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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, 1972 - June 30, 1973

Prepared by

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

Purdue University
West Lafayette, Indiana

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REPORT SUMMARY

A. 1. Project Title and Contract Number: Inheritance and Improvement of Protein Quality and Content in Maize; Contract Number AID/csd-2809

2. Principal Investigator, Contractor and Mailing Address:

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3. Contract Period (as amended): from July 1, 1970 to June 30, 1975

4. Period covered by Report: from July 1, 1972 to June 30, 1973 ?

5. Total A.I.D. funding of contract to date: $809,812 (through 3/31/74)

6. Total expenditures and obligations through previous contract year: $543,328.06 (through 3/31/73)

7. Total expenditures and obligations for current year: $39,520.69 (4/1/73 through 6/30/73 only)

8. Estimated expenditures for next contract year: $250,000

B. Narrative Summary of Accomplishments and Utilization

An "International Symposium on Protein Quality in Maize" was co-sponsored with CIMMYT in Mexico City in December, 1972, with 150 scientists participating.

Normal, opaque-2 and floury-2 maize, sorghum, wheat, and triticales were compared with reference casein in the rat, mouse, vole, and chick in a cooperative study involving five laboratories. The male weanling rat gave the best differentiation among the cereals tested.

Amino acid patterns of the Landry-Moureax fractions of normal and mutant endosperms revealed that the albumin-globulin fraction and the true glutetin fraction have high levels of lysine and tryptophan. Fractionation of protein from modified opaque-2 revealed an increase in zein and grain yield was not improved by selection of modified types.

The double mutant sugary-2/opaque-2 shows excellent promise for increasing the acceptance and utilization of high lysine maize because of its improved kernel density, vitreousness, enhanced caloric and nutritive value, improved digestibility and biological value.

Opaque-2, sugary-2/opaque-2 and waxy/opaque-2 versions of two temperate zone synthetics have been developed. A modified opaque-2 synthetic has been released to interested breeders. Three sugary-2 (Oh43, B37 and C103) and two waxy (Oh43 and C103) inbreds were released because of interest in their utilization to improve nutritional characteristics.

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Some 50 percent of human protein needs are supplied currently by cereal grains. Maize supplies an important portion of the total but is deficient in lysine and tryptophan. Continuation of adequate, systematic, and cooperative research, in maize protein quality will play an increasingly important role in solving the world's human and animal nutrition problems.

Problems associated with initial versions of high lysine maize have pointed up the necessity for continued basic research on genetics, breeding, analytical methods, and nutritional problems associated with quantity and quality of protein in maize. The full potential will be realized only through sustained basic, interdisciplinary research into these complex problems.

The specific objectives of this research are:

a. Evaluate and develop breeding procedures for efficient development through breeding of quality protein maize.

b. Study gene action and interactions with opaque-2 and other mutant endosperm genes, especially sugary-2 and waxy.

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

d. In cooperation with other world maize research centers, a search is being made for new genes and development of germplasm for improved protein quality and agronomic performance.

e. Verify biological value by rat and other animal feeding tests of new maize genotypes and germplasm.

f. Determine the relationship between environmental heat units, based on air temperature and net radiant heat at a number of latitudes and altitudes to determine their value in developing maize with broad adaptation.

A. New Genes and Germplasm that May Provide Improved Protein Types

1. Promising new mutants from Colombian or other germplasm

By extensive allele tests the phenotypically opaque mutants collected in Colombia in 1964 and 1965 have been placed in seven groups:

Group 1: 4915

Group 2: 4918, 4940, 4944, 5578, 5579, 5586, 5587, 5588, 5592, and 5593

Group 3: 4921

Group 4: 5595

Group 5: 4939

Group 6: opaque (Poey) and opaque-4

Group 7: 5590

In Group 7, 5590 is allelic to, and apparently identical to, opaque-2. Chemical analyses have shown that the amino acid profile is essentially identical to that of opaque-2.

Opaque-4 and opaque (Poey) were found to be recessive alleles at the floury-1 locus. So presumably they are identical to fl_a reported by Mazoti. Therefore the proposed designation opaque-4 has been withdrawn in favor of the prior floury-a.

Mutant 4939 (Group 5) is allelic to opaque-1 with which it is apparently identical. It is located on chromosome 4.

The mutants of groups 1, 5, 6, and 7 are basically monogenic although their expression may be modified by other genes. But the mutants of groups 2, 3, and 4 are basically two gene systems. They are duplicate recessive genes which generally give a 15:1 segregation in the F_2 and a 3:1 segregation in the backcross.

However, they are subject to modification by other genes in various genetic backgrounds.

The chromosomal location of 4915 is on chromosome 2 near floury-1. Group 2, as established by work primarily on 5586, has one gene on chromosome 7 and one on chromosome 3. Group 3, which contains only the mutant 4921, apparently has one gene on chromosome 1. The location of the second gene has not yet been established. Mutant 5595 (Group 4) has one gene on chromosome 2. The location of the other gene has tentatively been identified as chromosome 5.

In 1971, another round in the search for protein quality mutants was initiated by the inspection for segregation of several hundred self-pollinated ears from each of the following sources in Colombia: World Composite (16 possible mutants), Colombian Variety 254 (13 possible mutants), Yellow Eto (40 possible mutants), White Eto (1 possible mutant), Colombian Variety 206 (6 possible mutants), Colombian hybrid H105 (9 possible mutants), Tropical Coastal Flint (4 possible mutants), Yellow and White Mixture (3 possible mutants), Andaqui (7 possible mutants), Pira (1 possible mutant), Amagaceño (1 possible mutant), and Cariaco (11 possible mutants). These 112 possible mutants were planted in separate rows at CIAT (Centro International de Agricultura Tropical). Those which produced viable progeny were self-pollinated, outcrossed to Eto, and where possible outcrossed to the United States single cross hybrid A619 x A632.

Seed produced by the outcrosses to Eto was again planted at CIAT, and seed produced by the outcrosses to A619 x A632 was

planted at Purdue. Kernels of the self-pollinated ears will be analyzed for protein and lysine at Purdue.

A number of endosperm mutants had been isolated from the "Temp A" and "Temp B" synthetics, and allele and analytical tests are being conducted.

2. Temperate germplasm

Two synthetics have been developed with three generations of random mating (see 1972 report p. 5). These have been designated Temp HA o_2 and Temp HB o_2 Mild selection pressure has been applied visually for modified opaque types and grain quality. Little agronomic selection except for obviously undesirable types has been practiced to avoid narrowing the germplasm and adaption to United States cornbelt environment.

In the 1973 summer nursery approximately 350 to 400 full sibs will be made in each of these two synthetics. These blocks will be inoculated with northern leaf blight (Helminthosporium turcicum). Selections will be made for resistance, modified types, and grain quality. Approximately 150 to 200 full sib families in each synthetic will be evaluated at 5 or 6 worldwide locations in the Temperate Zone.

Temp HA and Temp HB synthetics were each crossed on endosperm mutants su_2o_2, wxo_2 and bt_2o_2 and separate BC_2 conversions made within each synthetic group (6 groups). Modified opaque-2 selections will be planted in an isolation in the 1973 summer nursery and allowed to random mate within groups. Present plans are to select for improved kernel size, weight, vitreousness, and protein quality in these double mutant versions.

Conversion of the temperate synthetic variety Colus to opaque-2 has been completed. A scheme to combine selection for yield, modified (more vitreous) kernel, and lysine content is being initiated with this variety.

3. Selection for high protein in opaque-2 population

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 some consideration for yield. Compared with previous generations, both protein and yield were lower in 1972 (Table 1). It should be noted that because of environmental conditions both yield and protein content values were lower in other experiments in 1972. Means and ranges in protein values indicate that considerable variation still exists within the population. The final generation of selection is being conducted in 1973. Also 50 of the selected ears from 1972 are being selfed to examine the possibility of deriving high protein inbred lines from this population.

The first cycle of a similar selection program was completed in an opaque-2 version of Iowa Super Stiff Stalk Synthetic. The mean percent protein for the 127 half-sib families analyzed in 1972 was 11.0 with a range of 9.7 to 12.1. Average yield was 101 Bu/A. The mean protein content for the 32 selected families was 11.3 with a range of 11.0 to 12.1. Mean yield for the selected families was 103 Bu/A. Eighty ears were selected from the selected families to plant in 1973. Their mean protein content was 12.2 with a range of 11.2 to 14.3.

Table 1. Means and ranges in percent protein and grain yield for the first three 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	
1972			
Mean of all half-sib families analyzed	11.6	10.7 - 12.4	86
Mean of selected families	11.9	11.6 - 12.4	87
Mean of selected ears from selected families	12.9	11.8 - 14.4	

B. Modified Opaque-2

1. Modified Temperate Synthetic HM o_2o_2

Synthetic HM o_2o_2, a population with a high percentage of modified (vitreous) opaque-2 phenotypes, was developed and released as source germplasm. The synthetic was developed by intercrossing in a diallel fashion nine lines homozygous for opaque-2 that exhibited a significant degree of endosperm vitreousness and were derived from United States Cornbelt germplasm. From these crosses 444 S_1 ears were produced, and on the basis of modification and ear-size and appearance, 70 ears were visually selected for endosperm protein and lysine content determinations. The protein content of these ears ranged from 8.5 to 13.7 percent with a mean of 10.6; while lysine as a percent of protein ranged from 2.01 to 3.34 with a mean of 2.75, and a percent of sample ranged from 0.223 to 0.349 with a mean of 0.289. Thirty-two ears with a lysine content expressed as percent of sample greater than 0.300 were intercrossed to form the synthetic.

2. Yield and protein quality relationships in modified opaque-2 types

Two experiments were conducted to study yield components and protein and lysine content of modified opaque-2 maize. Characters evaluated were: kernel yield per ear, kernel weight and density, protein percent, and lysine as percent of protein. "Normal"($+/o_2/o_2$) counterparts were obtained by outcrossing to +/+.

In Experiment I a diallel among six modified opaque-2 lines was compared to the "normal" counterpart diallel. The lines, their respective sources, and the types of endosperm modification are presented in Table 2. The "normal" phenotype was significantly higher

Table 2 . Origin of modified opaque-2 lines.

Inbred	Pedigree	Endosperm Modification
M-3	o_2 SynHA S_2	Sectored
M-4	(Flint "S"x(B37o_2xH60o_2)) S_4	1/2 : 1/2
M-5	[((B37xB14o_2)B14o_2)⊠(Syn60D)] S_4	1/2 : 1/2
M-6	o_2 SynHA High oil S_2	Sectored
M-7	((Pioneer 3306xB37o_2) x Pioneer 3306) S_5	Sectored (Near o_2)
M-8	((B14xIHP)⊠ x (B14o_2)) S_4	Sectored

than the modified phenotype for kernel yield, kernel weight, kernel density, and protein percent. (Tables 3 and 4) However, the modified opaque-2 exhibited a significantly greater lysine content than the "normal." (Table 5)

In Experiment II eight modified opaque-2 lines were crossed to an opaque-2 inbred. F_2 plants grown from selected opaque-2 and modified opaque-2 kernels were sibbed and outcrossed to normal pollen to compare opaque and its "normal," and modified and its "normal" phenotypes.

As in Experiment I the "normal" phenotypes exhibited significantly higher mean values than the modified phenotype for kernel yield per ear, kernel weight, and density. (Tables 6, 7 and 8) Modified opaque-2 was not significantly different from opaque-2 for kernel yield per ear, kernel weight, and protein percent; but it had significantly denser kernels. Opaque-2 had significantly higher lysine content than modified opaque-2. (Table 8) There was a significant negative correlation between lysine content and visual endosperm grade in the modified opaque.

Another study investigated the protein content and quality of the vitreous and opaque portions from modified opaque-2 endosperms. These portions were compared to whole modified endosperm as well as whole "normal" endosperm.

The vitreous portion had significantly greater protein content than the opaque portion. (Tables 9 and 10) Lysine content expressed both as percent of protein and percent of sample was significantly higher in the opaque portion. The vitreous portion had a higher lysine than the whole "normal" endosperm.

Table 3. Mean values of kernel yield per ear and 100-kernel weight for modified opaque-2 hybrids and normal counterparts.

Genotype	Kernel yield per ear (g)			100-kernel weight (g)		
	"Normal"*	Modified opaque-2	Percent of "Normal"	"Normal"*	Modified opaque-2	Percent of "Normal"
M-3 x M-4	162	153	94.0	24.59	22.77	92.6
M-3 x M-5	201	202	100.5	32.07	30.58	95.4
M-3 x M-6	178	172	96.6	27.45	26.25	95.6
M-3 x M-7	228	213	93.4	33.19	29.28	88.2
M-3 x M-8	208	180	86.5	31.94	28.09	87.9
M-4 x M-5	176	155	88.0	23.81	21.56	90.6
M-4 x M-6	162	159	98.1	22.62	21.60	95.5
M-4 x M-7	177	152	85.9	26.32	22.50	85.5
M-4 x M-8	180	166	92.2	27.37	24.19	84.4
M-5 x M-6	187	173	92.5	27.18	24.32	89.5
M-5 x M-7	231	215	93.1	31.08	29.40	94.6
M-5 x M-8	200	181	90.5	29.48	26.99	91.6
M-6 x M-7	178	167	93.8	29.05	25.89	89.1
M-6 x M-8	197	174	88.3	30.68	25.73	83.9
M-7 x M-8	229	199	87.0	34.09	30.26	88.8
Grand mean	193	177	91.7	28.73	25.96	90.4
SE of differences between means of Normal vs. Modified phenotypes in same hybrid:		3.78			0.44	
SE of differences between two hybrid means in either Normal or Modified phenotype:		5.50			0.49	

* Obtained by outcrossing the modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

Table 4. Mean values of kernel density for modified opaque hybrids and "normal" counterparts, and grade for modified opaque-2 kernels.

Genotype	Kernel density			Grade**
	"Normal"*	Modified opaque-2	Percent of "Normal"	
M-3 x M-4	1.32	1.24	93.9	3.0
M-3 x M-5	1.33	1.28	96.2	3.2
M-3 x M-6	1.32	1.27	96.2	3.0
M-3 x M-7	1.32	1.23	93.2	2.5
M-3 x M-8	1.32	1.23	93.2	2.7
M-4 x M-5	1.34	1.29	96.3	3.3
M-4 x M-6	1.31	1.28	97.7	2.8
M-4 x M-7	1.34	1.24	92.5	2.8
M-4 x M-8	1.32	1.16	87.9	2.5
M-5 x M-6	1.32	1.26	95.5	2.9
M-5 x M-7	1.30	1.24	95.4	2.3
M-5 x M-8	1.33	1.21	91.0	2.1
M-6 x M-7	1.28	1.20	93.8	2.0
M-6 x M-8	1.30	1.21	93.1	1.8
M-7 x M-8	1.25	1.19	95.2	1.5
Grand Mean	1.31	1.24	94.7	2.6

SE of differences between means of "Normal" vs. Modified phenotypes in same hybrid: 0.017.

SE of differences between two genotype means in same phenotype: 0.022.

* Obtained by outcrossing the modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

** Visual rating based on the proportion of the endosperm that is modified and on the frequency of endosperms showing modification.

Table 5. Mean values of protein percent and lysine as percent of protein in "normal" and modified opaque-2 kernels.

Genotype	Protein percent			Lysine as percent of Protein		
	"Normal"*	Modified opaque-2	Percent of "Normal"*	"Normal"*	Modified opaque-2	Percent of "Normal"*
M-3 x M-4	12.4	11.6	93.5	2.31	3.72	161.0
M-3 x M-5	11.6	11.1	95.7	2.39	3.54	148.1
M-3 x M-6	10.9	10.6	97.2	2.55	3.63	142.4
M-3 x M-7	11.7	11.2	95.7	2.46	3.62	147.2
M-3 x M-8	13.5	13.0	96.3	2.11	3.34	158.3
M-4 x M-5	11.7	11.2	95.7	2.46	3.59	145.9
M-4 x M-6	10.5	10.6	101.0	2.75	3.98	144.7
M-4 x M-7	11.6	10.8	93.1	2.61	4.03	154.4
M-4 x M-8	12.6	13.0	103.2	2.27	3.56	156.8
M-5 x M-6	11.6	11.5	99.1	2.66	3.74	140.6
M-5 x M-7	10.8	10.4	96.3	2.67	3.77	141.2
M-5 x M-8	13.1	12.6	96.2	2.29	3.43	149.8
M-6 x M-7	8.7	8.8	101.1	3.22	3.98	123.6
M-6 x M-8	12.6	12.2	96.8	2.33	3.69	158.4
M-7 x M-8	12.7	12.0	94.5	2.57	3.73	145.1
Grand Mean	11.7	11.4	97.4	2.51	3.69	147.0
SE of differences between means of Normal vs. Modified phenotypes in same hybrid:		0.24			0.08	
SE of differences between two genotype means in same phenotype:		0.30			0.10	

* Obtained by outcrossing the modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

Table 6 Mean values of kernel yield per ear and 100-kernel weight for opaque-2 outcrossed to normal and sibbed, and modified opaque-2 outcrossed to normal and sibbed.

Genotype	Opaque-2			Modified opaque-2		
	Outcrossed to normal	Sibbed	Percent of "normal"*	Outcrossed to normal	Sibbed	Percent of "normal"**
	Kernel yield per ear (g)					
A632o_2o_2xM-7	143	129	90.2	148	134	90.5
A632o_2o_2xM-5	125	111	88.8	130	122	93.8
A632o_2o_2xM-8	113	96	85.0	115	103	89.6
A632o_2o_2xM-2	133	115	86.5	137	115	83.9
A632o_2o_2xM-6	127	100	78.7	116	102	87.9
A632o_2o_2xM-9	122	106	86.9	118	121	102.5
A632o_2o_2xM-4	119	107	89.9	122	107	87.7
A632o_2o_2xM-3	126	114	90.5	125	109	87.2
Grand Mean	126a+	110b	87.3	126a	114b	90.5
	100-kernel weight (g)					
A632o_2o_2xM-7	30.17	25.43	84.3	30.58	26.50	86.7
A632o_2o_2xM-5	26.08	22.21	85.2	27.36	23.11	84.5
A632o_2o_2xM-8	28.00	22.73	81.2	28.37	24.68	87.0
A632o_2o_2xM-2	28.23	25.08	88.8	28.12	24.12	85.8
A632o_2o_2xM-6	24.75	20.82	84.1	25.33	19.86	78.4
A632o_2o_2xM-9	24.61	21.90	89.0	23.67	23.11	97.6
A632o_2o_2xM-4	21.49	18.56	86.4	21.26	18.55	87.3
A632o_2o_2xM-3	27.75	23.55	84.9	27.01	23.22	86.0
Grand Mean	26.39a+	22.54b	85.4	26.46a	22.89b	86.5

* and ** Obtained by outcrossing the opaque-2 and modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

\+ Means followed by the same letter do not differ significantly at 0.05 level (Duncan's new multiple-range test).

Table 7 Mean values of kernel density for opaque-2 outcrossed to normal and sibbed, modified opaque-2 outcrossed to normal and sibbed, and grade for modified opaque-2 kernels.

	100-kernel density						
	Opaque-2			Modified opaque-2			
Genotype	Outcrossed to normal	Sibbed	Percent of "normal"*	Outcrossed to normal	Sibbed	Percent of "normal"**	Grade+
A632o_2o_2xM-7	1.22	1.06	86.9	1.22	1.15	94.3	1.8
A632o_2o_2xM-5	1.27	1.08	85.0	1.27	1.19	93.7	2.7
A632o_2o_2xM-8	1.25	1.03	82.4	1.28	1.15	89.4	2.7
A632o_2o_2xM-2	1.26	1.10	87.3	1.26	1.14	90.5	2.5
A632o_2o_2xM-6	1.26	1.09	86.5	1.27	1.15	90.6	2.3
A632o_2o_2xM-9	1.25	1.08	86.4	1.25	1.14	91.2	2.5
A632o_2o_2xM-4	1.27	1.05	82.7	1.25	1.16	92.8	2.5
A632o_2o_2xM-3	1.25	1.09	87.2	1.28	1.13	88.3	2.5
Grand Mean	1.25a#	1.07c	85.6	1.26a	1.15b	91.3	2.4

* and ** Obtained by outcrossing the opaque-2 and modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

\+ Visual rating based on the proportion of the endosperm that is modified and on the frequency of endosperms showing modification.

\# Means followed by the same letter do not differ significantly at 0.05 level (Duncan's new multiple-range test).

Table 8 Mean values of protein and lysine for opaque-2 outcrossed to normal and sibbed, and modified opaque-2 outcrossed to normal and sibbed.

Genotype	Standard opaque-2			Modified opaque-2		
	Outcrossed to normal	Sibbed	Percent of "normal"*	Outcrossed to normal	Sibbed	Percent of "normal"**
	Protein percent					
A632o_2o_2xM-7	11.3	11.4	101.2	11.4	11.0	96.5
A632o_2o_2xM-5	12.6	12.0	95.2	12.0	12.3	102.5
A632o_2o_2xM-8	14.3	13.5	94.4	14.6	13.0	89.0
A632o_2o_2xM-2	12.5	13.1	104.8	12.2	13.1	107.4
A632o_2o_2xM-6	11.7	12.0	102.8	10.9	10.9	100.0
A632o_2o_2xM-9	12.1	12.4	102.5	12.9	12.3	95.3
A632o_2o_2xM-4	11.7	11.8	100.6	12.0	11.8	98.3
A632o_2o_2xM-3	11.7	12.5	106.6	12.6	12.3	97.6
Grand Mean	12.2a+	12.3a	100.8	12.3a	12.1a	98.4
	Lysine as percent of protein					
A632o_2o_2xM-7	2.66	4.17	156.8	2.64	4.07	154.2
A632o_2o_2xM-5	2.51	3.99	159.0	2.38	3.56	149.6
A632o_2o_2xM-8	2.27	4.08	179.8	2.17	3.55	163.6
A632o_2o_2xM-2	2.26	4.02	177.9	2.43	3.70	152.3
A632o_2o_2xM-6	2.49	4.21	169.1	2.71	4.08	150.6
A632o_2o_2xM-9	2.46	4.16	169.1	2.35	3.78	160.9
A632o_2o_2xM-4	2.52	4.08	161.9	2.47	4.08	165.2
A632o_2o_2xM-3	2.53	4.16	164.4	2.41	3.86	160.2
Grand Mean	2.46c+	4.11a	166.7	2.45c	3.84b	156.7

* and ** Obtained by outcrossing the opaque-2 and modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

\+ Means followed by the same letter do not differ significantly at 0.05 level (Duncan's new multiple-range test).

Table 9 Mean values of protein and lysine in modified opaque-2 maize endosperms for 1971 and 1972.

Genotype	Protein percent			Lysine content					
				Percent of protein			Percent of sample		
	Opaque portion	Vitreous portion	Whole endosperm	Opaque portion	Vitreous portion	Whole endosperm	Opaque portion	Vitreous portion	Whole endosperm
				1971					
M-1	8.6	10.6	10.9	4.52	2.76	3.19	0.39	0.29	0.35
M-2	9.8	11.2	11.5	3.74	2.22	2.54	0.37	0.25	0.29
M-3	8.7	11.5	11.2	5.04	2.80	3.25	0.44	0.32	0.37
Grand Mean	9.0b*	11.1a	11.2a	4.43a*	2.59c	2.99b	0.40a*	0.29c	0.34b
				1972					
M-1 x M-2	9.2	11.5	10.6	4.83	2.56	3.04	0.45	0.30	0.33
M-2 x M-5	10.2	13.4	12.8	3.29	2.09	2.36	0.34	0.28	0.30
M-4 x M-5	8.8	12.2	10.9	4.56	2.34	2.80	0.40	0.29	0.31
M-4 x M-6	8.4	12.1	11.2	4.73	2.44	2.83	0.40	0.30	0.32
M-4 x M-7	8.0	12.9	12.0	5.14	2.34	2.75	0.41	0.31	0.33
M-4 x M-8	7.7	12.1	11.1	4.40	2.30	2.62	0.34	0.28	0.29
Grand Mean	8.7c*	12.4a	11.4b	4.49a*	2.34c	2.73b	0.39a*	0.29c	0.31b

* Means followed by the same letter do not differ significantly at 0.05 level (Duncan's new multiple-range test).

Table 10 Mean values of protein and lysine in maize endosperms, 1973.

Genotype	Modified opaque-2 endosperm			"Normal" endosperm*
	Opaque portion	Vitreous portion	Whole endosperm	
	Protein percent			
M-3 x M-4	7.8	11.1	9.5	11.9
M-3 x M-8	8.1	14.6	11.0	12.8
M-4 x M-7	7.4	10.0	8.8	10.9
M-5 x M-6	7.7	10.2	9.1	9.9
Grand mean	7.8c**	11.5a	9.6b	11.4a
	Lysine as percent of protein			
M-3 x M-4	3.98	2.30	2.75	1.36
M-3 x M-8	3.48	1.82	2.44	1.27
M-4 x M-7	4.00	2.20	2.88	1.59
M-5 x M-6	3.18	2.13	2.60	1.60
Grand mean	3.66a**	2.11c	2.67b	1.46d
	Lysine as percent of sample			
M-3 x M-4	0.31	0.25	0.26	0.16
M-3 x M-8	0.28	0.27	0.27	0.16
M-4 x M-7	0.29	0.22	0.26	0.17
M-5 x M-6	0.24	0.22	0.24	0.16
Grand mean	0.28a**	0.24c	0.26b	0.16d

* Obtained by outcrossing the modified opaque-2 to normal pollen source, giving the endosperm genotype $+/o_2/o_2$.

** Means followed by the same letter do not differ significantly at 0.05 level (Duncan's new multiple-range test).

3. Amino acid profiles and protein fractionation of modified opaque-2

A complete amino acid profile of all four endosperm classes revealed that the opaque portion as compared to the vitreous portion was much higher in lysine, tryptophan, arginine, and glycine concentrations. (Table 11) The vitreous portion was much higher in lysine, tryptophan, cystine, histidine, arginine, and glycine as compared to the whole "normal" endosperm.

The results from endosperm protein fractionation (Table 12) showed that the opaque portion was lower in zein than any of the other endosperm classes. The zein content of vitreous portion was quite high compared to the opaque portion and was approaching values found in "normal."

Although these results were taken upon a small germplasm sample, it may be concluded that the modified opaque-2 maize as compared to standard opaque-2 expression did not increase yield and were poorer in protein quality.

C. Recurrent Selection for Increased Germ Size

1. Selection for larger germ size (higher oil) to maximize nutritional qualities

In the last annual report results of three methods of selection for larger germ size (percent oil) applied to opaque-2 Syn A were reported (pp. 12-17). The second cycle of selection by the S_1 progeny method has been completed. The derived populations previously obtained by half- and full-sib family selection (two cycles each), and those obtained by S_1 progeny selection (two cycles) are again being compared in a replicated field test in the summer of 1973.

A highly significant, positive correlation ($r = 0.48^{**}$) between oil and protein content was also reported for this population.

Table 11 Amino acid composition of defatted maize endosperms.

Amino Acid	Amino acid concentration (grams per 100 g of protein) in							
	M-3 x M-4				M-3 x M-8			
	Modified opaque-2 endosperm			"Normal" endosperm*	Modified opaque-2 endosperm			"Normal" endosperm*
	Opaque portion	Vitreous portion	Whole endosperm		Opaque portion	Vitreous portion	Whole endosperm	
Lysine	3.99	2.27	2.78	1.45	3.54	1.99	2.49	1.33
Tryptophan	1.11	0.71	0.74	0.35	0.98	0.39	0.71	0.37
Leucine	8.16	12.18	11.57	15.00	8.35	13.55	12.48	16.21
Isoleucine	3.25	3.48	3.61	3.65	3.20	3.74	3.66	4.02
Threonine	3.54	3.39	3.67	3.32	3.54	3.55	3.68	3.54
Methionine	1.72	2.02	2.12	2.20	1.69	2.10	2.12	2.30
Cystine	2.68	2.67	2.92	1.91	2.95	2.64	2.76	1.98
Phenylalanine	3.74	4.65	4.73	5.33	3.92	5.35	3.95	5.70
Tyrosine	3.84	4.43	4.58	4.94	3.96	4.72	4.52	5.26
Valine	5.33	4.84	5.32	4.84	5.34	4.90	5.32	4.74
Histidine	3.94	3.63	3.82	2.95	3.97	3.44	3.77	2.92
Arginine	5.79	4.30	4.78	3.32	5.25	4.08	4.52	3.07
Glycine	4.65	3.56	4.12	2.69	4.63	3.54	3.99	2.80
Alanine	5.47	6.76	6.74	8.22	5.65	7.43	7.11	8.75
Serine	3.90	4.19	4.47	4.80	4.05	4.75	4.50	5.16
Aspartic acid	7.84	7.07	7.78	5.78	7.14	6.84	7.45	6.12
Glutamic acid	17.24	22.47	22.22	25.19	18.36	24.28	23.60	27.77
Proline	8.38	10.41	10.34	9.98	8.96	10.59	11.01	10.67
Protein (percent)	7.8	11.1	9.5	11.9	8.1	14.6	11.0	12.8

Table 12 Nitrogen distribution in maize endosperms.

Fraction	Percent of soluble nitrogen*							
	M-3 x M-8				M-5 x M-6			
	Opaque portion	Vitreous portion	Whole endosperm		Opaque portion	Vitreous portion	Whole endosperm	
			Modified	"Normal"**			Modified	"Normal"**
I (Albumin and globulin)	17.02	10.11	7.34	4.98	16.95	10.05	13.63	6.08
II (Zein)	15.82	42.19	24.13	46.36	15.92	38.53	24.15	45.76
III (Glutelin-1)	9.06	20.28	20.80	20.30	10.31	15.75	15.87	20.37
IV (Glutelin-2)	15.46	9.72	16.20	9.45	22.69	12.90	14.22	14.41
V (Glutelin-3)	42.63	17.71	31.54	18.90	34.14	22.78	32.14	13.39

* Data adjusted for 100 percent recovery.

** Obtained by outcrossing the modified endosperm to a normal pollen source.

On the basis of this relationship a program of rather intense selection was begun to increase both oil and protein. The program was initiated by selecting from the derived populations eight individual ears having high oil content and intercrossing them in all combinations. These crosses were then ear-rowed and selfed. One hundred and eighty of the selfed ears were analyzed for oil and protein content. Eighteen of the ears whose oil content ranged from 6.45 to 8.18 percent with a mean of 7.20 and whose protein content ranged from 11.2 to 14.2 percent with a mean of 12.6 were selected and intercrossed in all possible combinations. These crosses will be selfed in the 1973 Summer Nursery and resulting ears analyzed for oil and protein.

Additionally, 12 of the 18 selected ears having an oil content above 7 percent and a protein level above 12 percent were selfed again. Five to eight S_2 progeny ears from each of the 12 selected S_1 ears are being grown to investigate the possibilities of deriving inbred lines having high oil and protein.

D. Genetic Interactions

1. Isolation of genetic lines and synthesis of specific endosperm mutant hybrids

A continued effort is being made to evaluate several endosperm mutant genes in double gene combinations with opaque-2 and floury-2. This is being done in an expanded number of inbred lines. Much of the progress in line conversions, synthesis of double gene combinations, and production of single-cross hybrids has been advanced by the use of the Florida and Hawaii winter nursery sites. Considerable emphasis is being placed on the opaque-2 double gene

combinations with the endosperm mutant genes sugary-2 and waxy because of their potential utilization to improve nutritional characteristics.

The initial phases of this program have involved backcrossing the mutant genes to the selected inbred backgrounds and recovering near isogenic lines for each mutant within an inbred. Several of the mutants have been recovered after six or more backcrosses within five backgrounds, while other background conversions are not as far advanced.

The following conversions (six backcrosses) were released because of the interest in their utilization to improve nutritional characteristics: Oh43 su_2/su_2, B37 su_2/su_2, C103 su_2/su_2, Oh43 wx/wx, C103 wx/wx. These releases have been distributed widely both in the United States and to other countries.

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--have been isolated in the Oh43,W64A, B37, and C103 backgrounds. Of these combinations the more promising double mutant genotypes are being or will be derived in additional backgrounds. The o_2 double combinations with bt_1 and bt_2 mutants are being isolated in the Oh43, W64A, B37, and C103 backgrounds.

The four near isogenic line 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 and/or fl_2 were crossed in a diallel series along with their normal counterparts to produce isogenic line single-cross hybrids.

A limited effort is being continued in the isolation of the opaque-7 mutant in a few inbred backgrounds (see 1971-72 Annual

Report, pp. 4-5) and the isolation of opaque-7 double mutant combinations with a select few endosperm mutant genes. Data from initial recoveries of o_7, o_2o_7 and fl_2o_7 from four different backgrounds gave mean values over the backgrounds of 11.4% protein and 2.8 lysine percent of protein for o_7; 10.4% protein and 3.6 lysine percent of protein for o_2o_7; and 12.0% protein and 3.3 lysine percent of protein for fl_2o_7. It would appear that the o_7 gene may affect lysine levels very near those of fl_2.

2. Mature grain characteristics in selected single endosperm mutant genes and their double mutant combinations with opaque-2 in hybrid backgrounds (1972)

(Part of Mr. G. A. Tosello's Ph.D. thesis program) Reported here will be the mature grain data of the second year (1972) evaluation of the near-isogenic hybrid lines. These hybrids were derived from a diallel series of crosses (see 1971-72 Annual Report, p. 18) from four inbreds (Oh43, W64A, B37, and C103) each nearly isogenic for the following single endosperm mutants--*ae*, *du*, *wx*, su_2, fl_2 and o_2--and their double combinations with opaque-2. The endosperm mutant hybrids and their normal hybrid counterparts were grown at the Purdue Agronomy Farm in 1972 in a split-split plot randomized complete block design with four replicates. The parental materials, although grown with these hybrid materials as one of the main plot effects, were analyzed separately from the hybrid data and will not be shown here.

One hundred-kernel weight and volume, kernel density, germ percent, and percent protein and lysine of the endosperm fraction were determined for all genotypes of the six hybrids. Percent

starch in the endosperm, amylose percent of the endosperm starch, and percent oil in the whole kernels were determined for selected genotypes of the six hybrids. The data are presented on the genotype means over hybrids.

The analyses of variance on the 1972 data indicated significant differences among hybrids for all variables except percent amylose and between the means of genotypes over hybrids for all characteristics. All hybrid x genotype interactions were significant for all variables except for percent starch and percent amylose. These data conform very closely to the 1971 data and indicate the importance of genetic background.

The 1972 means for 100-kernel weight and volume, kernel density, and germ percent of the whole kernel of the single endosperm mutants and their o_2 double combination lines over hybrids are presented in Table 13. One hundred-kernel weight and volume of all genotypes were significantly less than normal. The o_2 double combination lines were reduced in 100-kernel weight and volume in comparison to the respective nonopaque line; however, not all were significantly reduced. The wx o_2 double combination was higher in kernel weight while all other double combination genotypes were significantly lower in kernel weight than the o_2 single mutant line. The su_2 mutant when averaged over the hybrids was 16 percent lower in kernel volume. This reduced kernel size may also be principally responsible for the reduction in kernel weight.

The su_2 mutant gives a superior kernel density (Table 13). The most striking effect of the su_2 gene is that when it is combined with the o_2 gene to produce the su_2o_2 double combination, it

Table 13. One-year means of several single endosperm mutants and o_2 combination lines over hybrids for 100-kernel volumes, 100-kernel weight, kernel density, and germ percent--1972.

Genotype	100-Kernel Volume (ml)	100-Kernel Weight (g)	Kernel Density (g/ml)	Germ (%)
+/+	26.47	34.03	1.29	13.32
o_2/o_2	25.46	29.18	1.15	17.85
fl_2/fl_2	25.15	28.56	1.14	16.90
$fl_2/fl_2o_2/o_2$	22.56	26.09	1.16	17.38
ae/ae	25.05	31.19	1.25	15.85
ae/ae o_2/o_2	24.61	27.60	1.12	20.41
du/du	24.67	31.30	1.27	14.82
du/du o_2/o_2	23.47	27.26	1.16	18.87
wx/wx	25.63	33.48	1.31	12.94
wx/wx o_2/o_2	25.19	29.74	1.18	18.13
su_2/su_2	22.15	29.46	1.33	13.90
$su_2/su_2o_2/o_2$	21.30	26.93	1.26	17.54
LSD (0.01)	1.15	1.29	0.02	1.97
CV (%)	2.61	2.40	1.25	6.56

shows an apparent interaction to improve the kernel density and vitreousness. The kernel density of the su_2o_2 combination was significantly superior to the o_2 lines and all other o_2 double combinations.

The increase in germ percent of o_2 as has been shown in previous years and in other studies is evident in these materials (Table 13). The fl_2o_2 and su_2o_2 double combinations were reduced in germ percent compared to the o_2 genotype; however, not significantly so, whereas the other double combinations with o_2 resulted in increased germ percent. Even though the su_2 gene decreased kernel volume (seed size) as mentioned above, it is noted that the germ percent of the whole kernel was not significantly greater (<0.01) than the normal counterpart.

The 1972 means for percent protein and lysine as percent of protein in the endosperm fraction of the single endosperm mutants and their o_2 double combination lines over hybrids are presented in Table 14. The percent protein in the endosperm of the hybrid materials was lower across all genotypes in 1972 than those observed in the 1971 season.

The mean percent protein in the endosperm fraction of o_2 genotype over hybrids was significantly lower than the normal counterpart hybrids and the means of all o_2 double combination lines were significantly lower than their respective single non-opaque line. However, with the exception of the wx o_2 combination, all o_2 double combination lines were significantly greater in percent endosperm protein than the o_2 mean. When compared on a milligram of protein per endosperm basis over hybrids, there was

Table 14. One-year means of several single endosperm mutants and o_2 double combination lines over hybrids for percent protein and lysine percent of protein in the endosperm--1972.

Genotype	Endosperm Protein (%)	Endosperm Lysine (g/100g Pro.)
+/+	9.90	1.75
o_2/o_2	8.42	3.20
fl_2/fl_2	10.76	2.75
$fl_2/fl_2o_2/o_2$	9.08	3.19
ae/ae	11.24	2.06
ae/ae o_2/o_2	9.23	3.71
du/du	10.61	2.20
du/du o_2/o_2	8.76	3.80
wx/wx	10.20	1.82
wx/wx o_2/o_2	8.28	3.45
su_2/su_2	10.58	2.25
$su_2/su_2o_2/o_2$	8.87	3.73
LSD (0.01)	0.77	0.41
CV (%)	4.42	7.96

no significant difference among means of the o_2 double mutant combinations and the o_2 genotype nor among the o_2 double mutant combinations.

With the exception of the wx mean values for lysine as percent of protein (g/100 g protein) in the endosperm fraction of all single endosperm mutants and o_2 double mutant combination lines were significantly greater than the mean of the normal hybrid counterpart. In addition to the fl_2 mutant, the ae, du, and su_2 mutants showed some increase in lysine as percent of protein. Sugary-2 was 29 percent greater than the normal counterpart in lysine as percent of protein and 19 percent greater than the normal counterpart in actual milligrams lysine per endosperm. The fl_2o_2 double combination was not different from the o_2 genotype, while the mean of all other o_2 double combinations were significantly greater than o_2 in lysine as percent of protein in the endosperm fraction. The wx o_2 and su_2o_2 double combination means were 8 and 17 percent respectively greater than o_2 in lysine (g/100 g protein), and 7 and 14 percent respectively greater than o_2 when compared on a milligrams lysine per endosperm basis.

Percent starch of the mature endosperm fractions of two replications of each genotype in all six hybrids was determined by the procedure of Shuman and Plunkett (1964) which uses $CaCl_2$ to solubilize the starch. Percent amylose in the endosperm starch of all genotypes except fl_2, fl_2o_2, wx, and wx o_2 was determined. There were very small differences in percent starch among the genotypes. The mean values for the genotypes ranged from 65.89 to 62.72 percent starch in the endosperm fractions. The opaque-2

mean percent amylose value was not significantly different from the normal counterpart. The ae o_2 and du o_2 double combinations showed a slight nonsignificant decrease in percent amylose while su_2o_2 showed a significant (<0.05) increase in percent amylose over their respective nonopaque-2 single mutant lines.

The mean values for percent oil (whole kernel bases) and milligrams oil per whole kernel of the o_2, su_2, su_2o_2 hybrids and their normal counterpart hybrids are shown in Table 15. Significant differences were observed among all four genotypes for percent oil. Although the mean value of o_2 was greater than the normal counterpart hybrids in oil percent, o_2 did not show any significant difference in oil content per kernel (mg oil/kernel). As was observed in the 1971 results, the su_2 gene showed a significant increase in oil percent of the whole kernel (Table 15) while the germ percent was not siginificantly changed. However, the germ percent of the whole kernel did show a nonsignificant increase over normal in the 1972 data (Table 15). The su_2o_2 combination resulted in the greatest oil percent and was significantly higher than su_2. The mean values for actual oil content (mg oil/kernel) of the su_2 and su_2o_2 hybrids were 18 and 19 percent respectively greater than the mean of the normal counterpart hybrids.

3. Immature grain (green corn) characteristics in selected single endosperm mutant genes and their double combinations with opaque-2

(Part of Mr. G. A. Tosello's Ph.D. Thesis program) Reported here will be preliminary data on the immature (21- and 42-day postpollination) green corn protein quality of the first year's evaluation of the near isogenic hybrid lines. The derivation of

Table 15. One-year means of su_2, su_2o_2, o_2 and normal genotypes over hybrids for percent oil and oil content in the whole kernel--1972.

Genotype	Oil (%)	Oil/Kernel (mg)
$+/+$	4.27	14.47
o_2/o_2	4.96	14.48
su_2/su_2	5.71	17.01
$su_2/su_2o_2/o_2$	6.43	17.21
LSD (0.01)	0.17	1.45
CV (%)	8.96	4.86

the materials is the same as reported in the 1971-72 Annual Report, page 18 and section D-2 above. The hybrids each with the following single endosperm mutants--ae, wx, su_2, su_1, sh_2, fl_2, and o_2--their double combinations with opaque-2, and their normal hybrid counterparts were sampled at 21- and 42-days after pollination, representative of edible corns in the near green corn or late roasting ear stage of development. The kernels were immediately cut from the harvested ears, placed on dry ice, transferred to -29°C holding storage until freeze-dried for longer term preservation and until the samples could be prepared for protein and carbohydrate quality analyses.

The su_1, su_1o_2, sh_2, sh_2o_2 along with the o_2 and normal counterpart hybrids were statistically analyzed as a group within sample dates. Three hybrids and two replications were represented in the 21-day samples and four hybrids and two replications were represented in the 42-day samples. The ae, ae o_2, wx, wx o_2, su_2, su_2o_2, fl_2, fl_2o_2 along with the o_2 and normal counterpart hybrids were statistically analyzed as a group within sample dates. Six hybrids and three replications were represented in the 21-day samples, and six hybrids and two replications were represented in the 42-day samples.

Preliminary data on percent protein and lysine as percent of the protein in the endosperm fraction are presented on the genotype means over hybrids. Carbohydrate quality--i.e., reducing sugars, sucrose, water-soluble polysaccharide, starch content, and kernel component weights--will be reported later when the data are more complete from the two season trials in this study.

The analysis of variance of the 1971 data for the 21-day samples of the $\underline{su}_1$, $\underline{su}_1\underline{o}_2$, $\underline{sh}_2$, $\underline{sh}_2\underline{o}_2$, $\underline{o}_2$ and normal counterpart hybrids indicated significant differences among hybrids for protein percent and lysine as percent of protein in the endosperm and between the means for genotypes over hybrids for lysine as percent of protein. The hybrid x genotype interactions were not significant for either variable. In the 42-day samples of the same materials, significant differences among hybrids existed for protein percent in the endosperm fraction and between the means of these genotypes over hybrids for both protein percent and lysine as percent of protein. The hybrid x genotype interaction was significant for lysine as percent of protein.

The mean values of protein percent and lysine as percent of protein in the endosperm fraction of $\underline{o}_2$, $\underline{su}_1$, $\underline{su}_1\underline{o}_2$, $\underline{sh}_2$, $\underline{sh}_2\underline{o}_2$ and the normal counterpart hybrids for the 21- and 42-day samples are presented in Table 16. Percent protein in the endosperm fraction was not significantly different among genotypes at 21-days after pollination, but protein content at this immature stage of development was excellent. As development proceeded, the percent protein decreased considerably in the endosperm fraction at 42-days after pollination as one would expect, with the exceptions of $\underline{sh}_2$ and $\underline{sh}_2\underline{o}_2$ hybrids. The latter two genotypes were significantly greater than other genotypes in percent protein within the 42-day samples. The $\underline{su}_1$ and $\underline{su}_1\underline{o}_2$ genotypes were not significantly greater than normal, but they were significantly greater than $\underline{o}_2$ for percent protein at 42-days after pollination.

The mean values for lysine as percent of protein in the endosperm fraction at 21-days after pollination of the $\underline{o}_2$, $\underline{su}_1\underline{o}_2$,

Table 16. Mean values of endosperm protein percent and lysine as percent of protein for o_2, su_1, su_1o_2, sh_2, sh_2o_2 and the normal counterpart hybrids for 21- and 42-days after pollination--1971.

	Days after Pollination							
	21+				42++			
Genotype	Protein (%)		Lysine (g/100g protein)		Protein (%)		Lysine (g/100g protein)	
$+/+$	14.0	a*	3.87	b	11.5	bc	2.27	d
o_2/o_2	13.5	a	4.40	ab	10.0	c	3.27	b
su_1/su_1	13.9	a	3.92	b	12.3	b	2.71	c
$su_1/su_1o_2/o_2$	14.3	a	4.50	a	11.8	b	4.32	a
sh_2/sh_2	15.8	a	4.05	ab	15.1	a	3.53	b
$sh_2/sh_2o_2/o_2$	15.2	a	4.55	a	14.7	a	4.20	a

\+ Means of three hybrids and two replications.
++ Means of four hybrids and two replications.
* 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 Tests.

sh_2 and sh_2o_2 genotypes were not significantly different from each other, but all were higher than the normal counterpart. However, o_2, su_1 and sh_2 were not significantly greater than normal for lysine as a percent of protein. In the 42-day samples the su_1o_2 and sh_2o_2 genotypes were significantly superior in lysine as percent of protein to the respective nonopaque single mutants and o_2. All genotypes were significantly greater than normal in lysine as percent protein at this stage of development.

A field replicated trial of the bt_2 and bt_2o_2 genotypes in an early generation hybrid background was analyzed for percent protein and lysine as percent of protein in the whole kernel at near mature stage of development. The bt_2 had 15.6% protein and 3.81 lysine as percent of protein while bt_2o_2 ran 17.8% and 4.73 (g/100g protein) for protein and lysine as percent of protein respectively. The bt_2o_2 genotype compares favorably with the protein quality observed in sh_2o_2.

The analyses of variance of the 1971 data for the 21- and 42-day samples of the ae, ae o_2, wx, wx o_2, su_2, su_2o_2, fl_2, fl_2o_2, o_2, and normal counterpart hybrids indicated significant differences among hybrids and between the means of genotypes over hybrids for protein percent and lysine percent of protein in the endosperm fraction. With the exception of the variable protein percent in the 42-day samples, the hybrid x genotype interactions were significant.

The mean values of protein percent and lysine as percent of protein in the endosperm fraction of o_2, fl_2, fl_2o_2, ae, ae o_2, wx, wx o_2, su_2, su_2o_2, and the normal counterpart hybrids for the

21- and 42-day samples are presented in Table 17. Percent protein and lysine percent of protein (quality) at 21-days after pollination was at a high level in all genotypes, and few significant differences existed. As development proceeded (42-day samples), protein quality dropped; and the differences among genotypes became more apparent. The double combination genotypes fl_2o_2, $ae\ o_2$, $wx\ o_2$ and su_2o_2 were not statistically different from o_2 at 21-days after pollination, and with the exception of fl_2o_2 all were significantly greater than o_2 at 42-days after pollination in lysine as percent of protein. The $ae\ o_2$, $wx\ o_2$ and su_2o_2 genotypes appear to have favorable protein quality for edible corn maturity although they were not as high in protein quality as the su_1o_2 and sh_2o_2 genotypes (Table 16). Opaque-2 was reduced in percent protein in the endosperm fractions at 21- and 42-days after pollination compared to the normal counterpart and significantly so in the 42-day samples. The effect of o_2 on the double combinations was also reduced percent protein compared to the respective single non-opaque-2 gene in the combination. This result is consistent with what was found in the mature grain samples.

4. In vitro digestibility characteristics of endosperm mutants and their double combinations with opaque-2

In previous studies we have demonstrated the differences among several single endosperm mutants and their double combinations with opaque-2 in *in vitro* susceptibility of starch granules (raw and whole grain starch) to the action of amyloglucosidase using a reaction system adopted to a differential respirometer (see 1971-72 Annual Report, pp. 28-31). In cooperation with

Table 17. Mean values of endosperm protein percent and lysine as percent of protein for several single endosperm mutants, their o_2 double combinations and the normal counterpart hybrids for 21- and 42-days after pollination--1971.

	Days after Pollination			
	21+		42++	
Genotype	Protein (%)	Lysine (g/100g protein)	Protein (%)	Lysine (g/100g protein)
$+/+$	14.6 bcd*	3.87 e	11.6 abc	2.25 h
o_2/o_2	14.1 d	4.33 ab	10.4 de	3.36 de
fl_2/fl_2	16.1 a	4.12 bc	12.5 a	3.25 e
$fl_2/fl_2o_2/o_2$	15.1 bc	4.18 bc	11.8 ab	3.54 cd
ae/ae	15.2 b	4.23 abc	12.3 a	2.78 f
ae/ae o_2/o_2	14.6 bcd	4.39 a	10.9 bcde	4.10 a
wx/wx	14.7 bcd	3.93 de	11.5 abcd	2.25 h
wx/wx o_2/o_2	14.4 bcd	4.23 abc	10.3 e	3.77 bc
su_2/su_2	15.1 bc	4.09 cd	11.8 ab	2.54 g
$su_2/su_2o_2/o_2$	14.2 cd	4.31 ab	10.6 cde	4.03 ab

\+ Means of six hybrids and three replications.
++ Means of six hybrids and two replications.
* 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 Tests.

Dr. Fuwa, who spent some time in our laboratory, we have conducted further studies on the *in vitro* susceptibility of starch granules of the several endosperm mutant and combinations with o_2 in the Oh43 inbred to the action of fungal gluco-amylase, pancreatin, and bacterial α-amylase. We followed the method of Fukui, et al., 1964 (J. Agr. Chem. Soc., Japan 38:262), with slight modification. The contents of the solublized carbohydrates were determined by the phenol-sulfuric acid method (Dubois et al. 1956. Anal. Chem. 28: 350).

Starch granules of the *ae* and *ae* o_2 genotypes were very resistant to the action of amylases than those of normal. Starch granules of su_2 and su_2o_2 were digested by amylases very much faster than those of the normal counterpart. In general, the double mutant combinations with opaque-2 were digested by the amylases very similar to their respective single nonopaque mutant in the combination. The extreme differences in susceptibility of the starch granule to the action of the amylases disappeared when the starches were gelatinized with alkali.

5. Biological value of several single endosperm mutants and their double combinations with opaque-2 in hybrid backgrounds

Opaque-2, *wx*, *wx* o_2, su_2, su_2o_2, and their normal counterpart in the Oh43 x B37 and B37 x Cl03 single-cross hybrids plus casein as a control have been fed to weanling rats to compare their biological performance. Whole ground grain was fed in an isonitrogenous-isocaloric diet with mineral and vitamin supplement. Ten male rats were used in each group and fed for a duration of 28 days. The *wx* o_2 and su_2o_2 hybrids gave an excellent response in comparison

to the normal and o_2 hybrids. The su_2 hybrids gave better gains than normal but were not as good as o_2, while the wx hybrids did not give as good of gains as the normal counterpart hybrids. The results suggest that the change in the starch composition and vitreousness of the kernels in the su_2o_2 mutant combination has not affected the biological value of this high lysine grain.

Additional feeding tests in different animal species of the su_2, su_2o_2, wx, wx o_2, o_2, and their normal counterpart hybrids are underway.

6. Selection for higher protein content in sugary-2/opaque-2 and waxy/opaque-2 double mutant combinations

The su_2o_2 and wx o_2 double mutant genotypes have been crossed with at least two agronomically desirable selections of opaque-2, high protein line recoveries in each of the Oh43, B14, and B37 backgrounds. These higher protein recoveries were derived from crosses to the Illinois High Protein material and selecting for protein content and agronomic type. Selection will continue for protein quality and content, seed quality (vitreousness), and size within the su_2o_2 and wx o_2 double mutant recoveries.

7. Selection for protein content in multialeurone sugary-2/opaque-2 and waxy/opaque-2 double mutant combinations

A few initiatory crosses involving su_2o_2 and wx o_2 double mutant combinations by multialeurone layer stocks (obtained from M.S. Zuber, Mo.) were made in the 1972 summer and 1972-73 winter nurseries. Selection will be made to increase the proportion of aleurone tissue relative to the starchy portion of the endosperm in an additional effort to improve the protein nutritional quality.

8. Characterization of endosperm protein bodies and matrix in several single endosperm mutants and their double combinations with opaque-2

(Part of Mr. P. S. Baenziger's M.S. thesis program) Preliminary evaluation of the nature of the protein body development using interference contrast microscopy (Nomarski system) has shown that endosperm mutants high in lysine have greatly reduced protein bodies both in size and number. The endosperm mutants with low levels of lysine tend to have large protein bodies and are more numerous. Opaque-2 kernels from inbred lines unselected for modified vitreous opaque types but which had small patches of vitreous starch in the endosperm, and modified opaque-2 kernels with nearly all vitreous endosperm were examined. There appeared to be a strong correlation in the endosperm vitreousness fraction and the development of more numerous and larger protein bodies.

9. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins from developing normal, opaque-2, brittle-2 and brittle-2 opaque-2 endosperms

Dr. John Snyder, postdoctorate, initiated a study to compare protein development in normal, protein-modified (opaque-2) and carbohydrate modified (brittle-2) maize endosperms. C103, B37, W64A, and Oh43 normal inbred lines and Oh43 opaque-2, brittle-2, and brittle-2 opaque-2 near-isogenic lines were used as sources of endosperm material. The four Oh43 genotypes provided material for comparing effects of the endosperm mutant genes. The four normal inbreds provided material for comparison of different "normal" genotypic backgrounds. Representative ears were harvested at 7-day intervals beginning 14 days after pollination and concluding

at 42 days after pollination. This developmental period includes the maximum rates of carbohydrate and protein synthesis in the endosperm. The 1971-72 Annual Report presented evidence that some carbohydrate-modifying mutations, such as brittle-2, also affect lysine levels in endosperm tissue (p. 35).

Endosperm proteins were fractionated on the basis of molecular weight by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. SDS was also used for extraction of immature endosperm proteins. This detergent denatures the endosperm proteins, aids inhibition of hydrolytic enzyme activity, and provides single-step extraction of classical protein fractions--i.e., albumins, globulins, zeins and glutelins. Attempts to identify the subcellular source(s) of protein fractions obtained by SDS-polyacrylamide gel electrophoresis have been initiated.

Thirty-one bands or protein fractions were obtained from Oh43 normal endosperms at 21 days' post-pollination. This genotype and maturity was selected as a standard for all comparisons. All genotypes gave similar banding patterns at 14 days. The banding patterns at 14 and 21 days differed in both the mobilities and relative concentrations of bands. At 21-days banding patterns of the four normal genotypes were similar except for relative concentrations of the two zein bands. Among the four Oh43 genotypes at 21 days obvious differences were observed in concentrations of the two zein bands and one high molecular weight band. The high molecular weight band is more concentrated in $\underline{o}_2$ extracts than normal and absent from $\underline{bt}_2$ and $\underline{bt}_2\underline{o}_2$ extracts. The nature of this band is presently unknown. Banding patterns from later maturities

were similar to respective 21-day patterns except for changes in relative concentrations of bands. In general, both developmental and genotypic differences were observed.

The zein fraction of maize endosperm protein is separated into two distinct bands of approximately 22,000 and 25,000 MW. These bands have been identified as zein proteins by two different procedures: (1) The two bands are absent from $\underline{bt}_2\underline{o}_2$ endosperm extracts and reduced in $\underline{o}_2$ and $\underline{bt}_2$ endosperm extracts compared to normal (see also Annual Report, 1971-72, p. 36-37), and (2) when protein bodies--i.e., the "organelles" in which zein is accumulated--were isolated from Oh43 normal endosperms (21-days) and subsequently subjected to electrophoresis, most of the protein migrated as two bands that corresponded to the two bands missing from $\underline{bt}_2\underline{o}_2$ extracts.

This initial study provides a possible basis for identification of subcellular sources--e.g., ribosomes, membranes, enzymes, etc.-- of other protein bands, identification of protein bands that have high basic amino acid content, and survey of different endosperm mutants, inbred lines, or populations of maize for increased levels of lysine-rich proteins and normal zein content. In this regard preliminary experiments indicate that "microsomal" proteins-- ribosomes, associated proteins, and membranes--can be isolated, and after electrophoresis compared to the banding patterns of total protein extracts. Also acid orange-12, a dye which complexes specifically with the basic amino acids lysine, histidine, and arginine, has been used to stain proteins from both total protein and microsomal extracts. Additional work will be required to determine the significance of these two observations.

10. Effect of the opaque-2 gene and outcrossing on characteristics of the kernel, cob, protein, and lysine

In the last annual report (pp. 31-33) the influence of several mutant genes on kernel and cob weights was reported. It was found that the opaque-2 gene reduced kernel weight in the three hybrids used, but its effect on their cob weight varied with hybrids.

A further study of the effect of the opaque-2 gene on cob and kernel characteristics was conducted in 1972 on opaque-2 and normal versions in six hybrid backgrounds: "A619 x B37", "B14 x C123", "R177 x B37", "A632 x A619", "C123 x B37", and "R177 x C123". These were grown in a split plot design with the following pollinating applied in subplots: (1) normal sibbed, (2) opaque-2 sibbed, and (3) opaque-2 outcrossed to their normal counterpart (opaque-2 outcrossed).

Means for kernel characters are presented in Table 18. The average kernel yield of opaque-2 hybrids was significantly less than normal. "Opaque-2 outcrossed" yielded more than the "opaque-2 sibbed" but less than the "normal sibbed". A similar result was found for 100-kernel weight as for kernel yield per ear. All treatments behaved similarly for percent protein. "Opaque-2 sibbed" was significantly higher than "opaque-2 outcrossed" and "normal sibbed" for lysine as percent of protein and as percent of sample and weight of lysine per ear. "Opaque-2 outcrossed" and "normal sibbed" did not differ for these characters. The difference in cob weight between normal and opaque-2 versions depended on genetic background. Three of the hybrids with inbred line C123 as a common parent did not show any difference with respect to cob weight

Table 18. Means for several kernel characters for the opaque-2 and normal versions of six hybrids with three pollinating treatment.

Pollinating Treatments	Endosperm Genotype	Kernel Yield wt/ear	100-Kernel Weight	Protein	Lysine		Protein wt/ear	Lysine wt/ear
					% of Prot	% of Sample		
		g	g	%	%	%	g	g
Normal Sibbed	$\underline{+}/\underline{+}$	236.3 a[+]	33.1 a	11.72 a	2.62 b	.306 b	27.60 a	.723 b
Opague-2 Outcrossed	$\underline{o_2}\underline{o_2}/\underline{+}$	227.8 b	32.1 b	12.00 a	2.61 b	.312 b	27.61 a	.715 b
Opaque-2 Sibbed	$\underline{o_2}\underline{o_2}/\underline{o_2}$	200.1 c	28.1 c	11.86 a	3.99 a	.473 a	24.56 b	.977 a

+ Mean within a column followed by the same letter or letters do not differ significantly at .05 level of probability (Student Newman - Keuls' Test).

(Table 19). With hybrids "A619 x B37" and "R177 x B37", the cob weight of "normal sibbed" was significantly heavier than "opaque-2 outcrossed" and "opaque-2 sibbed".

11. Effects of the opaque-2 gene on kernel and cob development

Previous annual reports have presented results of comparisons of normal and opaque-2 versions of standard hybrids that indicated the opaque-2 gene reduced kernel yield and in some cases cob weight. It was also reported that the rate of dry matter accumulation by opaque-2 hybrids decreased significantly beginning 30 to 40 days after pollination and nearly ceased 7 to 10 days earlier than normal counterparts; yet black layer formation at the tip of the kernel, an indicator of physiological maturity, occurred at the same time. Development during the first 3 to 4 weeks after pollination was not investigated in these earlier studies.

With the above considerations in mind additional studies of kernel and cob development were made. One phase was to examine kernel and cob development in the opaque-2 and normal versions of four singlecross hybrids: (1) W64A x B37; (2) B37 x A239; (3) A239 x A545; and (4) B37 x C123. Sampling was done at 5-day intervals from 10 to 55 days after pollination. Kernel and cob weights and ear moisture were taken. Mean values expressed on a per ear basis are given in Table 20. Kernel weight values are plotted in Figure 1. The rate of dry matter accumulation in the opaque-2 decreased 30 to 40 days after pollination compared with the normal versions. Kernel weight of the opaque-2 at the last harvest was less than the normal. The magnitude of difference varied with hybrids. At 55 days kernel weight of the opaque-2

Table 19 . Cob weights for six hybrids with three pollinating treatments.

Hybrids	Pollinating Treatments		
	Normal Sibbed $+/+/+$	Opaque Out-crossed to Normal $+/o_2/o_2$	Opaque-2 Sibbed $o_2/o_2/o_2$
	g	g	g
A619 × B37	50.5 a[+]	44.5 b	42.5 b
B14 × C123	43.5 a	46.5 a	46.3 a
R177 × B37	54.0 a	48.8 b	46.5 b
A632 × A619	40.0 a	41.0 a	42.0 a
C123 × B37	53.3 a	53.8 a	53.8 a
R177 × C123	42.8 a	44.0 a	41.5 a
Mean	47.3	46.4	45.4

[+] Means within the same line followed by the same letter or letters do not differ significantly at the .05 level of probability (Student Newman-Keuls' Test).

Table 20 . Mean kernel and cob weight per plant and ear moisture of normal and opaque-2 versions of four hybrids harvested at 5-day intervals 10-55 days post-pollination.

Days Post-Pollination	Kernel Weight		Cob Weight		Ear Moisture	
	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2
	g		g		%	
10	6	5	21	23	86	84
15	14	15	30	30	82	80
20	39	40	34	33	74	74
25	80	77	37	36	64	64
30	120	115	41	39	57	56
35	153	138	42	40	49	51
40	169	155	42	39	45	47
45	192	168	42	40	40	44
50	204	175	44	40	38	41
55	211	176	44	40	35	38

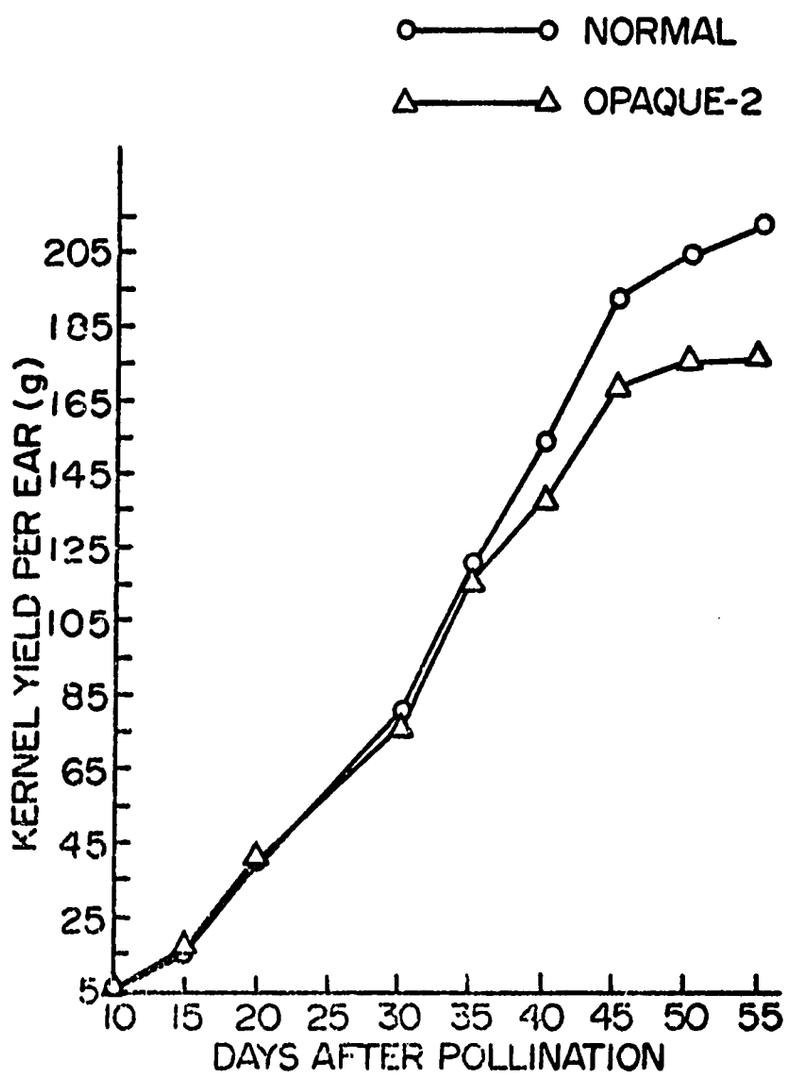

Figure 1. Comparison of Kernel Yield per Ear of Opaque-2 and Normal Versions Averaged Over Four Hybrids (Group I).

hybrids averaged 84 percent of normal with the range of 79 percent for B37 x A239 to 89 percent for B37 x C123.

Cob weight of the opaque-2 was less than for normal hybrids; however, the magnitude of difference depended on genetic background. The cob weight of opaque-2 hybrid B37 x C123 was only slightly less at most harvest dates, whereas the opaque-2 version of B37 x A239 was less than normal at all harvest dates (Figure 2). Cob weight of both opaque-2 and normal hybrids reached a maximum at 35 days after pollination. This is in agreement with our earlier results for pollinated ears. However, it is of interest to note from a previous study utilizing three of the same hybrids that maximum dry matter accumulation in the cobs of unpollinated ears occurred 15 days after silking.

As found in the earlier study, ear moisture was less in the normal version beginning 35 days after pollination.

With the interest in modified (vitreous endosperm) opaques and the desirability of developing high yielding opaque-2 hybrids, it is of interest to know whether opaque hybrids of these types have dry matter accumulation patterns that differ from hybrids made from converted standard inbred lines. It is also of interest to know how their rates vary from normal versions. With these thoughts in mind ears from two experimental modified opaque-2 hybrids (E-1 and E-2) and two experimental high-yielding opaque-2 hybrids (E-3 and E-4) were harvested at 5-day intervals 25 to 55 days after pollination. Because no normal counterparts existed for these hybrids, comparisons were made between sib pollinated ears and those outcrossed to a normal pollen source.

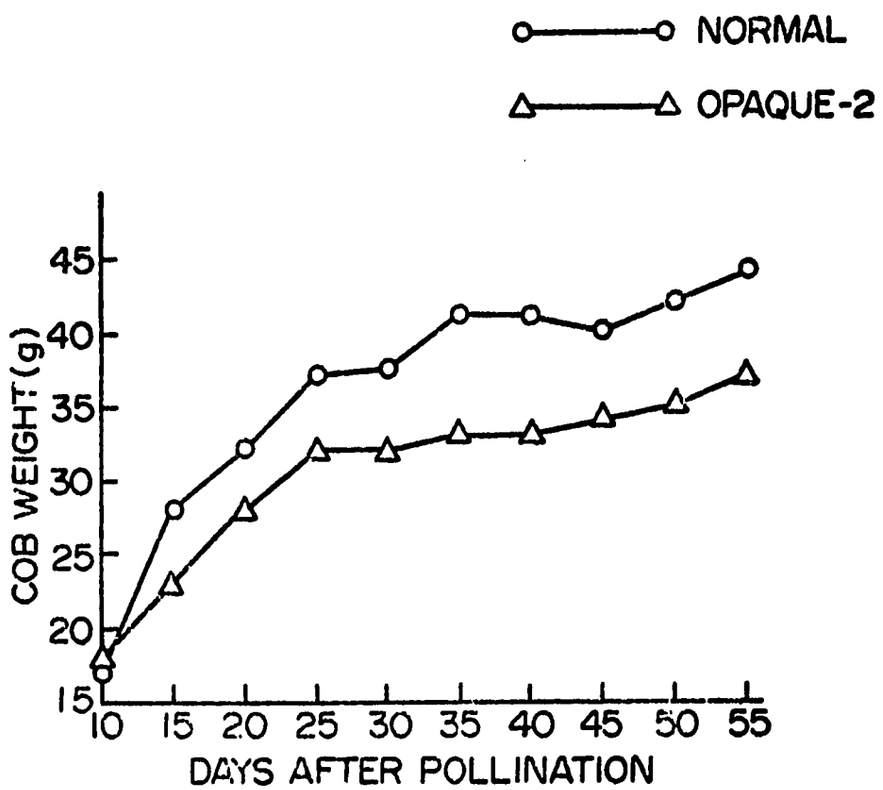

Figure 2. Comparison of Cob Weight of Opaque-2 and Normal Version of B37 x A239 (Group I).

Cob weights differed little between the sibbed and outcrossed of either type. There was also little difference in kernel weight between sibbed and outcrossed (Table 21). Ear moisture was 2 percent higher in the sibbed modified and 2 to 4 percent higher in the high-yielding at all harvest dates. Kernel dry matter accumulation curves for modified opaque-2 hybrid E-2, sibbed and outcrossed to normal, are shown in Figure 3, and for high-yield hybrid E-4 in Figure 4. The high-yielding opaque-2 hybrids were still accumulating dry matter at an appreciable rate 55 days after pollination. This is in sharp contrast to the converted hybrids and modified types that ceased dry matter accumulation well before this stage. This increased period of appreciable kernel dry matter accumulation could well account for the difference between hybrids that produce inferior or average yields and those that produce superior yields.

E. Research in Improved Analytical Methods

(Published new methods were monitored, but in our judgment were not considered superior to our present methods.)

F. Central Biochemical Laboratory

1. Cooperative research with Dr. E. Villegas (CIMMYT)

A cooperative nutritional testing program with several laboratories was developed by Dr. Villegas and Dr. Mertz and supervised by Dr. Mertz. This is reported under Section G.

2. Basic research on the nature of the proteins in developing and mature high lysine types of maize

a. Amino acid patterns of the Landry-Moureaux fractions

Tables 22 to 26 show the complete amino acid patterns (excluding tryptophan) of the five Landry-Moureaux fractions

Table 21. Mean kernel weight per plant and ear moisture for two modified and two high yielding opaque-2 hybrids sibbed and outcrossed to normal harvested at 5-day intervals 25-55 days post-pollination.

Days Post-Pollination	Modified				High-Yielding			
	Kernel Weight		Ear Moisture		Kernel-Weight		Ear Moisture	
	Sib	Outc*	Sib	Outc*	Sib	Outc*	Sib	Outc*
	g		%		g		%	
25	71	73	62	60	87	86	67	65
30	107	102	55	53	117	128	60	58
35	129	130	51	47	161	167	55	52
40	143	149	47	44	181	192	51	49
45	165	166	44	41	201	216	48	44
50	173	181	41	38	219	239	45	42
55	190	185	35	33	238	244	42	38

* Outcrossed to normal (+/+) pollen.

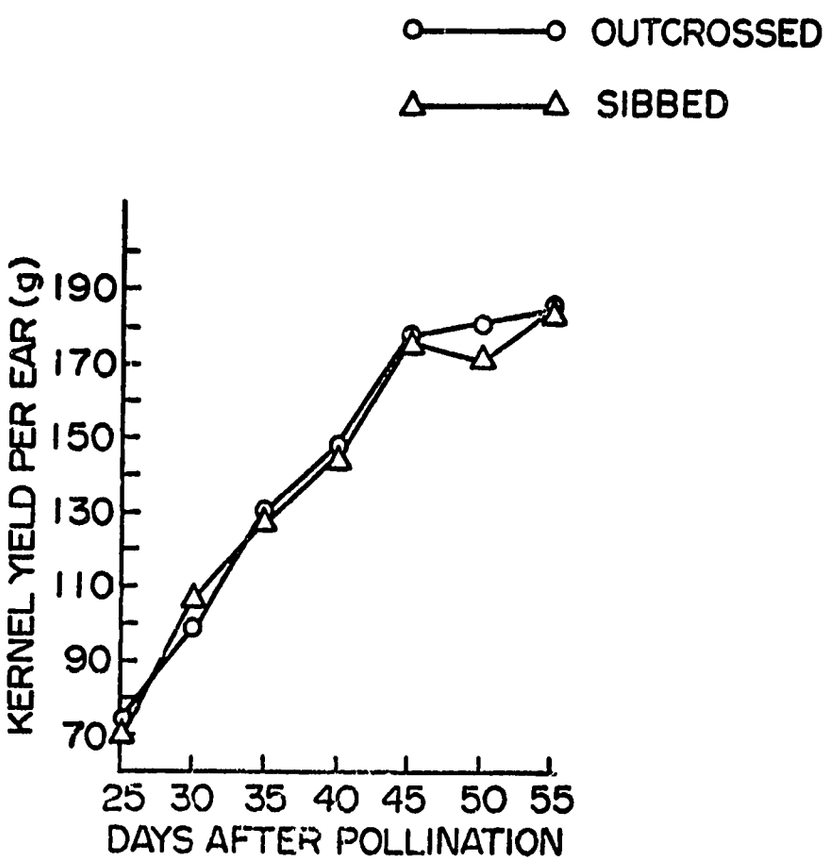

Figure 3. Comparison of Kernel Yield per Ear of Modified Opaque-2 Hybrid E-2 Sibbed and Outcrossed.

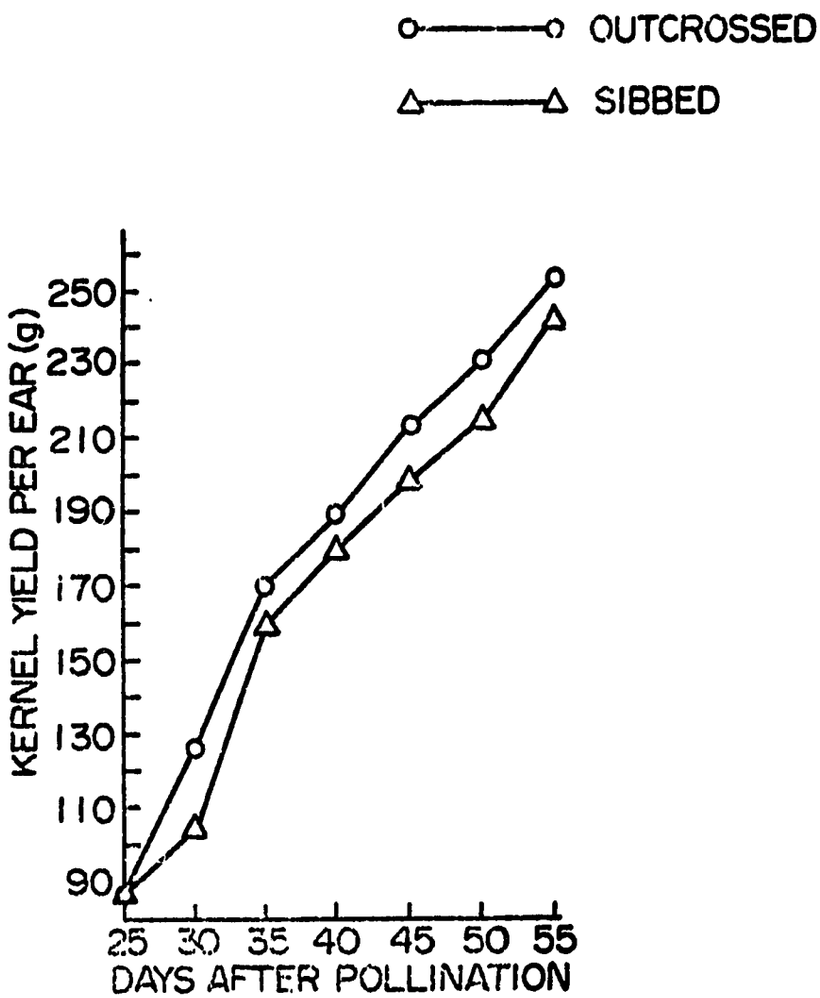

Figure 4. Comparison of Kernel Yield per Ear of High Yielding Opaque-2 Hybrid E-4 Sibbed and Outcrossed.

Table 22

Amino Acid Composition of Fraction I*

Inbred	Oh43					W22	
Genotype	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
Lysine	6.3	5.1	5.8	4.3	3.7	6.1	5.2
Histidine	3.2	2.4	3.3	2.2	2.2	2.5	2.3
Arginine	11.7	6.4	11.8	6.1	7.9	10.4	9.9
Aspartic Acid	11.1	9.6	9.2	9.2	7.4	7.9	7.6
Threonine	5.3	4.9	1.2	3.9	3.5	4.2	3.6
Serine	5.8	5.2	4.9	4.7	4.0	4.5	3.9
Glutamic Acid	18.5	14.3	13.8	13.7	9.2	10.9	14.8
Proline	6.4	5.1	5.3	7.4	6.3	4.8	4.5
Glycine	8.2	7.5	7.0	6.9	5.8	6.4	5.9
Alanine	8.8	7.7	7.2	8.5	7.7	7.0	5.8
Cystine	1.6	3.9	0.1	4.2	4.0	0.3	0.1
Valine	6.2	6.8	5.3	6.0	4.7	4.7	4.8
Methionine	2.5	3.0	1.5	1.1	0.8	1.7	1.8
Isoleucine	4.6	3.9	4.3	3.5	3.1	3.9	3.4
Leucine	6.4	4.6	6.1	4.4	4.5	5.4	5.2
Tyrosine	4.5	4.2	3.6	4.0	3.6	2.8	3.0
Phenylalanine	4.2	4.5	3.7	3.5	3.1	2.4	3.1
Total Recovery	115.3	99.1	97.1	93.6	81.5	85.9	84.9

*Amino acid levels as percent of total protein (N X 6.25).

Table 23

Amino Acid Composition of Fraction II*

Inbred	Oh 43					W22	
Genotype	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
Lysine	0.2	0.5	0.2	0.1	0.6	0.1	0.1
Histidine	1.6	2.4	1.4	1.0	1.5	1.6	1.7
Arginine	1.9	5.2	1.7	1.6	1.9	1.6	1.6
Aspartic Acid	5.9	6.8	5.8	6.1	1.5	6.0	5.8
Threonine	3.4	4.2	3.3	3.6	2.3	3.4	3.6
Serine	5.9	6.2	5.7	6.1	2.5	5.9	5.7
Glutamic Acid	32.8	33.3	30.6	31.7	14.3	30.5	30.3
Proline	11.0	11.2	10.5	10.4	7.0	10.9	10.8
Glycine	1.6	1.9	1.8	1.5	3.4	1.5	1.5
Alanine	11.2	11.3	10.4	11.0	3.4	10.4	10.2
Cystine	0.7	0.6	0.2	0.3	0.1	0.1	0.1
Valine	4.1	4.9	3.7	4.5	2.2	4.2	4.6
Methionine	2.2	4.0	1.7	1.9	1.2	1.9	2.3
Isoleucine	4.4	4.9	4.7	4.7	1.2	4.4	4.3
Leucine	20.5	20.2	21.1	19.6	5.1	20.3	19.9
Tyrosine	6.1	6.0	5.8	6.0	3.3	5.5	5.4
Phenylalanine	8.0	8.0	8.3	7.9	2.8	7.7	6.9
Total Recovery	121.5	131.6	116.9	118.0	54.3	116.0	114.8

*Amino acid levels as per cent of total protein (N x 6.25).

Table 24

Amino Acid Composition of Fraction III*

Inbred	Oh43					W22	
Genotype	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
Lysine	0.6	0.6	0.5	0.4	0.8	0.5	0.4
Histidine	8.0	0.3	3.3	1.9	3.8	4.8	4.2
Arginine	3.2	3.5	2.4	2.2	2.9	4.8	4.3
Aspartic Acid	3.1	5.2	3.9	4.9	1.6	2.8	3.7
Threonine	4.3	3.5	4.3	3.7	4.4	4.0	4.5
Serine	5.2	6.0	5.5	6.3	5.1	4.8	5.6
Glutamic Acid	25.7	27.0	30.9	31.8	31.2	29.3	31.1
Proline	15.6	12.4	16.2	13.5	15.4	17.1	17.2
Glycine	4.5	2.8	4.2	3.6	6.6	4.7	4.3
Alanine	7.5	9.8	8.4	10.1	6.5	8.2	9.2
Cystine	0.4	0.3	0.6	0.5	0.6	0.2	0.3
Valine	4.7	3.8	5.1	4.0	4.6	5.6	5.0
Methionine	4.3	8.9	6.2	9.2	2.5	5.7	8.0
Isoleucine	2.5	3.3	3.1	3.5	1.1	2.3	2.8
Leucine	13.7	16.1	15.5	17.7	10.5	13.9	16.0
Tyrosine	4.5	4.0	4.4	5.3	6.9	4.6	5.1
Phenylalanine	4.5	6.8	5.8	6.4	6.2	4.2	5.1
Total Recovery	112.3	114.3	120.3	125.0	110.7	117.5	126.8

*Amino acid levels as per cent of total protein (N x 6.25).

Table 25

Amino Acid Composition of Fraction IV*

Inbred	Oh 43					W22	
Genotype	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
Lysine	1.8	1.8	2.9	1.7	1.7	1.4	2.2
Histidine	8.2	8.0	7.6	8.4	8.9	9.6	9.0
Arginine	3.0	3.3	5.7	4.0	6.2	3.3	3.4
Aspartic Acid	2.7	3.3	4.0	2.6	2.6	2.2	2.3
Threonine	3.3	3.9	3.9	4.8	4.3	5.2	3.7
Serine	3.7	4.4	4.2	4.0	3.6	3.6	3.5
Glutamic Acid	25.1	30.0	22.6	25.0	21.6	23.7	26.3
Proline	13.7	14.5	13.9	16.8	17.3	17.4	16.3
Glycine	4.2	4.1	5.0	5.5	5.0	4.1	4.3
Alanine	4.1	3.8	4.4	4.2	4.5	3.9	4.0
Cystine	0.1	0.1	0.1	0.1	0.1	0.0	0.1
Valine	5.2	5.2	5.8	6.0	6.5	6.0	5.8
Methionine	1.4	1.5	1.5	1.1	1.0	1.1	1.4
Isoleucine	2.4	2.4	2.8	2.5	2.5	2.3	2.4
Leucine	7.9	7.1	8.4	8.6	9.1	8.3	8.3
Tyrosine	2.2	2.7	1.8	1.6	2.1	1.5	1.5
Phenylalanine	2.5	2.3	2.6	2.2	2.5	1.9	1.8

Total Recovery

*Amino acid levels as per cent of total protein (N x 6.25).

Table 26

Amino Acid Composition of Fraction V*

Inbred	Oh43					W22	
Genotype	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
Lysine	6.4	5.7	6.7	7.0	6.9	7.0	6.8
Histidine	3.6	2.8	4.8	3.9	3.7	3.8	3.3
Arginine	7.1	6.5	9.6	11.0	7.6	7.0	7.6
Aspartic Acid	9.4	11.2	10.7	8.9	10.0	8.8	9.3
Threonine	5.2	5.4	5.7	4.6	5.6	4.2	5.2
Serine	4.8	5.8	5.4	4.9	5.5	3.5	4.8
Glutamic Acid	15.2	19.5	15.7	17.1	15.5	13.0	14.4
Proline	5.3	6.2	5.6	6.7	6.4	5.3	5.4
Glycine	5.1	5.6	5.5	5.4	5.8	5.0	4.9
Alanine	7.0	8.0	7.3	6.8	7.2	6.8	6.4
Cystine	0.2	0.1	0.0	0.8	0.1	0.5	0.0
Valine	7.0	7.4	7.4	7.2	7.7	7.1	6.8
Methionine	2.4	3.1	3.2	2.4	2.4	4.0	2.4
Isoleucine	5.0	5.1	5.5	4.9	5.2	4.9	4.9
Leucine	10.1	10.4	10.4	10.1	10.1	9.4	9.1
Tyrosine	2.4	5.3	3.9	4.9	4.4	2.7	2.9
Phenylalanine	5.6	6.1	5.9	5.6	5.8	5.3	5.2
Total Recovery	101.8	114.2	113.3	112.2	109.9	98.3	99.4

*Amino acid levels as percent of total protein (N x 6.25).

of normal endosperms and of the mutants opaque-2, floury-2, brittle-2, brittle-2/opaque-2, and opaque-7. Representative tests indicate that the tryptophan levels rise and fall with the lysine levels. These data show that the albumin-globulin fraction (Fraction I) and the true glutelin fraction (Fraction V) are high in lysine. Table 27 shows the ranges obtained (Fraction I: 4.3-6.3% lysine, Fraction V: 5.7-7.0% lysine). Since these two fractions predominate in high lysine mutants (see pp. 36 and 37 of 1971-72 report), this accounts for the higher lysine content compared with the normal counterparts.

b. Zein formation in developing maize endosperms

Fraction II of the Landry-Moureaux fractionation scheme contains the true zein fraction of the maize endosperm. Figures 5 to 9 show the changes in the five Landry-Moureaux fractions 14 to 49 days postpollination in Ohio 43, and in the near-isogenic opaque-2, brittle-2, and brittle-2/opaque-2 mutants. The most outstanding finding is in Figure 6 where there is no evidence for zein formation in the double mutant brittle-2/opaque-2 at any time during development. Figure 5 shows that albumin and globulin formation occur at a faster rate and with slower fall-off in the double mutant, with the single mutants intermediate between the double mutant and the normal counterpart.

Figure 9 shows an unusually slow buildup of glutelin in the double mutant, but the level does not reach a plateau as it does in the single mutants and in the normal counterpart.

Ranges in Amino Acid Content* of Fractions I-V

Fraction	I	II**	III	IV	V
Lysine	4.3 - 6.3	0.1 - 0.5	0.4 - 0.8	1.4 - 2.9	5.7 - 7.0
Histidine	2.2 - 3.3	1.0 - 2.4	0.3 - 8.0	7.6 - 9.6	2.8 - 4.8
Arginine	6.1 - 11.8	1.6 - 5.2	2.2 - 4.8	3.0 - 6.2	6.5 - 11.0
Aspartic Acid	7.4 - 11.1	5.8 - 6.8	1.6 - 5.2	2.2 - 4.0	8.8 - 11.2
Threonine	3.5 - 5.3	3.3 - 4.2	3.5 - 4.5	3.3 - 5.2	4.2 - 5.7
Serine	3.9 - 5.8	5.7 - 6.2	4.8 - 6.3	3.5 - 4.4	3.5 - 5.8
Glutamic Acid	9.2 - 18.5	30.3 - 33.3	25.7 - 31.8	21.6 - 30.0	13.0 - 19.5
Proline	4.5 - 7.4	10.4 - 11.2	12.4 - 17.2	13.7 - 17.4	5.3 - 6.7
Glycine	5.8 - 8.2	1.5 - 1.9	2.8 - 6.6	4.1 - 5.5	4.9 - 5.8
Alanine	5.8 - 8.8	10.2 - 11.3	7.5 - 10.1	3.8 - 4.5	6.4 - 8.0
Cystine	0.1 - 4.2	0.1 - 0.7	0.2 - 0.6	0.0 - 0.1	0.0 - 0.8
Valine	4.7 - 6.8	3.7 - 4.9	3.8 - 5.6	5.2 - 6.5	6.8 - 7.7
Methionine	0.8 - 3.0	1.7 - 4.0	2.5 - 9.2	1.0 - 1.5	2.4 - 4.0
Isoleucine	3.1 - 4.6	4.3 - 4.9	1.1 - 3.5	2.3 - 2.8	4.9 - 5.5
Leucine	4.4 - 6.4	19.6 - 21.1	10.5 - 17.7	7.1 - 9.1	9.1 - 10.4
Tyrosine	2.8 - 4.5	5.4 - 6.1	4.0 - 6.9	1.5 - 2.7	2.7 - 5.3
Phenylalanine	2.4 - 4.5	6.9 - 8.3	4.2 - 6.8	1.8 - 2.6	5.2 - 6.1

*Based on data in Tables 5-9. Amino acid levels as percent of total protein (N X 6.25).

**o_2bt_2 not included.

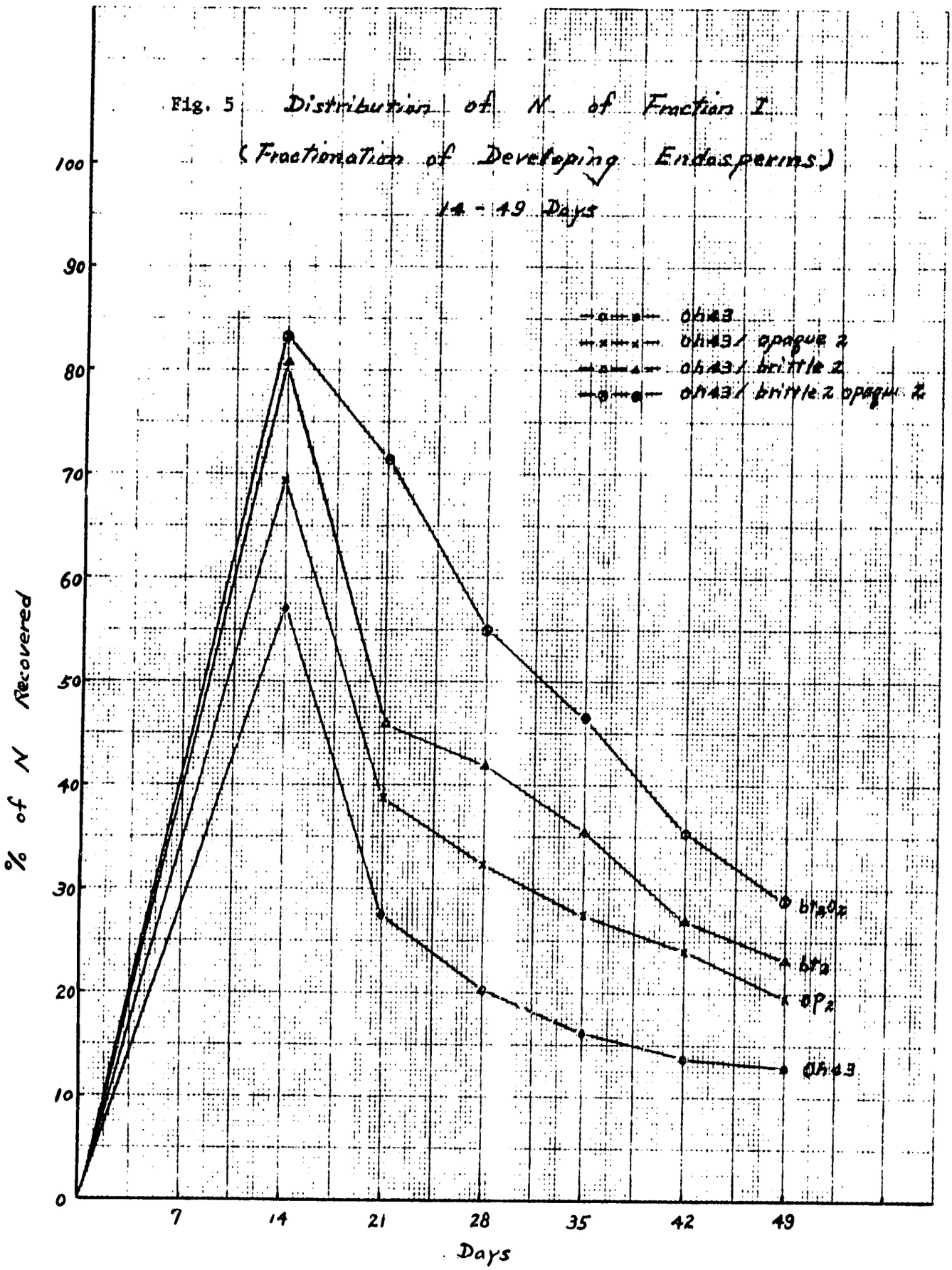
Fig. 5 Distribution of N of Fraction I
(Fractionation of Developing Endosperms)
14 - 49 Days
Oh43
Oh43 / opaque 2
Oh43 / brittle 2
Oh43 / brittle 2 opaque 2
br2Op2
br2
Op2
Oh43
% of N Recovered
Days
0
10
20
30
40
50
60
70
80
90
100
7
14
21
28
35
42
49

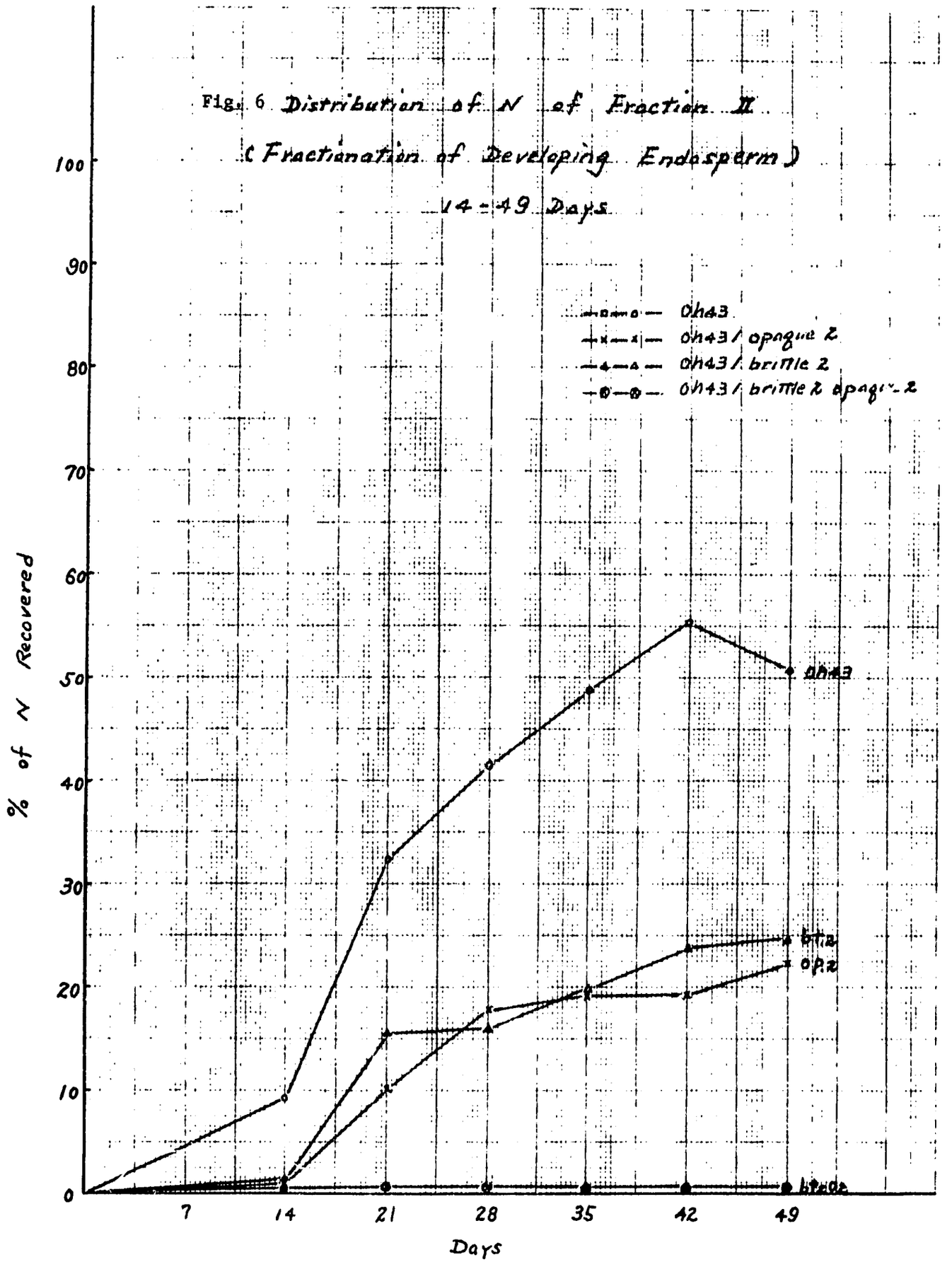
Fig. 6 Distribution of N of Fraction II
(Fractionation of Developing Endosperm)
14-49 Days
Oh43
Oh43 / opaque 2
Oh43 / brittle 2
Oh43 / brittle 2 opaque 2
% of N Recovered
Days
0
10
20
30
40
50
60
70
80
90
100
7
14
21
28
35
42
49
Oh43
bt2
op2
bt2op2

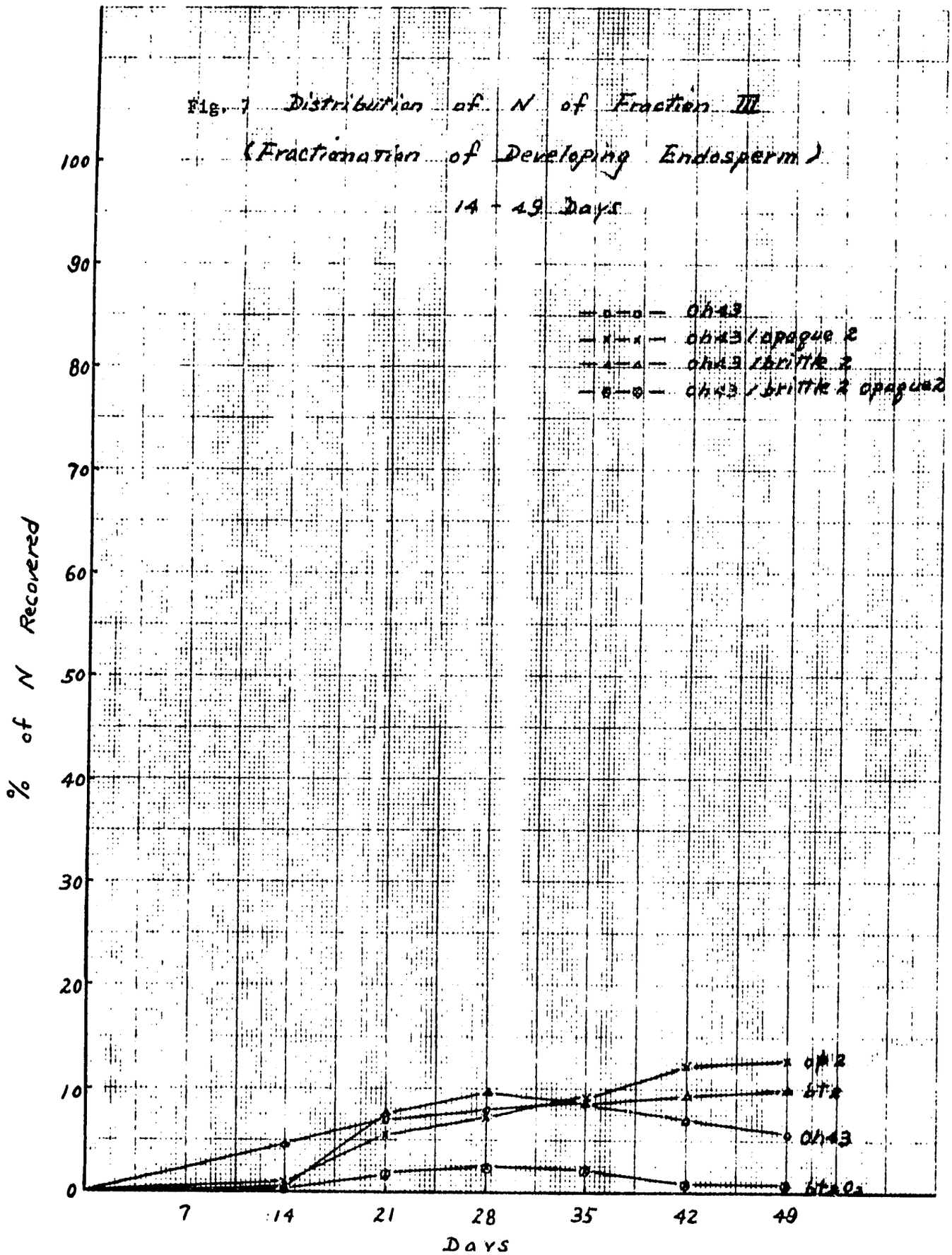
Fig. 7 Distribution of N of Fraction III
(Fractionation of Developing Endosperm)
14 - 49 Days
Oh43
Oh43 / opaque 2
Oh43 / brittle 2
Oh43 / brittle 2 opaque 2
% of N Recovered
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
49
Days
op 2
bt 2
Oh43
bt2 op2

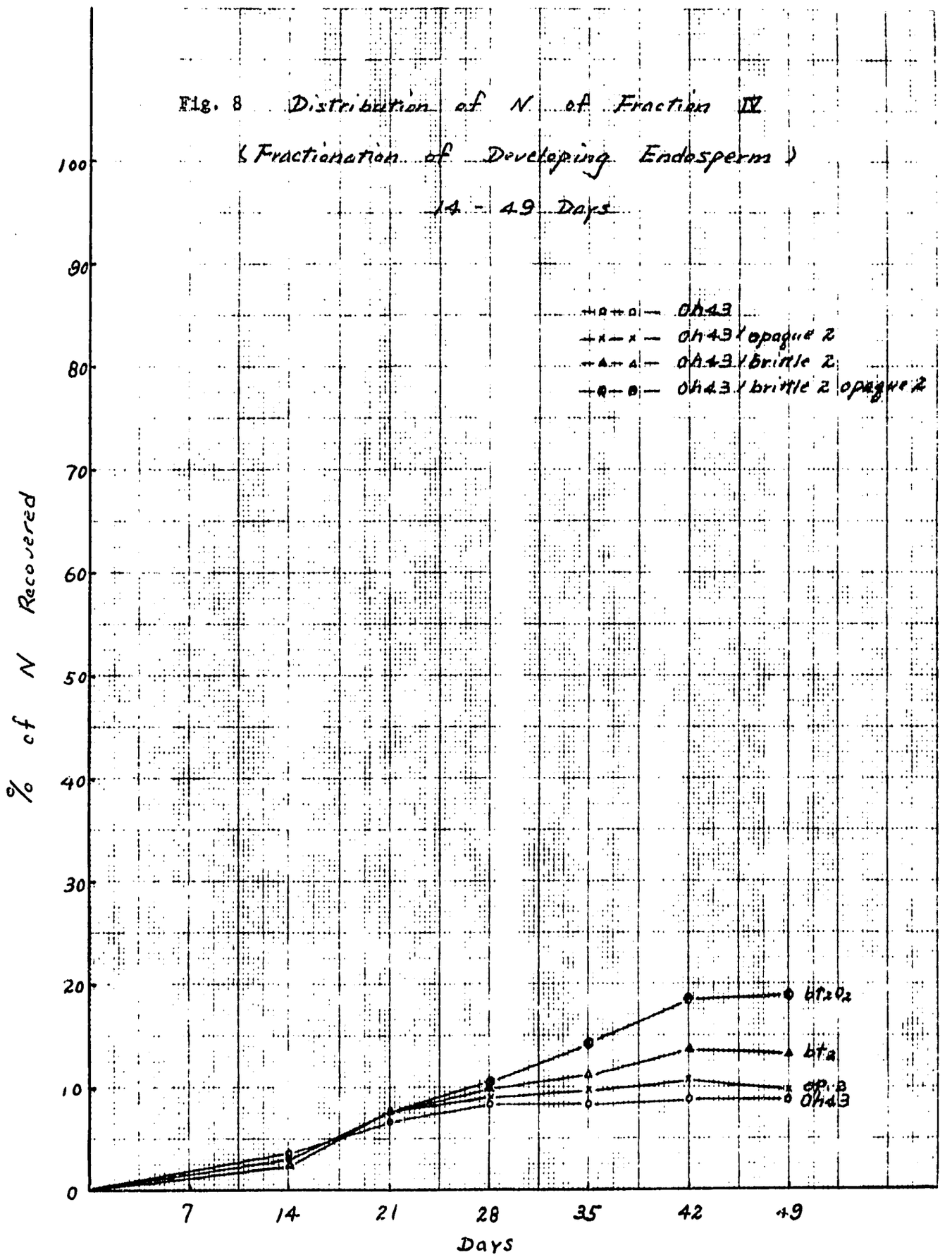
Fig. 8 Distribution of N of Fraction IV
(Fractionation of Developing Endosperm)
14 - 49 Days
Oh43
Oh43 / opaque 2
Oh43 / brittle 2
Oh43 / brittle 2 opaque 2
% of N Recovered
Days
0
10
20
30
40
50
60
70
80
90
100
7
14
21
28
35
42
49
bt2 o2
bt2
op. 2
Oh43

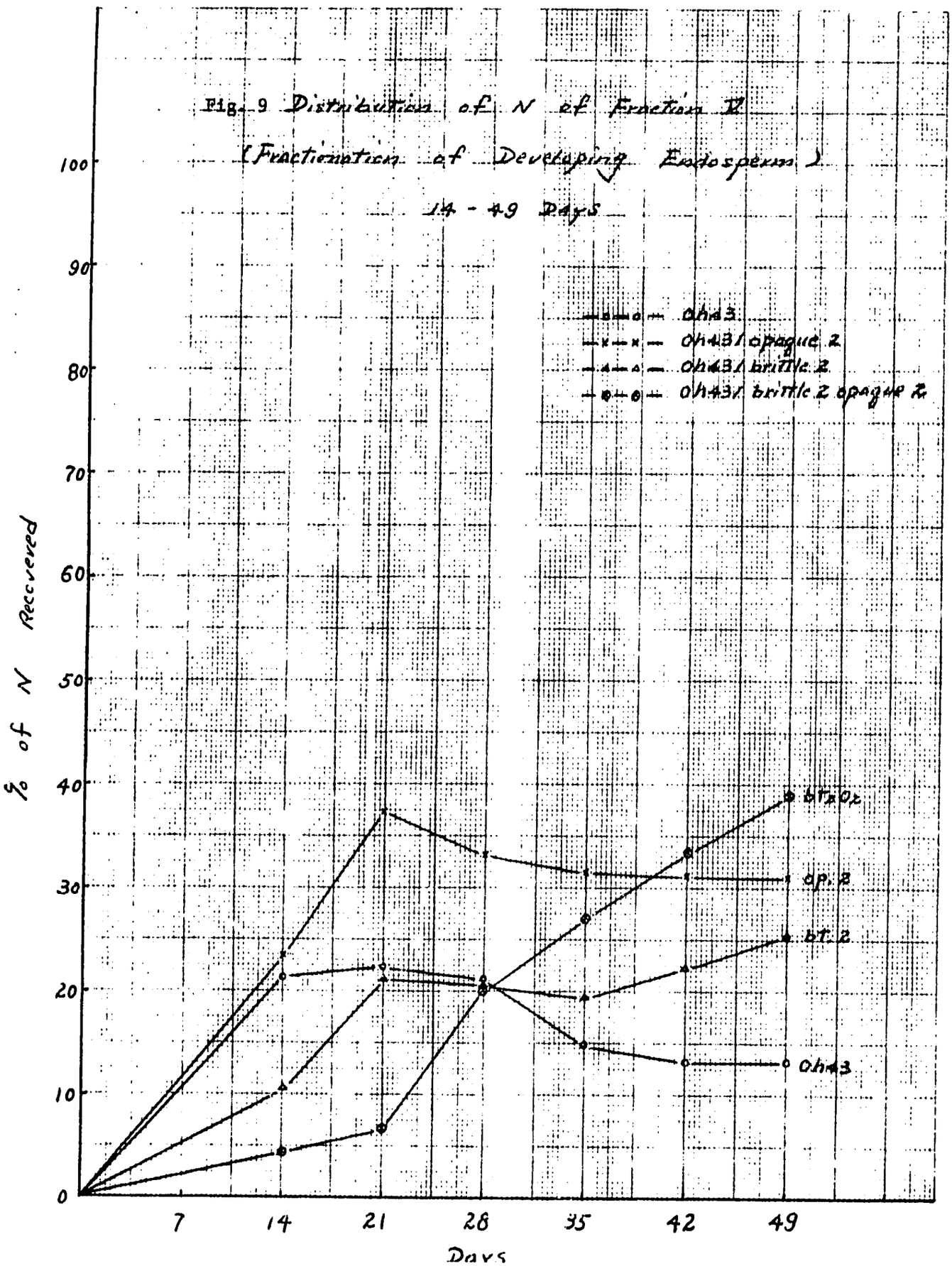
Fig. 9 Distribution of N of Fraction V
(Fractionation of Developing Endosperm)
14 - 49 Days
Oh43
Oh43/opaque 2
Oh43/brittle 2
Oh43/brittle 2 opaque 2
% of N Recovered
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
49
Days
bT2 O2
op. 2
bT. 2
Oh43

G. Protein Nutritive Value

1. Cooperative feeding tests

The Purdue maize research team and the maize research team at CIMMYT recommended in their annual planning session early in 1972 that a group of representative maize, wheat, triticale, and sorghum samples be sent to cooperating universities and tested for protein efficiency response in several species of small laboratory animals. The group at CIMMYT was to supply both the hard and soft endosperm types of opaque-2 maize and representative samples of wheat and triticales. The group at Purdue was to supply floury-2 maize and sorghum. The samples were to be prepared in a standard fashion and distributed among the cooperating laboratories. It was agreed that the same cereal samples and a reference casein would be fed to young weanling rats (Purdue), weanling voles (CIMMYT and Pennsylvania State University), weanling mice (University of Nebraska), and the chick (Washington State University). Dr. Mertz served as coordinator of the project.

The cooperative samples provided by CIMMYT and Purdue are described in Table 28. The ten CIMMYT cereal samples and the five Purdue cereal samples were ground in burr mills at CIMMYT and Purdue, respectively, and analyzed in duplicate by the State Chemist Laboratories at Purdue. The samples were analyzed for total nitrogen, moisture, fat, fiber, and ash. The results of these analyses are shown in Table 29. The moisture content of these samples ranged from a low of 4.8 percent in the casein to a high of 8.9 percent in sample CM-4854. Total protein ranged from a low of 9.3 percent in OH43 x B14 ($\underline{fl}_2$) to a high of 91.0 percent in the ANRC casein. Fat ranged from a low of 0 percent in the casein to a high of 4.6 percent in sample CM-4855, fiber

Table 28

Identification of Cooperative Cereal Samples

Sample No.	Identification
4854	CIMMYT Veracruz 181-Antiqua Gpo 2x Venezuela 1 - Normal Maize
4855	CIMMYT Veracruz 181-Antiqua Gpo 2x Venezuela 1 - Opaque-2 Maize (Hard)
4856	CIMMYT Veracruz 181-Antiqua Gpo 2x Venezuela 1 - Opaque-2 Maize (Soft)
4857	CIMMYT Tuxpeno - Opaque-2 Maize (Soft)
4858	CIMMYT Tuxpeno - Normal Maize (Hard)
4859	CIMMYT 7-Cerros-Wheat
4860	CIMMYT Inia-Wheat
4861	CIMMYT PM-132-Triticale
4862	CIMMYT PM-2-Triticale
4863	CIMMYT PM-15-Triticale
Oh43xB14(+)	Purdue - Single cross normal maize
Oh43xB14($\underline{fl}_2$)	Purdue - Near isogenic floury-2 version of Oh 43 x B14
IS 8165	Purdue - pigmented sorghum
IS 2319	Purdue - low pigment sorghum
S-0250421	Purdue - low pigment sorghum
ANRC-casein	Purdue - Animal Nutrition Research Council Reference Casein*

*Sheffield Chemical Co., 2400 Morris Ave. Union, N. J. 07083

Table 29

Proximate analysis of CIMMYT and Purdue Cereals*

Sample	Total N	Protein**	Moisture	Fat	Fiber	Ash
CM-4854	1.62	10.1	8.9	3.7	2.3	0.8
CM-4855	1.62	10.1	8.5	4.6	2.2	1.5
CM-4856	1.50	9.4	8.7	4.2	2.3	1.4
CM-4857	1.55	9.7	8.0	4.4	2.5	1.8
CM-4858	1.54	9.6	7.9	4.2	2.3	1.4
Oh43XB14(+)	1.52	9.5	8.5	4.1	2.5	1.1
Oh43XB14($\underline{fl}_2$)	1.49	9.3	8.5	4.2	2.4	1.7
CW-4859	1.95	11.1	5.9	1.5	2.9	1.6
CW-4860	1.98	11.3	6.7	1.4	2.6	1.3
CT-4861	2.56	14.6	6.4	1.6	2.6	1.9
CT-4862	2.60	14.8	7.3	1.9	2.7	2.3
CT-4863	2.74	15.6	5.9	1.6	2.5	2.3
IS-8165	1.57	9.8	8.5	2.7	2.5	1.8
IS-2319	1.87	11.7	6.7	4.3	2.0	1.7
S-0250421	1.86	11.6	6.4	3.0	2.3	2.3
ANRC-Casein	14.6	91.0	4.8	-	-	2.3

*State Chemist Laboratories, Purdue

**Maize, sorghum and casein: N x 6.25; wheat and triticales: N x 5.7.

ranged from 0 percent in the casein to a high of 2.9 percent in sample CW-4859, and ash varied from a low of 0.8 percent in sample CM-4854 to a high of 2.3 percent in casein, two triticale samples, and one of the sorghum samples.

On the basis of these analyses it was decided to set the total fat content of the diet at 5 percent, the total mineral plus ash content at 5 percent, to make no corrections for moisture, and to adjust the fiber content to a range of 2.0 to 2.9 percent. With the above parameters fixed the floury-2 sample established the maximum protein level at which all samples could be fed. This level was 8.8 percent protein, slightly below the 10 percent protein level usually used in protein efficiency (PER) determinations. Table 30 shows the composition of the diets used in the feeding tests on the rat and the mouse.

Table 31 shows the amino acid values obtained at Purdue on the cooperative maize samples and the ANRC casein used in these studies. The values in Table 31 are in good agreement with the values obtained by Dr. Villegas at CIMMYT on the same samples (CIMMYT data not shown). The floury-2 sample contained only 11 percent more lysine than its normal counterpart. The levels of lysine in the hard and soft endosperm of CM-4855 and CM-4856 did not differ significantly in lysine content. The tryptophan levels of the normal selections ranged from 0.5 to 0.7 percent, the floury-2 selection contained 0.7 percent and the opaque-2 selections varied from 0.9 to 1.0 percent. The casein carried an unusually high level of lysine (8.6 percent) and a high level of tryptophan (1.4 percent). It is also interesting to note the substantial drop in

Table 30

Proposed Diets Containing 8.8% Protein

Sample No.	Per Cent Sample	Per Cent Cottonseed Oil	Per Cent Mineral Mix**	Per Cent Vitamin Mix***	Per Cent Corn Starch
CM-4854	87.0	1.8	4.3	1.0	5.9
CM-4855	87.0	1.0	3.7	1.0	7.3
CM-4856	93.5	1.1	3.7	1.0	0.7
CM-4857	90.8	1.0	3.4	1.0	3.8
CM-4858	91.7	1.2	3.7	1.0	2.4
Oh43(+)	92.5	1.2	4.0	1.0	1.3
Oh43(fl_2)	94.6	1.0	3.4	1.0	0.0
CW-4859	79.3	3.8	3.7	1.0	12.2
CW-4860	77.8	3.9	4.0	1.0	13.3
CT-4861*	60.2	4.0	3.9	1.0	30.4
CT-4862*	59.4	3.9	3.6	1.0	31.6
CT-4863*	56.4	4.1	3.7	1.0	34.3
IS-8165	90.0	2.6	3.4	1.0	3.5
IS-2319*	75.2	1.8	3.7	1.0	17.8
S-0250421*	75.8	2.7	3.3	1.0	16.7
ANRC Casein*	9.7	5.0	4.8	1.0	77.5

*Add 2 percent cellulose (Cellu Flour or equivalent) for casein and 0.5% for starred (*) triticales and sorghum samples.

**Hawk-Oser Salt Mixture Number 3, Nutritional Biochemicals Corp., Cleveland, Ohio.

***Vitamin Fortification Mixture for the rat, Catalog No. 40060, General Biochemicals Corp., Chagrin Falls, Ohio.

the level of leucine as one goes from the normal maize selections to the opaque-2 selections. The range of protein in the maize selections varied from 10.1 to 11.0 percent.

In Table 31 are shown the amino acid levels in the cooperative sorghum, wheat, and triticale samples. Sorghum sample IS-8165 is a highly pigmented variety, with a lysine only slightly lower than that observed for the other two sorghum selections. The tryptophan values shown for sorghum were obtained with a colorimetric method. The values are uniformly low. Using another method which involves hydrolysis and ion exchange analysis, we have demonstrated that most sorghums run above 1 percent in tryptophan so that this is not a limiting amino acid in sorghum protein. Table 31 shows that the lysine values are slightly higher in the triticale samples than in the wheat samples, and that tryptophan is quite high in wheat and at a satisfactory level in triticales. It should be noted that the leucine is low and the leucine to isoleucine ratio low and favorable in wheat and triticales. This is in sharp contrast to the ratio of leucine to isoleucine in sorghum (approximately 3 to 1). It is believed that the high level of leucine in sorghum is responsible for the high incidence of pellagra in certain parts of India where sorghum supplies the major part of the protein intake. The protein level in these samples varied from a low of 10.3 percent in sorghum IS 8165 to a high of 15.7 percent in triticale PM-15. In the feeding tests, however, the protein was adjusted in all cases to 8.8 percent of the total diet (see Table 30).

Table 31

Amino Acid Composition of Cooperative Maize Samples*

	4854 Normal	Oh43xB14 Normal	4858 Normal	Oh43xB14 Floury-2	4855 Op-2(Hard)	4856 Op-2(Soft)	4857 Op-2(Soft)	ANRC Casein
Lysine	2.6	3.0	2.7	3.4	4.5	4.4	4.7	8.6
Tryptophan**	0.6	0.7	0.5	0.7	0.9	1.0	1.0	1.4
Isoleucine	3.5	3.6	3.5	3.7	3.5	3.5	3.5	5.2
Leucine	12.7	12.8	13.2	12.3	9.1	8.7	8.6	9.7
Cystine	1.6	1.9	1.6	1.4	2.2	1.9	2.1	0.1
Methionine	2.0	2.7	2.1	2.7	1.7	1.8	1.9	3.0
Histidine	3.1	2.9	3.1	2.9	3.7	3.6	3.6	3.0
Arginine	4.8	5.1	4.8	5.5	6.3	6.7	7.3	4.0
Tyrosine	4.3	4.5	4.4	4.4	3.7	3.7	3.9	5.7
Phenylalanine	4.8	5.1	5.0	5.2	5.2	4.1	4.3	5.3
Threonine	3.3	3.4	3.4	3.5	3.6	3.7	3.9	4.2
Valine	4.7	4.7	4.9	5.0	5.4	5.3	5.4	6.7
Aspartic Acid	5.8	6.5	6.2	6.9	8.7	8.9	9.9	7.2
Glutamic Acid	22.5	22.6	23.1	22.1	19.8	19.2	20.3	27.1
Proline	8.9	8.7	9.0	8.5	8.4	8.1	8.0	11.3
Alanine	7.3	7.5	7.7	7.7	6.0	5.9	6.4	3.1
Serine	4.6	4.8	4.6	4.9	4.3	4.5	4.6	5.7
Glycine	3.5	3.7	3.6	4.0	4.6	4.6	4.9	1.9
% Protein	11.0	10.2	10.5	10.4	10.9	10.7	10.1	88.1

* Grams per 100 gm protein (defatted samples)

** Colorimetric method (CIMMYT Bull. # 20). All others, ion exchange.

Table 31 (Continued)

Amino Acid Composition of Cooperative Sorghum Wheat and Triticales Samples*

	Sorghum (IS-8165)	Sorghum (025042-1)	Sorghum (IS-2319)	Wheat (7 Cerras)	Wheat (Inia)	Tritic. (PM-2)	Tritic. (PM-15)	Tritic. (PM-132)	ANRC Casein
Lysine	2.3	2.5	2.5	3.1	3.1	3.4	3.4	3.5	8.6
Tryptophan	(0.3)**	(0.6)	(0.5)	1.7	1.8	1.1	1.1	1.2	1.4
Isoleucine	4.2	4.7	4.3	3.8	3.9	3.9	3.7	3.8	5.2
Leucine	13.7	15.0	13.9	7.2	7.2	7.2	6.8	6.8	9.7
Cystine	1.0	1.6	1.0	2.0	2.2	1.8	2.0	1.5	0.[illegible]
Methionine	1.8	2.1	1.6	1.8	1.9	1.9	1.9	1.6	3.0
Histidine	2.1	2.5	2.1	2.5	2.6	2.6	2.4	2.5	3.0
Arginine	4.1	4.3	4.1	5.8	5.6	6.1	6.4	6.3	4.0
Tyrosine	4.4	5.1	4.9	3.6	3.7	3.4	3.3	3.2	5.7
Phenylalanine	5.3	6.1	5.5	4.8	4.9	5.0	4.9	4.8	5.3
Threonine	3.2	3.7	3.3	3.1	3.2	3.2	3.0	3.0	4.2
Valine	5.1	5.9	5.5	4.7	4.8	4.9	4.6	4.6	6.7
Aspartic Acid	7.3	7.4	7.5	5.5	5.6	6.5	6.3	6.3	7.2
Glutamin Acid	25.6	27.4	25.7	36.0	37.0	33.4	32.4	31.9	27.1
Proline	7.9	9.1	8.0	9.9	10.0	9.9	10.0	9.8	11.3
Alanine	9.9	10.3	9.7	3.9	3.9	4.2	4.0	4.0	3.1
Serine	4.4	4.9	4.3	5.0	4.9	4.6	4.5	4.4	5.7
Glycine	3.0	3.6	3.1	4.6	4.6	4.5	4.3	4.2	1.9
% Protein	10.3	12.2	12.7	11.0	11.5	14.9	15.7	14.8	88.1

*Grams per 100 gm protein (defatted samples)

**Colorimetric method (CIMMYT Bull. # 20). All others ion exchange. Tryptophan values for sorghum are too low. Ion exchange values obtained on other Purdue sorghum samples indicate that the tryptophan levels are above 1%.

2. Rat feeding tests

Table 32 summarizes the results obtained at Purdue when the collaborative maize samples were fed for 28 days. In these studies 10 male weanling rats were used for each cereal. The rats were housed in individual wire mesh cages and supplied with feed and water ad libitum. Special feed cups were used which minimized the scattering of food and permitted accurate recording of the feed consumed. It can be seen from Table 32 that there were no significant differences in average weight gained between the three normal maize samples and the floury-2 sample (25.8 to 36.9 grams). Also no significant differences were observed in the average weight gained between the three opaque-2 maize samples and the casein control, (82.7 to 87.1 grams) and the gains in weight of these last 4 groups were significantly higher at the 1 percent level than the gains in weight of the first 4 groups. The three normal maize diets and the floury-2 diet gave protein efficiency ratios which were not significantly different at the 1 percent level (1.59 to 1.83). The three opaque-2 maize diets also gave protein efficiency ratios which were not significantly different at the 1 percent level (2.83 to 2.90). However, the latter three gave significantly higher PER values than the three normal maize diets and the floury-2 maize diet. There was also a significant difference in the PER value between the three opaque-2 maize diets and the casein control. The opaque-2 maize diets gave an average PER that was 87 percent of the PER value (3.34) obtained with ANRC casein.

Table 33 summarizes the weight gains and protein efficiency ratio values obtained on the cooperative sorghum, wheat, and triticales samples. This was a separate feeding test for 28 days using

Table 32

PER Values of Cooperative Maize Samples[1,2]

	4854 Normal	Oh43XB14 Normal	4858 Normal	Oh43xB14 Floury-2	4855 Op-2 (Hard)	4856 Op-2(Soft)	4857 Op-2(Soft)	ARC Casein
Average Initial Weight (g)	49.6	49.6	49.6	49.5	49.6	49.5	49.6	49.5
Average Weight Gained (g)	25.8^{b}	31.4^{b}	32.6^{b}	36.9^{b}	82.7^{a}	87.1^{a}	84.4^{a}	85.1^{a}
Average Total Feed Consumed (g)	183.7^{c}	206.1^{c}	214.5^{c}	227.3^{c}	$333.5^{b,a}$	340.2^{a}	336.0^{a}	290.2^{b}
Protein Efficiency Ratio	1.59^{c}	1.71^{c}	1.72^{c}	1.83^{c}	2.83^{b}	2.90^{b}	2.84^{b}	3.34^{a}

1. Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of Mrs. L. Tanchoco, Dept. of Biochemistry, Purdue University, Lafayette, Indiana 47907. Duration of experiment: 28 days. Ten male rats in each group.
2. Values with the same superscript are not significantly different from each other at the 1% level.

Table 33

PER Values of Cooperative Sorghum, Wheat and Triticale Samples[1,2]

	Sorghum (IS-8165)	Sorghum (025042-1)	Sorghum (IS-2319)	Wheat (7 Cerros)	Wheat (INLA)	Triticale (PM-2)	Triticale (PM-15)	Triticale (PM-132)	AMRC Casein.
Average Initial Weight (g)	50.6	50.6	50.6	50.6	50.5	50.5	50.6	50.4	50.6
Average Total Weight Gained (g)	11.6^{e}	14.4^{e}	$19.6^{d,e}$	$27.0^{c,d}$	$26.7^{c,d}$	$32.7^{b,c}$	38.5^{b}	37.8^{b}	89.6^{a}
Average Total Feed Consumed (g)	$181^{c,d}$	165.2^{d}	171.6^{d}	$193.9^{b,c,d}$	$182.4^{c,d}$	$202.1^{b,c}$	221.9^{b}	213.3^{b}	314.2^{a}
Protein Efficiency Ratio	0.71^{f}	$0.97^{e,f}$	$1.28^{d,e}$	$1.57^{c,d}$	$1.66^{b,c}$	$1.81^{b,c}$	1.97^{b}	2.01^{b}	3.22^{a}

1. Data obtained by R. Jambunathan and E. T. Mertz, with the technical assistance of Mrs. L. Tanchoco, Dept. of Biochemistry, Purdue University, Lafayette, Indiana 47907. Duration of experiment: 28 days. Ten male rats in each group.

2. Values with the same superscript are not significantly different from each other at the 1% level.

ANRC casein as a control protein. The average total weight gain per animal on sorghum IS-8165 was substantially less than that on sorghum IS-2319, but the differences in weight gained among the 3 sorghums was not significant at the 1 percent level. Sorghum IS-2319 and the two wheat samples also did not differ significantly from each other in average total weight gained. The 3 triticales samples, however, were significantly better than the 3 sorghum samples, and triticale PM-15 and PM-132 were significantly better than the two wheat samples. However, triticale sample PM-2 was not significantly better than the 2 wheat samples. Weight gain on the control casein was more than twice that observed on the best triticale samples, and significantly higher than any of the cereal grain samples listed in Table 33.

If we now look at the PER values, it can be seen that the PER of the highly pigmented sorghum sample is significantly lower than that of sorghum IS-2319 (0.71 compared with 1.28). The PER values of the 3 triticale samples and the INIA wheat sample were significantly higher than the PER values of the three sorghum samples and the 7 Cerros wheat sample. The two wheat samples did not differ significantly from each other, and the 3 triticales samples did not differ significantly from each other. The highest PER value in this cereal group was 2.01 (PM-132), which was significantly lower than the PER value, 3.22, obtained on the control casein. In summary the average PER value of the triticales was about 62 percent of the casein control, the wheat samples about 52 percent, which is about equal to that of the normal corn shown in Table 32, and the sorghums varied from 25 to 40 percent of the casein control.

Data on the mouse, vole, and chick will be published in the Proceedings of the International Symposium on Protein Quality in Maize.

In summary, the weight gains and protein efficiency ratios of normal, floury-2, and opaque-2 maize and of sorghum, wheat, and triticales were compared with a reference casein in the rat, mouse, vole, and chick. With ten male weanling rats per test cereal, significant differences in protein quality were apparent in 7 days with values comparable to those obtained in 14 to 28 days. A 7-day test would require not more than 600 grams of each cereal. In the rat no significant differences were observed in either weight gain or PER between hard and soft endosperm opaque-2 maize. The 28 day PER of these two maizes was 85 percent of the casein control compared with 60, 50, 50, and 40 percent for triticales, wheat, normal and floury-2 maize, and sorghum, respectively. With five male weanling mice per test cereal for 28 days, floury-2, opaque-2, and triticales did not show significant PER differences and were about 60 percent of the control casein value. With 5 or 6 weanling voles per test cereal for 5 or 6 days, PER values were not significantly different between opaque-2 maize, wheat, and triticale samples. With four replicated groups of five-day old chicks per test cereal fed 6 percent of the cereal protein on top of an 8 percent balanced protein base, PER values distinguished hard endosperm opqaue-2 from soft endosperm but not hard endosperm from floury-2, wheat, or triticales.

The chick studies demonstrated the superiority of opaque-2 maize over normal even after lysine supplementation of both, and

a remarkable superiority of lysine-supplemented INIA wheat over lysine-supplemented 7-Cerros wheat.

Based on the present cooperative test, the male weanling rat gave the best differentiation between the cereals tested. Because of the chick's high protein requirement, it was used only to test the supplementary value of these cereal grains when fed on top of a well balanced protein base.

H. Factors Affecting Maize Adaptation

Problems with instrumentation have continued in Colombia and insects destroyed the 1972 experiment at Bogota. A satisfactory program has been developed to compute the units of environmental variables (Growing Degree Days, Corn Heat Units, solar radiation, net radiation, and evaporation) in each interval of plant development for each variety. The intervals being utilized are: (1) planting to floral initiation, (2) floral initiation to anthesis, (3) anthesis to silking, (4) silking to black layer formation (an indication of physiological maturity), and the combination intervals, (5) planting to anthesis, (6) planting to silking, (7) planting to black layer, and (8) anthesis to black layer.

It is anticipated the analysis of comparative stability of development over environments among the entries will be basically that developed by Eberhart. The comparison of environmental units as predictors of varietal performance is more complex as it will be necessary to transform the data to standardized units.

SUMMARY

1. An "International Symposium on Protein Quality in Maize" was co-sponsored with CIMMYT in Mexico City, December, 1972, with 150 scientists participating.

2. Twenty-one new endosperm mutants from Colombian varieties have been crossed to cornbelt stocks, then self-pollinated. Five or six genes controlling three new endosperm mutants found in the previous search cycle have been located to chromosome.

3. Opaque-2 synthetics Temp HA and Temp HB have been developed with random mating and mild selection for modified types. Full sib selection scheme with testing at 4 or 5 worldwide locations is planned.

4. Sugary-2/opaque-2, waxy/opaque-2, and brittle-2/opaque-2 versions of two temperate zone synthetics have been developed.

5. Evaluation of opaque-7 in different backgrounds indicate that this gene may affect lysine levels very near those of floury-2.

6. Three cycles of selections have been completed for higher protein content in two opaque-2 synthetics (syn HA_{02} and $SSSH_{02}$), and this germplasm released if analysis warrants.

7. Another cycle of selection for increased germ size (oil content) and the effect of selection on protein content will be evaluated in the 1973 season.

8. Selection for modified opaque types did not increase yield but did result in slight decrease in ear rot.

9. Landry-Moureax fractionation of protein from modified opaques revealed an increase in the zein fraction, which accounts for their lower protein quality.

10. Three sugary-2 (Oh43, B37, and C103) and two waxy (Oh43 and C103) inbreds were released because of interest in their utilization to improve nutritional characteristics. These releases have been distributed widely in other countries and the United States.

11. The second years' evaluation of mature grain characteristics of a diallel set of hybrids with specific starch modifying genes and their opaque-2 combinations showed considerable mean variation for background interaction for kernel weight, kernel volume, kernel density, percent germ, protein and lysine, percent oil, and oil content.

12. The double mutant sugary-2/opaque-2 shows excellent promise for increasing the acceptance and utilization of high lysine maize because of its improved kernel density, vitreousness, enhanced caloric and nutritive value, improved digestibility, and biological value. However, kernel size is reduced, though a full kernel, and must be improved through breeding.

13. In vitro susceptibility of starch granules of several endosperm mutant and double mutant combinations with opaque-2 to the action of several α-amylases showed that sugary-2 and sugary-2/opaque-2 were digested by the amylases much more rapidly than those of normal and other genotypes. Waxy and waxy/opaque-2 were also digested more rapidly than normal and opaque-2.

14. Rat feeding tests showed that su_2o_2 and $wx\ o_2$ hybrids had excellent biological value. Sugary-2 hybrids gave better gains than normal.

15. Endosperm protein quality of o_2, su_1, su_1o_2, sh_2, sh_2o_2, bt_2 and bt_2o_2 at 21-days after pollination is excellent. As development proceeds, the quality decreases; but it is maintained at a higher level.

16. Endosperm protein quality of o_2, fl_2, fl_2o_2, ae, ae o_2, wx, wx o_2, su_2, and su_2o_2 was high at 21-days after pollination. The double combinations ae o_2, wx o_2 and su_2o_2 genotypes had favorable protein quality for edible green corn maturity although the quality was not so good as su_1o_2, sh_2o_2, and bt_2o_2 genotypes.

17. A preliminary protein fractionation study (using SDS polyacrylamide gel electrophoresis) of isolated protein bodies of developing normal, o_2, bt_2, and bt_2o_2 endosperms has provided a possible basis for identification of subcellular sources of other protein bands, identification of subcellular sources of other protein bands, identification of protein bands that have high basic amino acid content, and survey of different endosperm mutants, inbred lines, or populations for increased levels of lysine rich proteins and normal zein content.

18. Normal, opaque-2 and floury-2 maize, and sorghum, wheat, and triticales were compared with reference casein in the rat, mouse, vole, and chick in a cooperative study involving five laboratories. The male weanling rat gave the best differentiation between the cereals tested.

19. Complete amino acid patterns of the Landry-Moureax fractions of normal and mutant endosperms revealed that the albumin-globulin fraction and the true glutelin fraction have high levels of lysine and tryptophan.

20. Three trainees completed the program on analytical techniques in maize protein.

21. The relationship between meteorological data and the development of widely differing strains of maize in Colombia, Mexico, and the United States in 1971 and 1972 are being analyzed.

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