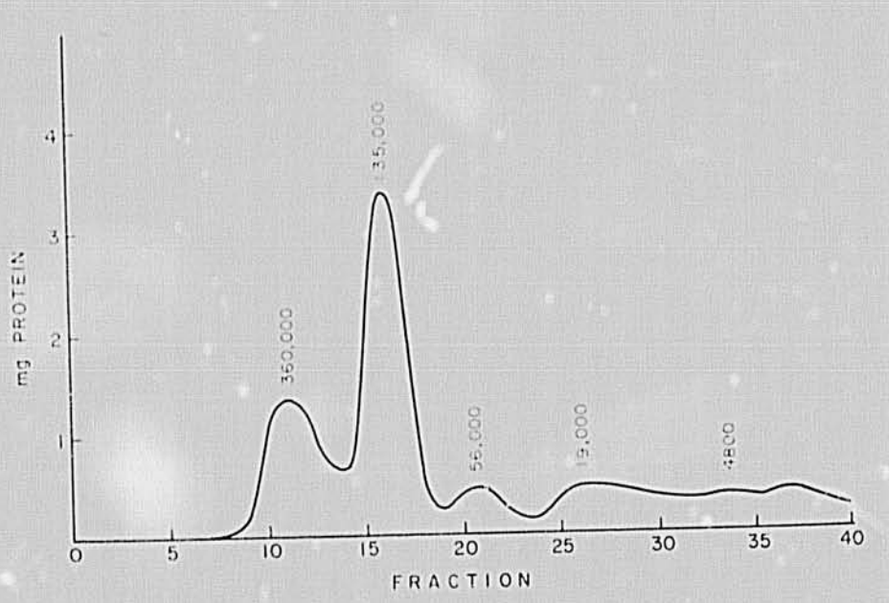

Figure 2. The protein constituents of U.I. 111 pinto seed separated by gel filtration on Sephadex G200.

Figure 3. Electrophoretic patterns of gel filtration fractions of U.I. 111 seed proteins. Banding patterns are as follows from top to bottom: 56,000 mol wt fraction; 135,000 mol wt fraction; 19,000 mol wt + 360,000 mol wt fraction; 19,000 mol wt fraction; 360,000 mol wt fraction; and RNA on treated unfractionated extract.

and disulfide groups. When the outer cell layers were removed and the protein extracted and compared to the protein of the remainder of the cells of the cotyledons, there was a decided shift in the quantity of the proteins of certain bands. Twice as much protein of the fast-moving bands was present in the outer cells; bands of regions 3 and 4 were most prevalent in the inner cells (Table III). High concentrations of sulfhydryl and disulfide in the fast-moving proteins were confirmed by labeling experiments, which showed that those proteins had about 10 times as much sulfur as the other bands. Lack of resolution in sectioning the gel made it difficult to state which bands of zone 6 of Figure 4 were high in sulfur.

We were able to demonstrate glycoproteins in bean cultivars, using the periodic-acid Schiff technique of Zacharius, et al. (1969), as shown in Figure 5. The presence of a sharply defined band near the front was most surprising, as a band in this position was not demonstrated with comassie blue staining.

We feel that our work has demonstrated that knowledge of the kinds of bean-seed proteins and their nutritional characteristics could be the basis of a very precise screening system. Genotypes would be screemed for the quantity and number of the seed proteins present and the nutritional value could be predicted, based on the knowledge of the component seed proteins. The plant breeder could carry the predictive process an additional step. Knowing the extent of the genetic variation for seed proteins, he could predict the genotype of the bean cultivar that could be developed with protein of the highest possible nutritional value.

TABLE II

Content of Bean Seeds of Different Varieties

Variety	Percent				mg Methionine / g Protein
	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Methionine	
Dark red kidney	4.35	.465	3.69	.442	0.87
Pinto, U.I. 111	4.29	.655	3.47	.442	0.95
Ventura	3.67	.426	3.17	.366	0.85

TABLE III

Comparison of Proteins Extracted from the Outer Cell Layers (0.5 - 1.0 mm) and the Remainder of the Cotyledons of the Bean Variety U. I. 111

Electrophoretic Zone	Outer			Inner		
	CPM ^{35}S	μg Protein	CPM / μg	CPM ^{35}S	μg Protein	CPM / μg
1	570	176	3.2	1100	173	6.4
2	921	150	6.1	836	153	5.5
3	3252	486	6.7	3622	522	6.9
4	737	100	7.4	1044	144	7.2
5	1305	26	50.2	666	17	39.2
6	4429	56	79.1	2471	25	98.8

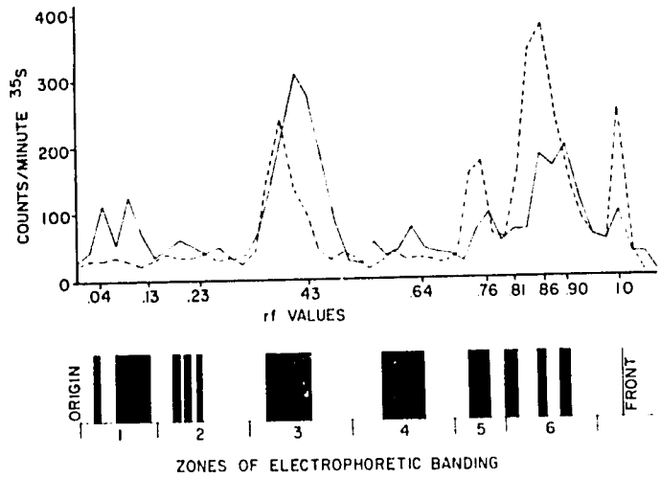

Figure 4. Comparison of ^{35}S activity of protein extracted from the outer cell layers of bean cotyledons (- - - -) and proteins from the remainder of the cotyledons (——).

Figure 5. Pattern of bean-seed proteins of different cultivars separated by polyacrylamide gel electrophoresis and stained for glycoproteins. The banding patterns are as follows from top to bottom: U.I. 74, N203, inter cell extract U.I. 111, outer cell extract U.I. 111, and U.I. 111.

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IV B. Useful Experience From Other Food Crops

Statement

ANALYTICAL PROCEDURES FOR SCREENING PROTEIN QUALITY IN CEREAL GRAINS
– Evangelina Villegas

Discussant
Earl R. Leng

Summary
L. Munck

Research Needed
Earl R. Leng

ANALYTICAL PROCEDURES FOR SCREENING PROTEIN QUALITY IN CEREAL GRAINS

Evangelina Villegas

Importance of Genetic Improvement of Protein in Crop Cultivars

A large proportion of the worldwide human protein needs is supplied by vegetal proteins. At present, only 29 per cent is supplied by animal protein and 71 per cent by vegetal proteins. In developing countries, where there is a dramatic protein-calorie deficiency, a critical urgency exists for increasing productivity and, especially, for improving the nutritional quality of the different crop cultivars.

Most of the expanded systematic and cooperative research needed to solve these problems involves the disciplines of breeding, genetics, biochemistry, and nutrition. Successful improvement of protein production and protein quality will permit the different crop cultivars to play an increasingly important role in solving the human and animal nutritional problems of most developing countries.

If supported financially and politically, programs for using highly nutritive supplements or prepared mixtures of proteins also could play a very important role in solving the nutritional problems of people living in the low-income, urban areas of the developing countries. They could also help the badly nourished, subsistence, rural families, which comprise 50 to 80 per cent of the total population of those countries. Certainly, all scientists working in plant breeding, genetics, biochemistry, nutrition, and related disciplines feel obligated to search for genetic improvement and for the nutritional quality of crop cultivars. The task we have imposed on the genetic-breeding programs is not easy, but neither is it impossible.

The discoveries of the effect of opaque-2 and floury-2 mutants (Mertz, et al., 1964; Nelson, et al., 1965) on the quality of the maize endosperm protein as it affects amino-acid balance, and later the effect of the "Hiproly" gene on the lysine level of barley endosperm (Hageberg and Karlson, 1969) have sparked the imagination and stimulated the researches of everyone concerned with genetic improvement of cultivars of the important crops. These discoveries offer a significant alternative for helping to solve nutritional problems of the less-developed nations.

Techniques for Evaluating New Germ Plasm in Cereal Breeding Programs

Rapid and reproducible analytical methods are an absolute necessity for guiding plant breeders in identifying and developing improved crop cultivars that combine good agronomic type and improved nutritional quality. Such tests also are useful in distinguishing between differences caused by environmental effects and those caused by genetic variability.

In the past, advances in breeding high-yielding, improved cultivars with outstanding nutritional properties have been hampered by the lack of rapid screening techniques capable of handling the thousands of samples that emerge each year from an aggressive breeding program.

What screening techniques can we recommend for evaluating the nutritional quality of legumes? The problem is unusually complex because of the diversity of the grain-legume species under consideration. Methods and analytical procedures that might be of value for one specie may be of only minor value for another. A critical study must be made of the nutritional properties — both weaknesses and strengths — of each species that is to be improved.

The problems encountered and methods needed for the improvement of grain legumes will, in general, be very different from those we have found effective for our work in cereals. Nonetheless, our experiences with the latter should prove useful in establishing parameters for grain legumes.

Maize

Maize varieties that possess the opaque-2 gene are called high-lysine maizes because they contain double or more the lysine level found in normal varieties. They also contain more tryptophan and a better amino-acid profile. The endosperm (kernel) characteristics of lines carrying this gene can be distinguished visually from most other types of maize endosperm because of their soft texture, low density, and dull appearance, which is the result of their not transmitting light. These characteristics make it possible to identify the opaque-2 gene in the segregating material even when it has been incorporated into a normal population through crossing. During the first few years of improving lysine and tryptophan levels in maize through the incorporation of this gene, only visual kernel selection was practiced in most breeding programs — a practice that precluded any opportunity to improve the grain type (i.e., by making it harder and denser), which was most necessary if these varieties were to be accepted by the farmer.

Recognizing these shortcomings, we at CIMMYT studied different procedures by which we would be able to circumvent them. Eventually, by employing simple and fast analytical methods, we were able to identify kernels with high contents of tryptophan and lysine and with almost normal endosperm types.

The opaque-2 and opaque-2 with modified kernel types are shown in Figure 1 A to E.

Methods

Currently, the procedures used routinely to evaluate thousands of popula-

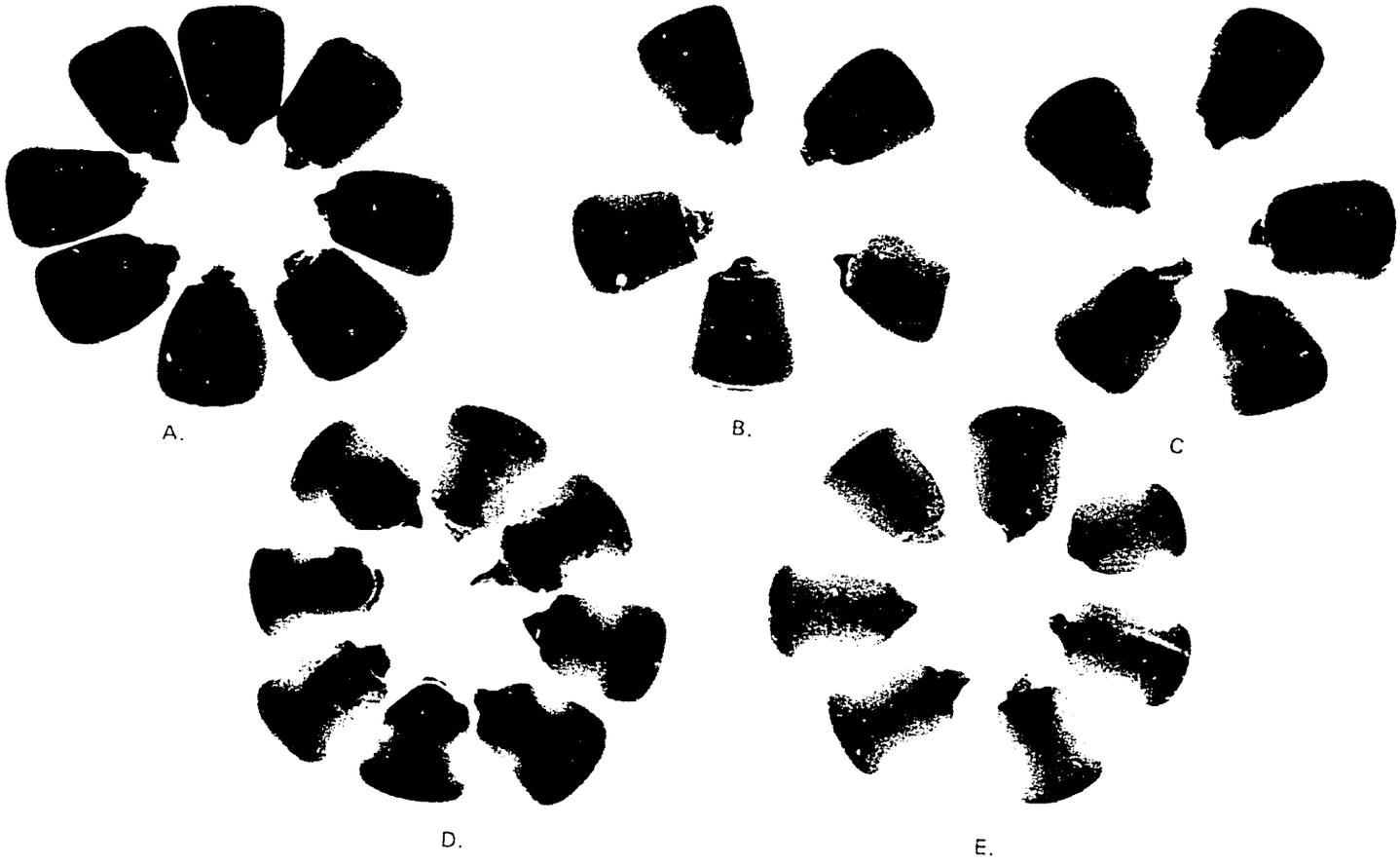

Figure 1. Opaque-2 maize and opaque-2 with modified kernel types. A: Opaque-2, soft endosperm kernels. B, C, D: Modified endosperm types derived from opaque-2 maize populations. E: High-lysine, modified opaque-2 maize with hard, nearly normal, type of endosperm.

TABLE I
Lysine and Tryptophan Content of Defatted Endosperm of Opaque-2 and Modified Opaque-2 Maize

	Common maize	Opaque-2 maize (soft endosperm) (4856)	Modified opaque-2 (hard endosperm) (4855)
Lysine	2.00	3.80	3.40
Tryptophan	0.40	1.00	0.85
Protein (% N x 6.25)	9.20	8.00	9.93

tions and to segregate materials from the maize-breeding program (Villegas and Mertz, 1971) are the following:

1. Sample preparation for endosperm analyses. In order to identify readily the materials with improved protein quality, analyses are restricted to endosperm material. This is necessary because the maize endosperm is deficient in lysine and tryptophan, while the embryo, on the other hand, has a relatively constant and well-balanced amino-acid composition, regardless of the genetic background.

2. The evaluation of genetic families is performed on the endosperm of 10 seeds taken from each ear.

3. The endosperm samples are defatted, using hexane.

4. The samples are dried and ground to a fine powder with a Wig-L-Bug amalgamator.

5. The protein is determined by estimating the nitrogen content, using the micro-Kjeldahl procedure.

6. In order to perform more analyses per day, we are also using Kjeldahl digestion for batches of 40 digests, after which we determine nitrogen colorimetrically, using the Technicon auto-analyzer. We usually analyze 200 samples daily.

7. Tryptophan. Because of the relationship observed by Hernandez and Bates (1969) between tryptophan and lysine in the maize endosperm protein (approximately 1 to 4), the tryptophan determination may be used as a single parameter for quality evaluation of maize. A modification of the colorimetric method of Opienska and Blauth has been used for this estimation. Up to 200 determinations can be performed in a working day.

8. Colorimetric lysine determination is slower and less reliable than is tryptophan determination. Lysine is determined only on those materials selected for high tryptophan values, or when the lysine value, as well as the tryptophan, is desired. We use the 2-chloro-3, 5-dinitropyridine method (Villegas and Mertz, 1971).

9. The complete amino-acid analyses, using the Beckman amino-acid analyzer, is performed only on the outstanding materials.

10. In certain cases, i.e., when one maize family with hard endosperm (normal endosperm type) is identified as possessing high levels of tryptophan in the protein, we perform a single-kernel analysis on a further subsample of the same ear, to identify which grains contain the high levels of tryptophan. This procedure does not destroy the embryo of the analyzed seed, and so allows the growing of those seeds with the highest levels of tryptophan.

Evaluation of Wheat and Triticale Proteins

In screening wheat and triticale materials emerging from breeding programs, different attempts have been made to develop a simple method for lysine determination. We consider that the pyridine method -- mentioned before for the maize evaluation -- is still very laborious when thousands of samples have to be screened.

At the present time, we are using the Dye Binding Capacity method (DBC), which has been used and recommended by Mossberg (1969). In the selected material -- that having high values of per cent of light transmittance the protein is determined by the Kjeldahl procedure. With the protein content known, a certain amount of wheat or triticale meal is weighed in order to have 60 mg of protein, and the DBC procedure is repeated. In this way, we are selecting simultaneously for high protein and high content of basic amino acids that reflect the higher contents of lysine, the most limiting amino acid in these cereals.

Criteria for Evaluating the Nutritional Quality of Promising Genotypes

Through the chemical analyses, we have selected materials with high potential nutritive value, such as the modified opaque-2 grains. However, this type of analysis does not allow us to determine if the potential quality is actually available to animals and humans. Therefore, it is also necessary to evaluate such materials in some type of biological assays.

Different types of bioassays are used successfully in testing the protein quality of many foods. But, again, the problems for the breeders are unique and difficult when they want to evaluate early-generation materials. The principal problems arise from the small size of samples available and the need to evaluate the large number of lines in a short period of time.

For these reasons, we have chosen the meadow vole *(Microtus pennsylvanicus)* as a test animal (Elliott, 1963) for our preliminary screening in the evaluation of promising cereal genotypes because:

1. The amount of sample required for the complete experiment is very small. One animal consumes approximately 30 g of diet in the test period. This is considerably less than that required for other experimental animals.

2. The voles complete an experiment in only five days. This is a shorter time than that commonly needed for other animals.

At present, in a cooperative study, we are comparing the voles with such other test animals as mice, rats, and chicks, in order to learn if they rank the materials with different protein quality (maize, wheat, and triticale lines) in the same order. As soon as we obtain the results from these feeding trials and evaluate the methodology used, we will select the most appropriate test animal for future quality evaluation of the genetic material. We consider this cooperative test an important step toward arriving at conclusions regarding future recommendations.

By using the aforementioned chemical methods for screening large numbers of samples, it has been possible for the protein-quality laboratory to help the cereal breeders concentrate their efforts on the most valuable materials.

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DISCUSSANT

Earl R. Leng

I will be very brief in these comments, first, because I am not an analytical chemist, and therefore am simply reporting the work of others who have been associated with me. Second, many of the major points and techniques already have been covered more than adequately.

However, I would like to make two observations about the work with soybeans. (I continue to put soybeans into the category of food legumes because of the possibility that they may be so used.) The first observation, which has not been mentioned until now, is the question of oil content. In our laboratory, as well as in other places around the world, the nuclear magnetic resonance, nondestructive technique has superseded all other methods for oil analysis. We used the technique on small samples, and as we have gained experience, we have used the instrument to referee a wide variety of crops.

There are many places in the world where nuclear magnetic resonance equipment cannot be set up. That is, it may not work reliably or stay in operation for a long period of time. However, the speed of the machine is such that cooperative possibilities exist whereby large numbers of samples can be screened with a minimum of waste time.

Second, although I agree that it may not, for the time being, be economical to attempt to breed out the trypsin inhibitors, some studies are worth attention. For instance, studies with starch gel electrophoresis, and the actual qualitiative identification of what appear to be different trypsin inhibitors or related items in soybeans, are of considerable interest. As yet, however, the approach is more theoretical than practical. For instance, we may find that we do not want to breed the inhibitors out in the first place. We all feel the need – if we are going to work with the sulfur-amino acids, say, or with some aspect of lysine – for a swift way to accomplish competent analysis.

SUMMARY

L. Munck

Dr. Carpenter pointed out that the first consideration in breeding food legumes should be their acceptance, because food is not food until it is eaten. Cooked legumes should be studied by more advanced analyses. The clear-cut assessment of nutrients should be of considerable complementary value in establishing the practical value of food legumes. Single-seed analyses should be avoided because of sampling difficulties; at least 10 seeds should be used. Publications should specify if dry-weight or wet-weight bases were used.

Protein Content

The Kjeldahl method is preferable in testing for protein content, and the Technicon auto-analyzer setup, complemented with tube or flash digestion stands, is good for about 200 samples a day. Dye-binding techniques are difficult to reproduce from one laboratory to the next, but are still faster and simpler than the Kjeldahl method, and could be used for preliminary screening.

Sulfur-Amino Acids

Total sulfur could be tested with the auto-analyzer to estimate cystine + methionine, but so far this method does not seem promising. Methionine could be run with a colorimetric technique. For cystine, only ion-exchange methods are available. Microbiological assays for these amino acids need little in the way of apparatus, but do require considerable skill. It is concluded that resources should be allocated to speed up and simplify methods for determining the sulfur amino acids.

Other Tests and Bioassays

Rapid and accurate methods to assess protein indigestibility and flatulence factors are not available and need further attention. Dr. Carpenter has pointed out that plant breeders may demand too much from the nutritionists when they ask for simple animal tests to establish complex "nutritional qualities." Rat tests are most widely used, and when the animals are fed legumes, the result is usually

a low growth rate caused by lack of methionine. Addition of methionine to diets should be considered, after which an assay may sum up digestibility, as well as some of the possibly deleterious factors of other limiting amino acids. Finally, cooked legumes could be tested as possible supplements to cereal and other diets.

Dr. Villegas gave a conclusive summary of the methods applied at CIMMYT in Mexico for the breeding of maize, wheat, and triticale. She pointed out the special value of advances in nutritional quality that can be obtained by genetic manipulation. If the new varieties could be introduced rapidly, poor urban and rural populations might be reached. However, the legume problem seems to be more diverse and, therefore, more complicated. In breeding cereal grains for high lysine content with the help of the opaque-2 gene in maize, the associated opaque character was used for selection in the early breeding. However, the opaque character was not accepted by the farmers; therefore, it was necessary for plant-breeding programs to screen for amino acids in order to modify that character and obtain nonopaque, high-lysine maize. This was accomplished by employing the 2-chloro-3,5-dinitropyridine, single-seed screening method. The dye-binding method was used for a crude screening of wheat and triticale.

Dr. Leng pointed out the advantages of the nuclear magnetic resonance (NMR) method for fat in soybeans. Dr. Wood used polyacrylamide electrophoresis to screen different varieties of *Phaseolus vulgaris*. When the nutritive characters of the different brands are known, this method could be used in plant-breeding programs.

Dr. Munck discussed dye-binding, which was introduced into the United States by Udy as a quick method for estimating crude protein by Kjeldahl techniques. However, studies carried out in cooperation with Dr. Mossberg at the Weihellyn Holm Plant Breeding Institute in Sweden showed that dye-binding was more effective in estimating basic amino acids. The method was used in Svalöf to isolate the high-lysine cultivar Hiproly in barley. In legumes, selection for high DBC per unit of Kjeldahl crude protein (basic amino acid per unit of crude protein) gave no response in a population of *Vicia faba*. It is possible that the dye precipitates only protein from the suspension, leaving the peptides and amino acids intact. This might be important when harvesting early or under off-climate conditions. The correlation between protein (nitrogen not soluble in trichloracetic acid) and DBC in *Vicia faba* is excellent at all developmental stages. Dr. Munck concluded that the use of the DBC method to screen for Kjeldahl protein, as is now done in the United States with maize, for example, is dependent on a good correlation between the basic amino acids and the crude protein. If high-lysine, opaque-2 varieties are released extensively in the market, the correlation between dye-binding and Kjeldahl protein tests will be poor, but the correlation between dye-binding and basic amino acids (lysine) will still be excellent.

The ways in which legumes are accepted by and affect the performance of birds and rats should be studied further, with an end view of developing simple, provisional bioassays of small samples that could be used in early stages in plant breeding.

RESEARCH NEEDED

Earl R. Leng

There is general agreement that total protein is the most important chemical determination to be made in rapid screening connected with grain-legume improvement programs. Dye-binding techniques are at present the most rapid, reasonably accurate methods available for determining total protein. The development of more accurate and/or faster techniques would be useful.

Research is needed on the relation of total sulfur content to sulfur-amino acids. Accurate methods that use such a relation could be highly useful in screening for methionine and cystine.

If "total S" methods of sufficient accuracy are not available, more accurate and rapid methods are needed for determining methionine and cystine content.

Further studies on application of electrophoretic methods should be conducted.

A search should be undertaken for correlations between simply inherited genetic traits and desirable chemical composition.

Nondestructive analytical methods (analogous to NMR for lipid content) would be highly desirable.

Simple (in vitro) methods, correlated with in vivo findings, are needed to study protein digestibility.

Bioassay, through the use of test organisms, appears essential for any final appraisal of improved varieties. The development of rapid, economical, and meaningful bioassay methods should have a high priority.

In bioassay and other nutritional studies, the consideration of total diets is much more significant than the evaluation of the grain legumes as sole diet.

V

A CALL FOR ACTION

PAG Statement 22

UPGRADING HUMAN NUTRITION THROUGH THE IMPROVEMENT OF FOOD LEGUMES

The food legumes are major sources of protein and other nutrients in the diets of many developing countries and they often have a significant role in desirable crop rotations. These important food species have been seriously neglected in terms of research necessary to increase their yield and to correct certain defects in the nutritional and food-use qualities.

The PAG recommends urgent research attention to six major species of food legumes: dry beans, pigeon peas, cow peas, chick peas, broad beans, and peas; and the two leguminous oilseeds, peanuts and soybeans. While increased yield and improved consumer-acceptance qualities are primary objectives, priorities are also proposed for genetic improvement of various nutritional factors. These include increased protein concentration, higher methionine and cystine levels (the sulfur-bearing amino acids are usually the first-limiting amino acids in legume proteins, cystine having a sparing effect on methionine), augmented lysine where feasible (legumes are primary sources of lysine in cereal-based diets),and protein digestibility.

The importance of specific nutrients is indicated and the need for careful nutritional evaluation is emphasized. Various techniques for evaluating nutritional properties in breeding programs are listed and critically compared. These include total protein, methionine, cystine, total sulfur, and other amino acids. Also listed and evaluated are methods for digestibility and for toxic constituents, which are classified under heat-labile and heat-stable types. These include trypsin inhibitor (whose effect on humans may not be significant), the hemagglutinins, antivitamins, goiterogens, cyanogens, alkaloids, lathyrogens, and factors leading to favism and flatulence. The applicability and interpretation of feeding tests for digestibility and for toxic constituents, which are classified under heat-labile and heat-stable types. These include trypsin inhibitor (whose effect on humans may not be significant), the hemagglutinins, antivitamins, goiterogens, cyanogens, alkaloids, lathyrogens, and factors leading to favism and flatulence. The applicability and interpretation of feeding tests for various factors, including protein efficiency ratio and digestibility, are discussed. Bioassays for growth-depressing factors are also listed. Recommendations are presented for needed nutritional and biochemical research.

The attention of legume breeders is directed to the need for solutions to storage, processing, and consumer-acceptance problems in legumes under the headings of cooking, milling quality, susceptibility to insect infestation, undesirable storage changes, and uniformity of time of maturation of legume crops.

Production problems and opportunities are reviewed under the headings of breeding objectives, study of growth processes, production and management policies, and production economics and utilization.

The statement closes with emphasis on the importance of a reinforced program of food-legume research and training and the need for greater research support and stronger international cooperation.

Background

The PAG has frequently emphasized the urgent need for improvement by agricultural and genetic means of the nutrtional value, and particularly the protein component, of staple food crops*. Recently the Group proposed that greater research effort should be devoted to upgrading the food legumes, which are major protein resources in many developing countries. Accordingly, the PAG Secretariat, with the cooperation of several governments and an international foundation, convened in July, 1972, at FAO in Rome, an invitational symposium on nutritional improvement of food legumes by breeding**. A group of internationally-recognized agronomists, plant breeders, geneticists, nutritionists, biochemists and food scientists reviewed the latest scientific and technical evidence regarding nutritional and food-use problems in food legumes and how these might be improved by breeding. The PAG will shortly publish the proceedings of this meeting.

In a related development, the PAG *ad hoc* Working Group on Guidelines for Legume Breeders met in Rome immediately following the symposium and drafted a statement subsequently approved by the PAG Steering Committee, which is the subject of this document. Members of the Working Group included Mr. P. Oram, Mr. J. Jalil, Dr. Bruce Nicol, Dr. P. Lunven, Dr. G.D. Kapsiotis (all of FAO), Dr. B. Sigurbjornsson (IAEA) and Dr. M. Milner and Miss E. van Beresteyn (PAG Secretariat).

1. Importance of Food Legumes

The food legumes+ are important and economical sources of protein and

*The following PAG documents and publications are pertinent:

Statement No. 8: Plant improvement by genetic means (1970)
Document 1.16/5: Need for nutritional and food quality guidelines for plant breeders (1971)
Document 1.16/4: Protein nutritional aspects of plant breeding (1970)
Document 1.16/3: Processing and food acceptance aspects of plant breeding (1970)

**PAG Bulletin Volume II, Number 4, page. 6.

+The following definition of food legumes has been proposed by Roberts (1970): "Food legumes as here defined include those species of the plant family Leguminosae (pea or bean family) that are consumed directly by human beings, most commonly as mature, dry seeds, but occasionally as immature, green seeds or as green pods with the immature seeds enclosed. It does not include species that provide leaf or stem tissue that is used as cooked or uncooked greens. Food legumes utilized as dry seed are often referred to as pulses or grain legumes."

calories as well as of certain vitamins and minerals essential in human nutrition. However, the significant role they play in the diets of many developing countries appears to be limited by their scarcity, caused in great part by their present low yields, their consequent cost, and certain defects in their nutritional and food-use qualities.

Information on regional and world production of food legumes is given in Table I. When analyzing these data, the reader should recognize that the species listed *other* than soybeans and peanuts are consumed directly as human food. The bulk of these two leguminous oilseeds, which are produced in far greater amounts, is processed for edible oil and for protein residues used largely for animal feeding, primarily in the industrialized countries. The availability of selected food legumes in terms of grams per caput per day for various countries in different regions is shown in Table II, and projections of demand for certain dry legumes between 1965 and 1980 appear in Table III. The increasing trend in demand in the developing countries is shown by two methods of projection.

Improvement of most of the food-legume crops used primarily and directly for human food has been greatly neglected. In fact, the "Green Revolution" will not be complete until breakthroughs are achieved with these food crops similar to those accomplished with the cereals (UN Strategy Statement, 1971). There is an obvious need for guidelines to stimulate efficient global progress towards realizing these objectives.

The PAG is primarily concerned with the improvement of diets deficient in protein, and stresses the unfulfilled nutritional potential of food legumes for improving human diets as well as their economic advantages, particularly in developing regions. The food legumes have primary importance for:

a) Complementing and supplementing the protein-deficient diets generally prevailing among populations in urban, suburban, and rural areas, including subsistence farmers, particularly in developing regions.

b) Providing economical protein concentrates as ingredients for processed, nutritious foods, including foods for weanling and preschool children.

c) Raising the income of agricultural producers by making these crops competitive with other food staples.

It must be emphasized that the first order of priority in approaching these objectives will be the improvement of productivity, adaptability, and yield stability. The second is improving the acceptability and food value of legumes as carriers of nutrients lacking in many diets, and reducing the concentration of certain undesirable constituents. The main protein-nutritional deficiencies of food legumes arise, in general, from a low sulfur-amino acid content. In addition, some of them contain toxic principles and some may cause flatulence. Limited digestibility may also be an important problem. Many are deficient in organoleptic qualities, such as flavor and color, and in desirable cooking characteristics. Factors requiring improvement should be considered in the context of total diets wherever legumes are accepted and where traditional home cooking practices are

used. Here, improved varieties can have an important impact on the total food supply in a country.

The PAG stresses that agronomic problems in food-legume production are very different from those with cereals, which were solved by the new high-yielding varieties. The Green Revolution in cereal production was based primarily on the favorable responsiveness of such varieties to nitrogen fertilization under irrigation, whereas legumes play a quite different but important complementary role in cropping systems by virtue of their capacity to fix nitrogen.

2. Priorities in Food-Legume Improvement

Considering the large number of existing food legumes, it is necessary to select those species which possess the greatest potential for significant contribution to the diets of people in the major ecological zones of the developing regions. The following are therefore recommended for urgent and immediate attention by all concerned agencies and institutions:

a) Dry beans (*Phaseolus vulgaris*) for intermediate and higher elevation tropics and drier irrigated conditions.

b) Pigeon peas (*Cajanus cajan*) for humid to semiarid, low to intermediate elevation tropics.

c) Cow peas, niébé, or Southern peas (*Vigna sinensis; V. unguiculata*) for semiarid to subhumid lowland tropics.

d) Chick peas (*Cicer arietinum*) for cooler environments, including the higher elevation tropics and Mediterranean winter-spring seasons.

e) Soybeans (*Glycine max*)* for the subhumid and humid, low and intermediate elevation tropics, and irrigated dry seasons.

f) Broad beans (*Vicia faba*) for higher elevations and Mediterranean winters.

g) Peas (*Pisum sativum*) for intermediate to high elevation tropics, and as a winter crop in Mediterranean-type ecologies.

h) Groundnuts (*Arachis hypogaea*)* for the semiarid and low and intermediate elevation tropics.

Other food legumes that have not yet achieved equally widespread importance are Asian grams (*Vigna radiata* and *V. mungo*), lentils (*Lens esculenta*), lupines (*Lupinus* spp.), yam beans (*Sphenostylis stenocarpa*), lima beans (*Phaseolus lunatus*), jack beans (*Canavalia ensiformis*), bambarra groundnuts (*Voandzeia subterranea*), and others. These species exhibit specific useful features of adaptation and nutritional value and should be given appropriate local

*The two leguminous oilseeds are considered here for their exceptional potential as food-protein sources (Noachovitch, 1969).

attention. As their value becomes better defined they may achieve major significance.

This statement seeks to identify and define those food-use and nutritionally related problems inherent in food legumes that plant breeders should consider when developing well-planned programs for genetic improvement of these crops, and it suggests techniques for evaluating and screening crops for these factors. It goes on to propose how production problems may be overcome through genetic improvement and better cultural practices, when such means are available, and indicates areas in which more research is necessary.

3. Genetic Improvement of Nutrient Composition and Digestibility

Within each of the important legume species, the range of genetic variability, as well as the effects of environment on this variability, should be determined in relation to various nutritional factors. The factors listed below in an order of priority varying with feasibility in individual species (Roberts, 1970) should be emphasized in breeding-improvement programs:

a) Protein concentration. This attribute should be increased or maintained at the highest level possible, while efforts are made to achieve higher crop yields and to retain or improve protein quality.

b) The first-limiting amino acids. These are usually methionine and cystine (except for *Cajanus* in which tryptophan is probably equally limiting), which should be increased if possible. These sulfur-bearing amino acids should be considered together because methionine may be utilized to supply cystine requirements.

c) Protein digestibility. This varies to some extent depending on species and is not poor overall, but for some food legumes it needs improvement.

d) Nonlimiting essential amino acids. The normal lysine content of legumes does not limit their nutritional value as does the characteristic methionine deficiency. However, inasmuch as lysine is generally the first-limiting essential amino acid in diets dominated by cereals, every effort should be made to retain or increase legume lysine levels.

e) Other essential amino acids (e.g., threonine). If they are likely to become limiting in the overall diet as the primary limiting amino acids are increased to the point of sufficiency, consideration should be given to increasing their level as well in the legumes.

f) Minerals (e.g., Zn, Ca, Fe) and vitamins should be maintained at the highest level possible.

Because legume species are used in many different diets, it may be necessary to reorder the above priorities to fit a particular regional dietary need. Thus, as indicated, where legumes are consumed with cereals it may be more important to improve lysine levels than methionine/cystine, but where legumes are consumed with starchy roots or tubers, methionine/cystine improvement would be more significant.

It must be established that any genetic changes introduced will not adversely affect other nutritionally beneficial constituents or increase antinutritional factors (e.g., excessive amounts of other essential amino acids, potentially competitive nonessential amino acids, and toxic inhibitors). Before incorporating any compositional change, except total protein, it should be established that the factor will be reflected in improved overall nutritional value of the diet (see paragraph 4) and that it is heritable in the legume species. Improvement of amino-acid balance and protein digestibility in legumes intended for animal feeding also should not be overlooked.

Breeding programs should emphasize improvement of yield, adaptability, total protein (for those crops in which significant protein variability has already been established), and consumer acceptability. Fundamental genetic studies for the selection of breeding populations with higher levels of total protein and amino acids should be conducted parallel to the agronomic improvement and breeding programs. These more nutritious lines can be introduced into the program as soon as the previously indicated requirements have been fulfilled and when enough is known about their mode of inheritance to facilitate rapid incorporation into otherwise superior agronomic types. Such studies may reveal undesirable linkages, quantitative inheritance patterns, or physiological associations that make the incorporation of the nutritional factors difficult or unfeasible.

Existing populations should be exploited wherever possible to introduce protein improvements into currently used varieties or to furnish substitutes for currently used varieties. Where suitable needed genotypes cannot be found in existing breeding stocks, attempts should be made to induce them by other known means. For each crop, goals for protein-nutritional improvement should be established and certain minimum levels of some amino acids should be set as practical objectives. In addition, standard reference varieties should be established and maintained for each crop. Biochemical studies to elucidate the nature and causes of differences in protein patterns, amino-acid balances, and protein digestibility should be encouraged.

4. Evaluating Nutritive Properties of Legumes

Because legumes are to be used as human food and because breeding programs can produce wide variation in the content of both nutrients and toxic substances, careful nutritional testing by reliable techniques must have high priority in any development program. Reference should be made to two comprehensive protocols, one dealing with preclinical testing of novel sources of protein (PAG Guideline 6) and the other with human testing of supplementary food mixtures (PAG Guideline 7). While Guideline 6 refers primarily to unconventional protein sources and food mixtures based on them, its principles are directly applicable to assessment of the safety and nutritional quality of new legume cultivars as well.

The objective of this portion of the statement is to indicate techniques for evaluating the specific nutritive properties which should be of concern to the plant breeder. Some of these are deemed important enough to merit a place in

routine programs for examination of new breeding lines. Other nutritive properties, although important, are not yet amenable to procedures that can be adapted readily to a rapid plant-breeding screening program. Some tests are recommended only at later stages of the breeding process, after selections have been made according to other criteria.

4.1 *Protein content*

This seems to be the only *nutritional* criterion that can be employed with early generation materials or with any very large series of samples. An attempt should be made to establish if a correlation between dye-binding capacity (DBC) and Kjeldahl nitrogen content (AOAC, 1950) exists (e.g., Hymowitz, et al., 1969; Mossberg, 1969, 1970). The DBC can be measured with the Udy Protein Analyzer (Boulder, Colorado, U.S.A.) and, when the samples are too numerous for classic Kjeldhal analysis, the Technicon or Carlo Erba (Milan) analyzers can be employed in micro-Kjeldahl nitrogen determination (e.g., Varley, 1966). In the latter case, a protein conversion factor of 6.25 is recommended for comparitive purposes only, and does not imply true protein content, which may be somewhat different (Thurman and Boulter, 1966; Smith, 1966). Consideration should also be given to the use of such techniques as infrared analysis when suitable instrumentation becomes available and its accuracy proved (Rosenthal, 1971). High nitrogen values per se may not be indicative of protein of desirable quality, as some species may contain disproportionate amounts of certain amino acids. Ultimately, a biological test for nutritive quality is essential in any screening program.

4.2 *Amino acids*

The deficiency or lack of availability of the essential amino acids methionine and cystine are of primary concern in most legumes. Appropriate analytical methods for these have been proposed. Sulfur content is under study as an indicator of these amino acids. Lysine and tryptophan are normally present in adequate amounts, but opportunities for increasing them should not be neglected. Analytical methods for these amino acids are available.

4.2.1 *Methionine.* This amino acid, together with cystine, constitutes the first-limiting amino acid of legumes (FAO, 1970; Swaminathan, 1967). Hence both these acids should be determined, if possible. Specific procedural recommendations, however, cannot be made because the method of choice will depend on the facilities available in each laboratory. The following assay methods are suggested:

a) Ion-exchange chromatography. For limited numbers of samples, this method is accurate and reproducible (Spackman, et al., 1958; Moore and Stein, 1963; Devenyi, 1971).

b) Gas-liquid chromatography of amino acids as N-trifluoroacetyl-n-butyl ester derivatives. This method was shown to be as accurate as ion-exchange chromatography, and it is cheaper and faster to run (Gehrke, et al., 1968, 1971; Mottershead, 1971; Basson and Bochmair, 1972).

c) Microbial assay. Although less precise, this method does have the advantage

of simplicity and speed, and, in the hands of experienced personnel, can yield very useful data (Kelly, et al., 1970).

d) Automated color assay. Although an automated color assay for methionine has been proposed, its validity remains to be established (Gehrke and Ussary, 1969; Ussary and Gehrke, 1970). Such a procedure would be extremely useful, and continued efforts to improve it should be strongly encouraged.

e) Other chromatographic techniques. Rapid techniques for the measurement of methionine by thin-layer chromatography (Ferenczi, et al., 1971) and paper electrophoresis are currently under investigation, but their applicability to the present problem remains to be determined.

4.2.2 *Cystine.* The total S-containing amino acids are of great importance in the diet, so a knowledge of the cystine content of legumes could be very desirable. Unfortunately, rapid, reliable assay methods for cystine are not available. Cystine may be determined as cysteic acid if an automated amino-acid analyzer is available (Spackman, et al., 1958; Hirs, 1967). It is hoped that more rapid techniques for the analysis of cystine will be forthcoming, and efforts in that direction should be strongly encouraged.

4.2.3 *Total sulfur.* If this analysis proves to be a useful, direct measure of methionine plus cystine, it will be a great convenience (Boulter, et al., 1973). Rapid automated techniques are available for total S analysis (Mottershead, 1971; Basson and Bochmair, 1972). Investigations are being carried out on the applicability of the Leco Sulphur Analyser for legume foods (Boulter, et al., 1973).

4.2.4 *Other amino acids.* Because legumes can serve as a valuable source of lysine for supplementing cereal-containing diets, a knowledge of the lysine content would be most valuable. Although not recommended on a routine basis, lysine analysis should be carried out at some later stage of the screening process. This analysis can be done by one of several available colorimetric methods. However, their convenience and accuracy may vary with the material analyzed (White and Gouger, 1967; Villegas and Mertz, 1971). Tryptophan, which may be a limiting amino acid in some diets, likewise can be assayed by simple colorimetric techniques (Spies, 1968; Villegas and Mertz, 1971) that also need improvement in accuracy and versatility.

4.2.5 *Digestibility.* Simple in vitro digestibility techniques are available (Sheffner, 1967) but cannot be recommended until such results are correlated with digestibility data obtained from animal experiments.

4.3 *Toxic constituents*

Although numerous toxic constituents are found in raw legumes (Liener, 1969), many of them are destroyed by adequate heat treatment, such as might be employed in common cooking procedures prior to consumption. It becomes important, therefore, to establish those processing conditions which are necessary to inactivate or eliminate these toxic constituents; this evaluation should be done as a minimum procedure on parental breeding material and at later stages of selection on more advanced material. Other toxic constituents may be heat-

stable and these also should be looked for. Specific recommendations for screening purposes are listed below with appropriate remarks.

4.3.1 *Heat-labile factors that need not be screened for initially:*

a)*Trypsin inhibitor.* (i) Normal cooking is considered sufficient to destroy the trypsin inhibitor. (ii) There is a strong indication that legume trypsin may not be physiologically active in man. This is based on recent in vitro evidence which shows that human trypsin, unlike bovine trypsin, is only weakly inhibited by the soybean inhibitor (Feeny, et al., 1969; Coan and Travis, 1971). (iii) Retention of trypsin-inhibitor activity in legumes might be desirable, because it may serve as a defense mechanism against insect infestation during storage (Birk, et al., 1963; Lipke, et al., 1954; Guen and Ryan, 1972). Further research is needed to establish the validity of this thesis. (iv) The trypsin inhibitor in its inactivated form may serve as a good source of the S-containing amino acids, as these proteins are known to be very rich in cystine, although it is unavailable in the raw bean (Kakade, et al., 1969).

b)*Hemagglutinins.* Routine examination for hemagglutinating activity is not recommended, but once selection of lines has been made, proper conditions of food preparation necessary to destroy the hemagglutinins should be established. The significance of these substances in human nutrition has not yet been established and they are definitely toxic to rats and chicks (Jaffé, 1969), so it is recommended that continuing efforts be made to breed legumes (particularly *Phaseolus*) which have low hemagglutinating activity.

c)*Antivitamins and metal-binding components.* The antinutritional effects of these components should be determined in the final selection procedures by biological assay. In some cases, these factors can be counteracted by dietary supplementation and/or cooking (Liener, 1969).

d)*Goiterogens.* There are no known screening methods for goiterogens. The goiterogenic effects produced by soybeans and peanuts (Van Etten, 1969) can be counteracted effectively by dietary iodine supplementation (e.g., in salt) and/or cooking.

4.3.2 *Heat-stable factors*

a) *Cyanogens.* The presence of cyanogenic glycosides in legumes (Montgomery, 1969) does not constitute a health hazard, with the possible exception of *Phaseolus lunatus.* The most commonly used edible varieties of this bean, however, are quite low in cyanide content. Any newly introduced varieties of this legume should be analyzed for their potential cyanide-producing capacity before being released for human consumption.

b) *Alkaloids.* Although relatively few legumes are known to contain toxic alkaloids (with the possible exception of *Lupinus*), their presence should be ascertained in the development or introduction of any new, improved varieties of legumes derived from parent material devoid of them (Lanners, et al., 1968).

c)*Unidentified factors.* See "Feeding tests" below.

4.3.3 *Toxic factors of special interest.*

a)*Lathyrogens.* Relatively simple techniques are available for destroying the neurotoxins of *Lathyrus sativus* (Sarma and Padmanaban, 1969). However, further research on the development of strains or varieties of *Lathyrus* deficient in the neurotoxin believed to be responsible for human lathyrism (β-N-oxalyl-α,β-diaminopropionic acid) should be encouraged. Further research on the causative factors of human lathyrism also is needed. During the course of such breeding studies, however, it should be remembered that other neurotoxins (e.g., α,γ-diaminobutyric acid and β-cyanoalanine) may be present.

b)*Favism.* The development of lines of *Vicia faba* that are free of the causative factor(s) of favism should be delayed, pending positive and complete identification of the toxic factors (Mager, et al., 1969). Efforts to identify the causative principle(s) of favism therefore should be continued. From such studies may also emerge a simple analytical technique which can be used for the purpose of screening strains of *Vicia faba.*

4.4 *Flatulence factors*

These do survive ordinary cooking and they might be considered by some consumers to be crucial factors limiting acceptance of increased proportions of certain legumes in diets (Berk, 1968). When legumes are consumed in large quantities, especially by young children, flatulence may cause considerable inconvenience. This limits their consumption but, if the legumes are well cooked, children can eat them. They should be introduced in small amounts and gradually increased as the child becomes adapted or accustomed to the new preparation (PAG, 1973; Narayana Rao, et al., 1972). It would be advantageous, however, to have varieties with lower flatulence activity, and research that leads to development of simple assay methods for this factor would be useful.

4.5 *Feeding tests*

The true nutritive value of any legume can be checked finally only by animal assay, taking into account the necessary prior chemical and biochemical evaluations recommended in PAG Guideline No. 6. It is recognized, however, that such assays cannot be part of a routine screening procedure. Until now, this type of assay has been done mainly with young rats. Other small animals, however, such as mice and voles (*Microtus pennsylvanicus*), may prove equally suitable for this purpose (Elliott, 1963; Rios Rirarte, et al., 1972), but before a species other than rats is selected for use with legumes, it should be clearly established by prior standardization that reliable results will be obtained and that responses are comparable. Recent Canadian studies suggest that the vole assay may be too insensitive to use as a screening method (private communication). Bioassays also have employed other organisms, such as bacteria (Ford, 1962), protozoa (Bayne, et al., 1967), insects, etc., for some types of tests, but these procedures may have limited usefulness in replacing tests with mammalian or avian species. The mean-

ing of these tests is often open to question, so more emphasis must be placed on the determination of available amino acids. However, they deserve to be investigated further as they might be developed as screening "indicators."

For any legume species for which there is evidence that cooking improves nutritional value (e.g., by inactivating toxic factors), feeding tests should be carried out with material subjected to a specific standardized cooking procedure adapted to each species or new strain of legume. While one convenient procedure suitable for *Phaseolus vulgaris* is to autoclave (in open steam) in shallow (2-cm) layers at 15-lb pressure for 15 minutes, such conditions do not simulate actual cooking procedures in developing countries, where beans are cooked in an open pot.

a) *PER*. The commonest procedure has been to measure *protein efficiency ratio* (gram weight gain per gram protein eaten) with rats (McLaughlan and Campbell, 1970). The test material is included in the diet at a level contributing 10 per cent protein to the diet (N x 6.25), and is the only source of protein. Thus a legume with a 25 per cent protein content is included as 40 per cent of the diet. The standard method requires 28 days; we recommend 10 days as being adequate for screening, using 6 rats per treatment. To reduce the possible effect of food intake, it might be advantageous to use the net protein ratio assay with a negative control group (NAS/NRC, 1963).

b) *Modified PER*. Because of low methionine content, the PER is always very low in legumes as compared to the result obtained with a protein source of well-balanced amino-acid composition and no toxic properties. If the methionine content of the material is being determined separately in any case, and one can afford only one feeding test for any particular sample, it may be preferable to carry out a PER determination with *added* methionine (or with methionine plus tryptophan, in cases where both are limiting). A high result from this type of test confirms *both* that no amino acid other than methionine (or methionine plus tryptophan) is seriously deficient in the protein and that the cooked legume contains nothing that seriously depresses a rat's growth or appetite. If the results are unusually low, it would indicate either a low level (or destruction) of another essential amino acid or the presence of a heat-stable toxic factor (Kakade and Evans, 1966; Bressani, et al., 1963).

c) *Digestibility*. This can be determined as part of a PER (or modified PER) assay (McLaughlan and Campbell, 1970). The overall digestibility of the legumes can be estimated from the weight of feed and fecal dry matter, and the digestibility of protein by feed and fecal analysis for N (McLaughlan and Campbell, 1970).

4.5.1 *Nutritional value in complete diets.* Another indication of the nutritional value of a food legume can be determined at a later stage in varietal testing with rats by feeding them a diet that contains the test legume in combination with other staple food components regularly available and used by populations in a given area. The nutrient content of the components of the test material should be utilized in formulating the diet, and any obvious or recognized defi-

ciencies should be corrected. Variations in the test could be made by feeding the diet with and without those supplementary nutrients suspected to be limited.

4.5.2 *Assays for growth-depressing factors.* These additional tests could be used for special investigations:

a) *Heat-stable, growth-depressing factors.* The biological test for presence of heat-stable, growth-depressing factors can be conducted by feeding at least a 50 per cent level of autoclaved test material (Kakade and Evans,1966; Bressani, et al., 1963). This should replace an equal weight of a standard mixture of casein and starch or sugar of the same protein content. The diet should be well balanced and adequate in all respects.

As a further extension of this test, a diet containing the test material could be fed with and without the supplement of an antibiotic such as penicillin or tetracycline (Goatcher and McGinnis, 1972). This would give an indication of the presence of growth-depressing factors that are counteracted by antibiotic supplements. Rats and chicks are suitable animals for this test.

b) *Heat-labile factors.* A biological test to ascertain the presence of heat-labile, growth-depressing factors can be conducted with rats, mice, or chicks. In effect, it is the same as that for heat-stable factors (see above). Forty to 50 per cent of the test diet should be autoclaved and replace that amount of sugar or starch. Use the same proportions with raw test material. Influences on growth as a result of the cooked and the raw material can then be measured.

4.6 *Relevant areas of nutritional and biochemical research*

a) *Methodology*

(i) *Protein.* The new infrared methods should be tested critically for their applicability to grain legumes of all types.

(ii) *Sulfur-amino acids and total S.* Intensive study is needed to find more sensitive procedures that can provide meaningful results with large numbers of samples.

(iii) *Rapid biological tests.* There is a need for reliable procedures that can use species that require less food than rats.

(iv) *In vitro digestibility tests.* In vitro tests of limited value are available (Sheffner, 1967). There is a need for studies on the correlations of results from these tests with those from in vivo tests or content of available essential amino acids.

(v) *In vitro assay for flatulence factors.* This would be of value, considering the desirability of increasing the consumption of legumes by younger age groups.

b) *Other research*

(i) *Identification of flatulence factors and growth-depressing factors for chicks.* Efforts should be expanded to identify the components in food

legumes that cause flatulence in man and cause growth depression and diarrhea in young chicks.

(ii) *Nutrient composition of legumes.* All opportunities should be used to increase our information on detailed nutrient content (amino acids, carbohydrates, fats, vitamins, minerals, energy) of legumes. Research should be encouraged to provide for this type of work.

(iii) *Relation of protease inhibitors and hemagglutinins to resistance of legume seeds to insect damage.* Reports have indicated that the presence in seeds of such proteins as the protease inhibitors reduces their susceptibility to attack by some insects (Birk, et al., 1963; Lipke, et al., 1954; Guen and Ryan, 1972). This should be investigated further to establish the validity of these reports and to determine whether insect- resistant properties might be utilized to enhance storage properties.

(iv) *Further studies of the causative factors for lathyrism and favism in man.* Even though progress has been made, the compounds so far identified may not be the only ones responsible.

5. Storage, Processing and Consumer Acceptance Properties of Food Legumes

Improving the home preparation and commercial processing characteristics and the consumer acceptance of legumes poses a series of problems for the plant breeder. These relate primarily to local food patterns and eating habits, which may vary not only from country to country but also within one country or within regions of a country. Conversely, they might be common for entire large ecological zones. This obviously implies that before any apparently desirable change can be introduced, it will be necessary to know in some depth the local food habits and preparation methods of food legumes. The contribution and cooperation of local food scientists, nutritionists, home economists and perhaps of social scientists are essential. The main considerations to which the plant breeder can make a contribution are: uniformity in crop maturation; cooking quality and acceptability; milling or processing characteristics; susceptibility to insect infestation; and storage stability.

5.1 *Cooking*

This appears to be the universal and preferred way of preparing legumes for consumption. Most food legumes require 3 to 4 hours of ordinary boiling to cook properly; others are adequately prepared in half that time. The long cooking required for most legumes necessitates the expenditure of fuel, which is frequently scarce, and probably causes significant destruction or modification of nutrients. Therefore, when breeding programs are evolving improved strains, they should include better cooking qualities and acceptability. The newly developed strains also should meet the organoleptic (taste and flavor) requirements of the population. Parameters to be considered in acceptance tests carried out with local consumers are: texture, taste, flavor, and color. In such efforts, plant breeders should work in close association with food technologists.

5.2 *Milling quality*

The milling quality of food legumes varies widely, due to varietal and agro-climatic characteristics. The presence of gums between the husk and cotyledons determines the extent to which the husk adheres to the endosperm. Large-seeded varieties of legumes can be milled more easily than small- and medium-seeded types and require less drastic premilling treatments. Milling characteristics and yields also appear to be better with loose-husked varieties. There seems to be a need, in areas where the legumes are consumed in the form of flour or split grains, to develop strains with better milling characteristics, specifically large, uniform seeds.

5.3 *Insect infestation*

Insect infestation and mold growth during storage of legumes are often major problems in those developing countries with warm and humid climates, and result in significant losses, both quantitative and qualitative. Insect infestation decreases the nutritive value of legume proteins and also reduces milling yields. Thus, breeding for resistance to insect and mold attack should be considered in cooperative efforts between entomologists and plant pathologists. Control of mycotoxin development, such as aflatoxin, may be possible by such genetic means.

5.4 *Changes on storage*

Undesirable changes in some legumes result from prolonged storage. Among these are (i) significant alteration in color, (ii) reduced digestibility and acceptability, and (iii) hardening of the seed, which requires increased cooking time. Attempts should be made to breed varieties that show minimal storage changes of this kind.

5.5 *Uniformity of maturation* Uniformity in time of maturation is important for the commercial producer, and availability of strains with this desirable property might reduce considerably the processing costs, increase processing yield, and provide products more acceptable to the consumer. On the other hand, food legumes produced and consumed by the subsistence farmer and his family may require varieties which yield edible products during an extended period.

6. Production Problems and Opportunities

Approaches recommended for overcoming various production problems are classified and discussed under the headings of breeding objectives, study of growth processes, production and management practices, and production economics and utilization.

6.1 *Breeding objectives*

The low productivity of most food legumes is generally recognized as a major deterrent to increasing their production and making them competitive with

other crops. It is therefore proposed that breeding efforts be concentrated in the following areas:

6.1.1 *Improving productivity.*

a) *Higher yield.* The first priority in all food-legume improvement is to increase yielding capacity per se. This may best be accomplished through changing the plant structure genetically and thereby increasing the harvest yield index (grain yield). Population improvement and recurrent selection should be used as tools in attaining such objectives.

b) *Stability of yield.* It will be essential to build into new, improved stocks the widest possible adaptation to prevalent soils and climates and broad-based resistance to pests and diseases to achieve a consistently high or dependable level of performance.

6.1.2 *Improving product quality.* Breeding objectives relating to acceptability, palatability, elimination of toxic principles, and increased nutritional value have been discussed previously, as have chemical and biological testing methods. In this context, it cannot be overemphasized that more rapid and efficient screening methods are needed urgently. Automation and increased capacity of present methods, and development of nondestructive single-seed analysis to facilitate early generation screening also are vital needs. It should also be noted that the most efficient screening methods for biochemically related factors would include those in which readily observable morphological characteristics of seed or green plants are closely linked to or pleiotropically controlled by common gene(s) that also affect the biochemical constituent. Examples of such characters are the opaque and floury endosperms, which are characteristic of high-lysine maize; large embryos and lowered specific gravities of high-oil seeds; and the dark-colored seed coats associated with tannins and other phenolic compounds in both cereals and legumes. Plant scientists should search for and apply such useful associated morphological characters.

A better understanding of the basic processes of the biosynthesis and translocation of plant and seed storage proteins could lead to more efficient screening techniques. Research in this area should be encouraged.

6.1.3 *New breeding methodology.* Breeders and their colleagues in related disciplines should keep abreast of the most advanced developments in breeding technology and improved screening methods, including:

a) *Better methods of population improvement* for self-pollinating species, with emphasis on genic male sterility or the use of chemosterilants to induce maximum recombination of characters. Entomologists should participate in the search for and utilization of more efficient pollen vectors.

b) *Broadening the genetic base.* Several methods are proposed and would include:

(i) As deemed appropriate, wide crossing at the intraspecific level (diverse and weedy types), and with wild relatives and other species and genera in special circumstances.

(ii) Induction of mutations by radiation and chemical mutagens, especially to improve specific simple characters in adapted, elite materials.

(iii) Chromosomal engineering, involving whole chromosome or segment transfer, ploidization, and other techniques.

c) *Advanced genetic manipulative techniques.* These should include induction of haploidy, redifferentiation processes, tissue culture, protoplast fusion, and other methods.

6.1.4 *Assembling, maintaining, and evaluating genetic resources.* Assembly, collection, maintenance, and evaluation of a complete range of the genetic diversity in the major food-legume species is of paramount importance. Such a project is essential to prevent loss of valuable traditional genetic materials through replacement with improved strains and varieties and the destruction of wild habitats through population pressure and other factors The collection of genetic material in previously neglected areas, such as tropical Africa, should be accorded the highest priority.

Legume improvement programs that are assigned the responsibility for particular ecological zones should collect, assemble, and maintain all available, genetically-diverse food legumes grown in that zone. However, they would not necessarily evaluate and develop the collections of minor or secondary species, which should be maintained in holding nurseries or placed in appropriate storage against possible future need. In "major" species, the following programs should be supported:

a) Assemble available genetic stocks from ongoing breeding programs.

b) Undertake the systematic collection of those species in important and growing regions and centers of genetic diversity.

c) Eliminate duplicates and evaluate for important agronomic, botanical, and biochemical characteristics over a broad range of environments.

d) Develop genetic pools and populations for specific objectives and uses, including yield, adaptation to specific soils and environments, increased protein content, improved nutritional properties, resistance to insects and diseases, etc.

e) Develop a uniform system for recording and retrieving information on genetic resources.

6.2 *Study of growth processes*

Supportive investigations on growth and development processes in food legumes are recommended as an essential adjunct to the overall process of improvement which plant physiologists, agronomists, biochemists, and breeders share in the search for more basic understanding of growth processes in relation to environmental influence. An important aspect of these investigations would be the systematic search for more efficient genotypes in terms of plant architecture, response to stress, and inherent photosynthetic and respirational efficiencies. Among the many kinds of investigation needed are those on a) carbo-

hydrate accumulation; b) nonphotorespiration; c) response to daylength, insolation and temperatures; d) monitoring respirational pathways; e) intermediate pathways in protein synthesis and translocation; f) moisture and plant development relationships; g) hormonal factors influencing vegetative/reproductive cycles and fruit abortion; h) rhyzobial nitrogen-fixing process; and other factors.

6.3 *Production and management practices*

Improvements in cultural practices and management are essential to sustain progress in legume improvement. Specifically, investigations in this field should include:

6.3.1 *Cultural practices.* These embrace studies on tillage practices, planting methods, population dynamics, intercultivation, cropping systems, and other factors under different environments and for different genotypes.

6.3.2 *Fertility aspects.* Realizing that the Green Revolution in cereals is related primarily to the responsiveness of high-yielding varieties to improved soil fertility, and especially high levels of nitrogen fertility, it should be stressed that legumes derive their nitrogen largely from symbiotic bacterial processes. Special attention must therefore be given to responses to bacterial inoculums; different major and minor plant nutrients; different soils and seasons; management factors; methods of placement; and genotypes.

6.3.3 *Control of pests and diseases.* Insects, diseases, and other pests are frequently major constraints to productivity. Effective and economical controls utilizing cultural practices, biological agents, and chemicals should be developed for different environments, seasons, genotypes, and other conditions.

6.3.4 *Harvesting, processing, and storage.* Efficient methods of harvesting and processing are required for developing regions. Inexpensive drying and storage will be essential in maintaining seed viability and food-use quality, and stabilizing the availability of food supplies throughout the year.

6.4 *Production economics and utilization*

Successful expansion of food-legume production will depend ultimately on incentives for producers, marketing economics, and product demand and utilization factors. These may be components of specific national policies relating to food production and nutrition objectives. Indirect benefits may include effects on soil fertility and tilth, crop rotation advantages, control of weeds, and availability and use of byproducts for animal food production. Economic studies should be carried out on cultural practices, including utilization of fertilizers, pesticides, and other direct inputs, relative to returns obtained from different legume genotypes grown in different environments and locales or countries. Reassessments will need to be made for new varieties as these are developed.

The introduction of new crops (e.g., soybeans in tropical Africa) and new types of familiar species will succeed only if these fit into local consumption patterns and food preferences. The exception would be those grown specifically for industrial purposes or export. Thus, it is considered most important, if not essential, to determine local consumer acceptability for these new food crops

and varieties. In analyzing these findings, a basic understanding of sociological factors could be highly important.

7. Possible Means to Achieve a Breakthrough in Food-Legume Improvement

The PAG recognizes the extraordinary success of the Green Revolution in cereal improvement and recommends the emulation of those features appropriate to improvement of the food legumes. It is sugested that the most efficient vehicle for achieving similar results would be a series of international technical networks (national research programs cooperating with international regional institutions and other international and national agencies) designed to collaborate with, strengthen, and serve all concerned national programs. One or more major centers for different ecological zones should be designated. There knowledge and resources could be pooled to catalyze research and training activities.

Organizing trials and experiments, distributing genetic stocks,disseminating information, holding regular meetings and workshops, training young scientists, and consulting on problems and future strategy as well as on nutrition are essential features of food-legume improvement programs in developing regions. Workshops (e.g., on regional crops) should be organized on a yearly basis; larger conferences and symposia may not be necessary more frequently than once every three to five years. The training of young scientists and production specialists is fundamental to the multiplication of efforts and the wider dissemination and adoption of specific research advances.

8. Recommendations on International Cooperation and Support

The PAG emphasizes the importance of a continuing and reinforced program of food-legume research and training and calls for international action involving cooperation among interested international and national agencies and programs, institutions in developed countries, and international and national programs in developing countries. It is further recommended that such programs be accorded high priority for support from the Consultative Group on International Agricultural Research and other interested research-fund donor agencies.

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TABLE I
Regional and World Production of Food Legumes*
(Figures = 1000 metric tons)

		Dry beans *(Phaseolus vulgaris)*	Dry broad beans *(Vicia faba)*	Dry peas *(Pisum sativum)*	Chick peas *(Cicer arietinum)*	Pigeon peas *(Cajanus cajan)*	Cow peas *(Vigna* sp.*)*	Soybeans *(Glycine max)*	Groundnuts in shell *(Arachis hypogaea)*
West Europe	1970	647	890	272	168	–	6	–	19
	1971	603	826	263	140	–	6	–	21
	1972	609	809	262	130	–	6	–	21
East Europe and USSR	1970	283	23	5319	1	–	–	707	3
	1971	320	23	5069	2	–	–	721	3
	1972	321	25	5405	2	–	–	810	3
North America	1970	836	–	223	–	–	18	30957	1351
	1971	814	–	277	–	–	20	32285	1363
	1972	896	–	154	–	–	20	35054	1492
Latin America	1970	3830	164	107	180	33	–	1966	1382
	1971	3725	168	106	183	33	–	2468	1541
	1972	3851	167	107	186	34	–	4038	1451
Near East	1970	182	360	7	187	–	13	19	461
	1971	192	343	9	217	–	10	19	519
	1972	186	347	10	235	–	10	20	508
Far East (incl. People's Rep. of China)	1970	4435	3311	4091	6173	1871	27	12804	11414
	1971	3958	3411	4180	5787	1871	27	12895	10021
	1972	3908	3510	4331	6530	1548	27	12740	8495

(continued)

Africa	1970	1320	373	381	358	64	1096	18	3950
	1971	1235	372	386	311	71	1078	14	4965
	1972	1237	428	392	332	66	1081	25	4534
Oceania	1970	1	–	73	–	–	1	3	43
	1971	4	–	70	–	–	2	9	33
	1972	2	–	70	–	–	2	25	28
World	1970	11534	5121	10473	7067	1968	1161	46474	17623
	1971	10851	5143	10360	6740	1975	1143	48410	18466
	1972	11010	5286	10731	7415	1648	1146	52712	16532

*Provided by courtesy of Food Policy and Nutrition Division, FAO

TABLE II

Availability of Selected Food Legumes*

(Grams per caput per day)

Country	Chick peas	Cow peas	Dry beans	Broad beans	Dry peas	Pigeon peas	Soy-beans	Ground-nuts	Un-specified	Total
Africa										
Algeria	2.6		0.7	3.6	0.7				3.3	10.9
Angola			20.0					3.9		23.9
Burundi			105.8		27.6			3.1		136.5
Cameroon			10.0					29.3	10.9	50.2
Central Africa Rep.								27.0	7.0	34.0
Chad								26.8	53.3	80.1
Congo (Braz.)								17.8	3.0	20.8
Dahomey			16.6					15.4	6.2	38.2
Egypt		0.6	0.8	8.6	0.1	–		3.5	8.2	22.6
Ethiopia	16.7		5.4	8.7	11.1	–		0.7	11.5	54.3
Gabon	–	–				–			0.8	0.8
Gambia	–			–	–	–	–	13.9	7.8	21.7
Ghana	–				–	–		6.4	2.7	9.1
Guinea	–			–	–	–		10.1	17.2	27.3
Ivory Coast	–	–	2.6	–	–	–		15.7	8.0	26.3
Kenya	–	–		–	–	–		0.8	64.9	65.7
Liberia	–	–		–	–	–		5.1		5.1
Libya	2.9	–		1.7	1.0	–		8.8	1.8	16.2
Madagascar	–	8.1	7.7	1.8	–			4.5	4.1	26.2
Malawi	–	4.2	4.2	–	–	3.5		12.5	0.7	25.1
Mali	–	5.5	–	–	–	–		15.3	6.7	27.5
Mauritania	23.7	–	–	–	–	–	–	0.9		24.6
Mauritius	–	–	0.7	–	–	–	–	2.8	4.5	28.0
Morocco	3.3	–	1.9	5.1	2.6	–	–	0.2	1.3	14.4
Mozambique	–	–	–	–	–	–	–		19.7	19.7
Niger	–	–	–	–	–	–	–	10.9	39.0	49.9
Nigeria	–	–	–	–	–	–	0.2	6.5	28.4	35.1

(continued)

Country	Chick peas	Cow peas	Dry beans	Broad beans	Dry peas	Pigeon peas	Soy-beans	Ground-nuts	Un-specified	Total
Rhodesia (S.)	-	-	7.1					20.0	4.1	31.2
Rwanda			101.3		33.8	-		7.0		142.1
Senegal		11.3	-	-	-			21.7	0.8	33.8
Sierra Leone		-	-	23.9	1.2			16.2		41.3
Somalia	-	-		-				2.2	12.1	14.3
Southern Africa	-	-	-	-				2.3	11.5	13.8
Sudan		-	2.0	1.8	-		-	1.2	11.3	16.0
Tanzania	-	-	-	-	-	-	0.5	1.1	32.6	34.2
Togo	20.1	-	-	-	-	-	-	11.7	14.5	46.3
Tunisia	4.7	-	0.6	6.6	-	-	-	-	0.8	12.7
Uganda	0.7	7.0	33.7	-	1.8	5.0	0.2	25.8		74.2
Upper Volta	-	-		-	-	-		19.8	55.3	75.1
Zaire	-		13.1		-	-	0.4	16.9	1.1	31.5
Zambia	-		-			-		20.7	45.5	66.2
AVERAGE**	1.8	0.6	5.2	2.2	1.6	0.2	0.1	7.0	15.5	34.3
Asia and Near East										
Afghanistan	-		-	-	-		-	-	8.0	8.0
Burma	2.9	0.4	6.1	-	0.7	1.6	-	5.0	-	16.7
Cambodia			7.6	-		-	0.6	3.9		12.1
Ceylon	2.9	0.5	-	-	5.9		-	1.0	11.1	21.4
China (Taiwan)	-	-	7.6	-	-	-	58.5	8.3	-	74.4
India	20.4		8.1	-	3.8	8.2	-	1.6	9.3	50.4
Indonesia		-	-	-	-	-	7.6	10.6	11.6	29.8
Iran	-	-	-	-	-	-			10.0	10.0
Iraq	-	-	3.0	4.7	7.7	-	-	-		15.4
Israel	-	-	-	-	-	-	-	6.3	9.6	15.9
Japan	-	-	-	-	-	-	76.5	-	14.3	90.8
Jordan	6.1	-	5.7	-	-	-	-		11.6	23.4
Korea (S.)	-	-	1.5	-	0.1	-	13.7	0.4	0.4	16.1
Korea (N.)	-	-	-	-	-	-		-	39.2	39.2
Laos	-	-	-	-	-	-	-	0.7	10.8	11.5
Lebanon	-	-	-	-	-	-	-	2.6	20.7	23.3
Malaysia	-	-	-	-	-	-	5.5	1.0	8.9	15.4
Nepal	-	-	-	-	-	-	-	-	11.6	11.6

(continued)

Philippines	–	–	0.6	–	–	–	0.5	1.1	2.6	4.8
Saudi Arabia	1.2	–	1.2	3.0	–	–	–	0.6	1.2	7.2
Syria	–	–	–	5.2	–	–	–	1.6	12.1	18.9
Thailand	–	–	–	0.9	–	–	1.5	3.9	6.6	12.9
Turkey	5.5	–	10.7	2.6	0.4	–	–	1.6	7.2	28.0
Vietnam (N.)	–	–	2.1	–	–	–	1.2	1.4	4.2	8.9
Vietnam (S.)	–	–	2.4	–	–	–	0.8	1.5	4.2	8.9
Yemen Arab Rep.	–	–	–	–	–	–	–	–	20.4	20.4
Yemen People's Rep.	–	–	–	–	–	–	–	0.3	10.5	10.8
AVERAGE	10.0	–	4.7	0.1	2.0	4.0	9.5	2.5	9.3	42.0
Europe										
Albania	–	–	–	–	–	–	–	–	25.0	25.0
Austria	–	–	–	–	–	–	0.4	–	2.4	2.8
Belgium-Luxembourg	–	–	–	–	–	–	–	–	7.2	7.2
Bulgaria	–	–	–	–	–	–	–	–	17.0	17.0
Cyprus	–	–	–	–	–	–	–	–	30.9	30.9
Czechoslovakia	–	–	–	–	–	–	–	–	3.9	3.9
Denmark	–	–	–	–	–	–	–	–	1.1	1.1
Finland	–	–	–	–	–	–	–	–	3.1	3.1
France	–	–	–	–	–	–	–	–	8.6	8.6
Germany (W.)	–	–	–	–	–	–	–	–	3.6	3.6
Germany (E.)	–	–	–	–	–	–	–	–	3.9	3.9
Greece	–	–	–	–	–	–	–	–	30.1	30.1
Hungary	–	–	–	–	–	–	–	–	23.8	23.8
Iceland	–	–	–	–	–	–	–	–	4.3	4.3
Ireland	–	–	–	–	–	–	–	–	7.0	7.0
Italy	–	–	–	–	–	–	–	–	16.2	16.2
Malta	–	–	–	–	–	–	–	–	87.7	87.7
Netherlands	–	–	–	–	–	–	–	–	5.7	5.7
Norway	–	–	–	–	–	–	–	–	5.1	5.1
Poland	–	–	–	–	–	–	–	–	9.0	9.0

(continued)

Country	Chick peas	Cow peas	Dry beans	Broad beans	Dry peas	Pigeon peas	Soy-beans	Ground-nuts	Un-specified	Total
Portugal	–	–	–	–	–	–	–	–	19.4	19.4
Romania	–	–	–	–	–	–	–	–	23.2	23.2
Spain	–	–	–	–	–	–	–	–	20.2	20.2
Sweden	–	–	–	–	–	–	–	–	3.9	3.9
Switzerland	–	–	–	–	–	–	–	1.8	2.7	4.5
United Kingdom	–	–	–	–	–	–	–	1.2	8.8	10.0
Yugoslavia	–	–	–	–	–	–	–	–	24.6	24.6
AVERAGE	–	–	–	–	–	–	–	–	11.3	11.3
Latin America										
Argentina	0.6	–	2.0	–	2.4	–	–	–	0.8	5.8
Bolivia	–	–	0.6	4.4	0.6	–	–	5.7	–	11.3
Brazil	–	–	1.7	–	0.2	–	3.2	12.8	0.1	18.0
Chile	0.9	–	13.2	–	1.9	–	–	–	0.3	16.3
Colombia	–	–	5.3	–	3.3	–	–	0.2	3.3	12.1
Costa Rica	–	–	29.6	–	–	–	–	–	–	29.6
Cuba	–	–	7.9	–	–	–	–	5.7	22.2	35.8
Dominican Rep.	–	–	26.5	–	–	14.9	–	–	–	41.4
Ecuador	–	–	13.1	9.9	8.5	–	–	1.6	3.1	36.2
El Salvador	–	–	28.1	–	–	–	–	0.3	–	28.4
Guatemala	–	–	28.6	–	–	–	–	0.3	–	28.9
Guyana	–	–		–	–	–	–	–	14.9	14.9
Haiti	–	–	24.3	–	–	1.9	–	1.7	21.2	49.1
Honduras	–	–	35.4	–	–	–	–	–	–	35.4
Jamaica	–	–		–	–	–	–	–	7.6	7.6
Mexico	6.8	–	48.9	2.2	0.3	–	0.8	2.8	0.3	64.1
Nicaragua	–	–	59.3	–		–	–	–	–	59.3
Panama	–	–	16.5	–	2.6	–	–	0.2	3.7	23.0
Paraguay	–	–	25.6	4.0	2.7	–	–	8.1	–	40.4
Peru	0.6	–	10.8	6.1	6.3	–	–	0.8	2.9	27.4
Puerto Rico	–	–	–	–	–	–	–	–	35.4	35.4
Trinidad and Tobago	–	–	–	–	–	–	–	3.2	28.1	31.3
Uruguay	–	–	3.0	1.0	3.0	–	–	–	–	7.0
Venezuela	0.6	–	17.3	–	4.3	1.6	–	0.7	0.5	25.0
AVERAGE	1.3	–	15.0	1.0	1.4	0.3	1.2	5.4	2.3	27.9

(continued)

North America										
Canada	–	–	4.2	–	1.8	–	–	4.2	–	10.2
United States	–	–	8.5	–	0.6	–	–	6.8	0.2	16.1
AVERAGE	–	–	8.1	–	0.7	–	–	6.5	0.2	15.5
Oceania										
Australia	–	–	–	–	–	–	–	2.9	4.8	7.7
New Zealand	–	–	–	–	–	–	–	–	3.9	3.9
AVERAGE**	–	–	–	–	–	–	–	2.3	4.6	6.9
China (Mainland)	–	–	3.9	8.4	6.7	–	18.4	1.5	–	38.9
USSR	–	–	–	–	–	–	–	–	12.3	12.3

*Provided courtesy of the Food Policy and Nutrition Division, FAO.
**Weighted regional averages.

Source: Food Balance Sheets 1964-1966, FAO, Rome, 1971.

TABLE III
Demand Projections for Beans, Peas, Lentils, Chick Peas and Similar Dry Leguminous Crops*

(Thousands of tons)

Country	1965	1970	1975T	1980T	1975H	1980H
Africa						
Algeria	47	57	68	82	70	86
Angola	38	42	46	52	47	53
Burundi	153	171	191	214	192	218
Cameroon	40	46	53	63	54	64
Central African Republic	3	4	4	5	4	5
Chad	64	68	74	82	76	85
Congo (Brazzaville)	1	1	1	1	1	1
Dahomey	16	18	21	25	21	25
Egypt, Arab Republic of	205	225	269	317	270	325
Ethiopia	444	497	572	660	597	711
Gabon	0	0	0	0	0	0
Gambia	1	1	1	1	1	1
Ghana	42	47	55	65	55	65
Guinea	22	23	26	30	27	33
Ivory Coast	16	20	24	30	25	32
Kenya	222	270	329	403	336	420
Libya	4	7	10	12	11	14
Madagascar	48	55	62	71	62	72
Malawi	18	22	26	31	26	32
Mali	20	22	25	28	25	29
Mauritania	9	11	12	14	12	15
Mauritius	7	8	9	10	9	10
Morocco	70	83	97	114	97	116
Mozambique	50	58	68	79	69	81
Niger	50	58	67	79	69	81
Nigeria	606	660	806	973	808	1016
Rhodesia (S.)	19	23	27	32	27	33

*Provided courtesy of the Food Policy and Nutrition Division, FAO.

Note: For high-income countries, *one growth rate* in Gross Domestic Product (GDP) and the associated Private Consumption Expenditure (PCE) was used to make demand projections at constant prices. For developing countries, *two alternative growth rates* were applied to demand. The first, the "Trend" (T) alternative, represents the FAO Secretariat interpretation of representative past trends. The other is a "High" (H) alternative based on targets established by the UN and its Regional Commissions for the Second UN Development Decade.

(continued)

Country	1965	1970	1975T	1980T	1975H	1980H
Rwanda	153	183	214	252	215	258
Senegal	15	16	18	20	18	20
Sierra Leone	22	24	27	30	27	30
Somalia	11	13	15	17	15	19
Southern Africa	85	99	115	134	115	134
Sudan	74	86	102	124	102	128
Tanzania	139	169	200	237	203	251
Togo	21	24	28	33	29	34
Tunisia	21	23	27	31	28	34
Uganda	133	154	182	215	183	227
Upper Volta	95	104	118	134	118	136
Zaire	81	92	108	128	112	134
Zambia	62	78	95	116	100	128
TOTAL	3130	3462	4192	4944	4235	5155
Asia & Near East						
Afghanistan	46	53	61	71	63	77
Burma	105	118	135	154	136	160
Ceylon	83	102	120	143	121	146
China (Taiwan)	36	44	55	67	55	69
India	8492	9957	11638	13705	11926	14384
Indonesia	446	512	587	681	594	698
Iran	91	117	149	189	149	189
Iraq	46	57	70	87	70	91
Israel	9	10	11	13	11	13
Japan	449	475	505	533	505	533
Jordan	16	20	24	28	24	30
Korea (S.)	20	26	33	40	33	40
Korea (N.)	173	203	237	276	240	284
Laos	10	12	14	16	14	16
Lebanon	17	21	25	29	25	31
Malaysia	28	33	38	45	39	46
Nepal	43	47	52	58	52	60
Philippines	36	43	52	63	53	65
Saudi Arabia	11	15	18	22	20	25
Syria	33	40	48	58	48	58
Thailand	84	102	124	149	127	156
Turkey	297	368	445	535	450	545
Vietnam (N.)	42	48	57	67	60	74
Vietnam (S.)	39	44	51	59	51	60
Yemen Arab Republic	37	42	47	54	49	58
Yemen People's Republic	4	4	5	5	5	6
TOTAL	10693	12113	14601	17147	14920	17914

(continued)

Country	1965	1970	1975T	1980T	1975H	1980H
Europe						
Albania	17	21	25	29	25	29
Austria	6	6	7	7	7	7
Belgium-Luxembourg	26	27	27	28	27	28
Bulgaria	51	53	55	57	55	57
Cyprus	7	7	8	8	8	8
Czechoslovakia	20	22	23	24	23	24
Denmark	2	2	2	2	2	2
Finland	5	5	5	5	5	5
France	154	156	159	162	159	162
Germany (E.)	24	26	28	30	28	30
Germany, Federal Rep.	77	77	76	75	76	75
Greece	93	98	101	104	101	104
Hungary	88	101	113	125	113	125
Iceland	0	0	0	0	0	0
Ireland	7	11	12	13	12	13
Italy	309	313	318	323	318	323
Malta	10	10	9	9	9	9
Netherlands	26	28	29	31	29	31
Norway	7	7	8	8	8	8
Poland	104	117	130	146	130	146
Portugal	54	59	64	70	64	70
Romania	161	173	187	201	187	201
Spain	235	260	284	311	284	311
Sweden	11	8	8	9	8	9
Switzerland	6	6	7	7	7	7
United Kingdom	178	186	196	208	196	208
Yugoslavia	175	185	195	205	195	205
TOTAL	1853	2964	2076	2197	2076	2197
Latin America						
Argentina	49	54	58	63	58	63
Bolivia	9	11	13	16	13	16
Brazil	1993	2309	2664	3074	2664	3074
Chile	52	61	70	80	70	82
Colombia	79	97	118	143	119	147
Costa Rica	16	24	30	37	30	38
Cuba	84	92	102	113	104	118
Dominican Republic	55	68	83	100	83	102
Ecuador	66	81	99	120	99	124
El Salvador	30	27	27	49	37	51
Guatemala	48	54	71	92	72	96
Guyana	4	4	5	6	5	6
Haiti	76	84	95	108	96	114
Honduras	28	38	48	59	48	61

(continued)

Country	1965	1970	1975T	1980T	1975H	1980H
Jamaica	5	6	6	7	6	7
Mexico	913	1049	1209	1393	1203	1378
Nicaragua	38	49	58	70	59	71
Panama	10	13	16	20	16	20
Paraguay	24	29	34	41	34	41
Peru	114	130	161	200	162	203
Puerto Rico	34	38	44	49	44	50
Trinidad and Tobago	10	11	12	14	12	14
Uruguay	7	7	8	9	8	9
Venezuela	85	103	125	151	126	156
TOTAL	3729	4439	5156	6014	5172	6041
North America						
Canada	41	43	44	45	44	45
United States	664	615	640	665	640	665
TOTAL	705	658	684	710	684	710
Oceania						
Australia	20	23	26	30	26	30
New Zealand	4	4	5	5	5	5
TOTAL	24	27	31	35	31	35
China (Mainland)	5307	5965	6729	7586	6822	7793
USSR	1031	1116	1200	1295	1200	1295
WORLD TOTAL	26472	29739	34669	39928	35140	41140

Source. FAO Agricultural Commodities Projections, FAO, Rome, 1971.

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