AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

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Batch #24

1. SUBJECT CLASSIFICATION	A. PRIMARY: Serials	Y-AF30-0120-0000
	B. SECONDARY: Agriculture--Plant breeding--Cereals--Maize	

2. TITLE AND SUBTITLE
Inheritance and improvement of protein quality and content in maize; annual report,1973/1974

3. AUTHOR(S)
(101) Purdue Univ. Dept.of Agronomy

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1974	131p.	ARC 633.15.P985

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Purdue

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(Research summary)

9. ABSTRACT

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-RAB-410	
12. DESCRIPTORS	13. PROJECT NUMBER
Maize Proteins	14. CONTRACT NUMBER CSD-2809 Res.
	15. TYPE OF DOCUMENT

AID 590-1 (4-74)

A N N U A L   R E P O R T

on

THE INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN MAIZE

Contract csd/2809

July 1, 1973 - June 30, 1974

Prepared by

Department of Agronomy
Department of Biochemistry
Agricultural Experiment Station
International Programs in Agriculture

Purdue University
West Lafayette, Indiana

Submitted to

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/csd-2809

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3. Contract Period (as amended): from July 1, 1970 to June 30, 1975

4. Period covered by Report: from July 1, 1973 to June 30, 1974

5. Total A.I.D. funding of contract to date: $1,019,390 (to March 31, 1975)

6. Total expenditures and obligations through previous contract year: $755,653 (through March 31, 1974)

7. Total expenditures and obligations for current year: $263,737 (4/1/74-3/31/75)

8. Estimated expenditures for next contract year: $291,100 (4/1/75-3/31/76)

B. <u>Narrative Summary of Accomplishments and Utilization</u>

Sixteen new mutants with higher than normal maize lysine content have been identified in a screening of Colombian germplasm. The ninhydrin test recently developed by this project provides a rapid and simple test to identify high lysine mutants which have increased levels of free amino acids. This test could prove valuable as a market place test and in the search for new mutants.

Modified (vitreous) opaque-2 varieties can be selected with good yield (generally not equal to normal), resistance to ear rots and insects and superior protein quality. To date this breeding approach has been the most practical in providing acceptable high quality protein varieties.

The double mutant sugary-2 opaque-2 shows excellent promise for increasing the acceptance and utilization of high lysine maize because of its vitreous kernel, ear rot resistance and enhanced nutritional and biological value. This combination may prove to be the most practical and successful in the future, especially if its yield, which is lower because of reduced seed size, can be improved by selection.

Six mutants have been found in addition to opaque-2, floury-2 and opaque-7 that influence lysine content: sugary-1, shrunken-1, shrunken-2, shrunken-4 brittle-1, and brittle-2, all of which decrease starch yield. All the mutants that increase lysine do so by decreasing zein and increasing other fractions that are higher in lysine content.

Feeding trials with samples of maize, sorghum, wheat and triticales were compared in the rat, mouse, vole, and chick in a cooperative study. The male weanling rat gave the best differentiation among the cereals.

Two varieties adapted to the temperate zone have been developed from diverse germplasm. Selection for agronomic and nutritional improvement is now being conducted in opaque-2 and sugary-2 opaque-2 versions of these two varieties.

A bulletin on "Progress in developing maize with improved protein quality" is now being printed. A brochure on "Simple chemical and biological methods used at Purdue" to evaluate cereals for protein quality has been prepared for publication. Seed of $\underline{su}_2$ and $\underline{wx}$ versions of inbreds and other germplasm has been widely distributed.

ANNUAL REPORT

on

THE INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN MAIZE

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A. GENERAL BACKGROUND

A large proportion of human protein needs are supplied by cereal grains. Maize supplies a principle source of energy and a major portion of protein for the human diet and for animal feed in many developing nations as well as in more developed areas of the world. Maize is, however, deficient in the amino acids, lysine and tryptophan. This deficiency constrains further improvement in the world's human and animal nutritional status.

Problems associated with initial versions of high lysine maize have pointed up the necessity of continued and expanded basic research on genetics, breeding, analytical methods and nutritional problems associated with improving the quantity and quality of protein in maize. The full potential will be realized only through sustained basic, interdisciplinary and cooperative research into these complex problems. Continuation of adequate, systematic, and cooperative research in maize protein quality will play an increasingly important role in solving the world's human and animal nutrition problems.

The purpose of this project is to develop source germplasm materials that will be capable of supplying adequate amounts of nutritionally balanced protein in the diets of monogastric animals, including man, and which have good agronomic characteristics and to contribute to the creation of and participate actively in a world wide network system of maize improvement research. This involves researching the most relevant and important fundamental problems inhibiting progress in this area, and widely disseminating the findings, providing a basic undergirding for the international centers, such as CIMMYT, and for institutional programs in developing countries.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

a. In cooperation with other world maize research centers, a search will be made for other genes and germplasm than those used in current research, that may provide improved protein nutritional characteristics of grain. Several new genes of this type have been found. These newly discovered genes and other promising germplasm will be evaluated for nutritive value, genetic mechanisms and agronomic potential.

b. Determine performance parameters, gene action and interactions with opaque-2 floury-2 and combined mutant hybrids to determine their relative value and to develop more efficient breeding procedures.

c. Evaluate effectiveness of various selection schemes for modifier gene(s) that improve yield, nutritive value and milling characteristics of new gene types.

d. Investigate maximum nutritional improvement with these new genes in conjunction with selection for embryo size and protein content.

e. Synthesize, through genetic manipulation, the several double and multiple mutant gene combinations of opaque-2 and floury-2 with other endosperm mutants (such as sugary-2, dull, waxy, sugary-1, shrunken-2, amylose extender, soft starch, floury-1 and others) and examine these for nutritional properties, digestibility and processing characteristics of the starch.

f. Examine extent and interactions of environment and cultural practices on amino acid and protein levels.

g. Determine adequate field testing and sampling procedures.

h. Establish a central biochemical unit at Purdue capable of instruction in and standardization of analytical procedures for maize protein samples in laboratories of other countries.

i. Conduct basic research on simple and accurate methods for protein and amino acid analyses.

j. Verify nutritive value by rat feeding tests of new maize genotypes shown by analysis to be high in protein quality.

k. Study factors responsible for increased susceptibility to ear and kernel rots of the opaque-2 and floury-2 genotypes.

l. Determine the relationship between environmental heat units, based on air temperature and net radiant heat, at a number of latitudes to determine their value in predicting adaptation to maize genotypes on a worldwide basis.

m. Develop a series of synthetic maize varieties with modified protein quality, which are nonsensitive to photoperiod and have different heat requirements for maturity, to achieve broad adaptation.

C. CONTINUED RELEVANCE OF OBJECTIVES

Two major reasons led to expanding efforts in our search for new mutants that improve protein quality. First, the genotypes currently available still have deleterious agronomic effects such as lower yield, poor grain quality, etc. Secondly, recent development of the rapid and simple ninhydrin test for identification of high lysine types adds a new dimension as a screening tool to our search for new and hopefully better mutants (objective a).

In our first coordinating conference with CIMMYT the serious need for developing source populations with improved protein quality for the more temperate areas of the world was discussed. This project then shifted more emphasis to the development of varieties i.e., Temp HA opaque-2 and Temp HB opaque-2 and an opaque-2 version of Colus (a synthetic developed at Purdue from Colombian and U.S. germplasm). When research revealed possible grain quality and nutritional improvement with the sugary-2 opaque-2 double mutant combination this genotype was introduced into the Temp HA and Temp HB varieties and subsequent agronomic and nutritional selection is being implemented in both the opaque-2 sugary-2 and opaque-2 versions. (objectives a, b, c, d, e and m).

Early in the period of this contract results were obtained showing the floury-2 gene alone or in double mutant combinations would not contribute significantly to improved protein quality. Similar data was rather quickly obtained on the new high lysine mutant opaque-7. Research effort on these two genes has been largely eliminated from objectives b, e and k.

Those specifically related to biochemistry and nutrition (i.e., a, d, e, f, g, h, i, j) are still very relevant. Biological assay of new maize genotypes is highly relevant, and tests need to be made to determine whether PER should be replaced by the newer rat assay method, relative protein value (RPV). Research has been expanded on the nature of the proteins and free amino acids in high lysine maize mutants.

Determining adequate field testing and sampling procedures (objective g) is not currently an area of active research. The study of environmental factors concerned with maize adaptation (objective l) has been discontinued. Active research is underway in the areas specified by the other research objectives.

D. <u>ACCOMPLISHMENTS TO DATE</u>

1. <u>Findings</u>:

In one or more analysis, eight putative new mutants from Colombian germplasm showed a high lysine content. (Objective a). An additional eight mutants had at least one family which was intermediate in lysine content. In a companion study eight Colombian maize populations were tested as sources of modifier genes for opaque-2. ETO X USA342, Pira, Puya and Comun were good sources of modifier genes. ETO X USA342 also has good agronomic performance in the mid-altitude tropics.

During the first coordinating conference with CIMMYT the urgent need for varieties adapted to the temperate areas of the world was recognized. (Objective a). This project developed two opaque-2 varieties Temp HA $\underline{o}_2\underline{o}_2$ and Temp HB $\underline{o}_2\underline{o}_2$ from diverse germplasm from around the world and the U. S. Cornbelt. These populations should permit development of nonmodified or modified opaque-2 varieties and are currently undergoing agronomic and protein quality selection at four worldwide test sites. To further fill the need for varieties adapted to temperate areas the variety Colus developed at Purdue from Colombian and U. S. germplasm has been converted to opaque-2 and is undergoing selection.

When research revealed the possible grain quality and nutritional improvements with the sugary-2 opaque-2 combination, this genotype was developed in these two temperate varieties. (Objectives a and e). Agronomic and nutritional selection is now being implemented in these varieties.

A modified opaque-2 synthetic HM $\underline{o}_2\underline{o}_2$ was developed from Corn Belt germplasm and has been released to corn breeders with commercial seed companies and to public breeders. (Objective c). This will provide a source of modifier genes for these breeding programs to facilitate development of improved modified opaque-2 varieties or hybrids.

A number of pertinent interactions and interrelationships involving opaque-2 have been elucidated. (Objectives b and f). For example opaque-2 kernels are higher than normal in percent of the mineral elements P, K, Mg, Fe and Zn, but on an amount per kernel basis, increases are significant only for K and Zn. Several significant correlations were detected between mineral elements and protein and lysine concentration but none were of sufficient magnitude to serve as selection criteria for lysine content. Several pertinent agronomic effects of opaque-2 are (a) dry matter accumulation essentially ceases at 45 days, about 7 to 10 days earlier than normal (b) black layer (indication of physiological maturity) occurs at same time (c) opaque-2 has higher moisture content at maturity and (d) cob weight is about 10 percent less than normal. In almost all opaque-2 expressions or effects there are significant interactions, indicating selection would be effective.

Findings of detailed genetic and selection studies involving the modified (vitreous) opaque-2 types may be summarized as follows: (a) Modified varieties can be selected from genetically diverse populations. (Objective c). However, some populations have a higher frequency of modifier genes than others as also shown by the detailed study of Colombian germplasm. (b) There is a negative relationship between modification and protein quality, but selection (based on analyses) can maintain high levels of protein quality. (c) These modified varieties are more acceptable for human food uses and more resistant to ear rots and grain insects. (d) The modified and opaque portions within a kernel differ greatly in protein with the modified portion being higher in quantity but lower in quality (lysine) than the opaque portion. Amino acid profiles of the modified portion show a shift in the pertinent amino acids toward the "normal" type of profile. (e) The modified expression (thereby probably protein quality also) varies with different environments, with lower temperatures enhancing the modified phenotype.

High and low lysine varieties (3.23 vs 2.35 g lysine/100 g protein) have been developed in modified and nonmodified versions of synthetic HM $\underline{o}_2\underline{o}_2$ for detailed electrophoretic and fractionation studies of their proteins. (Objective c). Modified and nonmodified varieties in both Temp HA $\underline{o}_2\underline{o}_2$ and Temp HB $\underline{o}_2\underline{o}_2$ have also been developed. These four varieties and the four from HM $\underline{o}_2\underline{o}_2$ described above have been random mated for three generations. The successive samples of the eight varieties will facilitate evaluation of genetic and environmental stability of modified and nonmodified types.

A major research achievement has been the discovery of a double mutant interaction, sugary-2 opaque-2 ($\underline{su}_2\underline{o}_2$) which holds promise for improving the acceptance of high lysine grain. (Objectives b and e). The sugary-2 opaque-2 double mutant shows promise as a superior food and feed grain for human and monogastric animals. The sugary-2 opaque-2 corn has very good kernel density and vitreousness in the endosperm which should improve acceptability, as well as ear rot and storage insect resistance and dry milling properties of the grain. Compared to the standard opaque-2 phenotype, the double mutant has improved protein quality, increased oil content and therefore possibly greater energy value, superior in vitro digestibility characteristics of the starch granules, and good biological value. The improved characteristic that sugary-2 opaque-2 has over "modified-opaque 2" corns is the improved vitreousness of the kernel that is not necessarily accompanied by a decrease in protein quality. Modified-opaque-2 corns have shown some decrease (without selection) in protein quality with modification to a more vitreous kernel type. One constraint associated with the sugary-2 opaque-2 grain is lower yield because of reduced seed size, though the grain is well filled.

Another double mutant combination waxy opaque-2 has shown good protein quality, good *in vitro* digestibility characteristics and good biological value. (Objectives b and e). The kernel vitreousness and density, however are not substantially improved over opaque-2. Waxy opaque-2 offers a dual purpose glutinous high-lysine type of corn for mature grain use or for fresh vegetable corn and may be used in other food preparations where glutinous starch properties are desirable.

The following conversions were released because of the interest in their use to improve nutritional characteristics: Oh43 su_2su_2, B37 su_2su_2, C103 su_2su_2, A632 su_2su_2, Oh43 *wx wx*, C103 *wx wx*, and A632 *wx wx*.

Protein and carbohydrate quality investigations of several endosperm mutants and their combinations with opaque-2 at vegetable corn maturities suggest that surgary-1 opaque-2 would offer a superior high-lysine high water-soluble polysaccharide corn conferring the desired textural properties for fresh eating or canned corn use. (Objectives b and e). Shrunken-2 opaque-2 and brittle-2 opaque-2 offer superior high-lysine high-sugar corns with potential for extending the harvest and storage quality of supersweet corns and may be a desirable snack food or used in dry cereal products. The amylose-extender opaque-2 double mutants offers a high quality protein intermediate sugar corn.

When this project was initiated in 1970, two high lysine maize mutants had been identified: opaque-2 and floury-2. (Objectives a, e and j). We have since identified six additional mutants which influence lysine: sugary-1, shrunken-1, shrunken-2, shrunken 4, brittle-1 and brittle-2. McWhirter in Australia has identified a seventh high lysine mutant, opaque-7. In addition we have demonstrated that double mutant combinations of these seven with opaque-2 have as high or higher lysine content than opaque-2 alone. Rat feeding tests have demonstrated the superior biological value of some of the double mutant combinations.

The Central biochemical unit has functioned as a center for instruction in and standardization of analytical procedures for determination of lysine, tryptophan and protein in maize samples. (Objective h). Four trainees (Colombia, Nigeria, Indonesia and Nepal) have received instruction in methodology and at least ten graduate students in agronomy have taken a graduate special problems course in amino acid and protein analysis of cereals.

Simple methods for determination of lysine, tryptophan and protein have been evaluated. (Objective i). This has been a cooperative project with Dr. E. Villegas at CIMMYT and has resulted in a publication (CIMMYT Research Bulletin No. 20) describing the best methods. In addition, a brochure on methods at Purdue has been prepared for publication.

In 1972 our laboratory organized a feeding test using a group of representative maize, wheat, triticales and sorghum samples which were sent to cooperating universities and tested for protein efficiency response in several species of laboratory animals. (Objective j).

These tests demonstrated that the rat was superior to other species tests (mouse, vole, chick) for evaluation of the protein quality of cereals.

Basic research on the nature of the proteins in high lysine types of maize has led to some very fruitful results. (Objectives e and i). A new method of fractionating the proteins (Landry-Moureaux) has increased our understanding of the nature of the protein and amino acid changes that occur in high lysine maize mutants. The findings are briefly summarized below.

Albumins, globulins and glutelins increase, and zein decreases in the nine mutants which influence lysine. (Objectives e and i). In normal maize, zein production was evident 14 days past pollination and reached a peak 42 days post pollination. Zein production is delayed in brittle-2 and opaque-2 and along with a slower rate of synthesis, the total production of zein is less than 50% of normal. In the double mutant of brittle-2 opaque-2, zein formation was not apparent at any time during development.

The level of free amino acids in opaque-2 alone and in all double mutant combinations with opaque-2, is two to ten times higher than the respective normal counterpart. (Objectives e and i). The marked increase in free amino acids with opaque-2 has been used as the basis of a rapid ninhydrin test for identification of seeds homozygous for this gene. The test also identifies high lysine mutants of barley and sorghum.

2. Interpretation of Data and Supporting Evidence:

a. New Genes and Germplasm That May Provide Improved Protein Quality

(1) Promising new mutants from Colombian or other germplasm (Objective a)

Fifteen sources of Colombian races, hybrids and composites were self-pollinated and each ear inspected to find putative new endosperm mutants (Table 1). The 116 found were planted ear-to-row, self-pollinated again and outcrossed to ETO (a Colombian commercial variety) and to A619 x A632 where possible. Whether the F_2 segregation fit the genetic ratios 3:1 and 15:1 was tested by Chi square. The expression of most of the mutants was subject to phenotypic modification by genes which were in the source population, in ETO, or in A619 x A632. Allele tests with four known mutants, $\underline{o}_2$, $\underline{o}_1$, $\underline{fl}^a$ and $\underline{h}$ (soft starch) were carried out for 15 of the mutants. Four mutants from World Composite showed allelism to $\underline{fl}^a$ and one from Andaqui was allelic to $\underline{o}_2$, the other mutants tested were not allelic to the four genes tested.

In one or more analysis, eight different mutants showed a high lysine content (Table 2). An additional eight mutants had at least one family which was intermediate in lysine content. Work is in progress on further allele tests and analyses for lysine content.

(2) Temperate Germplasm (Objectives a, b, c and k)

Two $\underline{o}_2$ varieties have been developed from the germplasm listed below and designated as Temp HA$\underline{o}_2\underline{o}_2$ and Temp HB$\underline{o}_2\underline{o}_2$.

TABLE 1. Putative New Endosperm Mutants from Self-pollinated Ears

Source	Number of self-pollinated ears examined	Number of putative mutants
Pira	219	1
Clavo	22	0
Andaqui	152	7
Amagaceno	240	1
Puya Grande	88	0
Cariaco	128	12
White ETO	669	1
Yellow ETO	400	42
Flint Costeno Tropical	605	4
White & Yellow Mixture	443	4
Variety 153	392	0
Variety 105	333	9
World Composite	205	16
Hybrid 254	195	13
Hybrid 206	715	6
TOTAL		116

TABLE 2. Promising New Endosperm Mutants.

Source	Mutant No.	Lysine (g/100g protein)			Probable F_2 Ratio
		S_3 Ears	F_2 with ETO	F_2 with A619 x A632	
World Composite					
	9	4.24	2.60	-	3:1
	9	4.12	-	-	-
	9	3.71	-	-	-
	11	4.41	2.62	-	1:1
	11	2.74	-	-	-
	11	2.28	-	-	-
	11	2.17	-	-	-
Yellow ETO	40	4.35	-	-	-
	40	4.38	-	-	-
	54	3.74	-	-	3:1
	58	4.61	-	-	15:1
	62	4.70	-	-	15:1
Flint Costeno Tropical					
	88	2.84	-	2.73	3:1
	88	4.87	-	2.76	15:1
Cariaco	106	4.86	-	2.22	3:1
	106	2.60	-	2.12	3:1
	106	4.43	-	-	3:1
	106	3.10	-	-	-
	106	2.64	-	-	-

Temp HAo_2o_2

Composite D
Syn 200
Syn 493
Syn 201
Samaru Comp.

crossed with Corn Belt Syn A o_2o_2

Temp HBo_2o_2

Composite CD
Composite CBD
Composite AC
Syn 494
Syn 2
Seleccion Nasal

crossed with Corn Belt Syn B o_2o_2

The original cross was followed by three generations of random mating. Mild selection pressure has been applied for modified opaque phenotype and resistance to Helminthosporium turcicum, smut (Utilago zea), and kernel rot (Fusarium moniliforme).

In the 1973 summer nursery about 350 full sibs were made in each variety. Based on disease resistance, modification, and grain quality, about 130 full sibs were selected and will be tested in Israel, Mexico, Pakistan and United States (Purdue). Phenotypically these two synthetics appear quite promising. Subsequent agronomic and protein quality evaluation and selection will determine their ultimate value. It may be an advantage to shift to reciprocal recurrent selection after several cycles of the full sib procedure.

Because of their potential value the su_2o_2, wx o_2, and bt_2o_2 combinations have been developed in these two varieties by backcrossing. Intensive selection is being conducted in the su_2o_2 which is the most promising version of these three combinations. A very preliminary sampling and chemical evaluation of the Temp HA +/$\underline{su}_2$ $\underline{o}_2\underline{o}_2$ and Temp HB +/$\underline{su}_2$ $\underline{o}_2\underline{o}_2$ synthetic varieties was conducted. This was done following the introduction of the $\underline{su}_2$ into the two $\underline{o}_2$ varieties, backcrossing twice to the respective $\underline{o}_2$ variety followed by two generations of random mating without selection within each variety. The mean and range in protein percent and lysine in the whole kernel of $\underline{o}_2$ and $\underline{su}_2$ $\underline{o}_2$ segregates from the same sampled ears from the Temp HA +/ $\underline{su}_2$ $\underline{o}_2\underline{o}_2$ variety are presented in Table 3 and those values from the Temp HB +/$\underline{su}_2$ $\underline{o}_2/\underline{o}_2$ variety are presented in Table 4. The data appear quite promising for $\underline{su}_2$ $\underline{o}_2$ when compared to the $\underline{o}_2$ counterpart. The protein quality is maintained in $\underline{su}_2$ $\underline{o}_2$, there is a range in variability and an examination of the distributions showed them to be normally distributed. Kernel vitreousness was markedly improved in the $\underline{su}_2$ $\underline{o}_2$ kernels compared to those of $\underline{o}_2$.

The brown-midrib-3 gene which decreases lignin content and thereby increases digestibility of corn stover (Table 5) offers tremendous promise to increase the nutritional value of the corn crop. Although research in this area is not supported by this project, it was thought wise to incorporate this gene into these varieties because the corn plant is fully utilized for animal feed in most of the developing countries. A promising short plant mutant is also being incorporated into these varieties.

Table 3. Temperate HA $\underline{su_2o_2}$ synthetic whole kernel protein percent, lysine as a percent of protein and lysine percent of sample.

Genotype		Protein (%)	Lysine as a percent of protein	Lysine as percent of sample
$\underline{o_2}$	$\bar{x}$ (25 ears)[+]	11.92	4.17	.497
	Range	(9.3 - 13.7)	(3.25 - 4.72)	(.38 - .59)
$\underline{su_2o_2}$	$\bar{x}$ (25 ears)	11.73	4.28	.500
	Range	(9.9 - 13.8)	(3.35 - 5.08)	(.40 - .58)

[+] Ears were segregating +/$\underline{su_2}$ $\underline{o_2}/\underline{o_2}$ - 25 random ears selected and $\underline{o_2}$ and $\underline{su_2o_2}$ kernels analyzed from some ears.

Table 4. Temperate HB $\underline{su_2o_2}$ synthetic whole kernel protein percent, lysine as a percent of protein and lysine percent of sample.

Genotype		Protein (%)	Lysine as a percent of protein	Lysine as percent of sample
$\underline{o_2}$	$\bar{x}$ (26 ears)[+]	12.3	4.17	.51
	Range	(10.5 - 14.8)	(3.80 - 4.54)	(.42 - .61)
$\underline{su_2o_2}$	$\bar{x}$ (26 ears)	12.2	4.29	.52
	Range	(10.3 - 16.2)	(3.75 - 4.69)	(.45 - .62)

[+] Ears were segregating +/$\underline{su_2}$ $\underline{o_2}/\underline{o_2}$ - 26 random ears selected and $\underline{o_2}$ and $\underline{su_2o_2}$ kernels analyzed from same ears.

Table 5. Daily gain, feed efficiency and intake of corn stover silages fed to cattle.

	Silage	
	$\underline{bm_3}$	Commercial hybrid
Average daily gain, lb.	2.39	1.16
Feed/gain, lb/lb.	5.36	7.43
Daily intake		
Dry matter, % BW[a]	2.06	1.47
Fiber, % BW	1.33	1.00
Digestible, DM, % BW	1.15	0.73
Digestible fiber, % BW	0.80	0.53

[a] BW = body weight

(3) Selection for high protein content in opaque-2 varieties (objectives a and c) - Three cycles of modified ear-to-row selection for increased protein percent along with mild agronomic selection for yield, lodging, etc., have been completed in CB Syn HAo_2o_2 (previously designated Syn Ao_2o_2) and Iowa Super Stiff Stalk. The average protein and ranges are 12.7% and 12.0-13.5% for CB Syn HAo_2o_2 and 11.7% and 11.1-12.5% for SSS. Two commercial o_2o_2 hybrids averaged 10.0% protein. In 1974 the selected families in each variety will be intercrossed for possible release as a source of germplasm for higher protein content for temperate areas.

(4) Opaque hybrids (objective a) - A uniform test of experimental o_2 hybrids was organized and conducted in four states in the U. S. cornbelt. The results are given in Table 6. The yields of o_2 hybrids ranged from 50 to 94 Q/hectare (140 bu./acre).1/ A number of these experimentals gave excellent yields that would be quite competitive with some of the better normal commercial hybrids. Protein content and quality was also quite good with ranges of 8.6 to 10.7 for protein percent, 3.94 to 4.74 for g lysine/100 g protein and .38 to .50 for lysine as percent of sample.

(5) Evaluation of independent opaque-2 mutant sources (objective a) - Seven mutant sources of the o_2 gene are being backcrossed into the common inbred backgrounds W64A and B37. These mutant sources will then be

1/ Q = Quintal = 100 kilo

TABLE 6. 1973 Data on Opaque-2 Hybrid Uniform Test

PEDIGREE	Yield					Moisture	Stalk Lodging		Stand
	Ill.[1/] Q/ha	Iowa Q/ha	Ind. Q/ha	Mo. Q/ha	Ave. Q/ha	%	%	L/P	%
Mo. 2^{Rf} o_2 x Mo. 20 W o_2	108	71	92	68	85.8	25.5	6.0	4.40	92
Mo. 2^{Rf} o_2 x K41 o_2	97	74	68	78	80.2	25.6	9.8	4 06	96
Mo. 2^{Rf} o_2 x 33-16 o_2	74	46	36	38	49.5	25.2	10.2	4.06	88
Mo. 2^{Rf} o_2 x K55 o_2	109	75	99	78	90.2	26.3	1.4	4.32	97
(Mo. 2^{Rf} o_2 x K55 o_2) x (Mo. 20 W o_2 x K41 o_2)	89	68	67	72	74.0	26.7	9.4	4.28	96
K55 o_2 x K41 o_2	92	68	70	77	76.8	26.0	1 [illegible]	4.54	94
Mo. 17 o_2 x N28 o_2	101	94	99	51	86.2	25.6	0.5	4.50	92
Va 35 o_2 x Mo. 17 o_2	84	80	66	53	70.8	23.5	3.1	3.94	92
R182 o_2 x N28 o_2	110	95	93	50	87.0	22.9	0.8	4.50	94
C123 o_2 x N28 o_2	109	75	80	60	81.0	23.3	6.7	4.22	90
R177 o_2 x N28 o_2	92	85	88	65	82.5	24.0	0.8	4.46	93
R177 o_2 x Va 43 o_2	105	80	97	65	86.8	22.7	4.7	4.63	94
Va 43 o_2 x N28 o_2	115	82	113	66	94.0	25.9	4.3	4.49	94
(B37 o_2 x B14A o_2) x B57 o_2	95	66	76	48	71.2	23.9	7.8	4.51	82
(R802 o_2 x R177 o_2 x C123 o_2	87	72	84	60	75.8	21.9	4.1	4.28	94
A619 o_2 x A632 o_2	75	64	65	39	60.8	19.3	1.1	4.66	90

(CONTINUED)

TABLE 6 (Continued)

Pedigree	Yield					Moisture %	Stalk Lodging %	L/P	Stand %
	Ill. Q/ha	Iowa Q/ha	Ind. Q/ha	Mo. Q/ha	Ave. Q/ha				
W64 o_2 x W117 o_2	73	60	41	28	50.5	18.5	11.5	4.74	86
(N28 o_2 x N31 o_2) x R Mo. 17 o_2	98	88	91	63	85.0	25.0	3.9	4.56	92
(A632 o_2 x B14A o_2) x C123 o_2	86	63	60	45	50.0	19.9	7.3	4.28	85
(R177^6 o_2 x C123^6 o_2) x 00387-1 o_2	114	78	82	68	85.5	22.0	8.5	4.59	94
(B14^6 o_2 x B37^6 o_2) x 00374-A o_2	106	69	85	60	80.0	19.6	8.9	4.44	96
(B14^6 o_2 x B37^6 o_2) x 00368-A o_2	95	72	98	53	79.5	21.9	3.6	4.69	84
(R177^6 o_2 x C123^6 o_2) x 00402-A o_2	94	78	91	60	80.8	21.5	6.4	4.51	94
(R177^6 o_2 x C123^6 o_2) x 00397-A o_2	95	79	72	59	76.2	23.3	4.8	4.55	92
(B14^6 o_2 x B37^6 o_2) x 00379-A o_2	99	81	97	68	86.2	20.7	4.0	4.72	96
(B37 o_2 x H84 o_2) x 00379-A o_2	109	83	102	74	92.0	22.5	5.1	4.64	96
B37^{Cm} s x N28 ^{Ht} check	107	87	98	59	87.8	24.6	5.6	3.21	92
H49^{Cm} s x N28 ^{Ht} check	125	96	125	68	103.5	25.0	9.2	3.07	94

[1]Q=Quintel=100 Kilo

intercrossed in all possible combinations to determine (a) if any o_2 sources are superior to others and (b) if the o_2 gene is a compound locus.

b. Modified Opaque-2

(1) Performance of modified and nonmodified selections in opaque-2 populations (objectives a, b, c and k) - (Mr. William Camerer's thesis program). Visual selections for kernels with modified and nonmodified phenotypes were made in four o_2 populations. These selected classes were then grown in a yield test with six replicates and data on performance obtained (Table 7). The selected versions did differ in modification factor and percent modified, although not by a large margin. The selected versions did not differ significantly for yield, kernel weight, percent protein, g lysine/100 g protein or kernel rot grade.

Skewed selections were made in two of the populations to further evaluate the effect on performance of selection for modified phenotype. The data in Table 8 show a much larger difference in modification factor and percent modified kernels (43.3 vs 8.8%). This skewed selection still did not result in any significant difference in yield, kernel weight or protein characteristics. Kernel rot grade was significantly less in the modified version.

(2) Evaluation of sources for modified opaque-2 (objectives a and c) - Eight Colombian maize populations were tested as sources of modifier genes for opaque 2. Seven race

Table 7. Characteristics of modified and nonmodified selections in four populations.

	Modified		Nonmodified
Yield (g/plant)	126.5		126.9
Kernel weight (g/50 K)	12.7		12.6
Modification factor1/	26.0	*	15.2
Percent modified	36.4	*	23.0
Percent protein	11.6		11.9
g lys/100 g protein	4.0		4.1
Kernel rot grade	1.2		1.3

* Indicates significant difference between modified and nonmodified classes.

1/ Based on combination of percent of kernel modified and the degree of modification.

Table 8. Characteristics of modified and nonmodified skewed selections in two populations.

	Modified		Nonmodified
Yield (g/plant)	137		139
Kernel weight (g/50 K)	12.0		12.0
Modification factor 1/	30.6	*	5.0
Percent modified	43.3	*	8.8
Percent protein	11.56		11.88
g lys/100 g protein	4.25		4.27
Kernel rot grade	1.21	*	1.42

* Indicates significant difference between modified and nonmodified classes.

1/ Based on combination of percent of kernel modified and the degree of modification.

collections and a population derived from ETO x USA342 were self-pollinated and outcrossed to the homozygous $\underline{o}_2$ lines 215 and 216 which have the soft endosperm expression. The F_2 segregation was classified in three- and four-class separations. Chi square tests indicated whether the intermediate classes II or II and III were modified $\underline{o}_2$ kernels or if they deviated significantly from 3:1 due to an excess of normal kernels, where possibly some homozygous $\underline{o}_2$ kernels were so modified that they were visually indistinguishable from normal kernels. The results are summarized in Table 9. A high percentage of intermediates and/or families with excess normals indicated that ETO x USA342, Pira, Puya and Común were good sources of modifier genes for $\underline{o}_2$. Amagaceño was less promising and, based on small samples, Clavo, Chococeño and Pollo were not promising sources of modifiers.

(3) <u>Genetic and environmental stability of modified (vitreous) varieties and their protein quality</u> (objectives a and c) - Research at CIMMYT and Purdue has demonstrated that (a) it is fairly easy to develop modified (vitreous) $\underline{o}_2$ varieties with good ear rot and grain insect resistance and (b) these varieties have excellent protein quality if selections are made based on analyses. A very critical question involving the practical utilization of selected high lysine "modified" $\underline{o}_2$ varieties is concerned with the stability of the vitreous expression and high lysine characteristics under random mating and various environmental conditions when used by the farmer.

TABLE 9. Summary of opaque-2 modification potential

Source	F_2 families from L215 Fit 3:1	PI*	Excess "Normals"	F_2 families from L216 Fit 3:1	PI*	Excess "Normals"
Amagaceno	2/6	34.2	1/6	6/9	35.3	1/9
Comun	6/17	44.8	0/17	14/23	57.7	4/23
Clavo	2/4	53.9	0/4	0/2	-	1/2
Chococeno	0/2	-	0/2	1/3	51.3	0/3
ETO x USA 342	18/30	54.2	5/30	10/19	45.8	7/19
Pira	18/36	56.4	12/36	9/17	35.9	3/17
Pollo	0/3	-	1/3	3/5	64.6	0/5
Puya	15/25	64.4	3/25	14/33	52.2	4/33

* PI = (intermediates / opaque + intermediates) x 100.

Two groups of materials are being developed to evaluate the genetic and environmental stability of "modified" opaque-2.

(a) Several years ago this project developed and released a highly modified $\underline{o}_2$ variety developed from cornbelt germplasm. During the final stage of this development analyses of a large number of families were made for endosperm lysine (g lysine/100 g protein) with values ranging from 2.02 to 3.34. Before making final intercross of selected families to make up HM$\underline{o}_2\underline{o}_2$, selections were made for modified and nonmodified versions and high and low lysine within each version. Selection was effective because the endosperm lysine values for the next generation were 3.23 and 2.35 for the high and low selections, respectively. Continued selection should further differentiate the levels of lysine. There is also a very distinct difference in the degree of modification due to selection. High and low lysine inbreds are also being developed from these populations for more theoretical studies.

The high lysine and low lysine selections in the modified and nonmodified versions of HM$\underline{o}_2\underline{o}_2$ will be subjected to detailed fractionation (Landry-Moureaux method) of protein, amino acid content of those fractions and electrophoretic separation of proteins. Hopefully these studies will reveal what changes in protein occur, which may enable us to achieve the modified types with high protein quality.

(b) Selection of modified and nonmodified subpopulations have also been made in Temp HA$\underline{o}_2\underline{o}_2$ and Tem HB$\underline{o}_2\underline{o}_2$. The eight populations (four from section (a) and four from section (b) above) have been random mated for two or three successive generations. The original and successive versions will be tested at a number of locations to determine genetic and environmental stability in regard to modification and protein quality.

c. Endosperm mutant interactions (objectives a, e, j) - Several double mutant combinations of opaque-2 ($\underline{o}_2$) with other endosperm mutants (e.g. sugary-2 ($\underline{su}_2$), waxy (wx), amylose-extender (ae), dull (du), floury-1 ($\underline{fl}_1$), floury-2 ($\underline{fl}_2$), soft starch (h), shrunken-2 ($\underline{sh}_2$), brittle-1 ($\underline{bt}_1$), brittle-2 ($\underline{bt}_2$), sugary-1 ($\underline{su}_1$) and others) have been developed and evaluated in isogenic line comparisons in some inbred lines, several single cross hybrids and a 3-way hybrid. To date, two double mutant combinations, sugary-2 opaque-2 ($\underline{su}_2\underline{o}_2$) and waxy opaque-2 (wx $\underline{o}_2$)appear superior for improving nutritional quality of mature corn grain. Sugary-2 opaque-2 offers the most potential. Supporting data is given for these two double mutant combinations in this section.

The kernel characteristics of $\underline{o}_2$, $\underline{su}_2$, $\underline{su}_2\underline{o}_2$, wx, and wx $\underline{o}_2$ genotypes in isoline comparisons to normal are shown in Table 10 for six singlecross hybrids grown for two years in replicated trials, four additional singlecross hybrids grown for one year and a 3-way hybrid grown for one year in

Table 10. Means from several singlecross hybrids and a 3-way hybrid comparing $\underline{o}_2$, $\underline{su}_2$, $\underline{su}_2\underline{o}_2$, wx, $\underline{wx}_2\underline{o}_2$ and the normal counterpart hybrids for kernel characteristics.

Genotypes	100 K weight (g)	100 K volume (ml)	Kernel density (g/ml)
Two-year means from six singlecross hybrids			
normal	34.52	26.81	1.29
o_2	29.00	25.52	1.14
su_2	29.87	22.44	1.33
su_2o_2	27.07	21.59	1.25
wx	33.51	25.50	1.32
wx_2o_2	29.65	25.16	1.18
One-year means from four singlecross hybrids			
normal	28.61	22.71	1.26
o_2	26.83	24.13	1.11
su_2	28.25	21.92	1.29
su_2o_2	25.79	21.06	1.22
One-year means from 3-way hybrids			
normal	34.64	28.00	1.24
o_2	30.26	27.25	1.12
su_2	31.59	24.50	1.30
su_2o_2	28.97	22.75	1.27
wx	34.86	27.38	1.27
wx_2o_2	31.97	27.25	1.18

production plots. Kernel density in the su_2o_2 hybrids was markedly improved over the o_2 hybrids. This interaction between the su_2 and o_2 genes gives a more vitreous type of kernel, more nearly like normal corn. This achievement should increase acceptance of the high lysine corn, improve the resistance to ear rots and storage insect problems as well as increase the grit fraction for improved dry milling characteristics. Kernel volume (seed size) of su_2 was reduced and the double mutant su_2o_2 was reduced in seed size compared to normal and o_2. This no doubt was responsible for the reduced kernel weight of su_2o_2 and consequently lower yield that should be improved by selection in su_2o_2 populations to maximize the potential of this double mutant. Waxy and wx o_2 hybrids showed very little reduction in kernel volume compared to normal hybrids and kernel weight and density of wx o_2 hybrids was not different from o_2 hybrids.

Expanded emphasis is being placed on the su_2 and su_2 o_2 double mutant to find out the barriers involved in lower yield because of reduction in kernel size. Considerable selection pressure is being made for increased yield, seed size, weight, carbohydrate and protein quality in su_2o_2 variety development and isoline conversions. Developmental and dry matter accumulation studies are in progress. Studies are in progress on su_2 dosage interaction and the effects of outcrossing on protein quality, kernel size and weight and related characters. Studies of the compensating effects and relationship between kernel characteristics, carbohydrate, protein and oil accumulation will be initiated. The nature of kernel density and vitreousness in su_2o_2 is being studied in relationship to the protein body size and distribution and protein matrix in the endosperm. Detailed genetic and agronomic evaluations of independent (mutant) sources of the su_2 gene are underway.

The two-year means for the six genotypes from six single-cross hybrids for endosperm protein quality are presented in Table 11. Table 12 shows the one year means from an additional four singlecross hybrids and Table 13 provides one-year means from a 3-way hybrid for whole kernel protein quality. Protein percent of su_2o_2 hybrids was above that of o_2 hybrids and the lysine concentration (as a percent of protein and as percent of sample) was 14 to 17 percent greater in the endosperm and 5 to 8 percent greater in the whole kernel than o_2 hybrids. The su_2 hybrids were consistently better in lysine as a percent of protein and as percent of sample than the normal hybrids, but had lower levels of lysine than o_2 hybrids. In previous nitrogen fractionation studies in an inbred background we found that with the introduction of the su_2 gene the zein (prolamine fraction) content decreases and the major glutelin fraction (glutelin-3, fraction V, Landry-Moureaux procedure) increases. When su_2 is combined with o_2, the double mutant supresses zein synthesis and shows a synergistic effect. Lysine content in the wx o_2 hybrids was increased over that of o_2 hybrids, though not as markedly as in the su_2o_2 hybrids.

Table 11. Two-year means from six singlecross hybrids comparing o_2, su_2, wx, $wx\ o_2$ and the normal counterpart hybrids for endosperm protein quality.

Genotypes	Protein (%)	Lysine as a percent of protein	Lysine as percent of sample
normal	10.41 a**	1.75 d**	.18 d**
o_2	8.98 b	3.25 b	.29 b
su_2	11.04 a	2.20 c	.24 c
su_2o_2	9.21 b	3.72 a	.34 a
wx	10.55 a	1.84 d	.19 d
wx o_2	8.73 b	3.51 a	.31 b

** Means within a column followed by a common letter do not differ significantly at the .01 level of significance as determined by Duncan's Multiple Range Test.

Table 12. One-year means from an additional four singlecross hybrids comparing o_2, su_2, su_2o_2, and the normal counterpart hybrids for whole kernel quality protein.

Genotypes	Protein (%)	Lysine as a percent of protein	Lysine percent of sample
normal	11.48 c**	2.65 c**	.304 d**
o_2	12.96 b	4.01 b	.518 b
su_2	13.39 a	2.72 c	.362 c
su_2o_2	13.23 ab	4.19 a	.554 a

** Means within a column followed by a common letter do not differ significantly at the .01 level of significance as determined by Duncan's Multiple Range Test.

Table 13. One-year means from 3-way hybrids comparing o_2, su_2, su_2o_2, wx, wx o_2 and the normal counterpart hybrids.

Genotypes	Protein (%)	Lysine as a percent of protein	Lysine as percent of sample
normal	9.68	2.85	.276
o_2	10.03	4.04	.405
su_2	10.75	3.01	.324
su_2o_2	10.58	4.30	.455
wx	10.77	2.50	.269
wx o_2	10.62	4.09	.434

These data suggest that an enhancement in protein quality is possible in the su_2o_2 and $wx\ o_2$ double mutants.

Selection for the possibilities of higher protein content in the su_2o_2 double mutant recoveries from crossing into agronomically desirable selections of "opaque-2, high protein" lines recoveries is underway. Some exploratory effort is in progress to evaluate the possibilities that the multiple-aleurone character may have to improve the protein nutritional quality of o_2 and su_2o_2 grain by increasing the proportion of aleurone tissue (high in protein) relative to the starch portion of the endosperm. Protein quality of su_2o_2 double mutants in specific selections for "modified-opaque-2" vitreous kernel phenotypes is being investigated.

Starch expressed on a percentage basis was not different among the mutant and double mutant hybrids and from normal hybrids, but when expressed on a weight basis the su_2o_2 hybrids were reduced in starch content an average of 15% below normal hybrid counterparts (Table 14). Percent amylose in the su_2o_2 hybrids (41%) was increased over that observed in su_2 hybrids (37%). There was a 16 and 18 percent increase in oil content in the su_2 and su_2o_2 hybrids, respectively, compared to their normal counterpart hybrids as shown in Table 15 for the mean of the six hybrids over 2 years. Though o_2 hybrids were greater in oil percent than normal, there was no difference in oil quantity. Germ weight and percent of germ in the whole kernel of o_2 hybrids was significantly greater than normal hybrids.

Table 14. Two-year means for six singlecross hybrids comparing o_2, su_2, su_2o_2, wx, wx o_2 and the normal counterpart hybrids for percent endosperm starch and amylose.

Genotypes	Starch (%)	Amylose (%)
normal	63.26 n.s.	26.21 b**
o_2	63.70	26.23 b
su_2	62.39	37.01 a
su_2o_2	62.20	40.50a
wx	60.96	-
wx o_2	60.50	-

n.s. Means not significantly different

** Means within a column followed by a common letter do not differ significantly at the .01 level of significance as determined by Duncan's Multiple Range Test.

Table 15. Means for oil percent and content in the whole kernel of $\underline{o}_2$, $\underline{su}_2$, $\underline{su}_2\underline{o}_2$ and normal genotypes over hybrids.

Genotypes	Oil (%)	Oil/ kernel (mg)
Two-year means for six singlecross hybrids		
normal	4.24 d**	14.55 b**
o_2	5.01 c	14.53 b
su_2	5.64 b	16.89 a
su_2o_2	6.39 a	17.20 a
One-year means from 3-way hybrids		
normal	4.15	14.38
o_2	4.85	14.68
su_2	5.52	17.44
su_2o_2	6.20	17.96

**Means within a column followed by a common letter do not differ significantly at the .01 level of significance as determined by Duncan's Multiple Range Test.

However, in su_2 hybrids germ weight was reduced and germ percent of whole kernel was not significantly greater than normal counterparts. The germ percent in the su_2o_2 hybrids was increased as a result of the o_2 gene but the oil content of su_2o_2 hybrids was 18 and 25% greater on the average, for the singlecross hybrids and 3-way hybrids respectively, than for normal hybrids. This particular attribute may contribute to increased energy content of the su_2o_2 double mutant corn. Studies are in progress with the su_2 gene and su_2o_2 double mutant in high and low oil selections to evaluate the effect of their interaction on caloric content and protein quality.

Mineral element (P, K, Mg, Fe and Zn) content among the o_2, su_2, su_2o_2, wx, wx_2o_2 isogenic versions and normal counterpart hybrids was not too different (Table 16), except for potassium content of o_2 and the double mutant combinations with o_2. Opaque-2 showed an increase in potassium content compared to normal and a similar effect of o_2 in the double mutant interactions was apparent also.

Because of the interest in their use to improve nutritional and grain quality characteristics the following su_2 conversions with six backcrosses have been released: Oh43 su_2su_2, B37 su_2su_2, C103 su_2su_2 and A632 su_2su_2. In addition Oh43 wx wx, C103 wx wx and A632 wx wx have been released because of their possible use to improve nutritional characteristics. We have distributed seed of the Temp HA su_2su_2 o_2o_2 and Temp HB su_2su_2 o_2o_2 varieties being developed as well as

Table 16. One-year means from three singlecross hybrids comparing *o_2*, *su_2*, *su_2o_2*, *wx*, *wx o_2* and the normal counterparts for mineral content.

Genotypes	P	K	Mg	Fe	Zn
	mg/k				
normal	97.25 a**	110.92 cd**	47.58 a**	7.15 a**	6.64 a**
o_2	87.00 c	129.75 ab	42.83 b	5.86 bc	6.04 abc
su_2	83.58 cd	98.67 d	40.08 bc	5.99 b	5.84 bc
su_2o_2	78.00 d	128.58 abc	37.75 c	5.37 c	5.54 c
wx	94.50 ab	116.33 bcd	47.25 a	6.43 b	6.41 ab
wx o_2	88.00 bc	146.33 a	43.83 ab	5.84 bc	6.61 a

** Means within a column followed by a common letter do not differ significantly at the .01 level of significance as determined by the Duncan's Multiple Range Test.

single cross hybrid $\underline{su}_2\underline{su}_2\underline{o}_2\underline{o}_2$ seed for sources of the $\underline{su}_2$ gene for breeding programs in many countries to facilitate development of $\underline{su}_2$ $\underline{o}_2$ varieties or hybrids.

The relative in vitro digestibility of the $\underline{o}_2$, $\underline{su}_2$, $\underline{su}_2\underline{o}_2$, wx, wx $\underline{o}_2$ and normal inbred Oh43 starch granules with three different amylase enzymes are presented in Table 17. These data suggest that $\underline{su}_2$ and $\underline{su}_2\underline{o}_2$ starches were digested by amylases much faster than those of the normal and $\underline{o}_2$ counterparts. The wx and wx $\underline{o}_2$ starches showed improvement in digestibility of the starches by amylases compared to normal and $\underline{o}_2$ counterparts, though the improvement was not as great as in the case of $\underline{su}_2$and $\underline{su}_2\underline{o}_2$. The highly digestible raw starches produced by the $\underline{su}_2\underline{o}_2$ and wx $\underline{o}_2$ double mutants suggests that they should serve to improve the feeding value of the corn grain for food and feed. However, it has not been established whether or not this characteristic will necessarily improve biological value of the corns.

The biological value of $\underline{su}_2$, $\underline{su}_2\underline{o}_2$, wx, wx $\underline{o}_2$, $\underline{o}_2$ isogenic versions, the normal Oh43 x B37 singlecross hybrid and a casein control diet were compared. Whole ground grain was fed as isonitrogenous-isocaloric diets with mineral and vitamin supplement. Ten weanling rats were used in each group. Table 18 shows the average feed/gain ratio, daily weight gain and protein efficiency ratio (PER) for the 28-day feeding trial. The $\underline{su}_2\underline{o}_2$ and wx $\underline{o}_2$ diets improved the feed/gain ratios, daily weight gain and PER values when compared to the $\underline{o}_2$ diet, but these increases were not

Table 17. Digestion by amylases of starch granules of o_2, su_2, su_2o_2, wx and wx o_2 and the normal counterpart in inbred Oh43 maize.

Genotypes	Glucoamylase Period 1 Hr.	Glucoamylase Period 5 Hr.	Enzyme Pancreatin Period 1 Hr.	Enzyme Pancreatin Period 5 Hr.	Bacterial α-amylase Period 1 Hr	Bacterial α-amylase Period 5 Hr.
	%					
normal	6.5	36.0	30.2	86.0	22.1	50.9
o_2	11.5	50.3	46.5	88.4	24.4	57.1
su_2	26.1	77.8	76.0	93.8	68.0	86.8
su_2o_2	31.4	82.2	74.2	91.6	72.2	90.7
wx	8.2	53.2	44.3	52.9	22.6	67.1
wx o_2	16.6	71.8	84.6	88.4	31.1	72.0

Table 18. Biological values of isonitrogenous and isocaloric diets prepared with o_2, su_2, su_2o_2, wx and wx o_2 isogenic versions and normal of the singlecross Oh43 × B37 hybrid in a rat feeding experiment.†

Genotypes	Feed/gain ratio	Daily weight gain (g)	PER
normal	7.43 a**	1.09 b**	1.37 c**
o_2	4.30 b	2.68 a	2.35 b
su_2	6.81 a	1.44 b	1.57 c
su_2o_2	3.92 b	3.17 a	2.57 ab
wx	7.98 a	0.96 b	1.27 c
wx o_2	4.11 b	3.20 a	2.45 b
casein	3.63 b	3.22 a	2.80 a

† Ten weanling male rats in each group: Duration 28 days

** Means within a column followed by a common letter do not differ significantly at the .01 level of significance as determined by Duncan's Multiple Range Test.

significant at the P$>$.01 level. The $\underline{su}_2\underline{o}_2$ diet did show a positive trend since there was a 12 percent improvement in gain over the $\underline{o}_2$ diet. Preliminary rat feeding studies were conducted previously comparing the biological value of these double mutants and others when fed as 95% of the diet (inherent grain protein levels) with mineral and vitamin supplement. Both $\underline{su}_2\underline{o}_2$ and $\underline{wx}$ $\underline{o}_2$ diets gave better gains than the $\underline{o}_2$ diets. These data suggest that the change in the starch composition and vitreousness, in the case of the $\underline{su}_2\underline{o}_2$ double mutant, has not negatively affected the biological value of these corns. The effect of these double mutants on the metabolism and performance of swine is being evaluated.

Preliminary studies on the effects of $\underline{su}_2$, $\underline{su}_2\underline{o}_2$, $\underline{wx}$, $\underline{wx}$ $\underline{o}_2$ and $\underline{o}_2$ isogenic versions and the normal 3-way hybrid in a high-concentrate diet on energy and nitrogen utilization in finishing beef cattle have been conducted with the cooperation of Mr. V. M. Thomas and Dr. W. M. Beeson of the Animal Science Department. Each t pe of corn was fed on an isonitrogenous basis at approximately 11 percent crude protein at 2.0 to 2.5% of their body weight per head per day. Steers fed the $\underline{su}_2\underline{o}_2$ diet retained significantly higher percent absorbed nitrogen (64.77) than did those steers fed the normal (47.20) or $\underline{su}_2$ (49.82) diets (Table 19). The $\underline{su}_2\underline{o}_2$ diet improved the levels of rumen ammonia, digestible and metabalizable energy when compared to the other corn diets, but these increases were not significant at the P$>$.05 level. Steers fed the $\underline{wx}$ corn diet tended to have improved nitrogen retentions when compared to the other diets (Table 20), although these improvements

Table 19. Effect of sugary-2, sugary-2 opaque-2 and waxy corn on nitrogen and energy utilization in finishing beef cattle.

Variable	Treatment: Regular Corn	su_2 Corn	su_2o_2 Corn	wx Corn	SEM[d]
Dietary N retained, %	29.68	32.51	45.45	35.34	3.61
Absorbed N retained, %	47.20[b]	49.82[b]	64.77[c]	53.99[bc]	3.14
Rumen ammonia[a]	17.11	11.79	19.61	17.20	3.26
Digestible energy, %	75.91	75.59	77.91	76.31	1.20
Metabolizable energy, %	73.14	73.22	75.86	73.92	1.31
Rumen VFA's, %					
Acetic	49.83	48.17	48.51	49.32	2.80
Propionic	32.99	33.21	33.64	23.80	2.22
Butyric	11.37	13.71	14.53	15.64	2.43
Isovaleric	3.86	2.71	3.79	3.23	.72
Valeric	2.45	2.22	2.52	3.00	.45
Rumen pH	6.22	6.30	6.37	6.38	.16

[a]Mg. NH_3-N per 100 ml. rumen fluid.

[bc]Means having the same superscript do not differ significantly ($P > .05$).

[d]Standard error of the mean.

Table 20. Effect of opaque-2, waxy and waxy opaque-2 corn on nitrogen and energy utilization in finishing beef cattle.

Variable	Treatment				SEM[d]
	Regular Corn	o_2 Corn	wx Corn	wxo_2 Corn	
Dietary N retained, %	39.33	40.02	42.60	40.31	3.33
Absorbed N retained, %	58.96	58.31	64.15	59.74	4.23
Rumen ammonia[a]	15.80	12.46	14.44	16.34	1.51
Digestible energy, %	78.21[b]	77.90[b]	74.67[c]	78.20[b]	.31
Metabolizable energy, %	75.64[b]	75.18[b]	72.45[c]	76.05[c]	.45
Rumen VFA's, %					
Acetic	47.60	44.91	47.64	47.26	3.64
Propionic	35.77	38.19	34.25	34.74	4.81
Butyric	11.84	10.58	13.64	13.78	1.92
Isovaleric	2.16	2.15	1.97	2.38	.14
Valeric	2.61	4.25	2.49	2.61	.89
Rumen pH	6.01	6.12	6.15	6.17	.21

[a]Mg. NH_3-N per 100 ml. rumen fluid.

[bc]Means having the same superscript fo not differ significantly ($P > .05$).

[d]Standard error of the mean.

were not significantly different ($P > .05$). Waxy corn was significantly lower ($P > .05$) in digestible and metabolizable energy than normal, $\underline{o}_2$ and $\underline{wx}$ $\underline{o}_2$ corns. There were no statistically significant differences in rumen ammonia and rumen volatile fatty acids. Sugary-2 opaque-2 was the only diet that consistently improved the nitrogen and energy utilization by the animals involved. It would appear from this data that the $\underline{su}_2\underline{o}_2$ double mutant shows the most promise for improving the nutritional balance of beef cattle. It is suggested that further studies with larger numbers of animals and under high intake feed conditions may show the $\underline{su}_2\underline{o}_2$ corn to be beneficial to improve feeding efficiency.

The data on $\underline{su}_2\underline{o}_2$ to date point to the fact that the improved characteristic which the $\underline{su}_2\underline{o}_2$ corn has over modified-opaque-2 corns is the improved vitreousness of the kernel that is not necessarily accompanied by a decrease in protein quality. Modified-opaque-2 corns have shown some decrease (without selection) in protein quality with modification to a more vitreous kernel type, and the standard opaque-2 endosperm phenotype is soft starch and nonvitreous.

Not unlike the normal varieties of the U. S. and tropics, the vegetable maize varieties have poor protein quality. The high-lysine mutant $\underline{o}_2$ has been almost unused in vegetable corn improvement. We began some preliminary studies to evaluate the kernel characteristics and endosperm protein and carbohydrate composition related to quality and content of selected endosperm mutants and their double-mutant combinations with opaque-2. Isogenic hybrids were sampled at 21 and 42 days after pollination, more or less representative of vegetable corn maturity.

The mean protein percent and lysine (expressed as a percent of protein) of genotypes sampled at 21 and 42 days after pollination (DAP) are presented in Table 21. The means are based on two replications and six isogenic hybrid backgrounds. At the early stage (21 DAP), protein quality was high and not too different. Sugary-1 opaque-2, $\underline{sh}_2\underline{o}_2$ and $\underline{ae}$ $\underline{o}_2$ had the highest levels of lysine in the endosperm (4.24, 4.23 and 4.26 g/100 g protein, respectively). As development proceeded protein quality differences become more apparent among the genotypes and from $\underline{o}_2$ and the normal. Again $\underline{su}_1\underline{o}_2$, $\underline{sh}_2\underline{o}_2$ and $\underline{ae}$ $\underline{o}_2$ had the highest endosperm lysine values (4.13, 4.16 and 3.94 g/100 g protein) at 42 DAP. As expected, protein percent dropped considerably from 21 to 42 DAP except in the $\underline{sh}_2$ and $\underline{sh}_2\underline{o}_2$ genotypes. The $\underline{sh}_2\underline{o}_2$ genotype compares favorably with protein quality of the $\underline{bt}_2$ (15.6% protein and 3.81 lysine as a percent of protein) and $\underline{bt}_2\underline{o}_2$ (17.8% protein and 4.73 lysine as a percent of protein) previously reported.

The moisture percentage and carbohydrate quality at 21 and 42 DAP are given in Tables 22 and 23, respectively. The means for the carbohydrate data are from two replications and two hybrids, while those for moisture percentage are from four replications and six hybrids. The $\underline{su}_1\underline{o}_2$ has high lysine and high water-soluble polysaccharide,

Table 21. Two-year means from six singlecross hybrids comparing selected mutant and double mutant genotypes and their normal counterpart hybrids for endosperm protein quality at 21 and 42 days after pollination.

	Days after pollination			
	21		42	
Genotypes	Protein (%)	Lysine as a percent of protein	Protein (%)	Lysine as a percent of protein
normal	14.8	3.73	10.9	2.24
o_2	13.9	4.16	10.0	3.31
su_1	15.1	3.86	11.8	2.67
su_1o_2	14.7	4.24	11.6	4.13
sh_2	16.2	3.94	15.4	3.46
sh_2o_2	15.1	4.23	15.0	4.16
fl_2o_2	14.7	4.09	10.9	3.36
ae o_2	14.6	4.26	10.7	3.94
su_2o_2	14.1	4.17	10.0	3.85
wx o_2	13.9	4.16	10.1	3.60
du o_2†	15.0	4.10	10.0	3.70

† One-year data

Table 22. Two-year means from six singlecross hybrids comparing selected mutant and double mutant genotypes and their normal counterparts for moisture percent and carbohydrate quality at 21 days after pollination.

Genotypes	Moisture (%)	Reducing-sugars/ endosperm (mg)	Sucrose/ endosperm (mg)	WSP/ endosperm (mg)	Starch/ endosperm (mg)
normal	73.7	4.2	6.8	.30	24.9
o_2	76.4	3.2	5.7	.23	22.2
su_1	77.4	4.4	7.0	12.16	11.6
su_1o_2	74.7	4.1	9.1	10.95	9.8
sh_2	76.7	6.0	20.9	.30	5.4
sh_2o_2	76.2	5.1	23.5	.31	4.6
fl_2o_2	76.2	2.9	5.6	.26	22.9
ae o_2	77.8	4.5	9.2	.31	20.1
su_2o_2	71.5	4.1	6.8	.29	23.1
wx o_2	77.4	4.0	6.4	.35	23.7
du o_2†	78.2	4.5	7.9	.28	22.9

† One-year data

Table 23. Two-year means from six singlecross hybrids comparing selected mutant and double mutant genotypes and their normal counterparts for moisture percent and carbohydrate quality at 42 days after pollination.

Genotypes	Moisture (%)	Reducing-sugars/ endosperm (mg)	Sucrose/ endosperm (mg)	WSP/ endosperm (mg)	Starch/ endosperm (mg)
normal	44.2	4.4	10.1	.81	154.0
o_2	50.8	2.8	7.2	.54	137.1
su_1	60.8	10.5	14.9	50.93	56.2
su_1o_2	64.8	8.5	14.2	45.15	39.5
sh_2	67.5	6.2	21.0	.63	28.8
sh_2o_2	69.3	6.1	17.7	.61	22.1
fl_2o_2	49.4	3.8	6.0	.61	128.3
ae o_2	56.6	4.5	8.7	.52	125.7
su_2o_2	52.1	4.3	8.1	.78	133.7
wx o_2	52.2	4.6	7.9	.66	128.1
du o_2†	52.2	5.1	5.8	.65	126.8

† one-year data

conferring the desired textural properties for fresh or canned corn. Shrunken-2 opaque-2 as well as bt_2o_2 (data not shown, but nearly the same carbohydrate content) would offer a high sugar high lysine corn with the potential for extending the harvest period and storage life of the supersweet corns. They also could make a desirable snack food or dry cereal breakfast food product. We have introduced the bt_2o_2 double mutant into the Temp HA and Temp HB varieties and some selection will be made for improved vegetable types. (objective a). The ae o_2 combination may offer a high quality protein corn with intermediate sugar levels compared to that of normal and sh_2o_2 or bt_2o_2. Perhaps ae o_2 could be used in certain food preparations and snack foods as well as a sweet corn type. The wx o_2 double mutant had a higher lysine content than o_2 at the later stage (42 DAP) Table 21. Waxy maize (glutinous) has been used widely in the food industry. The waxy starch has the special properties of solution stability, clarity of solution and thickening power. Waxy starch also has the modification which contributes to the fatting properties necessary in extrusion processes for making snack type foods. There are many people of the world who utilize the waxy varieties as fresh corn at a more mature stage of development. It appears that wx o_2 may offer a glutinous high-lysine corn for fresh corn or roasting ears and possibly other food preparations.

d. Lysine and protein distribution in single and double maize endosperm mutants (objectives a, b, and e) - Table 24 shows the chromosome location, increased lysine

Table 24. Single Endosperm Mutants of Maize [1/]

Mutant gene	Chromosome	Lysine (g/100 g protein)	Increased Lysine (% above normal)	Zein (%)	Glutelin (%)	Saline Soluble (%)
Normal	-	1.6	-	59	13.8	5.8
Opaque-2	7	3.5	118	26.9	29.2	13.6
Floury-2	4	2.7	69	49.1	22.0	9.2
Opaque-7[2/]	10	3.5	65	20.3	29.5	16.6
Sugary-1	4	1.8	12	27.1	22.8	11.9
Shrunken-1	9	1.9	19	43.7	16.3	8.2
Shrunken-2	3	2.7	69	29.4	23.6	12.3
Shrunken-4	5	3.0	87	30.8	23.6	25.7
Brittle-1	5	2.3	44	36.0	27.4	8.8
Brittle-2	4	3.3	106	26.1	27.9	12.1

[1/] Near-isogenic lines in Oh 43 inbred

[2/] Compared with isogenic inbred W22 (40.6% zein, 21% glutelin, 6.9% saline-soluble, and 2.3 g lysine/100 g protein) levels, and zein, glutelin and saline-soluble protein distribution in nine endosperm mutants with above normal lysine levels. Introduction of these mutant genes increases lysine 12 to 118 percent above the normal level. In all cases zein levels fall, and glutelin and saline-soluble proteins increase. Table 25 shows the effect of incorporating $\underline{o}_2$ along with one of eight other mutant genes to give the double mutants. Introduction of the $\underline{o}_2$ gene with one other gene affecting lysine content of the endosperm increases lysine 52 to 230 percent above the normal level. Zein levels fall to below 10 percent in all except $\underline{o}_2$ $\underline{fl}_2$, and glutelin and saline-soluble proteins increase to a greater extent than with the single mutants. These promising increases in lysine content are tempered by the fact that yield of some of the double mutants are reduced compared to normal or $\underline{o}_2$.

e. Zein levels in developing maize endosperms (objectives a, b and e) - Figure 1 shows that 14 days post-pollination 10% of the nitrogen is zein nitrogen in normal maize endosperm, whereas little or no zein has formed in $\underline{o}_2$, $\underline{bt}_2$, or the double mutant, $\underline{o}_2\underline{bt}_2$. By the 21st day, the normal endosperm proteins contain about 32 percent zein, the $\underline{bt}_2$ and $\underline{o}_2$ 10 to 14 percent, and $\underline{bt}_2\underline{o}_2$ zero percent. By the 42nd day normal endosperm proteins contain about 53 percent zein, and $\underline{o}_2$ and $\underline{bt}_2$ about 20 percent. At no time during development of the $\underline{o}_2\underline{bt}_2$ endosperm are zein proteins in evidence.

Table 25. Double Endosperm Mutants of Maize [1]

Double Mutant	Lysine	Increased Lysine in Endosperm (% above normal)	Zein (%)	Glutelin (%)	Saline Soluble (%)
Normal	1.6	-	59.0	13.8	5.8
o_2fl_2	2.7	69	25.0	24.8	17.0
o_2o_7	3.5	52[2]	8.7	33.3	17.6
o_2su_1	3.9	144	3.0	45.3	22.7
o_2sh_1	4.8	200	1.8	32.2	39.9
o_2sh_2	4.2	162	1.2	35.4	25.3
o_2sh_4	4.0	150	6.5	26.8	43.3
o_2bt_1	4.8	200	2.7	50.2	23.2
o_2bt_2	5.3	230	2.9	48.0	22.3

[1] Misra et al. (13,16)

[2] Compared with W22 normal endosperm (2.3 g lysine/100 g protein)

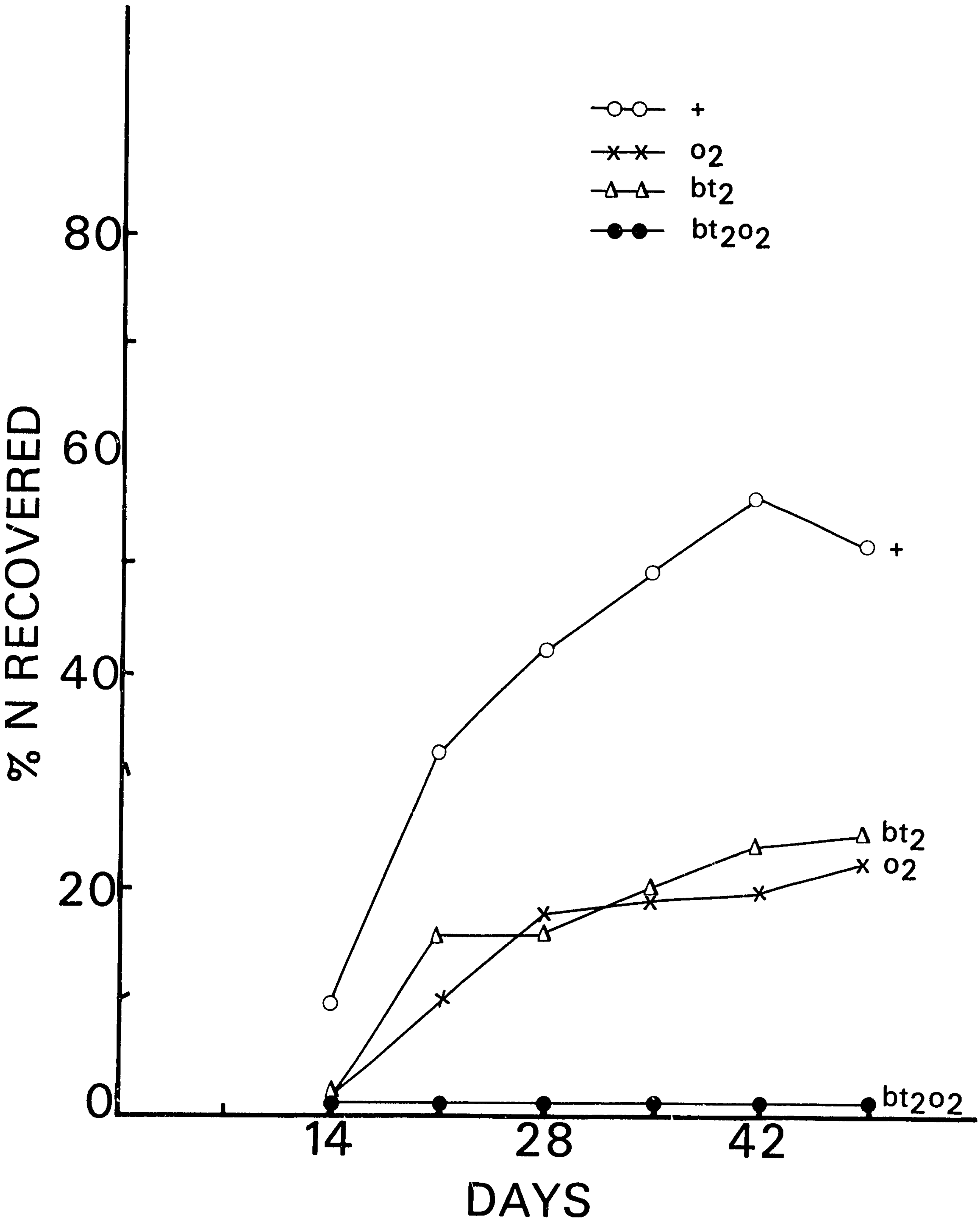

FIGURE 1. Zein levels in Developing Maize Endosperms.

f. <u>Total free amino acids in single and double mutant maize endosperms</u> (objectives a, b and e) - Table 26 shows the free amino acids in single and double mutant corn endosperms from four different inbred lines. Results indicate that $\underline{o}_2$ has a higher level of free amino acid in all the genetic backgrounds studied when compared with the respective normal counterpart. However, there are wide differences between inbred lines (2 - 5 fold). It is interesting to note that in the case of the double mutant, the introduction of $\underline{o}_2$ increases the free amino acid level of all of the genotypes again with wide differences between different inbreds

g. <u>Genetic control of ear rot resistance in opaque-2</u> (objective k) - Three sets (generation mean analysis) of parents, the F_1's, F_2's and backcrosses to both parents were evaluated for ear rot (<u>Fusarium</u> <u>moniliforme</u>) resistance (Table 27). The extreme differences in percent kernels infected was due to natural infection. In Set I both parents were relatively resistant and the F_1 and advanced generations are all resistant. In Sets II and III, the common parent Oh07 is susceptible and the other parents are resistant. Resistance is definitely inheritable and tends to be inherited as a recessive characteristic. These and other data indicate that it is relatively easy to develop nonmodified $\underline{o}_2$ varieties with very good resistance to ear rots.

h. <u>Factors affecting Maize Adaptation</u> (objective l) - Data on the time interval from planting to floral initiation, flowering, and maturity has been collected on a set of 12

Table 26. Total Free Amino Acids in Single and Double Mutant Maize Endosperms

	Inbred							
	Oh 43		W 64A		B 37		C 103	
Genotype	Lys.%[a]	Free Amino Acid[b]	Lys.%	Free Amino Acid	Lys.%	Free Amino Acid	Lys. %	Free Amino Acid
+ (Normal)	1.6	10.6	1.6	5.6	1.8	9.0	1.8	9.9
o_2	3.4	18.6	3.7	31.5	3.3	15.4	3.5	52.6
fl_2	2.5	16.0	3.0	16.2	2.8	7.9	2.6	12.2
fl_2o_2	3.2	37.8	4.5	40.3	3.1	20.7	3.0	21.1
ae	2.2	5.9	2.0	2.4	2.2	6.7	2.2	7.2
aeo_2	3.8	17.8	4.5	37.7	3.8	15.6	3.6	69.7
du	2.0	11.3	1.8	6.7	2.1	9.0	2.0	12.7
duo_2	3.9	28.7	4.5	40.5	3.7	23.9	3.6	95.7
su_2	2.2	9.4	1.9	6.1	2.0	8.0	2.0	12.5
su_2o_2	3.8	13.2	4.5	52.9	3.3	22.8	3.4	32.4
wx	1.7	8.8	1.7	7.5	2.0	14.1	1.7	11.2
wxo_2	3.9	21.4	4.3	49.6	3.8	22.1	3.1	104.8

[a] g lysine/100 g protein

[b] µM leucine/100 mg protein. Range of total protein (N x 6.25) = 8.6-12.2%. Opaque-2 mutants underlined in columns 3, 5, 7, and 9.

Table 27. Percent kernels infected with Fusarium moniliforme (Ear rot)

	Set I C123 o_2 (P_1) B14 o_2 (P_2)	Set II H84 o_2 (P_1) Oh07 o_2 (P_2)	Set III B14 o_2 (P_1) Oh07 (P_2)
P_1	5.8	5.2	8.2
P_2	8.2	25.8	25.8
F_1	2.0	47.6	18.1
F_2	4.6	20.2	28.5
Bc (P_1)	3.0	12.4	10.0
Bc (P_2)	5.3	42.9	33.0

strains of maize in Maturin, Venezuela; Palmira, Colombia; El Batan, Ilalticipan and Poza Rica, Mexico; Columbia, Missour; Ft. Collins and Rocky Ford, Colorado; Madison, Wisconsin and Lafayette, Indiana. The interrelationships of these intervals with accumulated units of heat as measured by air temperature, solar radiation and net radiation are being analyzed to learn more about the nature of adaptation and its prediction. (specific objective 1).

3. Research Design:

Development of the rapid ninhydrin staining technique that identifies high lysine mutants requires research to determine if this test may greatly increase our "screening power" to detect new mutants. It is hoped this test may be an aid in identifying "normal" phenotype high lysine mutants in Central American germplasm.

Biological value of protein sources has generally been expressed as a protein efficiency ratio (PER) index. A recently proposed new approach, "relative protein value" (RPV) has been suggested as a better indication of biological value. This method must be evaluated to determine its usefulness as an index of biological value.

E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results:

a. Research report.

The 1972-73 annual research report was sent to over 400 maize cooperators and interested centers, missions or institutions. For a bibliographic list of publications see Section G.

b. Status reports.

A status report on the development of maize with improved protein quality was distributed earlier (1972) to over 500 interested persons, institutions, centers and missions. There has been expressed need for an updating and expansion of this publication. This need has been met with a revision "Progress In Developing Maize With Improved Protein Quality" to include an update on analytical techniques, more recent findings on modifier genes of opaque-2, other high lysine genes, and the double mutants with opaque-2. This status report is in printing presently and will be distributed worldwide to interested persons.

A second status report "Simple Chemical and Biological Methods Used at Purdue to Evaluate Cereals for Protein Quality" has been produced and is in the final editing stage and will be distributed worldwide to interested maize workers.

c. Workshops, symposia, papers and seminars presented:

Papers were presented by project personnel at the American Society of Agronomy meetings; The American Association of Cereal Chemists; National Academy of Sciences International Workshop on Genetic Improvement of Seed Proteins, Wash. D.C.; Agricultural Chemical Society of Japan, Tokyo, Japan; VIIth International Symposium on Carbohydrate Chemistry, Bratislava, Czechoslovakia; U.S. AID Office of Nutrition-Third Agency-wide Workshop on Nutrition, Harper's Ferry, Va.; Inter-Asian Maize Conferences, Bangkok, Thailand; Sixth Meeting of Maize Breeders of the Andean Zone, Maracay, Venezuela; National

Corn Growers Workshop; Illinois Corn Breeders School, Urbana, Ill.; and Waterloo University, Waterloo, Canada. Dr. Mertz was a special participant in a workshop to develop a protein advisory group (PAG) guideline on chemical and biological methods for plant breeders, PAG (UNICEF) Obregon, Mexico; Committee member (2 mtgs) of U.S. National Committee of the International Union of Nutritional Sciences, Wash. D.C.; and visiting lecturer on genetic improvement of cereal grains, Macon Jr. College, Macon, Ga. Project personnel participated in the University of Illinois Workshop on "Corn Quality in World Markets" and the International Symposium on "Haploids in Higher Plants Advances and Potential", Guelph, Canada.

Within Purdue University project personnel gave seminars and participated in special field days and program activities in the departments of Agronomy, Biochemistry and Animal Science.

d. Germplasm distribution and utilization.

The following research workers in the respective countries have received germplasm of Purdue developed opaque-2, sugary-2 or waxy inbred lines, HMo_2o_2 Synthetic, Mod Syn Ao_2, Mod Syn Bo_2, Temperate HA and HB opaque-2 and sugary 2 opaque-2 and other sources of material for improvement of the protein quality of maize: S. Chang, Taiwan; Y. Efron, Israel; C. Lorenzoni, Italy, F. Salamini, Italy; A. Monteagudo, Madrid; W. DeSilva, Brazil; A. Vistasovic, Yugoslavia; C. Kiss, France; G. Mariani, Italy; I. Boulus, Egypt; A. Brandeline, Italy; I. Langer, Czechoslovakia; T. Izuno, West Pakistan; G. Tosello, Brazil; B. Pinot, France H. Semangoen, Indonesia; T. Ekpenyong, Nigeria; A. Ordas, Spain; S. Vasal, CIMMYT-Mexico;

K. Rosic, Yugoslavia; F. Rivera, Colombia; C. Kehlet, Mbeya; Cerrate, Peru; B. Abebe, Ethiopia; V. Asani, India; V. Carongal, Philippines; C. Cortaza, Ecuador; G. Francis, CIAT-Colombia; H. Barbosa, Brazil; S. Adisewojo, Indonesia, I. Soeharsono, Indonesia; A. Halin, Malaysia; I. Kovacs, Hungary; J. Monya, Tanzania; V. Long, Vietnam; A. Manrique, Peru; D. Malithano, Malawi; A. du Toit, South Africa; A. Maicado, Philippines; M. Misovii, Yugoslavia; L. Munck, Denmark; C. Moore, Thailand, E. Omolo, East Africa; E. Paterniani, Brazil; F. Poey, Mexico; A. Secundino, Brazil; J. Silva, Brazil; M. Splitter, Nepal; J. Singh, India; G. Semitti, Tanzania; K. Zima, USSR; H. Gevers, South Africa; T. Scheuch, Peru; U. Ribeiral, Brazil; D. Makonnen, Ethiopia; J. Wray, Scotland; K. McWhirter, Australia; L. Kaanenburg, Canada; S. Nelson, Rhodesia; A. Grobman, Peru; U. Rosbaco, Argentina; N. Janos, Hungary; A. Piouarci, Czechoslovakia; A. Balint, Hungary; Bulgaria; E. Johnson, Mexico.

In addition, germplasm for protein quality improvement was distributed to over 75 different private or public institutions and maize companies within the U.S.

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

There has been a continuing increase in interest and requests for seed, information, data, protein quality monitoring of seed, technical help and advice regarding protein quality improvement in maize and all aspects of cereal grain protein quality improvement. Over 100 different inquiries or requests for seed and information have been received from over 40 countries outside the U.S. Numerous maize researchers from the public and private sectors in the U.S. have made inquiries regarding our research in maize protein quality improvement. Increasingly wider requests have been made for opaque-2 maize materials, the modified opaque-2 and double mutant (sugary-2 opaque-2) germplasm, from over 43 different countries and 75 private or public institutions or commercial companies within the U.S. An increasing number of visitors concerned with production and the improvement of protein quality in maize have come to Purdue from numerous countries. A tremendous increase in the number of visitors from within the U.S. has also occurred. Information and germplasm requests and personal contacts have helped to increase involvement with the LDC's, and constitute a part of project efforts to disseminate and exchange information relevant to LDC programs and involvement in protein quality improvement.

Several graduate students have completed their M.S. or Ph.D. programs under the general direction of this project and returned to their countries. There have been several short term trainees and visiting scientists who have been associated with the project and returned to their respective maize research programs. Two post-doctorals have received training and have been employed by commercial companies in the U.S. and are presently involved in research programs to improve protein quality in maize.

The development of the rapid ninhydrin color test for screening of high lysine mutants in maize and other cereals will no doubt enhance the ability of researchers to screen their materials for mutants and will be particularly helpful to LDC's where less involved screening methods are needed. This technique will also provide for a rapid and economical way of identifying the high-lysine opaque-2 corns in the market places of the LDC's which heretofore has not been realizable.

A recent world-wide survey, conducted by Purdue, indicated some production of opaque-2 maize in Brazil, the U.S.S.R. and the U.S. and minor use in several other countries. Most researchers in the various countries estimated the future prospects of opaque-2 as good to excellent. Peru is reported to be making good progress in the utilization of the opaque-2 gene in their maize programs.

3. <u>Plans for Effective Ways to Expand Use of Research Results</u>:

   a. Continue cooperation with CIMMYT in the development of annual research work plans and to develop cooperative and complimentary maize programs for improvement of protein quality in maize for LDC's, particularly for more temperate regions. Undergirding with research and cooperating with the other research centers in the world-wide maize network will strengthen and improve the utilization of research information and new germplasm in LDC's.

   b. Continue to cooperate with the Andean zone breeders and CIAT in identifying and developing improved germplasm. Assist in training and advising on breeding methods for utilization of improved types of maize.

c. Set up cooperative testing of selections in Pakistan, Israel, Mexico and U.S. Cooperative testing of opaque-2 hybrids in uniform trials at several locations in the U.S. will continue.

d. Students from the LDC's involved in maize research will be trained in maize breeding and quality improvement at Purdue. Visiting scientists and researchers from LDC's will be trained in short-term programs in maize quality improvement at Purdue.

e. Participate in seminars and workshops related to protein quality improvement in maize.

4. Involvement of LDC or U.S. Personnel and Institutions

a. The project personnel have continued to meet annually with Dr. Ernest Sprague and Dr. Elmer Johnson and the maize research staff of CIMMYT to discuss research plans and coordinate efforts in the development of work plans for the project. The development of wide-based temperate germplasm materials for the more temperate regions of the world has been an outreach of this cooperation to compliment the varieties being developed for the more tropical areas of the world. Testing sites have been planned and coordinated through joint efforts of Purdue and CIMMYT staff. Project personnel have been involved in special workshops with the Andean corn breeders to guide the development and utilization of the high-lysine materials in their breeding programs.

b. Project personnel have had extensive contacts with maize breeders and other interested personnel from the LDC's, other countries and the U.S. An increasing number of visitors from LDC's come to our laboratories for direct information, and technical help and advice on utilization of high lysine maize materials in their breeding programs. Many come to acquaint themselves with the analytical and biological evaluation techniques used in protein quality improvement.

c. Extensive correspondence has also been an additionally important means of disseminating information, data, valuable advice and help and germplasm materials. Contact was made with over 43 countries and over half of the states in the U.S.

d. Cooperation with north central corn breeders (U.S.) in testing of high lysine, opaque-2 hybrids developed by station breeders has been initiated and will continue.

e. Graduate students Dr. Renato Ruschel (Brazil) Dr. Helio Barbosa (Brazil, Dr. Dejene MaKonnen (Ethiopia), Dr. Urbano Ribeiral (Brazil), Dr. Geraldo A. Tosello (Brazil) and Miss Nora D'Croz (Colombia) completed their Ph.D. or M.S. degree programs under the direction of this project and returned to their native country and respective research programs.

f. Postdoctorates Dr. Jerry Arnold and Dr. John R. Snyder have received training under the project and returned to major commercial company maize research programs in the U.S. and are concerned with the development and utilization of high lysine maize.

g. Four trainees from Indonesia, Columbia, Nigeria and Nepal received intensive training in analytical techniques for determining protein quality in maize and other cereals. The graduate students listed in (e) above also received this intensive training.

h. At present the following students are working toward graduate degrees and are being trained under the direction of the maize project: Alfredo Navarro (Mexico), Lourdes Nazarea (Philippines), Hugo Zorrillo (Colombia), Iffat Rahim (Pakistan), Weyman Fussell, P. Stephen Baenzier, and Theron E. Roudy (U.S.).

5. Material Representing Efforts to Disseminate Results of Research Project:

Under separate cover four copies of publications, reprints, status reports, seminar reports, and news articles representative of efforts to disseminate research findings have been forwarded. (See also Section G).

F. Specific Work Plans for 1975-76 and Projected General Plans for 1976-78

A coordinating conference with Drs. Sprague, Johnson and Villegas from CIMMYT and Dr. Litzenberger with AID was held on August 22-23, 1974.

Considerable discussion was devoted to breeding proposals of Drs. G. F. Sprague and S. A. Eberhart and their feasibility for developing countries and world wide germplasm improvement.

Specific cooperation was planned in the development of the two populations Temp. HA and Temp. HB for the temperate regions in modified opaque-2 and opaque-2 sugary 2 versions.

Further plans were made to screen Central American germplasm for vitreous high lysine types using the ninhydrin screening method on single kernel basis. Cooperation will continue on development of chemical and biological methods for evaluating protein quality.

Plans will be made to evaluate elite tropical germ plasm in temperate areas and conversely Corn Belt germplasm in tropical environment. Based on these trials, genotypes might be selected for introgression into the other areas. The high grain/stover ratio of cornbelt germplasm would be a desirable attribute to introduce into tropical varieties.

Specific work plans for 1975-76 are given below along with projected plans for 1976-78.

1. New genes and germplasm to provide improved protein quality.

   a. New mutants (Objectives a, c, and h)

      (1) Some of the new mutants have shown high lysine in preliminary analyses. Others have shown stable inheri-

tance in F_2 ratios but have not yet been analysed for lysine.

1975-76 - The mutants which have shown high lysine values will be retested in the subsequent generation. Those not yet analysed but with stable inheritance patterns will be analysed. Allele testing will continue against all known genes of similar phentype.

1976-78 - Continue allele and linkage tests and start introduction of any promising new mutants into elite germplasm. Combine and evaluate epistatic effects with other genes such as opaque-2, floury-2 and sugary-2.

(2) Use of ninhydrin technique adapted to screening hard endosperm types to search for opaque-2 with "normal" phenotype.

1975-76 - With tests of material from the Germplasm Bank (CIMMYT) as a base, the search will be broadened to new collections from the most promising areas indicated by the screening process.

1976-78 - The search will be further broadened and promising material analysed in more detail in a subsequent generation. Completely modified opaque-2 and/or new high lysine germplasm of "normal" phenotype will be the object of the search. Promising new mutants will undergo further evaluation as in a-1 above.

b. **Development of populations with improved protein or nutritional qualities for temperate areas.** (Objectives a, c, e, and h)

(1) Two opaque-2 synthetics designated Temp HA and Temp HB were developed and undergone mild selection pressure for modified kernel type and resistance to H. turcicum and smut (Ustilago zeae). In the summer 1974 full sibs were yield tested at four worldwide locations.

1975-76 - Based on agronomic performance and protein quality full sibs will be made among selected families in winter nursery. These will be yield tested at 4 or 5 world wide locations.

1976-78 - Continue full sib selection for agronomic performance, modified kernel types and protein quality. When either or both synthetics show promise at a location one may wish to practice intensive testing for more specific adaptation to that area. These populations could fit into a reciprocal recurrent selection program for development of a population cross hybrid.

(2) Temperate HA and Temperate HB sugary-2 opaque-2, waxy opaque-2 and brittle-2 opaque-2 synthetics - Maximum effort will be placed on the development of the Temp HA and Temp HB sugary-2 opaque-2 synthetic populations. A full-sib family selection method to improve these high-lysine sugary-2 opaque-2 maize populations is being used. Based on chemical analysis for protein quality, kernel

size, weight and vitreousness, family selections will be made. Plant-to-plant crosses will be made among selected families in the 1975-winter nursery.

1975-76 - Selected full-sib families will be yield tested and simultaneously grown in the 1975-summer breeding nursery to be self-pollinated. Based on performance, self-pollinated ears harvested from each family will be analyzed for protein quality, kernel size, weight and vitreousness and poorer families discarded. Selected families will be grown in the 1976-winter breeding nursery to make full sib matings among selected group.

On a smaller scale bulked equal quantities of seed of the selected S_1 families of the Temp HA and Temp HB wx o_2 synthetics from the 1974 summer breeding nursery will be grown in the 1975-winter breeding nursery and randomly mated. Selected ears (families) will again be self-pollinated in the 1976-summer breeding nursery and selections again made based on protein quality, and kernel quality.

In the Temp HA and Temp HB bt_2o_2 synthetics selected plants were randomly mated in the 1974-summer breeding nursery. A form of S_1 selection or mass selection will be used to improve the populations.

1976-80 - Continue full sib, S_1 or mass-selection as outlined above in the synthetics. After some progress has been realized these synthetics, particularly the sugary-2 opaque-2 synthetics may be introgressed into the opaque-2 Temp HA and Temp HB synthetics (sec. 1-b) which will have had some selection for agronomic quality and wide adaptation characteristics.

(3) Results from selection studies by Dr. M. S. Zuber at University of Missouri and discussion with CIMMYT breeders at recent coordinating conference point to the need for definitive data on selection for improved protein quality in normal maize. This approach would have the theoretical advantage of improving protein quality without the agronomic disadvantages of lower yield, ear rots and/or grains insect damage associated with use of the mutant genes.

1975-76 - Analyze normal kernels from segregating families (ears) of the original random mated versions of Temp HA and Temp HB. Approximately 100 ears in each variety will be analyzed for protein and lysine. If there are a promising number of high lysine families then 100 additional ears in each variety will be analyzed.

1976-77 - In winter nursery intercross the 25 families with highest lysine content.

1977-78 - Test 200 full sib families for another cycle of recurrent selection. A decision will be made at this time depending on results obtained whether to continue this approach.

(4) The brown midrib-3 gene which increases digestibility of the vegetative portion of the maize plant is being incorporated into the above populations.

c. Selection for higher protein content in double-mutant combinations. (Objectives d and e)

(1) Selection for protein content in sugary-2 opaque-2 and waxy opaque-2 double mutant combinations. The sugary-2 opaque-2 and waxy opaque-2 double mutant genotypes are being backcrossed into at least two agronomically desirable selections of "opaque-2, high protein" line recoveries in each of the Oh43, B14 and B37 inbred sources recovered from crosses to Illinois High Protein material. The first and second backcrosses are being made in the 1974-summer nursery.

1975-76 - Based on evaluations for agronomic performance, kernel quality, kernel size, and protein quality continue backcrossing and selection for increased levels of protein.

1976-78 - Continue backcrossing and selection for improvement in protein nutritional quality within the sugary-2 opaque-2 and waxy opaque-2 selected recoveries.

(2) Selection for protein content in multialeurone sugary-2 opaque-2 and waxy opaque-2 double mutant combinations.

1974-75 - Crosses involving multialeurone layer stocks with several sugary-2 opaque-2 and waxy opaque-2 inbreds are being made.

1975-76 - Selection and continued backcrossing to the recurrent double-mutant lines will be made to increase the proportion of aleurone tissue relative to the starch portion of the endosperm in an additional effort to improve the protein nutritional quality. Detailed genetic and agronomic evaluation of the increased aluerone layered recoveries.

1976-78 - Advanced generation recoveries will be selected for increased number of aleurone layers and protein quality in the endosperm as further backcrossing proceeds to develop isogenic material and eventual evaluation in hybrids.

d. Detailed genetic and agronomic evaluation of independent (mutant) sources of opaque-2 gene (Objective a)

Seven opaque-2 sources are being backcrossed into common inbred sources W64A and B37 to evaluate possible differences among the sources. The fifth or sixth backcrosses have been made.

1975-76 - In summer nursery evaluate preliminary crosses and inbred sources for agronomic performance, grain quality, and protein quality to determine possible differences among mutant sources. Continue backcrossing and make more advanced crosses.

1977-78 - Continue backcrossing and evaluation if there are differences among sources. Expand this phase if some sources are more promising, or there is evidence that opaque-2 is structurally a multiple allele.

2. Modifier genes - To achieve a more normal phenotype for consumer acceptability and ear rot and grain insect resistance detailed studies of modified types will be conducted. (Objectives b, c, and f)

a. A modified opaque-2 synthetic $HM\underline{o}_2$ has been developed from

Cornbelt germplasm and released as a source of modifier genes. Areas of research to answer some questions involving modified opaque-2 types are as follows.

(1) A very critical question involving the practical utilization of selected high lysine modified opaque-2 types concerns the stability of the characteristics under random mating and various environmental conditions when used by the farmer. If these populations tend to lose their modified or high lysine characteristics the farmer will be dissatisfied. Also if continued or frequent selection pressure is necessary to maintain high lysine and modified characteristics this approach may not be too practical.

Modified and non-modified version of synthetics Temp HA $\underline{o_2}$ and Temp HB $\underline{o_2}$ and high and low lysine selections of modified and non-modified versions of synthetic HM $\underline{o_2}$ have been random mated successively for two or three generations.

1975-76 - Evaluate the random mated generations of the eight populations described above in several environments.

1976-77 - Continue the testing for a second year as necessary to obtain definitive data.

(2) Data on relationships of agronomic performance to modified types and protein quality will also be obtained in the above (B -1a) experiments in 1976-77.

(3) Current research has shown that selection for modified opaque-2 types result in lower lysine levels and a shift in the protein fractions (Landry-Monreaux method) toward that found in normal maize. High protein quality types can be obtained in modified types by selection. However, it is not known what change(s) occurs in protein fractions or protein quality of those fractions to achieve those types. Understanding these changes could lead to more efficient selection criteria.

1975-76 - The high and low lysine version of the modified and non-modified versions selected in HM o_2 (sec 2 -al) will be subjected to protein fractionation, protein quality (amino acid patterns) of these fractions and electrophoretic separation of proteins.

b. The sugary-2 opaque-2 and waxy opaque-2 double mutant genotypes are being crossed with specific selections for modified opaque-2 vitreous kernel phenotype. (Objectives b, c, and e)

1975-76 - Continued backcrossing and selfing out in advanced generation materials and evaluation of the sugary-2 opaque-2 and waxy opaque-2 genotypes in a modified opaque-2 background for progress in protein quality improvement, kernel vitreousness, and seed size.

1976-78 - Evaluate for agronomic performance, protein quality, kernel size and degree of modification and determine if approach is practical before continued selection as outlined above.

3. Development of superior opaque-2, sugary-2 opaque-2 and waxy opaque-2 inbreds and hybrids. (Objective a)

Experimental hybrids are currently being tested by this project and in cooperative uniform tests with public corn breeders in the U. S. cornbelt region. Some of the better hybrids have been distributed for evaluation in other temperate areas of the world.

1975-76 - Continue inbred development and hybrid evaluation.

1976-78 - More promising hybrids evaluated in uniform tests in temperate areas in cooperation with CIMMYT, FAO and other organizations. Superior lines would be released to interested countries or seed companies.

4. Endosperm Mutant Interactions. (Objective e)

To explore the potential of genetic interactions among endosperm mutants for improving protein quality, kernel characteristics, digestibility and consumer acceptability, continued effort is being made in evaluating genetic interactions of endosperm mutants with opaque-2 and any promising new protein quality mutants.

1975-76 -

1. Continue endosperm mutant and opaque-2 double-mutant isogenic line development and hybrid evaluation.
2. Expand emphasis on the sugary-2 and sugary-2 opaque-2 and continue some emphasis on waxy and waxy opaque-2 ((see also section 1 -b2).
3. Because of the current possibilities that sugary-2 opaque-2 holds for improving kernel quality, digestibility,

caloric content and protein quality; and the digestibility and protein quality in waxy opaque-2 (glutinous corn) detailed studies are being conducted (see also sections 1-c1, 1 -c2, and 2 -c).

4. Continue study with sugary-2 and sugary-2 opaque-2 gene interactions in high and low oil selections to evaluate their effect on caloric content and protein quality. Evaluate agronomic performance, kernel, oil and protein quality in the sugary-2 and sugary-2 opaque-2 high and low oil background recoveries of F_2, F_3, Bc_1F_2, Bc_2F_2 generations in a replicated uniform nursery.
   Continue study of sugary-2 endosperm dosage interaction and effects of outcrossing on protein quality, kernel size and weight and related characters in sugary-2 and sugary-2 opaque-2 corn.
5. Developmental studies will be conducted with sugary-2 and waxy and their double-mutant combinations with opaque-2 in isogenic hydrids. Dry matter accumulation will be studied. The effects of plant stand, thinning date and kernel competition on seed size of sugary-2 and sugary-2 opaque-2 hybrids will be studied.
6. Detailed genetic and agronomic evaluation of independent mutant sources of the sugary-2 gene. Continue backcrossing into common inbred backgrounds to evaluate differences among sources.

7. Continued backcrossing of the brittle-2 opaque-2, brittle-1 opaque-2, sugary-1 opaque-2 and shrunken-2 opaque-2 into a few inbred lines and evaluation in hybrids for potential interest as nutritionally improved green corn (vegetable corn types) or in snack food types. Continue some selection in the Temperate HA and Temperate HB brittle-2 opaque-2 synthetics (see section 1- b2).

8. Basic research will be conducted on the nature of the proteins in certain key mutants and double-mutant combinations. The nature of the protein body characteristics, matrix protein, distribution and protein-profiles are being investigated.

1976-78 - The endosperm mutant interaction studies are complex and require a number of generations to develop. As new findings are discovered and new genotypes developed they will be examined for their agronomic quality, kernel characteristics and acceptability, protein, carbohydrate and oil quality and quantity, and biological value for potential food use as mature grain and vegetable types. Continue detailed agronomic, genetic and nutritional studies outlined above and in section G with continued evaluation as to practical value of new specialty corns. As new materials of promise are developed and if seemed practical they will be incorporated into synthetics or populations and long time breeding and selection programs employed to develop utilizable materials with wide adaptation.

5. Research on improved analytical methods. (Objective i)

1975-76 - The development of the ninhydrin method for screening both floury and hard endosperm types of maize has increased the need for a rapid method for determining the quantitative lysine level in ninhydrin-positive seeds. This laboratory will continue to search for better methods and will evaluate any new methods proposed by other laboratories. Two new methods to be tested are the ninhydrin colorimetric method for peptide bound lysine of Wall and coworkers, and the fluorometric dansyl chloride method of Kaul and coworkers.

1977-78 - Current methods of analysis of corn will be improved and new methods evaluated. This information will be provided to laboratories in the developing countries.

6. Central Biochemical Laboratory. (Objectives h and i)

   a. Cooperative research with Dr. E. Villegas (CIMMYT)

      1975-76 - Continue to cooperate with Dr. Villegas on development of improved simple analytical and biological methods for evaluating protein quality in maize.

      1977-78 - Distribute, with the cooperation of Dr. Villegas, the latest information on best simple analytical methods along with check samples of maize, to cooperating laboratories in the less-developed countries. Also, when requested, analyze their countrol samples.

      1977-78 - Continue studies on the mechanism of protein biosynthesis in opaque-2 maize.

   b. Basic research on maize proteins.

      1975-76 - Begin studies on amino acid pathways leading to lysine biosynthesis in developing opaque-2 maize endosperms. Several enzymes involved in lysine biosynthesis in maize are subject to feedback control by the end product, lysine. The levels of these enzymes in opaque-2 and normal endosperm will be determined to see if mutations have occurred.

1977-78 - Continue studies on the mechanism of protein biosynthesis in opaque-2 maize.

c. Analyses.

Samples from the genetic and breeding programs will be analyzed by the Biochemistry Laboratory for protein, lysine and tryptophan and complete amino acid profiles to the extent facilities and personnel permit. Additional analyses for protein quality and other characteristics will be on a contractual basis.

1977-78 - Continue protein fractionation, gel electrophoresis and other identification techniques on opaque-2, modified opaque-2 phenotypes, sugary-2, and other high lysine starch mutants in an effort to determine the mechanism of inhibition of zein synthesis which causes the elevation of lysine levels in maize.

7. Protein nutritive value (Objective j)

1975-76 - Feeding trials with rats and swine will be undertaken for initial evaluation of new genetic materials (sugary-2, sugary-2 opaque-2, waxy, waxy opaque-2, opaque-2 and the normal counterpart 3-way hybrids).

1977-78 -

a. A cooperative study will be carried out with the Department of Foods and Nutrition to:

(1) evaluate in adult human subjects the nutritional value

of the sugary-2 opaque-2 double-mutant corn compared to opaque-2 corn;

(2) to compare the response of human subjects to those sources of intact protein and

(3) to evaluate in feeding trials with rats combinations of nutritionally improved corns in food blends characteristic of those consumed in various parts of the world.

b. The new genetic types will be evaluated for milling quality. Food products and raw ingredients from genetically improved high lysine corns will be evaluated for

(1) keeping quality-factors,

(2) sensory evaluation and acceptance, and

(3) proximal composition of the products.

G. FURTHER INFORMATION ON DISSEMINATION OF RESEARCH RESULTS 1/

1. Bibliographic List and Short Abstracts of Research Reports for 1973-74 Representing Efforts to Disseminate the Results of the Research Project

Arnold, J. M., Albin Piovarci, L. F. Bauman and C. G. Poneleit. 1974. Weight, oil, and fatty acid content of components of normal, opaque-2, and floury-2 maize kernels. Crop Science 14:598-599.

Arnold, J. M. and L. F. Bauman. 1974. Combining ability for and interrelationships among kernel P, K, Mg. Fe, and Zn content and kernel weight, volume, and density in maize. Crop Science (Submitted to).

Arnold, J. M., L. F. Bauman and H. S. Aycock. 1974. Interrelationships among protein, lysine, oil, and certain elemental concentrations and physical kernel characteristics in two maize populations. Crop Science (Submitted to).

Bauman, L. F. 1972. Germ and endosperm variability, mineral elements, oil content, and modified genes in opaque-2 maize. Reprinted with permission from: High Quality Protein Maize, CIMMYT/Purdue University copyright (c) 1974. Dowden, Hutchinson & Ross, Inc., Publisher, Stroudsburg, Pennsylvania, U.S.A. *

Bauman, L. F., D. V. Glover, E. T. Mertz and P. L. Crane. 1974. Progress in developing maize with improved protein quality. Status Report. *

Baenziger, Stephen P. and D. V. Glover. 1974. Protein matrix and protein body size and distribution in various endosperm mutants in *Zea mays*, L. Agronomy Abstr. p 65.

D'Croz, Nora Elssy. 1974. A search for new endosperm mutants of maize and for genes to modify the expression of opaque-2. M.S. Thesis Purdue University Library.

D'Croz, N. E. and P. L. Crane. 1974. Colombian maize germplasm as sources of modifier genes for opaque-2. Crop Science (Submitted to).

Fuwa, H., M. Nakajima, A. Hamada and D. V. Glover. 1974. Comparative susceptibility to amylases of starches from different plant species and several single endosperm mutants and their double-mutant combinations with *opaque*-2 inbred Oh43 maize. Cereal Chemistry (In Press).

* No Abstract available.

1/ See also Section E. 1.

Fuwa, H. and D. V. Glover. 1974. Susceptibility of various starch granules to amylases as seen by scanning electron microscope. VII International Symposium on Carbohydrate Chemistry. Bratislava, Czechoslovakia, August 5-9.

Glover, D. V. and P. L. Crane. 1972. Genetics of endosperm mutants in maize as related to protein quality and quantity. Reprinted with permission from: High Quality Protein Maize, CIMMYT/Purdue University copyright (c) 1974. Dowden, Hutchinson & Ross, Inc., Publisher, Stroudsburg, Pennsylvania, U.S.A. *

Glover, D. V. and G. A. Tosello. 1973. Kernel characteristics, protein quality and biological value of the sugary-2 mutation and its combination with opaque-2 in Zea mays L. Agronomy Abstr. p. 5.

Helm, J. L., B. J. Donnelly and D. V. Glover. 1973. Corn cob hemicellulose composition of corn isolines involving several mutant genes. Agronomy J. 66:708-709.

Holder, D. G., D. V. Glover and J. C. Shannon. 1974. Interaction of shrunken-2 with five other carbohydrate genes in Zea mays L. endosperm. Crop Science 14: (No. 5).

Holder, David G., D. V. Glover and J. C. Shannon. 1974. Interactions of shrunken-2 and surgary-1 in dosage series in Zea mays L. endosperm Crop Science 14 (No. 5).

Makonnen, Dejene. 1973. Influence of mutant endosperm genes on kernels, cobs and other plant characters of corn (Zea mays L.) Ph.D. Thesis Purdue University Library.

Mertz, E. T. 1974. Simple chemical and biological methods used at Purdue to evaluate cereals for protein quality. Status Report. *

Mertz, E. T., Misra, P. S. and Ramamurthi Jambunathan. 1974. Rapid ninhydrin color test for screening high-lysine mutants of maize, sorghum, barley, and other cereal grains. Cereal Chemistry 51: 304-307. *

Mertz, Edwin T. 1973. The genetic improvement of cereal grain proteins. J. Jap. Soc. Food and Nutr., 26 (1), 1-13, 1973. *

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WEIGHT, OIL, AND FATTY ACID CONTENT OF COMPONENTS OF NORMAL, OPAQUE-2, AND FLOURY-2 MAIZE KERNELS

J. M. Arnold, Albin Piovarci, L. F. Bauman,
and C. G. Poneleit

ABSTRACT

Comparisons between normal and opaque-2 kernels from segregating maize (*Zea mays* L.) ears were examined for kernel components, oil content, and fatty acid composition. The normal kernels were higher in kernel and endosperm weight and embryo oil concentration, and lower in embryo weight. Oil from opaque kernels was higher in oleic acid and lower in linoleic acid concentration. Normal and floury-2 kernels from segregating ears were compared for kernel components. Kernel and endosperm weight of the normal kernels were higher than for floury-2 and embryo weight was similar or greater in the normal. Percent oil was greater in floury-2 which was largely a result of a greater proportion of embryo.

COMBINING ABILITY FOR AND INTERRELATIONSHIPS AMONG KERNEL P, K, Mg, Fe, AND Zn CONTENT AND KERNEL WEIGHT, VOLUME, AND DENSITY IN MAIZE

J. M. Arnold and L. F. Bauman

ABSTRACT

Combining ability for concentration and grams per 200 kernels of P, K, Mg, Fe, Zn, oil, and protein, and kernel weight, volume, and density in a six-parent diallel of maize (Zea mays L.) inbreds was examined. Correlations among certain variables were computed. Significant variation was detected among GCA effects for concentration and grams per 200 kernels of all kernel constituents except K concentration, but among SCA effects only for oil concentration. GCA and SCA effects were significant for kernel weight, volume, and density. Highly significant correlations were found between P and K, P and Mg, and K and Mg concentrations.

INTERRELATIONSHIPS AMONG PROTEIN, LYSINE, OIL, AND CERTAIN ELEMENTAL CONCENTRATIONS AND PHYSICAL KERNEL CHARACTERISTICS IN TWO MAIZE POPULATIONS

J. M. Arnold, L. F. Bauman, and H. S. Aycock

ABSTRACT

Interrelationships among protein, lysine, oil, P, K, Mg, Fe, and Zn concentrations, and physical kernel characteristics were studied in a heterozygous opaque-2 population and a homozygous opaque-2 maize (*Zea mays* L.) population.

Compared to normal the opaque-2 kernels were higher in percent of lysine, P, K, Mg, Fe, and Zn. However on an actual amount per kernel or given volume of kernels basis the opaque-2 kernels were significantly greater only for lysine, K and Zn. Differences between normal and opaque kernels for percent lysine and percent Zn were significantly correlated. Several significant correlations were detected between percent lysine and the elemental concentrations, but none were of a sufficient magnitude to indicate that the elemental concentrations would be of much value as a selection criteria for lysine content. Percent lysine was not correlated with kernel weight or volume, but was negatively correlated with kernel density. The coefficients for the correlation between percent lysine and percent protein are +.50 and +.70 for the normal and opaque kernels in the heterozygous population, respectively, and +.83 for the homozygous population.

PROTEIN MATRIX AND PROTEIN BODY SIZE AND DISTRIBUTION IN VARIOUS ENDOSPERM MUTANTS IN ZEA MAYS, L.

P. Stephen Baenziger and D. V. Glover

ABSTRACT

Kernels from thirteen endosperm mutants and their double combinations with opaque-2 (o_2), each nearly isogenic in Oh43 and the normal counterpart; and three mutants and their double combinations with o_2 each nearly isogenic in W64A, B37, and C103 and their normal counterparts were thin sectioned, destarched, and the endosperm protein body and matrix morphology was studied using interference-contrast light microscopy. Protein body size was significantly affected by mutants and inbred lines. The endosperm mutants with high-lysine levels had no visible protein bodies while the mutants with intermediate-lysine levels had fewer and generally smaller protein bodies than did the low-lysine mutants. No protein bodies were observed in the double-mutant combinations with o_2.

The data suggest that protein matrix may be involved in the expression of kernel vitreousness. The matrix of vitreous sugary-2 opaque-2 (su_2o_2) kernels resembled the matrix of vitreous sugary-2 (su_2) kernels and not that of o_2 kernels. Agronomy Abstr. p 65. (1974)

A SEARCH FOR NEW ENDOSPERM MUTANTS OF MAIZE AND FOR GENES TO MODIFY THE EXPRESSION OF OPAQUE-2

Nora Elssy D'Croz

ABSTRACT

Twelve sources of Colombian races, hybrids and composites were selfed and each ear inspected to find new putative endosperm mutants. The 116 found were planted ear-to-row, selfed again and outcrossed to ETO (a Colombian commercial variety) and to A619 × A632 where possible.

The F_2 segregation was tested for fitness to the genetic ratios 3:1 and 15:1 by Chi square. Allele tests with four known mutants o_2, o_1, fl^a and h (soft-starch) were carried out for 15 of the mutants. Four mutants from World Composite showed allelism to fl^a and one from Andaqui was allelic to o_2, the other mutants tested gave negative results.

In one or more analysis, eight different mutants showed a high lysine content. An additional eight mutants had at least one family which was intermediate in lysine content. However, more study is needed to determine if the higher lysine levels and a potential for nutritional improvement are characteristics of the new mutants.

The expression of most of the mutants was subject to modification by genes which were in the source population, in ETO or in A619 × A632.

The second objective of this study was to search for sources of modifier genes for a more normal (flint) expression of opaque-2. Seven Colombian races and a population derived from ETO × USA342 were self-

pollinated and out-crossed to the homozygous opaque-2 lines 215 and 216 which have the soft expression.

The F_2 segregation was classified in three- and four-class separation. Chi square tests were performed for the two ways of classification. These tests indicated whether the intermediate classes II and III were modified opaque-2 kernels or if they deviated significantly from 3:1 due to an excess of normal kernels, where possibly some homozygous opaque-2 kernels were so modified that they were visually indistinguishable from normal kernels.

This investigation showed that ETO × USA 342, Pira, Puya and Comun were good sources of modifier genes for opaque-2.

COLOMBIAN MAIZE GERMPLASM AS SOURCES OF MODIFIER GENES FOR OPAQUE-2

N. E. D'Croz and P. L. Crane

ABSTRACT

Seven Colombian maize races and a population derived from ETO × USA342 were self-pollinated and outcrossed to the homozygous opaque-2 lines 215 and 216 which have the soft endosperm expression. The F_2 kernels were classified into three classes over a light. One family of each group was reclassified into four classes on a black-topped table under intense light. Chi square tests indicated indirectly whether the intermediate classes II or II and III were modified opaque-2 kernels or if they deviated significantly from 3:1 due to an excess of normal kernels, where possibly some homozygous opaque-2 kernels were so modified that they were visually indistinquishable from normal kernels. A high percentage of intermediates indicated that ETO × USA342, Pira, Puya and Común were good sources of modifier genes for opaque-2. Amagaceño was less promising and conflicting results within our small samples of Clavo, Chococeño and Pollo made it impossible to draw definite conclusions about their potential.

COMPARATIVE SUSCEPTIBILITY TO AMYLASES OF STARCHES FROM DIFFERENT PLANT SPECIES AND SEVERAL SINGLE ENDOSPERM MUTANTS AND THEIR DOUBLE-MUTANT COMBINATIONS WITH OPAQUE-2 INBRED Oh43 MAIZE

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ABSTRACT

Several endosperm mutants each nearly isogenic in the maize inbred Oh43 (Zea mays L.), their double-mutant combinations with opaque-2 and the normal counterpart were studied for their relative susceptibility of granular and gelatinized starches to amylases.

When opaque-2 was combined with each of the ten endosperm genes, namely, amylose-extender, brittle-1, brittle-2, dull, soft-starch, shrunken-1, shrunken-2, sugary 1, sugary-2, and waxy it was observed that the starches of these double mutants were digested by fungal glucoamylase, pancreatin, and bacterial α-amylase to the extent very comparable to their respective nonopaque single-mutant counterpart. Starch granules of the amylose-extender mutant and its double combination with the opaque-2 were much more resistant to the action of amylases than those of normal maize. Starch granules of the sugary-2 mutant and its double combination with opaque-2 were digested much

faster than those of the normal counterpart by amylases. These differences among the endosperm mutants and their double-mutant combinations in susceptibility of starch granules to the action of amylases disappeared following gelatinization of starches with alkali.

SUSCEPTIBILITY OF VARIOUS STARCH GRANULES TO AMYLASES AS SEEN BY SCANNING ELECTRON MICROSCOPE

H. Fuwa and D. V. Glover

We confirmed that starch granules of tubers (potato, yamanoimo - Dioscorea batatas DECNE; and sweet potato) are in decreasing order more resistant to the attack of pancreatin, bacterial α-amylase, and fungal glucoamylase than those of cereals (maize and rice). Maize mutants with starch-modifying genes, namely, amylose extender (ae), brittle-1 (bt_1), brittle-2 (bt_2), dull (du), h, shrunken-1 (sh_1), shrunken-2 (sh_2), sugary-1 (su_1), sugary-2 (su_2), and waxy (wx) were concerned with changes influencing gelatinization temperature, viscosity, gel stability of starch, starch granule digestibility, and production of amylose, amylopectin, water-soluble polysaccharides, and sugars. In studies of starch-modifying genes of inbred maize Oh43 and their combination with opaque-2 gene (o_2), which produces the high lysine maize, the o_2 gene dose not change the production of amylose, amylopectin, water-soluble polysaccharides, and sugars, and susceptibility of starches to amylases. For example, starch granules of the ae mutant and its combination with the o_2 (ae o_2) are very much resistant to the action of amylases than those of the isogenic normal maize. Starch granules of the su_2 and su_2o_2 mutants are digested very much faster than those of the normal by amylases. By the examination with the scanning electron microscope, starch granules resistant to the action of amylases (tuber starches and starches of the ae and ae o_2 maize mutants) showed very similar intact shape and the surface layer to the original starch granules during digestion with either bacterial α-amylase or fungal glucoamylase. Starch granules susceptible to amylases showed pores on the surface layer and the pores penetrated into the inner layer of the granules during the attack of amylases.

KERNEL CHARACTERISTICS, PROTEIN QUALITY AND BIOLOGICAL VALUE OF THE SUGARY-2 MUTATION AND ITS COMBINATION WITH OPAQUE-2 IN *ZEA MAYS* L.

D. V. Glover and G. A. Tosello

Six hybrids from a diallel cross among four dent corn inbreds each nearly isogenic for the *sugary*-2 (su_2) and *opaque*-2 (o_2) single recessive endosperm mutations and the double mutant combination *sugary*-2/*opaque*-2 (su_2o_2) were compared with their normal counterpart for mature grain kernel characteristics, protein quality and oil content. The hybrids were grown in a replicated experiment in 1971-72. The su_2 gene when combined with o_2 improved the vitreous quality of the endosperm and kernel density in the double mutant combination su_2o_2. Endosperm lysine as percent of sample and lysine percent of protein were increased in su_2o_2 compared to the o_2 counterpart. The su_2 gene increased oil content with little change in the germ to endosperm ratio. However, kernel size was reduced in su_2 compared to the normal counterpart resulting in decreased 100-kernel weight. The su_2o_2 double mutant combination shows definite promise for increasing acceptability of the high lysine grain because of the improved protein quality, kernel density and increased oil content. We have also demonstrated superior digestibility characteristics and good biological feeding value of the su_2o_2 endosperm mutant combination.

CORN COB HEMICELLULOSE COMPOSITION OF CORN ISOLINES INVOLVING SEVERAL MUTANT GENES

J. L. Helm, B. J. Donnelly, and D. V. Glover

ABSTRACT

All economically successful xylose production procedures require a carbohydrate source rich in hemicellulose and with a minimum content of other pentose sugars. Mature corn (*Zea mays* L.) cobs are a good source of hemicellulose. However, birchwood sulphite liquor has been a more economical raw material for xylose production in recent years. A strong pleiotropic or linkage relationship between genes used in today's specialty corn programs and increased hemicellulose content or improved composition would facilitate a program to purchase corn cobs of known genotype. The inbred line Oh43 and its isogenic counterparts homozygous for the recessive endosperm genes *wx*, su_1, *du*, su_2, o_2, and *ae* were tested for hemicellulose content and composition. The results indicate no relationship between composition of mature cobs and any of the six recessive endosperm genes either in a pleiotropic or linkage sense. Additional visual marker genes should be evaluated so that corn cobs could be used as an economically suitable raw material in a xylose production program.

INTERACTION OF SHRUNKEN-2 WITH FIVE OTHER CARBOHYDRATE GENES IN *ZEA MAYS* L. ENDOSPERM

D. G. Holder, D. V. Glover and J. C. Shannon

ABSTRACT

The interactions of the *shrunken*-2 (sh_2) gene with *waxy* (*wx*), *amylose-extender* (*ae*), *sugary*-1 (su_1), *dull* (*du*) and *sugary*-2 (su_2) on the carbohydrate composition of maize (*Zea mays* L.) endosperm were determined. The contents of fructose, glucose, sucrose, water-soluble polysaccharide (WSP), and starch of whole kernel samples 21 and 28 days after pollination were determined. Percent starch and amylose of mature kernels were determined.

The sh_2 mutation reduced starch drastically concurrent with a large accumulation of sucrose as previously observed. In homozygous double and triple recessive genotypes sh_2 was epistatic over all other genes for the accumulation of sucrose and reduction of starch. Genotype combinations containing both su_1 and sh_2 produced the most extreme reduction in starch content and in all cases sh_2 inhibited the large WSP content normally caused by su_1. Starch from kernels homozygous for *ae* alone had 60% amylose. Presence of sh_2 in the multiple recessive genotypes inhibited the *ae* enhancement in percent amylose. With few exceptions those genotypes with *wx* produced no amylose; those which did were in combination with *ae*. Genotype combinations containing both wx and sh_2 had higher sucrose than normal and the starch, although reduced in quantity, was 100% amylopectin.

INTERACTIONS OF SHRUNKEN-2 AND SUGARY-1 IN DOSAGE SERIES IN ZEA MAYS L. ENDOSPERM

David G. Holder, D. V. Glover and J. C. Shannon

ABSTRACT

The interaction of gene dosage on *Zea mays* L. endosperm carbohydrate composition was studied by quantifying carbohydrates from the 16 genotypes of 21 day old endosperm resulting from all possible sib-pollinations and reciprocal crosses between lines homozygous for Su_1 Sh_2 (*normal*), su_1 Sh_2 (*sugary*-1), Su_1 sh_2 (*shrunken*-2), and su_1 sh_2 (*sugary*-1; *shrunken*-2). No dosage effects were produced by either the allele su_1 or the allele sh_2 for fructose and glucose sugars and starch content of the endosperm. There was a dosage effect of su_1 on sucrose content in the homozygous recessive sh_2 genotypes. Two and three doses of su_1 allele produced a 10% and 26% increase in sucrose content, respectively, over the zero or one dose level of su_1. Two doses of su_1 produced a very slight increase in water-soluble polysaccharide (WSP) and three doses produced large amounts of WSP typical of su_1. Two doses of sh_2 reduced the WSP content of the homozygous recessive su_1 genotype and the presence of three doses of sh_2 considerably decreased the amounts of WSP accumulated at 21 days after pollination. The interaction effects suggested that WSP accumulation in homozygous su_1 may be affected by the dosage level of the sh_2 mutant. Starch synthesis was markedly decreased in homozygous recessive su_1, sh_2 and su_1 sh_2 genotypes.

INFLUENCE OF MUTANT ENDOSPERM GENES ON KERNELS, COBS AND OTHER PLANT CHARACTERS OF CORN (*ZEA MAYS* L.)

Dejene Makonnen

ABSTRACT

The effects of endosperm mutants (opaque-2, waxy, amylose extender, and floury-2) on kernels and cobs were studied in near isogenic hybrids with their normal counterparts. Modified and high yielding opaque-2 and sweet corn hybrids were included in the study.

The reduction in kernel yield per ear of opaque-2 was 18.4%, amylose extender 10.9%, and floury-2 6.4% compared to normal. Waxy was approximately equal in kernel yield to normal. Outcrossing opaque-2 and amylose extender to the normal pollen of their counterparts increased kernel yield. At maturity the increase in kernel yield due to outcrossing in the standard opaque-2 hybrids was 12.8%. At 55 days after pollination no apparent increase in kernel yield was observed in modified opaque-2 to outcrossing, and the increase in kernel yield in high yielding opaque-2 hybrids was only 6.7%.

Outcrossed opaque-2 kernels weighed significantly higher than the sibbed ones, however, outcrossed kernels ($\underline{+}/\underline{o_2}/\underline{o_2}$) weighed less than normal ($\underline{+}/\underline{+}/\underline{+}$). Opaque-2 outcrossed and normal kernels do not differ with respect to percent protein, lysine as percent protein, lysine as percent of sample, protein weight per ear (g) and lysine weight per ear (g). Opaque-2 sibbed ($\underline{o_2}/\underline{o_2}/\underline{o_2}$) was higher than opaque-2 outcrossed ($\underline{+}/\underline{o_2}/\underline{o_2}$) and normal ($\underline{+}/\underline{+}/\underline{+}$) for the above protein characters.

Only opaque-2 has a marked influence on the cob weights of pollinated and nonpollinated ears. The increase in cob weight due to outcrossing was significant only in case of opaque-2.

A drastic reduction in cob weight was detected from nonpollinated ears as compared to those from pollinated ears. Nonpollinated cobs reached maximum dry weight at 15 days after silking, compared to pollinated ears at 35 days after pollination.

Beginning at 35 days after pollination the percent moisture content of opaque-2 was consistently higher than normal.

STUDIES ON CORN PROTEINS. V. REDUCED COLOR RESPONSE OF OPAQUE-2 CORN PROTEIN TO THE BIURET REAGENT, AND ITS USE FOR THE RAPID IDENTIFICATION OF OPAQUE-2 CORN

P. S. Misra, R. Barba-Ho, E. T. Mertz, and D. V. Glover

ABSTRACT

The biuret method of Johnson and Craney for the determination of protein in corn (maize) gave reduced color values when applied to *opaque*-2 corn, a mutant with low zein content. Further tests with normal and *opaque*-2 corn protein fractions showed that the specific intensity of the biuret reaction is nearly four times greater with zein than with glutelin. Because of the reversal in the zein:glutelin ratio in *opaque*-2 corn, the biuret absorbance per unit of Kjeldahl nitrogen is 50 to 80% of that in normal corn. This color reduction can be used for the rapid identification of *opaque*-2 corn in corn breeding programs.

TOTAL FREE AMINO ACIDS IN SINGLE AND DOUBLE ENDOSPERM MUTANTS OF MAIZE

P. S. Misra, E. T. Mertz and D. V. Glover

ABSTRACT

A simple ninhydrin test for free amino acids was devised to distinguish hard vitreous opaque-2 maize mutants from normal maize in the field and in the market place (Mertz, E. T., Misra, P. S. and Jambunathan, R., Cereal Chem. 51, 304-307 (1974). We have now determined the level of free amino acids in 14 endosperm mutants and their double mutant combinations with opaque-2 in the Oh43, W64A, C103 and B37 inbred lines, as well as certain hybrid combinations of these lines. In all double mutant combinations studied the presence of the opaque-2 gene invariably raised the level of free amino acids (as measured with ninhydrin) above that of the respective nonopaque counterpart. The quantitative ninhydrin assay can therefore be used to detect the presence of the opaque-2 gene in normal maize and in maize containing other endosperm mutants.

STUDIES ON CORN PROTEINS. VI. ENDOSPERM PROTEIN CHANGES IN SINGLE AND DOUBLE ENDOSPERM MUTANTS OF MAIZE

P. S. Misra, E. T. Mertz, and D. V. Glover

ABSTRACT

The endosperm proteins of the maize mutants, floury-2, opaque-2, opaque-7, sugary-1, shrunken-1, shrunken-2, shrunken-4 and brittle-1, and double mutant combinations of these with opaque-2, were separated into five soluble fraztions by the Landry-Moureaux mehtod. As compared to their isogenic normal counterparts, all single mutants had higher concentrations of albumin, globulin, and glutelin, and lower concentrations of prolamine. The combination of opaque-2 with floury-2 or opaque-7 did not increase lysine above that in the single mutants. The combination of opaque-2 with any of the other five mutants increased levels of albumin, globulin ard glutelin above those found in the single mutants. The double mutants showed an almost complete suppression of prolamine synthesis and the lysine levels were higher than in the single mutants.

STUDIES OF CORN PROTEINS VII. ENDOSPERM PROTEIN SYNTHESIS IN DEVELOPING MAIZE MUTANTS WITH INCREASED LYSINE CONTENT

P. S. Misra, E. T. Mertz and D. V. Glover

ABSTRACT

The endosperm protein of the near isogenic maize mutants, opaque-2, brittle-2, the double mutant of opaque-2 and brittle-2, and the normal counterpart were separated into five soluble gractions by the Landry-Moureaux method. As compared to the normal counterpart, all three mutants had higher concentrations of albumins and globulins during seed development. In all cases the highest concentrations were observed 14 days post-pollination, with a steady decrease 21, 28, 35, 42, and 49 days post-pollination. In the normal counterpart, zein production was evident 14 days post-pollination and reached a peak 42 days post-pollination. The zein level attained in the normal endosperm in 14 days was not reached until the 18th and 21st day, respectively, in brittle-2 and opaque-2. This delay in onset of zein synthesis, along with slower rates of synthesis, reduced total production of zein to less than 50% of the normal counterpart. In the double mutant of opaque-2 and brittle-2, zein formation was not apparent at any time during development.

STUDIES ON CORN PROTEINS VIII. TOTAL FREE AMINO ACIDS IN SINGLE AND DOUBEL ENDOSPERM MUTANTS

P. S. Misra, Edwin T. Mertz and D. V. Glover

ABSTRACT

With the development of a vitreous opaque-2, the usual methods of classification and identification could no longer be used very successfully. The simple ninhydrin method developed recently by Mertz, et. al. (1) was used to determine the level of free amino acids in 14 endosperm mutant and their double-mutant combinations with opaque-2 in the Oh43, W64A, C103 and B37 inbred lines, as well as certain hybrid combinations of these lines developed by Glover et. al. (2). In all combinations studied, the presence of the opaque-2 gene invaribly raised the level of free amino acids above that of the counterpart lacking this gene.

EFFECTS OF MODIFIED OPAQUE-2 KERNELS ON YIELD AND PROTIN QUALITY AND QUANTITY OF MAIZE (*ZEA MAYS*, L.)

Urbano Campos Ribeiral

ABSTRACT

Two experiments were conducted to study yield component and protein and lysine content of modified opaque-2 maize. Characters evaluated were: kernel yield per ear, kernel weight and density, protein percent, and lysine as percent of protein. "Normal" counterparts were obtained by outcrossing to $\underline{+}/\underline{+}$ pollen, which gave an endosperm genotype of $\underline{+}/\underline{o}_2/\underline{o}_2$.

In Experiment I, a diallel among six modified opaque-2 lines was compared to the "normal" counterpart diallel. The "normal" phenotype was significantly higher than the modified phenotype for kernel yield per ear, kernel weight, kernel density, and protein percent. However, the modified opaque-2 exhibited a significantly greater lysine content than the "normal".

In Experiment II, eight modified opaque-2 lines were crossed to an opaque-2 inbred. F_2 plants from selected opaque-2 and modified opaque-2 kernels were sibbed and outcrossed to normal pollen to compare opaque and its "normal," and modified and its "normal" phenotypes.

As in Experiment I, the normal phenotypes exhibited significantly higher mean values than the modified phenotype for kernel yield per ear, kernel weight and density. Modified opaque-2 was not significantly different from opaque-2 for kernel yield per ear, kernel weight and

protein percent, but it had significantly densor kernels. Opaque-2 had significantly higher lysine content than modified opaque-2. There was a significant negative correlation between lysine content and visual endosperm grade in the modified opaque.

Another study investigated the protein content and quality of the vitreous and opaque portions from modified opaque-2 endosperms. These portions were compared to whole modified endosperm as well as whole "norml" endosperm.

The bitreous portion had significantly greater protein content than the opaque portion. Lysine content expressed both as percent of protein and percent of sample was significantly higher in the opaque portion. The vitreous portion had a higher lysine content than the whole normal endosperm.

A complete amino acid profile of all four endosperm classes revealed that the opaque portion as compared to the vitreous portion was much higher in lysine, tryptophan, arginine, and glycine concentrations. The vitreous portion was much higher in lysine, tryptophan, cystine, histidine, arginine, and glycine as compared to the whole "normal" endosperm.

The results from endosperm protein fractionation showed that the opaque portion was lower in zein than any of the other endosperm classes.

Although these results were taken upon a small germplasm sample, it may be concluded that the modified opaque-2 maize as compared to the opaque-2 did not increase yield and had a negative influence on protein quality.

THE NUTRITIONAL QUALITY AND INHERITANCE OF VARIOUS ENDOSPERM MUTANTS IN MAIZE, ZEA MAYS L.

Thomas R. Stierwalt

ABSTRACT

Opaque "type" endosperm mutants were obtained from segregating ears of South American maize varieties to study the nutritional quality and inheritance. The mutants were placed in seven groups based on allele tests with known endosperm mutants and the selected mutants.

Amylose content of mutant stocks were considered to be in the range of normal maize.

Protein contents of mutants 4915, 4918, 5582 and 5587 had higher than usual levels for "normal" maize; however, no rigorous comparisons were made with isogenic lines. All mutants; with the exception of 5590, had lysine levels within the range of variations found in "normal" maize. Mutant 5590 had lysine content equal to that of opaque-2 which is in agreement with the fact that it is allelic to opaque-2.

Opaque-4 and opaque (Poey) were found to be allelic to floury-1; however, they were inherited as simple recessives so they are considered equivalent to fl^a at the floury-1 locus. Mutant 4939 is allelic to opaque-1.

Mutants were crossed with a series of multiple marked seedling mutants to study their inheritance and to locate the factor(s) responsible for their phenotypic expression. The hypothesis of one and two genes

was the basis of expression for mutant stocks studied. Mutant 4915, now tentatively designated as opaque-8 (o-8-4915), was located on chromosome-2. Mutant 5586, an allele of 4918, had duplicate recessive factors. Soft endosperm factor 1 (sen 2-5586) was located on chromosome-3 while factor 2 (sen 3-5586) was located on chromosome-7. Of the chromosome arms tested, mutant 4921 showed linkage on only one chromosome, but F_2 family segregations show two factors. Soft endosperm factor 1, for mutant 4921 located on chromosome-1, was given the genetic designation of sen 4-4921 and factor 2 was given a genetic designation of sen 5-4921. Mutant 5595 was segregating for duplicate recessive factors. Soft endosperm factor 1 (sen 6-5595) of Mutant 5595 was located on chromosome-2 and factor 2 (sen 7-5595) was located on chromosome-5.

Linkage analysis between opaque-1, (o_1-4939) glossy-3 and japonica-2 suggests that o_1-4939 was located at the terminal end of the long arm of chromosome-4.

THE NUTRITIONAL VALUE OF MUTANT GENE CORNS FOR FINISHING BEEF CATTLE

Verl Melvin Thomas

ABSTRACT

Four metabolism studies and one feedlot trial were conducted in an effort to study the effects of several mutant corns, one mutant corn silage and one processed corn in the rations of beef steers.

In metabolism study I, four 274-kg. Hereford steers were fed either normal or opaque-2 corn on an isonitrogenous basis (11% crude protein). Steers fed opaque-2 corn had non-significantly higher digestible energy value than did those steers fed normal corn. All other parameters were unchanged.

In metabolism study II, four 354-kg. Hereford steers were fed either 13.64 kg. of regular corn silage or opaque-2 corn silage plus 0.909 kg. of a 32% protein supplement per head daily. The digestible energy of regular corn silage (70.15) was significantly higher ($P< .05$) than opaque-2 corn silage (68.41) while there were no significant differences in the other parameters studied.

In the feedlot trial, twelve lots of six Hereford steers were fed a ration of 8.13 kg. of either opaque-2 or regular corn silage, 0.454 kg. of a 64% protein supplement per head daily and a full-feed of either regular, opaque-2 or roasted corn to appetite. Average initial and

final weights were 307 kg. and 534 kg., respectively. Steers fed the regular corn silage gained significantly faster ($P < .05$) and were more efficient (1.11 kg./day, 6.72 kg. dry feed/kg. gain, respectively) than were those steers fed the opaque-2 corn silage (1.06 kg./day, 6.98 kg. dry feed/kg. gain, respectively). Steers fed regular corn gained more rapidly ($P < .05$) than the steers fed the opaque-2 (1.10 kg. vs. 1.03 kg./day). Steers fed regular corn silage had significantly higher grades ($P < .05$) than steers fed opaque-2 corn silage. Roasted and regular corn produced significantly larger ($P < .01$) loin-eye areas than steers fed opaque-2 corn.

In metabolism study 3, four 205-kg. Hereford steers were fed either regular, opaque-2, waxy or waxy-opaque-2 corn on an isonitrogenous basis (11% crude protein), at 2.0 to 2.5% of their body weight per head per day. Steers fed the waxy corn diet tended to have improved nitrogen retentions when compared to the other diets, although these improvements were not significantly different ($P > .05$). Waxy corn was significantly lower ($P < .05$) in digestible and metabolizable energy than regular, opaque-2 and waxy-opaque-2 corns. There were no statistically significant differences in rumen ammonia and rumen volatile fatty acids.

The final metabolism study was conducted to compare the nutritional value of regular, sugary-2, sugary-2-opaque-2 and waxy corn in a high-concentrate diet for beef steers. Diets were fed on an isonitrogenous basis (11% crude protein) at 2.0 to 2.5% of their body weight per head per day. Steers fed the sugary-2-opaque-2 diet retained a significantly ($P < .05$) higher percent absorbed nitrogen (64.77) than did those steers fed the normal (47.20) or sugary-2 (49.82) diets. The sugary-2-opaque-2 diet improved the levels of rumen ammonia, digestible and metabolizable energy when compared to the other corn diets, but these increases were not significant at the $P < .05$ level.

EVALUATION OF PROTEIN AND CARBOHYDRATE QUALITY AND CONTENT IN SELECTED ENDOSPERM MUTANTS AND THEIR DOUBLE-MUTANT COMBINATIONS WITH OPAQUE-2 AT TWO IMMATURE STAGES OF DEVELOPMENT IN *ZEA MAYS* L.

Geraldo Antonio Tosello

ABSTRACT

Four near isogenic parental inbreds (Oh43, W64A, B37, C103) each with the follwing eight single endosperm mutants -- *ae*, *du*, *wx*, $\underline{su}_2$, $\underline{su}_1$, $\underline{fl}_2$ and $\underline{o}_2$ -- and their double-mutant combinations with $\underline{o}_2$ were crossed in a diallel series. The four parental inbred and six single-cross hybrid endosperm mutant genotypes and their normal counterparts were grown in 1971 and 1972. Ear harvests were made at 21 and 42 days after pollination (DAP). Kernel characteristics, endosperm protein and carbohydrate quantity and quality were determined to evaluate the differences among the several genotypes and their potential for nutritional improvement in vegetable corn types and specialty corns for new and novel food products.

The endosperm mutants and their double-mutant combinations with $\underline{o}_2$ were not too different in 30-kernel, 30-kernel endosperm and 30-kernel germ weight, and germ and moisture percent at 21 DAP. As the kernels developed in maturity (42 DAP) the $\underline{o}_2$ and double mutants with $\underline{o}_2$ gained relatively less weight, increased in germ weight and percentage and were higher in moisture than their respective nonopaque single mutants.

Protein content among the genotypes at 21 DAP was excellent and showed very few differences. Opaque-2 decreased protein percent and mg protein per endosperm compared to normal and o_2 in double mutants reduced protein levels compared to the respective nonopaque single mutants at both stages of maturity. Shrunken=2 and sh_2 o_2 means were maong the greatest in percent protein at both 21 and 42 DAP, but on a protein per endosperm basis were among the lowest at 42 DAP.

Protein yield and quality, expressed as lysine as a percent of protein, in the su_1 o_2 and sh_2 o_2 double mutants at 21 and 42 DAP were superior to their respective nonopaque single mutants as well as o_2.

In the hybrid backgrounds du o_2, fl_2, ae o_2, sh_2 o_2, su_1 o_2, su_2 o_2 and wx o_2 were greater in lysine per endosperm than o_2 (.34 mg) and all had higher lysine yields than their respective nonopaque single mutant counterpatts at 21 DAP. At 42 DAP o_2 was 21% greater than normal (.57 mg) in mg lysine per endosperm; and ae o_2 and su_2 o_2 were 23 and 17% respectively greater than o_2 (.69 mg). Floury-2, wx o_2, su_1 o_2 sh_2 o_2 and du o_2 were greater, though not significantly so, in lysine yield than o_2 at 42 DAP.

Among hybrids protein per endosperm was positively correlated with endosperm weight, and lysine per endosperm, and negatively correlated with lysine as a percent of protein; while protein percent was negatively correlated with endosperm weight. Lysine as a percent of protein was negatively correlated with endosperm weight and positively correlated with lysine per endosperm.

Reducing-sugar levels of o_2 were lower than normal. Opaque-2 showed an epistatic interaction to decrease reducing-sugars in double-mutant combinations. At 21 DAP, $\underline{sh}_2$, $\underline{sh}_2$ $\underline{o}_2$ and $\underline{ae}$ were 47, 21 and 23% respectively greater than normal. Sugary-1 and $\underline{su}_1$ $\underline{o}_2$ were 2.5 and 2 times greater than normal in reducing-sugars and $\underline{sh}_2$ and $\underline{sh}_2$ $\underline{o}_2$ were 1.4 and 1.38 times greater than normal at 42 DAP.

Sucrose contents of $\underline{sh}_2$ and $\underline{sh}_2$ $\underline{o}_2$ were increased three to four-fold over the normal hybrids at 21 DAP and were increased nearly two-fold at 42 DAP. Sucrose contents in $\underline{su}_1$ $\underline{o}_2$, $\underline{ae}$ and $\underline{ae}$ $\underline{o}_2$ hybrids at 21 DAP were increased 34, 31 and 35% respectively over normal, and at 42 DAP $\underline{su}_1$ and $\underline{su}_1$ $\underline{o}_2$ were 47 and 40% greater than normal.

The WSP contents of $\underline{su}_1$ and $\underline{su}_1$ $\underline{o}_2$ were increased 41 and 37-fold respectively at 21 DAP and 63 and 56-fold respectively over normal at 42 DAP. No differences existed among the other mutant genotypes and normal.

Starch contents at 21 DAP of $\underline{su}_1$, $\underline{su}_1$ $\underline{o}_2$ $\underline{sh}_2$ and $\underline{sh}_2$ $\underline{o}_2$ were reduced 60-80% and $\underline{ae}$ was reduced 30%; whereas there were no significant differences among the other genotypes and from normal. At 42 DAP, $\underline{su}_1$, $\underline{su}_1$ $\underline{o}_2$, $\underline{sh}_2$, and $\underline{sh}_2$ $\underline{o}_2$ were extremely reduced in starch content and $\underline{fl}_2$ $\underline{o}_2$, $\underline{ae}$ $\underline{o}_2$, $\underline{wx}$ $\underline{o}_2$, $\underline{du}$ and $\underline{du}$ $\underline{o}_2$ were reduced 18 to 19.5% below the normal hybrids. Other mutants were not significantly different from normal. Starch content was positively correlated with protein per endosperm.

Sugary-1 opaque-2 would offer a superior high-lysine high WSP corn conferring the desired textural properties for fresh eating or canned corn use. Shrunken-2 opaque-2 would offer a superior high-lysine high-sugar corn with potential for extending the harvest and storage quality of supersweet corns and may be a desirable snack food as raisin corn or used in dry cereal products. The $\underline{ae}$ $\underline{o}_2$ double mutant offers a high quality protein high sucrose corn and possibilities for use in vegetable corn improvement. Wacy opaque may offer a superior high-lysine glutinous corn for fresh corn-eating people and possibly may be used in other food preparations where glutinous starch properties are desirable.

THE EFFECT OF THE BRITTLE-1 SUGARY-1 DOUBLE MUTANT COMBINATION ON CARBOHYDRATE AND POST-HARVEST QUALITY OF SWEET CORN

C. Y. Tsai and D. V. Glover

ABSTRACT

Whole kernels of the double mutant combination of brittle-1 sugary-1 of maize (Zea mays L.) contain about 40% and 200% of the water-soluble polysaccharides and sucrose, respectively, of the sugary-1 mutant alone. More important, the double mutant preserves 90% of the sucrose even up to 4 days post-harvest at room temperature. These features of the double mutant may offer a desirable new high quality sweet corn with the added potential for improving post-harvest quality.

Search for Protein Crucial In Struggle Against Hunger

The New York Times/October 11, 1974

By JANE E. BRODY

Scientists are juggling plant genes, squeezing juice out of leaves and growing microorganisms on manure in a wide-ranging assault on one of the most fundamental problems of the world's food shortage—how to get more protein for more people.

The push for protein is a major part of the worldwide effort to fight the growing specter of hunger aggravated in recent years by unbridled population growth in already food-poor countries, crop failures caused by droughts and floods, fertilizer shortages and the energy crunch.

As a result, malnutrition is now epidemic in many countries. Experts estimate that nearly a third of the world's people are suffering from hunger and its consequences and that the diets of half the world's children lack adequate protein, the nutrient most essential to proper physical and mental development.

This is the first of two articles on the drive to increase the amount of protein in the human diet. The articles are part of a series that will appear from time to time examining the world food situation.

While carbohydrates and fats are most important as energy sources, protein is the core substance of the body's vital organs, including the brain. If a child's diet lacks sufficient protein during critical growth periods, body and mind may be permanently stunted. In adults, protein deficits prevent the proper rebuilding of body tissues.

Protein deficiency also increases susceptibility to infections which, combined with the stress of malnutrition, is the main cause of death among young children in developing countries.

Thus, in laboratories and farm fields throughout the world, scientists from government, industry and the universities are seeking to improve the protein quantity and quality of conventional foods as well as to develop novel sources of protein nutrition.

The approaches, besides extracting protein from leaves, using protein-rich wastes and breeding crops with more and better protein, include fortifying traditional foods and developing new foods from under-utilized proteins.

The United States, the world's major breadbasket, is the leading center for this research and the main source of funds for projects abroad. But the effort is worldwide, with considerable work under way in such industrialized countries as Japan, Scandinavia and England and at internationally supported research centers in Mexico, the Philippines, India, Nigeria and other developing areas.

Proteins (from the Greek for first or primary) are constructed out of about 20 different chemical building blocks called amino acids, all of which contain nitrogen. The human body is able to manufacture 12 of these amino acids from various sources of dietary nitrogen—proteins or parts of proteins that the body digests into molecules called amino groups.

But the remaining eight building blocks, called essential amino acids, cannot be made by human beings and must be supplied as such in the diet. In addition, in order for the body to make the proteins it needs, all the essential amino acids must be consumed in balanced amounts at approximately the same time.

Most Balanced Proteins

The most balanced proteins (that is, those that supply all eight essential amino acids in adequate amounts) come from animals—meat, fish and dairy products—but they provide less than a third of the world's protein.

Plants, which supply about 80 per cent of the protein for people in developing countries, are normally deficient in one or another essential amino acid. In order to obtain usable protein from vegetarian sources then, two different kinds of plant proteins that make up for each other's deficiencies—say, cereals and beans—must be eaten in the same meal.

Most of the current research has centered on plant proteins, which have the potential of adequately nourishing more people at less cost than meat proteins.

For a steer [illegible] to produce one p[illegible] requires the a[illegible] sumption over its lifetime of four to five pounds of grain. The pound of beef could provide protein for two persons a day, but the grain the animal consumed to produce that meat could theoretically feed four.

Despite all the research, science has not [illegible] nificant [illegible] world's pro[illegible] The complex[illegible] lems are such [illegible] tein foods can [illegible] even decade[illegible] the laboratory [illegible] mouths of the hung[illegible] factors as tas[illegible] centives and pr[illegible] the lack of techn[illegible] agricultural re[illegible] adequate number of [illegible] personnel all make [illegible]

Nonetheless, [illegible] are hopeful. Their [illegible] founded on the pr[illegible] if the earth's re[illegible] optimally used, [illegible] possible to provide adeq[illegible]

Above: dented kernels of high lysine corn. Below tropical type natives [illegible]

Protein Key in Fight Against Hunger

Continued

nutrition for the present world population and the more than six billion people who are expected to inhabit the earth in the year 2000.

A survey of scientific results to date has revealed a wide range of promising prospects—some that are now ready for application, others that need [illegible] further development and many that will require years more research.

Plant breeders, who by increasing productivity of crops have thus far kept the world from mass starvation, are now re-engineering some of the most fundamental characteristics of plants to improve their protein yield and quality. Breeding for better protein is one of the key efforts in the current protein thrust and one that is most likely to pay off in the near future.

Plant breeding is the painstaking, prolonged and somewhat unpredictable task of attempting to change the genes of one kind of plant by crossing it with close relatives that contain the desired genetic characteristics.

Perhaps the best known success of plant breeding toward boosting the world's protein supply is the development of the so-called miracle seed of the Green Revolution. These were semidwarf varieties of wheat (an achievement for which Dr. Norman Borlaug received the Nobel Peace Prize in 1970) and rice that, when properly cultivated with adequate fertilizer and water, can double the yield of grain per acre.

But the grain of the Green Revolution is still deficient in certain essential amino acids, and the emphasis of most of the current breeding work is on improving the amino acid balance of cereal crops.

Studied Protein in Corn

Twenty-eight years ago, when American grain elevators were bursting at the seams with excess grain, Dr. Edwin T. Mertz of Purdue University received a state grant "to figure out some way to use up all this surplus food in a nonfood manner." Since Dr. Mertz was a protein chemist, he began by studying the protein in corn.

Seventeen years later, this low-key effort led to an unexpected success—the discovery, with the plant geneticist Oliver E. Nelson (now at the University of Wisconsin), that certain varieties of corn contained twice as much of the amino acids lysine and tryptophan as are found in ordinary corn varieties.

Since these are the two deficient, or limiting, amino acids in corn, the discovery held the prospect of greatly improving the nutritive value of this grain, the principal staple in many countries of Latin America and Black Africa. Indeed, when young rats were raised on high-lysine corn, they grew nearly as well as rats raised on milk protein.

Piglets fed the new variety grew three and a half times faster than piglets fed ordinary corn. In fact, in Colombia a high-lysine corn diet was able to cure children of the severe protein deficiency disease, kwashiorkor, which they had developed from living on a diet of ordinary corn.

But the discovery of high-lysine corn was only the beginning. An incredible amount of work lay ahead to breed the high-lysine gene into otherwise successful native varieties without adversely affecting the desirable characteristics of those varieties.

The high-lysine gene changed the corn kernel from hard to soft, which affected milling characteristics. In addition, explained Dr. Loyal (Pete) Bauman, who heads the breeding work at Purdue, the softer kernels were more susceptible to ear-rot and, because the kernels weighed less, the yield per acre was lower.

Yields Almost Normal

Ten years of "backcrossing" have virtually solved the ear-rot problem and yields are now up to 90 per cent of normal, Dr. Bauman said. In backcrossing, the original high-lysine corn was crossed with standard high-quality varieties. The resulting new high-lysine hybrid was then repeatedly crossed with the original standard variety to breed out unwanted genetic characteristics while preserving the high level of lysine.

In each area where corn is grown, the breeding work must be repeated with local varieties in order to preserve the disease resistance and other agronomic characteristics necessary in those regions. High lysine corn is now being tested in many countries around the world, and American farmers grew some 200,000 acres of it last year for animal feed.

Purdue scientists have calculated that by eating only high-lysine corn plus a vitamin-mineral supplement, an adult could eat adequately on 10 cents a day.

As Dr. Mertz put it, the discovery of high-lysine corn "upset the apple cart of plant genetics," which had previously assumed that plant protein could not be changed by breeding. This inspired a search for better quality protein in other cereals.

Thus far, the search has paid off for barley, used mainly as animal feed in this country but an important food grain for about 200 million of the world's disadvantaged people in Eastern and Northern Europe, the Mediterranean and Near East, India and the Andean countries of South America. A high-protein, high-lysine barley strain from Ethiopia was found in screening 1,000 varieties in the World Barley Collection in Beltsville, Md.

Similar success has been achieved for sorghum, the world's fourth most important grain (after wheat, rice and corn), on which some 300 million of the very poorest people in the developing countries of Africa, East Asia and India depend.

High-Lysine Sorghum

Sorghum grows in towering stands, with heavy heads of edible seeds—produced atop tall, thick stalks.

High-lysine sorghum was discovered last year at Purdue by Drs. John D. Axtell, Dallas L. Oswalt and Rameshwar Singh. In six years of analyzing the protein of some 10,000 varieties of sorghum from all over the world under a grant from the Agency for International Development, the Purdue scientists found two varieties from Ethiopia that contain nearly a third more protein and twice as much lysine as other sorghum strains.

Tenant farmers of Ethiopia like to roast the nutty-flavored seeds of these two strains and eat them like nuts, Dr. Axtell discovered on a recent visit there. But only small amounts of these strains are grown because landlords, who get a share of what the farmer sells but not of what he eats, discourage their cultivation.

The varieties have been maintained largely because the tenant farmers plant them hidden in the middle of stands of other sorghum varieties so that landlords would be less likely

Protein Key in Fight Against Hunger
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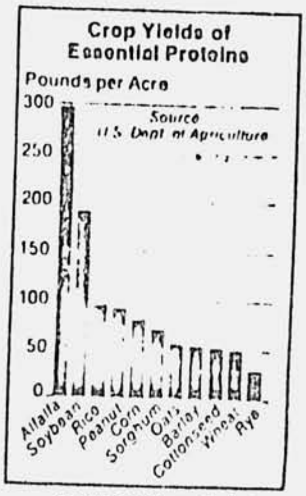

The New York Times/Oct. 11, 1974

to notice them, Dr. Axtell said.

Dr. Axtell, an enthusiastic but realistic scientist, estimates that a decade of work lies ahead to breed the high protein quality into native sorghums. Work is already under way toward this end in India, Lebanon and Mexico.

Once the scientists have completed their work, the task remains to convince farmers to grow the new varieties. "It's extremely difficult to get farmers to grow something you tell them is nutritionally superior when they've never even heard of nutrition," Dr. Axtell noted. "In Brazil, with high-lysine corn, they're telling farmers to feed it to their hogs. Once the farmers see how well the hogs thrive, it's easy to convince them that the corn is good for their children, too."

Protein in Oats Studied

A push is also on now to increase the protein content of oats, which have an exceptionally good balance of amino acids and already contain more protein than most cereals, said Dr. Vernon Youngs, a chemist who heads the Agriculture Department's National Oat Quality Laboratory at the University of Wisconsin. Last year, his laboratory analyzed the protein in 28,000 oat samples sent in by breeders throughout the country.

Until now, oat breeding emphasized larger kernels—which gives farmers bigger yields—but larger kernels meant a lower percentage of protein, Dr. Youngs said.

As a result of the new emphasis on protein, two new oat varieties containing about 22 per cent protein have already been released. Their protein content compares to the 17 per cent average of the World Oat Collection and rivals that of meat, which ranges from 20 to 30 per cent.

"Right now, even though oats are such a good food, only 5 to 10 per cent of the U. S. production goes into human foods," Dr. Youngs remarked. "We'd like to change this. We're feeding our animals better than our people."

Another cereal grain that has attracted much research interest is triticale (pronounced trit-i-kaley), a man-made hybrid of wheat and rye that surpasses wheat—the world's main cereal—in both total protein and lysine content. Its main drawback is that its hybrid genes are somewhat unstable, making it an unreliable producer of quality seed, according to Dr. Kenneth Lebsock, an Agriculture Department scientist who is overseeing Government-sponsored work on triticale in India.

Wheat itself appears to be resistant to much change in its protein quantity or quality. Thus far, 15,000 lines in the World Wheat Collection also housed in Beltsville, have been analyzed but nothing yet has come near the improvement in high-lysine corn.

However, steady progress has been made in developing hybrid wheat, which holds the promise of considerable increases in yield per acre. Scientists at Cargill Wheat Research Farms in Fort Collins, Colo., have just harvested their first crop of hybrid wheat, the seeds of which derived from two high-quality inbred lines.

Hybrid Vigor Concept

Theoretically, according to the concept of hybrid vigor, the hybrid seed should produce a crop better than either of its parents. The achievement depends on the development of one line in which the male part of the plant is sterile, preventing self-pollination, and a second line containing fertility-restoring genes so that fertile seeds will result from a cross with the male-sterile plant.

Beans, which Americans look upon as a source of carbohydrate but which much of the world uses for protein, have also begun to attract the interest of plant breeders. Although higher in total protein content than the cereal grains and adequately endowed with lysine, most beans are deficient in other essential amino acids, mainly methionine and cysteine.

At the University of Ife, Nigeria, Dr. Frederick Bliss, a University of Wisconsin plant breeder, helped develop a high protein (30 per cent) cowpea (also known as black-eyed or southern pea), an important protein source in many tropical and subtropical areas. According to what he calls his "sky-scraper" philosophy, he is now trying to improve the productivity of the African pea, in part by changing it from a vine, plant that grows along the ground to an upright one.

Dr. M. Wayne Adams at Michigan State University estimates that by changing the architecture of the field bean from a short bushy plant with lots of branches to a taller, nonbranching type that can produce the same number of pods in less space, the protein yield per acre could be increased by a third.

This season, Dr. Adams, who works under a grant from the Rockefeller Foundation, will examine "one by one" some 22,000 plants—the results of crosses he made last year—to select for those that grow tall and narrow.

Cross-pollinating bean plants is in itself an arduous, back-breaking task that requires the dissection and emasculation of flower buds one-sixteenth of an inch wide. Theodore Hymowitz, a plant geneticist at the University of Illinois, has enlisted bees to do the pollination for him. A table of soybean plants in a greenhouse is covered with a screened cage that is home for a hive of bees, whose diet of nectar and pollen from soybean flowers is supplemented with synthetic pollen. The result is thousands of hybrid seeds all year round, Dr. Hymowitz said.

Soybean Seed From China

He is seeking, among other things, to breed out some of the plant sugars that humans have difficulty digesting, resulting in the classic "gassiness" of beans. In the search for better soybeans, Dr. Hymowitz had been disturbed that no breeding material was available from the Chinese mainland, where the soybean originated. Last week however, American scientists returned from China with many native soybean seeds, including some wild soybeans, to test in American breeding programs.

Soybeans, with an average of 40 per cent protein (nearly twice that of most meats), contain more and better protein than any other edible plant. But like all beans, its yields are low compared to cereal crops such as corn and wheat, a fact that Richard Cooper, an Agriculture Department researcher in Urbana, Ill., is trying to change by developing a more efficient semidwarf soybean plant.

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As he explained it, when a lot of fertilizer is used to increase the yield of ordinary soybeans, they get so tall that they fall over, or "lodge," which diminishes yield. Using the approach that produced the greatly increased yields of miracle rice and wheat, Mr. Cooper has bred a 22-inch plant that produces as many beans per acre as the 44-inch plant. This year he has planted the short variety closer together to see if yields will increase.

Other scientists at the Regional Soybean Laboratory are trying to create soybeans that will bloom regardless of how long the day or night is, a characteristic that would allow the soybean to spread into the food-short tropics. One such "day-neutral" variety is now being tested in Puerto Rico.

Rather than wait for desired genetic variants to occur naturally and, following their discovery, breed them into native crops, some scientists are attempting to induce genetic change in existing crops with radiation or chemicals. This approach, which has already succeeded in producing a high-lysine barley and a high-yielding semidwarf rice, can potentially save the plant breeder much time and effort.