**BIBLIOGRAPHIC INPUT SHEET**

<table>
<thead>
<tr>
<th>A. PRIMARY SERIALS</th>
<th>Y-AF30-0120-0000</th>
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<tr>
<td>B. SECONDARY</td>
<td>Food production and nutrition--Plant breeding--Cereals--Maize</td>
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**1. SUBJECT CLASSIFICATION**

<table>
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</table>

**2. TITLE AND SUBTITLE**

Inheritance and improvement of protein quality and content in maize; annual report, 1975/1976

**3. AUTHOR(S)**

Purdue Univ. Dept. of Agronomy

**4. DOCUMENT DATE**

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April 1, 1975 to March 31, 1976
Contract AID/ta-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/ta-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. 1. Project title and contract number: Inheritance and Improvement of Protein Quality and Content in Maize; Contract number AID/ta-C-1211.

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


5. Total A.I.D. funding of contract to date: $250,000.00.
   (to March 31, 1976).

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

7. Total expenditures and obligations for current year: $246,336.57.


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 second group of 30 collections from the germplasm bank at CIMMYT has been screened for potential higher lysine with the ninhydrin test. In over half of the collections tested, a few positive reacting kernels were found. Studies are in progress using chemical mutagen treatments for potential upgrading of protein quality and content. Selection studies in two maize varieties adapted to the temperate areas has shown limited effectiveness of this approach in selecting for protein quality in these normal populations.

Modified (vitreous) opaque-2 varieties can be selected with good yield (not equal to normal), resistance to ear rots and grain insects and good protein quality. Random mating modified and non-modified opaque-2 populations for four generations without selection resulted in little or no change in nutritional value and the degree of modification was maintained. This indicates that modified opaque-2 varieties (except for outcrossing to normal) will tend to retain their desirable characteristics when grown by the farmer for successive generations.

During the past year we have identified a simply inherited ("single") gene that modifies the soft endosperm of opaque-2 giving it a more vitreous (normal) appearance without a serious reduction in lysine levels. This
would greatly simplify 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.

Feeding experiments with weanling rats using selections with high and low protein with high and low lysine in modified opaque-2 germplasm confirmed that with selection one could breed for modified phenotypes without losing superior protein quality and biological value of the opaque-2 phenotype. Protein quantity to some extent compensated for lower protein quality.

Isogenic line recoveries of the double mutant sugary-2 opaque-2 in several inbreds are being developed. Line recoveries with higher levels of protein are being selected. Though the sugary-2 opaque-2 materials have improved kernel vitreousness, kernel density, ear rot resistance, excellent protein quality, improved digestibility characteristics and biological value, like opaque-2, these materials suffer from reduced yields compared to their normal counterparts. Developmental studies have shown that dry matter accumulation is restricted compared to normal beginning about 38 days after pollination in these mutant systems. There are some gene dosage effects with sugary-2, particularly in oil content; and sugary-2 increases percent oil in both low and high oil lines. Further developmental studies are in progress to investigate the compensating effects in endosperm metabolism and constraints on dry matter accumulation. This is an extremely important area of investigation in order to enhance our progress in improving nutritional quality and grain production.

Two varieties adapted to the more temperate areas are being 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.

A growth and digestion trial with pigs to evaluate the biological value of normal, opaque-2, sugary-2, sugary-2 opaque-2, waxy, and waxy opaque-2 isogenic hybrid corns suggested that the structure of starch in these corns does not alter the energy available for optimum performance of growing pigs. Growth trials using the same corns plus lysine and tryptophan for pigs and rats showed rats fed corns with the opaque gene gained faster and more efficiently than others when supplemented with Try, Lys, or both. When both amino acids were supplemented, sugary-2 corn types produced superior feed gain ratios compared to the others. Performance of pigs fed the six corn types ranked between the positive and negative control and were not different.

A characterization of the polypeptides in the endosperm of normal, opaque-2 and double mutants with opaque-2 has indicated differences between zein and zein-like polypeptides of these mutants. An investigation of biosynthesis of lysine in normal and its opaque-2 counterpart during germination suggested that the lysine biosynthetic pathway is under feedback control in both systems.

Research information has been distributed through workshops, seminars, journals, publications, and annual reports. Graduate student and post-doctorate 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. Husun Tu* (Graduate Student)
Mr. Richard C. Ade** (Technician)
Mr. Terry R. Lemming** (Technician)

Dr. P. L. Crane
Mr. Hugo Zorrilla (Graduate Student)
Mr. Weyman P. Fussell* (Graduate Student)
Mr. H. Keith Kessler** (Technician)
Miss Nora D'Croz (Graduate Student-Technician)

Dr. D. V. Glover
Dr. James B. Barnett (Postdoctorate)
Mr. P. Stephen Baenziger (Graduate Student)
Mr. Steve G. Ballinger (Graduate Student)
Mr. Arthur H. Long* (Graduate Student)
Mr. Theron E. Roundy* (Graduate Student)
Mr. Richard S. Burns (Technician)
Mr. Wayne J. Whitlow** (Technician)
Mr. Terry R. Lemming** (Technician)
Miss Charlotte Allen** (Technician)

Department of Biochemistry

Dr. E. T. Mertz
Dr. Bakshy A. K. Chibber (Postdoctorate)
Mr. Mohammed M. Hassen (Technician)
Mrs. Gina H. Y. Lu (Technician)
Mr. John Tomich (Technician)
Mrs. Ecaterina Voicu (CIMMYT - UNDP Trainee)

*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
Mr. Juan C. 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. We now know of the 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 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 calories and protein 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.

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 a simply inherited gene that gives 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. This system 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 sizable 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.

Evaluation of the Ninhydrin technique to non-destructively test normal-looking vitreous kernels is continuing. The purpose is to screen for the possible occurrence of a new high-lysine mutant without the undesirable side effects on kernel texture and yield. The second group of 30 collections from the germplasm bank at CIMMYT has been tested. In over half of the collections tested, a few positive reacting kernels were found among the 300 kernels tested. These have not yet been further analysed. The selections from the initial group were increased by Dr. S. K. Vasal of CIMMYT and returned to us. Results of analyses from this second generation are not yet available.

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 will be intercrossed and further cycles of selection carried out. Preliminary data indicate limited effectiveness of this approach.
Table 1. Variation in protein quality in endosperm from 100 normal families (ears) each in Temp HA and Temp HB.

<table>
<thead>
<tr>
<th></th>
<th>Temp HA</th>
<th>Temp HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Protein</td>
<td>Mean</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>7.3-14.6</td>
</tr>
<tr>
<td>Lysine as a Percent of Protein</td>
<td>Mean</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.26-2.28</td>
</tr>
<tr>
<td>Lysine as a Percent of Sample</td>
<td>Mean</td>
<td>.187</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>.150-.238</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 HAO2 and Temp HBO2, 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 Mexico, Israel and Indiana. Percent protein and lysine as a percent of protein at the Indiana location were for Temp HA 10.2 to 12.9% and 3.21 to 4.76% and for Temp HB 9.0 to 12.5% and 3.01 to 4.80%, respectively. 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.

To further fill the need for varieties adapted to temperate areas, the variety Colus developed at Purdue from Colombian and U.S. germplasm has been converted to opaque-2 and is undergoing selection. Ranges in protein and lysine as a percent of protein for Colus Cycle 1 self-pollinated one generation were 9.4 to 15.7% and 3.63 to 5.07% with mean values of 12.3 and 4.28%, respectively.

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 \( su_2 o_2 \) and Temp HB \( su_2 o_2 \) is in progress. Full-sib family selection and evaluation of 270 families based on yield and protein quality was completed for cycle 1 in the 1975 summer nursery for both populations. Ranges in protein and lysine as a percent of protein for Temp HA \( su_2 o_2 \ C_1 S_1 \) (cycle 1, selfed one generation) were 8.5 to 16.2\% and 3.53 to 5.76\% with mean values of 12.5 and 4.29\%, respectively. Ranges in protein and lysine as a percent of protein for Temp HB \( su_2 o_2 \ C_1 S_1 \) were 10.4 to 16.1\% and 3.57 to 5.54\% with mean values of 13.05 and 4.30\%, respectively. Based on yield, kernel vitreousness, and quality full-sibs were made in each variety in the 1975-76 winter nursery. These will be yield tested and full-sib families will be selfed in the 1976 summer nursery. Full sib family selection will continue.

A parallel study using half sib family selection in the temp HB \( su_2 o_2 \) "highly modified opaque-2" material is in progress. Cycle 1 was completed in 1975. Protein and lysine as a percent of protein ranged from 9.7 to 18.0\% and 2.79 to 4.87\%, respectively with respective mean values of 13.11 and 4.01\% in the \( C_1 S_1 \) population. Based on kernel vitreousness and quality, half sibs (\( C_2 \)) were made in the 1975-76 winter nursery. These will be yield tested and half-sib families will be selfed in the 1976 summer nursery.

The waxy opaque-2 (\( wx o_2 \)) and brittle-2 opaque-2 (\( bt_2 o_2 \)) 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 \( wx o_2 \) populations have been advanced to cycle 2, and the \( bt_2 o_2 \) populations have been advanced through cycle 1 in the 1975 summer nursery. One season was lost with the \( bt_2 o_2 \) due to dryer difficulties following harvest of the 1974 materials.

**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, Bl4, and B37 inbred sources recovered from crosses to the Illinois High Protein (IHP) material. Most of these materials were advanced to backcross 5 during the 1975 summer nursery season. Protein values in the backcross four selfed on generation IHP recoveries from the 1975 Oh43 and B37 lines, averaged around 1.25 to 2.5 percentage points higher than the non-recurrent parents. However, in the Bl4 lines the increase in protein percent was not as great and ranged from 0 to 1 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 Bl4 inbred sources recovered from crosses to IHP material. These materials were advanced to backcross 4 in the 1975 summer nursery. Again the Oh43 IHP line recoveries averaged 2 percentage points greater in protein percent over the non-recurrent parent control while the Bl4 IHP line recoveries were not greater than its respective non-recurrent parentage.

Backcross 3 recoveries of IHP Oh43, Bl4 and B37 recoveries of opaque-2 and sugary-2 opaque-2 were test crossed to $su_{2}o_{2}$ single cross testers and the progenies of respective normal, opaque-2 and sugary-2 opaque-2 genotypes were grown in replicated nurseries in isolation. Mean protein percent over all IHP line recoveries were 11.66, 11.44, and 11.49% for the normal, opaque-2 and sugary-2 opaque-2 genotypes respectively. The mean protein percent for a commercial normal and opaque-2 counterpart hybrid was 8.9 and 9.8% in the same trial.

Multiple Aleurone Sugary-2 Opaque-2 Conversions.

The project is studying the feasibility of increasing the proportion of aleurone tissue relative to the starch portions of the endosperm in an
additional effort to improve the protein nutritional quality. In the 1975 nursery the backcross 3 and 4 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 $o_2$, $su_2$ and $su_2o_2$ with 2 and 3 aleurone layers and are continuing to select for increased layer number in the conversion program. Preliminary early generation recoveries of a few selected inbreds were intercrossed in the 1975-76 winter nursery and will be evaluated for inheritance of stability of the aleurone number and for protein content in the 1976 summer nursery in replicated trials.

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

Seven opaque-2 sources (mutational events) have been collected and are available for distribution to provide nonrelated or diverse sources of this gene. These sources have been backcrossed (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 mutational sources might be expected. The seven opaque-2 sources are being evaluated as inbreds, intercrosses with inbred backgrounds and single crosses among inbred backgrounds. These are being evaluated for agronomic and nutritional characteristics in the 1976 summer nursery.

Independent mutant sources of sugary-2 ($su_{2}^{std}$, $su_{2}^{i}$, $su_{2}^{PI}$, $su_{2}^{p}$) are being backcrossed into common inbred backgrounds Oh43, W64A, B37, C103, C123 and A632. Backcross conversions are complete to BC 6 and 7 generations for the sugary-2 standard allele, and the other $su_{2}$ alleles were recovered to backcross 2 and 3 in the 1975-76 winter nursery. These mutant sources will be evaluated in advanced recoveries in intercrosses within inbreds, as inbreds, and intercrosses among inbred backgrounds.
Genetic and Environmental Stability of Modified and Nonmodified Synthetics with Varying Levels of Lysine.

Genetic and environmental stability was studied in several opaque-2 synthetics. High and low lysine versions of HMO were selected for modification, and Temperate A and Temperate B synthetics were selected for both opacity and modification giving eight different synthetic versions. On each of these synthetics, random mating was simulated three times to give four generations (See Annual Report, April 1, 1974-March 31, 1975). These synthetics were studied in two different experiments in the summer of 1975.

In Experiment I (Tables 2-4), the fourth generation from each synthetic was planted at three locations: Evansville, Tipton, and Lafayette (all in Indiana). In Experiment II two different planting dates were studied, with all four generations included of each of the eight synthetics (Tables 5 and 6).

A randomized complete-block design was used in both experiments, with three replications in Experiment I and four replications in Experiment II. In Experiment II HMO-LL, HMO-HL, TA and TB were considered as whole units, selections as sub-units, and generations as sub-sub-units. Plant characteristics measured were ear height, percent lodged plants, and ear yield per plant. Kernel characteristics measured were percent modified kernels, modification index, weight and volume of 100 kernels, specific gravity, and percent protein and percent lysine in protein on a whole kernel basis. The following conclusions were made:

1. A location effect was found for percent modified kernels and modification index. Values were higher at Tipton and Lafayette, where cooler temperatures prevailed. Kernel weight and volume and lysine content were higher at Lafayette than at the other two locations.

2. The interactions location x population (Experiment I), and planting date x synthetic (Experiment II) were significant for both kernel weight
Table 2. Analyses of variance of percent modified kernels, modification index, weight and volume of 100 kernels, kernel specific gravity, and percent protein and percent lysine in protein whole kernel basis (Experiment I).

<table>
<thead>
<tr>
<th>Sources</th>
<th>D.F.</th>
<th>Modified</th>
<th>100 Kernel</th>
<th>Protein</th>
<th>Lysine (g/100 g Pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>Weight (g)</td>
<td>Volume (ml)</td>
<td>Specific Gravity (g/ml)</td>
</tr>
<tr>
<td>Locations</td>
<td>2</td>
<td>611.73**</td>
<td>50.558**</td>
<td>26.76**</td>
<td>0.0057**</td>
</tr>
<tr>
<td>Blocks/locat.</td>
<td>6</td>
<td>127.18*</td>
<td>0.510</td>
<td>0.41</td>
<td>0.0004</td>
</tr>
<tr>
<td>Populations</td>
<td>9</td>
<td>6364.57**</td>
<td>83.429**</td>
<td>104.75**</td>
<td>0.0227**</td>
</tr>
<tr>
<td>Locat. x Popul.</td>
<td>18</td>
<td>29.49</td>
<td>4.355**</td>
<td>3.31*</td>
<td>0.0006</td>
</tr>
<tr>
<td>Error</td>
<td>54</td>
<td>40.88</td>
<td>1.700</td>
<td>1.45</td>
<td>0.0004</td>
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<tr>
<td>Coef. of Variation</td>
<td></td>
<td></td>
<td>13.32%</td>
<td>15.81%</td>
<td>5.05%</td>
</tr>
</tbody>
</table>

*, ** Significant at the 0.05 and 0.01 levels, respectively.

Table 3. Percent modified kernels, modification index, weight and volume of 100 kernels, kernel specific gravity, and percent protein and percent lysine in protein whole kernel basis for locations (Experiment I).

<table>
<thead>
<tr>
<th>Location</th>
<th>Modified</th>
<th>100 Kernel</th>
<th>Protein</th>
<th>Lysine (g/100 g Pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Weight (g)</td>
<td>Volume (ml)</td>
<td>Specific Gravity (g/ml)</td>
</tr>
<tr>
<td>Evansville</td>
<td>42.93a</td>
<td>24.84a</td>
<td>21.33a</td>
<td>1.1681a</td>
</tr>
<tr>
<td>Tipton</td>
<td>51.60b</td>
<td>25.98b</td>
<td>21.93b</td>
<td>1.1875b</td>
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<tr>
<td>Lafayette</td>
<td>49.47b</td>
<td>26.66c</td>
<td>22.67c</td>
<td>1.1794b</td>
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<tr>
<td>LSD</td>
<td>3.98</td>
<td>0.57</td>
<td>0.53</td>
<td>0.0088</td>
</tr>
</tbody>
</table>

†Values with the same letter were not different with Tukey's procedure at p = 0.05.
Table 4. Percent modified kernels, modification index, weight and volume of 100 kernels, kernel specific gravity, and percent protein and percent lysine in protein whole kernel basis of populations (Experiment I).†

<table>
<thead>
<tr>
<th>Population</th>
<th>Modified</th>
<th>Modification Index</th>
<th>Weight (g)</th>
<th>Volume (ml)</th>
<th>Specific Gravity (g/ml)</th>
<th>Protein %</th>
<th>Lysine (g/100 g Pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMO-LLN</td>
<td>73.89f</td>
<td>1.592f</td>
<td>25.58c</td>
<td>21.11bc</td>
<td>1.2121cd</td>
<td>11.20bc</td>
<td>3.84abc</td>
</tr>
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<td>HMO-LLM</td>
<td>84.78g</td>
<td>1.913g</td>
<td>22.44a</td>
<td>18.25a</td>
<td>1.2293d</td>
<td>11.52cd</td>
<td>3.67a</td>
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<td>46.00c</td>
<td>0.874c</td>
<td>25.07bc</td>
<td>21.33bcd</td>
<td>1.1753b</td>
<td>10.60a</td>
<td>4.01abc</td>
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<td>HMO-HLM</td>
<td>59.33de</td>
<td>1.234de</td>
<td>23.32ab</td>
<td>19.50ab</td>
<td>1.1959bc</td>
<td>10.66a</td>
<td>3.90abc</td>
</tr>
<tr>
<td>TA-O</td>
<td>20.00b</td>
<td>0.344b</td>
<td>26.46c</td>
<td>23.17de</td>
<td>1.1420a</td>
<td>11.49bcd</td>
<td>4.03bc</td>
</tr>
<tr>
<td>TA-M</td>
<td>50.00cd</td>
<td>1.037cd</td>
<td>26.95c</td>
<td>22.78cde</td>
<td>1.1839bc</td>
<td>11.30bc</td>
<td>3.92abc</td>
</tr>
<tr>
<td>TB-O</td>
<td>17.33b</td>
<td>0.283b</td>
<td>26.55c</td>
<td>23.42e</td>
<td>1.1346a</td>
<td>11.53cd</td>
<td>4.17c</td>
</tr>
<tr>
<td>TB-M</td>
<td>61.78e</td>
<td>1.349e</td>
<td>25.35c</td>
<td>21.00bc</td>
<td>1.2073cd</td>
<td>11.86d</td>
<td>3.73ab</td>
</tr>
<tr>
<td>CH-1</td>
<td>64.00ef</td>
<td>1.141de</td>
<td>26.14c</td>
<td>22.14cde</td>
<td>1.1810bc</td>
<td>10.98ab</td>
<td>3.97abc</td>
</tr>
<tr>
<td>CH-2</td>
<td>2.89a</td>
<td>0.037a</td>
<td>30.39d</td>
<td>27.08f</td>
<td>1.1218a</td>
<td>11.21bc</td>
<td>4.18c</td>
</tr>
<tr>
<td>LSD</td>
<td>9.97</td>
<td>0.241</td>
<td>1.99</td>
<td>1.88</td>
<td>0.0314</td>
<td>0.53</td>
<td>0.35</td>
</tr>
</tbody>
</table>

†Values with the same letter were not different with Tukey's procedure at p = 0.05.
Table 5. Percent modified kernels, modification index, percent protein on whole kernel basis, and percent lysine in protein (Experiment II).

<table>
<thead>
<tr>
<th>Synthetic Generation</th>
<th>Modified</th>
<th>Protein</th>
<th>Lysine (g/100 g Pro.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMO-LLN 1</td>
<td>79</td>
<td>1.78</td>
<td>11.80</td>
</tr>
<tr>
<td>HMO-LLN 2</td>
<td>75</td>
<td>1.66</td>
<td>11.65</td>
</tr>
<tr>
<td>HMO-LLN 3</td>
<td>66</td>
<td>1.40</td>
<td>11.70</td>
</tr>
<tr>
<td>HMO-LLN 4</td>
<td>60</td>
<td>1.29</td>
<td>11.08</td>
</tr>
<tr>
<td>HMO-LLM 1</td>
<td>80</td>
<td>1.85</td>
<td>11.58</td>
</tr>
<tr>
<td>HMO-LLM 2</td>
<td>83</td>
<td>1.89</td>
<td>11.80</td>
</tr>
<tr>
<td>HMO-LLM 3</td>
<td>70</td>
<td>1.57</td>
<td>11.65</td>
</tr>
<tr>
<td>HMO-LLM 4</td>
<td>77</td>
<td>1.78</td>
<td>11.88</td>
</tr>
<tr>
<td>HMO-HLN 1</td>
<td>48</td>
<td>0.91</td>
<td>10.23</td>
</tr>
<tr>
<td>HMO-HLN 2</td>
<td>48</td>
<td>0.86</td>
<td>10.53</td>
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<td>HMO-HLN 3</td>
<td>40</td>
<td>0.76</td>
<td>10.70</td>
</tr>
<tr>
<td>HMO-HLN 4</td>
<td>39</td>
<td>0.67</td>
<td>10.30</td>
</tr>
<tr>
<td>HMO-HLM 1</td>
<td>65</td>
<td>1.31</td>
<td>10.68</td>
</tr>
<tr>
<td>HMO-HLM 2</td>
<td>56</td>
<td>0.93</td>
<td>10.58</td>
</tr>
<tr>
<td>HMO-HLM 3</td>
<td>55</td>
<td>1.07</td>
<td>10.45</td>
</tr>
<tr>
<td>HMO-HLM 4</td>
<td>49</td>
<td>0.92</td>
<td>10.65</td>
</tr>
<tr>
<td>TA-O 1</td>
<td>13</td>
<td>0.25</td>
<td>11.88</td>
</tr>
<tr>
<td>TA-O 2</td>
<td>9</td>
<td>0.14</td>
<td>11.48</td>
</tr>
<tr>
<td>TA-O 3</td>
<td>13</td>
<td>0.20</td>
<td>11.60</td>
</tr>
<tr>
<td>TA-O 4</td>
<td>14</td>
<td>0.23</td>
<td>12.00</td>
</tr>
<tr>
<td>TA-M 1</td>
<td>28</td>
<td>0.54</td>
<td>11.78</td>
</tr>
<tr>
<td>TA-M 2</td>
<td>34</td>
<td>0.71</td>
<td>11.65</td>
</tr>
<tr>
<td>TA-M 3</td>
<td>48</td>
<td>0.98</td>
<td>11.68</td>
</tr>
<tr>
<td>TA-M 4</td>
<td>43</td>
<td>0.76</td>
<td>11.93</td>
</tr>
<tr>
<td>TB-O 1</td>
<td>28</td>
<td>0.52</td>
<td>11.68</td>
</tr>
<tr>
<td>TB-O 2</td>
<td>16</td>
<td>0.26</td>
<td>11.38</td>
</tr>
<tr>
<td>TB-O 3</td>
<td>11</td>
<td>0.17</td>
<td>11.48</td>
</tr>
<tr>
<td>TB-O 4</td>
<td>10</td>
<td>0.17</td>
<td>11.58</td>
</tr>
<tr>
<td>TB-M 1</td>
<td>40</td>
<td>0.84</td>
<td>11.53</td>
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<td>11.50</td>
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<tr>
<td>TB-M 3</td>
<td>57</td>
<td>1.21</td>
<td>11.60</td>
</tr>
<tr>
<td>TB-M 4</td>
<td>64</td>
<td>1.43</td>
<td>11.58</td>
</tr>
</tbody>
</table>

| X        | 46 | 0.94 | 11.36 | 4.03 |
Table 6. Percent modified kernels, modification index, weight and volume of 100 kernels, kernel specific gravity, and percent protein and percent lysine in protein whole kernel basis for selections and generations (Experiment II).†

<table>
<thead>
<tr>
<th>Modified %</th>
<th>Index</th>
<th>100 Kernel</th>
<th>Weight (g)</th>
<th>Volume (ml)</th>
<th>Specific Gravity (g/ml)</th>
<th>Protein %</th>
<th>Lysine (g/100 g Pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SELECTIONS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N + O</td>
<td>35.50a</td>
<td>0.705a</td>
<td>25.94b</td>
<td>22.30b</td>
<td>1.165a</td>
<td>11.31a</td>
<td>4.12b</td>
</tr>
<tr>
<td>M + M</td>
<td>56.55b</td>
<td>1.183b</td>
<td>24.84a</td>
<td>20.75a</td>
<td>1.197b</td>
<td>11.40b</td>
<td>3.93a</td>
</tr>
<tr>
<td>LSD</td>
<td>2.21</td>
<td>0.069</td>
<td>0.57</td>
<td>0.44</td>
<td>0.005</td>
<td>0.08</td>
<td>0.04</td>
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<tr>
<td>GENERATIONS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>47.63a</td>
<td>1.000b</td>
<td>25.55a</td>
<td>21.55a</td>
<td>1.185a</td>
<td>11.39a</td>
<td>4.02a</td>
</tr>
<tr>
<td>2</td>
<td>47.19a</td>
<td>0.954ab</td>
<td>25.39a</td>
<td>21.52a</td>
<td>1.182a</td>
<td>11.32a</td>
<td>4.02a</td>
</tr>
<tr>
<td>3</td>
<td>44.94a</td>
<td>0.918ab</td>
<td>25.39a</td>
<td>21.57a</td>
<td>1.180a</td>
<td>11.36a</td>
<td>4.07a</td>
</tr>
<tr>
<td>4</td>
<td>44.34a</td>
<td>0.904a</td>
<td>25.24a</td>
<td>21.46a</td>
<td>1.178a</td>
<td>11.37a</td>
<td>4.02a</td>
</tr>
<tr>
<td>LSD</td>
<td>3.91</td>
<td>0.084</td>
<td>0.68</td>
<td>0.54</td>
<td>0.010</td>
<td>0.39</td>
<td>0.11</td>
</tr>
</tbody>
</table>

†Values with the same letter were not different with Tukey's procedure at p = 0.05.
and volume.

3. Selection for higher lysine content in HMO synthetics was effective, and this difference was constant throughout the subsequent random mating generations. Lysine values were also constant through these generations.

4. Selection for higher lysine content resulted in decreased protein content, frequency of modified kernels, modification index, and specific gravity, and increased kernel volume.

5. Selection for higher modification was effective in increasing both percent of modified kernels and modification index in all synthetics. Values for both characteristics tended to decrease in the subsequent random mating generations (Experiment II) in HMO's, and tended to increase in TA-M and TB-M.

6. Selection for higher modification resulted in increased specific gravity and decreased kernel volume in all synthetics. Protein content was increased in the HMO's. In the Temperate A and B synthetics the effect of this selection on protein content interacted with planting dates. Kernel weight was decreased as an effect of selection for high modification only in the HMO synthetics.

7. Lysine in protein was decreased as an effect of selection for higher modification and the change was larger in the Temperate A and B synthetics.
Feeding Experiments with High and Low Lysine with High and Low Protein in Modified Opaque-2 Germplasm.

Modified and opaque selections with varying levels of protein and lysine have been developed (see Annual Report April 1, 1974-March 31, 1975). These were increased for studies on environmental and genetic stability and for feeding trials with rats.

Among segregating modified opaque-2 maize, one generally observes an inverse relationship between values for g. protein/100 g. sample and g. lysine /100 g. protein. Considering these two values, two classes of materials may be selected: Class 1 made up of samples with less protein per unit of sample with more lysine in the protein (LP-HL); and, class 2 made up of samples with more protein per unit of sample but with less lysine in protein (HP-LL). Out of these materials, samples containing almost the same absolute amount of lysine/100 g. of sample may be selected.

A feeding experiment with rats to determine the biological value of some available materials falling into class 1 and class 2 was conducted. Maize samples used were \( S_2 \) families taken out of the high and low (modified selections) lysine intercrosses developed from HMO-opaque-2 synthetic. Two \( S_2 \) families were selected to represent each class (LP-HL1, LP-HL2, HP-LL1, HP-LL2). Two checks were also included: a three-way opaque-2 hybrid (OCK) and its normal counterpart (NCK). Casein was used as a check protein standard. Maize was used as the only protein source for each diet. Six weanling rats were assigned to each diet.

Table 7 shows values obtained for g protein/100 g sample, g lysine/100 g protein and g lysine/100 g sample for the six samples. The mean protein efficiency ratio (PER) and mean feed efficiency ratio (FER) for the seven diets are also included.
Table 7. Protein quality, protein efficiency ratio and feed efficiency ratio for modified opaque-2 corns with high and low lysine with high and low protein values compared with a casein control.

<table>
<thead>
<tr>
<th></th>
<th>g protein/100 g sample</th>
<th>g lysine/100 g protein</th>
<th>g lysine/100 g kernel</th>
<th>Mean PER</th>
<th>Mean FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP-HL1</td>
<td>9.08</td>
<td>4.07</td>
<td>.36</td>
<td>2.51</td>
<td>.22</td>
</tr>
<tr>
<td>LP-HL2</td>
<td>9.20</td>
<td>4.08</td>
<td>.38</td>
<td>2.66</td>
<td>.23</td>
</tr>
<tr>
<td>HP-LL1</td>
<td>11.48</td>
<td>3.49</td>
<td>.36</td>
<td>2.20</td>
<td>.24</td>
</tr>
<tr>
<td>HP-LL2</td>
<td>12.35</td>
<td>3.00</td>
<td>.40</td>
<td>2.14</td>
<td>.25</td>
</tr>
<tr>
<td>OCK</td>
<td>8.70</td>
<td>4.10</td>
<td>.36</td>
<td>2.40</td>
<td>.20</td>
</tr>
<tr>
<td>NCK</td>
<td>7.70</td>
<td>2.85</td>
<td>.22</td>
<td>1.24</td>
<td>.09</td>
</tr>
<tr>
<td>Casein</td>
<td></td>
<td></td>
<td></td>
<td>3.43</td>
<td>.30</td>
</tr>
</tbody>
</table>
No statistical test has been done so far to determine if differences among PER or FER values are significant.

The mean PER values for two modified samples, LP-HL1 and LP-HL2 are comparable to the opaque-2 check. This indicates that with selection, one could breed for modified phenotypes without losing the superior protein quality of opaque-2 phenotype. This study also shows that PER is influenced more by the quality rather than the quantity of protein that is in the diet.

FER values are almost the same for all the modified samples tested, including the opaque-2 check. This shows that for the two classes, the proportion of lysine relative to the amounts of the other amino acids supplied by the protein resulted in gains in weight which are equal or even a little bit better than that obtained from the opaque-2 check. It is difficult to predict, however, how far one could shift values for g protein/100 g sample and g lysine/100 g protein and still not produce an amino acid imbalance in the diet.

The amino acid profiles of the different protein fractions and carbohydrate contents, will also be determined in these materials.

In an additional study $S_5$ families which were selected for lysine content from the HMO synthetic were utilized. The objective is to determine if some other mechanisms, other than a shift in the relative proportion of zein to glutelin, could be involved in bringing about an increase in lysine content in the endosperm protein of highly modified opaque-2 materials.

Twelve families for each group, low lysine group (LL) and high lysine group (HL), were originally selected. Each year a sample of seeds from individual ears were analyzed for lysine content. Ears with the highest or lowest value (to whichever group they belong) were used to plant a row in the next season.

From the $S_5$ families, two samples were selected to represent each of
the two groups: LL had 11.3 percent protein and 1.99 percent of lysine (lysine as a percent of protein), LL had 12.0 percent protein and 1.92 percent lysine (lysine as a percent of protein), HL had 8.72 percent protein and 3.32 lysine as a percent of protein and HL had 9.10 percent protein and 3.17 lysine as a percent of protein. It is planned to fractionate the protein in these samples and determine the amino acid distribution in the different fractions of the protein.

Agronomic and protein quality selection is being conducted in the Temp HB su2o2 synthetic in highly modified opaque-2 selected material. The progress with this material was reported earlier (p. 6). The new single gene modifier of opaque-2 material has been crossed to selected su2o2 inbreds and is undergoing backcrossing to the su2o2 recurrent parent inbreds. Backcross one was completed in the 1975-76 winter nursery. These materials will be evaluated for agronomic performance, protein quality and the degree of modification and interaction with the su2 effect on opaque modification.

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

In 1973, a self-pollinated ear out of an opaque synthetic appeared to be segregating a "single" gene that modifies the soft endosperm of opaque-2 giving it a more vitreous (normal) appearance. Self-pollinated progeny from these two types of kernels on this ear showed that this trait was indeed heritable. The opaque kernels generally yielded plants that produced completely opaque ears. The modified opaque kernels yielded plants that produced either completely modified ears or ears that were segregating for modified opaque kernels.

This factor has been introduced into different genetic backgrounds. The phenotypic expression varies considerably with the different genetic backgrounds. Other factors also affect the expression of this trait.

This factor appears to be quite simply inherited with some degree
of dominance. This is shown by the fact that the $F_1$ ear of a modified opaque parent and an opaque parent will usually contain a degree of modification. After two self-pollinations it is possible to recover completely modified ears and completely opaque ears.

A limited number of samples have been analyzed to determine the effect of this modification on the lysine levels. Results showed a slight reduction in the lysine level. This reduction wasn't statistically significant for the small experiment that was conducted.

Currently more extensive work is being done to more precisely describe the mode of inheritance of this modifier. Its effect on the lysine level and other kernel characteristics is being further studied. The preliminary data indicate one major gene is involved.

Several new families segregating for a single gene controlling modified expression of opaque-2 were found in Temp HB. These simply inherited genes controlling modification are being introduced into opaque-2 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. The double mutant sugary-2 opaque-2 ($su_2^{2-2}$) has improved kernel vitreousness and kernel density similar to normal with improved protein quality, *in vitro* digestibility characteristics, and biological value. (See previous Annual Reports). It, however, suffers from reduced yield in those materials evaluated to date. The waxy opaque-2 double mutant has protein quality equivalent to opaque-2 and good biological value. It has a highly
modified starch type (all amylopectin), but is not improved in kernel vitreousness or density and also has reduced yields.

We have previously reported on the development of a restricted number of opaque-2, sugary-2, sugary-2 opaque-2, waxy and waxy opaque-2 inbreds and their hybrid kernel characteristics and protein quality. We have continued to backcross the o2, su2, su2o2, wx and wx o2 gene systems into 24 elite inbred backgrounds. Backcross 6 generation recoveries have been completed in approximately 16 inbreds and will be grown out and self pollinated to recover isogenic lines of the respective o2, su2, su2o2 genotypes and will continue in the 1976 summer and winter nurseries. The wx and wx o2 conversions are not as far advanced. A diallel set of crosses among near isogenic o2, su2, su2o2 inbred recoveries and their normal counterparts for 15 inbred lines are being made up in the 1976 summer and winter nurseries. The new isogenic line hybrids will be evaluated for agronomic performance, kernel characteristics and protein quality. The isogenic lines which have been developed and released 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.


Three endosperm mutants, sugary-2 (su2), waxy (wx), and opaque-2 (o2) and their double mutants with o2 each nearly isogenic in four hybrids, and the normal counterparts were sampled 10 days after hand pollination, on each succeeding 7 days until 73 days after pollination. Average fresh weight, dry weight, grain weight, and cob weight per ear and percent moisture were determined.

The hybrids were highly significantly different for all variables except percent moisture at 24 days after pollination and thereafter. While genotypes
were significantly different at 24 days after pollination and thereafter for
cob weight and percent moisture, significant differences in fresh weight,
dry weight, and grain weight of the ear were not found until 38 days after
pollination. There were no significant differences among sampling dates after
52 days. Hence the differences among genotypes that were first found at 38
days were fixed by 52 days after pollination. The hybrid x genotype interaction
were generally nonsignificant except for cob weight. The grain weight of \( o_2 \),
\( su_2 o_2 \), and \( wx o_2 \) hybrids were significantly less than \( wx \) and the normal counter-
part hybrids (Figures 1 and 2). The grain weight of \( su_2 \) was not significan-
tly less than normal and \( wx \) in some hybrids.

Six related single crosses from the diallel among Oh43, W64A, B37, and
C103 inbreds and the 3-way hybrid (Oh43 x B37) x C103 each near isogenic for
the \( o_2 \), \( su_2 o_2 \) and their normal counterparts were evaluated for agronomic
yield in a replicated yield test on the Purdue Agronomy Farm and only one
season. Three normal commercial single crosses and their \( o_2 \) counterpart
hybrids were also grown as checks. The six related single crosses and the
3-way normal hybrid ranged from 144 to 187 bu/acre; the opaque hybrids ranged
from 113-163 bu/acre; and the sugary-2 opaque-2 hybrids ranged from 113-143
bu/acre. The commercial normal hybrids ranged from 171 to 206 bu/acre and
their counterpart opaque-2 hybrids ranged from 136-177 bu/acre. Over all
the opaque-2 hybrids ranged from 74 to 93% of their normal counterpart hybrids,
while sugary-2 opaque-2 hybrids ranged from 74 to 87% of their normal counter-
part hybrids. The mean protein percent over all the hybrids for the normal,
opaque-2, and sugary-2 opaque-2 near isogenic varieties was 9.523, 9.831 and
10.584%, respectively; while mean lysine as a percent of protein values were
2.99, 4.29, and 4.50; and the mean lysine as a percent of sample values were
0.285, 0.419, and 0.476 for normal, opaque-2, and sugary-2 opaque-2, respect-
ively. In each case the genotypes were significantly different at the 0.01%
Figure 1. Grain weight per ear accumulation of \( su_2, su_2o_2, o_2 \) and normal averaged over hybrids.
Figure 2. Grain weight per ear accumulation of wx, wx O2, O2 and normal averaged over hybrids.

A study was conducted to evaluate the effect of population and differential thinning during both the vegetative and reproductive stages on the yield, ear and kernel characteristics of sugary-2 and normal maize. Varying the population may indicate more favorable population for influencing seed size development in sugary-2 and sugary-2 opaque-2 maize.

The single cross hybrid Oh43 su2 x B37 su2 and the normal counterpart were grown on the Purdue University Agronomy Farm and were used to study the effect of delayed thinning on ear and kernel characteristics, and on plot yield. The field was planted at a high population of 74,775 plants/hectare and thinned to medium (49,882 plants/hectare) and low (24,925 plants/hectare) populations at emergence, 30 days after emergence (DAE), 50 DAE, 10 days after midsilk (DAP), and 20 DAP.

Sugary-2 had significantly less grain weight per ear, and plot yield than did normal. Sugary-2 had larger cob weight per ear than did normal. Both genotypes responded similarly to the thinning treatments for all variables measured. The high population had the smallest and the thinned-to-low populations had the largest cob and grain weight per ear. There were non-significant decreases with delayed thinning among the thinned-to-medium population for cob, and grain weight per ear and plot yield. There were significant decreases among the thinned-to-low population for cob, and grain weight per ear and plot yield. There were significant decreases among the thinned-to-low population for grain weight per ear and plot yield if thinning was delayed until after pollination. Using the mean of the genotypes, the high population had the highest plot yield.

Sugary-2 had fewer kernels per ear and smaller 100-kernel volumes and weights, but su2 kernels were more dense than normal. Kernel number decreased
with increasing population density and with thinning after pollination. Kernel
density was constant for the genotypes and unaffected by the thinning treatment.
Very early and very late thinnings increased kernel weight and volume.

**Dosage Effects of the Sugary-2 Gene on Kernel Characteristics and Oil, Protein,
Lysine and Starch Content of Corn Grain.**

A diallel set of hybrids among four inbreds; Oh43, W64A, B37, and C103,
each nearly isogenic for the mutants sugary-2 (su_2), opaque-2 (o_2), and sugary-2
opaque-2 (su_2o_2) and their normal counterpart were grown on the Purdue University
Agronomy Farm in each of two years. Crosses among the four genotypes were
made in such a manner so as to produce a dosage series for su_2 in both an opaque
and nonopaque background with respect to the o_2 locus in each of the hybrids. A
significant decrease in weight and volume occurred between one, two, and three
su_2 doses while kernel density increased in the same dosage series. Percent oil
increased by 28% in a stepwise manner although the one and two dose intermediates
were not different from each other. Changes in total oil yield followed the
same trend as percent oil. Percent germ was increased by one percentage point in
homozygous su_2 genotypes. Percent protein, lysine percent of sample, lysine as
a percent of protein, and mg lysine in the endosperm were greater in the su_2
homozygote while other doses were not different from each other. Significant
interaction effects existed between su_2 doses and o_2 for kernel weight, volume
and density as well as percent protein of the endosperm. Significant differ­
ences between hybrids were observed for all variables measured.

**Effects of Outcrossing and Sibbing on Kernel Character and Oil Content
Conditioned by the Sugary-2 Gene.**

Effects of outcrossing and sibbing on kernel characteristics and oil
content conditioned by the su_2 gene were determined in the same diallel
set of hybrids as indicated above. Comparisons between homozygous and hetero-
zygous su_2 kernels on segregating ears eliminated sporophytic variation.
Kernel weights were not significantly changed by outcrossing regardless of the su$_2$ genotype. Volume was increased for outcrossed kernels produced on homozygous su$_2$ females. Kernel densities were generally increased with outcrossing. No differences between sibbed kernels on nonsegregating vs. segregating ears existed for kernel density while inconsistent differences were observed for weight and volume.

Outcrossing increased percent oil in all su$_2$ genotypes except from a heterozygous su$_2$ female in an opaque background. Average increases of 3 and 4% were observed for nonopaque and opaque kernels, respectively. Total oil per 100-kernels was increased by outcrossing but the percentage increase was dependent upon the su$_2$ genotype. Sibbed, normal kernels had greater weights and volumes but lower densities, percent oil, and oil per 100-kernels than either outcrossed or sibbed su$_2$ heterozygotes or recessive homozygote.

**The Influence of the Sugary-2 Gene on Kernel Characteristics and Oil Content of High and Low Oil Lines.**

The su$_2$ gene from B37 and C103 was crossed and backcrossed into R84, a high oil selection, and Hy2, an inbred with low oil content. Heterosis was present for kernel weight, volume, density, and oil yield in the F$_2$ seed. Successive backcrosses became more like the recurrent parent when the normal class of kernels was considered. Sugary-2 kernels were lighter, occupied less volume, and had greater kernel density than their normal counterparts. Endosperm weight decreased, percent germ increased, while germ weight remained nearly constant in su$_2$ backcross recoveries. The su$_2$ gene increased the percent oil in both low and high oil lines. A portion of this increase can be attributed to higher germ to endosperm ratios. Oil yield per 50-kernels was increased in the su$_2$ backcross recoveries compared to normal kernels.
Biological Value of Normal, Opaque-2, Sugary-2, Sugary-2 Opaque-2, Waxy, and Waxy Opaque-2 Corns in Protein Adequate Diets for Pigs.

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 (o\textsubscript{2}), (3) waxy (wx), (4) waxy-opaque-2 (wxo\textsubscript{2}), (5) sugary-2 (su\textsubscript{2}), and (6) sugary-2 opaque-2 (su\textsubscript{2}o\textsubscript{2}) 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 o\textsubscript{2} gene or to the starch type, but a significant o\textsubscript{2} 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 o\textsubscript{2}. 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 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. (Abstr. in print and papers submitted for Journal review).


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 (o\textsubscript{2}), waxy (wx), waxy opaque-2 (wxo\textsubscript{2}), sugary-2 (su\textsubscript{2}) and sugary-2 opaque-2 (su\textsubscript{2}o\textsubscript{2}) 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 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 o_2, su_2-o_2, and wxo_2 is due to more than Lys and Try, and that the superior quality of o_2 corn is maintained in the double mutants with different starch structure. (Abstr. in print and papers submitted for Journal review).

Nitrogen Distribution and Amino Acid Composition in the Protein Fractions of Near-Isogenic Opaque-2, Sugary-2, Sugary-2 Opaque-2 and the Normal Counterpart Endosperm.

The endosperm proteins of inbred C103 near isogenic for su_2, o_2, and su_2-o_2 and the normal counterpart were fractionated using the Landry-Moreaux procedure. Normal and su_2 had high zein levels (49.9% and 43.8%, respectively), whereas o_2 and su_2-o_2 had low zein levels (18.8% and 13.2%, respectively). opaque-2 had a high albumin and globulin level (24.1%) and su_2-o_2 had a high glutelin level (34.2%). The lysine levels of the mutants was explained by the relative abundance of the different protein fractions and not by changes in the amino acid composition within the protein fractions.

Characterization of Key Proteins in High Lysine Endosperms.

A detailed electrophoretic characterization of the polypeptides in the endosperm of normal, opaque-2 and double mutants with opaque-2 has indicated
significant differences between the zein and zein-like polypeptides of these mutants (papers in press).

Two polypeptides in Fraction II and one in Fraction III are of special interest because of their amounts in opaque-2 and opaque-2 genotypes. Preliminary efforts have been made to obtain purified preparations of these polypeptides in quantities large enough to determine their chemical composition, and the results have been encouraging. These polypeptides will now be isolated by a combination of ion exchange and gel filtration chromatography. The purified polypeptides will be characterized by amino acid analysis.

**Characteristics of the Lysine Biosynthetic Pathway in Normal and High Lysine Mutants.**

The biosynthesis of lysine in normal and its opaque-2 counterpart have been investigated during germination. The studies reveal that the lysine biosynthetic pathway is under feedback control in both systems (Studies on Corn Proteins XI: Distribution of lysine during germination of normal and high lysine maize. B.A.K. Chibber, E. Voicu, E. T. Mertz and D. V. Glover, Cereal Chem., Submitted).

**Climatological Factors in Maize Adaptation.**

In our last annual report we presented data showing that Corn Heat Units (CHU) were more consistent for a particular maize strain from environment to environment than other heat units based on air temperature. In Table 8, the consistency of accumulated Solar Radiation, expressed as coefficients of variability, may be compared with various heat units based on air temperatures. The accumulations used from each environment were for the entire period from planting to physiological maturity ("black-layer" formation) of each maize strain. Accumulated Solar Radiation was not as consistent as Effective Growing Degree Days nor Corn Heat Units. In a more limited number of environments, the coefficients of variability of Net Radiation may be compared with those of Solar
Table 8. C. V.'s over environments from planting to "black-layer" formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>No. Environ.</th>
<th>Days</th>
<th>GDD&lt;sup&gt;2&lt;/sup&gt;</th>
<th>EGDD&lt;sup&gt;3&lt;/sup&gt;</th>
<th>CHU&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Sol. Rad.</th>
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<tr>
<td>ICA-H208</td>
<td>18</td>
<td>20.3&lt;sup&gt;5&lt;/sup&gt;</td>
<td>14.0</td>
<td>12.3</td>
<td>8.6</td>
<td>19.3</td>
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<tr>
<td>ETO</td>
<td>12</td>
<td>22.3</td>
<td>18.4</td>
<td>16.1</td>
<td>10.7</td>
<td>21.7</td>
</tr>
<tr>
<td>Oaxaca 179</td>
<td>32</td>
<td>17.0</td>
<td>17.2</td>
<td>15.6</td>
<td>11.4</td>
<td>16.8</td>
</tr>
<tr>
<td>Antigua Gp. 2</td>
<td>24</td>
<td>19.4</td>
<td>14.4</td>
<td>12.7</td>
<td>8.8</td>
<td>17.5</td>
</tr>
<tr>
<td>Tuxpeno</td>
<td>17</td>
<td>20.5</td>
<td>17.9</td>
<td>15.6</td>
<td>9.8</td>
<td>17.6</td>
</tr>
<tr>
<td>H49xH55 (O&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>33</td>
<td>20.6</td>
<td>14.0</td>
<td>12.2</td>
<td>8.1</td>
<td>25.0</td>
</tr>
<tr>
<td>N28xMo17</td>
<td>32</td>
<td>20.9</td>
<td>14.1</td>
<td>12.4</td>
<td>8.1</td>
<td>17.7</td>
</tr>
<tr>
<td>A619xA632</td>
<td>32</td>
<td>20.9</td>
<td>13.6</td>
<td>11.6</td>
<td>6.8</td>
<td>17.0</td>
</tr>
<tr>
<td>USA 342xETO</td>
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<td>22.6</td>
<td>15.9</td>
<td>13.9</td>
<td>9.6</td>
<td>21.4</td>
</tr>
<tr>
<td>ICA-H302</td>
<td>12</td>
<td>22.4</td>
<td>17.4</td>
<td>15.2</td>
<td>9.6</td>
<td>20.7</td>
</tr>
<tr>
<td>Comp. K (O&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>21</td>
<td>20.4</td>
<td>14.9</td>
<td>13.0</td>
<td>8.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Colus</td>
<td>25</td>
<td>20.2</td>
<td>17.0</td>
<td>15.0</td>
<td>9.9</td>
<td>17.4</td>
</tr>
</tbody>
</table>

<sup>1</sup> Physiological maturity as indicated by "black layer" formation.

<sup>2</sup> Growing Degree Days = \( \sum \text{Daily Min + Max Air Temp} - 10^\circ\text{C} \) where Mins. less than \( 10^\circ\text{C} \) are set equal to 10.

<sup>3</sup> Effective Growing Degree Days, Same as above except Maximum Temperatures greater than \( 30^\circ\text{C} \) are set equal to 30.

<sup>4</sup> Corn Heat Units = \( \sum 1.85 (\text{Max} - 10) - 0.026 (\text{Max} - 10)^2 + \text{Min} - 4.4 \)

<sup>5</sup> Legitimate comparisons cannot be made between different varieties (horizontal lines) in this table because of differing combinations of environments.
Radiation and other heat units in Table 9. It was surprising to us that Net Radiation accumulations were generally less consistent than any of the other heat units compared.
Table 9. C. V.'s over environments from planting to anthesis.

<table>
<thead>
<tr>
<th>Entry</th>
<th>No. Environ.</th>
<th>Days</th>
<th>EGDD&lt;sup&gt;1&lt;/sup&gt;</th>
<th>CHU&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Sol. Rad.</th>
<th>Net Rad.</th>
</tr>
</thead>
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<tr>
<td>ICA-H208 (O&lt;sub&gt;2&lt;/sub&gt;)</td>
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<td>16.5&lt;sup&gt;3&lt;/sup&gt;</td>
<td>10.7</td>
<td>7.2</td>
<td>10.3</td>
<td>14.9</td>
</tr>
<tr>
<td>ETO</td>
<td>15</td>
<td>15.8</td>
<td>13.0</td>
<td>9.3</td>
<td>10.6</td>
<td>16.0</td>
</tr>
<tr>
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<td>8.6</td>
<td>9.0</td>
<td>15.4</td>
</tr>
<tr>
<td>Tuxpeno</td>
<td>19</td>
<td>12.4</td>
<td>19.3</td>
<td>13.2</td>
<td>10.0</td>
<td>16.1</td>
</tr>
<tr>
<td>H49xH55 (O&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>19</td>
<td>19.0</td>
<td>12.2</td>
<td>8.2</td>
<td>12.4</td>
<td>18.6</td>
</tr>
<tr>
<td>N28xMo17</td>
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<td>11.0</td>
<td>6.5</td>
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<td>20.2</td>
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<td>A619xA632</td>
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<td>10.6</td>
<td>6.5</td>
<td>12.0</td>
<td>18.1</td>
</tr>
<tr>
<td>USA 342xETO</td>
<td>10</td>
<td>17.5</td>
<td>12.3</td>
<td>8.0</td>
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<td>17.3</td>
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<td>12</td>
<td>13.8</td>
<td>15.6</td>
<td>10.6</td>
<td>8.6</td>
<td>17.6</td>
</tr>
<tr>
<td>Comp. K (O&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>16</td>
<td>14.1</td>
<td>18.0</td>
<td>11.8</td>
<td>9.8</td>
<td>17.6</td>
</tr>
<tr>
<td>Colus</td>
<td>16</td>
<td>16.7</td>
<td>13.4</td>
<td>7.7</td>
<td>10.3</td>
<td>17.8</td>
</tr>
</tbody>
</table>

<sup>1</sup> Effective Growing Degree Days = Σ Daily Min + Max Air Temp - 30°C where Mins. less than 30°C are set equal to 30.

<sup>2</sup> Corn Heat Units = Σ Daily 1.85 (Max-10) - 0.06 (Max-10)<sup>2</sup> + Min - 4.4

<sup>3</sup> Legitimate comparisons cannot be made between different varieties (horizontal lines) in this table because of differing combinations of environments.
E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results:

a. Research report. Approximately 500 copies of the 1974-75 annual research report were distributed to over 300 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. Continued requests are being made by interested maize and cereal workers throughout the world for the updated and expanded revision of the status report--Purdue University Agricultural Experiment Station Research Bulletin 914 (October 1974)--entitled "Progress in Developing Maize with Improved Protein Quality" and the second 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".

c. Workshops, symposia, papers and seminars presented. Papers were presented and project personnel participated in the following activities: American Society of Agronomy Annual Meetings; Tenth Inter-Asian Corn Improvement Workshop, Islamabad & Lehore, Pakistan; Rockefeller Foundation and Kasetsart University, Bankok, Thailand; Maize Research Institute, Belgrade-Zemun, Yugoslavia; Institut Sperimentale Cerealcoiltura, Bergamo, Italy; Osaka City University, Department of Foods and Nutrition, Osaka City, Japan; American Society of Animal Sciences Annual Meeting; Mid-West Section of the American Society of Animal Sciences Annual Meeting; US/AID International Workshop--Mississippi State; Xth International Congress on Nutrition, Kyoto, Japan; 29th Annual Mtg. Japanese Society of Food and Nutrition, Kyoto, Japan; The Agricultural Chemical Society of Japan, Sapporo, Japan; International Maize Symposium, Urbana, Illinois;
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, IMo$_2$o$_2$ Synthetic, Mod. Syn. Ao$_2$, Mod Syn Bo$_2$, 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; Urba/Czechoslovakia; Gunn/England; Pollmer/Fed. Rep. of Germany; Alber/Bundesrepublik Deutschland; Tzuno/Pakistan; House/Lebanon; Lai/Taiwan; Georgiev/Bulgaria; Dumitrue/Romania; Salamini/Italy; Ordas/Spain; Schoster/West Germany; Vasal/CIMMYT-Mexico; Read/Acores; Jovanović/Yugoslavia; Ottaviano/Milan; Navolotskiy/Russia; Balint/Hungary; Oaks/Canada; Nemith/Hungary; Petrović/Yugoslavia; Grobman/Peru; Misović/Yugoslavia; Sriwantanapongse/Thailand; Koshal/Napal; Gul/Afghanistan; Fuwa/Japan; Poey/Mexico; Munck/Denmark; Djamhuri/Indonesia; Nekez/Hungary; Kim/Korea; Tomov/Bulgaria.

Germplasm for protein quality improvement was distributed to over 30 different private or public institutions and maize companies within the U.S.

2. Utilization: Evidence and Cases Where Findings are Being Used in LDC's and U.S.:
Requests have continued for information, seed, analytical monitoring, technical help, 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 and reflects a continued interest in the high lysine materials. The number of visitors from LDC's and other countries to Purdue has continued. In a visit to Southeast Asia in connection with the Tenth Inter-Asian Corn Improvement Workshop, Dr. Glover consulted with maize workers from Thailand, Napal, India, Pakistan, Egypt, Africa, Australia, Papua New Guinea, Indonesia, Phillipines, Korea, Italy and Yugoslavia. In many areas (Thailand, Phillipines, Napal, Italy and Yugoslavia) there was some small part of the total maize program that was devoted to high lysine work. 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. Emphasis was given to the necessity of researching some of the yield constraints which are associated with breeding 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 Philippines and Thailand, East Africa, and Guatemala will strengthen and improve the utilization of fundamental knowledge and germplasm materials.

Dr. Bauman and Dr. Crane reviewed with US/AID and Guatemalan maize workers and government officials their corn program in prospects of aiding the Guatemalan maize program in a cooperative effort to improve corn production. Dr. Glover led a workshop and presented an invitational paper to the Asian maize workers. There has been some discussion with CIMMYT personnel and USAID to conduct a maize production and protein quality workshop in 1977 or 1978.

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.

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. Leng) and CIMMYT maize research staff (Drs. E. W. Sprague, G. C. Johnson, S. K. Vasal, E. Villegas M., P. R. Goldsworthy, A. Ortega C., J. C. Vessey) was held December 1-2, 1975 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.

Project personnel are 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, New Guinea, Nepal, Egypt, Africa, Pakistan and other countries in special visits, seminars, and workshop discussion groups to help guide the development and utilization of improved protein quality maize.

Project personnel (Dr. Bauman) have been involved in a special panel group on investigation of grain crops for NSF/MIT Protein Resources Study. A special report was submitted.

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

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

Students from Mexico (Alfredo Navarro), Phillipines (Lourdes Nazarea), Colombia (Hugo Zorillo), Taiwan (Husan Tu), and the United States (P. Stephen Baenziger, Theron E. Roundy, 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. Four trainees (Ecaterina Voicu, Romania; Dumitrue Runduinu, Romania; Ratko Petrović, Yugoslavia; and Chido Jovanović, Yugoslavia) received training in analytical techniques and/or breeding of protein quality maize.

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.

F. STATEMENT OF EXPENDITURES AND OBLIGATIONS AND CONTRACTOR RESOURCES:

<table>
<thead>
<tr>
<th>Category</th>
<th>Total Budget Amount (4/1/75-3/31/76)</th>
<th>Expenditures (4/1/75-3/31/76)</th>
</tr>
</thead>
<tbody>
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<td>Fringe Benefits</td>
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<td>Overhead</td>
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<td>Travel, Workshop &amp; Subsistence</td>
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<td>Other Direct</td>
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<td>Subcontracts</td>
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<td>Total</td>
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<td>246,336.57</td>
</tr>
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</table>

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) 1976-1977—The mutants which have shown high lysine values will be retested. Those with regular inheritance patterns shall be analyzed. Allele testing will continue with genes of similar phenotypes. Introduce promising new mutants into elite germplasm, and combine with other high lysine mutants and evaluate epistatic effects.
(2) 1977-1978—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) 1976-1977—Continue tests of material from the Germplasm Bank (CIMMYT), extend the search to new collections from the most promising areas indicated by the ninhydrin and other screening procedures. Continue search for and evaluation of new improved protein quality mutants in $M_2$ progeny of chemically induced mutants. Investigate other non-conventional ways of producing high quality protein maize such as screening for feedback insensitive mutants to lysine biosynthesis.

(2) 1977-1978—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) 1976-1977--Continue full sib selection for high lysine families in normal maize for another cycle of recurrent selection if there are any promising results in the 1975-1976 selections. A decision will be made at this time depending on results obtained whether to continue this approach.

(2) 1977-1978--Continue full sib 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 zaeae).

(1) 1976-1977--Cycle 3 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) 1977-1978--Test and analyze cycle 4 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) 1976-1977--270 selected full-sib families (Cycle 2) in each population shall be yield tested and analyzed for agronomic quality, kernel vitreousness and protein quality during the summer of 1976. Selected families will be intercrossed in the 1977 winter nursery.

(2) 1977-1978--Continue full sib selection as outlined above in the populations. Yield test and analyze full sibs Cycle 3.

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.


   (2) 1977-1978--Continue backcrossing and selection for improvement in protein quality within the sugary-2 opaque-2 selected recoveries. Evaluate for protein quantity in hybrids from early generation backcross recoveries of selected lines.

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

   (1) 1976-1977--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) 1977-1978--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). Seven opaque-2 sources and four sugary-2 sources are being backcrossed into common inbred sources to evaluate possible differences among the sources.

(1) 1976-1977--In summer nursery mutant opaque-2 sources backcrossed (7 backcrosses) into W64A and B37 backgrounds will be evaluated for agronomic performances, grain quality, and protein quality to determine possible difference among mutant sources. Continue backcrossing opaque-2 and sugary-2 sources into common inbred sources and make more advanced crosses of opaque-2 sources.

(2) 1977-1978--Continue backcrossing sugary-2 sources and continue to evaluate in second year the opaque-2 sources in hybrid and 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. A very critical question involving the practical utilization of selected high lysine modified opaque-2 types concerns the stability of the characteristics under random mating and various environmental conditions when used by the farmer. Modified and non-modified versions of synthetics Temp H\textsubscript{2}o\textsubscript{2} and Temp H\textsubscript{3}o\textsubscript{2} and high and low lysine selections of modified and non-modified versions of synthetic H\textsubscript{2}o\textsubscript{2} have been random mated successively for two or three generations.

(1) 1976-1977—Continue the testing and evaluation for a second year of the random mated generations of the eight populations described above in several environments as necessary to obtain definitive data. Data on relationships of agronomic performances to modified types and protein quality shall also be obtained in the above experiments.

b. 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) 1976-1977—Continue backcrossing of new single gene modifier into selected germplasm and evaluate in preliminary hybrids and populations.

(2) 1977-1978—Continue backcrossing and evaluation in hybrids and other selected germplasm for agronomic performance, modified kernel type, protein quality and biological value.

c. Current research has shown that selection for modified opaque-2 types results in lower lysine levels and a shift in the protein fractions (Landry-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) 1976-1977—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) 1977-1978—Continue evaluation of protein quality if additional information is needed.

d. 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) 1976-1977—Continue backcrossing the new simply inherited modifier gene of opaque-2 to sugary-2 opaque-2 material and selfing in advanced generation materials and continue half-sib selection in sub-population of Temp HB sugary-2 modified O₂. Evaluate the sugary-2 opaque-2 genotype in the modified opaque-2 backgrounds for agronomic performance, modified kernel types and protein quality.

(2) 1977-1978—Evaluate for agronomic performance, protein quality, kernel size and degree of modification and determine if approach is practical before continuing 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) 1976-1977—Continue backcrossing of the sugary-2 opaque-2 and
other double-mutants to elite inbred backgrounds. Backcrosses 6 and 7 should be completed in sugary-2 opaque-2 for many elite backgrounds. Evaluate in preliminary hybrids.

(2) 1977-1978--Continue hybrid evaluation of interactions 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) 1976-1977--Continue backcrossing to adapted cuzco synthetic backgrounds.


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) 1976-1977--Continue to characterize the developmental process of grain filling and associated compensation effects in endosperm and germ biosynthetic development.

(2) 1977-1978--Continue characterization of developmental processes of grain filling in high lysine genotypes.

d. Development of promising new genotypes.

(1) 1976-1977--As new mutants are described under Objective 1 and appear promising, intercross with selected endosperm mutants.
(2) 1977-1978--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) 1976-1977--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) 1977-1978--Continue studies on protein biosynthesis in double-mutants.

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

1976-1977--The biochemistry laboratory will continue to function as a center for screening maize and other cereals and check sample analyses for protein quality. The laboratory will continue on a limited scale to search for better methods to evaluate protein quality in maize breeding programs. This includes the development of new methods as well as evaluation of methods proposed by other laboratories. The laboratory will continue to cooperate with the Protein Quality Laboratory at CIMMYT in screening new analytical methods proposed for cereal protein analysis.

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

1976-1977--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.

A protein synthesizing system will be utilized to 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.

We plan to utilize antibiotics to retard early zein synthesis in normal maize as a possible means of creating an "opaque-2"-like effect during seed development. If successful, a vitreous endosperm with the amino acid pattern of opaque-2 corn may be obtained in a normal genotype.

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

a. Animal subjects.

(1) 1976-1977--Protein quality evaluation and energy utilization studies with rats and swine will continue in cooperation with the Department of Animal Sciences and Biochemistry for evaluation of selected promising genetic materials.

(2) 1977-1978--Continue animal nutrition evaluation.

b. Human subjects.

(1) 1976-1977--Depending upon results obtained from nutritional studies in adult human subjects (1975-1976), evaluate in feeding trials with rats combinations of nutritionally improved corns in food blends characteristic of those consumed in various parts of the world. If cooperative studies can be arranged with Dr. Graham of John Hopkins University or Dr. Young of MIT, it is proposed to evaluate in young children subjects the nutritional value of sugary-2 opaque-2 and modified opaque-2 corns.
c. The new genetic types will be evaluated for milling quality.

1976-1978—Food products and raw ingredients from genetically improved high lysine corns will be evaluated for (1) keeping quality factors, (2) sensory evaluation and acceptance, and (3) proximal consumption of the products.
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.


Cereal Chemistry 52:734-739.


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

J. M. Arnold and L. F. Bauman

ABSTRACT

Combining ability for concentration and grams per 200 kernels of P, K, Mg, Fe, Zn, oil, and protein, and kernel weight, volume, and density in a six-parent diallel of maize (Zea mays L.) inbreds was examined. Correlations among certain variables were computed. Significant variation was detected among GCA effects for concentration and grams per 200 kernels of all kernel constituents except K concentration, but among SCA effects only for oil concentration. GCA and SCA effects were significant for kernel weight, volume, and density. Highly significant correlations were found between P and K, P and Mg, and K and Mg concentrations.
INTERRELATIONSHIPS AMONG PROTEIN, LYSINE, OIL, 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 criteria for lysine content. Percent lysine was not correlated with kernel weight or volume, but was negatively correlated with kernel density. The coefficients for the correlation between percent lysine and percent protein 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.
THE EFFECT OF SUGARY-2, WAXY, AND OPAQUE-2 ENDOSPERM MUTANTS OF ZEA MAYS L.

ON DRY MATTER ACCUMULATION: THE RESPONSE OF SUGARY-2 TO POPULATION STRESS

AND CHARACTERIZATION OF SUGARY-2 ENDOSPERM PROTEINS

Peter Stephen Baenziger

ABSTRACT

Four hybrids, each nearly isogenic for sugary-2 (su-2), waxy (wx), opaque-2 (o-2), sugary-2 opaque-2 (su-2 o-2), and waxy opaque-2 (wx o-2), and their normal counterparts were grown on the Purdue University Agronomy Farm in 1973. Each genotype within a hybrid was sib pollinated and four ears were collected 10 days after the first hand pollination (DAP), and at 7 day intervals until 73 DAP. Fresh weight, dry weight, cob weight, and grain weight per ear and moisture percent were determined.

Opaque-2 had less fresh, dry, cob and grain weight per ear than normal and greater moisture percent during development than normal. Waxy, except for a higher moisture percent, was similar to normal for all variables measured. Waxy opaque-2, except for a higher moisture percent and cob weight, was similar to o-2 for fresh, dry, and grain weight per ear. Sugary-2 was similar to normal for cob weight, intermediate between normal and o-2 for fresh, dry and grain weight per ear and had a higher moisture percent than normal. Sugary-2 opaque-2 had less fresh, dry and grain weight per ear than o-2, was similar to o-2 for cob weight, and had a higher moisture percent than o-2 and normal.

Normal significantly exceeded all genotypes, followed by wx, su-2, and o-2 of the single endosperm mutant hybrids in order of decreasing final
grain weight. Opaque-2 was not significantly different from wx o-2 and both were significantly greater than su-2 o-2 for final grain weight. The decreases in final grain weight of su-2, o-2, wx o-2, and su-2 o-2 was due to shorter filling periods and less rapid dry matter accumulation. The decrease in final grain weight of wx was due to less rapid dry matter accumulation than normal.

The single cross hybrid Oh43 su-2 x B37 su-2 and the normal counterpart were grown on the Purdue University Agronomy Farm in 1974 and were used to study the effect of delayed thinning on ear and kernel characteristics, and on plot yield. The field was planted at a high population of 74,775 plants/hectare and thinned to medium (49,882 plants/hectare) and low (24,925 plants/hectare) populations at emergence, 30 days after emergence (DAE), 50 DAE, 10 days after midsilk (DAP), and 20 DAP.

Sugary-2 had significantly less fresh weight, dry weight, and grain weight per ear, and plot yield than did normal. Sugary-2 had larger cob weight per ear and moisture percent than did normal. Both genotypes responded similarly to the thinning treatments for all variables measured. The high population had the smallest and the thinned-to-low populations the largest fresh, dry, cob and grain weight per ear. There were nonsignificant decreases with delayed thinning among the thinned-to-medium population for fresh, dry, cob, and grain weight per ear and plot yield. There were significant decreases among the thinned-to-low population for fresh, dry, cob, and grain weight per ear and plot yield. There were significant decreases among the thinned-to-low population for fresh, dry, and grain weight per ear and plot yield if thinning was delayed until after pollination. Cob weight significantly decreased with thinning prior to pollination. Using the mean of the genotypes, the high population had the highest plot yield.
Sugary-2 had fewer kernels per ear and smaller 100-kernel volumes and weights, but su-2 kernels were more dense than normal. Kernel number decreased with increasing population density and with thinning after pollination. Kernel density was constant for the genotypes and unaffected by the thinning treatment. Very early and very late thinnings increased kernel weight and volume.

The endosperm proteins of inbred C103 nearly isogenic for su-2, o-2, and su-2 o-2 and the normal counterpart were fractionated using the Landry-Moreaux procedure. Normal and su-2 had high zein levels (49.9% and 43.8%, respectively), whereas o-2 and su-2 o-2 had low zein levels (18.8% and 13.2%, respectively). Opaque-2 had a high albumin and globulin level (24.1%) and su-2 o-2 had a high glutelin level (34.2%). The lysine levels of the mutants was explained by the relative abundance of the different protein fractions and not by changes in the amino acid composition within the protein fractions.
ABSTRACT

Three endosperm mutants, sugary-2 (su2), waxy (wx) and opaque-2 (o2) and their double mutants with o2 each nearly isogenic in four hybrids, and the normal counterparts were sampled 10 days after hand pollination on each succeeding 7 days until 73 days after pollination. Average fresh weight, dry weight, grain weight, and cob weight per ear and percent moisture were determined.

The hybrids were highly significantly different for all variables except percent moisture at 24 days after pollination and thereafter. While genotypes were significantly different at 24 days after pollination and thereafter for cob weight and percent moisture, significant differences in fresh weight, dry weight, and grain weight of the ear were not found until 38 days after pollination. There were no significant differences among sampling dates after 52 days. Hence the differences among genotypes that were first found at 38 days were fixed by 52 days after pollination. The hybrid x genotype interaction were generally nonsignificant except for cob weight. The grain weight of o2, su2o2, and wx o2 hybrids were significantly less than wx and the normal counterpart hybrids. The grain weight of su2 was not significantly less than normal and wx in some hybrids.
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 o2 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 o2 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 visi­
ble in o2 and the double mutants with o2. The data suggest that protein matrix
may be involved in the expression of kernel vitreousness. The matrix of
vitreous su2o2 kernels resembled the matrix of vitreous su2 kernels and not
that of o2 kernels.
NITROGEN RETENTION OF ADULT HUMAN SUBJECTS WHO CONSUMED 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 balance between leucine and isoleucine was improved because the concentration of leucine was decreased.
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 \([(Oh43^+ \times B37^+) \times C103^+)]\) and opaque-2 \([(Oh43o2 \times B37o2) \times C103o2]\) 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 [(Oh43 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 inherant 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 \(-0.04 \pm 0.25\) 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 of 8 men were in positive balance when opaque-2 corn was fed but only half of them when the other corns were tested.
COLOMBIAN MAIZE GERMPLASM AS SOURCES OF MODIFIER GENES FOR OPAQUE-2

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

ABSTRACT

Despite its proven nutritional superiority, opaque-2 maize (Zea mays L.) has not been generally accepted in tropical regions and for certain food uses because of its soft endosperm. Modified (more vitreous) opaque-2 types would be more acceptable. Seven Colombian maize races and a population derived from ETO x USA342 were outcrossed to the homozygous opaque-2 lines L215 and L216 which have the soft endosperm expression. The crosses were self-pollinated, and the F₂ kernels were classified into three classes over a light. One sub-family (single ear) of each family was reclassified into four classes on a black-topped table with a lighted magnifying glass. Chi square tests indicated indirectly whether the intermediate classes II or II and III were modified opaque-2 kernels or if the ratios deviated significantly from 3:1 due to an excess of normal kernels. In the latter case some homozygous opaque-2 kernels were so modified that they were phenotypically indistinguishable from normal. A high percentage of intermediates indicated that ETO x USA342, Pira, Puya and Común were good sources of modifier genes for opaque-2. Amagaceño was less promising and conflicting results within our smaller samples of Clavo, Chococeño and Pollo made it impossible to draw definite conclusions about their potential.
DIGESTIBILITY STUDIES WITH RATS OF NEAR ISOGENIC MUTANT HYBRID CORNS

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

ABSTRACT

Two rat digestion trials were conducted to compare a 3-way hybrid (1) normal corn (n) and the genetic single and double mutant near isogenic conversions (2) opaque-2 (o₂), (3) waxy (wx), (4) waxy opaque-2 (wxo₂), (5) sugary-2 (su₂), and (6) sugary-2 opaque-2 (su₂o₂) in protein adequate and protein limiting diets. In trial 1, all diets consisted of 78.5% of the respective corn and 21.5% supplement and contained about 16% protein. Eight individually caged rats were ad lib. fed each diet for 23 days. Energy digestibility (DE) was determined by the Cr₂O₃ indicator procedure, whereafter 5 rats per treatment were moved to metabolism cages for total fecal and urinary collections. No differences in average daily gain (ADG) or feed/gain (F/G) were found. DE's by the Cr₂O₃ procedure for treatments 1-6 were 87.5, 82.8, 87.4, 88.8, 84.5 and 84.3% (4, 1, 3 >5, 6, 2; p < .01). DE's by total collection for treatments 1-6 were 90.2, 87.3, 89.1, 89.4, 87.6, and 87.4%. Both methods found treatments n, wxo₂, and wx higher in DE than su₂, su₂o₂ and o₂. No differences in N digestibility, N retention or apparent biological value (BV) were observed in these protein adequate diets. In trial 2, 6 rats per treatment in metabolism cages were fed for 24 days isonitrogeneous diets (8% protein) containing the corns as in trial 1. ADG and F/G were best for su₂o₂ followed by wxo₂ and su₂ than wx and n fed rats. Dry matter digestibility was highest for wx and n and lowest for o₂ and su₂o₂ and N digestibility was highest for n and wxo₂ and lowest for o₂ and su₂o₂ fed rats. N retention and BV were highest for su₂o₂ and o₂ and lowest.
for n fed rats.
COMPARATIVE SUSCEPTIBILITY TO AMYLASES OF STARCH GRANULES OF SEVERAL ENDOSPERM MUTANTS AND THEIR DOUBLE-MUTANT COMBINATION WITH OPAQUE-2 OF FOUR KINDS OF INBRED MAIZE

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

ABSTRACT

Several endosperm mutants each nearly isogenic in the maize B37, C103, Oh43 and W64A (Zea mays L.), their double-mutant combinations with opaque-2 (o2) and the normal counterparts were studied for relative susceptibility of starch granules to amylases. When o2 was combined with each of the ten endosperm genes, namely, brittle-1 (bt1), brittle-2 (bt2), dull (du), flours-1 (fl1), flours-2 (fl2), soft-starch (h), shrunken-1 (sh1), shrunken-2 (sh2), shrunken-4 (sh4), and sugary-2 (su2), it was observed that the starches of these double-mutants were digested by fungal glucoamylase and pancreatin to the extent very comparable to their respective nonopaque single-mutant counterpart. Starch granules of the su2 and su2o2 mutants 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 glucoamylase or pancreatin disappeared following gelatinization of starches with alkali. After the enzyme attack, residual starch granules were examined under scanning electron microscopes. It was observed that starch granules of double-mutants attacked by glucoamylase or pancreatin were very comparable to those of their respective nonopaque single-mutant counterpart attacked by the enzyme.
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.
QUALITY PROTEIN MAIZE

David V. Glover

ABSTRACT

This is an update and review of the protein quality work in maize and to review recent findings of our recent research in improvement of protein quality in maize at Purdue University.

Current research is focusing upon (1) a search for and evaluation of new mutants and germplasm sources with improved protein quality and quantity, (2) selection for modified endosperm types with kernel characteristics similar to normal phenotypes and having high nutritive value of opaque-2, (3) selection of double-mutant endosperm combinations and (4) developing special varieties and source breeding materials with improved agronomic characteristics for use in the LDC's. Special emphasis is given to opaque-2 and sugary-2 opaque-2 materials adapted to more temperate regions. The central biochemical unit has functioned as a center for instruction in and standardization of analytical procedures for determination of the nutritional quality of maize samples by chemical and physical-chemical means.
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.
BREEDING FOR IMPROVED NUTRITIONAL VALUE IN CEREALS

Edwin T. Mertz

ABSTRACT

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

E. T. Mertz and B. A. K. Chibber

ABSTRACT

This is a review of the protein quality research on cereal grains starting with the discovery of the improved protein quality of opaque-2 corn in 1963. The authors propose that health ministries should subsidize the growing of opaque-2 maize and other high lysine cereals now available, or to be developed, when the low quality protein cereals from which these are derived constitute a major part of the diet of infants and preschool children.
The protein quality of a cereal grain can be determined only by chemical or biological methods. In this bulletin, the simple chemical and biological methods used at Purdue for determining protein quality of cereals are outlined in detail. These methods are suitable for a laboratory in the developing countries that is severely limited in supplies, equipment and trained personnel. The bulletin also lists the low cost equipment needed, and sources of supply.
STUDIES ON CORN PROTEINS X. POLYPEPTIDE MOLECULAR WEIGHT DISTRIBUTION IN INLANDRY-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, f12, fl2 o2, bt2 and bt2 o2, 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 bt2 o2, 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 fl2 o2 is reduced below that in normal, f12 and o7. The 44,000 dalton component of Fraction II is a unique component of fl2 and fl2 o2, 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.
STUDIES ON CORN PROTEINS VIII. TOTAL FREE AMINO ACIDS IN SINGLE AND DOUBLE ENDOSPERM MUTANTS

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

ABSTRACT

With the development of a vitreous opaque-2, the usual methods of classification and identification could no longer be used very successfully. The simple ninhydrin method developed recently by Mertz, et. al. (1) was used to determine the level of free amino acids in 14 endosperm mutant and their double-mutant combinations with opaque-2 in the Oh43, W64A, Cl03 and B37 inbred lines, as well as certain hybrid combinations of these lines developed by Glover et. al. (2). In all combinations studied, the presence of the opaque-2 gene invariably raised the level of free amino acids above that of the counterpart lacking this gene.
STUDIES OF CORN PROTEINS VII. ENDOSPERM PROTEIN SYNTHESIS IN DEVELOPING MAIZE MUTANTS WITH INCREASED LYSINE CONTENT

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

ABSTRACT

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

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

ABSTRACT

The endosperm proteins of the maize mutants, floury-2, opaque-2, opaque-7, sugary-1, shrunken-1, shrunken-2, shrunken-4 and brittle-1, and double mutant combinations of these with opaque-2, were separated into five soluble fractions by the Landry-Moureaux method. As compared to their isogenic normal counterparts, all single mutants had higher concentrations of albumin, globulin, and glutelin, and lower concentrations of prolamine. The combination of opaque-2 with floury-2 or opaque-7 did not increase lysine above that in the single mutants. The combination of opaque-2 with any of the other five mutants increased levels of albumin, globulin and glutelin above those found in the single mutants. The double mutants showed an almost complete suppression of prolamine synthesis and the lysine levels were higher than in the single mutants.
STAND, EARLY GROWTH AND PLANT AND EAR HEIGHT OF ENDOSPERM MUTANTS AND NORMAL MAIZE HYBRIDS UNDER FIELD CONDITIONS

A. Pucaric, P. L. Crane and D. V. Glover

ABSTRACT

Five endosperm mutants, waxy (wx), amylose extender (ae), opaque-2 (o2), floury-2 (f12) and floury-1 (f11), and normal endosperm corn in three single crosses and their reciprocals were planted at two depths (4-5 and 7-8 cm) on two dates (May 18 and June 7) and studied for emergence stand, early growth and plant and ear heights. The study was conducted at the Purdue University Agronomy Farm near Lafayette, Indiana, USA in 1972. The results can be summarized as follows.

1. Highly significant differences for early seedling emergence stand were obtained among endosperm characters. Floury-1 gave higher number of early emerged plants per plot than normal. Opaque-2, floury-2 and especially amylose extender had lower number of early emerged plants per plot than normal while waxy was very near to normal. Deeper planting at 7-8 cm reduced early seedling emergence stand with greater reduction for the second planting date when weather and soil conditions were less favorable. Amylose extender had the greatest and floury-1, normal and waxy the least reduction while opaque-2 and floury-2 intermediate reduction.

2. Final emergence stand also differed significantly among endosperm characters. Floury-1 had the highest emergence percent (97.8) and amylose extender the lowest (92.8) while other endosperm mutants had emergence percent practically same as normal (96.2). Planting depth had some influence.
on final emergence stand when planting was done on June 2.

3. **Floury-1** gave 1.0 cm taller seedlings than normal. **Waxy**, **floury-2** and **opaque-2** had small reduction of seedling height in comparison to normal while **amylose extender** had 4.9 cm lower seedling height than normal. Deeper planting reduced seedling height of **amylose extender**.

4. Plant height at the beginning of rapid vegetative growth also differed significantly among endosperm characters. **Floury-1** still had the tallest (4.0 cm taller than normal) and **amylose extender** the shortest (9.6 cm shorter than normal) plants. Other mutants had plant heights very near to normal. Deeper planting on the second planting date reduced plant height at the beginning of rapid vegetative growth in all endosperm characters.

5. Although differences for final plant and ear heights among endosperm characters were significant, they were of small magnitude.
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.
EFFECT OF ENDSPERM AND PROTEIN TYPE OF CORN ON PERFORMANCE OF RATS


ABSTRACT

Two growth trials were conducted with 230 rats to study the nutritional quality of three way hybrid (1) normal (n), (2) opaque-2 (o2), (3) waxy (wx), (4) waxy opaque-2 (wx o2), (5) sugary-2 (su2) and (6) sugary-2 opaque-2 (su2 o2) corns derived from near isogenic conversions of the same hybrid. In trial 1 diets were formulated with the corns, soybean meal and glucose to make isonitrogenous diets within 8, 11 and 14% protein levels (3 x 2 x 3 factorial arrangement of treatments). Rats fed diets containing o2 (diets 2, 4, 6), showed improved average daily gain (ADG) and feed efficiency (F/G) over those fed non-o2 (diets 1, 3, 5) at 8% protein (3.43, 4.34 vs 1.45, 7.29 g) and 11% protein (6.27, 2.96 vs 5.14, 3.26 g) (p < .01), but no significant differences were observed at 14% protein. In terms of endosperm type, at 8% protein rats fed su2 (diets 5, 6) had better ADG and F/G than wx (diets 3, 4) and n (diets 1, 2) with 2.84, 2.27 and 2.21 g for ADG and 5.00, 6.35 and 6.09 for F/G, respectively. At 11 and 14% protein there was no effect of endosperm type. An interaction (p < .05) was found between the n, wx and su2 endosperm types and those containing o2. Rats fed the su2 endosperm out-performed those fed wx and n in the absence of o2, but there were no differences in the presence of o2. In trial 2 the corns were compared on an equal weight basis in protein adequate (15-16%) diets. There were no differences in ADG or F/G. These results indicate that endosperm type (n, wx or su2) has little effect on carbohydrate availability, but does have an effect on protein quality of corn.
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 fed 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.) ENDOSPERM 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 [(Oh43 x B37) x C103] 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% 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 in waxy, sugary-2 and double mutants with the opaque-2 gene is as good as for normal corn for growing pigs.
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 (wx), waxy opaque-2 (wxo2), sugary-2 (su2) and sugary-2 opaque-2 (su2o2) 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 su2 outperformed those fed wx 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, su2o2, 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.
EFFECTS OF THE SUGARY-2 ENDOSPERM MUTANT ON KERNEL CHARACTERISTICS, OIL, PROTEIN, AND LYSINE IN ZEA MAYS L.

Theron E. Roundy

ABSTRACT

A diallel set of hybrids among four inbreds, Oh43, W64A, B37, and C103, each nearly isogenic for the mutants sugary-2 (su-2), opaque-2 (o-2), and sugary-2 opaque-2 (su-2o-2) and their normal counterparts, was grown on the Purdue University Agronomy Farm in 1973 and 1974. Appropriate crosses among the four genotypes were made to produce a dosage series for su-2 in both dominant and recessive homozygotes at the o-2 locus in each of the hybrids. Dosage effects on the physical characteristics, oil, protein, lysine, and starch content conditioned by the su-2 gene were determined.

The su-2 gene was partially recessive for lower kernel weight and volume and greater kernel density. Three doses of su-2 decreased endosperm weight but had no effect on germ weight. Percent germ values were increased by one percentage point in homozygous su-2 genotypes.

Sugary-2 dosage effects were found for whole kernel percent oil and percent oil in the germ although one and two dose intermediates were not significantly different from each other. The su-2 gene was partially recessive for total oil per kernel but completely recessive for greater percent oil in the endosperm. Increases in whole kernel percent oil associated with su-2 resulted from greater percent oil in both germ and endosperm tissue as well as slightly higher germ to endosperm ratios but did not result from increased germ weight.

Endosperm percent protein, lysine as a percent of protein, lysine percent of sample, and lysine per endosperm values were enhanced by three doses of su-2.
The su-2 allele was recessive for higher whole kernel percent protein, lysine as a percent of protein, lysine percent of sample, and lysine per kernel values. Protein per endosperm or kernel was not significantly altered by increasing doses of su-2. No differences among su-2 doses existed for germ protein or lysine.

Three doses of su-2 had greater amylose as a percent of starch values and more amylose per endosperm but less percent starch than the other doses. No differences existed among the other dosage levels. The amount of starch per endosperm decreased throughout the dosage series.

Effects of outcrossing and sibbing on kernel characteristics and oil content conditioned by the su-2 gene were determined in the same diallel set of hybrids. Comparisons between homozygous and heterozygous su-2 kernels on segregating ears eliminated sporophytic variation.

Kernel weights were not significantly changed by outcrossing regardless of the su-2 genotype. Volume was increased for outcrossed kernels produced on homozygous su-2 females. Kernel densities were generally increased with outcrossing. No differences between sibbed kernels on nonsegregating vs. segregating ears existed for kernel density while inconsistent differences were observed for weight and volume.

Outcrossing increased percent oil in all su-2 genotypes except from a heterozygous su-2 female in an opaque background. Average increases of 3 and 4% were observed for nonopaque and opaque kernels, respectively. Total oil per 100-kernels was increased by outcrossing but the percentage increase was dependent upon the su-2 genotype. Sibbed, normal kernels had greater weights and volumes but lower densities, percent oil, and oil per 100-kernels than either outcrossed or sibbed su-2 heterozygotes or recessive homozygote.

The su-2 gene from B37 and C103 was crossed and backcrossed into R84, a high oil selection, and Hy2, an inbred with low oil content. Heterosis was
present for kernel weight, volume, density, and oil yield in the F_2 seed. Successive backcrosses became more like the recurrent parent when the normal class of kernels was considered. Sugary-2 kernels were lighter, occupied less volume, and had greater kernel density than their normal counterparts. Endosperm weight decreased, percent germ increased, while germ weight remained nearly constant in su-2 backcross recoveries. The su-2 gene increased the percent oil in both low and high oil lines. A portion of this increase can be attributed to higher germ to endosperm ratios. Oil yield per 50-kernels was increased in the su-2 backcross recoveries compared to normal kernels.
DOSAGE EFFECTS OF THE SUGARY-2 GENE IN ZEA MAYS L.

Theron E. Roundy and D. V. Glover

ABSTRACT

A diallel set of hybrids among four inbreds, Oh43, W64A, B37, and C103, each nearly isogenic for the mutants sugary-2 (su₂), opaque-2 (o₂), and sugary-2 opaque-2 (su₂o₂) and their normal counterpart were grown on the Purdue University Agronomy Farm in 1973 and 1974. Crosses among the four genotypes were made in such a manner so as to produce a dosage series for su₂ in both an opaque and nonopaque background with respect to the o₂ locus in each of the hybrids. A significant decrease in weight and volume occurred between one, two, and three su₂ doses while kernel density increased in the same dosage series. Percent oil increased by 28% in a stepwise manner although the one and two dose intermediates were not different from each other. Changes in total oil yield followed the same trend as percent oil. Percent germ was increased by one percentage point in homozygous su₂ genotypes. Percent protein, lysine percent of sample, lysine as a percent of protein, and mg lysine in the endosperm were greater in the su₂ homozygote while other doses were not different from each other. Significant interaction effects existed between su₂ doses and o₂ for kernel weight, volume and density as well as percent protein of the endosperm. Significant differences between hybrids were observed for all variables measured.
NUTRITIONAL VALUE OF NEW ENDOSPERM TYPES OF CORN
FOR GROWING-FINISHING BEEF CATTLE

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

ABSTRACT

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

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

ABSTRACT

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