AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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Batch #24

1. SUBJECT CLASSIFICATION

A. PRIMARY: Serials — YAAF30-0120-0000

B. SECONDARY: Agriculture--Plant breeding--Cereals--Sorghum

2. TITLE AND SUBTITLE

Inheritance and improvement of protein quality and content in maize; annual report,1971/1972

3. AUTHOR(S)

(101) Purdue Univ. Dept.of Agronomy

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1972	53p.	ARC 633.15.P985

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Purdue

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

(Research summary)

9. ABSTRACT

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-RAB-408	
12. DESCRIPTORS	**13. PROJECT NUMBER**
Maize Proteins	**14. CONTRACT NUMBER** CSD-1175 Res.
	15. TYPE OF DOCUMENT

AID 590-1 (4-74)

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

on

THE INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN MAIZE

Contract csd/2809

July 1, 1971 - June 30, 1972

Prepared by

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Submitted to

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

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Introduction

During the second year of this contract further progress and some significant results have been achieved toward the original research objectives. No serious problems arose during the year, but there were some minor difficulties with some material in the winter nursery program. Analytical determinations of protein and lysine were quite accurate and of sufficient number at this stage of the research project.

Based on (1) limited but reasonably successful commercial introduction of opaque-2 types in several countries and (2) recent research results utilizing background interactions and modified types, one can certainly be more optimistic about the successful development of acceptable opaque-2 germplasm in the near future.

Coordination with CIMMYT

A third planning conference was held in March 1972 with CIMMYT personnel in Mexico City. The major conclusions and plans developed during these meetings were as follows:

1. Symposium and Workshop Planning. The Purdue group met with CIMMYT personnel March 6 and 7, 1972 in El Batan, Mexico and drew up a preliminary program for the International Symposium on Protein Quality in Maize to be held at El Batan on December 4-8, 1972. Dr. E. W. Sprague met with the Purdue group in Lafayette on May 10, 1972 to finalize the program for the Symposium. The final program is shown in Appendix A. All speakers have accepted their assignments. A deadline has

been set for receipt of manuscripts and prompt publication of the Proceedings is planned.

In addition to the speakers on the program, invitations have been sent to key representatives from all major countries in Central and South America, Asia, the Middle East, Africa, Europe, Canada and the United States. The total number attending the Symposium will be limited to the seating capacity of the CIMMYT auditorium (143).

2. Status Report. The great need for an up-to-date evaluation and status report on quality protein in maize has been met with the publication: "The Status of Development of Maize with Improved Protein Quality", L. F. Bauman and E. T. Mertz; Research Progress Report 405, Purdue University. Approximately 500 copies have been distributed to interested persons worldwide.

3. Modifiers. Continued emphasis will be given to develop modified or more vitreous opaque-2 germplasm. Detailed research on the effect of these modified phenotypes on yield, protein quality, ear rots and insect resistance is being conducted.

4. Cooperative feeding tests of maize samples. A cooperative feeding test on several different maize, wheat and triticale samples was planned. Several test animals (rat, mouse, chick and meadow vole) were to be used in a study to compare their relative ranking within the several cereal samples.

5. Training Program - Workshop on Analytical Techniques in Maize Improvement. One trainee (Mr. Ricardo Barba-Ho from CIAT, Cali, Colombia) was enrolled in the workshop training program at Purdue the fall semester (1971-72). Three trainees have

been accepted for the school year 1972-73: Mr. Purna B. Shakya from Khumal TAR Government Agricultural Education and Research Department, Nepal (July 8 - December 31, 1972); Mr. Benjamin Nana from IITA, Ibadan, Nigeria (December 9, 1972 - March 9, 1973); and Professor Soeharsono from Gadjah Mada University, Jogjakarta, Indonesia (August 28 - December 31, 1972).

In addition to the above training program, the protein laboratory (Biochemistry Department) provided an amino acid methodology course (Biochemistry 695, Special Topics in Biochemistry) the fall semester, 1971-72 for five foreign graduate students in agronomy who are conducting maize research.

Progress Report on Research

Research progress and initiation of research efforts under this contract are discussed in this section. The main points (capital letters) correspond to the objectives and work plans as originally submitted.

A. New genes and germplasm that may provide improved protein types.

1. Promising new mutants from Colombian and other germplasm. Samples from the generation following that reported last year have again been analyzed for protein and lysine. Other than the one allelic to, and apparently identical with opaque-2, none of this initial group of mutants show promise of being useful in protein improvement breeding programs.

It has now been determined that the gene designated opaque-4 is a recessive allele at the fl_1 locus on the short arm of chromosome 2. A comparison is being made between this allele

and the one recently reported at the same locus and designated $\underline{fl}^a$ by a worker from Argentina.

A new cycle of mutant search has been initiated with CIAT.

2. Opaque-7. The original opaque-7 (W22 inbred) mutant identified by McWhirter [Maize Genetic Cooperation News Letter 45,184 (1971)] was analyzed for amino acid composition and the endosperm proteins were separated into five soluble fractions by the Landry-Moureaux method [Bull. Soc. Chim. Biol. 52 1021 (1970)]. The results have been published [Misra, P. S., Jambunathan, R., Mertz, E. T., Glover, D. V., Barbosa, H. M. and McWhirter, K. S., Endosperm protein synthesis in maize mutants with increased lysine content. Science 176, 1425 (1972)]. The opaque-7 mutant had a vlaue of 3.8 compared to 2.3 lysine as percent of protein for the isogenic normal counterpart. The methionine level was rather high (3.2 methionine as percent of protein) in opaque-7; however, the normal counterpart gave the same value. Opaque-7 causes a repression of zein synthesis and a stimulation of albumin, globulin and glutelin synthesis similar to that found in opaque-2. The original protein value in the W22, opaque-7 isogenic line was low (7.3 percent protein).

We have initiated backcrossing of opaque-7 to several inbred backgrounds. Three different F_2 recoveries of opaque-7 following crosses to the inbreds Oh43, W64A, and B37 were analyzed for lysine and protein content and each gave values of 2.77, 2.77 and 3.22 lysine as percent of protein and 10.4, 10.0 and 9.0 percent protein, respectively. The lysine as percent of protein values are intermediate between normal and opaque-2 and similar to floury-2.

The double mutant combination opaque-2; opaque-7 is not readily identifiable by phenotypic expression of the endosperm. Furthermore, the single gene opaque-7 recovery in various inbred backgrounds is subject to considerable modification and does not give clear-cut expressivity in all cases.

3. Temperate Germplasm. The five populations--Composite D, Syn 200, Syn 493, Syn 201, and Samaru Composite 1--were crossed with Purdue opaque Syn A in the 1971 summer nursery. The opaque Syn A was used as male, with multiple planting dates to assure crossing with various maturities. Populations Composite CD, Composite CBD, Composite AC, Syn 494, Syn 2 and Seleccion Nasal were similarily crossed with Purdue opaque Syn B. The two opaque Syn A and Syn B are made up of opaque-2 versions of U.S. cornbelt inbreds.

These two groups of crosses were random mated within groups in the winter nursery. They are again being random mated in isolated plantings in the 1972 summer nursery. Present plans are to select modified homozygous opaque versions of these populations at Purdue with little agronomic or adaptive selection at possibly three locations in the temperate zone for widespread adaptation.

In the 1972 winter nursery, endosperm mutants su_2, wx, bt_2 ae and du were planted in the isolations and detassled to introduce these genes into these populations. A second intercrossing of these population isolations will be made in the 1972 summer nursery.

4. Selection for high protein in opaque-2 populations. Modified ear-to-row selection is being conducted in opaque-2 Syn A. One cycle of selection is being completed each generation. Selection has been primarily for high protein but with pressure also being applied for increased grain yield. Means and ranges in protein values indicate that considerable variation exists for selection (Table 1).

In 1971, composite samples were taken from all families from the first generation (1970) and from selected families that were used to produce half-sib families for the second cycle of selection. These two composite samples plus five opaque-2 single crosses were evaluated in 1971 in a randomized complete block design with four replications. Results from this evaluation are presented in Table 2. Protein values for the 1970 composite and select composite were greater-than or equal-to protein values for the opaque single crosses. Lysine values for the two composites were equal to values for all single crosses except H55 X A632. The large differential between the composite of all families and the composite of selected families for percent protein and grain yield is of particular interest. This indicates that the selection techniques were effective for increasing percent protein.

Table 1. Means and ranges in percent protein and grain yield for the first two generations of selection.

	Percent Protein	Range in Percent Protein	Yield Bu/A
	1970		
Mean of all half-sib families analyzed	11.2	9.8 - 12.5	87
Mean of selected families	11.6	11.0 - 12.5	90
Mean of selected ears from selected families	13.7	12.2 - 15.9	
	1971		
Mean of all half-sib families analyzed	11.8	10.8 - 12.8	101
Mean of selected families	12.2	11.8 - 12.8	100
Mean of selected ears from selected families	13.3	12.1 - 15.1	

Table 2. Means for percent protein, lysine as percent of protein and grain yield for two composites and opaque single crosses.

	Percent Protein	Lysine g/100g Protein	Grain Yield Bu/A
1970 composite	12.3	4.20	104
1970 select composite	13.4	4.20	110
B37 X W64A	12.6	4.14	99
B37 X A619	11.4	4.36	108
C123 X R177	11.2	4.12	138
H55 X C123	11.1	4.38	146
H55 X A632	11.3	4.87	119
LSD (0.05)	0.7	0.38	8
CV (%)	3.8	6.1	5.0

The third generation of selection in Syn A is being conducted this summer. In addition, the first cycle of modified ear-to-row selection will be completed in an opaque-2 version of Iowa Super Stiff Stalk Synthetic.

B. Modifier Genes

1. Recurrent selection for opaque-2 and floury-2 kernel weight. In the last annual report (pp. 4-7) results of selection for greater kernel weight ratio (mutant/normal) in heterozygous opaque-2 and floury-2 populations were reported. Comparisons of the derived populations per se indicated that selection had increased the mutant to normal kernel ratios, but the improvement was the result of a decrease in the weights of the normal class.

Some of the heterozygous opaque-2 populations have subsequently been evaluated in top crosses to three opaque-2 single cross hybrids. Results of this evaluation are presented in Table 3. The selected populations had higher mutant to normal kernel weight ratios than the unselected. The higher ratios for the selected were the result of the increased weight of the opaque-2 class. Similar results were also found in top crosses with the heterozygous floury-2 populations (Table 4).

Both methods of evaluation indicated that selection improved the mutant to normal kernel weight ratio. In top cross evaluations, the weight of the mutant classes increased while that of the normals were unchanged, but in the population evaluations per se, the weights of the mutant classes changed

Table 3. Mean 200-kernel weights for the opaque-2 and normal classes from segregating ears and their relative weight ratio expressed on a percentage basis from five of the heterozygous opaque-2 populations crossed with three opaque-2 single cross testers.

Testers	Populations				
	Original Synthetic	Select Cycle 2	Control Cycle 2	Select Cycle 3	Control Cycle 3
	Relative Weight Ratio of Opaque-2 to Normal (%)				
H55o_2 x R181Bo_2	85.00	88.16	86.47	88.16	85.41
Oh43o_2 x W64Ao_2	85.46	87.84	85.95	87.80	86.18
A239o_2 x C123o_2	85.82	88.15	85.46	85.70	85.31
Mean	85.43	88.05	85.96	87.22	85.63
	Weight of 200 Opaque-2 Kernels (grams)				
H55o_2 x R181Bo_2	52.82	55.87	52.65	54.27	51.97
Oh43o_2 x W64Ao_2	49.96	50.48	51.34	52.09	51.26
A239o_2 x C123o_2	49.75	52.74	50.34	51.29	49.78
Mean	50.84	53.03	51.44	52.55	51.00
	Weight of 200 Normal Kernels (grams)				
H55o_2 x R181Bo_2	61.66	63.41	61.60	61.63	60.94
Oh43o_2 x W64Ao_2	58.44	57.46	59.80	59.34	59.49
A239o_2 x C123o_2	58.05	59.90	58.91	59.87	58.37
Mean	59.38	60.26	60.10	60.28	59.60

Table 4. Top cross means for 100-kernel weights of Floury-2 and normal classes and their relative weight ratio for the third cycle select and control heterozygous floury-2 populations.

Top Crosses		Genotypic Class	Weight of 100-Kernels (g)	Weight Ratio of Floury-2 to Normal (%)
(Oh 43 $\underline{fl}_2$ x W64A $\underline{fl}_2$)	x Select	$\underline{fl}_2\underline{fl}_2/\underline{fl}_2$	25.86	91.88
(Oh 43 x W64A)		+ +/+	28.26	
(Oh 43 $\underline{fl}_2$ x W64A $\underline{fl}_2$)	x Cont 1	$\underline{fl}_2\underline{fl}_2/\underline{fl}_2$	23.82	82.44
(Oh 43 x W64A)		+ +/+	28.90	
(H55 $\underline{fl}_2$ x B14 $\underline{fl}_2$)	x Select	$\underline{fl}_2\underline{fl}_2/\underline{fl}_2$	26.90	95.42
(H55 x B14)		+ +/+	28.22	
(H55 $\underline{fl}_2$ x B14 $\underline{fl}_2$)	x Control	$\underline{fl}_2\underline{fl}_2/\underline{fl}_2$	23.71	87.86
(H55 x B14)		+ +/+	27.07	

little while the weights of the normals decreased. Some inbreeding may have been present in the derived populations evaluated, since relatively small populations were used in the recurrent selection programs. The greater decrease in the normals compared with the mutants might suggest a differential effect of inbreeding on normal and mutant kernel weights.

2. Comparisons of modified and vitreous portions from modified ears. Phenotypically modified kernels from six opaque-2 ears were degermed. A sample of modified endosperms from each ear was then separated into opaque and vitreous portions. In addition, a sample of whole endosperms was also taken from each ear. These various samples were analyzed for protein and lysine. Results of these analyses are presented in Table 5. For the ears sampled, the opaque portion was lower in percent protein but higher in lysine than the vitreous portion. However, both protein and lysine values obtained for the vitreous portion were more reflective of values obtained for whole endosperms.

3. Genetic study of specific modifying genes on opaque-2 kernel appearance. This study is continuing under the leadership of a graduate student (Mr. Urbano Ribeiral), and a recurrent S_1 selection study for modified "normal" opaques is in progress.

C. Recurrent selection for increased germ size.

1. Selection for larger germ size (higher oil) to maximize nutritional qualities. Three methods of selection for larger germ size (percent oil) were applied to opaque Syn A population. These methods were half- and full-sib family selection (two cycles each) and S_1 progeny selection (one cycle). Two sub-selection

Table 5. Comparison of protein and lysine values from opaque and vitreous portions of modified kernels.

Ear	Component Analyzed	Percent Protein	Lysine g/100 g Protein
1	opaque portion	9.2	4.82
	vitreous portion	11.5	2.55
	whole endosperm	10.5	3.03
2	opaque portion	10.2	3.28
	vitreous portion	13.3	2.08
	whole endosperm	12.7	2.36
3	opaque portion	8.8	4.56
	vitreous portion	12.2	2.34
	whole endosperm	10.9	2.80
4	opaque portion	8.4	4.73
	vitreous portion	12.1	2.44
	whole endosperm	11.2	2.83
5	opaque portion	8.0	5.14
	vitreous portion	12.9	2.33
	whole endosperm	12.0	2.75
6	opaque portion	7.6	4.40
	vitreous portion	12.1	2.30
	whole endosperm	10.0	2.62

procedures were applied in the second cycle of the half-sib method: (1) mass selection, i.e., selection of 30 ears highest in percent oil; (2) combined selection, i.e., selection of three highest ears from each of ten highest families in percent oil. In addition to these methods, progeny from the ten ears highest in percent oil from all 944 ears analyzed in the first cycle were random mated.

The first generation for each of the selection schemes was grown in Indiana and the second in Hawaii. Composite samples from each cycle were evaluated in a randomized complete block design with four replications.

Results from the various cycles of selection are presented in Tables 6 and 7. All selected populations except A3 and A4 were significantly higher in percent oil than the base populations (A1 and A2). Population A10, where the highest intensity of selection was applied, had the highest oil level.

Gain in percent oil per generation (measured from the mean of A1 and A2) for full-sib, half-sib and S_1 progeny selection resulted in a greater gain per cycle since two generations were required for each cycle.

For populations A2 and A9 the germ as percent of kernel and percent of oil in the germ were determined. Results from this analysis indicated that S_1 progeny selection resulted in a statistically significant increase in the germ as percent of the kernel but did not change percent of oil in the germ. Thus it appears that the germ as percent of the kernel was the oil component most affected by selection for oil.

Table 6. Population means for 100-kernel weight, percent of oil and protein in kernel, and lysine as a percent of protein in kernel.

Method of Selection	Cycle	Population Designation	Characters			
			100-Kernel Weight	Percent Oil in Kernel	Percent Protein in Kernel	Lysine as Percent Protein
(Base pop.)	C0	A1	24.080	5.709 d*	12.062 ab	3.891
(Base pop.)	C0	A2	24.063	5.959 c	12.212 ab	3.881
HS**	C1	A3	24.326	6.089 bc	12.500 ab	3.864
HS (combined) 1	C2	A6	24.384	6.343 ab-	12.400 ab	3.892
HS (mass)	C2	A11	23.817	6.344 ab-	12.637 ab	3.782
FS	C1	A4	24.132	6.077 bc	12.675 ab	3.816
FS	C2	A8	23.542	6.348 ab-	12.825 ab	3.649
S_1 progeny	C1	A9	24.625	6.297 ab-	12.887 a	3.795
Mass (10 highest ears)	C1	A10	23.621	6.453 a	12.262 ab	3.775

* Means followed by same letter or letters do not differ significantly 0.05 level of probability (Student - Newman - Keul's test).

** HS and FS indicates half-sib and full-sib, respectively.

Table 7. Mean squares from the analysis of variance from data on 100-kernel weight, percent of oil and protein in kernel, and lysine as a percent of protein in kernel.

Sources of Variation	Mean Squares		Mean Squares	
	100-Kernel Weight (g)	Percent Oil in Kernel	Percent Protein in Kernel	Lysine as Percent Protein
Replications	0.716	0.148	0.553	0.489
Populations	1.229^{ns}	0.558*	0.755*	0.048^{ns}
(A1 + A2) vs (A6 + A8 + A9 + A10 + A11)	0.031^{ns}	1.563*	0.824*	0.044^{ns}
Experimental error	0.901	0.074	0.249	0.036
Sampling error	0.185	0.023	0.096	0.052

* Indicates significance at 0.01 level of probability

ns Indicates nonsignificant difference

Mean values for percent protein in the kernel for the populations are given in Table 6. The difference of 0.46 percent protein between the unselected (A1 and A2) and the selected populations (A6 + A8 + A9 + A10 + A11) was highly significant (Table 7). This significant correlated response can be attributed to the positive correlation (r = 0.48**) between oil and protein.

As protein content increased with selection for oil, lysine values expressed as percent protein were not significantly changed (Tables 6 and 7). Thus, the increase in protein quantity was achieved without a loss in protein quality. In addition, 100-kernel weight and grain yield were not affected by oil selection.

Further selection cycles are being continued with the S_1 progeny procedure.

D. Genetic interactions.

1. Isolation of genetic lines. The study to evaluate several endosperm mutant genes in double and multiple gene combinations with opaque-2 and floury-2 is continuing (see 1970-71 Annual Report, pp. 11-12). This has involved backcrossing the mutant genes to selected inbred backgrounds and recovering near isogenic subline materials for each mutant within an inbred. Most of the mutants have been recovered after 5 to 6 backcrosses within the Oh43, W64A, B37 and C103 backgrounds. The line conversions to the A632 inbred are at the third or fourth backcross generation. Several double mutant genotypes involving the o_2 or the fl_2 gene with the following mutants ae, du, wx, su_2, sh_2, su_1 and fl_2

were each isolated in the Oh43, W64A, B37 and C103 backgrounds. The bt_1 and bt_2 mutants and their double combinations with o_2 or fl_2 have been isolated in Oh43.

Initial crosses have been made among selected endosperm mutant genes and the opaque-2 mutant and the double gene combinations have been isolated. These combinations will be testcrossed in the summer of 1972 and the protein quality analyses made.

2. Evaluation of protein, carbohydrate and nutritive quality in selected single endosperm mutant genes and their double mutant combinations with opaque-2 (part of Mr. G. A. Tosello's Ph.D. thesis program). Four near isogenic subline sets (Oh43, W64A, B37 and C103) each with the following single mutants--ae, du wx, su_2, su_1, sh_2, fl_2 and o_2--and their double combinations with o_2 were crossed in a diallel series and their normal counterparts and the parental subline sets were grown in 1971 and will be repeated in 1972 in a split-split plot randomized complete block design. Ear harvests were made at the 21- and 42-day post pollination and at maturity. The two immature stages are representative of green or edible stages of development. One-hundred kernel weight, shelled weight per ear, kernel density and germ percent were determined for the mature grain samples. Percent protein, lysine and amylose in the endosperm fraction, and percent oil and oil content of whole kernels have been determined on selected genotypes for the mature grain samples. Analyses for protein content and quality, and carbohydrate quality have not been finished for su_1 and sh_2 and their o_2 double

combinations at the mature stage and for all genotypes at both immature stages. The data for the $\underline{+}$, $\underline{o}_2$, $\underline{fl}_2$, $\underline{o}_2\underline{fl}_2$, $\underline{ae}$, $\underline{ae}$ $\underline{o}_2$, $\underline{wx}$, $\underline{wx}$ $\underline{o}_2$, $\underline{su}_2$ and $\underline{su}_2\underline{o}_2$ genotypes were statistically analyzed separately since there were fewer replications and some hybrids missing in the diallel series. The parental (inbred kernels produced on inbred plants by sib-pollination) and hybrid (F_2 kernels produced on F_1 plants by sib-pollination) data were handled separately for the statistical analyses of genotypes, parents or hybrids and their interactions.

The analyses of variance on the one year's data indicated significant differences between genotypes ($\underline{+}$, $\underline{o}_2$, $\underline{fl}_2$, $\underline{o}_2\underline{fl}_2$, $\underline{ae}$, $\underline{ae}$ $\underline{o}_2$, $\underline{wx}$, $\underline{wx}$ $\underline{o}_2$, $\underline{su}_2$ and $\underline{su}_2\underline{o}_2$) within the parental and hybrid sets, among parents for all variables and among hybrids for all variables except kernel density. All interactions were significant for all variables in the parental analyses and, with the exception of kernel density, for all variables in the hybrid analysis indicating the importance of genetic background.

Considering the genotype means over all parents and hybrids, kernel weight and shelled weight per ear of all genotypes, except the $\underline{wx}$ kernel weight in the parental analysis and all genotypes in the hybrid analysis, were less than normal. With few exceptions, the $\underline{o}_2$ gene significantly reduced kernel weight and shelled weight per ear of the double combination lines, as compared to the single non-opaque line. The $\underline{wx}$ $\underline{o}_2$ genotype mean in the hybrid set was the only double combination line that was not significantly lower in shelled weight per

ear than the mean of the o_2 line. The shelled weight per ear comparisons among the several genotypes within one of the better single crosses B37 X C103 were the following percent of the normal line.

o_2	93%	fl_2	68%
$o_2 fl_2$	79%	ae	88%
ae o_2	78%	wx	103%
wx o_2	87%	su_2	93%
$su_2 o_2$	88%		

The mutant gene su_2 had the most pronounced effect on kernel density in both the parental and hybrid lines. The $su_2 o_2$ combination was significantly superior in kernel density to the o_2 and all other o_2 double combinations. Apparently su_2 interacts with o_2 to improve the kernel density of the $su_2 o_2$ combination to near normal quality.

Percent germ in wx was significantly lower than normal in both the parental and hybrid sets and su_2 was not significantly different from normal. The effect of o_2 was pronounced in increasing percent germ. The $su_2 o_2$ in the parental set and $su_2 o_2$ and wx o_2 in the hybrid set were the only double combinations in which the mean values for percent germ were not significantly different from the o_2. The percent germ of all other double combinations was significantly greater than o_2.

The mean percent protein and lysine (g/100 g protein) in the endosperm fraction of the single mutant lines and o_2 double combination lines over parents and hybrids are presented in Tables 8 and 9, respectively. Percent protein of the o_2 genotype mean was significantly lower than the mean of the normal over parents and hybrids. The effect of the o_2 gene was pronounced in significantly reducing the percent protein of all the o_2 double combination lines below that of the comparative single non-opaque line; however, none were significantly lower than the o_2 line itself in either the parent or hybrid set. On an actual mg protein per endosperm basis, wx and ae were the only lines with comparable protein yields to normal with su_2 and fl_2 being very close. The su_2o_2, wx o_2 and o_2fl_2 genotype means over all hybrids were not significantly different in actual protein yield from the single o_2 line.

All mutant and o_2 double combination genotypes, except wx in the parental set, were significantly higher than normal in lysine as percent of protein (Tables 8 and 9). The su_2 genotype produced a 34 and 24 percent increase over normal in lysine percent of protein over parents and hybrids, respectively. The o_2fl_2 genotype was significantly greater than fl_2 in lysine percent of protein, but not significantly different than o_2. The o_2fl_2 genotype over parents and hybrids was significantly inferior to all other double combinations with o_2. Over the parents there was little or no difference (in lysine percent of protein) between the double combinations

Table 8. One-year means of several single endosperm mutant and o_2 double combination lines over parents for percent protein and lysine percent of protein in the endosperm.

Genotype	Endosperm Protein* (%)	Endosperm Lysine (g/100 g Pro.)
+/+	11.50 b**	1.74 e
o_2/o_2	9.33 e	3.40 b
fl_2/fl_2	11.75 b	2.82 c
o_2/o_2 fl_2/fl_2	9.68 cd	3.45 b
ae/ae	12.38 a	2.08 d
ae/ae o_2/o_2	9.50 de	3.91 a
wx/wx	11.53 b	1.81 e
wx/wx o_2/o_2	9.20 e	3.78 a
su_2/su_2	12.43 a	2.34 d
su_2/su_2 o_2/o_2	9.95 c	3.75 a
du/du	12.03***	2.00**
du/du o_2/o_2	9.55	3.86

* Fat free, dry basis.

** Means within a column followed by a common letter do not differ significantly at the 1 percent level of significance as determined by Duncan's Multiple Range Test. Mean values are from four parental lines and four replications. All means were statistically analyzed on inverse sine transformed means.

*** Single gene mutant significantly different from its respective o_2 double combination at the 0.01 level. Mean values over four parental lines and two replications for du and du o_2

Table 9. One-year means of several single endosperm mutant and o_2 double combination lines over hybrids for percent protein and lysine percent of protein and endosperm.

Genotype	Endosperm Protein* (%)	Endosperm Lysine (g/100 g Pro.)
+/+	10.90 c**	1.74 b
o_2/o_2	9.53 e	3.30 c
fl_2/fl_2	12.25 a	2.67 d
o_2/o_2 fl_2/fl_2	10.13 d	3.24 c
ae/ae	12.05 a	1.99 f
ae/ae o_2/o_2	9.30 e	3.90 a
wx/wx	10.88 c	1.86 g
wx/wx o_2/o_2	9.30 e	3.63 b
su_2/su_2	11.50 b	2.16 e
su_2/su_2 o_2/o_2	9.55 e	3.70 b
du/du	11.42***	2.04*
du/du o_2/o_2	10.13	3.56

* Fat free, dry basis.

** Means within a column followed by a common letter do not differ significantly at the one percent level of significance as determined by Duncan's Multiple Range Test. Means are from six hybrid lines and four replications. All means were statistically analyzed on inverse sine transformed means.

*** Single gene mutant significantly different from its respective o_2 double combination at the 0.05 level. Mean values over three hybrids and two replications for du and du o_2.

with o_2, other than $o_2 fl_2$ (Table 8). In the hybrid set, however, ae o_2 was significantly superior to the other double combinations with o_2. The $su_2 o_2$ and wx o_2 genotypes were not significantly different from one another, while du o_2 was lower than either of the latter two genotypes, but greater than $o_2 fl_2$.

The amylose content of mature endosperm starch samples from two replications of the normal (+), o_2, su_2, $su_2 o_2$, ae and ae o_2 genotypes of the parental and the hybrid lines from the experiment described above were determined and statistically analyzed as a group. The analyses of variance indicated significant differences between genotypes and nonsignificant differences among parents and among hybrids as well as no interaction effects in either set. Percent amylose for the o_2 genotype in both sets was not significantly different from normal (Table 10). The effect of the o_2 gene on percent amylose in the o_2 double combination lines was different. The ae o_2 showed some decrease in percent amylose while $su_2 o_2$ resulted in significant increase in percent amylose over their respective non-opaque-2 single mutant lines.

The percent oil and oil content of whole kernels from the su_2, $su_2 o_2$, o_2 and normal (+) genotypes of four parental lines and their six single-cross hybrids were determined. Significant differences existed between genotypes, among parents and among hybrids for both variables. All interactions were significant indicating the effect of genetic background. A comparison is given among percent oil and oil content per kernel in Table 11 along with percent germ and lysine content

Table 10. One-year means of selected single endosperm mutants and their o_2 double combination lines over parents and hybrids for percent amylose in the starch.

Genotype	Amylose Percent	
	Parental Means	Hybrid Means
+/+	24.68 d*	26.49 e
o_2/o_2	25.73 d	26.37 e
su_2/su_2	35.44 c	35.58 d
su_2/su_2 o_2/o_2	39.55 b	39.97 c
ae/ae	57.11 a	59.50 a
ae/ae o_2/o_2	56.96 a	54.93 b

* Means within a column followed by a common letter do not differ significantly at the one percent level of significance as determined by Duncan's Multiple Range Test. Mean values are from four parental lines and six single-cross hybrids and two replications in each set.

Table 11. One-year means of su_2, su_2o_2, o_2 and normal genotypes over parents and hybrids for percent oil and oil content in the whole kernel, lysine (g/100 g pro.) in the endosperm, and percent germ - 1971.

Genotype	Parental Means				Hybrid Means			
	Oil (%)	Oil/Kernel (mg.)	Germ (%)	Endosperm Lysine (g/100 g Pro.)	Oil (%)	Oil/Kernel (mg.)	[illegible] (%)	Endosperm Lysine (g/100 g Pro.)
$+/+$	4.43 d*	10.67 b	13.73 b	1.74 d	4.21 d	14.63 b	13.99 b	1.74 d
o_2/o_2	5.32 c	11.19 b	17.77 a	3.40 b	5.05 c	14.58 b	18.14 a	3.30 b
su_2/su_2	5.81 b	12.49 a	13.87 b	2.34 c	5.57 b	16.78 a	13.99 b	2.16 c
su_2/su_2 o_2/o_2	6.42 a	12.47 a	18.20 a	3.75 a	6.36 a	17.19 a	18.10 a	3.70 a

* Means within a column followed by a common letter do not differ significantly at the one percent level of significance as determined by Duncan's Multiple Range Test. Mean values are from four parental lines and six single-cross hybrids and four replications in each set.

previously discussed for these genotypes. It is apparent that the $\underline{su}_2$ gene significantly increased percent oil. What is even more important is the fact that actual oil yield per kernel (mg oil/kernel) in $\underline{su}_2$ and the double combination with $\underline{o}_2$ was significantly increased over both normal and $\underline{o}_2$ while the percent germ in the $\underline{su}_2$ remained the same as normal. Overall hybrids $\underline{su}_2$ and $\underline{su}_2\underline{o}_2$ were 15 and 18 percent respectively, better in oil yield than their normal counterpart. Even though the percentage of oil was significantly increased in $\underline{o}_2$, the actual oil yield per kernel was not significantly different than normal. This is apparently due to the fact that the percent germ in $\underline{o}_2$ was increased by 30 percent and perhaps no significant change in actual oil concentration occurred.

The $\underline{su}_2$ gene and its $\underline{o}_2$ double combination ($\underline{su}_2\underline{o}_2$) have definite possibilities of improving the feeding value of grain for human and animal consumption with its improved protein and caloric value along with superior digestibility characteristics (see section D-3).

Analysis were not completed as of this report on the $\underline{su}_1$ and $\underline{sh}_2$ genotypes and their double combinations with $\underline{o}_2$ over parents and hybrids. However, preliminary data from the protein quality analyses of the 21- and 42-day old post-pollinated materials substantiate last year's findings, i.e., these genotypes are exceptionally high in protein quality and offer potential for their food value as edible corns in the green corn or roasting ear stage of development. A field replicated trial of the $\underline{bt}_2$ and $\underline{bt}_2\underline{o}_2$ genotype in the Oh43

inbred background was repeated again this year and the percent protein and lysine (g/100 g protein) in the mature endosperm of bt_2 was 15.2 and 3.07, respectively, and that of bt_2o_2 was 14.9 and 4.93, respectively. We have reported the endosperm proteins of near isogenic (Oh43 inbred) su_1, sh_2 and bt_2 as having increased lysine, albumins, globulins and glutelin-3, and a reduction in zein, when these genes are either present singly or combined with o_2 [Science 176:1425 (1972)].

These results in the single-cross hybrid backgrounds, even though from one-year data and thus preliminary in nature, are in agreement with the parental data and those data for the near isogenic materials (Oh43) reported in last year's annual report. The data suggest the potential for producing grain with improved protein quality and digestibility characteristics (see section D-3), higher caloric content and modified starch types which could be of considerable value in their application in foods and feeds for human and animal consumption.

3. In vitro digestibility characteristics of endosperm mutants and their double combinations with opaque-2. Dr. Linda Bartolome, Postdoctorate, initiated a study to evaluate the digestion rate of raw-starch and whole kernel sample preparations by respirometry. The respirometry method developed by Dr. Bartolome is based on the measurement of the loss of oxygen during yeast fermentation of the sugars produced by amyloglucosidase digestion using a reaction system adapted to a differential respirometer. The normal, 13 endosperm mutants and their double combinations with o_2 in the Oh43 inbred

were analyzed. Each genotype was replicated seven times in the respirometry analyses for both the raw-starch and the whole kernel preparations. The data were subjected to curvilinear regression analysis and the best fit model for each genotype was found. Regression analysis was also used to test differences among genotypes and among groups of genotypes.

A comparison of digestion rates from the raw-starch samples for the single and double combination genotypes is presented in Table 12. There were very wide differences in the digestion rate of the raw-starch samples. All of the raw-starches, except ae, ae o_2 and du, digest more rapidly than the normal corn. The du raw-starch digestion rate was slower but not significantly different from the normal starch. The ae and ae o_2 were extremely resistant to digestion. The su_2o_2, bt_2o_2, wx o_2, su_1 and su_2 raw-starches far exceeded the digestion rate of the normal raw-starch. The o_2 was 42 percent higher in digestibility than the normal raw-starch and the combinations of o_2 were significantly higher in digestion rate than the single non-opaque-2 gene in the combinations, except for the ae, su_1 and bt_1 combinations.

The wx o_2, su_2 and su_2o_2 produced the highest whole kernel digestion rates (Table 12). The differences among the genotypes for the whole kernel samples were not as great as they were for raw-starch samples and there are some differences in digestion rate patterns from those observed in raw starches. For example, ae, bt_1 and bt_2 digest more rapidly than their respective combination with o_2. The su_1 and su_1o_2 digestion

Table 12. The comparisons of susceptibility of several mutant raw-starch and whole-kernel samples to hydrolysis by amyloglucosidase enzyme.

Raw-Starch			Whole Kernel		
Group* No.	Genotype	Percent of Normal at 6-hr. Digestion Period**	Group* No.	Genotype	Percent of Normal at 6-hr. Digestion Period
1	ae, aeo_2	12.9	1	aeo_2	35.3
2	du	98.1	2	su_1	51.8
3	fl_2, duo_2	110.8	3	du	63.9
4	fl_1, h	121.3	4	fl_2, sh_1, fl_2o_2	74.5
5	ho_2	132.5	5	fl_1	80.8
6	sh_1	134.0	6	h_1duo_2	86.5
7	bt_1, sh_2, wx, o_2, fl_1o_2, fl_2o_2, su_1o_2, bt_1o_2	141.6	7	ae	88.2
8	sh_2o_2	152.6	8	sh_2, bt_2o_2, wx	88.5
9	bt_2	155.9	9	su_1o_2	91.0
10	sh_1o_2	166.5	10	bt_1, bt_2o_2	96.0
11	su_1, su_2, bt_2o_2, wxo_2	184.5	11	sh_1o_2	101.0
12	su_2o_2	205.5	12	bt_2	105.9
			13	o_2, fl_1o_2, ho_2, sh_2o_2	107.9
			14	su_2, su_2o_2	125.4
			15	wxo_2	139.5

* The model curve for each group is significantly different from every other group at the 0.01 level.

** Total μl of oxygen consumed for the mutant genotype in question divided by the μl of oxygen consumed by the normal sample.

rates were significantly lower than the normal sample in the early periods of digestion, but were much more rapid in the mid-to-late periods of the digestion trial. More inherent variability in the whole kernel analysis would be expected because of differences among genotypes for hydrolytic enzymes, carbohydrates and sugars, as well as other factors. Nonetheless, of considerable interest is the fact that su_2, su_2o_2 and $wx\ o_2$ were consistently superior in producing the highest digestion rates in both types of sample preparation.

4. Influence of mutant endosperm genes on kernel and cob weights. Opaque-2, waxy, amylose-extender and normal versions of three inbred lines (A632, A619 and B37) constituted the source material for this study. A diallel set ($3F_1$ hybrids) was made for each version. Three treatments, (1) sibbing, (2) outcrossing to the normal version and (3) no pollination, were assigned at random to each sub-plot.

Kernel weight per ear and 100-kernel weight of opaque-2 hybrids were 18.4 and 18.2 percent less than the normal, respectively (Table 13). For amylose-extender hybrids these two characters were about 10 percent less than for normal. Kernel weight per ear for waxy was not significantly different from normal; whereas for 100-kernel weight, normal was slightly greater than waxy.

Outcrossing increased kernel weight per ear (when compared to sibbing) by 14.6 and 10.0 percent for opaque-2 and amylose-extender, respectively. The increase in 100-kernel weight due

Table 13. Kernel weight per ear, 100-kernel weight, and cob weight means of sibbed and outcrossed ears from four endosperm mutant versions of three single cross hybrids.

	Sibbed				Outcrossed to Normal		
Hybrids	+	o_2	wx	ae	o_2	wx	ae
			Kernel Weight Per Ear				
A632 x B37	205.3	172.2	219.9	185.5	201.1	213.5	211.9
A632 x A619	219.8	188.4	226.2	210.2	224.1	215.7	221.3
A619 x B37	248.0	188.3	240.2	204.1	204.0	247.9	226.7
			100-Kernel Weight				
A632 x B37	31.1	24.7	29.0	25.9	26.7	30.0	29.9
A632 x A619	31.5	26.0	28.5	32.5	30.9	29.7	31.8
A619 x B37	32.3	26.8	30.0	27.9	28.0	30.4	32.0
			Cob Weight				
A632 x B37	43.8	40.4	52.0	44.5	42.6	49.4	46.7
A632 x A619	42.4	39.5	42.8	41.2	41.5	42.8	43.3
A619 x B37	55.2	39.6	53.3	50.4	42.2	53.9	52.7

to outcrossing was 10.4 percent in opaque-2, 8.6 percent in amylose-extender and 2.8 percent in waxy.

Cob weights of opaque-2 hybrids were 15.4 percent less than the normal counterparts. For amylose-extender and waxy hybrids, cob weights were approximately equal to the normal counterpart. No effect of outcrossing on the cob weight of waxy was detected. Outcrossing in opaque-2 and amylose-extender resulted in approximately a 5 percent increase in cob weight compared to sibbing.

The effect of opaque-2 gene on cob weight was dependent upon the genetic background. In A632 X A619, cob weights of opaque-2 and normal were about the same; whereas in A619 X B37, cob weight of the mutant was 29.8 percent less than the normal counterpart. There was no significant difference in cob weights between the other mutants (wx and ae) and their normal counterparts. Thus, from the mutants sampled it appears that only opaque-2 resulted in a significant decrease in cob weight. Measurement of length and diameter of cobs from the opaque-2 and normal hybrids revealed no significant difference between the two.

Opaque-2 and normal versions of four standard single cross hybrids plus four opaque-2 experimental hybrids--two opaque and two phenotypically modified--will be evaluated for the rate of dry matter accumulation in the kernel and cob.

E. Research on improved analytical methods.

1. Evaluation of new assay for total protein in grains. Johnson and Craney [Johnson, R. M. and Craney, D.E., Rapid biuret

method for protein content in grains. Cereal Chem. 48:277 (1971)] proposed a new rapid biuret method for the determination of protein levels in barley, corn, oats, sorghum and wheat. The Biochemistry Laboratory has evaluated this method for the rapid determination of protein in high lysine maize. Our group found that the absorbence values for the opaque-2 maize samples were consistently lower at a given nitrogen level than the normal isogenic maize counterpart. Protein fractionation studies revealed that the biuret color intensity in maize samples varies with the level of zein. Zein protein gives four times more biuret color per unit of nitrogen than glutelin protein.

Because of the reversal in the zein to glutelin ratio in opaque-2 corn, the biuret absorbence per unit of Kjeldahl nitrogen is 50 to 80 percent of that in normal corn. Since both the biuret reaction and the determination of protein are simple rapid procedures requiring a minimum of equipment and technical skills, estimation of the biuret absorbence per unit of Kjeldahl nitrogen should be useful as a qualitative screening tool in the rapid identification of the opaque-2 character for high lysine in corn breeding programs. This may prove especially important now that opaque-2 endosperm is being altered by modifying genes to the extent that the high lysine grain is not easily visibly differentiated from normal vitreous-type endosperm. A manuscript on these findings has been accepted by Cereal Chemistry (Barba-Ho, R., Misra, S., Mertz, E.T., and Glover, D.V., Studies on Corn Proteins V. Reduced

Color Response of Opaque-2 Corn Protein to the Biuret Reagent, and its Use for the Rapid Identification of Opaque-2 Corn).

F. Central biochemical laboratory.

1. Cooperative research with Dr. E. Villegas (CIMMYT). A research bulletin on simple methods for determining nitrogen, tryptophan and lysine in maize screening programs has been published (see p. 44). This was coauthored by Dr. Mertz and Dr. Villegas.

   At the March planning session in El Batan, a cooperative nutritional testing program with several laboratories was agreed upon. This is discussed under section G.

2, Basic research on the nature of the proteins in high lysine types of maize. Dr. P. S. Misra has completed the fractionation of the endosperm proteins in three floury mutants with increased lysine (o_2, fl_2 and o_7) and ten starch modifying mutants (su_1, sh_1, sh_2, sh_4, bt_1, bt_2, ae, du, wx, su_2 in the Oh43 inbred background). The first six starch-modifying mutants in parentheses have increased lysine levels in the endosperm. The ae, du and su_2 mutants show some increased lysine levels in the endosperm while wx above is not different from normal. All ten have been crossed with o_2 to form the double mutants and the endosperm proteins in the double mutants have been fractionated using the Landry-Moureaux method.

   Table 14 summarizes the protein distribution in the single mutants and their isogenic controls. Table 15 shows the protein distribution in the double mutants.

Table 14. Nitrogen distribution in maize endosperms of single mutants.

Fraction	Soluble nitrogen (per cent of total)														
	Oh43													W 22	
	+	fl_2	o_2	su_1	sh_1	sh_2	sh_4	bt_1	bt_2	ae	du	wx	su_2	+	o_7
I (saline)	5.8	9.2	13.6	11.9	8.2	12.3	25.7	8.8	12.1	7.3	6.8	7.8	7.2	6.9	16.6
II (zein)	59.0	49.1	26.9	27.1	43.7	29.4	30.8	35.9	26.1	34.7	39.2	46.1	45.6	40.6	20.3
III (glutelin-1)	5.8	9.0	8.4	21.9	12.3	9.4	7.7	16.4	15.4	17.6	13.3	11.4	13.5	15.3	12.0
IV (glutelin-2)	12.7	7.6	14.0	9.1	14.3	15.0	8.3	8.2	8.7	11.7	14.0	10.8	10.8	12.8	18.8
V (glutelin-3)	13.8	22.0	29.2	22.8	16.3	23.6	23.6	27.4	27.9	19.9	18.4	13.4	16.6	21.0	29.5
Total nitrogen extracted	97.1	96.9	92.1	92.8	94.8	89.7	96.1	96.7	90.2	91.2	91.8	89.5	93.7	96.6	97.2

Table 15. Nitrogen distribution in maize endosperms of Oh 43 double mutants.

	Soluble nitrogen (percent of total)											
	+	fl_2o_2	su_1o_2	sh_1o_2	sh_2o_2	sh_4o_2	bt_1o_2	bt_2o_2	$ae\ o_2$	$du\ o_2$	$wx\ o_2$	su_2o_2
I (saline)	5.8	17.0	22.7	39.9	25.3	43.3	23.3	22.3	13.5	14.3	14.0	15.3
II (zein)	59.0	26.0	3.0	1.8	1.2	6.5	2.7	2.9	17.3	12.1	20.6	16.3
III (glutelin-1)	5.8	15.2	9.0	1.6	1.1	3.9	2.5	5.5	11.3	6.6	9.0	13.8
IV (glutelin-2)	12.7	9.9	14.2	16.4	26.1	8.7	13.1	12.2	13.1	15.9	13.6	11.4
V (glutelin-3)	13.8	24.8	45.3	32.2	35.4	26.8	50.2	48.0	30.8	33.7	29.5	31.4
Total nitrogen extracted	97.1	92.9	94.2	91.9	89.1	89.2	91.8	90.9	86.0	82.6	86.7	88.2

In Table 15 it can be seen that all double mutants (o_2o_7 is not included) show some reduction in zein greater than that with o_2 alone, except for fl_2. Reduction is so marked with several of the combinations that the zein almost disappears. Part of these data have been published in Science (see p. 44, Misra, et. al., 1972). In the Science article we propose the hypothesis that the mechanism of inhibition of zein synthesis in the floury mutants such as o_2 and fl_2 is different from that in the starch-modifying mutants such as bt_2.

Complete amino acid patterns (except for tryptophan) have been obtained on the Landry-Moureaux fractions from several high lysine single mutants. The range of lysine values in these fractions was as follows (grams of lysine per 100 grams of protein): I, 4.3-6.3; II, 0.1-0.5; III, 0.4-0.6; IV, 1.4-2.9; and V, 5.7-7.0. The gel electrophoresis patterns of these fractions from different mutants are being examined to detect differences in protein composition.

G. Protein nutritive value.

1. Cooperative feeding tests. Dr. Mertz has agreed to serve as coordinator for cooperative feeding tests in different species on ten cereal samples supplied by CIMMYT through Dr. Villegas (opaque-2, both vitreous and floury, normal maize, wheat and triticales) and five cereal samples supplied by Purdue (floury-2, normal maize and sorghums). All samples have been distributed and analyses have been made at Purdue for moisture, protein, fiber, oil and ash. A uniform diet

has been formulated for testing the PER (Protein Efficiency Ratio) of these samples in the chick (Washington State), the rat (Purdue and INCAP), the meadow vole (CIMMYT), the mouse (Nebraska) and the cricket (Missouri). It is hoped that the results of these feeding tests can be reported at the International Symposium on Protein Quality in Maize in December 1972.

H. Factors affecting maize adaptation.

During their first year of operation, various problems of instrumentation, plant data recording and communication were encountered. However, most difficulties have been solved and data collection is believed to be progressing satisfactorily at Maracay, Venezuela; Bogota, Palmira and Monteria, Colombia; Mexico City, Cuernavaca and Poza Rica, Mexico; Portageville and Colombia, Missouri; Fort Collins and Rocky Ford, Colorado; Madison, Wisconsin; and Lafayette, Indiana. Four substitutions were made in the 1971 uniform group of 12 corn entries for 1972 use. A computer program for the initial summary of the data has been written and de-bugged. The remaining 1971 data are being punched on cards.

Summary

1. Two modified opaque-2 synthetic populations are being developed from several temperate germplasm sources.

2. Large variations were observed for percent protein in the opaque-2 Syn A population.

3. In opaque-2 Syn A the germ as percent of the kernel was the oil component most affected by S_1 progeny selection for percent oil.

4. Selection for percent oil in opaque-2 Syn A resulted in a significant increase in percent protein.

5. Full sib, half-sib and S_1 progeny selection for percent oil in an opaque-2 population resulted in an equal gain in percent oil per generation.

6. One year means of a diallel set of hybrids with specific starch modifying genes and their opaque-2 double combinations showed considerable variation for background interaction for kernel weight, kernel weight per ear, kernel density, percent germ, protein and lysine, percent oil and oil yield.

7. Waxy-opaque-2, su_2o_2, $ae\ o_2$ and $du\ o_2$ hybrids were superior to o_2fl_2 in kernel weight (except $du\ o_2$), kernel weight per ear and lysine as percent of protein. Percent protein was greater in the o_2fl_2 and $du\ o_2$ hybrids.

8. The sugary-2 gene interacts with opaque-2 to improve the kernel density of the su_2o_2 combination.

9. The su_2o_2 and $wx\ o_2$ hybrids show definite promise for increasing lysine percent of protein. The $ae\ o_2$ combination was greater in lysine percent of protein but was inferior in yield in the better hybrids.

10. The *in vitro* digestibility rates of su_2, su_2o_2 and $wx\ o_2$ were consistently superior to other genotypes in both raw-starch and whole kernel sample preparations.

11. Oil yields per kernel of su_2 and su_2o_2 hybrids averaged 15 and 18 percent better, respectively, than normal. In the better producing hybrids, oil yields were 20 and 25 percent better, respectively. These genotypes show particular promise for improving total caloric and nutritive value along with improved kernel density and digestibility.

12. Cob weights of opaque-2 hybrids were 15 percent less than normal, and this differential was not significantly changed upon outcrossing with normal pollen.

13. Cob weights of amylose-extender and waxy hybrids were not significantly different from the normal counterparts.

14. A simple rapid procedure for determining protein requiring a minimum of equipment and technical skills has been described and will be published in Cereal Chemistry (in press). The procedure should be useful as a qualitative screening tool in rapid identification of the opaque-2 and modified "normal" opaque-2 character for high lysine in corn breeding programs.

15. Endosperm protein fractionation studies of ten starch modifying mutants and their double combinations with opaque-2 have shown that six of the starch modifying mutants have increased lysine levels and marked reduction in zein content. The mechanism of inhibition of zein synthesis in the floury mutants such as opaque-2 and floury-2 may be different from that in the starch-modifying mutants with reduced zein content.

The research activities of the second year of this project have generated considerable optimism for the successful development and utilization of acceptable opaque-2 and modified "normal" opaque-2 germplasm in the near future. Preliminary one-year tests show that some opaque-2 materials developed at Purdue do have comparable yield levels to competitive normal hybrids. The opaque-2 kernel types are being modified by genetic modifying factors to produce a much harder endosperm type with a vitreous appearance. This is an added bonus which will enhance even further the usefulness of this high-quality corn. These types will not only improve appearance and acceptance of the grain, but most likely improve grain quality for handling and storage in addition to reducing ear rots and insect damage.

Promising methods of selection and breeding procedures for the long-range nutritional improvement of maize are being continued. Significant increases in protein content have been obtained through selection.

Special types of corn with characteristic high-quality protein may be produced in the future from combining specific starch modifying

mutants with opaque-2. The waxy-opaque-2 and sugary-2-opaque-2 combinations show promise for increasing total caloric value and digestibility of the grain, and improving biological value and kernel characteristics.

A simple rapid procedure for identifying opaque-2 corn has been described and tested at Purdue. This should be useful as a qualitative screening tool in rapid identification of the opaque-2 and modified "normal" opaque-2 character for high lysine in corn breeding programs and as an aid in the identification and handling of this high-quality modified "normal" opaque-2 grain in commercial seed channels.

Optimism is further expressed in the accumulation of information from feeding trials with humans, swine and now with cattle that show better gains with opaque-2 corn compared with normal corn. This, coupled with the fact that comparable yield levels may be obtainable through utilization of background interactions and modified types, encourages confidence that this concerted research effort will have its impact in world maize production and improvement of nutrition in LDCs.

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APPENDIX A

PROGRAM FOR
CIMMYT - PURDUE INTERNATIONAL SYMPOSIUM
ON PROTEIN QUALITY IN MAIZE

CIMMYT-PURDUE

INTERNATIONAL SYMPOSIUM

ON PROTEIN QUALITY IN MAIZE

INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER

El Batan, Mexico

MONDAY
December 4, 1972

9:30-9:45	Welcoming Address	Haldore Hanson, CIMMYT
9:45-10:15	Worldwide Needs for Quality Protein	A. M. Altschul, U.S.A.

NUTRITIONAL STUDIES ON QUALITY PROTEIN MAIZE

Chairman: J. Cravioto

10:15-10:30	Current Issues and Problems	J. Cravioto, Mexico
10:30-11:00	Quality Protein Maize in Human Nutrition	A. G. Pradilla, Colombia
11:00-11:30	Improving Maize Diets with Amino Acid and Protein Supplements	R. Bressani, INCAP
11:30-12:00	Quality Protein Maize in Swine Nutrition	J. Maner, CIAT
12:00-1:00	Group Discussion	
1:00-2:30	Lunch	

BREEDING FOR PROTEIN QUALITY IN MAIZE - I

Chairman: D. E. Alexander

2:30-2:45	Current Issues and Problems	D. E. Alexander, U.S.A
2:45-3:15	Selection Systems for Increasing Protein Quality with the Opaque-2 Gene in Flint and Dent Maize	V. L. Asnani, India J. Singh, India

3:15-3:30	Coffee	
3:30-4:00	Selection Systems for Increasing Protein Quality with the Opaque-2 Gene in Floury Maize	C. A. Francis, CIAT F. Scheuch, Peru
4:00-4:30	Selection for Lysine and Yield in Opaque-2 and Floury-2 Synthetics	J. W. Dudley, U.S.A.
4:30-5:30	Group Discussion	

TUESDAY
December 5, 1972

BREEDING FOR PROTEIN QUALITY IN MAIZE - II

Chairman: V. Carañgal

9:30-9:45	Current Issues and Problems	V. Carañgal, Philippines
9:45-10:15	Systems for Rapid Development of Quality Protein Maize Varieties	E. C. Johnson, CIMMYT M. N. Harrison, IITA
10:15-10:45	Agronomic Aspects of Producing Quality Protein Maize	D. Sperling, U.S.A P. R. Goldsworthy, CIMMYT
10:45-11:00	Coffee	
11:00-11:30	Disease Insect Interactions in Quality Protein Maize	A. Ortega, CIMMYT
11:30-12:30	Group Discussion	
12:30-2:00	Lunch	

BREEDING FOR PROTEIN QUALITY IN MAIZE - III

Chairman: O. E. Nelson

2:00-2:15	Current Issues and Problems	O. E. Nelson, U.S.A
2:15-2:45	Use of Genetic Modifiers to Obtain Normal Type Kernels with the Opaque-2 Gene	S. K. Vasal, CIMMYT

2:45-3:15	Germ and Endosperm Variability as Related to Oil Content and Modifier Genes in Opaque-2 Maize	L. F. Bauman, U.S.A.
3:15-3:30	Coffee	
3:30-4:00	Genetics of Endosperm Mutants in Maize as Related to Protein Quality and Quantity	D. V. Glover, U.S.A. P. L. Crane, U.S.A.
4:00-4:30	Approaches to Improving Protein Quality in Maize without the Use of Specific Mutants	M. S. Zuber, U.S.A.
4:30-5:30	Group Discussion	

WEDNESDAY
December 6, 1972

COMMERCIAL PRODUCTION OF QUALITY PROTEIN MAIZE

Chairman: F. Poey

9:30-9:45	Current Issues and Problems	F. Poey, Mexico
9:45-10:15	Worldwide Seed Industry Experience with Opaque-2 Maize	W. L. Brown, U.S.A.
10:15-10:45	Production and Acceptance of Opaque-2 Maize in Colombia	C. Casselett, Colombia
10:45-11:00	Coffee	
11:00-11:30	Production and Acceptance of Opaque-2 Maize in Brazil	A. Secundino, Brazil
11:30-12:00	Utilizing Opaque-2 Maize in Food Products	D. Robinson, U.S.A.
12:00-1:00	Group Discussion	
1:00-2:30	Lunch	

CHEMICAL AND BIOLOGICAL EVALUATION OF MAIZE PROTEIN QUALITY

Chairman: J. Wall

2:30-2:45	Current Issues and Problems	J. Wall, U.S.A.
2:45-3:15	Characteristics of Proteins in Single and Double Endosperm Mutants of Maize	P. S. Misra, U.S.A.
3:15-3:30	Coffee	
3:30-4:00	Use of Small Animals for Evaluation of Protein Quality in Cereals	E. T. Mertz, U.S.A.
4:00-4:30	An Integrated System for Chemical Screening of Quality Protein Maize	E. Villegas, CIMMYT
4:30-5:30	Group Discussion	
7:00	Symposium Banquet	
	Master of Ceremonies	H. F. Robinson, U.S.A.
	Meeting World Food Needs	N. E. Borlaug, CIMMYT

THURSDAY
December 7, 1972

ECONOMIC IMPLICATIONS OF QUALITY PROTEIN IN MAIZE

Chairman: O. J. Kelley

9:30-9:45	Current Issues and Problems	O. J. Kelley, U.S.A.
9:45-10:15	Cost-Benefit Relationships in Quality Protein Maize Production	Per Pinstrup-Anderson, CIAT
10:15-10:45	The Economic and Technical Feasibility of Improving Protein Quality of Food Through Fortification and Supplementation	M. J. Forman, U.S.A.
10:45-11:00	Coffee	
11:00-11:30	Systems for Rapid Introduction of Quality Protein Maize Varieties into National Production Programs	E. W. Sprague, CIMMYT

11:30-12:30	Discussion	
12:30-2:00	Lunch	

PROGRESS IN BREEDING FOR QUALITY PROTEIN IN OTHER CEREALS

Chairman: H. Doggett

2:00-2:15	Current Issues and Problems	H. Doggett, Canada
2:15-2:35	Status of Protein Quality Improvement in Sorghum	J. D. Axtell, U.S.A. R. C. Pickett, U.S.A.
2:35-2:55	Status of Protein Quality Improvement in Wheat	P. Mattern, U.S.A.
2:55-3:15	Status of Protein Quality Improvement in Oats	W. L. Clark, U.S.A.
3:15-3:30	Coffee	
3:30-3:50	Status of Protein Quality Improvement in Triticales	F. J. Zillinsky, CIMMYT
3:50-4:10	Status of Protein Quality Improvement in Barley	L. Munck, Sweden
4:10-5:30	Group Discussion	

FRIDAY
December 8, 1972

WORKSHOPS

9:30-12:30

		Leaders:
Group 1	Breeding Methodology	E. C. Johnson, CIMMYT S. K. Vasal, CIMMYT
Group 2	Chemical and Biological Analytical Techniques	E. Villegas, CIMMYT E. T. Mertz, U.S.A.
Group 3	Methods of Introducing Improved Protein Varieties at the Farm Level	I. Narvaez, Mexico A. Secundino, Brazil

		Leaders:
Group 4	Economic and Social Factors in Acceptance of Improved Protein Varieties	D. Winkelmann, CIMMYT D. W. Thomas, U.S.A.

12:30-2:00 Lunch

2:00-3:00 Plenary Session - Workshop

Summaries by Leaders

Chairman: R. Osler, CIMMYT

2:00-2:15	Breeding Methodology
2:15-2:30	Chemical and Biological Analytical Techniques
2:30-2:45	Methods of Introducing Improved Protein Varieties at the Farm Level
2:45-3:00	Economic and Social Factors in Acceptance of Improved Protein Varieties