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Introduction

The initiation of research under this contract was rapidly accomplished and significant progress has been achieved during the first year. This was possible in many areas because research or background development was already underway at Purdue.

To delineate pertinent research areas and develop cooperative efforts, two coordinating conferences have been held with CIMMYT personnel (May 1970 and February 1971). The more important points derived from these meetings are as follows:

1. Symposium - There is an urgent need for a thorough and updated presentation of research information in the area of protein quality and quantity in maize. Definite time and place for this cannot be decided until needed financial support is obtained. Sources of financial support are currently being investigated.
2. Analytical methods - Development of satisfactory analytical techniques has been made and a publication covering these procedures is in press (more details given in results section).
3. Current Status Report - There is a great need to keep researchers informed of recent results and procedures. A report has been prepared and is presently being reviewed by CIMMYT and CIAT personnel.
4. Superiority of opaque-2 - Based on research to date, the opaque-2 offers more promise than floury-2 or the double mutant in improving protein quality.
5. Modifiers - A number of modified opaque-2 types have been detected. Research should be expanded on those that give opaque-2 with more

normal phenotype to improve both agronomic performance and acceptability for use in the human diet.

6. Training - The need for a training program in analytical techniques was emphasized and Purdue will offer this in cooperation with CIMMYT in 1971-72.

7. Optimum levels of Lysine, Tryptophan and Protein - Considerable discussions in this area failed to produce any definite conclusions. It was felt that analytical values are good indicators of the biological value of opaque-2 since no great differences in digestibility have been demonstrated among maize types.

These conferences with CIMMYT have been quite productive in establishing research objectives, cooperation and the exchange of germplasm and should be continued.

Progress Report on 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 of sixteen putative new opaque mutants were analyzed in Dr. Mertz's laboratory for lysine content by the rapid colorimetric TNBS (Trinitro benzene sulfonic acid) method. The results are presented in Table 1.

In the meantime, we discovered that o_{5590} is allelic to opaque-2, a finding consistent with the lysine values shown for it. Also,

Table 1. Preliminary analyses of new opaque mutants

Mutant	Ear	Lysine (% of Protein) TNBS*	P*	AA*	Tryptophan
o_{4915}	1026-3	3.82			
	1026-6	3.64			
o_{4918}	1028-5	3.63			
	1028-6	3.89			
o_{4921}	1031-7	3.37			
o_{4939}	1176-2	3.5			
	1176-4	3.5			
	1178-1	3.4			
o_{4940}	1036-3	4.63	4.1	3.2	.6
	1036-5	4.15			
o_{4944}	1038-8	4.0			
	1039-1	4.15			
o_{5578}	1054-1	3.3			
	1054-3	4.2			
o_{5579}	1058-5	4.0			
	1058-6	2.8			
o_{5582}	1062-4	3.5			
	1062-5	3.8			
o_{5586}	1063-1	4.5	4.9	3.7	.8
	1063-2	3.6	3.7	2.8	.6
o_{5587}	1066-5	4.5	3.8	2.7	.6
	1067-2	3.79	4.0	3.6	.6
o_{5588}	1069-2	3.2			
	1069-4	4.0			
o_{5590}	1072-1	4.85			
	1073-1	4.4			
o_{5592}	1075-3	2.9			
	1075-4	3.7			
o_{5593}	1078-2	4.7	4.0	2.8	.6
o_{5595}	1082-7	3.9	4.5	3.3	.8
	1083-3	4.9	4.1	3.4	.6

* Trinitro benzene sulfonic acid, pyridine and amino acid analyzer.

o_{4939} is allelic with opaque-1 and has essentially the same lysine content as normal corn.

Eight samples representing five of the remaining putative new mutants were resubmitted for analysis. These were analyzed for lysine by the pyridine method and by the amino acid analyzer; also, for tryptophan by the colorimetric Hernandez-Bates method. None of these mutants appear to have particularly promising protein quality on the basis of these limited samples, but some will be rechecked in another generation.

Preliminary segregation ratio counts indicate that of the group of 16, only o_{4939} and o_{5590} are single gene mutants. Ratios from the others suggest that each is controlled by two genes.

Limited F_2 linkage data suggest that gene opaque-4 is on the short arm of chromosome 2. The coupling phase backcross is being set up to more accurately determine the chromosomal locus of this gene.

2. Temperate Germplasm - Thirteen composites from more temperate zones of adaptation (Pakistan, Argentina and Africa) were obtained from CIMMYT. These were divided into two groups in the summer nursery and are being crossed with cornbelt opaque-2 Syn A and Syn B.

B. Modifier Genes

1. Recurrent selection for greater kernel weight ratio (Mutant/normal) in heterozygous opaque-2 and floury-2 populations. An approach to improvement of kernel weight of opaque has involved a recurrent selection program. On about 200 segregating ears from a synthetic ($B37o_2$ and $B14o_2$ x Syn 60D), 50 kernel samples of opaque

and normal were weighed. Fifteen to twenty ears which showed the least difference in kernel weight between normal and opaque were selected. Normal kernels (to maintain a segregating population) were planted and the resulting plants intercrossed. Plants from the intercrosses were selfed, the opaque and normal kernels from segregating ears were weighed and 15-20 ears showing the least difference were again selected for another cycle. For comparison purposes a control population was handled in a similar manner but with no selection.

Results for three cycles of the select and control populations are given in Table 2. These data are based on five replications with approximately 15 segregating ears per plot. For the select population after the third cycle the opaque-2 kernel weight expressed as a percent of normal on the same ear was 94.59 compared to 88.20 for the control population. This is a significant difference; however, most of the improvement was made in the first cycle. Also, the weights of the opaque kernels changed little, and most of the improvement can be accounted for by a decrease in the weights of the normal. It should be emphasized that little selection for kernel weight per se was practiced, but that the primary criteria for selection was the weight ratio between opaque and normal on the same ear to eliminate environmental effects.

Whole kernel protein, determined on a fat free basis, tended to decline in the select and the control populations with each cycle. Lysine values expressed as a percent of protein declined somewhat with each cycle of selection.

Table 2. Kernel weight, protein and lysine content of normal and opaque-2 kernels for three cycles of recurrent selection for weight of opaque-2 as expressed in percent of normal kernel weight.

	Cycle 1		Cycle 2		Cycle 3	
	Select	Control	Select	Control	Select	Control
50 kernel wt. o_2	12.00	11.63	12.08	12.84	11.91	11.82
50 kernel wt. +	13.32	14.01	12.95	14.73	12.68	13.51
o_2 as % of normal	91.04	85.14	93.53	86.84	94.59	88.20
% protein of o_2 (fat free)	12.53	13.29	12.30	12.49	11.97	12.38
Lysine as % of protein	4.57	4.53	4.29	4.55	4.18	4.34

Table 3. Kernel weight, protein and lysine content of normal and floury-2 kernels for the synthetic and three cycles of recurrent selection for weight of floury-2 as expressed in percent of normal kernel weight.

	Original Synthetic	Cycle 1		Cycle 2		Cycle 3	
		Select	Control	Select	Control	Select	Control
25 kernel wt. fl_2	6.42	6.43	5.74	6.42	5.62	5.93	5.89
25 kernel wt. +	7.06	6.86	6.23	6.79	6.15	6.03	6.44
fl_2 as % of normal	91.11	92.45	92.45	96.19	91.74	98.69	91.80
% protein of fl_2 (fat free)	12.09	11.21	11.91	11.12	12.23	11.47	12.35
Lysine as % of protein	3.56	3.59	3.58	3.46	3.47	3.35	3.33

A similar recurrent selection program was also practiced on a heterozygous floury-2 synthetic [(SSS x fl_2) intercross]. Twelve families were used for each advanced cycle. Selection is somewhat more difficult because three doses floury-2 kernels cannot be distinguished from those with two doses with absolute accuracy because of the nature of gene expression involved. The experimental design was the same as the previous experiment. The original population was also included. The results are presented in Table 3. The responses obtained were very similar to that of the opaque-2 experiment since the improvement in floury-2 kernel weight expressed as percent of normal did increase with selection. However, again this was mainly due to a decrease in the normal kernel weight. No significant trends in protein or lysine values were apparent.

A more practical program would involve selection for kernel weight per se as well as weight ratio expressed as mutant in percent of normal. Also, the effect of this selection on total yield has not been determined.

2. Genetic Study of specific modifiers. Another promising approach to modifying the opaque-2 phenotype involves visual selection of more specific modifiers in segregating families during selfing. This appears promising and these modified expressions may be classified under the following categories:

   a. Kernels with ½:½ phenotype with top or crown part normal or vitreous endosperm and the base part opaque. On the ear these give a normal phenotypic appearance. This type occurs rather

frequently and lysine as a percent of protein values are good ranging from 3.6 to 4.0.

b. A saddle pattern where the soft or opaque starch is limited to the center of the abgerminal side of the kernel.

c. Kernels with a vitreous base portion and the crown is opaque starch.

d. A sectored or diffuse type where the opaque or soft starch is distributed irregularly in otherwise vitreous starch.

These modifier genes acting on the expression of opaque-2 are definitely heritable and are probably recessive. A graduate student (Mr. Urbano Ribeiral) is studying their inheritance in testcrosses, diallel set and making up a synthetic.

C. Recurrent selection for increased germ size

1. Selection for larger germ size (higher oil) to maximize nutritional qualities. A selected portion (50 ears) of opaque Syn A with a mean oil content of 5.83 served as the parent population. Three breeding schemes; i.e., half-sib, full sib and S_1 performance, are being used to select for larger germ size (percent oil). Since the germ has a relatively high proportion of high quality protein, this approach may tend to maximize nutritional properties. Also, it may lead to greater caloric yield per area with opaque-2 varieties since we have shown the mutant compensates for reduced endosperm yield with larger germs.

The 50 selected families, grown at the Purdue Agronomy Farm in 1970, produced 950 progeny ears ranging in oil from 3.58% to

8.35% with a mean of 5.90%. Fifteen percent of these 950 ears with the highest oil content (mean 6.77%) were planted in a winter nursery.

The 650 ear progenies harvested from the winter nursery for the half-sib and full-sib populations had an average oil percent of 6.31. The highest select ten percent from these second cycle populations had a mean of 7.27% oil. Composite samples from the various selected populations and cycles were planted this summer to evaluate efficiency of selection methods and determine correlated response in lysine and protein levels.

2. Variation and Correlations of Protein and Lysine in Germ and Endosperm. Thirty kernel samples from 45 ears (random) from an opaque-2 synthetic were analyzed for oil content. The kernels were then dissected into germ and endosperm portions and protein, lysine and oil content determined. The means and ranges are given in Table 4. Surprisingly large variations were observed for all characteristics and one might question whether "endosperm only" analyses are sufficiently accurate in a breeding program, especially if maximum nutritional improvement is a primary goal. Of particular interest is that the quantities and variation of lysine in the endosperm and germ are nearly equal.

All possible correlations among the characteristics given in Table 4 gave many high and significant values. The more pertinent ones of interest in this brief discussion are as follows:

Table 4. Variation in oil, protein and lysine content in endosperm and germ portions of 45 random ears (30 kernel samples) from an opaque-2 synthetic.

	$\overline{X}$	Range
% oil in kernel	6.05	3.58-8.35
% oil in germ	32.36	25.09-42.46
% prot. in N.D.* germ	26.78	22.92-31.72
Lys. g./100 gr. prot. in germ	5.80	4.99-6.90
% prot. in endosperm	10.3	6.4-14.1
Lys. g./100 g. prot. in endosperm	3.36	2.66-5.02
Lys. g. in 30 germs	.01944	.01018-.02838
Lys. g. in 30 endosperms	.02169	.01302-.02986
Lys. g. in 30 kernels	.04113	.02517-.05734
% Lys. in kernel	.538	.424-.636
Lys. g./100 g. prot. in kernel	4.17	3.54-5.30
% prot. in kernel	12.97	9.79-16.42
g. prot. in 30 kernels	.985	.590-1.340

* Non-defatted.

percent oil in kernel vs. percent lysine in kernel	r = .12
percent oil in kernel vs. percent protein in kernel	r = .44*
percent oil in kernel vs. percent protein in endosperm	r = .39*
percent oil in germ vs. percent protein in germ	r = -.43*
percent oil in germ vs. percent protein in endosperm	r = .22
g. lys./100 g. prot. in endo vs. percent protein in germ	r = .41*
percent protein in germ vs. percent protein in endosperm	r = -.01

Although the data must be subjected to more critical statistical analysis, it appears that selection for higher oil content in opaque-2 would (1) not have an adverse effect on protein quality, (2) produce grain with higher caloric content and (3) high oil genotypes may compensate for reduced endosperm yield to make total caloric yield per unit area more comparable to normal corn. Selection presently underway will give data bearing on this last point.

D. Genetic interactions

1. Isolation of genetic lines. A study was initiated to evaluate several endosperm mutant genes in double and multiple gene combinations with opaque-2 and floury-2. The initial phases involved backcrossing the mutant genes to five inbred backgrounds (Oh43, W64A, B37, C103 and A632) and recovering near isogenic line materials for each mutant within an inbred. Most of the mutants have been recovered after 3 to 6 backcrosses within the Oh43, W64A, B37 and C103 backgrounds. The line conversions to the A632 inbred

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* indicates significant r values at .01 (.36) level.

are in the initial phases and the recovery of advanced backcross lines will be obtained by use of the Florida and Hawaii winter nursery sites.

All possible double mutant genotypes involving either the o_2 or the fl_2 gene with the following mutants ae, du, su_1, su_2, wx, sh_1, bt_1, bt_2, fl_1, h and fl_2 were each isolated in the Oh43 inbred background. Part of this work was the Ph.D. thesis study of Helio M. Barbosa.

2. Chemical analyses of double mutant lines. The normal, single mutants, double combinations with o_2, and double combinations with fl_2 in the Oh43 inbred were analyzed in a replicated experiment and 100-kernel weight, germ weight, germ percent of whole kernel, kernel density, percent protein and lysine in the endosperm and germ fractions, reducing sugars, water soluble polysaccharide (WSP), starch, amylose and the mineral elements P, K, Mg, Cu, Fe, and Zn were determined.

The genes o_2 and fl_2 decreased kernel weight in the double combinations with the other endosperm mutants. The wx o_2 line was the only combination which resulted in higher kernel weight than the single o_2 line. With the exception of the du, du o_2 and wx lines, all single and o_2 combination lines were lower in germ weight than the o_2 line. The germ weight of h fl_2 was the only double combination with fl_2 exceeding that of floury-2. The effect of o_2 in increasing percent germ was more pronounced than fl_2. Percent germ in the o_2 combination lines ranged from 14-23% with a mean of 18% while the fl_2 combination lines had a range from 12-20% and a mean of 14%. The su_2 line had greater kernel density

than normal and this effect was also apparent in its combinations with o_2 and fl_2. As expected, the bt_2, bt_2o_2 and bt_2fl_2 lines were the lowest in kernel density.

The percent protein, lysine and lysine as a percent of protein in the endosperm of the single mutant lines and double mutant combination lines with opaque-2 and floury-2 are presented in Tables 5, 6 and 7, respectively. With few exceptions, the o_2 gene decreased percent protein in the endosperm of the double combination lines, as compared to the single non-opaque line. The fl_2 line was no different than normal in percent protein, but fl_2 tended to increase endosperm protein in the double combinations. The bt_2 gene, singly or in double combinations, substantially increased percent protein; however, on the basis of milligrams of protein per degermed kernels, these lines were low due to reduced kernel weight and density.

Most of the double combinations with o_2 were higher in the percent of lysine than either of the respective single mutants. The combination of fl_2o_2 resulted in a small increase in percent lysine over that of fl_2, but was not higher than o_2. The combination bt_2o_2 had nearly twice as much lysine (percent of sample) as the single mutant o_2 line. The lines su_1o_2, su_2o_2 and bt_1o_2 were noticeably higher in percent lysine than o_2. The fl_2 gene also increased percent lysine when combined with other genes but its effect was not as pronounced as that of o_2.

All single mutants, except wx and h, were higher than normal in lysine as percent of protein. The double mutant fl_2o_2 was lower in lysine as percent of protein than any other double mutant with o_2 or the o_2 line. All other double mutants with o_2 were

Table 5. Summary of percent protein, lysine and lysine percent of protein in the endosperm of Oh43 isogenic lines for several single mutants -- 1970 replicated nursery.

Genotype	Protein %	Lysine % of Sample	Lysine % of Protein
++	11.45	.197	1.72
ae/ae	11.65	.273	2.34
du/du	10.95	.251	2.32
wx/wx	10.00	.171	1.71
su_1/su_1	12.40	.278	2.25
su_2/su_2	11.45	.21	1.87
sh_1/sh_1	11.95	.266	2.22
bt_1/bt_1	12.35	.319	2.59
bt_2/bt_2	14.90	.462	3.10
h/h	11.35	.192	1.69
fl_1/fl_1	10.90	.222	2.04
o_2/o_2	9.40	.310	3.32
fl_2/fl_2	11.45	.261	2.28
Mean	11.55	.263	2.26
Range	9.40-14.90	.171-.462	1.68-3.32
Genotype M.S. sign.	**1/	**	**
$S_{\bar{x}}$	.5253	.0201	.1583
$S_{\bar{d}}$	.7428	.0285	.2238
C.V.	6.43	10.84	9.89

1/ ** significant at the .01 level.

Table 6. Summary of percent protein, lysine and lysine percent of protein in the endosperm of Oh43 isogenic lines for several double mutant combinations with opaque-2 -- 1970 replicated nursery.

Genotype	Protein %	Lysine % of Sample	Lysine % of Protein
o_2/o_2	9.40	.310	3.32
ae/ae o_2/o_2	10.90	.421	3.86
du/du o_2/o_2	9.65	.354	3.67
wx/wx o_2/o_2	9.60	.357	3.72
su_1/su_1 o_2/o_2	13.25	.569	4.28
su_2/su_2 o_2/o_2	10.70	.429	4.01
fl_1/fl_1 o_2/o_2	10.05	.407	4.05
h/h o_2/o_2	9.85	.357	3.62
fl_2/fl_2 o_2/o_2	11.25	.315	2.80
bt_1/bt_1 o_2/o_2	10.25	.512	5.00
bt_2/bt_2 o_2/o_2	15.60	.798	5.11
Mean	10.96	.439	3.95
Range	9.40-15.60	.310-.798	2.80-5.11
Genotype M.S. sign.	**1/	**	**
$S_{\bar{x}}$	.4451	.0039	.1635
$S_{\bar{d}}$	.6295	.0479	.2312
C.V.	5.75	10.93	5.86

1/ ** significant at the .01 level.

Table 7. Summary of percent protein, lysine and lysine percent of protein in the endosperm of Oh43 isogenic lines for several double mutant combinations with floury-2 -- 1970 replicated nursery.

Genotype	Protein %	Lysine % of Sample	Lysine % of Protein
o_2/o_2	9.40	.310	3.32
fl_2/fl_2	11.45	.261	2.28
fl_2/fl_2 o_2/o_2	11.25	.315	2.80
ae/ae fl_2/fl_2	12.05	.364	3.02
du/du fl_2/fl_2	13.30	.338	2.55
wx/wx fl_2/fl_2	11.60	.308	2.66
su_1/su_1 fl_2/fl_2	13.45	.374	2.79
su_2/su_2 fl_2/fl_2	12.20	.297	2.44
sh_1/sh_1 fl_2/fl_2	12.60	.363	2.89
fl_1/fl_1 fl_2/fl_2	10.85	.312	2.88
h/h fl_2/fl_2	10.90	.282	2.58
bt_1/bt_1 fl_2/fl_2	12.45	.361	2.90
bt_2/bt_2 fl_2/fl_2	17.30	.584	3.36
Mean	12.22	.344	2.80
Range	9.40-17.30	.261-.584	2.28-3.36
Genotype M.S. sign.	** 1/	**	ns
$S_{\bar{x}}$	.7396	.0365	.2009
$S_{\bar{d}}$	1.0460	.0517	.2841
C.V.	8.56	15.03	10.14

1/ ** significant at the .01 level.

higher than o_2 in lysine as percent of protein, with ae o_2, su_1o_2, su_2o_2, fl_1o_2, bt_1o_2 lines being significantly greater. The fl_2 double combinations were not significantly different from the o_2 or fl_2 lines, nor were there significant differences among these doubles. However, all fl_2 combinations, except fl_2o_2, were higher in lysine as percent protein than the respective non-opaque-2 single mutant lines. Milligrams of lysine per degermed kernel in ae o_2, ae fl_2, su_2o_2, wx o_2, wx fl_2, bt_1o_2 and h fl_2 were equal to or better than o_2. The double combinations ae o_2, su_2o_2 and wx o_2 were significantly higher than o_2.

The percent protein in the germ of the single mutant lines ranged from 21.7-28.1% with a mean of 25.2%. Germs of o_2 and fl_2 were the highest with 28.1 and 27.6%, respectively. The double combination lines with o_2 did not differ significantly in germ protein from o_2; however, all were higher than the respective non-opaque-2 single mutant lines. The percent of lysine in the germ of o_2, fl_2, su_2 and bt_1 was significantly higher than normal. The percent of lysine in the germ in o_2 double combinations ranged from 1.31-1.92% and tended to be higher than the respective non-opaque-2 single mutant lines. When lysine was expressed as percent of protein in the germ, there was very little difference among the o_2 combination lines.

Opaque-2 increased reducing sugars, sucrose, and WSP 1.9, 2.1 and 1.5 times the level found in the normal counterpart, respectively. The same carbohydrates were increased 1.8, 1.6 and 1.2 times respectively in fl_2. Sugary-1, singly or in combination with o_2 or fl_2, produced extremely high amounts of reducing sugars, sucrose

and WSP as expected based on the effect of $\underline{su}_1$ on carbohydrate synthesis. The reducing sugar and sucrose content of $\underline{bt}_2$, $\underline{bt}_2\underline{o}_2$ and $\underline{bt}_2\underline{fl}_2$ were approximately 3, 4 and 2 times higher, respectively, than $\underline{o}_2$. An interaction for sugar level was apparent since the $\underline{su}_2\underline{o}_2$ line produced a marked increase in reducing sugars and sucrose compared to the single mutants. The $\underline{su}_2\underline{fl}_2$ line, on the other hand, did not produce any significant increase in sugar content over the respective single mutants. The percent starch content of the double combinations was not materially affected by the presence of $\underline{o}_2$ or $\underline{fl}_2$. The amylose content of the $\underline{ae}$ $\underline{o}_2$, $\underline{du}$ $\underline{o}_2$, $\underline{su}_1\underline{o}_2$, $\underline{ae}$ $\underline{fl}_2$, $\underline{du}$ $\underline{fl}_2$, $\underline{su}_1\underline{fl}_2$ and $\underline{su}_2\underline{fl}_2$ lines was reduced by the presence of $\underline{o}_2$ or $\underline{fl}_2$ compared with these starch modifying genes alone. These results suggest a possible interaction of the $\underline{o}_2$ and $\underline{fl}_2$ genes on percent amylose.

Significant differences among genotypes were found for the mineral element quantities per kernel for P, K, Mg, Cu and Fe within the single mutant lines and the $\underline{o}_2$ combinations; and for P, Mg, Cu, Fe and Zn within the $\underline{fl}_2$ combinations. Within the single mutant lines, milligrams of protein per endosperm, kernel weight and kernel density were positively and significantly correlated with quantities per kernel of P, Mg, Cu, Fe and Zn. Significant negative correlations existed between percent germ and quantities per kernel of P, Mg, Cu, Fe and Zn. Within the $\underline{o}_2$ combination genotypes there were significant positive correlations for milligrams of lysine per endosperm, milligrams of protein per endosperm, kernel weight and kernel density with quantities of P, Mg, Cu, Fe and Zn, with the exception of kernel density and Cu.

Sugary-2 and its double combinations with o_2 and fl_2 were most interesting since they were relatively high in most of the mineral elements compared to the normal and o_2.

As genetic material becomes available the double mutant combinations will be studied under hybrid conditions. Nonetheless, these results in the Oh43 inbred background point out that there is potential for modifying high protein corns which may have special application in foods for human comsumption. At present, some of the genotypes are not agronomically desirable. It may well be that some combinations, such as bt_2o_2, may be feasible in meeting the basic protein requirements for non-ruminant weanling individuals since they have high lysine and protein, a very high glutelin fraction (high quality; see Section G) and have an excellent biological feeding value (see Section H). There are a variety of protein and lysine levels in these modified protein corns and they are being tested for their relative digestibility and nutritive value. Preliminary tests suggest that some may give better gains and feed efficiencies than opaque-2 (see Section H). Our preliminary results on relative *in vitro* digestibility rates of the double combinations suggest that the su_1o_2, su_2o_2 and $wx\ o_2$ lines (section D-3), for example, have high starch digestibility combined with good protein quality. The su_2o_2, $wx\ o_2$, $ae\ o_2$ and $du\ o_2$ genotypes may be of special interest for food purposes, since they possess the potential of a variety of water absorption, gelatinizing, viscosity and cross-linking properties. The su_2o_2 combination is particularly interesting because of its kernel characteristics, high lysine, sucrose and mineral levels. The su_1o_2, bt_2o_2 and bt_1o_2

combinations, for example, offer potential for their food value as edible corns in the roasting ear stage of development. Although they are lower in kernel weight they may provide corns of greater nutritional value.

3. In vitro digestibility characteristics of double mutant combinations. Dr. Linda Bartolome, a postdoctorate research fellow, has initiated a study to evaluate the digestibility of isolated corn starch and starch in the whole kernel by respirometry. Much of the preliminary evaluation of the methodology has been accomplished and the evaluation of the genetic materials under test conditions is now in progress. 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. Preliminary data suggest very wide differences in digestibility of isolated starch. Since starch is usually consumed as the whole grain, it is of interest to show whether the differences in digestibility will be existent and comparable to that of the isolated starch. If so, digestion measurements on the whole grain will provide a faster and more convenient method since the time-consuming process of starch isolation can be omitted. These studies will be compared with digestion rates determined by using rumen fluid as the enzyme source (Osman et al., J. of Nutrition 100 1133, 1970).

4. Preliminary feeding tests of double combinations. A limited number of double combinations were evaluated for biological value. These tests were preliminary in nature because of small quantities of seed available. The results are shown in Table 13 (Section II).

The results suggest that all the double combinations have remarkably good biological value, especially the bt_2o_2. The latter double combination contains a very different protein profile (Table 12, Section G) which explains why it has a high lysine and good biological value. Further feeding tests will be conducted on these and other double combinations and correlated with the *in vitro* digestibilities.

5. Gene interaction dosage studies. Studies were conducted to characterize the gene action and interaction effects of the genes o_2 and fl_2 on endosperm composition and kernel characteristics. In addition, it was of interest to characterize the gene action of these two genes with other genes which (1) may be in the backgrounds of indigenous varieties of corn such as the floury types (floury-1 and soft-starch) or (2) have indicated an interaction effect for endosperm composition or kernel characteristics.

The 16 possible dosage combinations involving the o_2 and fl_2 genes each with the floury-1 (fl_1) and soft-starch (h) genes were produced in the Oh43 background. The dosage combinations involving fl_2 with o_2 and amylose extender (ae) and o_2 with ae were produced in the W64A background. Kernel phenotype, 100-kernel weight, kernel density, protein, lysine and lysine percent of protein were determined for the dosage experiments involving the o_2 and fl_2 genes each with the fl_1 and h genes in the Oh43 inbred. In addition to the above determinations, percent germ, amylose and oil were determined for the dosage experiments involving the o_2 and fl_2 genes together and each with the ae gene in the W64A inbred.

The o_2 gene showed no consistent trends for dosage effects in any of the dosage combination experiments. The fl_2 gene showed a definite dosage effect for kernel density, percent germ, lysine, oil, amylose and lysine as percent of protein. There was a trend for kernel weight to be decreased with increased doses of the fl_2 allele, particularly at the two and three dose levels of fl_2 in the endosperm. There was an apparent interaction of the fl_2 and ae genes on percent amylose in the endosperm. Floury-1 showed a dosage effect for kernel appearance in the dosage combinations with o_2. The fl_1 gene decreased kernel weight at the two and three dose levels of the recessive allele in the dosage combinations with fl_2.

6. Maturity interaction and black layer occurrence in opaque-2 and normal hybrids. (Dejene MaKonnen, M.S. Thesis) Six opaque-2 lines were crossed in a diallel series and their normal counterparts were grown in 1970 in a split plot randomized complete block design. Harvests were made at 7-day intervals starting at 28 days post pollination and continuing through 63 days.

The opaque-2 hybrids with normal cytoplasm and those with Texas cytoplasm along with their normal counterparts were compared on the basis of kernel dry matter curve. The result revealed that H. Maydis race T. probably had little effect on the performance of the opaque-2 hybrids with Texas cytoplasm.

Kernel weight of opaque-2 hybrids was inferior to the normal. The opaque-2 gene performed differently in different hybrids over harvest dates. In H60 x A239 and B14 x B37 the mutants had kernel weight similar to their normal counterparts in the first and second

harvest dates. In contrast, a wide difference was found between opaque-2 and normal, opaque-2 being inferior, both at early and late stages of development in A239 x A545 and W64A x A545 single crosses.

Analyses of variance indicated significant differences between genotypes, among hybrids, and harvest dates for all variables. All interactions were significant for kernel weight indicating the importance of genetic background.

The mutant gene had a pronounced effect on cob weight. The normal hybrids had 9.7-11.8% greater cob weight. This difference in cob weight was constant over different harvest dates and genotypes.

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

Black layer formed at about the same time in the opaque-2 and in the normal despite the earlier nearly complete cessation of dry matter accumulation in the opaque.

7. Interrelationships among protein, lysine, oil and certain mineral element concentrations in two maize populations. The concentration of lysine, protein, oil, P, K, Mg, Fe and Zn was determined on normal and opaque kernels from 60 segregating ears from a heterozygous population and kernels from 62 ears from a homozygous opaque-2 population. In the heterozygous population the correlation between percent protein and percent lysine was +.50 and +.70 for the normal and opaque kernel types, respectively, (Tables 8 and 9). In the

Table 8. Correlation coefficients involving protein, lysine, oil and mineral elements of opaque-2 kernels from 60 segregating ears of opaque Sys KW.

	Protein %	Lysine %	Lysine g/100 g Pro.	P %	K %	Mg %	Fe PPM	Zn PPM	Oil %	Ker. Wt.	Ker. Vol.	Ker. Density
Protein %		.70**	-.12	.45**	.32**	.53**	.37**	.48**	.22	.01	.03	-.05
Lysine %			.62**	.25*	.20	.36**	.30*	.33**	.24	.00	.07	-.25*
Lysine g/100 g Pro.				.13	-.07	-.05	.01	.08	.10	-.01	.07	-.29*
P %					.64**	.74**	.54**	.39**	.25*	.07	.03	.05
K %						.44**	.52**	.48**	.07	-.25*	-.24	-.23
Mg %							.57**	.31*	.21	.06	.04	.08
Fe PPM								.52**	.16	-.20	-.13	-.20
Zn PPM									.06	-.12	-.14	-.12
Oil										.04	-.01	.11
Ker. Wt. g											.91**	.23
Ker. Volume												-.08
Ker. Density g/ml												

Table 9. Correlation coefficients involving protein, lysine, oil and mineral elements of normal kernels from 60 segregating ears of opaque Syn KW

	Protein %	Lysine %	Lysine g/100 g Pro.	P %	K %	Mg %	Fe PPM	Zn PPM	Oil %	Ker. Wt.	Ker. Vol.	Ker. Density
Protein %		.50**	-.62**	.59**	.26*	.53**	.29*	.36**	.20	.11	.09	.04
Lysine %			.36**	.35**	.33**	.23	.43**	.25*	.23	-.06	.00	-.31*
Lysine g/100 g Pro.				-.32**	.01	-.36**	.08	-.16	-.01	-.19	-.11	-.34**
P %					.59**	.70**	.43**	.35**	.14	.07	.07	-.13**
K %						.27*	.41**	.32**	.01	-.19	-.13	-.53**
Mg %							.49**	.26**	.14	.04	.03	.07
Fe PPM								.41**	.11	-.16	-.14	-.28*
Zn PPM									.01	-.19	-.16	-.33**
Oil %										-.03	-.01	.09
Ker. Wt. g											.98**	.38**
Ker. Volume												.24
Ker. Density												

homozygous population this correlation was +.83 (Table 10). Correlations between protein content and content of the various mineral elements were all positive and significant for both kernel types in the heterozygous population. Significant positive correlations were detected between lysine content and P, K, Fe and Zn content in the opaque kernels. Lysine and protein concentration were positively correlated with P, Zn and oil concentration in the homozygous population, while protein concentration was also correlated with Mg concentration. The results indicate a strong tendency for lysine concentration to increase as protein concentration is increased, especially in an opaque-2 population. The low or nonsignificant correlations between K and protein and lysine were disappointing. Kernel density showed a low but significant relationship to lysine percentage in the opaque-2 kernels.

E. Genetic test procedures in populations

1. Feasible breeding system for introducing opaque-2 into floury populations. This procedure was investigated in various combinations of selfed progeny, testcross and backcross ratios. The correct and most efficient procedure is outlined below. The four possible genotypes in the backcross generation are given. These plants are outcrossed as males to an o_2o_2 tester and these same plants are pollinated (females) with a bulk sample of pollen from the floury population. The desired heterozygote $+/fl_1$ $+/o_2$ is easily identified by those plants which segregate 1:1 on testcross

Table 10. Correlation coefficients involving protein, lysine, oil and mineral elements in grain of 62 ears of opaque Syn A.

	Protein %	Lysine %	Lysine g/100 g Pro.	P %	K %	Mg %	Fe PPM	Zn PPM	Oil %	Ker. Wt.	Ker. Density
Protein %		.83**	.33**	.65**	.17	.42**	.18	.61**	.25*	-.11	-.14
Lysine %			.25**	.43**	.01	.24	.19	.49**	.39**	-.01	-.12
Lysine g/100 g Pro.				-.39**	-.29*	-.33**	.01	-.25*	.23	.16	.05
P %					.23	.69**	.36**	.66**	.06	.04	.03
K %						.15	.24	.23	-.18	.04	-.39**
Mg %							.28*	.50**	.06	-.01	.01
Fe PPM								.54**	-.21	.01	-.12
Zn PPM									.04	.03	.06
Oil %										.17	.09
Ker. Wt.											.06
Ker. Density											

ears to o_2o_2 tester and also segregate 1:1 when pollinated by pollen from floury population.

Expected Ratios On Cross to o_2o_2 Tester	Embryonic Genotype Of Backcross Plants	Expected Ratios On Backcross with fl fl
all "normal"	$+/fl_1$ $+/+$	1 normal : 1 floury
1 normal : 1 opaque	$+/fl_1$ $+/o_2$*	1 normal : 1 floury
all "normal"	fl_1fl_1 $+/+$	all floury
1 normal : 1 opaque	fl_1fl_1 $+/o_2$	all floury

* Desired genotype or backcross plant

Continuous backcrosses can be made with this procedure and it will probably work equally well if a floury-2 or floury-1 population is involved. This breeding scheme works quite well in providing adequate sampling of the population or variety since bulk pollen can be used to make the backcrosses. After a desired number of backcrosses then selfing and further testcrossing will be necessary to isolate the double recessive types.

F. Research on improved analytical methods.

1. The first six months were spent comparing two simple colorimetric methods for lysine, the TNBS (Trinitro benzene sulfonic acid) and the pyridine (2-chloro-3,5 dinitro pyridine) methods. An abstract of the TNBS method has been published by this laboratory [Simple methods for determining lysine and tryptophan in high lysine and normal maize. S. S. Subramanian, R. Jambunathan, J. M. Concon and E. T. Mertz, Fed. Proc. 29 761 (1970)]. The pyridine

method was first applied to corn by Dr. Charles Tsai, Botany and Plant Pathology, Purdue. It was improved by Dr. Eva Villegas, CIMMYT, and her modification was used in these studies.

Dr. Villegas spent one week in October 1970 at Purdue learning the details of the TNBS method. The same check samples of corn were then analyzed for lysine at CIMMYT and at Purdue using the TNBS and pyridine methods.

At a meeting in Mexico City, it was concluded that more samples could be analyzed per day using the pyridine method than with the TNBS method, and the pyridine method was therefore selected as the recommended method for laboratories in the developing countries. In the same manner, the rapid tryptophan method of Hernandez and Bates [Research Bull. No. 13, CIMMYT (1969)] was recommended.

Data in Table 11 show the excellent agreement between the basic ion-exchange method and the pyridine method in the analysis of Colombian whole kernel and endosperm corn samples segregating for vitreous and opaque types of endosperm. The tryptophan values (Hernandez-Bates method) vary directly with the lysine values, which suggests that they are accurate measures of the relative tryptophan contents.

G. Central biochemical laboratory

1. Cooperative research with Dr. E. Villegas (CIMMYT). As a result of the cooperative study by Dr. Mertz and Dr. Villegas on methodology reported under Part F, CIMMYT is publishing a research bulletin (in press) entitled "Chemical Methods Currently Used at

Table 11. Comparison of ion-exchange and CIMMYT-Purdue colorimetric methods.

Sample*	Protein %	Lysine (Ion-Exchange) g/100 g Prot.	Lysine (Pyridine) g/100 g Prot.	Tryptophan Hernandez-Bates g/100 g Prot.
H-208 Opaque (WK)	11.1	4.7	4.3	1.0
H-208 Vitreous (WK)	12.0	4.2	4.1	0.9
H-255 Opaque (WK)	10.6	4.3	4.2	1.1
H-255 Semi-Opaque (WK)	12.2	3.8	3.9	0.9
H-255 Vitreous (WK)	12.3	2.7	2.5	0.6
H-208 Opaque (E)	8.9	4.0	4.1	0.9
H-208 Vitreous (E)	9.6	3.7	3.7	0.8
H-255 Opaque (E)	10.3	4.1	4.1	0.9
H-255 Semi-Opaque (E)	12.0	3.2	3.0	0.7
H-255 Vitreous (E)	11.0	1.8	2.2	0.5

* Samples sent to E. T. Mertz by C. A. Francis, CIAT, Bogota, Colombia, South America. WK = whole kernel. E = endosperm.

Table 12. Protein distribution in normal and mutant maize endosperms*.

Maize Endosperm	Total Protein Extracted (%)	Acid-Soluble Proteins (%)	Zein (%)	Glutelins (%)
Normal Hybrid	98	25	47	15
Opaque-2 Hybrid	93	32	30	29
Oh43 ($\underline{o}_2/\underline{o}_2/\underline{bt}_2/\underline{bt}_2$)	90	30	9	61
Oh43 ($\underline{bt}_2/\underline{bt}_2$)	91	25	35	36

* Copper fractionation method. All values in duplicate.

CIMMYT for Screening Maize Populations for Protein Quality" by Dr. E. Villegas and Dr. E. T. Mertz.

2. Basic research on the nature of the proteins in high lysine types of maize. Dr. P. S. Misra is comparing the proteins of normal and mutant types of endosperm using the copper fractionation method of Mertz, Lloyd and Bressani [Cereal Chem. 35 146 (1958)] and the fractionation method of Landry and Moureaux [Bull. Soc. Chim. Biol. 52 1021 (1970)].

Table 12 shows that the double mutant, o_2/o_2 bt_2/bt_2, (supplied by Dr. Glover) contains four times more glutelin and one-fifth as much zein as normal maize. The mutant, bt_2/bt_2, has a level of zein which is intermediate between that of normal and opaque-2 maize. These fractionation studies explain why o_2/o_2 bt_2/bt_2 endosperm has such a high lysine level (about 4.5% of the protein). Analyses of these and other double mutants using the copper and the French methods are in progress.

H. Protein nutritive value. Promising new maize genotypes from the Purdue program and elsewhere, shown by chemical analysis to be high in protein quality, have been evaluated for biological efficiency in in the weanling albino rat.

1. Comparison of isogenic lines of hybrid normal, opaque-2 and floury-2 maize. The PAG Hybrid Corn Company, Aurora, Illinois, provided us with isogenic hybrids of opaque-2 and floury-2 maize derived from their normal hybrid SX-52. Samples of these three versions of the hybrid were ground and incorporated in diets containing 95% of the ground corn, 4% of the mineral mixture and 1% of a vitamin

fortification mixture. Each of the diets was fed to 10 rats for 21 days. Table 13 (Experiment 1) shows the total protein content, lysine as a percent of total protein, daily average gain and average feed efficiency. The lysine content of the floury-2 was intermediate between that of the opaque-2 and the normal. The biological response agrees with the chemical data since the daily gain and feed efficiency of the rats on floury-2 was intermediate between normal and opaque-2 maize.

Dr. O. E. Nelson, University of Wisconsin, provided two floury-2 hybrids containing approximately 17% protein and 3.7 grams of lysine per 100 grams of protein. In feeding tests with rats (3 rats per diet, 21 days), these hybrids gave the same weight gains and feed efficiencies as the PAG opaque-2 shown in Table 13 (Experiment 1), which contained 5% less protein. These results suggest that floury-2 proteins have a lower biological value than opaque-2 proteins, and support our belief that major emphasis should be placed on breeding improved varieties of opaque-2 in preference to floury-2 maize.

2. Biological value of double mutants containing the opaque-2 gene. Some of Dr. Glover's double mutants which are high in protein quality based on amino acid analyses have been fed to weanling rats. The biological responses of several double mutants in the same genetic background (Oh43) are compared in Table 13 (Experiment 2). Because of the small amounts of seeds available, the feeding test was reduced to ten days with only 2 animals in each group (4 animals in the control groups). These preliminary data suggest that the double mutant of bt_2/bt_2 o_2/o_2

Table 13. Gains of weanling rats on isogenic lines of mutant and normal maize.

Maize Type	Protein (%)	Lysine (g/100g protein)	Average Daily Gain*	Feed/Gain**
		Experiment		
PAG SX-52 normal	13.1	2.6	1.0	7.4
PAG floury-2	12.0	3.3	1.8	5.1
PAG opaque-2	11.4	3.8	3.5	3.7
		Experiment 2		
Oh43 bt_2/bt_2 o_2/o_2	17.0	4.4	4.8	2.2
Oh43 bt_2/bt_2	15.7	3.8	3.0	2.7
Oh43 su_2/su_2 o_2/o_2	13.8	4.1	3.5	2.8
Oh43 ae/ae o_2/o_2	13.6	4.6	3.6	3.1
Oh43 wx/wx o_2/o_2	11.9	3.6	3.2	3.0
Oh43 o_2/o_2	10.6	4.3	3.0	3.2
PAG SX-52 normal	13.1	2.6	0.4	11.1

* Grams per day. Experiment No. 1: 10 rats on each type of maize for 21 days. Experiment No. 2: 2 rats on double mutants, and 4 rats on o_2/o_2 and PAG normal, for 10 days.

** Grams of feed required for 1 gram of gain.

has remarkably good biological value. Even $\underline{bt}_2/\underline{bt}_2$ was as effective as the Oh43 opaque-2 control (3 gm. daily for both). All of the opaque-2 double mutants gave better gains than the single mutant opaque-2.

I. Factors affecting maize adaptation.

Four net radiometers of the Fritschen type and four Leeds and Northrup strip chart recorders were purchased; also, four Belfort mechanical pyrheliographs to record total incoming solar radiant heat. One of each has been installed near Bogota, Palmira and Monteria, Colombia and Mexico City, Mexico. The instruments at Palmira have been steadily in operation since their installation in February, but operation of the net radiometer and recorder at Bogota and Mexico City has been delayed for lack of electrical wiring in new meteorological stations. Also, problems with fluctuating current may force us to modify the equipment at Monteria. However, plant data and data from the pyrheliograph and other more standard meteorological data are being collected.

A uniform group of twelve maize types, four supplied by Purdue, four by CIMMYT and four by CIAT are being grown at the Colombian and Mexican locations, two locations in Missouri, two in Colorado, one in Wisconsin and one in Indiana. Floral initiation, silking, anthesis and black-layer formation dates are being recorded, as well as plant and ear node heights and total leaf number.

Summary

1. Specific modifier genes that give a more normal phenotype with good levels of lysine have been isolated.

2. Large variations were observed for percent protein and percent lysine in the germ.

3. The $\underline{su}_2\underline{o}_2$ double combination shows particular promise because of its good kernel characteristics and high lysine, sucrose and mineral levels.

4. The $\underline{bt}_2\underline{o}_2$ double combination may be feasible in meeting the basic protein requirements for non-ruminant weanling individuals because of its very high quality protein and excellent biological feeding value.

5. The floury-2 gene showed dosage effects for kernel density, percent germ, lysine, oil and amylose and lysine as percent of protein.

6. The floury-2 gene showed an interaction effect with the amylose extender gene on percent amylose.

7. The high protein quality derived from the opaque-2 gene can be enhanced further by introducing a second mutant gene from the starch-modifier group.

8. Preliminary fractionation studies reveal that wide variations exist in the distribution of endosperm proteins in single and double maize mutants when compared with normal maize.

9. The opaque-2 gene appears, at this time, more promising than floury-2 or the double mutant.

10. Dry matter accumulation in grain of opaque-2 and normal diallel showed (a) cob weight of opaque-2 was 10 percent less than normal, (b) there were interactions of opaque-2 with genetic background of dry matter curves starting at 35 days past pollination and (c) black layer formed at the same time in opaque and normal.

11. Analyses of random ears from opaque-2 synthetics revealed significant correlation between lysine content and mineral elements P, Mg, Fe and Zn.

12. Highly significant correlations were observed between percent lysine and percent protein.

13. Simple, fast methods for quantitative determination of lysine and tryptophan in maize have been described in a CIMMYT research bulletin (in press).

The research activities of the first year of this project have been particularly significant in that a simple, rapid and easily repeatable analytical method for lysine and tryptophan determination in maize has been agreed upon in cooperation with the chemical research staff of CIMMYT. The simple, fast pyridine method should be suitable for use in field laboratories throughout the world. The central biochemical unit at Purdue has been strengthened and is now capable of providing instruction in and standardization of analytical procedures for maize protein samples.

Specific modifier genes acting on the expression of opaque-2 have been found and appear particularly promising in improving the kernel characteristics and nutritive value of the grain.

Improved methods of selection and breeding procedures for the long-range nutritional improvement of maize have been suggested from these investigations and these promising schemes are being further evaluated.

Our studies have revealed large variations for percent protein, percent lysine and percent oil in the germ fraction, that the high protein quality derived from the opaque-2 gene can be enhanced further by introducing a second mutant gene from the starch modifier group, and that wide variations exist in endosperm proteins and in their *in vitro* digestibility properties.

These results point out the potential for producing grain with higher caloric content with improved protein quality and modified starch types which may have special application in foods for human consumption.

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