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Annual Report
on

Inheritance and Improvement of Protein Quality and Content in Maize

April 1, 1974 – March 31, 1975
Contract csd/2809
Agency for International Development
Department of State
Washington, D.C.

ANNUAL REPORT

on

THE INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN MAIZE

Contract csd/2809

April 1, 1974 - March 31, 1975

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 Improvement of Protein Quality and Content in Maize; Contract number AID/csd-2809.

2. Principal investigator, contractor and mailing address:

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3. Contract period (as amended): from July 1, 1970 to March 31, 1975.

4. Period covered by report: from April 1, 1974 to March 31, 1975.

5. Total A.I.D. funding of contract to date: $1,011,631. (to March 31, 1975).

6. Total expenditures and obligations through previous contract year: $975,900.88 (through March 31, 1975).

7. Total expenditures and obligations for current year: $218,498.22.

8. Estimated expenditures for next contract year: $250,000.00.

B. Narrative Summary of Accomplishments and Utilization

Most new mutants with higher lysine content identified in Colombian germplasm have proved to be allelic to opaque-2. About 30,000 individual kernels from 30 collections from Central American areas have been screened with the ninhydrin test. A number of high protein kernels have been identified but few have high lysine content. The ninhydrin test could also prove valuable as a market place test to identify high lysine varieties

Modified (vitreous) opaque-2 varieties can be selected with good yield (not equal to normal), resistance to ear rots and grain insects and good protein quality. A recently discovered "single" gene modifier system

that in preliminary tests does not reduce protein quality offers considerable potential. To date the modified opaque 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 of high lysine maize because of its vitreous kernel, ear rot resistance and good nutritional value as shown by feeding trials. This type may prove useful in the future, especially if its lower yield 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 yield. All the mutants improve protein quality by decreasing zein and increasing other fractions that are higher in lysine.

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

Feeding trials with maize, sorghum, wheat and triticales showed the weanling rat was superior to the mouse, vole and chick as a test animal. Two bulletins on "Simple chemical and biological methods used at Purdue University to evaluate cereals for protein quality" and "Progress in developing maize with improved protein quality" have been distributed. Seed of various mutants and varieties has been widely distributed to other breeding projects.

Diverse genotypes grown at a number of locations from tropic to temperate areas revealed some interesting interrelationships between growth and heat units as measured by temperature, solar radiation and net radiation.

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Dr. Helen E. Clark - Department of Foods and Nutrition

A. GENERAL BACKGROUND

In the poorer countries of the world cereals provide a major portion of the protein in human diets. Maize is one of these cereals providing both calories and protein for millions of people. This is particularly true in the Central American area, Africa, and other regions of the world. Maize, however, is deficient in the essential amino acids: lysine and tryptophan. This deficiency is a major constraint on improvement of human and animal nutrition.

Problems associated with high lysine maize have pointed up the necessity of continued basic and applied research on genetics, breeding, analytical methods, and nutritional characteristics associated with improving quality of protein in maize. Utilization of high lysine maize in developing countries will be realized only through sustained interdisciplinary and cooperative research into these complex problems. Maize with improved protein quality and agronomic characteristics can play an increasing role in solving the world's human and animal nutrition problems.

The purpose of this project is to develop source germplasm that will be capable of supplying more adequate amount of nutritionally balanced protein in the diets of monogastric animals, including man, and contribute to the creation of and participate actively in a world-wide system of maize improvement research. This involves researching the most relevant and important fundamental problems in this area, widely disseminating the findings, providing basic undergirding for the international centers and breeding programs in developing countries.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

1. 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.
2. 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.
3. Evaluate effectiveness of various selection schemes for modifier gene(s) that improve yield, nutritive value and milling characteristics of new gene types.
4. Investigate maximum nutritional improvement with these new genes in conjunction with selection for embryo size and protein content.
5. 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.
6. Examine extent and interactions of environment and cultural practices on amino acid and protein levels.
7. Determine adequate field testing and sampling procedures.
8. 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.

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

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

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

12. 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.

13. 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. ACCOMPLISHMENTS TO DATE

Evaluation of New Mutants.

Mutants 40, 58 and 62, which we reported last year to be high in lysine have been found allelic to opaque-2. Also, the high lysine sub-families of mutants 11, 95, 102 and 106 are allelic to opaque-2. The frequency of occurrence of opaque-2 among the putative new mutants suggests that contaminant outcrosses from the opaque-2 breeding programs may have occurred in the source populations. Mutants 89, 103, and 106 are allelic to each other, but are not allelic with opaque-2, opaque-1, floury-2 nor soft starch (h).

Use of The Ninhydrin Test in Screening For New Mutants.

We are using a Ninhydrin technique to non-destructively test normal-looking vitreous kernels for the possible occurrence of a new high-lysine mutant without the undesirable side effect on kernel texture

and grain yield. Our initial effort was to test 30 collections from the germplasm bank sent to us by Mario Gutierrez of CIMMYT. Approximately 300 kernels of each collection were tested on an individual kernel basis. In 27 of the 30 collections, one or more kernels gave a positive or intermediate reaction. From six collections we were able to pool small endosperm pieces from six positive reacting kernels each and they were analyzed in Dr. Mertz's laboratory for total protein and for lysine. None of the six were high in lysine as a percent of protein, but all six were high in protein, from 12.19 to 20.02%. This is our first preliminary evidence that free amino acid levels may also rise with total crude protein. Since total crude protein content of kernels on individual ears may vary greatly with environmental factors, including poor seed set, we are not greatly optimistic about the practical implications of this evidence at present. The positive reacting kernels are being grown by S.K. Vasal at CIMMYT.

A better controlled study of the usefulness of variations of the Ninhydrin technique has been initiated.

Selection For Improved Protein Quality in Normal Maize.

Endosperms of 200 normal families (ears) from Temp HA and Temp HB populations were analyzed for protein and lysine content. Variations found were 7.3 to 14.6 for percent protein, 1.26 to 2.28 for lysine as percent of protein and .150 to .238 for lysine as percent of sample (Table 1). This variation is probably sufficient to assure some progress in selecting for protein quality in these normal populations.

Table 1. Variation in protein quality in endosperm from 100 normal families (ears) each in Temp HA and Temp HB.

		Temp HA	Temp HB
Percent protein	Mean	11.1	10.5
	Range	7.3-14.6	7.9-14.3
Lysine as a percent of protein	Mean	1.71	1.74
	Range	1.26-2.28	1.43-2.13
Lysine as a percent of sample	Mean	.187	.181
	Range	.150-.238	.154-.226

Temperate Germplasm.

During the first coordinating conference with CIMMYT the urgent need for varieties adapted to the temperate areas of the world was recognized. This project developed two opaque-2 varieties, Temp HAo_2o_2 and Temp HB o_2o_2 from diverse germplasm from around the world and the U.S. Cornbelt. These populations should permit development of non-modified or modified opaque-2 varieties. Approximately 150 full sib were tested at four worldwide locations. Because of warfare, drought and frost damage, data was obtained only from the Indiana location. That location gave ranges for percent protein and lysine as a percent of protein for Temp HA of 9.5-12.7 and 3.75-4.61 and for Temp HB of 10.1-13.2 and 3.62-4.59 respectively. Selection should be quite effective in establishing excellent protein quality in these two populations. Several cycles of selection will be required to improve agronomic performance.

To further fill the need for varieties adapted to termperate areas the variety Colus developed at Purdue from Colombian and U.S. germplasm has been converted to opaque-2 and is undergoing selection.

Maximum effort is being placed on the improvement of the populations Temp HA su_2o_2 and Temp HB su_2o_2. In the 1974 summer nursery over 300 ear families were selfed out to produce S_1's in each variety. The mean

and range of protein and lysine content represented by selected S_1 ears in the 300 families of the Temp HB su_2o_2 variety is shown in Table 2.

Table 2. Temp HB su_2o_2 synthetic whole kernel protein percent and lysine as a percent of protein from S_1 ears.

Protein composition	Mean	Range
Protein (%)	12.6	8.8 - 15.9
Lysine as a percent of protein	4.30	2.70 - 6.10

Based on kernel vitreousness and quality 270 full sibs were made in each variety in the 1974-75 winter nursery. These will be yield tested and full-sib families will be selfed in the 1975 summer nursery. Full sib family selection will be continued.

The waxy opaque-2 (wxo_2) and brittle-2 opaque-2 (bt_2o_2) double mutant combinations are also being developed in the Temp HA and Temp HB synthetic background. A mass selection or half sib mating system of selection is being used. Plants from 300 Temp HA wxo_2 and Temp HB wxo_2 families were self pollinated in the 1974 summer nursery to produce S_1's. The protein and lysine content of S_1 seed from these two populations is shown in Table 3.

Table 3. Temp HA wxo_2 and Temp HB wxo_2 synthetic whole kernel protein and lysine as a percent of protein from S_1 ears.

	Waxy opaque-2 Synthetic			
	Temp HA		Temp HB	
Protein Composition	Mean	Range	Mean	Range
Protein (%)	11.3	9.4-15.1	11.6	8.9-14.8
Lysine as a percent of protein	4.15	3.47-5.08	4.21	2.65-5.48

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

The sugary-2 opaque-2 double mutant genotype is being backcrossed into 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 the Illinois High Protein (IHP) material. In addition sugary-2 opaque-2 genotype is being backcrossed into one agronomically desirable selection of "modified-opaque-2, high protein" line recoveries in the Oh43 and B14 inbred sources recovered from crosses to IHP material. Protein values in the backcross 3 generation recoveries from the 1974 nursery materials averaged around 1 to 2.5 percentage points higher than the non-recurrent parents.

Multiple Aleurone Sugary-2 Opaque-2 Conversions.

The project is studying the feasibility of increasing the proportion of aleurone tissue relative to the starch portions of the endosperm in an additional effort to improve the protein nutritional quality. In 1974 we completed the backcross 2 or 3 generation conversion of the multiple aleurone characteristic to about 15 elite inbred backgrounds. The multiple aleurone character is not simply inherited and thus not easy to transfer. Through selective screening for the multiple aleurone characteristic we have established lines of o_2, su_2 and su_2o_2 with 2 and 3 aleurone layers and hope to increase this number by selection as backcrossing is continued. The stability of the aleurone number and effectiveness of this approach will be determined in hybrid evaluation and testing.

Evaluation of Independent Mutant Sources of Opaque-2 and Sugary-2.

Seven opaque-2 sources (mutational events) have been collected and are available for distribution to provide nonrelated or diverse sources of this gene. These sources have been backcrossed into inbreds B37 and

W64A for detailed studies on possible allelic differences in inbred and hybrid backgrounds. If opaque-2 is a compound locus then differences among the mutational sources might be expected.

Independent mutant sources of sugary-2 ($\underline{su}_2^{std}$, $\underline{su}_2^{i}$, $\underline{su}_2^{PI}$, $\underline{su}_2^{P}$) are being backcrossed into common inbred backgrounds Oh43, W67A, B37, C103, C123 and A632. These mutant sources will be evaluated in intercrosses within an inbred to determine if any $\underline{su}_2$ sources are superior to others and the nature of the $\underline{su}_2$ locus. Backcross conversions are complete for the sugary-2 standard allele, and is continuing with other alleles.

Modified and Nonmodified Opaque-2 Germplasm.

Studies of the modified (vitreous) opaque-2 types may be summarized as follows: (a) modified varieties are more acceptable for human food uses and more resistant to grain insects and ear rots, (b) modified varieties can be selected from any heterozygous population, (c) there is a negative relationship between vitreousness and protein quality, but selection (based on analyses) can maintain higher levels of protein quality, and (d) the modified phenotype varies with different environments.

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 $\underline{o}_2\underline{o}_2$. 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 $\underline{o}_2\underline{o}_2$ have been random mated for three generations. The successive samples of these eight varieties will be grown to evaluate genetic and environmental stability of modified and nonmodified types.

Detailed fractionation (Landry-Moureaux) studies have been made of high and low lysine types in both modified (vitreous) and opaque versions.

The distribution of protein fractions in low lysine types either modified or opaque phenotype is quite similar to that found in normal. Selection for the high lysine modified phenotype shifts the protein fraction pattern back toward that found in the opaque phenotypes.

Modified and opaque selections with varying levels of protein and lysine have been developed (Table 4). These were increased for studies on environmental and genetic stability and for feeding trials with rats to be conducted in 1975-1976. Because of the variation in lysine and protein these feeding trials may provide some guidance for selection criteria in high lysine varieties.

Table 4. Variations in protein and lysine of modified and opaque selections in HM o_2o_2.

Selection	Percent protein	Lysine as a percent of protein	Lysine as a percent of sample
Modified-High Lysine			
Range	7.3-9.9	2.72-3.45	
Mean	8.94	3.04	.272
Modified-Low Lysine			
Range	8.4-12.3	2.29-2.82	
Mean	11.02	2.59	.285
Opaque-High Lysine			
Range	7.0-9.7	3.11-3.81	
Mean	8.76	3.51	.307
Opaque-Low Lysine			
Range	7.7-12.3	2.19-2.92	
Mean	10.66	2.54	.271

Progress in protein quality improvement, kernel vitreousness and seed size is being evaluated in the Temp HB su_2o_2 synthetic in modified opaque-2 backgrounds. In the 1974 summer nursery 500 random ear families were selfed and plants from selected S_1 ears were half sib mated in the 1974-1975 winter nursery.

Single Gene Modifier of Opaque-2

A very promising, apparently "single", gene modifier has been found that gives a vitreous kernel in combination with the opaque-2 gene. This modifier gene is particularly promising because it does not reduce lysine levels as is true with most modified opaque-2 varieties (Table 5). The gene is being introduced into opaque-2 inbreds and populations Temp HA o_2o_2 and Temp HB o_2o_2 to further evaluate its potential value.

Table 5. Kernel weight, protein and lysine of modified and opaque kernels.

Phenotype	Kernel wt. (g)	Percent protein	Lysine as a percent of protein
Vitreous *	10.7	11.8	3.7
Opaque *	10.6	11.7	3.6

* based on analysis of 12 families

Double Mutant Combinations.

We have been studying the modification of kernel characteristics and nutritional quality in maize through the genetic interactions which result from double mutant combinations of endosperm mutants. For the high lysine maize to become more acceptable, particularly in the more tropic areas, a hard endosperm, high lysine type is needed. An important finding has been the discovery that the double mutant sugary-2 opaque-2 (su_2o_2) has improved kernel vitreousness and kernel density similar to normal.

Kernel density ranged in normal from 1.24-1.29 g/ml in opaque-2 from 1.11-1.14 g/ml while that of sugary-2 opaque-2 ranged from 1.22-1.27 g/ml. Sugary-2 opaque-2 has shown some important improved characteristics over standard opaque-2 and modified opaque-2 corn. The improved vitreousness of sugary-2 opaque-2 is not necessarily accompanied by a decrease in protein

quality (Table 6). When sugary-2 is combined with opaque-2, the double mutant supresses zein synthesis and shows a synergistic effect.

Table 6. Protein and lysine concentration of endosperm fraction samples from six hybrids of $\underline{su}_2\underline{o}_2$, $\underline{su}_2$, $\underline{o}_2$ and the normal counterpart hybrids.

Genotypes	Protein (%)	Lysine as a percent of protein	Lysine as percent of sample
+	10.41	1.75	.18
$\underline{o}_2$	8.98	3.25	.29
$\underline{su}_2$	11.04	2.20	.24
$\underline{su}_2\underline{o}_2$	9.21	3.72	.34

Baenziger and Glover demonstrated experimentally with use of interference-contrast light microscopy that sugary-2 opaque-2 maize has a more extensive protein matrix than observed in opaque-2. The improved kernel vitreousness appeared to be associated with a more extensive thick matrix from the sub-aleurone layer which is continuous into the center of the endosperm with many subcellular enclosures that surrounded the starch granules. Thus there are perhaps fewer air spaces resulting in refractive indices similar to endosperm of normal maize.

The double mutant waxy opaque-2 has protein quality equivalent or better than opaque-2, improved *in vitro* digestibility characteristics and good apparent biological value. Kernel quality is not as good as sugary-2 opaque-2 but more nearly like opaque-2. It is not improved in vitreousness and density. 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.

Not unlike the normal varieties of the U.S. and tropics, the vegetable maize varieties have poor protein quality. The high lysine mutant opaque-2 has been almost unused in vegetable corn improvement. Protein and carbohydrate quality studies (G.A. Tosello's thesis) of several endosperm mutants and their combinations with opaque-2 at vegetable corn maturities suggest that sugary-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. Shrunken-2 opaque-2 and brittle-2 opaque-2 offer improved 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.

Development of Sugary-2 Opaque-2 Inbreds and Evaluation of Hybrids.

The project has reported on the development of a restricted number of opaque-2, sugary-2, sugary-2 opaque-2, waxy and waxy opaque-2 inbreds and their hybrids and these have been evaluated for kernel characteristics and protein quality (See Annual Report July 1, 1973 - June 30, 1974). Seed has been released and distributed widely of the Oh43 su_2su_2, B37 su_2su_2, C103 su_2su_2, A632 su_2su_2, Oh43 wxwx, C103 wxwx, and A632 wxwx. Because of the superior kernel characteristics and improved protein quality of the double mutant sugary-2 opaque-2, the su_2 and su_2o_2 genes are being backcrossed into 16 additional elite inbred backgrounds. Emphasis is being placed on evaluation of the hybrids in respect to improved kernel size as it affects yield potential along with improved kernel vitreousness and protein quality. These isogenic lines are being used in developmental studies to determine the interactions of genotype on the germ and endosperm storage and compensating effects involved in protein, starch and oil accumulation in the kernel.

Biological Value of su_2o_2 and wxo_2 Double Mutants.

Two growth trials were conducted with rats comparing the nutritional quality and carbohydrate availability of near-isogenic 3-way hybrid normal, opaque-2, waxy, waxy opaque-2, sugary-2 and sugary-opaque-2 corns. Trial 1 diets were fed with 8, 11, and 14% protein levels. Rats fed diets containing opaque-2, waxy opaque-2 and sugary-2 opaque-2 showed improved average daily gain (AGD) and feed efficiency (F/G) over those fed normal, waxy and sugary-2 at 8% protein and 11% protein, but no significant differences were observed at 14% protein. In terms of endosperm type, at 8% protein rats fed sugary-2 and sugary-2 opaque-2 diets had better ADG and F/G than waxy, waxy opaque-2, and normal and opaque-2. At 11 and 14% protein there was no effect of endosperm type. In trial 2 the corns were compared on an equal weight basis in protein adequate (15-16%) diets. There were not differences in ADG or F/G. Two rat digestion trials were conducted to compare the same corns mentioned above in protein adequate and protein limiting diets. In trial 1 all diets consisted of 78.5% of the respective corn and 21.5% supplement and contained about 16% protein. No differences in ADG or F/G were found. Normal, waxy opaque-2 and waxy were higher in digestible energy than sugary-2 and sugary-2 opaque-2 and opaque-2. No differences in nitrogen digestibility, nitrogen retention or apparent biological value were observed in protein adequate diets. In trial 2 rats were fed isonitrogeneous diets at 8% protein containing the same corns. ADG and F/G were best for sugary-2 opaque-2 and opaque-2 followed by waxy opaque-2 and sugary-2 then waxy and normal fed rats. Dry matter digestibility was highest for waxy and normal and lowest for opaque-2 and sugary-2 opaque-2 and nitrogen digestiblity was highest for normal and waxy opaque-2 and lowest for opaque-2 and sugary opaque-2 fed rats.

Nitrogen retention and biological value were highest for sugary-2 opaque-2 and opaque-2 and lowest for normal fed rats. These results indicate that endosperm type has little effect on carbohydrate availability, but does have an effect on protein quality of corn. Several swine feeding trials are underway as well as evaluations of sugary-2 opaque-2 and opaque-2 corn diet comparisons in human subjects.

Two metabolism studies were conducted to evaluate the nutritive values of these same corns for beef cattle. In experiment 1 (isonitrogenous basis-11% crude protein diets) waxy corn (74.67%) was lower ($P<.05$) in digestible energy than normal (78.21%), opaque-2 (77.90%) or waxy opaque-2 corn (78.20%). In experiment 2 (isonitrogenous basis - 11% crude protein diets) steers fed the sugary-2 opaque-2 diet had higher ($P<.05$) absorbed, nitrogen retained (64.77%) than steers fed normal (47.20%) or sugary-2 (49.82%) corn diets, but dietary nitrogen retained was not significantly different among diets. The sugary-2 diet produced a nonsignificant higher (77.91%) digestible energy than the normal corn diet (75.91%). All other parameters studied indicated no significant differences between corns.

Improved Analytical and Biological Methods.

The central biochemical unit has functioned as a center for instruction in and standardization of analytical procedures for determination of the nutritional quality of maize samples by chemical and physical-chemical means. Each semester a special topics course (Biochemistry 695) has been offered to graduate students in our Agronomy, Plant Pathology and other agricultural disciplines on amino acid and protein analysis of cereals. During this period we published a bulletin entitled "Simple Chemical and Biological Methods Used at Purdue University to Evaluate Cereals for Protein Quality". It contains directions on sample preparation, protein

determination, the ninhydrin test, tryptophan analysis, lysine analysis, the dye binding method and the biological assay for protein quality in rats.

Characterization of Proteins in High Lysine Endosperms.

The level of free amino acids in opaque-2 alone and in all double mutant combinations with opaque-2, is 2 to 10 times higher than the respective normal counterpart. 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. Quantitative determinations of the total free amino acid level in single and double mutant endosperms from 4 different inbred lines showed that opaque-2 had a higher level of free amino acid in all the genetic backgrounds studied when compared with the respect normal counterpart. However, there were wide differences between inbred lines (2-5 fold). In the case of the double mutant, the introduction of opaque-2 increases the free amino acid level of all the genotypes again with wide differences between different inbreds. In the present reporting period, these studies were extended to whole kernel analysis with similar results, and also to whole kernel analysis of hybrid combinations, again with similar results.

These studies raised the question as to whether similar differences in the total free amino acid levels existed in the early developing kernel. To study this, whole kernels of opaque-2, brittle-2, the double mutant brittle-2 opaque-2 and the normal counterpart of the Oh43 background were analyzed, 10, 14, 21, 28, 35, 42 and 49 days post-pollination. It was found that the total level of free amino acids was similar in these four selections and very high, representing about 30 to 40% of the total nitrogen in the developing kernel, ten days postpollination. At fourteen days

postpollination, there was a drop in the level of total free amino acids but little or no difference in level between the mutants and the normal counterpart. From the 21st to the 49th day postpollination there was a gradual drop in the level of free amino acids with a separation between the mutants and the normal counterpart. The normal counterpart dropped more rapidly than did the mutants, resulting in a higher level of free amino acids in the mutants at the 49th day and at maturity.

In addition to following the level of free amino acids in the developing whole kernels described above, each of the amino acids present in the amino acid pool was determined on the same postpollination dates. This study was carried out primarily to determine whether the level of lysine was exceptionally high in the mutants as compared to the normal counterpart during early development of the seed. The results were similar for each of the amino acids and showed that there were no major differences in total percent of each amino acid in the pool on the six postpollination dates tested. These data suggest that lysine is not being overproduced due to the mutations studied, but rather that there is an interference with the disposal of the amino acid pool with maturation in the mutants. This could be related to the blockage of synthesis of zein in the mutant endosperms.

The Landry-Moureaux method of fractionation which has been used in studies at Purdue has been compared with the protein fractions obtained by Wall and Paulis in studies at the Northern Regional Laboratory in Peoria, Illinois. These studies showed that each of the Paulis-Wall fractions could be related to one of the Landry-Moureaux fractions based on amino acid composition. The basic difference between the two fractionation methods is the sequential extraction of the proteins from the

endosperm with various reagents leaving a starch residue behind (Landry-Moureaux method) as compared with removal of albumins, globulins and zein first, followed by removal of starch with a starch-splitting enzyme, and then sequential fractionation of the glutelin fraction with special reagents (Paulis-Wall method).

The proteins present in the Landry-Moureaux fractions have been separated and their molecular weights determined by acrylaminde gel electrophoresis. Using this technique, we have demonstrated that the zein fraction of opaque-2 endosperm contains a lower level of the 25,000 dalton polypeptide than is found in the normal counterpart and in other high lysine mutants. In addition, the opaque-2 mutant endosperm contains in its zein-like fraction a 14,000 dalton component which is not present in the normal counterpart or in other mutant endosperms. These are the first major differences found between mutant endosperms and their normal counterpart other than the well known shifts in proportions of the major protein fractions which was reported previously.

Climatalogical Factors in Maize Adaptation.

Length of time from planting to maturity is one of the key components of adaptation of maize to seasonal climatic changes and/or the desired cropping sequence. Heat unit accumulations expressed as Effective Growing Degree Days have been shown to be a more consistent expression of time to maturity of maize varieties than number of days in the Cornbelt. Table 7 is a summary of heat unit data collected at CIAT, Cali, Colombia; CIMMYT's stations El Batan, Tlalticipan, and Posa Rica (high, medium and low altitude, respectively); Columbia and Portageville, Missouri; Ft. Collins and Rocky Ford, Colorado; Madison, Wisconsin; and Lafayette, Indiana during 1971, 1972, and 1973. The consistency of response can best be measured by

the coefficient of variability (C.V.) since the units differ in magnitude. The data show that Corn Heat Units (CHU's) is more consistent in the diverse climatic situations encountered in international maize varietal development. The possible contribution of accumulated solar radiation and/or net radiation to such a predictive formula is still being analyzed.

Table 7. Comparison of varietal maturity[1/] rating over diverse environments by four different units.

	Days		GDD[2/]		EGDD[3/]		CHU[4/]	
Entry	Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
ICA-H208	138	20.2[5/]	1666	14.4	1592	12.3	2444	8.5
ICA-H255 (o_2)	166	11.6	1421	14.5	1400	12.5	2332	9.8
Eto	140	22.2	1686	17.3	1612	14.9	2475	9.2
Harinoso Mosquero	191	4.4	1459	6.0	1458	5.9	2528	4.9
Oaxaca 179	109	22.0	1305	16.4	1250	14.7	1919	11.9
Antigue Group 2	129	23.7	1605	14.5	1532	12.6	2335	10.4
St. Croix	176	11.2	1515	13.9	1492	11.7	2487	6.5
Tuxpeno P.B.	135	23.9	1698	15.0	1621	12.9	2456	8.7
H49 x H44 (o_2)	118	21.8	1430	14.6	1370	12.7	2096	9.3
H71 x H60 (o_2)	133	21.1	1367	6.8	1334	6.2	2116	6.8
N28 x Mo17	122	24.1	1446	13.4	1388	11.7	2130	9.3
A619 x A632	114	23.0	1362	12.8	1305	10.6	2003	7.1
USA 342 x Eto	134	21.7	1708	15.0	1622	13.1	2464	10.1
ICA-H302	135	20.8	1737	16.0	1656	14.0	2497	8.9
Composite K (o_2)	125	21.5	1652	12.7	1573	11.2	2362	8.3
Colus	116	22.7	1461	14.9	1393	13.2	2112	10.1

1/ Planting to physiological maturity as indicated by "black layer" formation.

2/ Growing Degree Days = Σ Daily $\frac{\text{Min + Max Air Temp}}{2}$ - 10C where Mins. less than 10°C are set equal to 10.

3/ Effective Growing Degree Days, Same as above except Maximum Temperatures greater than 30°C are set equal to 30.

4/ Corn Heat Units = Σ Daily $\frac{1.85\ (\text{Max}-10) - .026\ (\text{Max}-10)^2 + \text{Min} - 4.4}{2}$

5/ Legitimate comparisons cannot be made between different varieties (horizontal lines) in this table because of differing combinations of environments.

D. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results:

a. Research report. Approximately 600 copies of the 1973-74 annual research report were distributed to over 500 maize cooperators and interested centers, missions or institutions representing over 35 countries and many institutions within the U.S.

b. Status report. Over 1,000 copies of an updated and expanded revision of a status report--Purdue University Agricultural Experiment Station Research Bulletin 914 (October 1974)--entitled "Progress In Developing Maize With Improved Protein Quality" has been distributed world-wide to interested persons. The report includes 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.

Approximately 900 copies of a second status report - International Programs in Agriculture, Agricultural Experiment Station, Purdue University Station Bulletin No. 70 (March 1975) - entitled "Simple Chemical and Biological Methods Used at Purdue University to Evaluate Cereals for Protein Quality" has also been distributed worldwide to interested maize workers.

c. Workshops, symposia, papers and seminars presented. Project personnel presented papers and/or participated in the following activities: American Society of Agronomy Annual Meetings; The American Association of Cereal Chemists; VII International Symposium on Carbohydrate Chemistry, Bratislavia, Czechoslovakia; Sixth Meeting of Maize Breeders of the Andean Zone, Maracay, Venezuela; Protein Advisory Group (PAG) participant in workshop to develop guidelines on chemical and biological methods for plant breeders, PAG (UNICEF) Obregon, Mexico;

Committee member of U.S. National Committee of the International Union of Nutritional Sciences, Washington, D.C.; NSF Special Study Panel on Cereal Proteins, Massachusetts Institute of Technology; International Symposium on "Haploids in Higher Plants - Advances and Potential", Guelph, Canada; member evaluation team for proposed East African coordinated cereal breeding and protein quality evaluation project. Project personnel gave several seminars and participated in special field day and program activities in the Agronomy, Biochemistry and Animal Science departments at Purdue University and at other institutions.

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: Balint/Hungary; Nemeth/Hungary; Grobman/Peru; Gul/Afghanistan (Kanbul); Dunford/U.S.A.; Poey/Mexico; Kabore/Upper Volta, Africa; Fuwa/Japan; Bonucci/U.S.A.; Tomov/Bulgaria; Kim/Korea; Pollmer/Germany; Iudy/Pakistan; Gaova/USSR; Boehm/Germany; Nekez/Hungary; Munck/Denmark; Djamhuri/Indonesia; Yang-Lai/Taiwan; House/Lebanon; Izuno/Pakistan; Alber/Germany; Vrba/Czechoslovakia; Lorenzoni/Italy; Radic/Yugoslavia; Rivera/Colombia; Rosic/Yugoslavia; Vasal/Mexico; Ordas/Spain; Chang/Taiwan; Pinot/France; Ekpenyong/Nigeria; Marani/Italy; and Vitosovic/Yugoslavia.

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

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

Requests have continued for information, seed, analytical monitoring, technical help and advice concerning protein quality improvement in maize. Interest was especially strong in modified opaque-2 and double mutant (sugary-2 opaque-2) germplasm. The number of visitors from LDC's and other countries to Purdue continued to increase. These personal contacts have helped guide numerous protein quality improvement programs along more productive approaches.

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

3. Plans for Effective Ways to Expand Use of Research Results:

The project continues to coordinate with CIMMYT on an annual basis to develop work plans and cooperative and complimentary programs for maize protein quality improvement for LDC's, particularly for the more temperate regions. The linkage with CIMMYT, Andean Zone breeders, East Africa, Guatemala and other research centers in the world-wide maize network appears essential in providing of backstopping basic research, fundamental knowledge and multi-disciplinary scientific capacity to international centers and LDC institutions.

The project is planning to assist the Guatemalan maize workers to identify problems and develop programs related to the production of maize and improved protein quality maize.

Plans are underway to assist in a maize and sorghum workshop planned for Africa to identify problems, provide training, and

exchange information related to utilization of improved protein quality maize in that region.

Cooperative testing of temperate selections continues in Pakistan, Israel, Mexico, and U.S.

Continued training of students, visiting scientists and researchers (short-term) from LDC's on problems related to maize production and protein quality improvement facilitates exchange of information among maize workers and provides trained leadership for conducting maize breeding and production programs for national programs.

The Project personnel continue to participate in seminars and workshops, share germplasm and data related to protein quality improvement, and cooperate with maize workers in the world-wide network.

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

An annual workshop and planning conference with CIMMYT maize research staff (Drs. E. W. Sprague, E. Johnson and E. Villages) was held August 21-23, 1974 to exchange information, discuss research plans and coordinate efforts. Testing of wide-based temperate germplasm materials for the more temperate regions of the world has been coordinated by CIMMYT-Purdue staff. These materials are being developed to compliment the varieties developed for the more tropical areas of the world. Project personnel are involved in cooperative work to screen the Central American maize germplasm from the germplasm bank, Mexico City, for vitrious high lysine types. Project personnel have been involved with the Andean corn breeders in special workshops to guide the development and utilization of improved protein quality maize. Project personnel have participated in an evaluation team for the proposed establishment of a cereal protein quality laboratory,

extension of maize breeding methods study and coordinated disease resistance and protein quality breeding project for East Africa.

Students from Mexico (Alfredo Navarro), Philippines (Lourdes Nazarea), Colombia (Hugo Zorillo, Nora D'Croz), Brazil (Geraldo Tosello), Pakistan (Iffat Rahim) and the United States (P. Stephen Baenziger, Theron E. Boundy) were in graduate training during the project year. Dr. P. S. Misra (India) received training as a post-doctorate during the year.

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

Extensive correspondence has also been an important means of disseminating information, data, technical help and germplasm source of the improved quality maize materials to the LDC's.

E. WORK PLANS FOR 1975-76 AND PROJECTED GENERAL PLANS FOR 1976-78 UNDER AID CONTRACT AID/ta-c-1211

OBJECTIVES:

1. In cooperation with other maize breeding programs (particularly that of CIMMYT), expand the search for and evaluation of new mutants and germplasm sources with improved protein quality and quantity, both in existing maize populations and by means of chemical mutagens and to introduce these new mutant genes into lines and populations of use to breeders in LDC's.
2. Concentrate on the development of double and multiple combinations of endosperm mutants and determine the effect of associated interactions on nutritional quality, physical properties of the kernel and agronomic characteristics, with a view to the improvement of grain type and yield as well as protein content and quality.
3. Determine the extent of interactions of both genetic backgrounds and environment with individual or combinations of endosperm mutants and how such interactions may influence protein quality and breeding methods.
4. In cooperation with CIMMYT and other maize workers, develop special varieties and source breeding materials with improved nutritional and agronomic characteristics for use in the LDC's. Special emphasis will be given to opaque-2 and sugary-2 opaque-2 materials adapted to more temperate regions.

WORK PLANS:

1. New genes and germplasm to provide improved protein quality (Objective 1).

   a. Some of the new mutants have shown high lysine in preliminary analyses. Others have shown stable inheritance in F2 ratios but have not yet been analyzed for lysine.

(1) 1975-1976 - The mutants which have shown high lysine values will be retested. Those with regular inheritance patterns shall be analyzed. Allele testing will continue against genes of similar phenotype.

(2) 1976-1978 - Continue allele and linkage tests and start introduction of any promising new mutants into elite germplasm, and combine and evaluate epistatic effects with other genes such as opaque-2, floury-2 and sugary-2.

b. Screening for high lysine mutant types.

(1) 1975-1976 - With tests of material from the Germplasm Bank (CIMMYT) as a base, the search shall be broadened to new collections from the most promising areas indicated by the ninhydrin and other screening procedures. Search for and evaluation of new high lysine mutants in chemical mutagenized maize lines.

(2) 1976-1978 - The search shall be further broadened and promising material analyzed in more detail. 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 1a above.

c. Definitive data is needed 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 rot and/or grain insect damage associated with the use of mutant genes.

(1) 1975-1976 - 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 shall be analyzed for protein

and lysine. If there are a promising number of lysine families then 100 additional ears in each variety will be analyzed.

1975-1976 - In winter nursery intercross the 25 families with highest lysine content.

(2) 1976-1978 - Test 100 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.

2. Develop populations with improved protein or qualities for temperate areas (Objective 4).

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

(1) 1975-1976 - Based on agronomic performance and protein quality 150 full sibs will have been made among selected families in each population in the winter nursery. These shall be yield tested at 4 or 5 worldwide locations.

(2) 1976-1978 - Continue full sib selection for agronomic performance, modified kernel types and protein quality. When either or both populations 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.

b. Maximum effort will be placed on the development of the Temp HA and Temp HB sugary-2 opaque-2 populations. A full-sib family selection method to improve these high lysine sugary-2 opaque-2 maize populations is being used.

(1) 1975-1976 - 200 selected full-sib families in each population shall be yield tested and simultaneously grown in the 1975-summer breeding nursery to be self-pollinated. 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 200 full sibs.

(2) 1976-1978 - The contractor shall continue full sib selection as outlined above in the populations.

3. Selection for higher protein content in double-mutant combinations (Objectives 2 and 4).

a. Selection for protein content in the sugary-2 opaque-2 double mutant combination. The sugary-2 opaque-2 double mutant genotype is 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.

(1) 1975-1976 - Continue backcrossing and selection for increased levels of protein based on evaluations for agronomic performance, kernel quality, kernel size, and protein quality.

(2) 1976-1978 - Continue backcrossing and selection for improvement in protein nutritional quality within the sugary-2 opaque-2 selected recoveries.

b. Selections for protein content in the multiplealeurone sugary-2 opaque-2 inbreds.

(1) 1975-1976 - Selection and continued backcrossing to the recurrent double-mutant lines shall 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 aleurone layered recoveries.

(2) 1976-1978 - Advanced generation recoveries shall 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.

4. Detailed genetic and agronomic evaluation of independent (mutant) sources of opaque-2 and sugary-2 (Objectives 1 and 2). Seven opaque-2 sources and four sugary-2 sources are being backcrossed into common inbred sources to evaluate possible differences among the sources.

(1) 1975-1976 - in summer nursery evaluate preliminary crosses and inbred sources for agronomic performances, grain quality, and protein quality to determine possible differences among mutant sources. Continue backcrossing and make more advanced crosses.

(2) 1976-1978 - Continue backcrossing and evaluation in hybrid and inbred backgrounds to determine if there are any differences among the sources.

5. Modifier genes - To achieve a more normal phenotype for consumer acceptability and ear rot and grain insect resistance detailed studies of modified types shall be conducted (Objective 3).

a. A modified opaque-2 synthetic HMo_2 has been developed from Cornbelt germplasm and released as a source of modifier genes. 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.

Modified and non-modified versions of synthetics Temp HA o_2 and Temp HB o_2 and high and low lysine selections of modified and non-modified versions of synthetic HMo_2 have been random mated successively for two or three generations.

(1) 1975-1976 - Evaluate the random mated generations of the eight populations described above in several environments.

(2) 1976-1977 - Continue the testing for a second year as necessary to obtain definitive data.

b. Data on relationships of agronomic performances to modified types and protein quality shall also be obtained in the above experiments in 1976-1977.

c. Current research has shown that selection for modified opaque-2 types results 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.

(1) 1975-1976 - The high and low lysine version of the modified and non-modified versions selected in HMo_2 (Sec. 5-a-1) shall be subjected to protein fractionation, protein quality (amino acid patterns) of these fractions and electrophoretic separation of proteins.

d. Conduct detailed genetic and agronomic studies of new single gene modifier that gives a vitreous opaque-2 phenotype with little if any reduction in protein quality.

(1) 1975-1976 - Backcross into selected materials.

(2) 1976-1978 - Continue backcrossing and evaluate in hybrids.

e. The sugary-2 opaque-2 double mutant genotype has been crossed with specific selections of modified opaque-2 vitreous kernel phenotype (Objectives 2 and 3).

(1) 1975-1976 - Continue backcrossing and selfing in advanced generation materials and continue half-sib selection in subpopulation of Temp HB sugary-2 modified $\underline{o}_2$, and evaluate the sugary-2 opaque-2 genotype in the modified opaque-2 backgrounds.

(2) 1976-1978 - Evaluate for agronomic performance, protein quality, kernel size and degree of modification and determine if approach is practical before continuing selection as outlined above.

6. Development of double and multiple combinations of endosperm mutants (Objective 2). A continued effort is being made in evaluating genetic interactions of endosperm mutants with opaque-2 and other promising new protein quality mutants which may be discovered.

a. Endosperm mutant and opaque-2 double and multiple mutant isogenic line development.

(1) 1975-1976 - Continue backcrossing of the sugary-2 opaque-2 and other double mutants to elite inbred backgrounds. Backcrosses 5 and 6 should be completed in sugary-2 opaque-2.

(2) 1976-1978 - Hybrid evaluation of interactions for improved protein quality, kernel and nutritional characteristics and agronomic quality.

b. Study of sugary-2 and sugary-2 opaque-2 gene interaction effects in high lysine and low oil selections on total caloric content.

(1) 1975-1976 - Conclude study on evaluation of agronomic performance, kernel, oil and protein quality in sugary-2 and

sugary-2 opaque-2 high and low oil background recoveries.

1975-1976 - Conclude study on sugary-2 endosperm dosage interaction and effects of outcrossing on protein quality, caloric content, kernel size, weight and density.

c. Detailed genetic and agronomic evaluation of the sugary-2 and sugary-2 opaque-2 system in large seeded and flint backgrounds to study associated interactions on nutritional quality, physical properties of the kernel and agronomic characteristics.

(1) 1975-1976 - Continue backcrossing to adapted cuzco synthetic and to flint backgrounds.

(2) 1976-1978 - Continue backcrossing and evaluate sugary-2 and sugary-2 opaque-2 segregates.

d. Studies will be conducted to determine the developmental constraints on seed size in the sugary-2 and sugary-2 opaque-2 double mutant.

(1) 1975-1976 - Characterize the developmental process of grain filling and associated compensation effects in endosperm and germ biosynthetic development.

(2) 1976-1978 - Continue developmental studies as leads are discovered.

e. Development of promising new genotypes.

(1) 1975-1976 - As new mutants are described under Objective 1 and appear promising, intercross with selected endosperm mutants.

(2) 1976-1978 - Evaluate double mutant segregates for agronomic quality, kernel characteristics, protein and nutritional quality.

f. Conduct basic research on the nature of proteins in double mutant combinations.

(1) 1975-1976 - Characterize the nature of the protein bodies and matrix protein distribution in selected key mutants. Characterize the protein-profiles in investigations to elucidate the genetic control of protein quality.

(2) 1976-1978 - Continue studies on protein biosynthesis in double mutants.

7. Improved analytical and biological methods (Objectives 1, 2 and 3).

1975-1978 - The biochemistry laboratory will continue to function as a center for instruction in analytical procedures for screening maize for protein quality. A special course on this subject will be offered to Purdue graduate students in plant breeding and related areas each semester, and trainees supported through CIMMYT funds will be taught our methods. The laboratory will continue to search for better methods to evaluate protein quality in maize breeding programs. This includes the development of new methods as well as evaluation of methods proposed by other laboratories.

The laboratory will continue to cooperate with the Protein Quality Laboratory at CIMMYT in screening new analytical methods proposed for cereal protein analysis.

8. Characterization of key proteins in high lysine endosperms (Objectives 1, 2 and 3).

1975-1976 - The individual basic polypeptides in zein and a zein-like fraction in opaque-2, brittle-2 and $\underline{bt}_2$ $\underline{o}_2$ show characteristic differences. These polypeptides will be isolated and their amino acid patterns determined.

9. Mechanism of biosynthesis of protein in high lysine mutants (Objectives 1, 2 and 3).

1975-1976 - Enzymes involved in lysine biosynthesis in maize will be determined quantitatively and their sensitivity to feedback control tested in both developing and germinating normal and opaque-2 seeds.

10. Protein nutritive value (Objectives 2, 3 and 4).

a. Animal subjects.

(1) 1975-1976 - Feeding trials with rats and swine will be undertaken with the cooperation of the Department of Animal Sciences, 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).

(2) 1976-1978 - Continue animal nutrition evaluation.

b. Human subjects.

(1) 1975-1976 - A cooperative study will be carried out with the Department of Foods and Nutrition to (a) evaluate in adult human subjects the nutritional value of the sugary-2 opaque-2 double mutant corn compared to opaque-2 corn and (b) compare the response of human subjects to those sources of intact protein.

(2) 1976-1977 - Depending upon results obtained above, evaluate in feeding trials with rats combinations of nutritionally improved corns in food blends characteristic of those consumed in various parts of the world.

c. The new genetic types will be evaluated for milling quality.

1975-1978 - 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 consumption of the products.

F. INVOLVEMENT OF MINORITY PERSONNEL AND WOMEN

Women:

Graduate Students:

Nora D'Croz

Lourdes Nazarea

Iffat Rahim

Laboratory Assistants:

Dorcas Carter

Susan Lessman

Gina H.Y. Lu

Field Assistants:

Catherine Axtell

Debbie Magaw

Jayne Daughtery

Sheron Mecham

Blacks:

Dorcas Carter (above)

Spanish:

Nora D'Croz (above)

Lourdes Nazarea (above)

Orientals:

Wen Yang Lai - Laboratory and Field Technician

Bakshy A. Chibber - Post doctorate

G. FURTHER INFORMATION ON DISSEMINATION OF RESEARCH RESULTS

Bibliographic List and Short Abstracts of Research Reports Representing Efforts to Disseminate the Results of the Research Project.

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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.

PHYSICAL AND CHEMICAL KERNEL CHARACTERISTICS OF NORMAL AND OPAQUE-2 MAIZE HYBRIDS

J. M. Arnold, L. F. Bauman, and Dejene MaKonnen

ABSTRACT

Six normal maize (Zea mays L.) hybrids and the opaque-2 counterparts were compared for kernel weight, volume, density, and number and whole kernel concentration and content of P, K, Mg, Fe, Zn, protein, and oil on six dates at 7-day intervals beginning 28 days after pollination. At 56 days after pollination, kernel weight, volume, and density of the opaque-2 hybrids were 17.0, 6.9 and 10.2% less, respectively, than for the normal hybrids. Kernel weight was only slightly greater for the normal kernels at 28 and 35 days after pollination, but the difference between the kernel types increased after the 35-day harvest. Kernel volume was similar at 28, 35, 42 and 49 days but greater for the normal kernels at 56 and 63 days. Kernel density of the normal kernels was higher at all harvests with the difference between kernel types being similar at all harvests. At 56 days after pollination, the opaque-2 kernels were significantly greater in P, K, Mg, Fe, Zn, and oil concentration. When expressed as mass per 200 kernels, the normal kernels were higher than or similar to opaque-2 kernels in all constituents except K at 56 days after pollination. The opaque-2 kernels were higher in concentrations of all constituents at all harvests and were higher in content of K per 200 kernels at all harvests.

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

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

ABSTRACT

Normal and opaque-2 kernels from segregating maize (*Zea mays* L.) ears were examined for kernel components, oil content, and fatty acid composition. Normal kernels were higher in kernel and endosperm weight and embryo oil concentration, and lower in embryo weight than opaque-2 kernels. Compared to normal, the oil from opaque-2 kernels was slightly but significantly higher in olcic acid and lower in linolcic acid composition. Comparison of normal and floury-2 kernels from segregating ears showed kernel and endosperm weights of the normal kernels were greater than for floury-2, and embryo weight was similar. In a comparison of near-isogenic floury-2 and normal single-cross hybrids, the kernel components were heavier in normal, while percent oil was greater for floury-2 owing largely to a greater proportion of embryo. Percent oil in embryo was less in floury-2 hybrids.

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 x 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 x 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 x USA342 were self-pollinated and outcrossed 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 homosygous opaque-2 kernels were so modified that they were visually indistinguishable from normal kernels.

This investigation showed that ETO x USA342, 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

Despite its proven nutritional superiority, opaque-2 maize (*Zea mays* L.) has not been generally accepted in tropical regions and for certain food uses because of its soft endosperm. Modified (more vitreous) opaque-2 types would be more acceptable. Seven Colombian maize races and a population derived from ETO x USA342 were outcrossed to the homozygous opaque-2 lines L215 and L216 which have the soft endosperm expression. The crosses were self-pollinated, and the F_2 kernels were classified into three classes over a light. One sub-family (single ear) of each family was reclassified into four classes on a black-topped table with a lighted magnifying glass. Chi square tests indicated indirectly whether the intermediate classes II or II and III were modified opaque-2 kernels or if the ratios deviated significantly from 3:1 due to an excess of normal kernels. In the latter case some homozygous opaque-2 kernels were so modified that they were phenotypically indistinguishable from normal. A high percentage of intermediates indicated that ETO x USA342, Pira, Puya and Común were good sources of modifier genes for opaque-2. Amagaceño was less promising and conflicting results within our smaller samples of Clavo, Chococeño and Pollo made it impossible to draw definite conclusions about their potential.

DIGESTIBILITY STUDIES WITH RATS OF NEAR ISOGENIC MUTANT HYBRID CORNS

D. M. Forsyth, Kathleen Fleck, Ronda Gretebeck
and D. V. Glover

ABSTRACT

Two rat digestion trials were conducted to compare a 3-way hybrid (1) normal corn (n) and the genetic single and double mutant near isogenic conversions (2) opaque-2 (o_2), (3) waxy (wx), (4) waxy opaque-2 (wxo_2), (5) sugary-2 (su_2) and (6) sugary-2 opaque-2 (su_2o_2) in protein adequate and protein limiting diets. In trial 1, all diets consisted of 78.5% of the respective corn and 21.5% supplement and contained about 16% protein. Eight individually caged rats were ad lib. fed each diet for 23 days. Energy digestibility (DE) was determined by the Cr_2O_3 indicator procedure, whereafter 5 rats per treatment were moved to metabolism cages for total fecal and urinary collections. No differences in average daily gain (ADG) or feed/gain (F/G) were found. DE's by the Cr_2O_3 procedure for treatments 1-6 were 87.5, 82.8, 87.4, 88.8, 84.5 and 84.3% (4,1,3 > 5,6,2; $P < .01$). DE's by total collection for treatments 1-6 were 90.2, 87.3, 89.1, 89.4, 87.6 and 87.4%. Both methods found treatments n, wxo_2 and wx higher in DE than su_2, su_2o_2 and o_2. No differences in N digestibility, N retention or apparent biological value (BV) were observed in these protein adequate diets. In trial 2, 6 rats per treatment in metabolism cages were fed for 24 days isonitrogeneous diets (8% protein) containing the corns as in trial 1. ADG and F/G were best for

su_2o_2 followed by wxo_2 and su_2 than wx and n fed rats. Dry matter digestibility was highest for wx and n and lowest for o_2 and su_2o_2 and N digestibility was highest for n and wxo_2 and lowest for o_2 and su_2o_2 fed rats. N retention and BV were highest for su_2o_2 and o_2 and lowest for n fed rats.

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

H. Fuwa, M. Nakajima, A. Hamada,
and D. V. Glover

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

ABSTRACT

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 does 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.

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.

MATURITY INTERACTION AND BLACK LAYER OCCURRENCE IN OPAQUE-2 AND NORMAL HYBRIDS IN CORN (*Zea mays* L.)

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ABSTRACT

Six opaque-2 lines and their normal counterparts were crossed in diallel crosses. The crosses were grown in 1970 in a split plot randomized complete block design. Harvests were made at 7-day intervals starting at 28 days after pollination and continuing through 63 days.

The average kernel weight of opaque-2 hybrids was inferior to that of the normal. Nevertheless, the opaque-2 gene performed differently in different hybrids. In B14 x B37 single cross the mutant had similar kernel weight as its normal counterpart in the first and second harvests. In contrast, a wide difference was found between the opaque-2 and the normal, both at early and late stages of development in W64A x A545 background.

The normal hybrids had greater cob weight, ranging from 9.7 to 11.8% more than the opaque-2. The difference in cob weight of the opaque-2 and the normal remained constant over the different harvest dates.

At physiological maturity, the opaque-2 hybrids averaged 3.5% higher moisture content than the normal. In general, a slower accumulation of dry matter in the kernels was accompanied by a retention of more moisture.

Shelling percentage was higher for the normal hybrids. Black layer, an indicator of physiological maturity, was formed at about the same time in the opaque-2 and normal.

BREEDING FOR IMPROVED NUTRITIONAL VALUE IN CEREALS

Edwin T. Mertz

ABSTRACT

Cereal proteins are inferior nutritionally to the proteins in milk, meat, and eggs because of inadequate levels of the amino acid lysine. With the discovery ten years ago by Mertz et al. (1964) that the maize mutant opaque-2 had nearly twice as much lysine as ordinary maize, scientists realized that cereal grain proteins could be improved in quality by genetic manipulation. The recent discovery of high lysine mutants of barley and sorghum raises the hope that all cereals can be improved in protein quality. If we assume that the FAO (1973) pattern of 5.2% lysine is the ideal level of lysine for the infant, high lysine maize and barley approach or equal this ideal, oats has approximately 73%, rice 71%, high lysine sorghum 63%, normal maize, barley, and wheat 50%, and normal sorghum and millet 35% of this level. Triticale, an artificial genus synthesized by combining the genomes of wheat and rye, has a substantially higher level of lysine per unit of protein than wheat. Other high lysine wide crosses between cereals are possible in the future. As the plant breeder selects for better cereal grains, it is necessary that the chemist carefully monitor the seeds used in the breeding program to insure that the level and quality of protein is maintained.

GENETIC IMPROVEMENT OF CEREALS

E. T. Mertz

ABSTRACT

This is a review of the nutritional implications of the genetic improvement of protein quality in cereals. The amino acid pattern of the economically important cereals is compared with that of the "ideal" pattern proposed by FAO for infants. On this basis, the best of the cereal grains, oats, has about 73 percent of the ideal level, and the poorest of the grains, sorghum and millet, 34 percent of the ideal level. The opaque-2 gene in maize raises the lysine level from 52 percent of the ideal patterns to about 88 percent, the high lysine gene in sorghum from 34 percent to 63 percent, and the high lysine gene in barley from 63 percent to 100 percent. As the search for high lysine mutants accelerates, it is hoped that eventually all cereal species will carry more lysine, thus closing the quality gap which now exists between cereal and animal proteins.

SIMPLE CHEMICAL AND BIOLOGICAL METHODS USED AT PURDUE UNIVERSITY TO EVALUATE CEREALS FOR PROTEIN QUALITY - STATION BULLETIN NO. 70

E. T. Mertz, R. Jambunathan, P. S. Misra

ABSTRACT

The protein quality of a cereal grain can be determined only by chemical or biological methods. In this bulletin, the simple chemical and biological methods used at Purdue for determining protein quality of cereals are outlined in detail. These methods are suitable for a laboratory in the developing countries that is severely limited in supplies, equipment and trained personnel. The bulletin also lists the low cost equipment needed, and sources of supply.

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 fractions by the Landry-Moureaux method. 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 and 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 fractions 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 DOUBLE 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 invariably raised the level of free amino acids above that of the counterpart lacking this gene.

STUDIES ON CORN PROTEINS IX. THE SIMILAR AMINO ACID COMPOSITION OF LANDRY-MOUREAUX NORMAL AND HIGH LYSINE MUTANT ENDOSPERM PROTEIN FRACTIONS AND PAULIS-WALL NORMAL ENDOSPERM PROTEIN FRACTIONS

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

ABSTRACT

Comparison of the average amino acid composition of the Landry-Moureaux (LM) endosperm fractions of two normal corn inbreds and five high lysine mutants with the amino acid composition of the Paulis-Wall (PW) endosperm fractions of a normal corn hybrid show marked similarity between LM Fraction II and PW alkylated-reduced zein, LM Fraction III and PW guanidine and 70% ethanolsoluble alkylated-reduced glutelin, LM Fraction IV and PW guanidine-insoluble alkylated-reduced glutelin, and LM Fraction V and PW guanidine-soluble 70% ethanol-insoluble alkylated reduced glutelin. Both the normal and mutant LM Fractions I and V contain high levels of lysine, LM Fractions II and III low levels of lysine, LM Fraction III high levels of methionine, and LM Fraction IV high levels of histidine. The corresponding PW fractions show these same differences even though the glutelins are separated in a different manner. Since the mutant fractions resemble the normal fractions, this is further evidence that the high lysine levels in the five mutants is due to the previously reported increase in LM Fractions I and V, not to new proteins high in lysine.

STUDIES ON CORN PROTEINS X. POLYPEPTIDE MOLECULR WEIGHT DISTRIBUTION IN LANDRY-MOUREAUX FRACTIONS OF NORMAL AND MUTANT ENDOSPERMS

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

ABSTRACT

The endosperm proteins of normal corn inbred Oh 43 and mutants $\underline{o}_2$, $\underline{fl}_2$, $\underline{fl}_2\underline{o}_2$, $\underline{bt}_2$ and $\underline{bt}_2\underline{o}_2$, as well as normal corn inbred W22 and its mutant $\underline{o}_7$, were separated into fractions by the Landry-Moureaux method. Based on molecular weights determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis, Fraction I (saline-soluble) had major polypeptides with average molecular weights of 58,000; 24,500; 22,000; and 13,400 daltons. Fraction II (zein), with the exception of $\underline{bt}_2\underline{o}_2$, contained major polypeptides with average molecular weights of 25,000 and 21,800 daltons. Fraction III (zein-like) had major polypeptides with average molecular weights of 26,000; 23,000 and 18,000 daltons, and Fraction IV (glutelin-like) had major polypeptides with average molecular weights of 61,000; 58,000; 25,700 and 19,000 daltons. Fraction V (true glutelin) polypeptides, did not separate clearly on the gel. The 25,000 dalton component of Fraction II in $\underline{o}_2$ and $\underline{fl}_2\underline{o}_2$ is reduced below that in normal, $\underline{fl}_2$ and $\underline{o}_7$. The 44,000 dalton component of Fraction II is a unique component of $\underline{fl}_2$ and $\underline{fl}_2\underline{o}_2$, as in the 14,000 dalton component of Fraction III in $\underline{o}_2$ and $\underline{o}_7$.

EFFECT OF ENDOSPERM AND PROTEIN TYPE OF CORN ON PERFORMANCE OF RATS

J. G. Rose, D. M. Forsyth, D. V. Glover and T. R. Cline

ABSTRACT

Two growth trials were conducted with 230 rats to study the nutritional quality of three way hybrid (1) normal (n), (2) opaque-2 (o_2), (3) waxy (wx), (4) waxy opaque-2 (wx o_2), (5) sugary-2 (su_2) and (6) sugary-2 opaque-2 (su_2o_2) corns derived from near isogenic conversions of the same hybrid. In trial 1 diets were formulated with the corns, soybean meal and glucose to make isonitrogenous diets within 8, 11 and 14% protein levels (3 x 2 x 3 factorial arrangement of treatments). Rats fed diets containing o_2 (diets 2,4,6), showed improved average daily gain (ADG) and feed efficiency (F/G) over those fed non-o_2 (diets 1,3,5) at 8% protein (3.43, 4.34, vs 1.45, 7.29 g) and 11% protein (6.27, 2.96 vs 5.14, 3.26 g) ($P < .01$), but no significant differences were observed at 14% protein. In terms of endosperm type, at 8% protein rats fed su_2 (diets 5,6) had better ADG and F/G than wx (diets 3,4) and n (diets 1,2) with 2.84, 2.27 and 2.21 g for ADG and 5.00, 6.35 and 6.09 for F/G respectively. At 11 and 14% protein there was no effect of endosperm type. An interaction ($P < .05$) was found between the n, wx and su_2 endosperm types and those containing o_2. Rats fed the su_2 endosperm outperformed those fed wx and n in the absence of o_2, but there were no differences in the presence of o_2. In trial 2 the corns were compared on an equalweight basis in protein adequate (15-16%) diets. There were no differences in ADG or F/G. These results indicate that endosperm type (n, wx or su_2) has little effect on carbohydrate availability, but does have an effect on protein quality of corn.

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.

NUTRITIONAL VALUE OF NEW ENDOSPERM TYPES OF CORN FOR GROWING-FINISHING BEEF CATTLE

V. M. Thomas, D. V. Glover and W. M. Beeson

ABSTRACT

Two metabolism studies were conducted to evaluate the nutritional value of a number of different corn varieties for growing-finishing beef cattle. The first experiment was conducted with normal, opaque-2, waxy and waxy opaque-2 corn; and the second experiment was conducted with isogenic backgrounds of normal, sugary-2, sugary-2 opaque-2 and waxy corn. Results from experiment 1 indicate that waxy corn was lower ($P < .05$) in digestible energy than normal, opaque-2 or waxy opaque-2 corn. In experiment 2, steers fed the sugary-2 opaque-2 diet had higher ($P < .05$) absorbed nitrogen retained than steers fed normal and sugary-2 corn diets, but dietary nitrogen retained was not different ($P < .05$) among diets. The sugary-2 opaque-2 diet produced 2% higher digestible energy than the normal corn diet but it was not significant ($P < .05$). All other parameters studied indicated no significant differences between corns.

NUTRITIONAL VALUE OF NEW ENDOSPERM TYPES OF CORN FOR STEERS

V. M. Thomas, D. V. Glover and W. M. Beeson

ABSTRACT

Two metabolism studies were conducted to evaluate the nutritive value of a number of different corn endosperm types for beef cattle. The experimental design in both studies was a 4 x 4 latin square. In experiment 1, four 205-kg Hereford steers were fed either normal, opaque-2, waxy, or waxy opaque-2 corn on an isonitrogenous basis (11% crude protein). Results of experiment 1 indicate that waxy corn (74.67%) was lower ($P < .05$) in digestible energy than normal (78.21%), opaque-2 (77.90%) or waxy opaque-2 corn (78.20%). In experiment 2, four 271-kg Hereford steers were fed sugary-2, sugary-2 opaque-2, and waxy corns in near isogenic backgrounds and the normal counterpart on an isonitrogenous basis (11% crude protein). Steers fed the sugary-2 opaque-2 diet had higher ($P < .05$) absorbed nitrogen retained (64.77%) than steers fed normal (47.20%) or sugary-2 (49.82%) corn diets, but dietary nitrogen retained was not significantly different among diets. The sugary-2 opaque-2 diet produced a nonsignificant higher (77.91%) digestible energy than the normal corn diet (75.91%). All other parameters studied indicated no significant differences between corns.

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 following eight single endosperm mutants -- *ae*, *du*, *wx*, su_2, su_1, fl_2 and o_2 -- and their double-mutant combinations with 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 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 o_2 and double mutants with 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 the

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_2o_2 means were among 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_1o_2 and sh_2o_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 counterparts 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, sh_2, sh_2 o_2 and ae were 47, 21 and 23% respectively greater than normal. Sugary-1 and su_1 o_2 were 2.5 and 2 times

greater than normal in reducing-sugars and sh_2 and sh_2 o_2 were 1.4 and 1.38 times greater than normal at 42 DAP.

Sucrose contents of sh_2 and sh_2 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 su_1 o_2, ae and ae o_2 hybrids at 21 DAP were increased 34, 31 and 35% respectively over normal, and at 42 DAP su_1 and su_1 o_2 were 47 and 40% greater than normal.

The WSP contents of su_1 and su_1 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 su_1, su_1 o_2 sh_2 and sh_2 o_2 were reduced 60-80% and ae was reduced 30%; whereas there were no significant differences among the other genotypes and from normal. At 42 DAP, su_1, su_1 o_2, sh_2, and sh_2 o_2 were extremely reduced in starch content and fl_2 o_2, ae o_2, wx o_2, du and du 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 ae o_2 double mutant offers a high quality protein high sucrose corn and possibilities for use in vegetable corn improvement. Waxy 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.

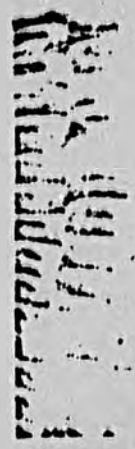
Plants, which supply about 80 per cent of the protein for people in developing countries, are normally deficient in one or another essential amino acid. In order to obtain usable protein from vegetarian sources then, two different kinds of plant proteins that make up for each other's deficiencies—say,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

Most of the current research has centered on plant proteins which have the potential to supplying essential [illegible]

[illegible] for a steer to produce [illegible] requires the [illegible] consumption 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 that [illegible] tein foods can [illegible] even decade[illegible] the laboratory [illegible] mouths of the hungr[illegible] factors as taste, [illegible] centives and pr[illegible] the lack of techno[illegible] agricultural re[illegible] adequate numbers of personnel all make [illegible]

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



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

Protein Key in Fight Against Hunger

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 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 [illegible] 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 [illegible] 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

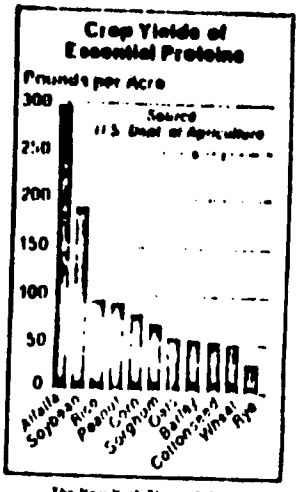
Sorghum grows in towering stands, with heavy heads of edible seeds—produced atop tall, thick stalks.

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 [illegible] [illegible] would be [illegible]

Protein Key in Fight Against Hunger
Continued



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 18 to 40 per

"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, in 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. Around [illegible]

trying to improve the product[illegible]ity 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.

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.