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FINAL REPORT

on Inheritance and Improvement of Protein Quality and Content in Maize

April 1, 1977 to March 31, 1978
Contract AID/ta-C-1211
Agency for International Development
Department of State
Washington, D. C.

F I N A L R E P O R T

on

THE INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN MAIZE

Contract AID/ta-C-1211

Prepared by

Department of Agronomy
Agricultural Experiment Station
International Programs in Agriculture

Purdue University
West Lafayette, Indiana

Submitted to

United States Agency for International Development
Department of State
Washington, D. C.

Report Summary

A. 1. Project title and contract number: Inheritance and Improvement of Protein Quality and Content in Maize; Contract number AID/ta-C-1211.

2. Principal investigator, contractor and mailing address:

Dr. D. V. Glover
Department of Agronomy
Purdue University
West Lafayette, IN 47907

3. Contract period (as amended): from April 1, 1975 to March 31, 1978.

4. Period covered by report: from April 1, 1977 to March 31, 1978.

5. Total A.I.D. funding of contract to date: $760,029.69 (to March 31, 1978).

6. Total expenditures and obligations through previous contract year: $494,494.31 (through March 31, 1977).

7. Total expenditures and obligations for current year: $265,535.38.

8. Estimated expenditures for next contract year: Project terminated.

B. Narrative Summary of Accomplishments and Utilization

Maize is a major source of calories and protein for many millions of people in several areas of the developing world. Though maize is considered an excellent source of carbohydrates and a particularly efficient cereal species, the protein quality is relatively low and thus a constraint on improvement of human and animal nutrition where maize is used as a staple in the diet.

Research over the eight year period of this project has led to expanding efforts in search for and evaluation of new mutants and germplasm sources that improve protein quality. The task of obtaining opaque-2 or other genes with similar effects on quality of protein with yields equal to "normal" has proven more difficult than originally envisioned. The opaque-2 genotype still has the serious drawback of lower agronomic yield.

Searches from Colombian and other maize germplasm, screening of over 55 collections of maize from the CIMMYT world germplasm bank with the ninhydrin test, and screening of germplasm following seed and pollen treatment with chemical mutagens have resulted in the isolation of several

endosperm mutants with opaque phenotype and/or alteration in protein quality. None of the mutants isolated thus far have proved to be a viable alternative to opaque-2.

Selection of opaque-2 in various diverse genetic backgrounds reveal a relatively frequent occurrence of vitreous (modified) opaque-2 phenotype. A vitreous opaque-2 synthetic HM o_2 o_2 was developed and released. It can serve as source germplasm for vitreous kernel type. Several simply inherited genes that modify the soft endosperm of opaque-2 giving it a more vitreous (normal) appearance have been identified. The inheritance of a single gene modifier for vitreous opaque-2 was studied and was found to be dominant and simply inherited. The modifier system significantly decreased kernel volume, increased kernel density, decreased lysine content as percent of protein, and decreased lysine content as percent of sample. The kernel weight and protein content were not significantly affected. The modifier decreased the lysine content but not to the level of normal maize. It should be easier to select for higher lysine content in vitreous varieties for LDC's due to the simple inheritance of the modifier system. A dominant gene that completely suppresses the opaque-2 phenotype has been isolated. The proposed phenotype o_2 o_2 Sp Sp is indistinguishable from "normal". The modifier systems that give vitreous opaque-2 phenotypes could lead to opaque-2 varieties that provide a more acceptable product for food use and more resistants to ear rots, grain insects, kernel damage, and storage loss, thought they may have lower levels of lysine, vary some in frequency and degree of vitreousness with environment, and the grain yield may average 10 to 15 below normal maize.

Selection and improvement of two sugary-2 opaque-2 varieties has continued. In addition, 28 elite corn belt inbred line sugary-2 opaque-2 conversions have been developed and available. The sugary-2 opaque-2 double mutant has improved kernel vitreousness and density. The serious shortcoming of sugary-2 opaque-2 is the reduced kernel size and yield characteristics, though there is some background genotype interaction. Sugary-2 opaque-2 has improved protein quality resulting from some increase in lysine, an increased content of tryptophan, a better leucine to isoleucine balance and there is some evidence that sugary-2 opaque-2 has a net shift of increased protein synthesis into the glutelin fraction. Sugary-2 opaque-2 has an increase in oil content. The starch granules of sugary-2 opaque-2 are digested much more rapidly than normal or opaque-2. We have shown that it has good biological feeding value for adult humans and swine. Recent evidence suggests the superior rates of starch digestion and other inherent characteristics of this double combination appear to be significant for improving biological value of corn diets for infants.

In additon to opaque-2, floury-2, and opaque-7 mutants which influence lysine content, the mutantssugary-1, shrunken-1, shrunken-2, shrunken-4, brittle-1, and brittle-2, all of which are starch deficient, also increase lysine content by decreasing zein and increasing other fractions that are higher in lysine content. These genes, the simple modifier genes of opaque-2, and the dominant suppressor gene of opaque-2 are useful in understanding the

phenotypic and biochemical action of starch and protein interactions in maize endosperm development and quality protein improvement.

Germplasm containing opaque-2 in several different backgrounds and a number of other endosperm genes, along with various modifying genes in various backgrounds have been developed by the project and distributed to a large number of LDC's, European countries, and corn breeders in the U.S. and Canada. Selection has been quite effective in improving yield, agronomic characteristics, vitreous kernel type and nutritional properties of two broad based temperate synthetics, Temp HA o_2 o_2 and Temp HB o_2 o_2, and Colus o_2 o_2 variety. These populations could prove quite valuable with further selection and adaptation to specific areas as sources of improved varieties and hybrids.

Although the chief aim of the cooperative undertaking, i.e. increasing the quantity and nutritional value of maize in developing countries, was not attained, the expenditure of A.I.D. funds can nevertheless be considered worthwhile. Much knowledge has been added to the field of the breeding, genetics, and biochemistry of endosperm of maize; quality and quantity of protein: deposition of starch; associated characteristics such as oil content and quality, mineral content, starch composition, and insect and disease resistance; effects of associated genes both singly and in combinations with opaque-2; and the nutritional value of maize with various qualities of protein.

There have been a total of 120 publications and abstracts of research reports, symposia, and workshops by the project staff members during the period the project was supported by A.I.D. (see section G). In addition, there has been an annual distribution of 700 copies of the annual research report to cooperators and interested centers, missions, and institutions representing over 35 countries and many institutions in the U.S. This extensive store of knowledge will be of value not only for the continuing research at Purdue but also at other institutions in the world wide maize network and others doing research on cereal grains.

The Purdue Project staff have worked with maize scientists in over 13 different LDC's as well as with a number in the Eastern European countries. The project personnel have trained 21 graduate students, nine postdoctorals, and 10 trainees representing seven different countries.

Personnel

Department of Agronomy

Dr. L. F. Bauman

Mr. Rodney L. Tietz (Graduate Student)
Mr. Hsun Tu (Graduate Student)
Mr. Terry R. Lemming** (Technician)

Dr. P. L. Crane

Mr. Hugo Zorrilla (Graduate Student)
Mr. Adeleke A. Ojo* (Graduate Student)
Mr. H. Keith Kessler** (Technician)

Dr. D. V. Glover

Dr. Karl Pederson (Postdoctorate)
Mr. Steve G. Ballenger (Graduate Student)
Mr. Arthur H. Long (Graduate Student)
Mr. Mohammed M. Hassen (Technician)
Ms. Charlotte Allen (Technician)
Mr. Wayne J. Whitlow* (Technician)

*Graduate students that are conducting research pertaining to the objectives, but not financially supported by this contract.

** Technicians associated with project but not financially supported by this contract.

***Other professional staff associated with project but not financially supported by this contract:

Dr. E. T. Mertz - Department of Biochemistry (Emeritus)
Dr. A. W. Kirleis - Department of Agronomy
Dr. D. M. Forsyth - Department of Animal Sciences
Dr. C. Y. Tsai - Department of Botany and Plant Pathology
Dr. Helen E. Clark - Department of Foods and Nutrition

A. GENERAL BACKGROUND

There has been a growing awareness of the world food supply situation. There is an urgent need for massive efforts to increase agricultural productivity and improve the nutritional value of plant materials for human and animal food, particularly in developing countries and simultaneously to raise the incomes of hundreds of millions of their farmers and other rural people. Cereals provide a major portion of the calories and proteins in human diets in many developing countries. Maize is the third most important cereal crop grown for human consumption in the world, being surpassed only by rice and wheat in worldwide importance. Maize is a principal source of food for many millions of people particularly in the Latin American area, Africa, and other regions of the world. Though maize is considered an excellent source of carbohydrates, the protein quality is relatively low since it is deficient in the essential amino acids, lysine and tryptophan. This deficiency is a major constraint on improvement of human and animal nutrition where maize is a significant part of the diet.

The principal purpose of this project was to research and develop source germplasm materials for improvement of protein quality and content in maize in order to improve the nutritional quality of maize for use in the LDC's. The project has contributed to the creation of and participated actively in a worldwide system of maize improvement research. This involved researching the most relevant and important fundamental problems in this area, widely disseminating the findings, providing basic undergirding for the international centers and breeding programs in developing countries.

Since the beginning of support of this project by A.I.D. there has been some shift in thinking on the part of plant scientists, nutritionists, and others on the relative importance in the human diet of protein vis-a-vis calories. The emphasis has shifted in part from protein to calories. This general development is evidenced in numerous technical reports, conferences, and other similar sources. It is a consensus of opinion that improvements in the quality or quantity of cereal grains should be directed toward the use by intended populations or specific target groups.

The shortcomings associated with high lysine maize have pointed up the necessity of continued basic and applied research on genetics, breeding, analytical methods, and nutritional characteristics associated with improving quality of protein in maize. Utilization of high lysine maize in developing countries will be realized only through sustained interdisciplinary and cooperative research into these complex problems. Present recommendations for utilization must recognize that there may have to be a trade off of some yield for quality, i.e. high lysine corn with a somewhat lower yield may have to be grown only as a specialty crop and the cost benefits considered in those recommendations. Maize is a particularly efficient crop species and with improved protein quality and agronomic characteristics can play an increasing role in solving the world's human and animal nutrition problems.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

1. In cooperation with other maize breeding programs (particularly that of CIMMYT), expand the search for and evaluation of new mutants and germplasm sources with improved protein quality and quantity, both in existing maize populations and by means of chemical mutagens and

2. To concentrate on the development of opaque-2 and double and multiple combinations of endosperm mutants and determine the effect of associated interactions on nutritional quality, physical properties of the kernel and agronomic characteristics, with a view to the improvement of grain type and yield as well as protein content and quality.
3. To determine the extent of interactions of both genetic backgrounds and environment with individual or combinations of endosperm mutants and how such interactions may influence protein quality and breeding methods.
4. In cooperation with CIMMYT and other maize workers, develop special varieties and source breeding materials with improved nutritional and agronomic characteristics for use in the LDC's. Special emphasis will be given to opaque-2 and sugary-2 opaque-2 materials adapted to more temperate regions.

C. CONTINUED RELEVANCE OF OBJECTIVES

Research over the eight year period of this project has led to expanding efforts in search for and evaluation of new mutants and germplasm sources that improve protein quality. The task of obtaining opaque-2 or other genes with similar effects on quality of protein with yields equal to "normal" has proven more difficult than originally envisioned. The opaque-2 genotype still has the serious drawback of lower agronomic yield. We have discovered modifying genes (or gene systems) such as "modified opaque-2" and sugary-2 opaque-2 which improve the kernel hardness and vitreousness. Protein quality in the "modified opaque-2" can be maintained with selection. In sugary-2 opaque-2 there are improvements in nutritional quality. However, both systems have not resulted in a yield

improvement over opaque-2. Opaque-2 shows the least yield reduction (about 10-12%) of the high lysine genotypes.

We have identified and characterized some simply inherited gene systems that give a modified and vitreous kernel with opaque-2 without a serious reduction in lysine levels. These systems are much more easily handled in the breeding of more acceptable vitreous opaque-2 varieties and should provide modified types with good nutritional quality. To date these systems have not resulted in substantial yield improvement of opaque-2 varieties.

The major challenge has been to obtain and develop high lysine germplasm without the serious yield constraints and with other acceptable agronomic and nutritional qualities for the LDC's and more temperate areas of the world.

At the very onset of our collaboration with CIMMYT the serious need for developing source populations with improved protein quality for the more temperate regions of the world was discussed. We shifted more emphasis to the development of special varieties and source breeding materials for use in the LDC's and more temperate regions. A rather sizeable portion of our total effort was, therefore, expanded in development of varieties, i.e., Temp HA opaque-2, Temp HB opaque-2, an opaque-2 version of Colus (a synthetic developed at Purdue from Colombian and U.S. germplasm), Temp HA sugary-2 opaque-2, and Temp HB sugary-2 opaque-2. Agronomic and nutritional selection is being implemented in these populations. These commitments to germplasm development were long range commitments and have contributed strongly to the relevance of the project.

Although the overall aim of the cooperative undertaking, i.e. increasing the quantity and nutritional value of maize with acceptable

agronomic yield characteristics for utilization in developing countries, was not attained, the expenditure of A.I.D. funds can nevertheless be considered as worthwhile. The purpose of this contract was to advance further the research already underway with the opaque-2 gene and its interaction with modifiers and other endosperm genes, calculated further to improve the nutritional quality as well as the yield, and other agronomic and market characteristics, and to search for other genes which may provide improved protein content and quality without the undesireable soft endosperm character of opaque-2. Much knowledge has been added to the field of the breeding, genetics, and biochemistry of endosperm of maize; quality and quantity of protein; deposition of starch; associated characteristics such as oil content and quality, mineral content, starch composition, and insect and disease resistance; effects of associated genes both singly and in combinations with opaque-2; and the nutritional values of maize with various qualities of protein.

Germplasm containing opaque-2, modified opaque-2, and a number of other endosperm mutants and combinations of mutants, in various genetic backgrounds have been distributed to a large number of LDC's, European countries, and corn breeders in the U.S. and Canada. We have worked with maize scientists in 13 different LDC's as well as with a number in the Eastern European block. We have trained 21 graduate students, nine postdoctorals, and 10 trainees representing seven different countries.

There have been a total of 120 publications and abstracts of research reports by the project staff members during the period the project was supported by A.I.D. This extensive store of knowledge will be of value not only for the continuing research at Purdue but also at other institutions over the world doing research on cereal grains.

D. ACCOMPLISHMENTS TO DATE

Search for New Mutants From Colombian and Other Germplasm.

None of the endosperm mutants isolated during the term of this project has proved to be a viable alternative to opaque-2. Some are opaque without alteration in protein quality, some were new occurrences of opaque-2, and some were non-heritable variations.

Use of the Ninhydrin Test in Screening for New Mutants.

The "ninhydrin reagent solution" which chemically reacts with the free amino acids present in the exposed maize endosperm rendering a blue color has been used to search for new mutants. Testing in normal looking vitreous kernels and screening for the possible occurrence of high lysine mutants has been conducted in over 55 collections of maize from the CIMMYT world germplasm bank. To date none of the "putative" mutants have proved to be of value.

Use of Chemical Mutagens to Produce New Mutants.

Both seed and pollen treatments have been made using the chemical mutagens ethyl methanesulfonate (EMS) and diethylsulfate (DES). Both of these chemicals are alkylating agents.

We screened some 12,000 M_2 ears from normal seed treated with EMS and DES. We observed 56 families which segregated for an endosperm mutant type in addition to numerous seedling and defective endosperm mutants. Twelve of these segregating endosperm mutant families segregated for a "putative" opaque kernel type. Allelism tests confirmed six positive tests with the opaque-2 allele. These also showed similar amino

acid lysine values in comparison to opaque-2. The remaining six "opaque-like" mutants did not show positive allelism tests to opaque-2, floury-2, opaque-1, floury-1, soft starch nor any of the other known endosperm mutant genes. Some of these appeared as though some modification of an "opaque-like" characteristic were present. Some were shrunken or defective and "opaque-like" in appearance. However, amino acid lysine values of these types was not encouraging.

We treated pollen from both normal and opaque-2 plants with EMS and DES (both at concentrations of .011% in light paraffin oil for 50 minutes) and pollinated several hundred ears of each kernel genotype. Kernels from these pollinations were grown out in the genetics nursery, self-pollinated and the M_2 ears were screened for endosperm mutant types. Thus far we have grown out only a portion of the M_1 treated (pollen) materials. From several hundred families of the normal background pollen treated material, 51 families showed segregation for endosperm mutant types, 21 families of which were putative "opaque" phenotypes. None of these proved to be promising alternatives to opaque-2. Many were opaque-2 genotypes as confirmed by allele tests and chemical evaluation. From over 2,000 families of the opaque background pollen treated material, 86 families segregated for endosperm mutant types, 23 of which segregated for a "normal" phenotype in the background of opaque-2. These segregates were chemically evaluated, none had significant increases in lysine levels to be of importance. Five additional families segregated for normal phenotype, but they were suspect from contamination. In both backgrounds treated there were several hundred families which segregated for a seeding mutant character (s). To date it appears that the pollen treatment method is more productive than seed treatment for inducing endosperm and

seeding mutants, but unfortunately none of these mutants isolated thus far has proved to be a viable alternative to our known lysine genes.

Temperate Germplasm.

Development of Temp HA o_2 o_2 and Temp HB o_2 o_2 Varieties. During the early years of this project the urgent need for opaque-2 varieties adapted to temperate areas of the world was recognized. This project developed two broad based synthetics, Temp HA o_2 o_2 and Temp HB o_2 o_2 from diverse germplasm from around the world and the U.S. Cornbelt. Approximately 150 full sib families have been evaluated at four worldwide locations. Agronomic data was obtained from Mexico, Israel and Indiana. Yield data from Pakistan was not obtained and some data from some years was not obtained because of frost, flood and other problems.

Three cycles of selection have been completed. The protein and lysine value of selected families are given in Table 1.

Table 1. Variation in protein quality in whole kernel from selected families (ears) each in Temp HA and Temp HB.

		Temp HA	Temp HB
Percent Protein	Mean	13.4	12.6
	Range	12.5 - 14.2	11.7 - 13.2
Lysine as a Percent of Protein	Mean	3.90	4.20
	Range	3.61 - 4.13	3.94 - 4.38
Lysine as a Percent of Sample	Mean	.52	.53
	Range	.50 - .54	.51 - .56

Selection has been quite effective in improving yield, agronomic characteristics, vitreous kernel type and nutrition properties. These populations could prove quite valuable with further selection and adaptation to specific areas as source of improved varieties and hybrids.

Development and Evaluation of the Colus Variety. The variety Colus was developed at Purdue from Colombian and U.S. germplasm and has been converted to opaque-2 and has undergone selection. The broad based population Colus-opaque was used to test the effectiveness of a scheme of improvement. Selection for yield was among full-sib families and selection for more vitreous kernels which were free from ear and kernel rots and had good lysine levels was primarily within full-sib families. Cycles of selection 1 through 4 were performed in 1974, 1975, 1976 and 1978. Because of a freeze in the winter nursery, the 4th cycle could not be completed in 1977. This was also the reason that only three cycles of improvement could be simultaneously evaluated in 1978 for actual gain achieved.

The cycles *per se* were tested in six replications at each of three locations in 1978. In addition, each cycle was topcrossed to two homozygous opaque inbred tester lines. These topcross hybrids were tested in three replications at each of three locations in 1978. The results are shown in Table 2. For the cycles *per se*, topcrosses to B37-o_2, and topcrosses to Mo17-o_2, the gains were 16.6, 15.9 and 9.7 bushels respectively for the three cycles. This equals 20.9, 15.1 and 9.8 percent or 7, 5, and 3.3 percent per cycle of selection respectively. These gains are comparable to results of similar selection studies in normal corn.

Development and Evaluation of the ETO x CBC and Antigua 2D x (B10 x B14) Varieties. These two populations were evaluated for their potential as opaque varieties (relative to normal counterparts). The two opaque corn populations used for this study were derived from (1) CBC (Corn Belt Composite) x ETO and (2) Antigua 2D (B10 x B14). Full-sib families of the two

Table 2. Evaluation in 1978 of Gain from Three Cycles of Full-sib Family Selection in the Colus-opaque Population.

	Cycle 0	Cycle 1		Cycle 2		Cycle 3		Total
	Yield bu.	Yield bu.	Gain bu.	Yield bu.	Gain bu.	Yield bu.	Gain bu.	Gain bu.
Cycles of Colus-o_2 *per se*	79.3	95.7	16.4	91.5	-4.2	95.9	4.4	16.6
Topcrosses to B37-o_2	104.9	---	2.7*	110.3	2.7	120.8	10.5	15.9
Topcrosses to Mo17-o_2	98.9	100.4	1.5	108.4	8.0	108.6	0.2	9.7

*In the absence of data for this topcross, it is assumed that the gains in cycles 1 and 2 were equal.

populations crossed to two unrelated pollinators heterozygous for the opaque-2 gene were compared for 100 kernel weight in a randomized complete block design using four replications. The weights of the opaque kernels were 5 to 27% less than normal kernels from the same ears.

Analysis of variance of the proportionate kernel weights (opaque/normal = PKW) showed significant family, pollinator and pollinator x family effects. Similar analyses on normal kernel weights and opaque kernel weights separately indicated that whereas pollinator effects were not significant on normal phenotype, they were highly significant on the opaque phenotype. It was, therefore, inferred that the significant pollinator effects on PKW was wholly due to the effects of the pollinators on opaque kernel weight. For pollinator 1, (Oh43-o_2 x W64A-o_2) x (A619 x A632), the PKW's were .82 for both populations. For pollinators 2, (C103-o_2 x B37-o_2) x (N28 x M17), the PKW's were .86 and .87 for populations 1 and 2, respectively.

Although the populations being tested did not differ, this is a dramatic demonstration of the interaction of other genes with opaque-2 resulting in different levels of opaque kernel weight relative to normal kernels. Considering that the pollinator only contributes one of three sets of chromosomes in the endosperm the 5 to 6% kernel weight difference is substantial.

Development of Temp HA su_2 su_2 o_2 o_2 and Temp HB su_2 su_2 o_2 o_2 Varieties. These two varieties have undergone full-sib family selection for yield, kernel vitreousness, and protein quality improvement through two cycles (C_1 and C_2) and C_3 have been made in each variety.

A related variety using half sib family selection on the Temp HB su_2 su_2 o_2 o_2 "highly modified opaque-2" germplasm has been selected through cycle 2 (C_2) and half sibs cycle 3 (C_3) families have been made.

The varieties Temp HA o_2 o_2, Colus o_2 o_2, ETO x CBC o_2 o_2, Antigua 2D x (B10 x B14) o_2 o_2, Temp HA su_2 su_2 o_2 o_2, Temp HB su_2 su_2 o_2 o_2, and HM o_2 o_2 varieties are being selected to further fill the need for varieties adapted to temperature areas.

Development of Specialty Varieties. The waxy opaque-2 (wx wx o_2 o_2) and brittle-2 opaque-2 (bt_2 bt_2 o_2 o_2) populations are being developed in the Temp HA and Temp HB synthetic backgrounds. The wx o_2 populations have been advanced to cycle 4, and the bt_2 o_2 populations have been advanced through cycle 3 through half sib family selection.

Protein Content in the Sugary-2 Opaque-2 Double Mutant.

Sugary-2 opaque-2 versions (backcross 6 conversions) of two agronomically desirable selections of "opaque-2, high protein" line recoveries in each of the Oh43, B14, and B37 elite inbred sources were testcrossed in the 1977 nursery to nonrelated sugary-2 opaque-2 single cross testers for performance evaluation.

Evaluation of the Multiple Aleurone Characteristics to Improve Protein Quality and Content.

The project has studied the feasibility of increasing the proportion of aleurone tissue relative to the starch portions of the endosperm in an additional effort to improve the protein nutritional quality. We have developed backcross 4 to 6 recoveries of o_2, su_2 o_2, and normal versions of 15 elite inbreds. These versions possess from 2 to 3 aleurone layers.

Evaluations of this characteristic thus far indicate that it is of limited practical value for improving protein quality and content. We have found that the multiple aleurone layer characteristic increased lysine concentration without altering protein percentage in both opaque-2 and normal kernels though this increase was small [Agron. Abstr. p. 47, (1978), previous Annual Report].

Evaluation of Independent Mutant Sources of Sugary-2.

Five independent mutant sources of sugary-2 are being backcrossed into common elite inbred backgrounds Oh43, W64A, B37, Cl03, Cl23 and A632. Backcross conversions are complete to BC6 to 8 generations for the sugary-2 standard allele, and the remaining four su_2 alleles were recovered to backcross 3 and to 6, depending upon the line in the 1977-78 winter nursery. These mutant sources will be evaluated in advanced recoveries in intercrosses within inbreds, as inbreds, and intercrosses among inbred backgrounds for detailed studies on possible allelic differences in inbred and hybrid backgrounds.

Development of Opaque-2 Germplasm with Vitreous Kernel Phenotype.

Selection of opaque-2 in various diverse genetic backgrounds reveal a relatively frequent occurrence of vitreous (modified) opaque-2 phenotype. These vitreous opaque-2 types would be extremely desirable for acceptance of the opaque-2 for food use, resistance to ear-rots, grain insects, improved handling, and storage characteristics.

Vitreous Opaque-2 Synthetic HM o_2 o_2.

This was developed by visually selecting vitreous kernels from a corn-belt opaque-2 population. Mild selection was made for lysine content. This synthetic was released and can serve as source germplasm for vitreous

Single Gene Modifier for Vitreous Opaque-2.

The inheritance of this opaque-2 modifier system in maize (*Zea mays* L.) was studied by determining the proportion of crossed, F_2, bc_1, and bc_2 kernels that were modified. To further substantiate the results, the ears bearing F_3, bc_1F_2, and bc_2F_2 kernels were classified as homozygous modified, segregating modified, or homozygous nonmodified. The effect of this modifier system on kernel characteristics was studied by comparing modified and nonmodified F_3 kernels.

The system was found to be dominant and simply inherited. A single gene model is proposed. Although the single model does not explain all of the results, the classification on an ear basis of ears bearing F_3, bc_1F_2, and bc_2F_2 kernels gives a strong indication of one major gene. The deviations could be due to interactions with the environment and other minor modifier genes.

The modifier system significantly decreased kernel volume, increased kernel density, decreased lysine content as percent of protein, and decreased lysine content as percent of sample. The kernel weight and protein content were not significantly affected. The modifier decreased the lysine content but not to the level of normal maize. It should be easier to select for higher lysine content in vitreous varieties for Latin America due to the simple inheritance of the modifier system.

Dominant Opaque-2 Suppressor Gene.

A dominant gene that completely suppresses the opaque-2 phenotype has been isolated. The proposed genotype o_2o_2 Sp Sp is indistinguishable from "normal." Lysine content will probably be at normal levels also

although this will not be confirmed until samples with identified genotypes are analyzed from the winter nursery. This gene may prove useful in understanding the phenotypic and biochemical actions of the opaque-2 gene.

The modifier systems that give vitreous opaque-2 phenotypes could lead to opaque-2 varieties that (1) provide a more acceptable product for certain food uses, (2) are more resistant to ear rots and grain insects, (3) tend to have lower levels of lysine, (4) frequency and degree of vitreous kernels will vary with environments and (5) grain yield relative to standard opaque phenotype will not be improved and will average 10 to 15% below normal corn.

Double Mutant Combinations of Endosperm Genes With Opaque-2.

This project has been evaluating and studying the modification of kernel characteristics and nutritional quality in maize through the genetic interactions which result from double mutant combinations of endosperm mutants. For the high lysine maize to become more acceptable, particularly for human consumption in the more tropic areas, a hard vitreous endosperm, high lysine type with good agronomic yield and biological value is needed. We have previously reported that the double mutant sugary-2 opaque-2 (su_2 o_2) has improved kernel vitreousness and kernel density similar to normal maize. It has improved protein quality resulting from some increase in lysine, an increased content of tryptophan, and a better leucine to isoleucine balance (see previous Annual Reports). There is some evidence that su_2 o_2 has a net shift of increased protein synthesis into the glutelin fraction in terms of yield per endosperm, whereas an increased yield of glutelin was not observed in o_2 [R. S. Luce 1978. Protein

Synthesis in the Endosperm of Normal, Opaque-2, Sugary-2, and Sugary-2 Opaque-2 Corn (*Zea mays* L.) by Electrophoretic Analysis, M. S. Thesis, Cornell University Library, Seed Stocks from Purdue Project]. We reported su_2 o_2 to have an 18% increase in oil content over normal on a weight basis whereas o_2 showed an 18% percent increase on a percentage basis but no increase in oil content on an actual weight basis. We discovered that the starch granules of su_2 and su_2 o_2 had superior *in vitro* digestibility characteristics. Their granules were digested much more rapidly than those of normal and any other mutant or mutant combination studied thus far. (See previous Annual Reports and several publications cited in section G). The su_2 o_2 corn has good biological feeding value for adult humans [J. of Nutrition 107:404-411 (1977)] and swine [J. of Animal Sci. 44:1004-1010 (1977); 44:1011-1019 (1977)]. The digestibility characteristics of su_2 o_2 corn appears to be significant in improving biological value of corn diets for human food, particularly in infants (See preliminary report given later in this section). The serious shortcoming of the su_2 o_2 double mutant materials is the reduction in yield in those materials evaluated to date. The kernel size is reduced due to the introduction of the su_2 mutant and as a consequence yields generally have been reduced greater than even opaque in comparison to their normal counterpart hybrids. We have observed some background genotype variation for yield characteristics.

Selection and improvement of two sugary-2 opaque-2 varieties Temp HA su_2 o_2 and Temp HB su_2 o_2 have continued (see previous section). Because of the potential usefulness of su_2 o_2 germplasm as a specialty high lysine corn, the project has continued to develop corn belt germplasm materials. Approximately 28 elite inbred near isogenic conversions to su_2 o_2, su_2,

and o_2 are being developed. Sixteen of these conversions have been advanced to the backcross 6 to 8 generations. The remaining conversions are advanced to the backcross 5 and 6 generations. These conversions will be grown out and selfed in the 1978 summer nursery and will be available for release. A few sources already have officially been released.

Diallel analysis of agronomic quality, kernel characteristics, protein and carbohydrate quality of su_2 o_2, o_2, su_2, and their normal counterpart hybrids is in progress (S. G. Ballenger, Ph.D. Thesis). This study involves a 10 parent diallel of diverse parentage selected from the broad genetic relationship groups. The experiment will be conducted in replicated trials in each of two locations in 1977-1978. Preliminary data from the first trial showed that 100-kernel weights of the 45 su_2 o_2 hybrids ranged from 8% better to as low as 33% less than their respective normal counterpart hybrids. The mean 100-kernel weights of the 45 su_2 o_2 hybrids were 19% lower than the mean of the normal counterpart hybrids. The 100-kernel weight gives a good approximation of potential yield differences. The means and ranges of protein percent of whole kernel for the 45 normal, o_2, su_2, and su_2 o_2 hybrids were 9.5% (7.4 to 12.9), 9.8% (7.6 to 12.7), 10.6% (8.5 to 13.8), and 10.5% (8.6 to 12.4), respectively. The means and ranges of the whole kernel lysine as percent of protein for the 45 normal, o_2, su_2, and su_2 o_2 hybrids were 2.86 (2.33 to 3.46), 3.99 (2.83 to 4.93), 3.36 (2.67 to 3.86), and 4.56 (3.75 to 6.38), respectively.

Evaluation of an additional 214 singlecross hybrids involving the o_2, su_2 o_2, and normal genotypes for agronomic quality will be made in the 1978 nursery.

In dry matter accumulation studies in four maize hybrids near isogenic for endosperm mutants conditioning protein quality, we have found that on

the average su_2, o_2, and su_2 o_2 accumulated 13, 18, and 25% less grain weight than normal. The reduction in kernel dry matter accumulation was the result of shorter grain filling periods and less rapid rates of grain filling during the reproductive phase (Crop Sci. In Press; previous Annual Reports). Previous reports have suggested that the development of the kernel black layer (an indication of physiological maturity or lack of further increase in weight) in o_2 occurred at about the same time as in the normal counterpart hybrids. More recent preliminary evidence gathered from a number of o_2 and su_2 o_2 near isogenic hybrids suggests that the kernel black layer does develop earlier in o_2, and su_2 o_2 genotypes than in the normal counterpart. There appears to be some environmental hybrid interaction for this character. Opaque-2 and su_2 o_2 showed a 9 and 11% reduction of cob weight, respectively compared to the normal hybrids throughout development, whereas su_2 hybrids were similar to normal. Opaque-2, su_2 and su_2 o_2 retained a higher moisture percent during development than their normal counterpart hybrids. However, on the average o_2 was not significantly different in moisture than normal at maturity (Crop Sci. In Press). Developmental studies of dry matter accumulation during the grain filling period and associated studies of metabolic properties of endosperm proteins and carbohydrates and their interrelationships with dry matter accumulation in high lysine maize genotypes is underway. Knowledge of the intercompensating effects involved in endosperm development and storage metabolism may help to establish the major restraints in our progress in improving nutritional quality and yield (A. H. Long and T. C. Lee, Ph.D. Theses).

We have previously reported that the waxy opaque-2 (wx o_2) double mutant has protein quality equivalent to opaque-2 and good biological value (previous Annual Report). It has a modified starch type (all

amylopectin) but it is not improved in kernel vitreousness or density and also has reduced yield compared to normal counterpart hybrids. This double mutant offers a glutinous and high lysine specialty type of corn and may have utility as a fresh edible corn when eaten at a more mature stage than conventionally thought of in the U.S. The waxy's are eaten to some extent in some countries such as the Philippines, and other south-eastern Asia, and Far East countries.

In addition to the Temp HA wx o_2 and Temp HB wx o_2 populations being developed (see previous section) we are developing 25 elite inbred near isogenic conversions to waxy (wx) and waxy opaque-2 (wx o_2). The materials have been advanced to the backcross 6 to 8 generations and are being selfed and these conversions will be available for release.

Whole Kernel Corn Meal and Degerminated Corn Flour Protein Quality and Digestibility of Carbohydrate in Infant Children.

Although very far advanced, these cooperative studies with Dr. George Graham, M. D., Department of International Health, The John Hopkins University, are not quite complete but some preliminary results are quite interesting. Whole kernel corn meals and degerminated corn flour (essentially endosperm) meals of the three near isogenic o_2, su_2 o_2, and normal hybrids are being evaluated for protein quality and digestibility of carbohydrate in infant children. Nitrogen absorption, nitrogen retention, stool weights, stool energy (by bomb colorimetry), fasting and post-prandial plasma free amino acids are being determined.

The superior rates of starch granule digestion and other inherent characteristics of the su_2 o_2 double combination appear to be significant for improving biological value of corn diets for human food, particularly infants.

Stool wet weights and energy, both good approximators of undigested carbohydrate are almost identical for the endosperm meals from normal and o_2. However, in the su_2 o_2 endosperm meal they seem to be about 15% higher than in the o_2 and normal. For the whole kernel meals stool wet weight and energy seem to be superior for su_2 o_2. Sugary-2 opaque-2 is higher than o_2 and o_2 is again a little higher than normal in stool wet weight and energy.

Apparent nitrogen absorption is similar for the three endosperm meals and for the three whole kernel meals but it looks thus far as if it will be better from whole kernel meals than from endosperm.

Apparent nitrogen retentions from su_2 o_2 endosperm meal are about 35% higher than those from the normal. The retensions from the normal whole kernel meal, though lower than from o_2 and su_2 o_2 whole kernel meals, seem to be about 20 to 25% lower. The retentions from the three whole kernel meals in each case are higher than from the endosperm meals and it appears quite significantly so.

Susceptibility of Starch Granules to Degradation by Amylases.

We have continued our cooperative studies with Dr. Fuwa's laboratory on the relative rates and patterns of starch granule degradation by use of several different amylase enzymes and by observation of starch granules following enzymatic degradation with the use of the scanning electron microscope. We have extended the comparative studies to several additional gene combinations and to additional inbred backgrounds. We have compared *in vivo* vs. *in vitro* rates and patterns. Several publications have resulted (See section G and previous Annual Reports).

Comparative Susceptibility to Amylase of Starch Granules of Double and Triple-Mutants Containing Amylose-extender, Dull, Waxy, Sugary-1, and Sugary-2 Genes of Maize. We studied the relative susceptibility and extent of enzyme degradation in yet other endosperm mutant combinations which have been synthesized in the maize inbred Oh43, but not previously reported.

Starch granules were prepared from 14 double- and 26 triple-mutants containing amylose-extender (ae), 14 double- and 18 triple-mutants containing waxy (wx), 15 double- and 20 triple-mutants containing sugary-1 (su_1), 13 double- and 23 triple-mutants containing sugary-2 (su_2), and 14 double- and 19 triple-mutants containing dull (du) of maize inbred Oh43 (*Zea mays* L.). The relative susceptibilities of these starch granules were examined by scanning electron microscopy. A commercial normal maize starch was used as a control.

Table 3 shows the data on relative susceptibility to glucoamylase of the several starch granule types. Of interest are the su_2 mutant and mutant combinations. Starch granules of 11 double- and 16 triple-mutants containing su_2 were digested 2.8 to 8.4 times faster than those of normal (Table 3). Starch granules of the double- and triple-mutants containing su_1 were digested 3.0 to 7.9 time faster than those of normal . The ae gene reduced susceptibility and seems to be epistatic to su_1 and su_2. Starch granules of the double- and triple-mutants containing wx were digested about two times faster than those of the normal and those containing shrunken-2 (sh_2) were digested 1.2 to eight times faster than the normal. Starch granules of triple-mutant combinations with opaque-2 (o_2) showed digestion properties which were very comparable to

Table 3. Relative susceptibility[1] to glucoamylase of starch granules of several double- and triple-mutant combinations with amylose-extender, dull, waxy, sugary-1, or sugary-2 gene of inbred maize Oh43.

	ae	du	wx	su_1	su_2
du	82.7	-	-	-	-
wx	125.0	273	-	-	-
su_1	65.9	321	232	-	-
su_1^{st}	51.4	215	151	-	-
su_2	39.4	227	344	312	-
bt_1	89.0	108	213	627	431
bt_2	100.0	97	236	-	563
fl_1	26.9	91	168	304	342
fl_2	23.3	101	195	437	277
h	32.9	106	156	778	352
o_2	35.3	89	234	373	353
sh_1	44.2	107	199	390	401
sh_2	119.0	122	250	-	514
sh_4	71.9	142	217	455	328
du fl_2	79.1	-	-	-	-
du o_2	91.6	-	-	-	-
du sh_1	58.6	-	-	-	-
du sh_2	113.0	-	-	-	-
du su_1	183.0	-	-	-	-
du su_2	54.9	-	-	-	-
du wx	267.0	-	-	-	-
o_2 fl_2	23.1	-	193	368	-
o_2 sh_2	163.0	-	-	504	433
sh_1 fl_2	52.2	-	254	600	516
sh_1 o_2	69.5	113	255	-	487
su_1 fl_2	72.7	292	-	-	-
su_1 o_2	78.7	-	-	-	-

Table 3. Continued

	ae	du	wx	su_1	su_2
su_1 sh_1	73.1	318	-	-	-
su_1 sh_2	169.0	-	-	-	-
su_1 su_2	69.9	299	-	-	-
su_1 wx	169.0	-	-	-	-
su_2 fl_2	41.4	303	-	545	-
su_2 o_2	41.4	229	-	573	-
su_2 sh_1	77.1	280	-	788	-
su_2 sh_2	258.0	275	-	788	-
su_2 wx	81.1	517	-	-	-
wx fl_2	34.7	-	-	541	-
wx o_2	81.1	321	-	296	602
wx sh_1	95.6	238	-	433	835
wx sh_2	198.0	227	-	-	839
(normal	24.9 ± 7[2]	14.4[3], 14.2[3]	14.3 ± 0.2[2]	8.3[3]	5.1[3])

[1]Susceptibility of normal maize starch granules = 100.

[2]Degradation, mean ± standard deviation, n = 3.

[3]Degradation, single determination.

those of their respective non-opaque double-mutant counterparts as was previously reported. (J. of Nutri. Sci. and Vitaminol: In Press)

Degradation of Various Starch Granules by Glucoamylases of Rhizopus Amagasakiens, Rhizopus Niveus, and Endomyces. The action of four preparations of glucoamylase (α-1, 4-glucan glucohydrolase, E.C. 3.2.1.3), crude glucoamylases of Rhizopus amagasakiens and Endomyces and crystalline glucoamylases of Rhizopus niveus and Endomyces, on starch granules of less susceptible types [potato, amylose-extender (ae) mutant of maize and heat-moisture treated potato], more susceptible [sugary-2 (su_2) and sugary-2 opaque-2 (su_2 o_2) mutants of maize] and normal maize as a control was studied to characterize the action of these enzymes on native starch granules. The extent of hydrolysis (% degradation) was followed by measuring the amount of glucose liberated. Patterns of degradation were observed by scanning electron microscopy (SEM). Fig. 1 shows time courses of degradation of starch granules of less susceptible types (potato, ae mutant and potato treated with heat-moisture), more susceptible (su_2 and su_2 o_2 mutants of maize) and the normal maize used as a control by four preparations of glucoamylase. Each preparation showed qualitatively similar time courses of degradation specific for each respective type of starch granules although each preparation contained different amounts of α-amylase. Relative rate of digestion by glucoamylases of the six types of starch granules showed that su_2 and su_2 o_2 mutants of maize were most rapid, followed by normal maize, heat-moisture treated potato, ae maize, and potato in decreasing order respectively. In Table 4 are summarized the relative susceptibility of these starch granules by the four preparations of glucoamylase when

Fig. 1 Time courses of degradation of several starch granules by four preparations of glucoamylase. a, R. amagasakiens (crude) 2.1 x 10^3 units/2.0 ml of the reaction mixture); b, Endomyces (crude, 4.0 x 10^3 units); c, R. niveus (crystalline, 2.4 x 10^3 units); d, Endomyces (crystalline, 1.2 x 10^3 units); O, sugary-2 (su_2); and ●, sugary-2 opaque-2 (su_2 o_2) mutants of Oh43 maize; Δ, the normal counterpart of Oh43 maize; ▲, potato treated with heat-moisture; ▭, amylose-extender (ae) mutant of Oh43 maize; ▬, potato.

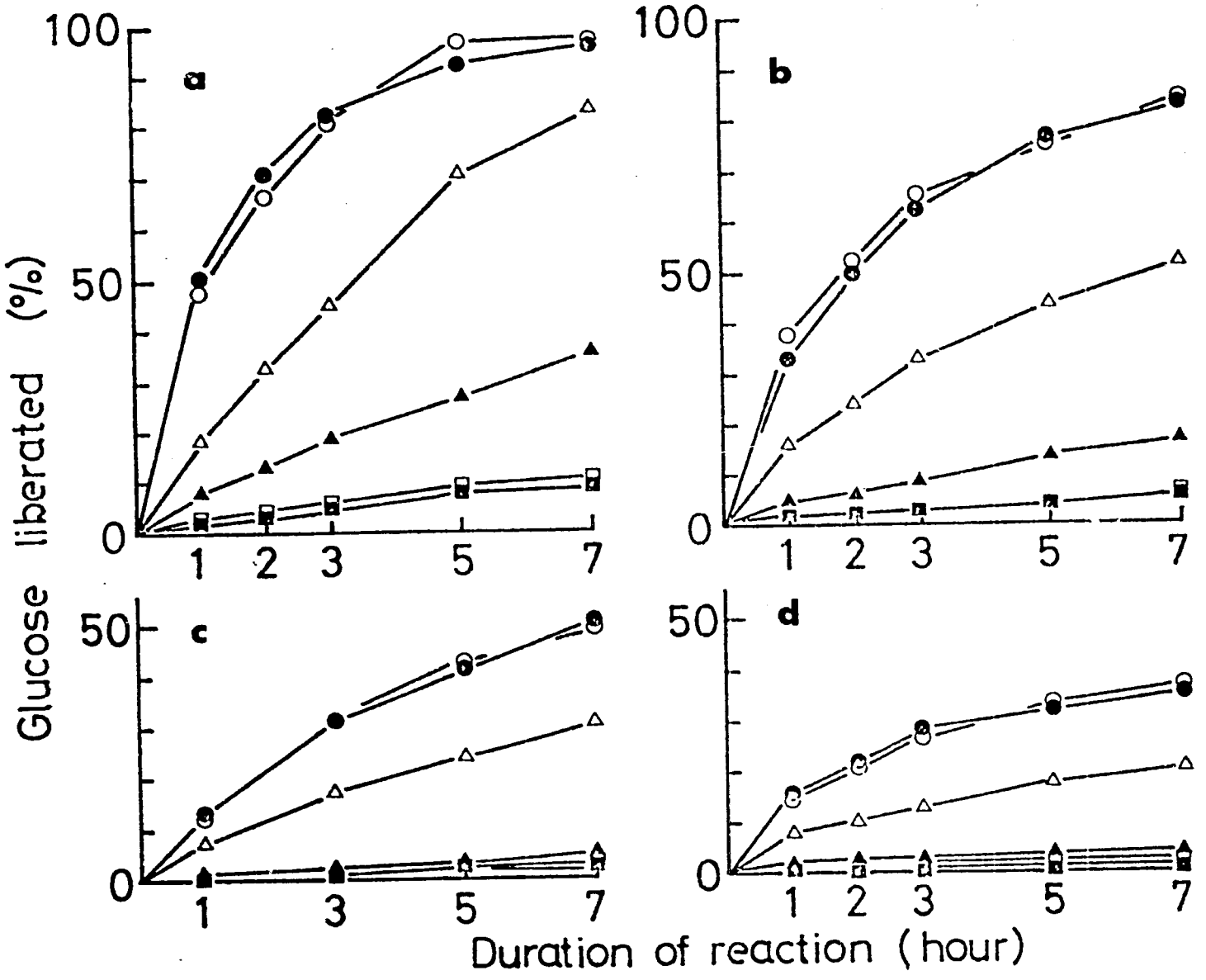
Glucose liberated (%)
100
50
0
a
b
c
d
1
2
3
5
7
Duration of reaction (hour)

Table 4. RELATIVE SUSCEPTIBILITY[a] OF SEVERAL STARCH GRANULES BY FOUR PREPARATIONS OF GLUCOAMYLASE

Starch granules	*R. amagasakiens* (crude)		*Endomyces* (crude)		*R. niveus* (cryst.)		*Endomyces* (cryst.)	
	b	c	b	b	b	c	b	c
	%	%	%	%	%	%	%	%
Potato	2.2	12.6	1.3	8.0	1.1	6.4	0.57	3.2
Oh43/*ae*	2.9	16.6	1.5	9.3	2.2	12.9	2.0	11.4
HM Potato[d]	6.9	39.4	4.1	25.3	2.4	14.0	3.3	18.8
Oh43/normal	17.5	100	16.2	100	17.1	100	17.6	100
Oh43/su_2	44.0	251	37.8	233	31.4	184	33.4	190
Oh43/su_2 o_2	47.8	273	32.8	203	31.4	184	31.2	180

[a]The data were expressed on a relative percentage of the Oh43/normal control enzyme degradation.

[b]% degradation.

[c]Relative susceptibility.

[d]Heat-moisture treated potato.

percent degradation of the normal maize starch granules was in a range of 16.2 to 17.6%. Four preparations of glucoamylase showed, in general, similar patterns of attach on the normal maize starch granules. Namely, small pits appeared on the surfaces of the granules, pits increased in number and size and holes penetrated into the inner portions of the granules toward the center of the granules. Patterns of attach were uniformly distributed on the surfaces of the granules. J. of Applied Biochem. Submitted)

SEM Observations of Degradation of Starch Granules in Germinating Kernels of Several Maize Endosperm Mutants in Four Inbred and One Hybrid Background and Their Normal Counterpart. The objective of this study was to make SEM observations of starch granules of several different maize endosperm mutants following the early germination period and to compare the relative patterns and digestibility by starch-degrading enzymes. Mature kernels of the *ae* endosperm mutant in four inbred lines and in a single cross hybrid and of five endosperm mutants in an inbred and a single cross hybrid, and their normal counterparts were used.

Enzymatic erosion of the starch granules was visible in the disected endosperm of the normal maize following germination 4, 6 and 8 days after planting. The types of the enzymatic erosion were similar to those of the *in vitro* attack by α-amylase on normal starch granules. Namely, numerous pin holes were visible on the surfaces of the granules and the pores penetrated into the granules and the inner portion, which appeared to be terraced or step-shaped indicative of layered internal structures. Some types of enzymatic erosion suggested the possibility of enzymatic

attack other than that of α-amylase. Starch granules of the germinating Oh43, Cl03, W64A, and Oh43XCl03 ae endosperm mutant lines scarcely showed enzymatic erosion on their surfaces. These observations were similar to the in vitro amylase attach of ae starch granules. On the other hand, enzymatic erosion of starch granules of the B37 ae endosperm was similar to the germinating kernels of the normal B37. The types of enzymatic erosion of endosperm starch granules of the germinating su_2, su_2 o_2, wx, and o_2 mutants were similar to those of their normal counterparts. [J. Japan. Soc. Starch Sci. 24:99-111 (1977)].

Interaction of the Opaque-2 Gene with Starch-Forming and Starch-Deficient Mutant Genes on The Synthesis of Zein in Maize Endosperm.

In an effort to better understand the regulation of zein synthesis in maize endosperm, we have continued, as one approach, to investigate the interactions between starch-forming and/or starch-deficient mutants and opaque-2 on zein synthesis. The protein synthetic machinery and levels of RNase activity in the normal, o_2, bt_2, and bt_2 o_2 double mutant were also examined in this experiment.

The combination of opaque-2 with starch-modified or starch-deficient mutants produced a cumulative and synergistic effect, respectively, in regulating zein synthesis. The double mutant, bt_2 o_2, which almost completely prevented the synthesis of Z1 and Z2, had high RNase activity. Presumably, amplification of RNase activity in the bt_2 o_2 double mutant may completely prevent the syntehsis of Z1 and Z2 by preferentially degrading zein mRNA's. [Biochem. Genetics 16:883-896 (1978)].

Messenger RNA for Zein Storage Protein in Maize Endosperm.

The major storage protein in maize endosperm is a prolamine known as zein. Analysis of this protein on SDS gels reveals two principle components of 22,000 and 19,000 daltons. This protein is synthesized on membrane-bound polysomes in the developing endosperm and deposited in protein bodies. The opaque-2 (o_2) mutant lacks the larger zein component. Total membrane-bound polysomes were isolated from developing endosperm from the inbred line W64A 22 days after pollination, mRNA's were isolated, and separated on linear log sucrose gradients. The polysomes isolated from the total membrane fraction or from purified protein body membranes showed no qualitative differences in size and translation products. When mkNA's were translated in the cell-free wheat germ system and the products analyzed by fluorography, both zein components were 2,000 daltons larger than the native proteins. The mRNA's sedimented at 13S in sucrose gradients but gave molecular weights of 500,000 in dimethyl sulfoxide-sucrose gradients and formamide containing gels. Complementary DNA was synthesized for o_2 mRNA by AMV reverse transcriptase and the R_0t 1/2 was slightly larger than that of ovalbumin determined under identical conditions. [Suppl. Plant Physiol. 61:611 (1978) and Abstr. J. Biol. Chem. (1978)].

E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results:

a. Research report. The project distributed 650 copies of the 1976-77 annual research report to over 400 maize cooperators and interested centers, missions, or institutions representing over 35 countries and many public and private institutions within the U. S. The project

has had continued request for the project annual reports during the list of the project and has distributed between 600-700 copies each year from 1971 to present.

b. Research publications and abstracts of research reports. There have been a total of 120 publications and abstracts of research reports by the Purdue project staff during the period the project was supported by A.I.D. This extensive store of knowledge will be of value not only for the continuing research at Purdue but also at other institutions and centers over the world doing research on cereal grains, protein quality, and nutrition.

For a bibliographic list of publications and research abstracts see Section G.

c. Status report. The status report--International Programs in Agriculture, Agricultural Experiment Station, Purdue University Station Bulletin No. 70 (March 1975)--entitled "Simple Chemical and Biological Methods Used at Purdue University to Evaluate Cereals for Protein Quality" has been of continued interest. Interested maize and other cereal workers as well as many nutritionists and biochemists throughout the world have surprisingly made continued requests for copies of this bulletin.

d. Workshops, symposia, papers and seminars presented. Papers were presented and project personnel participated in the following activities: American Soceity of Agronomy Annual Meetings; Fifth International Congress of Food Science and Technology; Agricultural Chemical Society of Japan; The Annual Meeting of the American Society of Biological Chemists; American Society of Plant Physiology, Blacksburg, VA; Planning and Coordinating Conference and Workshop

for Title XII--Maize Commodity Group from 10 Universities, representatives of Industry, and CIMMYT, St. Paul, MN; Planning and Coordinating committee meeting, and conference with BIFAD board members for Title XII-Maize Commodity Group Activities, Washington, D.C.; American Seed Trade Association Conference-Planning and coordinating committee meeting and discussion with maize workers, Chicago, IL; Mid-America International Agricultural Consortium (MIAC)-Maize Commodity Group meeting with invited adjunct university members and the Planning and Coordinating Committee and representatives for the 10 University-Industry-CIMMYT Maize Commodity Group, St. Louis, MO. Project personnel gave several seminars and participated in special field day and program activities in several departments at Purdue. They conducted several informal discussions and tours of the breeding material and laboratory with foreign and national visitors.

e. Germplasm distribution and utilization. The Project has continued to distribute germplasm materials containing opaque-2 and a number of other endosperm genes, along with various modifying genes, in various genetic backgrounds to a large number of LDC's, European countries, and corn breeders in the U. S. and Canada.

The following list is representative of Purdue germplasm which has been available and distributed as source materials for improvement of protein quality in maize:

Synthetics

Syn A o_2o_2

HM o_2o_2

TEMP HA o_2o_2

TEMP HB o_2o_2

Colus o_2o_2

ANTIGUA 2D x (B10 x B14) o_2o_2

ETO x CBC o_2o_2

TEMP HA $su_2su_2o_2o_2$

TEMP HB $su_2su_2o_2o_2$

TEMP HA $wx\ wx\ o_2o_2$

TEMP HB $wx\ wx\ o_2o_2$

TEMP HA $bt_2bt_2o_2o_2$

TEMP HB $bt_2bt_2o_2o_2$

Genetic and Breeding Source Material

Modified - o_2o_2 (HM o_2o_2 and other simply inherited gene sources)

Opaque-2 (several inbred lines and populations)

Sugary-2 (several inbred lines and populations)

Waxy (several inbred lines and populations)

Brittle-2 (several inbred lines and populations)

Other Endosperm Types (several inbred lines)

The following list is representative of those countries where germplasm distribution has been made:

Latin America:

Mexico
Guatemala
Honduras

South America:

Argentina
Brazil
Chile
Colombia
Ecuador
Peru
Venezuela

Africa:

Egypt
Nigeria
Kenya
Ethiopia
Tansania
Malawi

South Africa
Rhodesia
Upper Volta
Ivory Coast
Ghana

Near-East:

Israel
Lebanon

South-East Asia:

Taiwan
Pakistan
India
Nepal
Indonesia

Philippines
Malaysia
Vietnam
Thailand
Afghanistan

Japan
Korea
Papua New Guinea

Europe:

Bulgaria
Czechoslovakia
Hungary
Romania
Yugoslavia
Greece
U.S.S.R.

Italy
France
Germany
Spain
Acores
Denmark
England

U.S., Canada and Australia

2. Utilization: Evidence and Cases Where Findings are Being Used in LDC's and U.S.:

Though these opaque-2 and other high lysine germplasm have been utilized as source materials in breeder programs, to date, there is little evidence of significant commercial production of high lysine maize. If the yield limitations are solved the pay-off from such maize genetic materials would be very high, particularly where in some areas at least quality protein is limiting in the diets. Also, it should be kept in mind, that there is a possibility of growing high lysine maize (in spite of reduced yields) as a specialty crop. A.I.D. should keep in mind a possible program to extending

information and helping to make germplasm available to appropriate LDC's where quality protein is limiting in the diets. It may be noted that these yield differences between opaque-2 conversions and their normal counterparts may not be of great significance in LDC's where yield levels are on the order of 20-30 q/ha. At that yield level, a 10-20% loss is much simpler to overcome by varietal germplasm introduction along with a basic agronomic package. We do now have the potential of modified opaque types which should be much more resistant to insect attack, kernel injury, and disease problems. Purdue and CIMMYT materials are available but must be selected and further developed on a national level to become acceptable. Once these materials become acceptable they could be directly introduced as a new maize with a gain in yield and quality. In some cases the yield level of the varietal introductions is quite comparable or better than that of the locally bred improved maize and better than that being used under the traditional agricultural system. There is real difficulty, however, in devising a mechanism by which this material can be introduced at the farmer level. This calls for programs of practical demonstration of introduction and utilization of acceptable materials in certain key target LDC areas. It also suggests a seed industry of some order must be functional in order to guarantee the continued utilization of acceptable high lysine materials.

The project has made some recent attempts to gain information on commercial utilization of the high lysine character both in some LDC's and in the U.S.

CIMMYT has continued to have a strong protein improvement program (personal communication with Dr. S. K. Vasal, Dr. R. L. Paliwal, and other CIMMYT personnel; IAEA-SM-230/33 Quality Protein Status Report-Neurenberg, Germany; CIMMYT Annual Progress Report's to UNDP-1976 and 1977; CIMMYT Review 1978). CIMMYT has developed 34 gene pools and 5 advanced populations of opaque-2 and is presently selecting for hard endosperm and agronomic performance. Emphasis has been to produce quality protein maize with respect to yield, acceptability, ear rots, etc., considering quality protein as a bonus. With the use of modifiers of opaque-2 a number of the maize problems that at one time acted as a major hurdle in the promotion of quality protein maize have practically been overcome. CIMMYT has identified a number of materials that they would like to suggest to different national programs to evaluate and make recommendations on. In fact there are one or more materials that will perform reasonably well in some of the Latin American, South American, and African countries. CIMMYT has set up a new trial OMPT (opaque-2 maize population trial) that consists of hard endosperm opaque-2 versions of all advanced unit normal materials in the program. The trial has 25 entries and most of the entries look relatively stable in Mexico for kernel hardness. This trial is being sent to over 40 locations. For the Andean countries, CIMMYT is selecting and developing opaque-2 floury maize materials. One population of su_2 o_2 is being improved. The population as a whole looks very good and has a good potential for future selection.

Seven national maize programs are each developing from CIMMYT populations open-pollinated maize varieties with improved protein

quality for their area. These are Guatemala, Ghana, Ecuador, Nepal, Philippines, Tanzania, and Zaire.

It seems that CIMMYT is practically the only institution doing breeding work on high lysine maize in Latin America. The national programs for the most part do the testing for CIMMYT. In Guatemala, for example, emphasis is on synthetics and synthetic crosses, mostly in normal corn. There is some selection work in high lysine hard endosperm materials. The most promising are the white dent-LaPosta HE o_2 and Mezcla Tropical Blanca H.E. o_2. To our knowledge there is no production of high lysine corn on a commercial scale in Latin America. In Mexico, outside CIMMYT's work, other high lysine work seems to be very limited or non-existant.

In South American the work on high lysine maize is greatly reduced and rather insignificant compared to the total corn breeding effort. The work in Venezuala and Colombia is very insignificant. Again mostly testing of CIMMYT materials. There is some suggestion that there will be some extension-demonstration-testing type programs developed in the Maize Cooperative Project for the Andean Region with CIMMYT high lysine materials for selected areas in Colombia (personal communication, Dr. J. B. Barnett). Ecuador has a small laboratory for protein quality evaluation and is doing some work on opaque-2. They are developing one composite for the highlands and one for the lowlands. Bolivia has a high lysine selection, Aycha Sava-2, which appears promising and is being developed at the Institute Fitotecnico, Cochabamba, Bolivia. In Peru there is a very small amount of opaque-2 grown in a limited area in the north. Monnico o_2 is apparently one of their principal varieties. Argentina has virtually no high lysine maize program.

Brazil has limited programs in development of high lysine maize, for example, at Piracicaba and Viçosa. Agroceres has about eight inbred lines of o_2 and one hybrid (AG504) which comprise about 90% of the opaque-2 seed planted. About 10% is planted with synthetic varieties. Agroceres, which is by far the main group involved with opaque-2, is producing about 1,000 (60 kg) bags this year. There were about 7,200 (2,900 ha) acres planted with opaque-2 corn in Brazil this year.

Presently, quality protein maize is not being produced commercially in any of the African countries. There are a number of countries that have substantial programs. The national maize programs of Tanzania and Zaire are developing and testing CIMMYT high lysine maize materials (personal communication CIMMYT personnel, CIMMYT Review 1978, CIMMYT Annual Reports to UNDP, 1976 and 1977). The Kenya project has had a quality breeding program. Two populations, KSII o_2 (F25) and EC573 o_2 (F26), are being developed using full-sib family selection (Report of a Technical Consultation in Kenya 1978, Dr. L. L. Darrah). There is some concern about the fate of these two populations because of possible population mixing and/or insufficient backcrossing to the recurrent parent during gene transfer. It has been recommended that a new o_2 gene transfer series should begin using R11C6 and R12C6 as the recurrent parents. Full-sib method of selection in KCB o_2 and KCE o_2 has been successful. A selection trial KCA (E28 HP) for high protein in this improved normal population will get underway in 1979. Present work with su_2 o_2 has been dropped until convincing signs of progress are found in the single mutant populations.

The Cooperative Maize Project (CIMMYT/FF) in Egypt began work on opaque maize in 1975 (personal communication, Dr. W. L. Haag). Since that time the entire effort has been toward developing and improving three opaque-2 populations. There has been no release for commercial production to date. Expectations are that the population AED x (Tuxpeño x La Posta) opaque to be competitive with some of the better normal materials. It is a soft endosperm material and the highest yielding of the three opaque populations. There is selection for a hard endosperm opaque composite in one of the populations. There is optimism that the program will soon have materials to move commercially. There is cooperative work among the breeders, the quality protein laboratory and National Nutrition Institute in conducting acceptability trials in various villages, feeding trials, etc. in preparing the way for possible commercial utilization.

In South Africa at present all production of opaque-2 hybrids is experimental and geared towards a comprehensive evaluation of this new maize type by millers, breeders, agronomist, feed manufacturers, nutritionists, and farmers (personal communication, Dr. H. O. Gevers; representative reports and publications, Potchefstroom, S. Africa). Their experimental white opaque-2 hybrids (NO 61 series) and yellow opaque-2 hybrids are agronomically equal or better than the best commercial hybrids. Though the white hybrids in particular, have the soft endosperm type, they are the highest yielding and the best plant types compared with the proven and high yielding commercial hybrids SR 52 and PNR 353. In experimental white hybrid trials, in some places growers expected up to 10 tons/ha (metric) this past season.

A number of Eastern European countries have devoted a portion of their breeding effort to development of quality protein maize (Bulgaria, Hungary, Romania, U.S.S.R., and Yugoslovaia). There has been some commercial production reported in such countries as Yugoslovia (personal communication, Dr. J. Dumanovic, Maize Research Institute, Beograd-Zemun) and U.S.S.R. (Krosnodar). However to date perhaps less than 1% of the corn hectarage in Yugoslovia is devoted to opaque-2 maize production. The opaque-2 hybrids are reportedly lower yielding compared to best normal maize hybrids.

In the U.S. there has not necessarily been a need for selecting for the modified opaque-2 materials, since rather satisfactory disease quality can be obtained in some corn belt germplasm materials. Also there are opaque-2 hybrids that have quite satisfactory agronomic and kernel quality as well as the improved protein quality characteristic. Though there are several commercial companies which have available opaque-2 hybrids which have good agronomic and protein quality (though their yields may range from 8-15% lower than the best normal hybrids) there is very little hybrid production of opaque-2 maize in the U.S. It is estimated at considerably less than 1% of the acreage.

The Project has received continued request over the life of the project for information, seed, technical help, advice concerning protein quality improvement in maize and all aspects of cereal grain protein quality improvement. There have been numerous visitors from LDC's and other countries to Purdue for consultation and advice. Information and germplasm requests and personal contacts have helped to increase involvement with the LDC's, helped to understand their

real problems, and constitute a part of project efforts to disseminate and exchange information relevant to LDC programs and involvement in protein quality improvement.

The graduate students trained under the general direction of this project, short term trainees, and visiting scientists consitute a vital part of the project efforts to disseminate information relevant to LDC and other programs in protein quality improvement.

3. Involvement of LDC or U.S. Personnel and Institutions:

Over the life of the project there has been an annual workshop and planning conference with U.S./AID-TA/AGR and CIMMYT maize research staff to discuss program development and research plans, exchange information and coordinate efforts in the development of improved protein quality maize. Testing of wide-based temperate germplasm materials has been conducted in Pakistan, Israel, Mexico, and U.S. although there have been some serious limitations involved in obtaining adequate evaluations and coordination of the test materials.

Project personnel have been involved in screening Colombian x U.S. germplasm material, and in cooperative work to screen the Central American maize germplasm from the germplasm bank, Mexico City for vitreous high lysine types.

Listed below are those countries which the Purdue Project has worked more extensively with:

Colombia	Ethiopia	Many Eastern European Countries and South Africa
Brazil	Kenya	
Guatemala	Nepal	
Mexico	Philippines	
Peru	Pakistan	
Venezuela	Taiwan	
	Thailand	

Project personnel have been involved in special visits, seminars, and workshop discussion groups to help guide the development and utilization of improved protein quality maize. The following listing gives some overview of a few of the significant workshops, symposia, and planning conferences the project has participated in over the duration of the project:

--Purdue-CIMMYT-US/AID - Workshops and Coordinating Conferences, El Baton, Mexico and West Lafayette, In., 1970-1977.

--CIMMYT-Purdue International Symposium - December 1972. El Baton, Mexico Published Proceedings In: High-Quality Protein Maize. Dowden, Hutchinson and Ross. Stroudsburg, Pa. (1975) p. 524.

--National Academy of Sciences International Workshop, Washington, D.C., on Genetic Improvement of Seed Proteins. NAS

--U.S. AID Office of Nutrition - Third Agency-wide workshop on Nutrition, Harper's Ferry, Va.

--Inter-Asian Maize Conference, Bangkok, Thailand.

--Andean Zone Maize Breeders Conference, Colombia.

--Protein advisor group (PAG-UNICEF) Obregon, Mexico.

--U.S. National Com. of the International Union of Nutritional Sciences, Washington, D.C.

--NSF/MIT Protein Resources Study Panel on Cereal Proteins, Boston, Mass.

--East African Coordinated Cereal Breeding and Protein Quality Evaluation Project, Kenya.

--Tenth Inter-Asian Corn Improvement Workshop, Islamabad and Lehore, Pakistan.

--US/AID. International Workshop - M.S.U., Mississippi State. US/AID - Guatemalan Maize Workers Conference.

--Xth International Congress of Nutrition, Japan.

--First Chemical Congress of North American Continent, Mexico City, Mexico.

--Workshop on Breeding and Fortification - Improving the Nutritional Quality of Cereals, II. AID/LIFE, Boulder, Co.

--Other National and International Meetings, Conferences, Symposia, Seminars, and Planning Meetings.

Contacts by visitors from LDC's, other countries and the U.S. to the project and its personnel have provided continued opportunity to exchange information, stimulate research, give technical help on breeding and analytical procedures and advice on utilization of high lysine maize materials in their breeding programs.

Correspondence has also been an additionally important means of disseminating information, data, valuable advice and help.

The project scientists have trained 21 graduate students (12 foreign and 10 U.S.), nine postdoctorates, and 10 trainees representing 7 different countries over the life of the project. Many of these individuals have gone back to their respective countries and are actively engaged in corn improvement programs.

In section G is given a bibliographic list of scientific publications, abstracts, bulletins, symposium and workshop reports representing efforts of the Purdue project staff to disseminate results of the research project during the period the project was supported by A.I.D.

G. FURTHER INFORMATION ON DISSEMINATION OF RESEARCH RESULTS

Bibliographic List of Scientific Publications, Abstracts, Bulletins, Symposium, and Workshop Reports Representing Efforts to Disseminate the Results of the Research Project.

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