Inheritance and improvement of protein quality and content in maize; annual report, 1976/1977

(101) Purdue Univ. Dept. of Agronomy

Purdue

(Research summary)

A PROJECT: To research and develop source germplasm materials for improvement of protein quality and content in maize in order to improve the nutritional quality of maize for use in LDCs.


DEVELOPMENTS: The project is screening for new high-lysine mutants and identifying problems associated with acceptance of high lysine maize through an interdisciplinary and cooperative approach. A group of 55 collections of maize from the world germplasm bank have been screened for potential higher lysine with the ninhydrin test. Lysine content of positive reacting kernels was at the normal level but protein showed a tendency toward higher levels. Two low lysine-high protein populations and two high lysine-low protein populations were fractioned using the Landry and Moureaux method. The extreme difference in lysine content was strictly a function of varying amounts of protein present in the various fractions. Lysine content in the various fractions was not changed by selection. Developmental studies are in progress to determine the interactions of genotype on germ and endosperm storage and compensation effects involved in constraints to yield, protein, starch, and oil accumulation in the kernel. Two varieties adapted to the more temperate areas have been developed from diverse germplasm. Selection for improved agronomic and nutritional characteristics is being conducted in opaque-2 and sugary-2 opaque-2 versions of these two varieties. The variety Colus from Colombian and U.S. germplasm has been converted to opaque-2 and is undergoing selection. Maize is a particularly efficient crop species and with improved protein quality and agronomic characteristics can play an increasing role in solving the world's human and animal nutrition problems.
Annual Report on Inheritance and Improvement of Protein Quality and Content in Maize

April 1, 1976 to March 31, 1977
Contract AID/TS-C-1211
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 AID/tas-C-1211

Prepared by

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

Purdue University
West Lafayette, Indiana

Submitted to

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

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

2. Principal investigator, contractor and mailing address:
Dr. D. V. Glover
Department of Agronomy
Purdue University
West Lafayette, IN 47907


5. Total A.I.D. funding of contract to date: $531,890.00 (to March 31, 1977).

6. Total expenditures and obligations through previous contract year: $245,777.57 (through March 31, 1976).

7. Total expenditures and obligations for current year: $252,120.55.


B. Narrative Summary of Accomplishments and Utilization

Maize is a major source of calories and protein for many millions of people in several areas of the developing world. Though maize is considered an excellent source of carbohydrates and a particularly efficient cereal species, the protein quality is relatively low and thus a constraint on improvement of human and animal nutrition where maize is used as a staple in the diet. This project is screening for new high-lysine mutants, developing source germplasm materials for improvement of protein quality and content in maize and identifying some of the problems associated with acceptance of high lysine maize through an interdisciplinary and cooperative approach.

A group of 55 collections of maize from the CIMMYT world germplasm bank have been screened for potential higher lysine with the ninhydrin test. In general, there was a lack of ninhydrin penetration on vitreous kernels, but there were exceptions. Sources with floury endosperm type, or a soft crown showed a good ninhydrin penetration. Lysine content of positive reacting kernels was at the normal level, but protein showed a tendency toward higher levels. The potential effectiveness of the ninhydrin test in locating any new high-lysine mutant is subject to interactions between that specific mutant gene and its genetic background. Eleven "opaque" phenotype endosperm mutant segregates have been identified from screening over 20 thousand M2 ears following EMS chemical mutagen treatment of seeds.
During the past two years we have identified several simply inherited ("single") genes that modify the soft endosperm of opaque-2 giving it a more vitreous (normal) appearance without a serious reduction in lysine levels. This greatly simplifies the breeding of more acceptable vitreous opaque-2 varieties with good nutritional qualities, however, this system will probably not result in yield improvement of opaque-2 varieties. These genes are being incorporated into elite inbreds and populations.

Experimental opaque-2 testcross hybrids of elite inbreds with Illinois high protein background were yield tested and a number of these hybrids were equal or superior to their elite counterpart parent. Some of these hybrids were equal to one of the better commercial opaque-2 hybrids included as a check.

Early generation hybrid recoveries segregating for both normal and opaque-2, and for single and multiple aleurone layers in the endosperm were evaluated in a replicated field trial. The multiple aleurone layer characteristic increased lysine concentration without altering protein percentage in both normal and opaque-2 kernels, however, its practical value in improving protein quality is questionable.

Seven opaque-2 sources (mutational events) have been recovered in advanced backcross recoveries to elite inbreds and are available for distribution to provide nonrelated or diverse sources of this gene. Tests revealed no differences for yield and grain quality.

Two low lysine-high protein populations and two high lysine-low protein populations were fractioned using the Landry and Moureaux method. The extreme difference in lysine content was strictly a function of varying amounts of protein present in the various fractions. Lysine content in the various fractions was not changed by selection.

Several advanced backcross generation isogenic line recoveries of the double mutant sugary-2 opaque-2 in elite inbreds are being developed. Developmental studies are in progress to determine the interactions of genotype on germ and endosperm storage and compensation effects involved in constraints to yield, protein, starch, and oil accumulation in the kernel.

Two varieties adapted to the more temperate areas have been developed from diverse germplasm. Selection for improved agronomic and nutritional characteristics is being conducted in opaque-2 and sugary-2 opaque-2 versions of these two varieties. The variety Colu: from Colombian and U.S. germplasm has been converted to opaque-2 and is undergoing selection.

A study of nitrogen retention of young men who consumed isonitrogenous diets containing normal, opaque-2, or sugary-2 opaque-2 corn revealed that although sugary-2 opaque-2 as well as opaque-2 corns were not significantly different in mean daily nitrogen balances they tended to be superior to normal corn at 5.5g of nitrogen and opaque-2 corn caused improvement even at 4.4g of nitrogen. Protein quality and digestibility studies of these corns in infants is underway.
The combination of opaque-2 with starch-modified or starch-deficient mutants produced an additive and synergistic effect, respectively, in regulating zein synthesis. The double mutant, brittle-2 opaque-2, which almost completely prevented the synthesis of Z1 and Z2, had high RNase activity. We are investigating the possible involvement of RNase and high sucrose concentration in affecting zein synthesis.

Research information has been distributed through workshops, seminars, journals, publications, and annual reports. Graduate student and postdoctorate training programs continue to supply trained scientists for maize improvement programs in temperate and tropical areas of the world.
Personnel

Department of Agronomy

Dr. L. F. Bauman
- Mr. Alfredo Navarro* (Graduate Student)
- Miss Lourdes Nazarea (Graduate Student)
- Mr. Rodney L. Tietz (Graduate Student)
- Mr. Hsun Tu (Graduate Student)
- Mr. Terry R. Lemming** (Technician)

Dr. P. L. Crane
- Mr. Hugo Zorrilla (Graduate Student)
- Mr. Adeleke A. Ojo* (Graduate Student)
- Mr. H. Keith Kessler** (Technician)

Dr. D. V. Glover
- Dr. James B. Barnett (Postdoctorate)
- Mr. Steve G. Ballinger (Graduate Student)
- Mr. Arthur H. Long* (Graduate Student)
- Mr. Richard S. Burns (Technician)
- Mr. Wayne J. Whitlow** (Technician)
- Miss Charlotte Allen** (Technician)
- Mrs. Betty Lee (Technician)

Department of Biochemistry and Agronomy

Dr. Bakshy A. K. Chibber (Postdoctorate)
- Mr. Mohammed M. Hassen (Technician)

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

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

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

- Dr. D. M. Forsyth - Department of Animal Sciences
- Dr. Helen E. Clark - Department of Foods and Nutrition
- Dr. C. Y. Tsai - Department of Botany and Plant Pathology
- Dr. E. T. Mertz - Department of Biochemistry (Emeritus)
- Mr. Juan G. Rosa - Department of Animal Sciences
- Miss Jennie L. Betz - Department of Foods and Nutrition
A. GENERAL BACKGROUND

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

The purpose of this project is to research and develop source germplasm materials for improvement of protein quality and content in maize in order to improve the nutritional quality of maize for use in the LDC's. The project will 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.
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 is a particularly efficient crop species and with improved protein quality and agronomic characteristics can play an increasing role in solving the world's human and animal nutrition problems.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

1. In cooperation with other maize breeding programs (particularly that of CIMMYT), expand the search for and evaluation of new mutants and germplasm sources with improved protein quality and quantity, both in existing maize populations and by means of chemical mutagens and to introduce these new mutant genes into lines and populations of use to breeders in LDC's.

2. To concentrate on the development of opaque-2 and double and multiple combinations of endosperm mutants and determine the effect of associated interactions on nutritional quality, physical properties of the kernel and agronomic characteristics, with a view to the improvement of grain type and yield as well as protein content and quality.

3. To determine the extent of interactions of both genetic backgrounds and environment with individual or combinations of endosperm mutants and how such interactions may influence protein quality and breeding methods.
4. In cooperation with CIMMYT and other maize workers, develop special varieties and source breeding materials with improved nutritional and agronomic characteristics for use in the LDC's. Special emphasis will be given to opaque-2 and sugary-2 opaque-2 materials adapted to more temperate regions.

C. CONTINUED RELEVANCE OF OBJECTIVES

Research has led to expanding efforts in search for and evaluation of new mutants and germplasm sources that improve protein quality. The opaque-2 genotype still has the serious drawback of lower agronomic yield. In the past years we have discovered modifying genes (or gene systems) such as "modified opaque-2" and sugary-2 opaque-2 which improve the kernel hardness and vitreousness. Protein quality in the "modified opaque-2" can be maintained with selection. In sugary-2 opaque-2 there appears to be some nutritional improvement. However, both systems have not resulted in a yield improvement over opaque-2. Opaque-2 shows the least yield reduction (about 10-12%) of the high lysine types.

During the past year we have identified several simply inherited gene systems that give a vitreous kernel with opaque-2 without a serious reduction in lysine levels. This would greatly simplify the breeding of more acceptable vitreous opaque-2 varieties with good nutritional qualities. These systems will probably not result in yield improvement of opaque-2 varieties.

The major challenge now is to develop high lysine germplasm without the serious yield constraints and with other acceptable agronomic and nutritional qualities for the LDC's and more temperate areas of the world.
In our first coordinating conference with CIMMYT the serious need for developing source populations with improved protein quality for the more temperate areas of the world was discussed. We shifted more emphasis to the development of special varieties and source breeding materials for use in the LDC's and more temperate regions. A rather sizeable portion of our total effort will, therefore, be expended in development of varieties, i.e., Temp HA opaque-2, Temp HB opaque-2, an opaque-2 version of Colus (a synthetic developed at Purdue from Colombian and U.S. germplasm), Temp HA sugary-2 opaque-2 and Temp HB sugary-2 opaque-2. Agronomic and nutritional selection is being implemented in these populations. These commitments to germplasm development are long range commitments and contribute strongly to the continued relevance of the project.
D. ACCOMPLISHMENTS TO DATE

Use of the Ninhydrin Test in Screening for New Mutants

The "ninhydrin reagent solution" chemically reacts with the free amino acids present in the exposed maize endosperm rendering a blue color. This study utilized that reaction to search for new mutants and also tested the effectiveness of the use of "ninhydrin reagent solution" applied directly to exposed maize endosperm for protein mutant screening by the use of known (opaque-7) material.

In the search for new mutants, 300 kernels each of 55 collections of maize from the CIMMYT world germplasm bank were tested. In general, there was a lack of ninhydrin penetration on vitreous kernels, but there were exceptions. Sources with floury endosperm type, or a soft crown, or "cap" showed good ninhydrin penetration. Lysine content of positive reacting kernels was at the normal level, but protein showed a tendency to higher levels.

In the test of this ninhydrin technique, opaque-7 ears at different moisture levels were tested and homozygous opaque-7 genetic testers were used to verify the presence of the opaque-7 gene. A very weak ninhydrin reaction on exposed maize endosperm of opaque-7 ears was generally observed at moisture levels above 35 percent. Usually differentiation between normal and opaque-7 kernels was observed at any moisture level below 30 percent.

Some of the numerous exceptions in this experiment were apparently due to a variety of modifier genes not only masking the opaque-7 phenotypic
expression in varying degrees, but suppressing its protein altering functions too, sometimes resulting in normal levels of free amino acids and lysine. The potential effectiveness of the ninhydrin test in locating any new high-lysine mutant would be subjected to any interaction between that mutant gene and its genetic background.

The Use of Chemical Mutagens to Produce High Lysine, High Tryptophan Maize Germplasm

We are looking at other non-conventional ways of producing high lysine, high tryptophan maize endosperm. We have initiated a study to induce with chemical mutagens point mutations which may deactivate the feedback inhibitory systems controlling the levels of lysine and/or tryptophan in maize seed or which may give rise to a 'second step' mutation in maize which will reduce the prolamine content. Both seed and pollen treatments have been made using the chemical mutagens ethyl methanesulfonate (EMS) and diethylsulfate (DES).

From seed treated in 1975 of two different heterotic hybrid groups we grew out some 4000 families (2000 of each group) or about 20,000 individual plants of the M1 material in 1976 and self-pollinated them to produce the M2 generation which we have screened for endosperm and seedling mutations. In the (B14 x B37) group we obtained 47 ears which segregated for an endosperm mutant type, seven of which segregated an opaque phenotype; 141 ears segregating for a defective endosperm type, and 352 families which segregated for different seedling mutant characters. These were from a total of 4,293 ears screened. In the (C123 x Mo17) group from about 7,644 ears screened thus far, we have observed fewer mutants. We have obtained
seven families segregating for an endosperm character, four families segregating for endosperm defects, and 52 families which segregated for different seedling mutant characters. The endosperm mutant types will be allele tested in the 1977 summer nursery.

In 1976 we treated pollen from both normal and opaque-2 plants with EMS and DES and pollinated several hundred ears of each genotype. Each kernel produced represents a separate potential gamete affected, thus we produced several thousands of gametes (M₁ plants). These materials are being grown out in the 1977 summer nursery and will be self-pollinated to produce M₂ progeny which will be screened for mutant types.

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 limited progress in selecting for protein quality in these normal populations. Twelve families were selected within each of these two synthetics. Selected S₁'s have been intercrossed and further cycles of selection carried out in the 1976 nursery. Preliminary data indicate limited effectiveness of this approach.

<table>
<thead>
<tr>
<th>Percent Protein</th>
<th>Mean</th>
<th>Temp HA</th>
<th>Temp HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>11.1</td>
<td>7.3-14.6</td>
<td>7.9-14.3</td>
</tr>
<tr>
<td>Lysine as a Percent of Protein</td>
<td>Mean</td>
<td>1.71</td>
<td>1.74</td>
</tr>
<tr>
<td>Range</td>
<td>1.26-2.28</td>
<td>1.43-2.13</td>
<td></td>
</tr>
<tr>
<td>Lysine as a Percent of Sample</td>
<td>Mean</td>
<td>.187</td>
<td>.181</td>
</tr>
<tr>
<td>Range</td>
<td>.150-.238</td>
<td>.154-.226</td>
<td></td>
</tr>
</tbody>
</table>
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 HA_{2,0} and Temp HB_{2,0} 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 families were tested at four worldwide locations. Agronomic data was obtained from only Mexico and Indiana. Selection should be quite effective in establishing excellent protein quality in these two populations and additional cycles of selection will be conducted to improve agronomic performance.

Three varieties developed at Purdue from Tropical and U.S. cornbelt germplasm have been converted to opaque-2 by backcrossing. One of these, Colus, has undergone three cycles of full-sib family selection. The among-family selection was primarily for yield and secondarily for lower grain moisture at harvest and less lodging. Within-family selection was for kernel vitreousness, lysine content, and disease and insect resistance. Evaluation of the progress of selection is projected for 1978. In the other two populations, Eto x CBC and Antigua 2D x (B10 x B14), no selection has been done since their conversion to opaque. A preliminary evaluation of their potential as opaque varieties (relative to normal counterparts) will be conducted in 1977. These varieties are being selected to further fill the need for varieties adapted to temperate areas.

Considerable effort is being concentrated on the development of special varieties and source breeding materials using the sugary-2 opaque-2 double mutant. Selection and improvement of two sugary-2 opaque-2 varieties, Temp HA_{2,0} and Temp HB_{2,0} is in progress. Full-sib family selection and
evaluation of 270 families based on yield and protein quality was completed for cycle 2 in the 1976 summer nursery for both populations. The protein quality is maintained in the Temp HA su2o2 C2S1 (cycle 2, selfed one generation) varieties and kernel vitreousness and Temp HB su2o2 is markedly improved in the su2o2 kernels compared to those of o2. Based on yield, kernel vitreousness, and quality full-sibs were to be made in each variety in the 1976-77 winter nursery. However, due to severe frost damage these families were not made and thus one selection cycle was lost. These will be made in each variety in the 1977 summer nursery and yield tested and full-sib families will be selfed in the 1978 summer nursery. Full sib family selection will continue.

A parallel study using half sib family selection on the Temp HB su2o2 "highly modified opaque-2" material is in progress. Cycle 2 was completed in 1976. A generation was lost during the 1976-77 winter nursery due to severe frost damage. Based on kernel vitreousness and quality, half sibs (C3) will be made in the 1977 summer nursery. These will be yield tested and half-sib families will be selfed in the 1978 summer nursery.

The waxy opaque-2 (wxo2) and brittle-2 opaque-2 (bt2o2) double mutant combinations are also being developed in the Temp HA and Temp HB synthetic backgrounds. Half sib and/or mass selection mating system of selection is being used. The wxo2 populations have been advanced to cycle 3, and the bt2o2 populations have been advanced through cycle 2 in the 1976 summer nursery.

Experimental Opaque-2 Hybrids with Ill. High Protein Background.

Opaque-2 versions of the elite inbreds Oh43, B14 and B37 were outcrossed to Illinois High Protein and backcrossed once to the elite inbred parent.
These were selfed for 8 to 10 generations with selection and testcross evaluation. The surviving recoveries were testcrossed onto elite opaque-2 testers. The opaque-2 versions of the elite inbreds were also testcrossed and included as checks.

The sixty hybrids were evaluated in replicated yield trials in 1976 (Table 2). A number of testcross hybrids of these inbred recoveries were equal or superior to their elite counterpart parent. Several Sx29 opaque-2 inbreds gave excellent performance. Some of these testcross hybrids were equal to Pioneer 3369L, one of the better commercial opaque-2 hybrids on the market.

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 elite inbred sources recovered from crosses to the Illinois High Protein (IHP) material. Most of these materials were advanced to backcross 6 during the 1976 summer nursery season. Protein values in the backcross five selfed one generation sugary-2 opaque-2 IHP recoveries from the 1976 Oh43 and B37 lines, ranged from 0.51 to 4.13 percentage points higher than the respective non-recurrent parents. However, in the B14 lines the increase in protein percent was not as great and ranged from 0.25 to 1.11 percentage points difference. These values must be viewed with considerable caution due to environmental influence on protein content.

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. Most of these materials were advanced to backcross 5 in the 1976
Table 2. Performance of Experimental Opaque-2 Hybrids, 1976.

<table>
<thead>
<tr>
<th>Hybrid Pedigree</th>
<th>Acre Yield</th>
<th>Moisture</th>
<th>Lodging</th>
<th>Ear Height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bu.</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Tester I* X Sx2902 (00377-A)</td>
<td>84</td>
<td>19.3</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>&quot; X Sx2902 (00380-A)</td>
<td>60</td>
<td>23.7</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>&quot; X Sx2902 (39217)</td>
<td>66</td>
<td>20.7</td>
<td>16</td>
<td>6</td>
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<tr>
<td>&quot; X Sx2902 (39283)</td>
<td>86</td>
<td>20.1</td>
<td>18</td>
<td>5</td>
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<td>&quot; X Sx2902 (39453)</td>
<td>114</td>
<td>21.8</td>
<td>2</td>
<td>6</td>
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<tr>
<td>&quot; X Sx2902 (39453-A)</td>
<td>86</td>
<td>21.0</td>
<td>19</td>
<td>4</td>
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<tr>
<td>Tester I X Sx2902</td>
<td>80</td>
<td>21.9</td>
<td>2</td>
<td>5</td>
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<tr>
<td>&quot; X Sx2902 (39016-1)</td>
<td>48</td>
<td>21.4</td>
<td>2</td>
<td>6</td>
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<tr>
<td>&quot; X Sx2902 (39017-1)</td>
<td>83</td>
<td>22.4</td>
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<td>5</td>
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<tr>
<td>&quot; X Sx2902 (20141-1)</td>
<td>82</td>
<td>21.7</td>
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<td>1</td>
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<tr>
<td>&quot; X Sx2902 (39018-1)</td>
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<tr>
<td>Tester I X Sx2902</td>
<td>65</td>
<td>21.5</td>
<td>15</td>
<td>9</td>
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<tr>
<td>Tester II* X Ob4302 (500152 x 137)</td>
<td>108</td>
<td>22.8</td>
<td>10</td>
<td>1</td>
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<tr>
<td>&quot; X IHP4302 (500152 x 138)</td>
<td>100</td>
<td>20.9</td>
<td>15</td>
<td>3</td>
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<tr>
<td>&quot; X IHP4302 (500152 x 139)</td>
<td>76</td>
<td>24.2</td>
<td>29</td>
<td>1</td>
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<tr>
<td>&quot; X IHP4302 (500152 x 140)</td>
<td>67</td>
<td>23.0</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>&quot; X IHP4302 (500152 x 141)</td>
<td>101</td>
<td>23.8</td>
<td>28</td>
<td>5</td>
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<tr>
<td>&quot; X IHP4302 (500152 x 142)</td>
<td>113</td>
<td>23.4</td>
<td>8</td>
<td>3</td>
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<tr>
<td>&quot; X IHP4302 (500152 x 143)</td>
<td>112</td>
<td>23.0</td>
<td>8</td>
<td>3</td>
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<tr>
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<td>6</td>
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<td>19.7</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Tester II X Ob4302 (500181 x 161)</td>
<td>103</td>
<td>19.1</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

* Tester I pedigree is C12302 x Ob4302; Tester II pedigree is B8402 x B3702.
<table>
<thead>
<tr>
<th>Hybrid Pedigree</th>
<th>Acre Yield</th>
<th>Moisture</th>
<th>Lodging</th>
<th>Stand</th>
<th>Ear Height</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Bu.</td>
<td>%</td>
<td>%</td>
<td></td>
<td>In.</td>
</tr>
<tr>
<td><strong>Tester I</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>*X IH91402 500181 x 162</td>
<td>89</td>
<td>20.8</td>
<td>1</td>
<td>17</td>
<td>99</td>
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<tr>
<td>&quot; X IH91402 500181 x 164</td>
<td>93</td>
<td>19.5</td>
<td>1</td>
<td>2</td>
<td>100</td>
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<tr>
<td>&quot; X IH91402 500181 x 165</td>
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<tr>
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<td>96</td>
</tr>
<tr>
<td>&quot; X IH91402 500181 x 167</td>
<td>89</td>
<td>19.2</td>
<td>3</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>&quot; X IH91402 500181 x 168</td>
<td>93</td>
<td>21.6</td>
<td>2</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S5 500181 x 169</td>
<td>64</td>
<td>20.9</td>
<td>3</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S5 500181 x 170</td>
<td>91</td>
<td>20.3</td>
<td>3</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S6 500181 x 171</td>
<td>92</td>
<td>17.5</td>
<td>0</td>
<td>9</td>
<td>95</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S6 500181 x 172</td>
<td>57</td>
<td>20.0</td>
<td>1</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S6 500181 x 173</td>
<td>59</td>
<td>20.9</td>
<td>2</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S6 500181 x 174</td>
<td>63</td>
<td>20.3</td>
<td>6</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S4 500181 x 175</td>
<td>88</td>
<td>20.6</td>
<td>4</td>
<td>1</td>
<td>93</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S4 500181 x 176</td>
<td>74</td>
<td>21.7</td>
<td>4</td>
<td>0</td>
<td>89</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S4 500181 x 177</td>
<td>111</td>
<td>18.1</td>
<td>0</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S4 500181 x 178</td>
<td>86</td>
<td>20.6</td>
<td>5</td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S5 500181 x 179</td>
<td>63</td>
<td>19.4</td>
<td>3</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>&quot; X (IHP91402 x IHP91402) S5 500181 x 180</td>
<td>94</td>
<td>21.8</td>
<td>4</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td><strong>Tester III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*X B33702 500219 x 186</td>
<td>107</td>
<td>24.2</td>
<td>13</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 187</td>
<td>77</td>
<td>24.3</td>
<td>19</td>
<td>14</td>
<td>96</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 189</td>
<td>66</td>
<td>22.1</td>
<td>8</td>
<td>8</td>
<td>97</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 190</td>
<td>92</td>
<td>21.3</td>
<td>4</td>
<td>16</td>
<td>93</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 191</td>
<td>92</td>
<td>22.6</td>
<td>7</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 192</td>
<td>101</td>
<td>21.0</td>
<td>4</td>
<td>8</td>
<td>83</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 193</td>
<td>90</td>
<td>23.2</td>
<td>11</td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 197</td>
<td>75</td>
<td>21.4</td>
<td>3</td>
<td>29</td>
<td>93</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 198</td>
<td>76</td>
<td>20.7</td>
<td>8</td>
<td>11</td>
<td>94</td>
</tr>
<tr>
<td>&quot; X IH93702 500219 x 201</td>
<td>80</td>
<td>21.9</td>
<td>6</td>
<td>8</td>
<td>95</td>
</tr>
<tr>
<td>&quot; X (IHP93702 x IH93702) S6 500219 x 202</td>
<td>81</td>
<td>23.1</td>
<td>3</td>
<td>13</td>
<td>80</td>
</tr>
<tr>
<td>&quot; X (IHP93702 x IH93702) S6 500219 x 203</td>
<td>78</td>
<td>19.9</td>
<td>0</td>
<td>16</td>
<td>93</td>
</tr>
<tr>
<td>&quot; X (IHP93702 x IH93702) S6 500219 x 204</td>
<td>104</td>
<td>20.6</td>
<td>3</td>
<td>28</td>
<td>86</td>
</tr>
<tr>
<td>&quot; X (IHP93702 x IH93702) S4 500219 x 212</td>
<td>79</td>
<td>21.0</td>
<td>1</td>
<td>16</td>
<td>98</td>
</tr>
<tr>
<td>&quot; X (IHP93702 x IH93702) S5 500219 x 215</td>
<td>86</td>
<td>19.2</td>
<td>8</td>
<td>6</td>
<td>81</td>
</tr>
<tr>
<td><strong>FL3369A</strong></td>
<td>111</td>
<td>22.7</td>
<td>9</td>
<td>4</td>
<td>92</td>
</tr>
</tbody>
</table>

**Average of all entries**: 85 21.4 8 6 90 46

*Tester I pedigree is CL2302 x OH4302; Tester III pedigree is CL03TR+02 x CL2302."
summer nursery. The Oh43 and B14 IHP line recoveries averaged 2.89 and 0.36 percentage points greater in protein percent than the respective non-recurrent parent control. These recoveries will be selfed and test crossed to non-related sugary-2 opaque-2 singlecross testers for selection and evaluation, in the 1977 and 1978 summer nurseries.

Multiple Aleurone Opaque-2, Sugary-2, Sugary-2 Opaque-2, and Normal Counterpart 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 the 1976 nursery the backcross 4 and 5 generation recoveries of the multiple aleurone characteristic to some 15 elite inbred backgrounds were completed. Through selective screening for the multiple aleurone characteristic, we have established lines of o_2, su_2, su_2o_2 and their normal counterpart with 2 and 3 aleurone layers and are continuing to select for increased layer number in the conversion program.

Four hybrids were produced from crosses between individual plants from early generation backcross (BC1 to 3) inbred recoveries of the multiple aleurone layer characteristic. The hybrids were grown in a split plot, randomized complete block, replicated field trial in 1976. Three hybrids (A619 x A632, Oh43 x B37, and C103 x Mo17) segregated for both normal and opaque-2, and for single and multiple aleurone layers in the endosperm, whereas the other hybrid (H93 x H95) segregated for single and multiple aleurone layers only. The endosperm materials of kernels from two crosses of the same genotype within each hybrid were evaluated for protein percentage and lysine concentration (grams lysine per 100 grams protein).
Crosses of the same genotype within hybrids were significantly different from each other for lysine concentration. Furthermore, the difference between normal and opaque-2 kernels for protein percentage in the endosperm wasn't consistent for crosses within hybrids. This indicates there was a large amount of genetic variability within the multiple aleurone inbred conversions used to produce the hybrids.

The multiple aleurone layer characteristic increased lysine concentration without altering protein percentage in the endosperm of both normal and opaque-2 kernels. Opaque-2 kernels with multiple aleurone layers had 6.2 percent greater lysine concentration in the endosperm than opaque-2 kernels with a single aleurone layer (Table 3). Normal kernels with multiple aleurone layers had 4.2 percent greater lysine concentration in the endosperm than normal kernels with a single aleurone layer. This indicates that the multiple aleurone layer characteristic may be useful for improving the protein quality of normal and opaque-2 maize, although its practical value is questionable.

Table 3. Mean lysine concentration (g lysine/100g protein), averaged over crosses, of endosperms from normal and opaque-2 kernels with single and multiple aleurone layers for the four hybrids.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Kernel phenotype</th>
<th>Aleurone cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single</td>
</tr>
<tr>
<td>A619ma x A632ma</td>
<td>normal</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td>3.35</td>
</tr>
<tr>
<td>Oh43ma x B37ma</td>
<td>normal</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td>3.03</td>
</tr>
<tr>
<td>C103ma x Mol7ma</td>
<td>normal</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td>3.25</td>
</tr>
<tr>
<td>H93ma x H95ma</td>
<td>normal</td>
<td>1.81</td>
</tr>
</tbody>
</table>
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. Five of these sources have been backcrossed (7 or 8 backcrosses) 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 independent mutational origins might be expected.

The five opaque-2 sources were evaluated as inbreds (5 each in B37 and W64A), intercrosses within inbred backgrounds 15 each in B37 and W64A and 25 single crosses among inbred backgrounds. These were evaluated for agronomic and nutritional characteristics in the 1976 summer test plots. The data from these replicated test plots revealed no difference for yield and grain quality among these mutant opaque-2 sources in the inbred and hybrid (isogenic) pedigrees. This indicates the opaque-2 gene is probably not a compound locus.

These independent mutants of opaque-2 could serve equally well as diverse sources of the opaque-2 gene for breeding programs. Also these data indicate attempting to improve performance of opaque-2 varieties with new mutants at the opaque-2 locus would not likely be successful.

Four independent mutant sources of sugary-2 (su\textsubscript{2}\textsuperscript{std}, su\textsubscript{2}, su\textsubscript{2}^{PI}, su\textsubscript{2}^{P}) are being backcrossed into common inbred backgrounds Oh43, W64A, B37, C103, C123 and A632. Backcross conversions are complete to BC 6 to 8 generations for the sugary-2 standard allele, and the other su\textsubscript{2} alleles were recovered to backcross 2 and to 4, depending upon the line, in the 1976 summer nursery. The 1976-77 winter nursery materials were damaged by frost. These mutant
sources will be evaluated in advanced recoveries in intercrosses within inbreds, as inbreds, and intercrosses among inbred backgrounds.

Lysine in Protein Fractions from Populations Differing in Lysine and Protein Levels.

Two high lysine and two low lysine populations, designated HL1, HL2, LL1, and LL2, were developed from Syn HMo2O2 for contrasting lysine levels. The values for percent protein and grams lysine per 100 grams protein obtained from dried and defatted endosperm samples are given in Table 4. The criterion for selecting these high and low lysine populations was grams of lysine per 100 grams protein.

Table 4. Percent protein and grams lysine per 100 grams protein in endosperm of maize (dried and defatted).

<table>
<thead>
<tr>
<th>Population</th>
<th>Protein %</th>
<th>g Lysine per 100 g Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL1</td>
<td>10.9</td>
<td>1.93</td>
</tr>
<tr>
<td>LL2</td>
<td>11.6</td>
<td>1.86</td>
</tr>
<tr>
<td>HL1</td>
<td>8.1</td>
<td>3.93</td>
</tr>
<tr>
<td>HL2</td>
<td>8.1</td>
<td>3.44</td>
</tr>
</tbody>
</table>

^LL = low lysine and HL = high lysine

This selection resulted in a very definite negatively correlated response in percent protein. Endosperms of the HL1 and HL2 populations contained 8.1 and 8.1 percent protein and 3.93 and 3.44 grams lysine per 100 grams protein, respectively. The LL1 and LL2 populations contained 10.9 and 11.6 percent
protein and 1.93 and 1.86 grams of lysine per 100 grams protein, respectively. Selection decreased lysine levels in LL1 and LL2 almost to the level found in normal maize.

Five soluble fractions were extracted from endosperms of LL1, LL2, HL1, and HL2 using the method of Landry and Moureaux.

The distribution of protein (endosperm) among the five fractions (expressed as percent) and the amount of lysine in the protein of each fraction (expressed as grams of lysine per 100 grams protein) are given in Table 5. Of the four selections, LL1 and LL2 contained more protein in fractions II and III while HL1 and HL2 contained more protein in fractions I, IV and V.

Grams lysine per 100 grams protein for each fraction was not influenced by selection. However, there were some large shifts in the distribution of protein among the fractions as a response to selection. The percentage of protein in fractions which had higher grams lysine per 100 grams protein were increased in the HL selections and decreased in the LL selections. Similarly, the percentage of protein in fractions which had lower grams lysine per 100 grams protein increased in the LL selections and decreased in HL selections.

These results indicate that selection for grams lysine per 100 grams protein changed the percentage of protein in the various fractions. The grams lysine per 100 grams protein in the various fractions was not changed.

The grams lysine contributed by each fraction to the grams lysine per 100 grams protein of the LL1, LL2, HL1 and HL2 selections are given in Table 6. The grams lysine per 100 grams protein in these selections came mainly from the contributions of Fractions V and I, and to a lesser degree of Fraction IV. The difference in grams lysine per 100 grams protein between
Table 5. Percentage of protein in the five fractions of endosperm protein in low and high lysine maize selections.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Selection*</th>
<th>LL1</th>
<th>LL2</th>
<th>HL1</th>
<th>HL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>13.2 (4.52)**</td>
<td>11.9 (2.98)**</td>
<td>19.8 (4.80)**</td>
<td>22.8 (3.72)**</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>30.1 (0.15)</td>
<td>36.3 (0.12)</td>
<td>12.6 (0.20)</td>
<td>9.2 (0.30)</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>21.4 (0.09)</td>
<td>15.2 (0.12)</td>
<td>14.8 (0.17)</td>
<td>12.6 (0.09)</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>13.3 (1.30)</td>
<td>9.3 (2.03)</td>
<td>17.0 (1.86)</td>
<td>14.9 (1.53)</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>16.2 (6.02)</td>
<td>17.8 (5.22)</td>
<td>33.3 (6.19)</td>
<td>29.4 (6.01)</td>
</tr>
<tr>
<td>Percent recovery</td>
<td>94.2</td>
<td>90.5</td>
<td>97.5</td>
<td>88.9</td>
<td></td>
</tr>
</tbody>
</table>

*LL = low lysine and HL = high lysine.

**= grams lysine per 100 grams protein of each fraction.
the LL and HL selections could be attributed largely to the increase in the contributions of Fractions V, I and IV.

Table 6. Grams lysine per 100 grams protein for each fraction.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Selections†</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL1</td>
<td>LL2</td>
<td>HL1</td>
<td>HL2</td>
</tr>
<tr>
<td>I</td>
<td>0.60</td>
<td>0.35</td>
<td>0.95</td>
<td>0.85</td>
</tr>
<tr>
<td>II</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>III</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>IV</td>
<td>0.17</td>
<td>0.19</td>
<td>0.32</td>
<td>0.23</td>
</tr>
<tr>
<td>V</td>
<td>0.98</td>
<td>0.93</td>
<td>2.06</td>
<td>1.77</td>
</tr>
<tr>
<td>Total</td>
<td>1.81</td>
<td>1.53</td>
<td>3.37</td>
<td>2.89</td>
</tr>
</tbody>
</table>

†LL = low lysine and HL = high lysine.

Although based on limited data, an important point from this experiment might be of value to corn breeders developing maize with improved protein quality. Selection for percent lysine may be a more effective selection criterion than percent protein or grams lysine per 100 grams protein. This is true apparently, because it does not matter greatly how the two components, percent protein and grams lysine per 100 grams protein, vary to determine a particular level of lysine. Therefore, selection for modified types which tend to decrease grams lysine per 100 grams protein and increase percent protein may not have a serious adverse effect on nutritional quality. However, one would expect some practical limitations that may be imposed by protein level in the maize and adequacy of protein content in the diet. More detailed data is certainly needed to support this interpretation.
Selection for lysine content as measured by grams lysine per 100 grams protein resulted in 93 percent increase for this characteristic. Sixty-three percent of this increase was contributed by an increase in protein in Fraction V which is high in lysine. If a simple method for determining percent protein in Fraction V could be devised, this might be used to effectively select for improved protein quality.

**Single Gene Modifiers of Opaque-2.**

Genes which change the phenotypic expression of the opaque-2 gene to a vitreous phenotype is one approach to improving the agronomic characteristics of high lysine opaque-2 maize. Many such modifier genes exist. This is shown by the presence of partially vitreous kernels in different opaque-2 lines and populations. Most of these modifiers are polygenic and selection pressure must be maintained to retain the vitreous phenotype.

We have isolated another modifier that appears to be more simply inherited (see Annual Reports 1975 and 1976). The expression of this modification could be explained by the action of one or two partially dominant genes. This modifier gives kernels that vary in vitreousness depending on environmental conditions. The expression is stable from one generation to the next.

An attempt is being made to determine the effect of this modifier on kernel weight, kernel density, endosperm protein content, and endosperm lysine content. Four classes are being analyzed. They are homozygous modified, homozygous nonmodified, modified kernels from segregating families, and nonmodified kernels from segregating families. These four classes have analyzed under different environmental conditions and in different genetic backgrounds.
Preliminary results are shown in Table 7. The data was not analyzed statistically since the experiment is not yet complete. The data shows a trend toward higher kernel weight and density for the modified classes. The effect on protein percentage is not consistent. Lysine content shows a slight decline for the modified classes which however may be an improvement over most modifier types which have greater reduction in protein quality. More simply inherited modifier (vitreous) types would be more stable and easier to manipulate in the breeding operation.

These simply inherited genes controlling modification (vitreousness) in the opaque-2 types are being introduced into inbreds and populations.

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

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 vitreous endosperm, high lysine type with good agronomic yield is needed. We have previously reported that the double mutant sugary-2 opaque-2 (s_u<sub>2</sub>o<sub>2</sub>) has improved kernel vitreousness and kernel density similar to normal maize; and has improved protein quality, increased oil content, superior in vitro digestibility characteristics, and good biological feeding value. (See previous Annual Reports). It, however, suffers from reduced yield in those materials evaluated to date.

Considerable effort is being concentrated on the development of special varieties and source breeding materials using the sugary-2 opaque-2 double mutant. Selection and improvement of two sugary-2 opaque-2 varieties Temp HA s_u<sub>2</sub>o<sub>2</sub> and Temp HB s_u<sub>2</sub>o<sub>2</sub>, is in progress (see previous section).
Table 7. Kernel weight, volume, density, and protein quality in modified and non-modified families.

<table>
<thead>
<tr>
<th>Classification of families*</th>
<th>Weight (g)</th>
<th>Vol (ml)</th>
<th>Density (g/ml)</th>
<th>Protein %</th>
<th>g Lysine/100g Protein</th>
<th>Lysine %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous modified</td>
<td>10.474</td>
<td>8.7</td>
<td>1.204</td>
<td>9.6</td>
<td>2.86</td>
<td>.275</td>
</tr>
<tr>
<td>Homozygous non-modified</td>
<td>10.416</td>
<td>9.2</td>
<td>1.132</td>
<td>10.0</td>
<td>3.06</td>
<td>.306</td>
</tr>
<tr>
<td>Modified from segregating ears</td>
<td>10.952</td>
<td>9.2</td>
<td>1.196</td>
<td>10.0</td>
<td>2.96</td>
<td>.296</td>
</tr>
<tr>
<td>Non-modified from segregating ear</td>
<td>10.368</td>
<td>9.1</td>
<td>1.139</td>
<td>9.7</td>
<td>3.18</td>
<td>.308</td>
</tr>
</tbody>
</table>

*Each of the entries is a mean of 40 families.
The development of sugary-2 opaque-2 corn belt germplasm is continuing. Sixteen elite inbred near isogenic conversions to the mutants su\textsuperscript{2}o\textsubscript{2}, su\textsubscript{2} and o\textsubscript{2} have been advanced to the backcross 6 generation or further in the 1976 summer nursery. Additional elite inbred conversions of these mutants have been advanced to backcross 4 and 5. The 1976-77 winter nursery was severely frost damaged and therefore progress in growing out selection for agronomic type, protein quality, and seed quality was curtailed. A selected set of 12 inbred line conversions to su\textsubscript{2}o\textsubscript{2}, su\textsubscript{2}, and o\textsubscript{2} crossed in a diallel to produce all possible near isogenic single cross hybrids for each of the respective mutants will be evaluated for agronomic quality, kernel characteristics, and protein quality. Poor 1976-77 winter nursery conditions has forced a critical evaluation of these materials until the 1977-78 growing seasons.

Selected isogenic lines of the su\textsubscript{2}o\textsubscript{2}, su\textsubscript{2}, and o\textsubscript{2} conversions and their normal counterparts are being used in developmental studies to determine the interactions of genotype on the germ and endosperm storage and compensation effects involved in yield, protein, starch, and oil accumulation in the kernel.

Twenty-five elite inbred near isogenic conversions to waxy and waxy opaque-2 (wx o\textsubscript{2}) have been advanced to the backcross 4 to 6 generations. The waxy opaque-2 double mutant has protein quality equivalent to opaque-2 and good biological value. It has a modified starch type (all amylopectin), but is not improved in kernel vitreousness or density and also has reduced yields. These materials offer a glutinous and high lysine specialty type of corn.

One metabolism and two growth trials involving 432 rats and one trial with 64 pigs were conducted to evaluate the protein quality of six near isogenic versions of the maize 3-way hybrid [(Oh43 x B37) x C103] in protein-limiting diets. In 8% crude protein diets, rats fed opaque maize types (opaque-2, waxy opaque-2 and sugary-2 opaque-2) gained 143% faster and were 68% more efficient in converting feed to gain than rats fed non-opaque maize types (normal, waxy and sugary-2). These improvements were accompanied by a 5.5% lower dry matter digestibility, but a 10.5% improvement in biological value for opaque compared with non-opaque corns. Supplementation of the diets with lysine, tryptophan or both amino acids failed to eliminate the differences observed; diets containing opaque corns still supported higher rate of gain and improved feed efficiency as compared with those rats fed non-opaque corns. Pigs fed diets containing non-opaque corns supplemented with lysine and tryptophan showed 10.5% inferior feed efficiency, but 15% higher rate of gain than those fed opaque corns. In 11% crude protein diets, the differences observed in rat performance were smaller; at 14% protein, no differences were observed. It was found that higher contents of lysine and tryptophan in opaque maize are not the only reason for its superiority. Sugary-2 was found to be superior to normal corn; however its improvement was less than for opaque-2. These experiments indicated that the double mutants waxy opaque-2 and sugary-2 opaque-2 are similar to opaque-2 in protein quality. J. of Animal Science. 44:1011-1019, 1977.

One metabolism and two growth trials involving 140 rats and one growth and digestion trial with 36 growing pigs were conducted to compare six near isogenic versions of the maize 3-way hybrid [(Oh43 x 837) x C103] fed in protein adequate diets. No differences in rat or pig performance were obtained comparing three endosperm starch types: waxy (100% amylopectin), sugary-2 (40% amylose, 60% amylopectin) and normal (26% amylose, 74% amylopectin). It was found that opaque-2, sugary-2 opaque-2 and sugary-2 corns contained from 3.0 to 3.3% lower digestible energy than normal corn for rats. In addition, all corns with the opaque-2 gene contained, on the average, 2.65% lower digestible nitrogen than non-opaque types. With pigs it was found that the double mutants sugary-2 opaque-2 and waxy opaque-2 did not show lower apparent digestion coefficients than normal corn as did opaque-2 corn. These experiments indicated that energy utilization from waxy, sugary-2 and double mutants with the opaque-2 gene is as good as for normal corn for growing pigs. J. of Animal Science. 44:1004-1010, 1977.

Nitrogen Retention of Young Men Who Consumed Isonitrogenous Diets Containing Normal, Opaque-2, or Sugary-2 Opaque-2 Corn.

Three isogenic lines of corn provided two levels of nitrogen for young men, but the diets were isonitrogenous at 5.5 g. Mean daily nitrogen balances were -0.28 ± 0.34, +0.31 ± 0.23 and 0.40 ± 0.08 g, respectively, when normal, opaque-2 and sugary-2 opaque-2 corn furnished 5.5 g of nitrogen; and -0.07 ± 0.17, +0.38 ± 0.21 and -0.04 ± 0.25 g when corn supplied only 4.4 g of nitrogen. Although these values were not significantly different, both high-
lysine varieties tended to be superior to normal corn at 5.5 g of nitrogen and opaque-2 corn caused improvement even at 4.4 g of nitrogen. Lysine and tryptophan were higher and leucine lower in both opaque-2 and sugary-2 opaque-2 than in normal corn. Coefficients of apparent digestibility were between 65.5% and 68.8%. J. Nutr. 107:404-411, 1977.

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

Cooperative studies with Dr. A. Kirleis (AGRY) and Dr. George G. Graham (Dept. of International Health, The John Hopkins University) are in progress to evaluate the whole corn meal and degerminated corn meal protein quality and digestibility of carbohydrate in infant children. The three types of corn being evaluated are o₂, su₂o₂, and normal. In these studies we are looking at nitrogen absorption, nitrogen retention, stool weights, and stool energy (by bomb calorimetry). In addition we are taking a look at fasting and postprandial plasma free amino acids. We are particularly interested in seeing if lysine or tryptophan show up as first-limiting or if both amino acids suffer a fall in their plasma level postprandially. In addition we have studied the milling properties of the mutant genotypes in processing the flours for infant feeding.

**Effects of Actinomycin-D Treatment on the Developmental Pattern in Maize Kernels.**

Actinomycin-D is a known inhibitor of RNA, and consequently, protein synthesis. We treated developing maize kernels of opaque-2, the double mutants bt₂o₂, sh₂, o₂, and their normal counterpart in the inbred Oh43.
background with this antibiotic at dose levels ranging from 5 - 150 μg/ear on the 5th, 6th, 7th, 9th, and 11th days post pollination, and harvested the treated ears 5, 10, 15, 20, 25, 30, 40, and 50 days after treatment. This experiment was designed to test whether artificially blocking zein synthesis would give rise to a high lysine phenotype in normal maize. Difficulty was experienced in actual injection of the antibiotic and known distribution in the developing kernels. Our preliminary results indicated that either the highest dose used in our experiments does not block zein synthesis, or that zein synthesis in developing corn kernels is immune to inhibition by actinomycin D.

Chemical Nature of the Zein Proteins from Normal and Opaque-2 Maize.

We investigated the chemical nature of the zein proteins from normal and opaque-2 maize kernels. Large scale crude preparations of zein were obtained from ground, defatted seeds by extended (2 days) extraction with 70% (v/v) isopropanol. The crude extracts were dialysed extensively against distilled water to remove isopropanol and to precipitate the crude zein. The precipitated protein was freeze dried and dissolved in a minimum amount of dimethyl formamide (DMF), following which it was subjected to gel permeation chromatography on a sephadex G-75 column (2.5 x 90 cm) equilibrated in 90% DMF. The zein eluted from this column as a sharp, well defined peak, substantially free of contaminating material. The purified zein protein fractions were dialysed against distilled water, the flocculant protein precipitate collected, freeze dried, and stored at -20°C.

The constituent peptides of the zein preparations were prepared by reduction (mercaptoethanol) followed by treatment with iodoacetic acid.
Homogeneous zein protein treated in this manner provided three well defined protein peaks on G-75 chromatography, each of which was collected and freeze dried following dialysis. Zein proteins from both normal and opaque-2 kernels, as well as their constituent peptides were subjected to amino acid analyses. Our data thus far suggests that the zein protein from both normal and opaque-2 maize consists of three polypeptide chains linked together by disulphide bonds, and that one of those polypeptides is devoid of lysine. Two of the three peptides make up a majority of the protein in zein, while the third peptide is small by comparison. There are no significant differences in the proteins from normal and opaque-2 maize.

We also developed a rapid method for determining glutamine and asparagine content of these zein preparations. This method was based upon estimation of ammonia released from these amino acids upon dilute acid treatment.

**Distribution of Lysine During Germination of Normal and Opaque-2 Maize.**

Levels of total and free (pool) lysine in germinating normal [(Oh43+ x B37+) x C103+] and opaque-2 [(Oh43o2 x B37o2) x C103o2] maize were monitored over an 11-day period. Germination at 28°C in the dark (without supplemental carbon or nitrogen) was accompanied by an increase in total and free lysine levels in normal maize seedlings while, in opaque-2 seedlings, total lysine declined over an 11-day period, accompanied by an increase in the free lysine pool. Total nitrogen content remained constant in both experiments, while there was a 15% loss in total dry weight over the 11-day period. Both normal and opaque-2 seedlings developed comparable levels of aspartokinase activity which, reached a maximum at 10 days post germination. Aspartokinase preparations from both normal and high-lysine maize appeared to be subject to feedback inhibition by lysine. Cereal Chem. 54(3):558-564, 1977.
Interaction of Carbohydrate Mutants on Zein Synthesis in High Lysine Mutants

Opaque-2 and Flouiry-2.

The carbohydrate mutants sh1, sh2, sh4, bt1, bt2, ae, du, wx, su1 and su2 and their interactions with the o2 high lysine mutant; and the sh2 and bt2 mutants and their interactions with the f12 high lysine mutant were studied for their genetic regulation on storage protein content in maize endosperm. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and [14C]-leucine incorporation studies revealed that the inability of the starch deficient double mutants sh2o2, bt1o2, and bt2o2 to produce the two predominant zein protein species (Z1 and Z2) appears to be associated with the fact that membrane-bound polyribosomes are not able to synthesize both components from [14C]-leucine incorporation. An interesting observation was that sh2f12 and bt2f12 was much like f12 in zein protein components in that Z1 and Z2 species were present but in reduced amounts.

Comparative Susceptibility of Amylases of Starch Granules of Several Single Endosperm Mutants Representative of Floury-Opaque, Starch Deficient, and Modified Starch Types and Their Double-Mutant Combinations with Opaque-2 in Four Inbred Lines of Maize.

Starch granules were prepared from kernels of eight single endosperm mutants, brittle-1 (bt1), brittle-2 (bt2), floury-1 (f11), floury-2 (f12), soft-starch (h), opaque-1 (o1), shrunken-2 (sh2), and sugary-2 (su2), their double-mutant combinations with opaque-2 (o2) of four inbred lines of maize (Zea mays L.), B37, C103, Oh43 and W64A. We compared the susceptibility of various starch granules to Rhizopus glucoamylase and pancreatin. Starch granules of the su2 and su2o2 mutants were digested by amylases much faster
than those of the normal counterparts. Starch granules of the $bt_{1}$, $bt_{2}$, $o_{1}$ and $sh_{2}$ mutants tended to be digested by amylases faster than those of the normal. Starch granules of double-mutant combinations with the $o_{2}$ gene were, in general, digested to the extent very comparable to their respective nonopaque single mutant counterparts in each of their four inbred backgrounds. We followed the relative digestion of starch granules by using scanning electron microscopy (SEM). Starch granules of endosperm mutants susceptible to amylases showed numerous pin holes on the surface layer and the pores penetrated into the inner layers of the granules during the attack with amylases. For some of the granules the inner portion which appeared terraced or step-shaped could be seen. This may be indicative of layered internal structures of the granules.

E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results:

a. Research report. Approximately 650 copies of the 1974-75 annual research report were distributed to over 400 maize cooperators and interested centers, missions or institutions representing over 35 countries and many institutions within the U.S.

For a bibliographic list of publications see Section H.

b. Status report. Over one-hundred requests have been received by interested maize and other cereal workers as well as many nutritionists and biochemists throughout the world for the status report—International Programs in Agriculture, Agricultural Experiment Station, Purdue University Station Bulletin No. 70 (March 1975)—entitled "Single Chemical
and Biological Methods Used at Purdue University to Evaluate Cereals for Protein Quality". Continued requests are being made for the status report--Purdue University Agricultural Experiment Station Research Bulletin 914 (October 1974)--entitled "Progress in Developing Maize with Improved Protein Quality".

c. **Workshops, symposia, papers and seminars presented.** Papers were presented and project personnel participated in the following activities: American Society of Agronomy Annual Meetings; League for International Food Education and USAID Offices of Nutrition and Agriculture Workshop on breeding and fortification-Improving the Nutritional Quality of Cereals, Boulder, Colorado; American Society of Animal Sciences Annual Meeting; Mid-West Section of the American Society of Animal Sciences Annual Meeting; The Annual Meeting of the Japanese Society of Starch Science, Tokyo, Japan; The Annual Meeting of the American Society of Biological Chemists, San Francisco, CA; Symposium on Molecular Biology of Plants, St. Paul, MN; American Society for Experimental Biology.

Project personnel gave several seminars and participated in special field day and program activities in Agronomy, Animal Sciences, Agricultural Economics, Biochemistry, and Foods and Nutrition 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, H*O*O*O*, Synthetic, Mod. Syn. A*O*, Mod Syn B*O*, Temperate HA and HB opaque-2 and sugary-2 opaque-2, sources of modifier genes for opaque-2, and other sources of material for improvement of the protein quality of maize: Lorenzoni/Italy;
Germplasm for protein quality improvement was distributed to over 25 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, technical help, advice concerning protein quality improvement in maize and all aspects of cereal grain protein quality improvement. Requests continue for opaque-2, "modified opaque-2", and the double mutant, sugary-2 opaque-2, germplasm. The number of visitors from LDC's and other countries to Purdue has continued though there were fewer than in the past year or so. Information and germplasm requests and personal contacts have helped to increase involvement with the LDC's, helped to understand their real problems, and constitute a part of project efforts to disseminate and exchange information relevant to LDC programs and involvement in protein quality improvement.
The graduate students trained under the general direction of this project, short term trainees, and visiting scientists constitute a vital part of the 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. Continued emphasis must be given to overcoming the associated yield constraints of most high lysine types of maize.

Undergirding with research and cooperating with other research centers in the world-wide maize network such as CIMMYT, Andean Zone breeders, Brazilian maize workers, South East Asian maize workers in the Phillipines and Thailand, East Africa, and Guatemala will strengthen and improve the utilization of fundamental knowledge and germplasm materials.

Dr. Glover participated in a workshop and presented an invitational paper to the Second International Workshop on Breeding and Fortification.

Cooperative testing of temperate germplasm 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 USAID-TA/AGR (Dr. Kenneth McDermott) and CIMMYT maize research staff (Drs. E. W. Sprague, R. L. Paliwal, E. C. Johnson, S. K. Vasal, E. Villegas M., A. Ortega C., K. S. Fisher) was held January 25-27, 1977 to discuss program developments and research plans, exchange information and coordinate efforts in the development of improved protein quality maize. Considerable discussion centered upon the development and production of improved protein quality maize. The development of wide-based temperate germplasm materials for the more temperate regions of the world has been an outreach of this cooperation to complement the varieties being developed for the more tropical areas of the world. Testing of wide-based temperate germplasm materials is being conducted in Pakistan, Israel, Mexico, and U.S. although there have been serious limitations involved in obtaining adequate evaluations and coordination of the test materials.

Project personnel have been involved in cooperative work to screen the Central American maize germplasm from the germplasm bank, Mexico City, for more vitreous high lysine types.

Project personnel have been involved with breeders from the Andean region, Brazil, Guatemala, Thailand, India, Indonesia, Phillipines,
Korea, Nepal, Egypt, Africa, Pakistan, Yugoslavia, and other countries in special visits, seminars, and workshop discussion groups to help guide the development and utilization of improved protein quality maize.

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.

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

Students from Mexico (Alfredo Navarro), Nigeria (Adeleke A. Oho), Phillipines (Lourdes Nazarea), Colombia (Hugo Zorillo), Taiwan (Husan Tu), and the United States (Arthur H. Long, Steve G. Ballinger, Rodney L. Tietz) were in graduate training during the project year. Dr. Bakshy A. K. Chibber (India) and Dr. James B. Barnett (U.S.) received training as post-doctorates during the year or part of the year.

In section H is given a bibliographic list with abstracts of publications, papers, seminar or workshop reports representing efforts to disseminate results of the research project.


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. Chemical and genetical analyses will continue of new mutants which show improved protein quality and quantity.
      (1) 1977-1978--The mutants which have shown high lysine values from screening tests of populations will be allele tested, inheritance analyzed, and any promising new mutants will be introduced into elite germplasm, and combined with other high lysine mutants and evaluate epistatic effects.
      (2) 1978-1979--Continue allele and linkage tests and introduction of any promising new mutants into elite germplasm, and combine and evaluate epistatic effects with other high lysine mutants.
   b. Screening for mutant types with improved protein quality and quantity from the Germplasm Bank (CIMMYT) and chemically treated germplasm.
      (1) 1977-1978--Continue search for and evaluation of new improved protein quality mutants in M$_2$ progeny of chemically induced
germplasm materials. Investigate seed and pollen treatment methods of chemical treatment for producing "high lysine mutants". Allele test "high lysine" mutant recoveries and study their inheritance patterns is non-allelic to known high lysine genes. New types will be introduced into elite germplasm.

(2) 1978-1979--The search shall continue in promising source populations and induced mutants, and promising material analyzed in more detail. Completely modified opaque-2, new high lysine germplasm of "normal" phenotype, and mutants which further reduce the prolamine content with further increases in protein quality will be the object of the search. Promising new mutants will undergo further evaluation as in 1 a 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 new mutant genes. Normal kernels from 100 segregating families (ears) of the original random mated versions of Temp HA and Temp HB have been analyzed for protein and lysine.

(1) 1977-1978--Continue full sib selection for high lysine families in normal maize for another cycle of recurrent selection.

(2) 1978-1979--Continue full sib family cycle selection if feasible.
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. turcicum* and smut (*Ustilago zeae*).

   (1) 1977-1978--Cycle 4 of 150 full sibs will be yield tested at 4 or 5 worldwide locations and evaluated for agronomic performance and protein quality. Selected families will be intercrossed in the winter nursery.

   (2) 1978-1979--Test and analyze cycle 5 full sib families. 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) 1977-1978--Selected families based on yield tests, agronomic quality, kernel vitreousness and protein quality from 270 selected full-sib families (Cycle 2) tested in the summer of 1976 will be intercrossed in the 1977 summer nursery. These were grown out to intercross in the 1977 winter nursery but due to frost damage these materials were lost.
2. 1978-1979--Continue full sib selection as outlined above in the populations. Yield test and analyze full sibs Cycle 3 in summer of 1978 and then continue with the use of the summer and winter nursery to advance the material.

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 two 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) 1977-1978--Grow out advanced generation recoveries from backcrossing (BC5 and 6) and selection for increased levels of protein based on evaluations for agronomic performance, kernel quality, kernel size and protein quality. Make up test cross progeny of the selected materials to be grown out and evaluated for advance in protein content. Intercross improved line recoveries.
      (2) 1978-1979--Evaluate for protein quantity in test cross progeny and in hybrids from intercrosses of selections from backcross 5 and 6 recoveries of selected lines.
   b. Selections for protein content in the multiple aleurone sugary-2 opaque-2 inbreds.
      (1) 1977-1978--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. Conduct detailed genetic and agronomic evaluation of the increased aleurone layered recoveries. Grow out early backcross recovery lines and evaluate for stability of multiple aleurone characteristic. Intercross selected line early generation recoveries.

(2) 1978-1979--Advanced generation recoveries shall be selected for increased number of aleurone layers and protein quality in the endosperm. Evaluate for protein quantity and quality improvements in hybrids from early generation backcross recoveries of selected lines.

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

(1) 1977-1978--In summer and winter nursery continue backcrossing mutant sugary-2 sources into common inbred sources. Program is one generation behind because of 1977 winter nursery loss.

(2) 1978-1979--Continue backcrossing sugary-2 sources in inbred backgrounds and begin preliminary evaluation of sugary-2 sources 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 are being conducted (Objective 3).
a. Conduct detailed genetic and agronomic studies of new single gene modifiers that gives a vitreous opaque-2 phenotype with little if any reduction in protein quality.

(1) 1977-1978--Continue backcrossing of new single gene modifier into selected germplasm and evaluate in preliminary hybrids and populations.

(2) 1978-1979--Continue backcrossing and evaluation in hybrids and other selected germplasm for agronomic performance, modified kernel type, protein quality and biological value.

b. Current research has shown that selection for modified opaque-2 types results in lower lysine levels and a shift in the protein fractions (Landry-Moureaux 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. Recently we have found a new single gene modifier (5-b above).

(1) 1977-1978--Selected germplasm converted to the new single gene modifier shall be subjected to protein fractionation, protein quality (amino acid patterns) of these fractions and electrophoretic separation of proteins.

(2) 1978-1979--Continue evaluation of protein quality if additional information is needed.

c. The sugary-2 opaque-2 double-mutant genotype has been crossed with specific selections for modified opaque-2 vitreous kernel phenotype (Objectives 2 and 3).
1. Continue backcrossing the new simply inherited modifier gene of opaque-2 to sugary-2 opaque-2 material and selfing in advanced generation materials. Make up half-sib cycle 3, continue half families in sub-population of Temp HB sugary-2 modified o$_2$ in summer nursery. Evaluate the sugary-2 opaque-2 genotype in the modified opaque-2 backgrounds for agronomic performance, modified kernel types and protein quality.

2. Evaluate for agronomic performance, protein quality, kernel size and degree of modification and continue advanced backcrossing and half-sib family selection.

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. Continue backcrossing of the sugary-2 opaque-2 and other double-mutants to elite inbred backgrounds where necessary. Backcrosses 6 and 7 should be completed in sugary-2 opaque-2 for many elite backgrounds. Make up hybrids and begin evaluation.

2. Continue evaluation of double mutant hybrids for improved protein quality, kernel and nutritional characteristics, and agronomic quality.
b. Detailed genetic and agronomic evaluation of the sugary-2 and sugary-2 opaque-2 system in large seeded backgrounds to study associated interactions on nutritional quality, physical properties of the kernel and agronomic characteristics.

(1) 1977-1978--Continue backcrossing to adapted cuzco synthetic backgrounds and being random mating of sugary-2 conversions.


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

(1) 1977-1978--Characterize the developmental process of grain filling and associated compensation effects in endosperm and germ biosynthetic development.

(2) 1978-1979--Continue characterization of developmental processes of grain filling in high lysine genotypes.

d. Development of promising new genotypes.

(1) 1977-1978--As new mutants are described under Objective 1 and appear promising, intercross with selected endosperm mutants.

(2) 1978-1979--Evaluate double-mutant segregates for agronomic quality, kernel characteristics, protein and nutritional quality.
e. Conduct basic research on the nature of proteins in double-mutant combinations.

(1) 1977-1978--Characterize the nature of the protein bodies and matrix protein distribution in selected key mutants. Characterize the protein-profiles in the double-mutant types to determine what change(s) occurs in protein fractions or protein quality of those fractions which give modified kernel characteristics in investigations to elucidate the genetic control of protein quality and explain variations in high lysine mutant types.

(2) 1978-1979--Continue studies on protein biosynthesis in double-mutants.

7. Regulation of zein synthesis in maize endosperm and characterization of key proteins in high lysine endosperms (Objectives 1, 2, and 3).

1977-1978--Continue protein characterization of key mutant and mutant combinations by using the Landry-Moureaux method of fractionation. The amino acid patterns of the fractions will be determined. Acrylamide disc gel electrophoretic patterns will be obtained on the protein fractions to explain variations from normal and opaque-2 genotypes. Unique proteins will be isolated and characterized. Isolate-free and membrane-bound polyribosomes and carry out protein synthesis and characterize the nature of zein syntheses.

Determine the efficiency of the zein and glutelin synthesizing ability of the high lysine mutants during development in order to ascertain the possible mechanism by which zein synthesis is retarded in the developing high lysine mutants.
9. Protein nutritive value (Objectives 2, 3, and 4).

a. Animal subjects.

(1) 1977-1978--Protein quality evaluation and energy utilization studies with rats will continue in cooperation with the Department of Animal Sciences and Biochemistry for evaluation of selected promising genetic materials.

(2) 1978-1979--Continue animal nutrition evaluation where needed.

b. Human subjects.

(1) 1977-1978--Cooperative studies have been arranged with Dr. Allen Kirleis of Purdue University and Dr. Graham of John Hopkins University. It is proposed to evaluate in young children subjects the nutritional value of sugary-2 opaque-2, opaque-2, and normal endosperm and whole corn flour meal preparations. Both protein quality and the digestibility of the carbohydrates will be studied simultaneously.

In these studies we will look at nitrogen absorption, nitrogen retention, stool weights and stool energy (by bomb calorimetry). Fasting and postprandial plasma free amino acids will be determined.
H. 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.


Ikawa, Yoshiko, D. V. Glover, Y. Sugimoto, and H. Fuwa. 1977. Amylase percentage and distribution of unit chair length of maize starches with defined genetic background. Carbohydrate Research (Accepted for publication).


ABSTRACT

Combining ability for concentration and grams per 200 kernels of P, K, Mg, Fe, Zn, oil, and protein, and kernel weight, volume, and density in a six-parent diallel of maize (Zea mays L.) inbreds was examined. Correlations among certain variables were computed. Significant variation was detected among GCA effects for concentration and grams per 200 kernels of all kernel constituents except K concentration, but among SCA effects only for oil concentration. GCA and SCA effects were significant for kernel weight, volume, and density. Highly significant correlations were found between P and K, P and Mg, and K and Mg concentrations.
INTERRELATIONSHIPS AMONG PROTEIN, LYSINE, OIL, CERTAIN MINERAL ELEMENT CONCENTRATIONS AND PHYSICAL KERNEL CHARACTERISTICS IN TWO MAIZE POPULATIONS

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

ABSTRACT

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

Compared to normal kernels the opaque-2 kernels were higher in percent lysine, P, K, Mg, Fe, and Zn. However, on a kernel weight or kernel volume basis, the opaque-2 kernels were significantly greater only for lysine, K and Zn. Our results indicate that the opaque-2 gene, or closely linked genes, had a direct influence on K and Zn content, as well as on lysine content.

The correlation of the differences between the normal and opaque-2 kernels for percent lysine and percent Zn was significant. This relationship may indicate that modifier genes that are influencing the effect of the opaque-2 gene on lysine concentration may also be influencing the effect of the opaque-2 gene on Zn concentration in a similar manner.

Several significant correlations were detected between percent lysine and the mineral element concentrations, but none was of sufficient magnitude to be of value as a selection criterion for lysine content. Percent lysine was not correlated with kernel weight or volume, but was negatively correlated with kernel density. The coefficients for the correlation between
percent lysine and percent protein were +.50 and +.70, respectively, for the normal and opaque-2 kernels in the heterozygous population and +.83 for the homozygous opaque-2 population. The magnitude of these correlation coefficients indicated a rather strong tendency for lysine concentration to increase as protein concentration increased, especially in the opaque-2 kernels. Protein concentration was found to be the most efficient selection criteria for lysine concentration, except for possibly the lysine concentration per se.
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 amount per 200 kernels, the normal kernels were higher than or similar to opaque-2 kernels in all elements except K which was higher in opaque-2. The opaque-2 kernels were higher in concentrations of elements at all harvests and were higher in content of K per 200 kernels at all harvests.
PROTEIN BODY SIZE AND DISTRIBUTION AND PROTEIN MATRIX MORPHOLOGY IN VARIOUS ENDOSPERM MUTANTS OF ZEA MAYS, L.

P. S. Baenziger and D. V. Glover

ABSTRACT

Maize (Zea mays, L.) protein body size and distribution and matrix morphology were studied in 13 near-isogenic (Bc 6) endosperm mutant genotypes, amylose-extender (ae), dull (du), waxy (wx), sugary-2 (su₂), opaque-2 (o₂), flours-2 (fl₂), flours-1 (fl₁), soft starch (h), sugary-1 (su₁), shrunken-1 (sh₁), shrunken-2 (sh₂), brittle-1 (bt₁), and brittle-2 (bt₂), their double mutants with opaque-2 and the normal Oh43 inbred. Opaque-2 and two endosperm mutants, su₂ and wx, and their double mutant with o₂ each near-isogenic (Bc 6) in inbreds B37, C103, and W64A and their normal counterparts were also studied. Mature kernels of these genotypes were thin sectioned, destarched and studied using interference-contrast light microscopy.

Protein bodies were observed and measured in ae, du, fl₁, h, su₂, and wx endosperm of Oh43 and in the normal counterpart. Protein body size was significantly different among genotypes and decreased going into the center of the kernel from the aleurone layer.

No protein bodies were visible in o₂, fl₂, su₁, sh₁, sh₂, bt₁, and bt₂. 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 mutants with the lower lysine levels.
Opaque-2 and the double mutants with α₂ did not have visible protein bodies and the matrix was generally thinner than in the single mutants. Sugary-2 opaque-2 had a thicker, more extensive matrix than observed in α₂ endosperm.

Protein bodies were visible in the su₂ and wx isogenic conversions of Oh43, B37, C103 and W64A and their normal counterparts. Inbreds, genotypes, and inbred x genotypes were highly significant. No protein bodies were visible in α₂ and the double mutants with α₂. The data suggest that protein matrix may be involved in the expression of kernel vitreousness. The matrix of vitreous su₂α₂ kernels resembled the matrix of vitreous su₂ kernels and not that of α₂ kernels.
NITROGEN RETENTION OF ADULT HUMAN SUBJECTS WHO CONSUME ISONITROGENOUS DIETS CONTAINING NORMAL, OPAQUE-2 OR SUGARY-2/OPAQUE-2 CORN

Jennie Lee Betz

ABSTRACT

Because of the important role of corn as a major protein source for many people in the world, the present experiment was conducted to determine the adequacy of two varieties of high lysine maize, opaque-2 and sugary-2 opaque-2, in relation to normal maize of the same genetic background. Eight young healthy adult males served as experimental subjects for 56 days of study which included an adjustment period and 6 experimental periods of 7 days each. A crossover design was used to assign treatments. The three varieties of whole ground corn were evaluated in amounts to furnish two levels of nitrogen, 5.5 and 4.4 g per day; and the diets were made isonitrogenous by adding 1.1 g of supplemental nitrogen to the diets that provided 4.4 g of nitrogen from corn. Total energy value was modified for individual subjects to maintain their initial body weights which ranged from 59.9 to 74.0 kg.

Mean daily nitrogen balances were \(-0.28 \pm 0.34\), \(+0.31 \pm 0.23\) and \(+0.40 \pm 0.08\) g, respectively, when normal, opaque-2 and sugary-2 opaque-2 corn supplied 5.5 g of nitrogen; and \(-0.07 \pm 0.17\), \(+0.38 \pm 0.21\) and \(-0.04 \pm 0.25\) g at the 4.4 g level of nitrogen from corn. A significant difference was not detected between the nitrogen balances of subjects consuming these diets but there was a trend toward improved nitrogen retention with the two varieties of high lysine maize when 5.5 g of nitrogen were consumed as corn.
Urinary nitrogen loss was significantly higher \((p < 0.01)\) when sugary-2 opaque-2 maize furnished 4.4 g versus 5.5 g of nitrogen from corn; and when subjects consumed 5.5 g of nitrogen from normal corn versus sugary-2 opaque-2 corn at the same nitrogen level. Fecal nitrogen excretion was significantly higher \((p < 0.01)\) at 5.5 g versus 4.4 g nitrogen for both normal and opaque-2 corn.

Both the lysine and tryptophan contents of opaque-2 and sugary-2 opaque-2 corn were increased, and the balanced between leucine and isoleucine was improved because the concentration of leucine was decreased.
The nutritional quality of four selected modified opaque-2 populations, designated as LL-HP1 (low lysine-high protein), LL-HP2, HL-LP1 (high lysine-low protein), and HL-LP2 was evaluated in a rat feeding test (Experiment I). Diets made with the four modified opaque-2 populations and their respective percent protein and percent lysine were as follows: LL-HP1 with 10.91 and 0.38; LL-HP2, 11.73 and 0.35; HL-LP1, 8.74 and 0.36; and HL-LP2, 8.63 and 0.34. Diets made with a normal hybrid, its counterpart opaque-2 version, and casein were also included to give a total of seven diets. Percent protein and percent lysine of these diets were 7.32 and 0.21 for NORMAL, 8.26 and 0.34 for OPAQUE, and 8.83 and 0.76 for casein. Percent protein and grams lysine per 100 grams protein were determined for each fraction.

A limited feeding test was conducted for a period of 14 days with six rats for each diet. The variable measured were total feed consumed, total weight gained, PER and FER.

Selection for high or low values of grams lysine per 100 grams protein resulted in a very definite negatively correlated response for lower or high protein, respectively.
Higher percent lysine in the diet was the most important measure of nutritional quality and this was true regardless of whether it was contributed by higher percent protein or higher grams lysine per 100 grams protein. Among diets with the same level of percent lysine, rather large variations in percent protein did not affect nutritional quality.

Two high lysine and two low lysine populations, designed as LL1, LL2, HL1 and HL2, were selected for Experiment II. Endosperms of the HL1 and HL2 populations contained 8.1 and 8.1 percent protein and 3.93 and 3.44 grams lysine per 100 grams protein, respectively. The LL1 and LL2 populations contained 10.9 and 11.6 and 1.93 and 1.86 grams lysine per 100 grams protein, respectively.

Five soluble fractions were extracted from endosperms of LL1, LL2, HL1 and HL2 using the method of Landry and Moureaux.

Selection for higher or lower grams lysine per 100 grams protein resulted in large shifts in distribution of protein among the five fractions but did not consistently or greatly change grams lysine per 100 grams protein within the various fractions. Selection for higher grams lysine per 100 grams protein considerably increased the protein in Fraction V, the fraction that had the highest grams lysine per 100 grams protein.

Increased protein in Fraction V accounted for about 60 percent of the increase in grams lysine per 100 grams protein. If a simple method for determining percent protein in Fraction V could be devised, this might be used to effectively select for improved protein quality.
STUDIES ON CORN PROTEINS. XI. DISTRIBUTION OF LYSINE DURING GERMINATION OF NORMAL AND OPAQUE-2 MAIZE

Bakshy A. K. Chibber, Ecaterina Voicu, Edwin T. Mertz, and D. V. Glover

ABSTRACT

Levels of total and free (pool) lysine in germinating normal [(Oh43 x B37 x C103] and opaque-2 [(Oh43 x B37 x C103] maize were monitored over an eleven day period. Germination (at 28° in the dark without supplemental carbon or nitrogen) was accompanied by an increase in total and free lysine levels in normal maize seedlings, while in opaque-2 seedlings, total lysine declined over an eleven day period, accompanied by an increase in the free lysine pool. Total nitrogen content remained constant in both experiments, while there was a 15% loss in total dry weight over the eleven day period. Both normal and opaque-2 seedlings developed comparable levels of aspartokinase activity which reached a maximum at 10 days post germination. Aspartokinase preparations from both normal and high lysine maize were subject to feed back inhibition by lysine.
NITROGEN RETENTION OF YOUNG MEN WHO CONSUMED ISONITROGENOUS DIETS CONTAINING NORMAL, OPAQUE-2 OR SUGARY-2 OPAQUE-2 CORN

Helen E. Clark, David V. Glover, Jennie L. Betz and Lynn B. Bailey

ABSTRACT

The purpose of the experiment was to evaluate three isogenic lines of corn, normal, opaque-2, and sugary-2 opaque-2, in the 3-way hybrid [(0h43 x B37) x C103] background, at two levels of protein in healthy young men.

The three varieties of corn were consumed in amounts to provide 5.5 and 4.4 g of nitrogen per day. The experiment continued 56 days and included 12 days adjustment period and six experimental periods of 7 days each. A crossover design was used to assign corn varieties to individuals in the same sequence at both nitrogen levels and the diets were made isonitrogenous by adding 1.1 g of supplemental nitrogen to the diets that provided 4.4 g of nitrogen from corn. Urinary nitrogen values for the last six days of each period are reported, and fecal samples were pooled for each period.

Data pertaining to the nitrogen balance were analyzed in a randomized complete block design. A repeated measures, one-way analysis of variance was followed by Duncan's Multiple Range Test, and individual subject differences were eliminated.

Variety of corn within a particular nitrogen level did not alter fecal nitrogen significantly, but reduction in amount decreased fecal nitrogen (p < 0.01), presumably because the corn was replaced by an easily digested nitrogenous supplement.
When corn furnished 5.5 g of nitrogen, coefficients of apparent digestibility were: normal, 66.0 ± 3.3%; opaque-2, 68.8 ± 2.1%; and sugary-2 opaque-2 65.5 ± 2.3%. Digestibility therefore was not changed significantly by modifying kernel density inherent in the sugary-2 opaque-2 corn.

When corn furnished 5.5 g of nitrogen, the urinary nitrogen value of 3.42 ± 0.17 g that resulted from sugary-2 opaque-2 corn was lower (p < 0.01) than 4.13 ± 0.27 g due to normal corn, and opaque-2 corn (3.71 ± 0.14 g) did not differ significantly from either of the other corns. Since urinary nitrogen reflects absorbed amino acids utilized for protein synthesis, these results imply superiority of the amino acid composition of sugary-2 opaque-2 corn to normal corn. Although nitrogen balances were not influenced significantly by corn variety at this nitrogen level, some trends are evident. In contrast to a mean nitrogen balance of -0.28 ± 0.34 g due to the normal corn, which permitted nitrogen retention in only 50% of the men, mean balances resulting from the other varieties were positive. Opaque-2 corn induced a daily retention 0.31 ± 0.23 g and 75% of the subjects retained nitrogen; sugary-2 opaque-2 corn caused a positive balance of 0.40 ± 0.28 g and all values were within the range 0.77 to -0.02 g.

Reduction of nitrogen furnished by corn from 5.5 to 4.4 g per day increased urinary nitrogen (p < 0.01) although total nitrogen intake was unchanged. Urinary nitrogen due to opaque-2 corn increased from 3.71 ± 0.14 g to 4.06 ± 0.25 g, which approximated values of 4.13 ± 0.27 and 4.31 ± 0.11 g that resulted from 5.5 and 4.4 g, respectively, of nitrogen from normal corn. Urinary nitrogen resulting from sugary-2 opaque-2 corn also was increased to 4.06 ± 0.30 g by limiting corn to 4.4 g of nitrogen.
Nitrogen retention was essentially the same whether opaque-2 corn furnished 5.5 or 4.4 g of nitrogen in these isonitrogenous diets; but it was affected adversely when sugary-2 opaque-2 corn was reduced to 4.4 g and the balance of -0.04 ± 0.25 g was similar to that resulting from the normal corn. At an intake of 4.4 g of nitrogen from corn, variety did not have a significant effect on either urinary nitrogen or retention. However, 7 or 8 men were in positive balance when opaque-2 corn was fed but only half of them when the other corns were tested.
CLIMATOLOGICAL FACTORS IN MAIZE ADAPTATION

P. L. Crane, P. R. Goldsworthy, R. L. Cuany, M. S. Zuber and C. A. Francis

ABSTRACT

Data are presented from seven maize varieties and five hybrids representing U.S., Mexican, Caribbean and Colombian germplasm. Phenological and meteorological data from Madison, Wisconsin; Lafayette, Indiana; Portageville and Columbia, Missouri; Fort Collins and Rocky Ford, Colorado; CIMMYT’s stations El Batan, Tlalticapan and Posa Rica in Mexico; and at CIAT near Cali, Colombia. Four intervals of plant development were recorded: (1) Planting to floral initiation, (2) Floral initiation to mid-anthesis, (3) Mid-anthesis to mid-silking, and (4) Mid-silking to physiological maturity as indicated by "black-layer" formation. Heat accumulation units recorded or calculated were Days, Growing Degree Days (GDD), Effective Growing Degree Days (EGDD), Corn Heat Units, Solar Radiation (SR), and Net Radiation (NR). The coefficient of variability (c.v.) was used to measure the consistency of response of each strain of maize to the wide range of environments.

CHU’s were found to be the most nearly constant within varieties over environments. SR was intermediate and NR was the least consistent of the units compared.

The relationship of CHU’s from planting to anthesis with photoperiod was examined for Eto, Oaxaca 179, and A619 x A632. With observations from 52 environments, there was a slope of 77.8 CHU’s per additional hour of day-
length at the approximate date of floral initiation for Oaxaca 179, $r = 0.78$; 124.8 CHU's for Eto, $r = 0.76$; and 2.06 for A619 x A632, $r = 0.02$. Therefore, prediction of maturity adaptation based on CHU's would be limited to sites with similar day-length in the case of day-length sensitive strains.
COMPARATIVE SUSCEPTIBILITY TO AMYLASE OF STARCH GRANULES OF DOUBLE- AND TRIPLE-MUTANTS CONTAINING AMYLOSE-EXTENDER, WAXY, SUGARY-1, SUGARY-2 AND DULL GENE OF MAIZE INBRED Oh43 (Zea mays L.)

H. Fuwa, D. V. Glover and Y. Sugimoto

ABSTRACT

Starch granules were prepared from 15 double- and 26 triple-mutants containing amylose-extender (ae), 15 double- and 18 triple-mutants containing waxy (wx), 16 double- and 20 triple-mutants containing sugary-1 (su$_1$), 14 double- and 23 triple-mutants containing sugary-2 (su$_2$), and 15 double- and 20 triple-mutants containing dull (du) of maize inbred Oh43 (Zea mays L.) and the relative susceptibility to fungal glucoamylase of these starch granules were examined with the use of commercial normal maize starch granules as a control. Starch granules of the double- and triple-mutants containing su$_1$ and su$_2$ were digested two to eight times faster than those of the normal. The ae gene seems to be epistatic to su$_1$ and su$_2$. Starch granules of the double- and triple-mutants containing wx were digested about two times faster than those of the normal and those containing shrunken-2 (sh$_2$) were digested 1.2 to eight times faster than the normal. Starch granules of triple-mutant combinations with opaque-2 (o$_2$) were, in general, digested to the very comparable to those of their respective nonopaque double-mutant counterpart.
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.
ABSTRACT

This is a review of protein quality research in maize and an update on the state of progress in breeding for improved quality high-lysine maize. Results are reviewed in the following areas: (1) the selection for high-lysine types in normal maize; (2) improving the kernel characteristics of opaque-2 types through genetic modifiers and the development of double-recessive combinations with opaque-2; (3) the development of high-lysine source germplasm materials, and (4) to explore the yield potentials and variability among high-lysine opaque-2 germplasm developments and the relationship of protein to calorie production.
AMYLOSE-PERCENTAGE AND DISTRIBUTION OF UNIT CHAIN LENGTH OF MAIZE STARCHES WITH SPECIFIC GENETIC BACKGROUND

Y. Ikawa, D. V. Glover, Y. Sugimoto, and H. Fuwa

ABSTRACT

Starch granules prepared from kernels of the single mutants, amyllose-extender (ae), waxy (wx) and opaque-2 (o₂), and the double-mutant combinations ae o₂ and wx o₂ from four maize (Zea mays L.) inbred lines, Oh43, B37, C103 and W64A were fractionated with gel-filtration after debranching by isoamylase. The contents of each fraction and distribution of the linear α-1, 4-linked unit chains were investigated. There were no remarkable differences in above-mentioned criteria between single-mutants and their respective double-mutant combinations with o₂, and among four inbred lines. It was confirmed that starch granules of the ae mutants had intermediate fraction(s) different from typical amylose and amyllopectin.
MATURITY INTERACTION AND BLACK LAYER OCCURRENCE IN OPAQUE-2 AND NORMAL HYBRIDS IN CORN (ZEA MAYS L.)

D. Makonnen and L. F. Bauman

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 the B14 x B37 single cross the opaque-2 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 ranged from 9.7 to 11.8% greater in cob weight 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.
STUDIES ON CORN PROTEINS X. POLYPEPTIDE MOLECULAR 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 Oh43 and mutants o2, fl2, fl2o2, bt2 and bt2o2, as well as normal corn inbred W22 and its mutant o7, 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 bt2o2, 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 o2 and fl2o2 is reduced below that in normal, fl2 and o7. The 44,000 dalton component of Fraction II is a unique component of fl2 and fl2o2, as in the 14,000 dalton component of Fraction III in o2 and o7.
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% ethanol-soluble 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.
GENETIC AND ENVIRONMENTAL STABILITY OF MODIFIED OPAQUE-2 POPULATIONS

Navarro Ruiz De Las Cuevas, Alfredo Angel Domingo

ABSTRACT

Genetic and environmental stability was studied in the modified opaque-2 synthetics: HMO: High Lysine, HMO-Low Lysine, Temperate HA and Temperate HB. Two selections for modification were made on each, making eight final synthetic versions. On each version, random mating was simulated three times to give four successive generations. This research involved two experiments. The fourth generation from each version was planted at Evansville, IN; Tipton, IN; and Lafayette, IN in Experiment I. All four generations were included in Experiment II on which two planting dates were studied. A randomized complete-block design with three and four replications was used in Experiments I and II, respectively. Data was taken for (a) the plant characteristics ear height, percent lodged plants, and ear yield per plant and (b) the kernel characteristics percent modified kernels, modification index, weight and volume of 100 kernels, specific gravity, and percent protein and percent lysine whole kernel basis.

Locations affected percent modified kernels and modification index, with higher values at Tipton and Lafayette (locations with cooler temperatures). Kernel weight and volume as well as lysine content were higher at Lafayette. Location X population and planting date X synthetic interactions in Experiments I and II, respectively, were significant for both kernel weight and volume.
Selection for higher lysine content (in HMO's) was effective, and persisted throughout the subsequent random mating generations. This resulted in decreased protein content, frequency of modified kernels, modification index, and specific gravity, and increased kernel weight and volume.

Selection for higher modification was effective in increasing both percent modified kernels and modification index. Selection (for modification) X synthetics X generation interaction was significant. Throughout generations, a negative linear trend for percent modification and modification index was detected for the HMO's while values tended to be increased in TA-M and TB-M.

Selection for higher modification resulted in increased specific gravity and decreased kernel volume. Increased protein content and decreased kernel weight were detected in the HMO's as an effect of this selection. Lysine in protein was consistently decreased in all synthetics, but this effect was significant only in the Temperates.
NORMAL, OPAQUE-2, WAXY, WAXY OPAQUE-2, SUGARY-2 AND SUGARY-2 OPAQUE-2 MAIZE ENDOSPERM TYPES FOR RATS AND PIGS

Juan G. Rosa

ABSTRACT

The following three-way hybrid corns each near isogenic, opaque-2, waxy, waxy opaque-2, sugary-2, sugary-2 opaque-2 and their normal counterpart were compared. Their protein quality was compared in three experiments involving 396 rats and one with 64 growing pigs with isonitrogenous diets at suboptimal protein levels. Two experiments involving 110 rats and one with 36 pigs were conducted with protein adequate diets, with the corns compared on an equal weight basis. The results were analyzed as a factorial (three types of starch by two types of protein; presence or absence of the opaque-2 gene in the endosperm) arrangement of treatments.

At 8% crude protein (experiments 1 and 2), rats fed opaque corns showed 143 to 110% higher rate of gain and 68 to 61.5% superior feed efficiency compared to rats fed non-opaque corns. A significant interaction starch by protein types was obtained for feed efficiency. Rats fed sugary-2 corn were more efficient than those fed normal or waxy corns. The same corn types with the opaque-2 gene were not different. Apparent dry matter digestibility was 7.5% lower for opaque corns, however their biological value was 10.1% higher than for non-opaque corns (experiment 2). Higher digestible nitrogen was obtained for normal and waxy corns than for sugary-2 corns. Supplementation of the diets with lysine, tryptophan or both amino
acids (experiment 3) failed to eliminate the differences observed in experiments 1 and 2. Corns containing the opaque-2 gene, still supported greater rate of gain and improved feed efficiency, although the differences were not as great as without amino acid supplementation. With the corns supplemented with lysine, an interaction was obtained for rate of gain and feed efficiency. Rats fed sugary-2 corn showed higher rate of gain and improved feed efficiency than those fed waxy and normal corns, but no differences were obtained between their counterparts containing the opaque-2 gene. With pigs fed the corns supplemented with lysine and tryptophan, the improvement in feed efficiency was 10.5% but rate of gain was 15% lower than those pigs fed non-opaque corns (experiment 4). No differences were observed between pigs fed normal, waxy or sugary-2 corns.

At 11% crude protein (experiment 1), the improvements in rate of gain and feed efficiency obtained with the rats fed opaque-2 corns were smaller (23.5 and 10.2%, respectively) than at 8% crude protein. The differences observed at 8% crude protein between normal, waxy and sugary-2 corns were no longer observed.

At 14% crude protein (experiment 1), none of the parameters measured showed differences for the six corn endosperm types.

No differences in rat or pig performance were obtained among the six corns fed in protein adequate diets (experiments 5, 6 and 7). However, in experiment 7, interactions in the digestion coefficients for dry matter, nitrogen, energy and ether extract were obtained. Opaque-2 corn showed the lowest coefficients, but waxy opaque-2 and sugary-2 opaque-2 corns showed similar coefficients as normal, waxy and sugary-2 corns.
The results from these experiments showed the superior protein quality of opaque-2 corn compared to normal corn. Sugary-2 corn was also found to contain superior protein quality, but not as good as opaque-2 corn. The double mutants, waxy opaque-2 and sugary-2 opaque-2 were similar to the opaque-2 corn. These results suggest that it is possible to combine the other mutant genes into the endosperm with the opaque-2 gene, while maintaining its biological value.
NORMAL, OPAQUE-2, WAXY, WAXY OPAQUE-2, SUGARY-2 AND SUGARY-2 OPAQUE-2 CORN (ZEA MAYS L.) ENDOSPERM TYPES FOR RATS AND PIGS. STUDIES ON PROTEIN QUALITY

Juan G. Rosa, Dale M. Forsyth, David V. Glover and T. R. Cline

ABSTRACT

One metabolism and two growth trials involving 432 rats and one trial with 64 pigs were conducted to evaluate the protein quality of six near isogenic versions of the maize 3-way hybrid [(Oh43 x B37) x C103)] in protein-limiting diets. In 8% crude protein diets, rats fed opaque maize types (opaque-2, waxy opaque-2 and sugary-2 opaque-2) gained 143% faster and were 68% more efficient in converting feed to gain than rats fed non-opaque maize types (normal, waxy and sugary-2). These improvements were accompanied by a 5.5% lower dry matter digestibility, but a 10.5% improvement in biological value for opaque compared with non-opaque corns. Supplementation of the diets with lysine, tryptophan or both amino acids failed to eliminate the differences observed; diets containing opaque corns still supported higher rate of gain and improved feed efficiency as compared with those rats fed non-opaque corns. Pigs fed diets containing non-opaque corns supplemented with lysine and tryptophan showed 10.5% inferior feed efficiency but 15% higher rate of gain than those fed opaque corns. In 11% crude protein diets, the differences observed in rat performance were smaller; at 14% protein, no differences were observed. It was found that higher contents of lysine and tryptophan in opaque maize are not the only reasons for its superiority.
Sugary-2 was found to be superior to normal corn; however, its improvement was less than for opaque-2. These experiments indicated that the double mutants waxy opaque-2 and sugary-2 opaque-2 are similar to opaque-2 in protein quality.
NORMAL, OPAQUE-2, WAXY, WAXY OPAQUE-2, SUGARY-2 AND SUGARY-2 OPAQUE-2 CORN (ZEA MAYS L.) ENDOSEPM TYPES FOR RATS AND PIGS. STUDIES ON ENERGY UTILIZATION

Juan G. Rosa, Dale M. Forsyth, David V. Glover and T. R. Cline

ABSTRACT

One digestion, one metabolism and two growth trials involving 140 rats and one trial with 36 growing pigs were conducted to compare six near isogenic versions of the maize 3-way hybrid [(0h43 x B37) x CI03] fed in protein adequate diets. No differences in rat or pig performance were obtained comparing three endosperm starch types: waxy (100% amyllopectin), sugary-2 (40% amylose, 60% amyllopectin) and normal (26% amylose, 74% amyllopectin). It was found that opaque-2, sugary-2 opaque-2, and sugary-2 corns contained from 3.0 to 3.3% lower digestible energy than normal corn for rats. In addition, all corns with the opaque-2 gene contained, on the average, 2.65% lower digestible nitrogen than non-opaque types. With pigs it was found that the double mutants sugary-2 opaque-2 and waxy opaque-2 did not show lower apparent digestion coefficients than normal corn as did opaque-2 corn. These experiments indicated that energy utilization in waxy, sugary-2 and double mutants with the opaque-2 gene is as good as for normal corn for growing pigs.
MUTANT HYBRID CORNS PLUS LYSINE AND TRYPTOPHAN FOR PIGS AND RATS

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ABSTRACT

Growth trials were conducted with 180 rats and 64 pigs to study the effect of lysine (Lys) and tryptophan (Try) supplementation of three-way hybrid, normal (n), opaque-2 (o2), waxy (w), waxy opaque-2 (wxo2), sugary-2 (s2) and sugary-2 opaque-2 (s2o2) corns in near isogenic backgrounds. With rats, the diets contained 8% protein and were supplemented with Lys and/or Try to equal levels. With pigs, the diets were similar to the rat diets with both amino acids, and a positive and negative control were included. Rats fed corns with the opaque gene gained faster and more efficiently (p < .01) than the others when supplemented with Try, Lys or both; the differences were 108, 60 and 20% for rate of gain and 76, 36 and 8% for F/G, respectively. Rats fed waxy corns gained slower when supplemented with Try alone. Those fed s2 out-performed those fed w or n when supplemented with Lys. When both amino acids were supplemented, sugary-2 corn types produced superior F/G compared to the others.

Performance of pigs fed the six corn types ranked between the positive and negative control and were not different (p > .05). These experiments indicated that, for rats, the improvement in protein quality of o2, s2o2, and wxo2 is due to more than Lys and Try, and that the superior quality of o2 corn is maintained in the double mutants with different starch structure.
DIFFERENT ENDOSPERM TYPE CORNS IN PROTEIN ADEQUATE DIETS FOR PIGS


ABSTRACT

A growth and digestion trial involving 36 growing pigs was conducted to study the nutritional quality of the starch of three-way hybrid (1) normal (n), (2) opaque-2 (o2), (3) waxy (wx), (4) waxy opaque-2 (wxo2), (5) sugary-2 (su2) and (6) sugary-2 opaque-2 (su2o2) corns derived from near isogenic conversions of the same hybrid. The 12 kg pigs were fed the six (2 x 3 factorial arrangement) protein adequate diets ad-libitum for 31 days. The soybean meal level was held constant in all diets and all diets were fortified with vitamins and minerals. The results (not significantly different) for rate of gain (Kg/day), and feed/gain were: .53, 2.51; .52, 2.41; .52, 2.32; .54, 2.35; .55, 2.31 and .53, 2.23 for diets 1 through 6, respectively. Chromic oxide was added to the diets from day 25 and feces were collected from each pig twice a day on days 29, 30 and 31. No significant digestibility differences were found due to the presence of o2 gene or to the starch type, but a significant o2 gene by starch type interaction was obtained due to the relatively lower apparent dry matter (DM), energy (DE) and nitrogen (DN) digestibilities for pigs fed o2. The digestibility coefficients for DM, DE and DN were: 81.8, 75.9, 80.4; 77.7, 72.3, 75.9; 78.9, 71.9, 77.2; 82.5, 79.9, 80.9; 79.6, 76.0, 78.5 and 81.2, 76.0, 80.0 for diets 1 through 6 respectively. It is concluded that the structure of starch in these corns does not alter the energy available for optimum performance of growing pigs.
NUTRITIONAL VALUE OF NEW ENDOSPERM TYPES OF CORN FOR GROWING-FINISHING BEEF CATTLE

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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.
DIRECT APPLICATION OF THE NINHYDRIN REAGENT SOLUTION TO EXPOSED MAIZE ENDOSPERM FOR PROTEIN MUTANT SCREENING

Hugo Leon Zorrilla

ABSTRACT

The "ninhydrin reagent solution" chemically reacts with the free amino acids present in the exposed maize endosperm rendering a blue color. This study utilized that reaction to search for new mutants and also tested the effectiveness of the use of "ninhydrin reagent solution" applied directly to exposed maize endosperm for protein mutant screening by the use of known (opaque-7) material.

In the search for new mutants, 300 kernels each of 55 collections of maize were tested. In general, there was a lack of ninhydrin penetration on vitreous kernels, but there were exceptions. Sources with floury endosperm type, or a soft crown, or "cap" showed good ninhydrin penetration. Lysine content of positive reacting kernels was at the normal level, but protein showed a tendency to higher levels.

In the test of this ninhydrin technique, opaque-7 ears at different moisture levels were tested and homozygous opaque-7 testers were used to verify the presence of the opaque-7 gene. A very weak ninhydrin reaction on exposed maize endosperm of opaque-7 ears was generally observed at moisture levels above 35 percent. Usually differentiation between normal and opaque-7 kernels was observed at any moisture level below 30 percent.
Some of the numerous exceptions in this experiment were apparently due to a variety of modifier genes not only masking the opaque-7 phenotypic expression in varying degrees, but suppressing its protein altering functions too, sometimes resulting in normal levels of free amino acids and lysine. The potential effectiveness of the ninhydrin test in locating any new high-lysine mutant would be subjected to any interaction between that mutant gene and its genetic background.