

SOME ASPECTS OF HIGH LYSINE MAIZE BREEDING USING OPAQUE-2

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## ABSTRACT

Several field and sweet corn varieties from several sources were crossed with a variety carrying the opaque-2 gene to determine the phenotypic interactions in the breeding of high lysine maize.

The  $F_2$  segregation ratios for normal and opaque-2, 100-kernel weights, percentage seed set, opaque-2 phenotype, disease susceptibility, and the relationship between protein and lysine content of normal and opaque-2 were investigated. The determinations and observations were made on the  $F_2$ ,  $F_3$  and  $BC_1$ . Lysine content was determined by the ion exchange resin combined with paper chromatography method.

Even though opaque-2 lines showed lower protein content than the corresponding normal varieties, there was no correlation between the protein levels of the two types. Opaque-2 maize contained more lysine, but no relationship was found between the protein content and the lysine content of either normal or opaque-2 types, suggesting that high lysine corn using the opaque-2 gene may be developed independently from the protein content.

Most crosses segregated in a 1-opaque-2 : 3-normal ratio as expected. Opaque-2 segregates were lighter than the normal type and smaller in size in certain crosses. A mottled phenotype of opaque-2 was observed in the Philippines yellow endosperm. In some varieties opaque-2 maize was very susceptible to the ear and kernel rot disease.

## INTRODUCTION

Maize, Zea mays L. is grown throughout the world. In the United States, field corn, the second most important crop after wheat, is mostly grown for animal feed, while in Asia or in Latin America, maize is the third most important crop after rice and wheat and is mostly grown for human food. Maize protein is lacking in nutritional value for human consumption. It is deficient in two essential amino acids, lysine and tryptophane. The major component of maize protein, zein(41 percent), is very low in lysine and tryptophane.

Many attempts have been made to improve the nutritional value of maize. Experiments for the improvement of maize protein may be considered in two parts; quantitative increase of protein and qualitative improvement of protein. Attempts at the quantitative increase of protein started earlier than for the qualitative improvement of protein. At present, the high protein maize lines developed at the Illinois Agricultural Experiment Station are a good example, for they contain more than 20 percent protein compared with 11 percent in the original population. Even though high protein maize was successfully developed, the fundamental problem of the low nutritional value of the maize was not solved. The amounts of lysine and tryptophane, which are two of the eight essential amino acids for human beings, are still deficient in the high protein maize (see Table 1, page 4). A few trials were made, but no noteworthy results were found until Nelson's group (71, 72) reported that two genes, opaque-2 and floury-2, were responsible for increased lysine and tryptophane content.

Since the discovery of the effects of these genes by Nelson's group, many people have confirmed their value. Very few investigations reported on the problems raised in a breeding program with these genes. Breeders in many parts of the world are incorporating these genes in their breeding programs. More fundamental information is needed on the problems involved.

The objectives of this investigation, initiated in 1966, were to incorporate the opaque-2 gene into various maize varieties, to determine the phenotypic interactions of this gene with other endosperm genes, and to compare the relationship between lysine content and protein content among normal and opaque-2 versions of the same lines.

## LITERATURE REVIEW

Differences among proteins and amino acids in maize are presented in Table 1. Since Gorham (36) reported the content of zein in the maize kernel for the first time in 1832, many people have made chemical and genetic studies of the maize protein and its amino acid pattern. The major parts of maize protein are zein (alcohol soluble), glutelin (alkali soluble) and unnamed fractions (acid soluble) as shown in Table 1. Osborne, et al. (75) analyzed maize kernels and found that there is very little lysine and tryptophane, and a high percentage of leucine and aspartic acid in maize kernels. Frank (31) showed that the deficiency of tryptophane and lysine which are absent in zein, is not sufficiently corrected by the rest of the maize proteins. Miriam et al. (66) stated that differences of protein composition tended to be greatest between genetically diverse varieties. These differences were not due to variations in the proportions of zein in the various seed proteins. In earlier studies of maize protein, Hansen (38) and Frey (32) had shown that zein and total protein in the maize kernel are correlated with each other,  $r = .92$ . Miller et al. (65) determined the percentages of protein and lysine in the grain of nine single crosses. Percentage of crude protein and lysine varied from 8.95 to 13.0 percent and from 0.29 to 0.37 percent, respectively. Lysine and protein percentages were positively correlated,  $r = .85$ . Their data indicated that the ratio of lysine to protein was the same in both high and low protein maize.

Flynn et al. (30) analyzed individual selfed ears from an open-pollinated variety for crude protein, niacin, and certain amino acids, and found that as the percentage of crude protein increased, there was

Table 1. Composition of main protein fractions and amount of lysine and tryptophane in normal and opaque-2 maize

Amino acids	Total protein	Protein fractions			References
		Zein (alcohol-soluble)	Glutelin (alkali-soluble)	Acid-soluble	
Normal	8.9%	37 <sup>a</sup>	29 <sup>a</sup>	34 <sup>a</sup>	(60)
	10.0%	65 <sup>a</sup>	30 <sup>a</sup>	b	(102)
	Lysine 2.4 <sup>c</sup>	0-0.3 <sup>c</sup>	3.6 <sup>c</sup>	1.8 <sup>c</sup>	(60)
	Tryptophane 0.1 <sup>c</sup>	b	b	b	(60)
Opaque-2	11.2%	26 <sup>a</sup>	39 <sup>a</sup>	35 <sup>a</sup>	(60)
	13.6%	32 <sup>a</sup>	51 <sup>a</sup>	b	(102)
	Lysine 3.6 <sup>c</sup>	1.0 <sup>c</sup>	3.7 <sup>c</sup>	5.9 <sup>c</sup>	(60)
	Tryptophane 1.5 <sup>c</sup>	b	b	b	(60)

a percentage of total soluble nitrogen

b not reported

c grams per 100 grams of protein

a marked increase in lysine and a marked decrease in niacin when these nutrients were expressed as percentages of the whole corn. If these nutrients are expressed as percentages of protein, however, an increase of protein content from 8 to 16 percent resulted in a decrease of 12.5 percent for lysine. Miller et al. (64) also observed the same relationships. As the total maize protein increased, the percentage of lysine decreased. An increase in percentage of lysine was slightly associated with an increase in zein and other amino acids. Hansen et al. (38) pointed out that sweet corn endosperm contained an average of 1 percent less zein than dent corn of equal protein content.

Bressani and Mertz (13) observed no major differences in amino acid composition among several U.S. and Guatemalan maize selections, even though there were differences among acid-soluble proteins, alkali- and alcohol-soluble proteins, alkali-soluble protein (glutelin) and alcohol-soluble protein (zein).

Boundy et al. (11) examined protein content and composition of dent, waxy, and high-amylose corns with otherwise identical genetic backgrounds by using starch gel electrophoresis. They found no differences in the electrophoretic patterns of the proteins from the different genotypes. Tello et al. (96) studied racial and varietal characteristics in relation to lysine content of maize. They found varietal differences in lysine content among varieties and races using 20 varieties selected out of about 5,000 collections from Mexico, Central America, and the Caribbean.

In the past, nitrogen fertilizers have been tried in an effort to

increase the protein content of maize kernels. Arbuckle et al. (7) and Dotty et al. (26) hinted that varieties of maize might show different protein content due to the soil fertility and other environmental factors. They were convinced that climate was more important than soil fertility in influencing protein content. Schneider et al. (90) investigated the effects of selection and nitrogen fertilization of the soil on the weight of the component parts of the maize kernel, the percent of total nitrogen in the kernel and its parts, and the percent of nitrogen in the various solubility fractions of the whole kernel and its parts. They said that all nitrogen fractions of the whole kernel usually increased when the total nitrogen of the kernel was increased by breeding or by nitrogen fertilization of the soil. However, they found that the alcohol-soluble nitrogen (zein) increased at the fastest rate. They explained that the protein of high protein maize has a lower biological value than the protein of low protein maize, since zein is a low quality protein.

Even though the protein and amino acid contents were increased by nitrogen fertilization, Sauberlich et al. (88) indicated that there were considerable differences in the rate of increase among the individual amino acids. With other investigators, they also found that the increase of protein and amino acids due to nitrogen fertilization was primarily an increase in zein.

Pierre, et al. (79) indicated that nitrogen fertilization does not improve the amino acid balance in maize protein. They commented that the amino acid balance may be even poorer in nitrogen fertilized maize, since the amino acids, lysine and tryptophane, present in low proportions

do not increase with fertilization as rapidly as does total protein.

#### Breeding for increased protein

More attempts have been made to increase the protein content of maize than to improve the quality of maize protein. In 1911, Sure (95) first indicated that protein content in maize is genetically determined. East and Jones (28) compared protein content of self-pollinated and open-pollinated ears of high (13 percent) protein maize and found that self-pollinated ears of high protein varieties contained more protein than open-pollinated ears of high protein varieties. Hayes and Garber (39) noted that there was a high inverse correlation between the number of seeds and the protein content per ear. Hayes (43) and Jones (49) found that inbred strains showing the highest percentage protein were weak and unproductive. They said that protein content was controlled by a large number of inherited factors. Frey (35) suggested that the protein content of the maize kernel is influenced by at least 22 genes and dominance is toward low protein.

In 1896, breeding experiments designed to influence the protein content of the maize kernel began at the Illinois Agricultural Experiment Station where selection for low and high protein was carried out using the ear-to-row method. In 45 generations the protein percentage of the Burr White variety was modified from the original 10.9 percent to 7.72 percent in the low protein strains and to 17.2 percent in the high protein strains. Later, East and Jones (28), and Hayes (43) proposed a system of breeding whereby high protein strains would be crossed and selections made in the F<sub>2</sub> for high protein.

Breeding for improved protein quality

In 1957, Mertz et al. (58) developed a new method of extracting proteins of maize embryo and maize endosperm: the copper extraction-fractionation method. Since then, Mertz et al. (59) have been searching for a maize with a lower zein and a higher lysine content. They reported that the endosperms of corn seeds homozygous for the opaque-2 mutant gene have a higher lysine content than normal kernels. Mertz et al. (60) reported that protein from opaque-2 endosperm contained 15.7 percent zein and 42.3 percent glutelin compared with 41 to 52 percent zein and 17 to 28 percent glutelin in normal North American and Guatemalan maize lines. There is a reversal in the ratio of zein to glutelin in the opaque-2 endosperm when compared with normal maize lines. In opaque-2 endosperms the ratio of glutelin to zein is greater than one, and in normal endosperms it is less than one. They studied the lysine content of opaque-2 endosperms from three strains of differing genetic background. In the 3 strains, they found that the lysine content (3.3 - 4.0 percent) was more than twice that of a normal strain used as a control (1.3 percent). As a critical test, they divided the segregating backcross progeny into opaque-2 and normal kernels, and found that the opaque-2 endosperm had a different amino acid pattern and 69 percent more lysine than the normal endosperm. They also reported on other amino acids. The opaque-2 endosperm contained less glutamic acid, alanine, methionine, leucine, and tyrosine, and more lysine, histidine, arginine, aspartic acid, glycine, and cystine than the normal endosperm (Table 2). On the basis of their findings, they

concluded that the increased content of lysine in the opaque-2 can be attributed to three factors: (1) increased lysine in the acid-soluble fraction, (2) increased lysine in the zein fraction and (3) reduction in the ratio of zein to glutelin.

Nelson et al. (72) reported a second mutant gene affecting the amino acid pattern of maize endosperm proteins, floury-2. They compared the amino acid patterns of five different mutants of similar phenotype: opaque-1, opaque-2, floury-1, floury-2, and soft-starch (h) (Table 2). The floury-2 mutant also had a higher lysine content. Floury-2 differed from opaque-2 in that it also had a higher methionine concentration. The lysine content was almost the same as in the opaque-2 maize, and the protein content of this floury-2 line was higher than that of the opaque-2 line. Compared with the opaque-2 line, floury-2 maize had only one-third the zein of opaque-2. They suggested the possibility that, in the double mutant stocks, there may be higher amounts of lysine than in either mutant alone.

From the above considerations it may be concluded that breeding programs to improve protein quality in the maize kernel might proceed along two lines: (a) Decreasing the proportion of zein and (b) Increasing the percentage of tryptophane and lysine. Frey (35) indicated that the protein of the low protein maize samples was more nearly balanced nutritionally than that of the high protein samples. If protein quality is of prime importance, it might be well to select and grow low protein strains of maize. However, the protein content would have to be maintained at a level high enough to meet the amino acid requirements of animals in relation to the amount of energy food consumed.

Table 2. Amino acid composition of endosperms from normal and mutant stocks of maize (expressed as grams per 100 grams of protein)<sup>a</sup>

Amino acids	Normal	o <sub>2</sub>	o <sub>1</sub>	fl <sub>1</sub>	fl <sub>2</sub>	h
Lysine .....	1.6	3.7	1.7	1.8	3.2	1.8
Tryptophane.....	0.3	0.7	0.6	0.6	0.6	0.5
Histidine.....	2.9	3.2	2.3	3.2	2.0	2.7
Arginine.....	3.4	4.2	3.3	3.7	4.6	3.8
Aspartic acid ....	7.0	10.8	6.1	4.9	7.4	7.0
Glutamic acid ....	26.0	19.8	21.5	20.2	17.5	23.7
Threonine .....	3.2	3.7	3.3	3.1	3.0	3.6
Serine .....	5.6	4.8	9.4	11.9	7.6	10.4
Proline.....	8.6	8.6	9.4	4.9	7.6	5.7
Glycine .....	3.0	4.7	2.9	2.8	3.0	3.4
Alanine .....	10.1	7.2	8.2	7.2	7.3	9.4
Valine.....	5.4	4.3	4.7	4.4	4.8	5.0
Cystine.....	1.8	0.9	2.2	...	2.0	1.5
Methionine .....	2.0	1.8	2.2	2.5	3.0	2.8
Isoleucine .....	4.5	3.9	4.2	3.8	3.8	4.3
Leucine.....	18.8	11.6	15.4	15.1	12.7	16.8
Tyrosine.....	5.3	3.9	5.0	4.9	4.3	5.6
Phenylalanine.....	6.5	4.9	5.7	5.3	4.8	6.2
Protein (%).....	12.7	11.1	10.4	10.8	13.6	10.8

a/ This table is taken from the paper by Nelson et al. (71) in the proceedings of the 20th Annual Hybrid Corn Industry-Research Conference, 1965.

Frey et al. (34) suggested that maize with a moderate protein content having a larger tryptophane-protein ratio and a lower protein-zein ratio is to be desired rather than merely higher protein maize. They described the effectiveness of recurrent selection for high lysine maize. The amount of non-zein protein appears to be a better criterion in the selection for increased lysine content in the maize kernel than the amount of total protein.

Mertz et al. (61) said that if lysine content is considered as the criterion for protein quality, the percent of lysine in the whole kernel can be increased either by breeding for larger germ size or by decreasing the relative percentage of zein and increasing glutelin fractions of the endosperm.

#### Genetics of opaque-2 and floury-2 genes

The opaque-2 gene was first identified by Singleton in 1935 (93). Opaque-2 endosperm has very little or no c orneous starch. On segregating ears, 25 percent of the seeds will be homozygous for opaque-2. Classification of opaque-2 is easy in flinty stocks. When placed over a light, normal seeds are translucent, while the receissive are opaque. Opaque-2 is located on chromosome VII, probably beyond  $v_5$ , and its locus is 16. The mutation rate of opaque-2 among several inbred lines is  $15 \times 10^{-6}$  to  $2 \times 10^{-6}$  according to Lambert (51).

The floury-2 gene was first reported by Mumm in 1929 (69), and was located by Cornu in 1962 (21) on chromosome IV, very near the

lazy locus (exact locus is unknown). It is phenotypically indistinguishable from opaque-2.

#### Studies on opaque-2 and floury-2

Nelson et al. (72) studied the biological value of the opaque-2 phenotype in feeding experiments with rats. They found that the average weight gain during a 28 day period was 97 grams in the rats fed opaque-2 and 27 grams in the rats fed normal maize. They indicated that in young rats opaque-2 maize proteins have a feed value equal to that of heat-treated soybean meal (previously the best feed known) and superior to any cultivated cereal grain. Others, Beeson et al. (9) on pigs, Pickett et al. (78) on swine, Clark (19) on human beings, Bressani (14) on children, and Rogler (84) on chicks, have demonstrated the superior quality of the proteins in opaque-2 endosperm. Beeson et al. (8) indicated that weanling pigs fed opaque-2 maize gained 3.6 times faster than pigs fed normal maize (0.94 vs. 0.26 lb/day) and similar (0.94 vs. 0.93 lb/day) to pigs fed a corn-soybean meal diet at the same protein level (11.6 percent).

Concon (19), Jiménez (47), Watson et al. (102), and Wilson (103) studied the morphological and chemical characteristics of the high-lysine maize. Problems associated with breeding the opaque-2 gene were discussed by Alexander (2). Alexander asked the following questions. (a) Do epistatic effects exist? (b) Do modifiers of opaque-2 influence lysine concentration or phenotypic appearance of the kernel? and (c) Should breeding work on high lysine content be conducted on corns of 9-10 percent protein exclusively, or should higher protein levels be produced as well? He indicated that opaque-2 kernels, borne

on segregating ears, are usually lighter in weight than normal kernels. In certain genetic backgrounds, opaque kernels are equal in mass to the normal kernels. Out-crossing to an appropriate tester may be necessary, because of the difficulty in classifying opaque-2 kernels in some backgrounds. Alexander (3) reported that the yield of the opaque-2 version of normal maize was almost equal to normal maize. He also indicated that opaque-2 stands in the field generally are poorer than normal, and ear and kernel rots are also higher than in normal. He emphasized that great differences in disease susceptibility existed within the opaques. Further studies on opaque-2 versions of standard lines are being conducted at various institutes, i.e. production of opaque-2 versions of standard inbreds, mutation at the opaque-2 locus, allelism tests for opaque-2 and floury-2 genes in U.S. floury maize, epistatic effects on the opaque-2 phenotype, creation of high protein and high lysine synthetics at the Maize Genetics Laboratory at the University of Illinois, and incorporation of opaque-2 into various germplasm complexes at the International Maize and Wheat Improvement Center in Mexico.

## MATERIALS AND METHODS

Thirty one maize varieties and one opaque-2 line were selected for this study. The varieties were grouped as follows:

High lysine line (SuSuAeAeWxWxO <sub>2</sub> O <sub>2</sub> )	:	opaque-2
Starchy lines (SuSuAeAeWxWxO <sub>2</sub> O <sub>2</sub> )	:	Philippines No.1, No.3, No.5, No.7, and No.9 Guam No.1, No.2, No.3 Cuzco, Korean No.1, Korean No.1-1, Korean No.5, Korean No.5-1, Korean No.6 and No.25
Sugar lines (susuAeAeWxWxO <sub>2</sub> O <sub>2</sub> )	:	Chiripo Dulce Pajimaca Winter Green Hawaiian Hybrid Synthetic (XH) Southern Belle Hawaiian Sugar (HS) Iobelle Golden Bounty Hawaii 68 (H68)
High sucrose line (SuSuAeAeWxWxO <sub>2</sub> O <sub>2</sub> )	:	<u>aeWx</u>
Glutinous lines (SuSuAeAeWxWxO <sub>2</sub> O <sub>2</sub> )	:	Glutinous No.1, No.2, No.3, No.4, No.5, and No.6. (G-1, G-2, G-3, G-4, G-5, and G-6)

Description of varieties

The opaque-2 line used as high lysine stock was received from R.J. Lambert, Illinois University. Philippine varieties were open-pollinated yellow flint types of maize. Guam varieties were open-pollinated white flint type maize. The Cuzco variety was obtained from D.L. Shaver, Cornnuts Inc. and was a white open-pollinated dent type maize with a large kernel. The Korean varieties were open-pollinated white flint type maize varieties selected at the Crop Experiment Station, Suwon, Korea.

Chiripo Dulce and Pajimaca were open pollinated sweet corns originally obtained from the mainland U.S.A. Wintergreen, Southern Belle, Iobelle, and Golden Bounty were hybrid sweet corns received from mainland seed companies. Hawaiian Hybrid Synthetic, Hawaiian Sugar, and Hawaii 68 were developed in Hawaii. Hawaii 68, developed by J.L. Brewbaker, University of Hawaii, is the predominant sweet corn variety grown in the Hawaiian Islands. A breeding line from the University of Illinois with the genes aewx was used as a high sucrose line. This double mutant was received from R. J. Lambert. Six glutinous lines with white flint type corn were received from the Philippines. Except for the Korean varieties, all seed were obtained by J. L. Brewbaker for his corn breeding program.

#### Crossing procedures

In the production of  $F_1$ 's, the opaque-2 line was used as the male and no reciprocal crosses were made. In backcross generations the normal varieties were used as recurrent parents. From 15 to 29 plants of each line were grown and hand pollinated. Each recurrent parent was planted a week earlier and later than the  $F_1$  parent to allow for differences in flowering. Standard cultural practices were used. In order to make backcrosses to sweet corn, a test cross was made to the homozygous opaque-2 line, since the opaque-2 gene was not identifiable in the sweet corn varieties.

Data recorded

The following data were recorded for each parent, F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub> selfed:

Height of plants - in cm. at maturity

Days to 50 percent silking and tasselling

Height of first ear - in cm.

Disease - Helminthosporium turcicum infected leaves were graded 1 (resistant) to 5 (susceptible).

Total ear number per plant

Direction of leaves - classified as upright or spreading

Sterility - percentage of seeds set and pollen fertility

Maturity - days from planting to harvest

F<sub>2</sub> segregation of homozygous opaque-2 versus normal - when the segregation ratio of opaque-2 and normal were recorded for an ear, the color of kernels and the following were recorded at the same time:

Ear or kernel rot susceptibility - recorded in grades 1 to 5 (1 - resistant, 5 - susceptible), when shelling.

Kernel size of opaque-2 type - estimated in comparison with parental lines. (smaller than normal, etc.)

Weight of kernel - recorded in grams per 100 seeds after all seed was oven dried for 7 days at 80° C.

Protein content - percent of nitrogen analyzed by the modified micro-Kjeldahl method (9).

Lysine content - grams per 100 grams of protein

### Analytical methods

Iodine staining - For glutinous (waxy) maize, homozygous waxy kernels were identified by iodine staining and homozygous opaque-2 kernels were identified by illumination over an electric lamp. The tip of each F<sub>2</sub> kernel from crosses of glutinous with opaque-2 was cut by a razor blade and stained with iodine plus potassium iodide mixed equally in volume. The homozygous waxy type stained red, the non-waxy type stained dark-blue. Homozygous opaque-2 kernels were opaque over an electric lamp, normal kernels were translucent.

Protein analysis - The air dried maize samples obtained from segregating progenies and their parental varieties were ground to pass through a 40 mesh screen and defatted by refluxing with petroleum-ether (b.p. 40.8 - 56.4° C.) for 24 hours at about 50° C. in a soxhlet. 50 mg. of air-dried, defatted samples were weighed and analyzed by the modified micro-Kjeldahl method (8).

Lysine determination - The methods of Thompson et al. (97, 98), Sibalic and Radej (92), and Roberts et al. (81, 82) were adapted and modified for determination of lysine content. A flow diagram for the analysis of protein and lysine is presented on page 19.

Materials: Ion exchange resin. Dowex 50-8x, 100-200 mesh in the H<sup>+</sup> form was soaked in water overnight and stirred with an equal volume of water in a cylinder. The fines were removed by decanting the supernatant after 30 minutes. The process was repeated three times.

Glass columns for Dowex 50 - 8x. Columns are glass tubes (30-50 cm. x 0.9 cm.) with capillary tubes (8 x 0.2 cm.) attached at the bottom. A small plug of glass wool was placed in the column and the ammonium

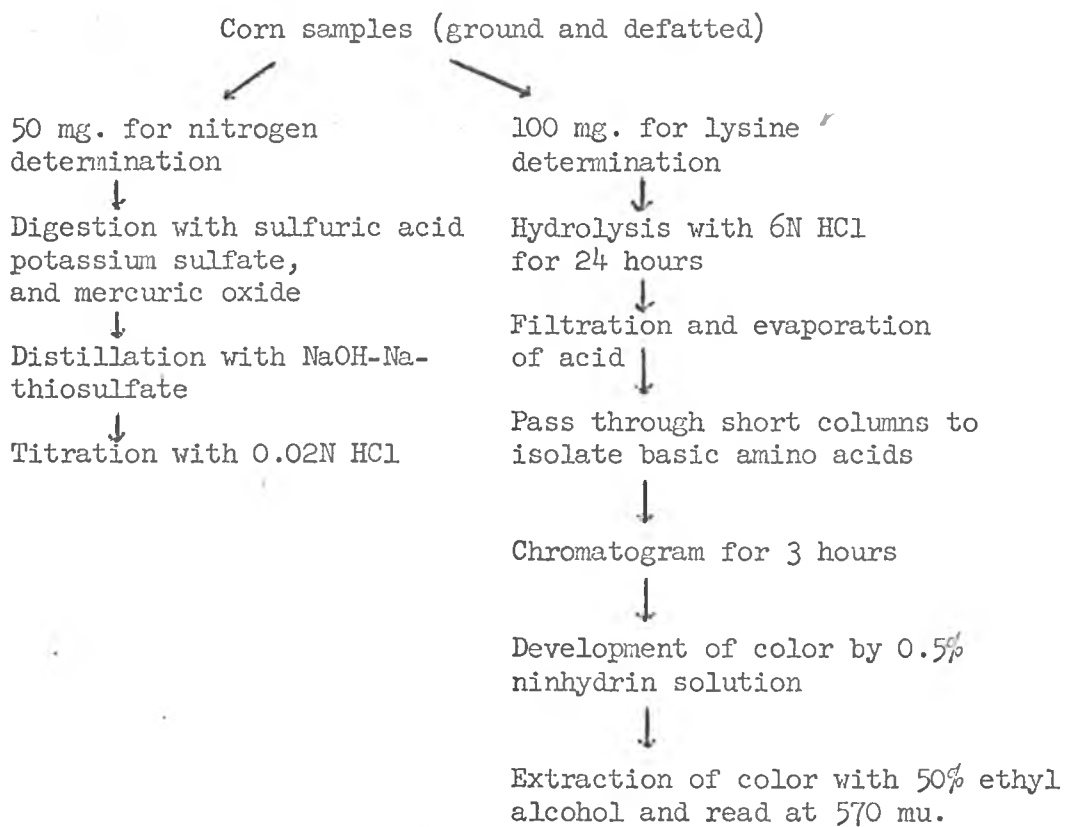
form of resin was added to a depth of 7 to 15 cm. The columns were reused a number of times. After replacing the top few cm. of resin with new resin, it was regenerated by passing through the columns 150 ml. of 2N NaOH, 150 ml. of water, 15 ml. of 3N HCl and 150 ml. of water in that order.

Ammonium form of resin. Dowex 50 - 8x was treated, in a column, with 10 volumes of 2N ammonium hydroxide made with distilled water. The resin column was washed with distilled water (10 to 20 column volumes) until the effluent reached a pH of 8 to 9.

Chromatographic chamber. For the development of the chromatograms, a glass-paned chamber 24 x 11.5 x 11.5 inches, whose edges were sealed by a mixture of water glass and talcum powder, was used. A glass pan, 11.5 x 11.5 x 0.025 inches, with four openings, 1 cm. in diameter, was glued to the top of the chamber. Below each hole there was a glass trough, 10 x 1.4 x 0.7 inches, fastened 19 inches from the bottom of the chamber. Two glass rods, used to support the filter paper, were fixed parallel to each trough at a distance of 4 cm. The chamber was placed in a room where the temperature was about 30° C.

Filter paper. Whatman No.1 chromatographic paper was soaked in buffer solution at pH 6.8. The paper was dried at room temperature, and cut into 20 x 28 cm. sheets.

Methods: Sample preparation. Materials were finely ground, defatted, air dried, and 50 mg. samples were placed in 5 ml. ampules, 5 ml. of 6N hydrochloric acid were added and each ampule was sealed over the bunsen burner while being evacuated. Sealed samples were hydrolyzed in the 105° C. oven for 25 hours. The hydrolysate was

General steps for the analysis of protein and lysine

of solvent, the residue was diluted to 20 ml. with pH 6.8 phosphate buffer. (The pH 6.8 phosphate buffer was made of 200 ml. of 0.067 M.  $\text{Na}_2\text{HPO}_4$  and 250 ml. of 0.067 M.  $\text{KH}_2\text{PO}_4$ ). The pH of the residue was brought to 7, and then it was passed through the column. Samples were run in duplicate.

Separation of basic amino acids. After the hydrolyzed sample was poured into the column, the column was eluted with 150 ml. distilled water, to pass neutral and acidic amino acids through the column. The retained basic amino acids, including lysine, arginine, and histidine were then eluted with 150 ml. of 3N ammonium hydroxide. The eluate was vacuum dried over a water bath at  $60^\circ\text{C}$ ., then diluted with 5 ml. of 10 percent isopropyl alcohol.

Isolation of lysine. For standardization, 2 mg. of lysine (hydrochloric form) were dissolved in 10 ml. of water, and serially diluted 1:10, 1:20, and 1:30, and then passed through the column. On the buffered paper, 6 cm. from the narrower edges, the three concentrations of standard solutions and 6 spots of samples were applied with 0.01 ml. micropipettes. The volume of each spot was 0.02 ml. The spots were prevented from spreading to more than 6 mm. in diameter by drying successive small applications. Chromatograms were developed in the chromatography chamber for about 3 hours. Then the chromatograms were removed, left at room temperature for about 7 hours and pulled through a solution of ninhydrin, which was made of 0.5 g. ninhydrin, 98 ml. acetone and 2 ml. glacial acetic acid. The chromatograms were then left in the dark at room temperature for about 7 hours. The colored spots were cut out, cut into strips, and placed in colorimeter

tubes. 2.5 ml. of 50 percent ethyl alcohol were added, the tubes were shaken for 30 minutes, and the color intensity was read at 570 mu. on a Bausch and Lomb Spectronic 20 spectrophotometer.

## RESULTS AND DISCUSSION

Protein determination

Results of protein determinations in the parental varieties used in the crosses and the opaque-2 segregates of  $F_3$  and  $BC_1$  selfed are presented in Table 3. It was assumed that the endosperm of opaque-2 type of  $F_2$  had the  $o_2o_2o_2$  genotype. No separation of germ and endosperm was made, even though there is a great difference in protein content between germ and endosperm. The protein content in normal maize ranged from 6.0 to 13.6 percent. The lowest protein content was found in the Ko. No.1-1, which was similar in appearance to the opaque-2 type, but probably was not genetically opaque-2. The lysine content was also very low. This indicates that not all opaque-appearing types necessarily have a high lysine content. A comparatively large amount of protein was found in the starchy-non-floury type of maize. In a preliminary experiment, it was found that sweet corn varieties, which generally had a larger proportion of germ than non-sweet corn, also had a high protein content. When the endosperm of sweet corn was subjected to the protein analysis, the protein percentage was very low compared with that of the endosperm of non-sweet corn.

The protein content among opaque-2 types differed with varieties. The lowest protein content, 5.8 percent, was observed in the opaque-2 version of Ko. No:5-1. Less protein was observed in opaque-2 maize than in normal maize. No large differences in protein content were found between floury and non-floury kernels in opaque-2 maize as was found in the normal maize.

Table 3. Protein content of normal maize and opaque-2 as percentage of air dry weight of defatted, ground kernels.

Varieties	Normal	Opaque-2	Kernel types
Glutinous - 1	10.2	8.3	waxy
Glutinous - 2	10.2	10.6	"
Glutinous - 3 <sup>a</sup>	-	8.1	"
Glutinous - 3 <sup>b</sup>	-	7.6	"
Phil. No.1	13.0	10.6	starchy and non-floury
Phil. No.3	-	8.3	"
Phil. No.5	13.6	-	"
Phil. No.9	11.6	13.4	"
Guam No.2	9.5	9.5	starchy and floury
Korean No.5	8.9	11.1	"
Korean No.5-1 <sup>c</sup>	-	5.8	"
Korean No.6	-	7.9	"
Korean No.25	-	9.0	"
Korean No.1	11.0	-	"
Korean No.1-1 <sup>c</sup>	6.0	-	"
Golden Bounty	11.2	-	sweet corn
Pajimaca	10.3	-	"
HS	13.1	-	"
XH	10.0	-	"
H68	12.5	-	"
Opaque-2	-	9.3	starchy and floury

a opaque but not waxy type

b opaque and waxy

c opaque appearing kernels from a normal variety

Lysine determination

Lysine determinations of the normal parental lines and opaque-2 types are presented in Table 4. Opaque-2 types were obtained from the F<sub>2</sub> selfing (o<sub>2</sub>o<sub>2</sub>o<sub>2</sub>) and selfing of BC<sub>1</sub>. No separation of germ and endosperm to show the dosage effects was made. Even though several apparently opaque samples from the Guam varieties, Cuzco, and sweet corn lines were analyzed for lysine content, they all showed low lysine content, indicating that they were normal types. There were large differences in lysine content among normal varieties used. Lysine content, expressed as grams per 100 grams of protein, ranged from 1.2 grams in Iobelle to 3.6 grams in Ko. No.1. These figures suggest that, even though they have not previously been selected for high lysine, some common varieties may have high lysine content, such as in Ko.No.1 and Ko. No.5. Some of the normal sweet corn varieties like H66, Hawaiian Sugar, Goldenbounty, and Pajimaca showed high lysine contents, almost the same as the amount found in the opaque-2 version of normal corn. From the distribution of lysine content in the normal varieties it may be concluded that floury types of kernel have higher lysine than the corneous or non-floury types.

No large differences in the lysine content were observed among opaque-2 versions of the 10 lines analyzed except for Glutinous No.3<sup>b</sup> and Korean No.25. Lysine content varied from 2.4 grams per 100 grams of protein in Glutinous No.3<sup>b</sup>, which was a double mutant for waxy and opaque-2, to 4.2 grams in Korean No.25. The average percentage increase of lysine content from normal to opaque-2 maize was about 70 percent. This is almost the same value obtained by Nelson et al. (71).

Table 4. Lysine content of normal and opaque-2 lines in grams  
per 100 grams of protein.

Varieties	Normal	Opaque	Kernel types
Glutinous - 1	-	3.8	waxy
Glutinous - 2	2.4	3.8	"
Glutinous - 3 <sup>a</sup>	-	3.6	"
Glutinous - 3 <sup>b</sup>	-	2.4	"
Phil. No.1	1.8	3.8	starchy and non- floury
Phil. No.3	-	3.8	"
Phil. No.5	2.6	3.6	"
Phil. No.9	2.6	-	"
Guam No.2	2.0	-	starchy and floury
Korean No.5	3.2	3.6	"
Korean No.5-1 <sup>c</sup>	2.4	-	"
Korean No.25	-	4.2	"
Korean No.1	3.6	-	"
Korean No.1-1 <sup>c</sup>	3.0	-	"
Korean No.6	-	3.0	"
Golden Bounty	3.6	-	sweet corn
Pajimaca	3.0	-	"
HS	1.6	-	"
Iobelle	1.2	-	"
H6E	3.6	-	"
XH	3.0	-	"
Opaque-2	-	3.4	starchy and floury

a opaque but not waxy

b opaque and waxy

c opaque appearing kernels from a normal variety

No relationship was found between the lysine content of normal and opaque-2 versions of the same line.

From Table 4 and Figure 1, it may be concluded that the lysine content of opaque-2 types was higher than that of the normal types, while the total protein content of opaque-2 was less than the normal (except Phil. No.9 and Korean No.5).

Attempts to determine the relationship between lysine and protein content in both normal and opaque-2 maize were made (Figure 1). It is interesting to note that opaque-2 versions have lower percentage of total protein than their normal parents. In normal maize the total protein percentage was not significantly correlated to lysine content with a  $r = -.38$  (d.f. = 10). This is in contrast to the finding of Miller et al. (65) who indicated that increases in protein content were generally associated with increase in lysine percentage in normal corn. The lysine content in the opaque-2 maize was almost constant throughout the varieties, while the protein content varied with variety (Table 4). This suggests a further limitation of the lysine producing capacity of the opaque-2 gene. No relationship was obtained between the lysine content of opaque-2 and normal maize. In conclusion it may be inferred that high lysine maize may be developed independently from the protein content or lysine content of normal maize by using the opaque-2 gene. The general decrease of protein content in opaque-2 in this study was not in agreement with the finding of Nelson et al. (72,73), who found a higher protein content in opaque-2 maize than in normal maize. However, the observations are in agreement with the

finding of Sauberlich (86) who said that the percentage of some amino acids in the total protein (like lysine and tryptophane) became smaller as the percentage of protein in the maize became larger.

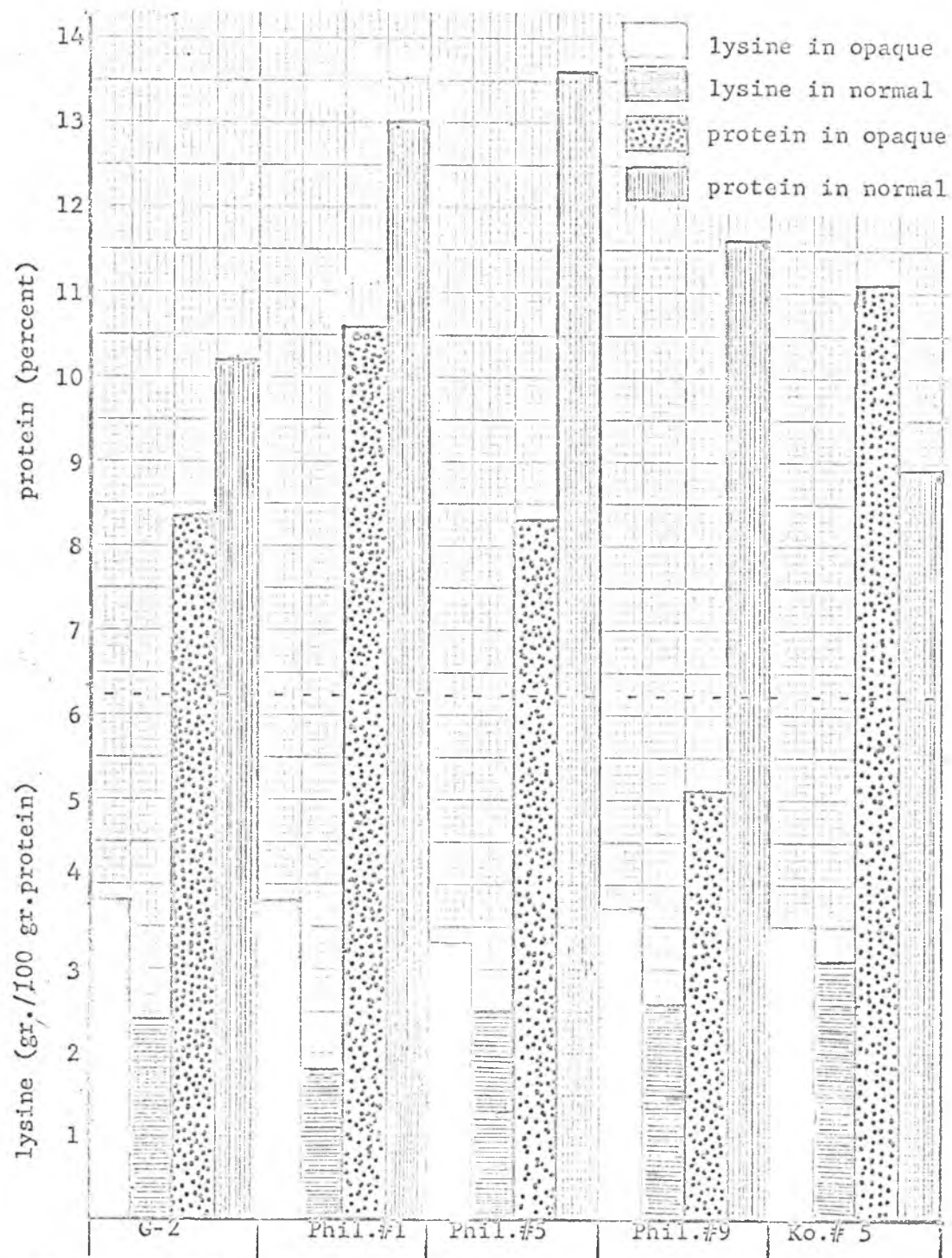


Figure 1. Relative content of protein and lysine in opaque-2 and normal defatted maize.

Table 5. Protein and lysine content of normal and opaque-2 defatted maize. (Lysine is expressed in grams per 100 grams of protein)

Varieties	Normal		Opaque	
	Protein (percent)	Lysine (grams)	Protein (percent)	Lysine (grams)
Glutinous-2	10.2	2.4	8.4	3.8
Phil. No.1	13.0	1.8	10.6	3.8
Phil. No.5	13.6	2.6	8.3	3.6
Korean No.5	8.9	3.2	11.1	3.6

## Segregation of F<sub>2</sub>

Results of segregation ratios of F<sub>2</sub>'s between normal varieties and opaque-2 are presented in Table 6. In most crosses the homozygous opaque-2 type had typical opacity and was easily identified. Even though the opaque-2 segregation was observable in the Phil. No.1, Cuzco, Hawaiian Hybrids, Golden Bounty, Pajimaca, and Southern Belle, the observed segregation ratios did not fit expected 3:1 ratios. In the crosses between Guam varieties (which have white, semi-opaque endosperm) and opaque-2, a very indistinct segregation was obtained. In the aewx cross, the homozygous opaque-2 was identifiable only in the yellow endosperm kernels. In the segregation of the F<sub>2</sub> of sweet corn crossed with opaque-2 maize, no segregates of sweet corn with opaque-2 background were observable. It may be necessary to make test crosses with a proper tester in order to identify the genotype of sweet corn progenies as far as the opaque-2 gene is concerned. It was noted that in the cross between Phil. No.3 (which has yellow endosperm) and opaque-2, a mottled kernel instead of opaque-2 kernel was observed (Figure 2). With the above mentioned exceptions, homozygous opaque-2 kernels segregated in the expected 3 (normal) : 1 (opaque) ratio.

The weights of opaque-2 and normal kernels on the same ear were compared (Table 7). The weights of opaque-2 kernels were usually lighter than the corresponding normal type. The kernel weights of the normal parents and their opaque-2 versions were correlated. ( $r = .94$ , d.f. = 10 for yellow endosperm; and  $r = .98$ , d.f.=18 for white endosperm). The average kernel weight was about 5 to 15 percent lighter in opaque-2 than in normal. The greatest difference in kernel weight

between opaque-2 and normal was found in Phil. No.1 (more than 20 percent) and the lowest difference was found in the Winter Green and Pajimaca varieties (less than 5 percent). In some varieties there was little or no difference in kernel weight between opaque-2 and normal kernels. This was found in the cross with Southern Belle and in the aeWX cross. This suggests the possibility of selecting modifiers which may reduce the gap between the mutant and the normal phenotype. The weights of normal and their opaque-2 version kernels were previously discussed by Alexander (3), Costa-Rodrigues (29), and Salamini (86). They found the same tendency for kernels carrying opaque-2 to be lighter in weight.

Among the  $F_2$  kernels on  $F_1$  plants of the cross, Golden Bounty sweet corn x opaque-2, the homozygous opaque-2 field corn type appeared smaller in kernel size on the same ear than its normal counterpart. Reduced kernel size was observed upon selfing of  $BC_1$  of Guam No.2 and H68 with opaque-2 background (Figure 3).

Male sterile plants were found in the  $F_1$  of Ko. No.5 crossed with opaque-2. Further studies were conducted in the  $F_2$ , but insufficient plants were grown to establish genetic ratios. In later trials it was found that the plants of  $BC_1$  crossed to the opaque-2 male were all male sterile, while the plants of  $BC_1$  crossed to the opaque-2 female were all male fertile. From the 10 ears of glutinous No.5 female crossed with opaque-2 as male, no  $F_1$  seeds were obtained. No reciprocal crosses were made at the time the original cross was made. It was later found that the crosses of male Glutinous No.5 with female opaque-2 set normal seeds. From these crosses it was suggested that some kind

Table 6. Segregation of F<sub>2</sub> kernels with respect to endosperm color (yellow and white with normal and opaque)

Varieties	Observed phenotypes				Total	Expected ratio	chi-square value
	Number	of Kernels in Class					
	Y-O <sub>2</sub> -	Y-o <sub>2</sub> o <sub>2</sub>	yyO <sub>2</sub> -	yyo <sub>2</sub> o <sub>2</sub>			
Phil. No.1	277	48	63	19	407	9:3:3:1	24.4**
Phil. No.3	362	104	85	32	583	9:3:3:1	9.6*
Phil. No.5	402	122	145	37	706	9:3:3:1	2.9
Phil. No.7	280	84	99	30	493	9:3:3:1	1.3
Phil. No.9	461	155	148	56	845	9:3:3:1	1.4
-----							
	O <sub>2</sub> -	o <sub>2</sub> o <sub>2</sub>					
Korean No.5	187	59			246	3:1	0.13
Korean No.6	52	14			66	3:1	0.25
-----							
	Su-//	Su-//	Su-//	Su-//	susu//	susu//	
	Y-O <sub>2</sub> -	Y-o <sub>2</sub> o <sub>2</sub>	yyO <sub>2</sub> -	yyo <sub>2</sub> o <sub>2</sub>	Y-O <sub>2</sub> -	yyO <sub>2</sub> -	
XH	377	125	131	35	163	63	894 27:9:9:3:12:4 7.5
Iobelle	250	87	99	33	118	36	623 27:9:9:3:12:4 2.6
Winter Green	398	112	126	32	160	67	895 27:9:9:3:12:4 7.6
Golden Bounty	308	176	104	60	144	37	829 27:9:9:3:12:4 66.2**
HS	447	138	178	49	207	60	1079 27:9:9:3:12:4 7.1
Pajimaca	344	73	129	26	96	52	722 27:9:9:3:12:4 35.2**
Southern Belle	491	59	190	19	159	52	970 27:9:9:3:12:4 78.8**
-----							
	O <sub>2</sub> -	o <sub>2</sub> o <sub>2</sub>					
Glutinous No.1	504	134			638	3:1	5.4*
Glutinous No.2	647	192			839	3:1	2.0
Glutinous No.3	326	102			428	3:1	0.3
Glutinous No.4	393	150			543	3:1	2.0
Glutinous No.6	450	138			588	3:1	0.7

Heterogeneity chi-square (d.f. = 14)

\* P less than 0.05

\*\* P less than 0.01

Y=yellow (dominant) endosperm

o<sub>2</sub>=opaque-2 (recessive) endosperm

su=sugary (recessive) endosperm

23.8\*

Table 7. One hundred kernel weights in F<sub>2</sub> progenies

Varieties	Yellow endosperm		White endosperm	
	Normal	Opaque	Normal	Opaque
	gm.	gm.	gm.	gm.
Phil. No.1	32.4	26.8	31.6	26.3
Phil. No.3	25.8	24.0	24.4	22.8
Phil. No.5	26.2	25.0	26.5	25.1
Phil. No.7	19.6	19.5	21.0	26.5
Cuzco			42.8	41.6
Korean No.5			27.6	26.1
Korean No.6			28.5	25.0
Hawaiian Synth.	22.9	21.8	22.9	20.8
Iobelle	21.6	20.2	19.9	23.4
Winter Green	21.1	20.9	22.5	26.8
Golden Bounty	20.9	17.3	20.6	16.5
Hawaiian Sugar	21.3	20.1	21.3	20.4
Pajimaca	20.8	19.7	20.3	19.2
Southern Belle	22.0	22.2	22.5	22.1
<u>aeWX</u>	13.5	13.9	12.9	13.7
Glutinous No.1			30.6	27.0
Glutinous No.2			31.5	26.3
Glutinous No.3			31.3	26.2
Glutinous No.4			18.8	18.5
Glutinous No.6			22.0	21.7
mean	22.4	21.0	23.4	23.2
correlation coefficient	.94** (d.f..10)		.98** (d.f..18)	



Figure 2. Upper left: normal Phil. No.3, upper right: normal opaque-2, lower left: mottled opaque-2 in Phil. No. 3, and lower right: opaque type of Phil. No.1.

of gametophytic relationship might be involved (Lambert, personal communication). Different degrees of seed set were found in the segregates of ae wx cross with opaque-2. The semi-sterility of pollen and scattered kernel set on the ear of *AeaeWxwx* genotypes was discussed previously by Snyder (94).

#### Segregation of F<sub>3</sub> and BC<sub>1</sub>

From F<sub>2</sub> plants which had opaque-2 background and from selfing the BC<sub>1</sub>, homozygous opaque-2 plants were selected. In selecting double mutants of waxy and opaque-2, it was necessary to use the electric lamp and iodine staining techniques. Homozygous opaque-2 types were first selected by the electric lamp from mixed kernels. Opaque-2 types were dark under the lamp, while other non-waxy and waxy types were translucent. It must be emphasized that the double mutant type of *wxwx/o<sub>2</sub>o<sub>2</sub>* kernel was not translucent over the lamp, but dark like the nonwaxy type. Therefore, it was necessary to select the waxy type of maize from homozygous opaque-2 types of corn by using iodine stain, however, homozygous waxy types were red instead of dark blue. A close fit to the theoretical ratio of 3 nonwaxy to 1 waxy and 3 normal to 1 opaque-2 was obtained. For instance, in the F<sub>2</sub> of Glutinous No.3 crossed with opaque-2 the following ratio was obtained for double genotypes of waxy and opaque-2.



Figure 3. Reduced kernel size of opaque type from normal kernels of the Guam No.2 variety (right:opaque-2, left: normal)

Genotype	--O <sub>2</sub> *	Wx-o <sub>2</sub> o <sub>2</sub>	wxwxO <sub>2</sub> o <sub>2</sub>
Observed number of kernels	235	66	23
Expected number of kernels	243.8	60.9	20.3
Ratio fitted	12	:	3 : 1
chi-square value (d.f. 2)	1.1		

\* includes 9 Wx-O<sub>2</sub>- and 3 wxwxO<sub>2</sub>-

Even though non-waxy and waxy types were not examined in the --O<sub>2</sub>- phenotype, it is assumed that they looked the same under the lamp. It is not known why there was a yellowish homozygous opaque-2 type of kernel in the selfing of BC<sub>1</sub> of the waxy Glutinous No.6 x opaque-2. In this case both parents have white endosperm.

In the F<sub>2</sub> of Philippine varieties with opaque-2 it was found that several lines did not appear opaque, but had mottled kernels. Such a mottled type of kernel was also found in the F<sub>1</sub> of some Philippine varieties (Figure 2). When yellow endosperm kernel types, as in the Philippine varieties, were homozygous for opaque-2, they were a lighter shade of yellow than the parent.

It was observed that the opaque-2 kernels of Philippine varieties were greatly damaged by ear and kernel rot, while normal kernels were not damaged in the same ear. Ear and kernel rot damage was found in varieties such as Hawaiian Sugar sweet corn. However, some varieties like Korean No.5 had very good resistance to this fungus even in the homozygous opaque-2 type (Figure 3). Kernels of varieties susceptible to the fungus were all completely rotted, while a resistant variety

like Korean No.5 had only a small black spot around the remnant part of style. Considering the damaged part of the resistant variety, i.e. remnant part of style, it was assumed that the fungus entered through the styles at pollination.

In early studies of opaque-2 and floury-2, it was proposed by Alexander (2), that opaque-2 and floury-2 may be alleles. In order to test allelism of these genes, reciprocal crosses of opaque-2 and floury-2 were made. When opaque-2 plants were used as females and floury-2 as males, the  $F_1$  was normal; in the reciprocal cross the  $F_1$  was floury indicating a dosage effect of floury-2 gene in the endosperm. Since endosperm of  $Fl_2fl_2fl_2$  genotype is floury, an  $F_2$  ratio of 6 : 10 is expected instead of 9 : 7 because three individuals heterozygous for  $Fl_2$  would have two doses of  $fl_2$  in the endosperm and be floury.

Table 8.  $F_2$  segregation of opaque-2 and floury-2 in endosperm types.

	Normal types	Opaque-2 plus floury-2
Observed number of kernels	385	550
Expected number of kernels	350.7	584.3
Expected ratio	6	: 10
chi-square value (d.f. 1)		5.3*

Even though the chi-square value is significant at the 5 percent level, they are not alleles because there is segregation. If they were alleles, there would be no segregation. In the above test it should be mentioned that opaque type and floury type look the same phenotypically.

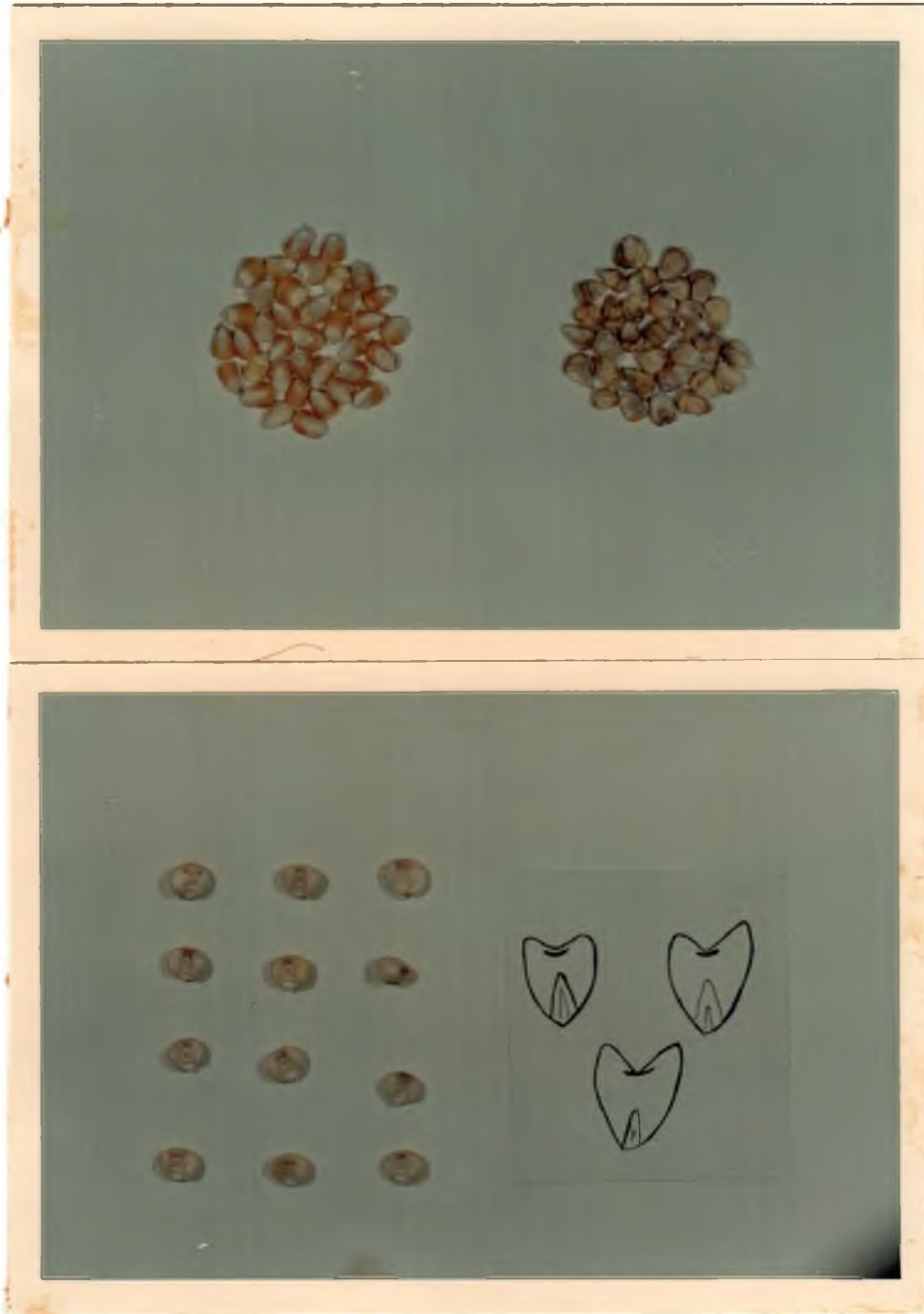


Figure 4. Comparison of kernel rot disease of normal and opaque-2 (above) and homozygous opaque-2 of Ko. No.5 showing resistance to kernel rot disease (below)

### General observations

Height of plants, silking and tasselling dates, height of ear, disease, ear number, direction of leaves, sterility, and maturity of plants are presented in Table 8. Under Hawaiian conditions, where several dates of planting are possible in a year, the plant heights, dates of silking, maturity, etc. are different due to the planting time and or location. For instance, Iobelle, Wintergreen, ae wx, Southern-belle, and Golden Bounty are early varieties which mature in about 90 to 100 days, while Pajimaca, Hawaiian Sugar, Hawaiian Hybrid Synthetic, and Chiripo Dulce are late varieties maturing in 100 to 105 days.

It was found that the opaque-2 parent used in the experiment was very susceptible to mosaic virus in the first planting of September, 1967. The Cuzco variety was very susceptible to ear rot and kernel rot disease. Korean No.25 was particularly susceptible to Helminthosporium turcicum. The glutinous varieties all had upright leaves, which allow greater light penetration directly and indirectly by reflection. A few varieties among the Philippine varieties, like Phil. No.3, showed a certain degree of male sterility.

The following problems were raised in the present study: 1) the mechanism of kernel rot disease infection in opaque-2 maize, 2) the mechanism of male sterility found in the Korean No.5 crossed with opaque-2, 3) gene interaction among ae wx, opaque-2, sugary (su<sub>1</sub> or su<sub>2</sub>), 4) the ear size of opaque-2 maize.

## SUMMARY

## 1. Protein

Although lower protein content was observed in the opaque-2 maize than in the normal maize, there was no relationship between protein contents of normal and opaque-2 versions of the same lines. The protein content among opaque-2 types differed with varieties.

## 2. Lysine

The lysine content among normal types differed with varieties, while the lysine content among opaque-2 types was almost constant. No relationship was found between protein and lysine content of normal or opaque-2 maize. Also, no relationship was found among lysine contents of normal and opaque-2, Opaque-2 lines, however, had higher lysine contents than their normal versions.

## 3. Segregation

Opaque-2 types segregated in most of the crosses in the expected ratio of 3 normal to 1 opaque. In segregating  $F_1$  ears, and in  $BC_1$  selfing, homozygous opaque-2 kernels were often smaller in size than normal types. The kernel weight of opaque-2 maize was usually lighter than that of normal kernels. However, a possibility of selecting heavier kernels of opaque-2 maize was suggested. Phenotypic interaction was observed in the crosses between the Philippine varieties and opaque-2. Mottled kernels instead of opaque kernels were observed in the opaque-2 with Philippine varieties background. It was found that homozygous opaque-2 kernels in segregating  $F_1$  ears were very susceptible to kernel rot disease. An apparently resistant type was observed in the Korean No.5.

#### 4. Others

Opaque-2 and floury-2 were not alleles. Different percentage of seed set were observed in the segregation of aewx crossed with opaque-2. An unusual gametophytic relationship was involved in a cross between Glutinous No.5 (female) and opaque-2 (male).

Selection of a double mutant of waxy and opaque-2 by using the iodine technique and electric lamp was discussed.

## APPENDIX

Table 8. General plant characters of varieties used

Varieties	Plant height	Ear height	Disease (1)	Tassel date (2)	Leaf direction (3)	Male sterility	Days to harvest (4)	Prot. (5)	Lys. (6)
	ft.	ft.		days			days	%	gr.
Cuzco	7.2	2.8	4	50	sp.	none	100	-	-
Guam No.1	7.5	3.6	1	50	sp.	none	100	-	-
Guam No.2	7.5	3.2	1	50	sp.	none	100	9.5	2.0
Guam No.3	7.5	3.1	1	50	sp.	none	100	-	-
Phil. No.1	8.5	3.9	2	45	sp.	none	90	13.0	1.8
Phil. No.3	7.1	3.3	1	44	sp.	30%	90	8.3	-
Phil. No.5	7.6	2.6	3	39	sp.	30%	90	13.6	2.6
Phil. No.7	6.8	2.8	3	39	sp.	none	90	13.3	2.6
Phil. No.9	6.9	2.9	2	40	sp.	none	90	11.6	-
Chiripodulce	7.6	4.0	1	45	sp.	none	105	-	-
Pajimaca	7.5	3.6	1	48	sp.	none	105	-	-
XH	6.9	2.9	1	43	sp.	none	80	10.0	3.0
HS	7.2	3.2	1	43	sp.	none	80	13.1	3.0
Wintergreen	5.8	1.4	1	40	sp.	none	78	-	-
Southern.	5.3	1.6	1	40	sp.	none	78	-	-
Iobelle	5.9	1.6	1	43	sp.	none	78	-	1.2
Golden Bounty	6.0	1.4	1	43	sp.	none	78	11.2	3.6
aewx	5.5	1.7	1	54	sp.	none	78	-	-
G-1	4.0	1.4	1	50	up.	none	90	-	-
G-2	4.6	1.5	1	49	up.	none	90	-	-
G-3	4.9	1.9	1	48	up.	none	90	8.1	2.4
G-4	6.6	2.6	1	48	up.	none	90	7.6	-
G-5	5.5	2.0	1	47	up.	none	-	-	-
G-6	6.3	2.3	1	49	up.	none	90	-	-
Ko. No.5	5.6	2.0	1	50	sp.	none	95	8.9	3.2
Ko. No.6	4.7	1.5	1	55	sp.	none	95	7.9	-
Ko. No.25	4.5	1.7	5	45	sp.	none	90	9.0	-
Ko. No.1	4.6	4.7	1	55	sp.	none	90	11.0	3.6
Opaque-2	5.5	1.8	2	47	sp.	none	90	9.5	3.4

- (1) 1 - resistant      5 - susceptible  
(2) from planting to 50% tasselling  
(3) sp. - spreading      up. - upright  
(4) year-round average on Oahu at low elevation (Manoa campus)  
(5) percent of total dry weight  
(6) grams per 100 grams of protein

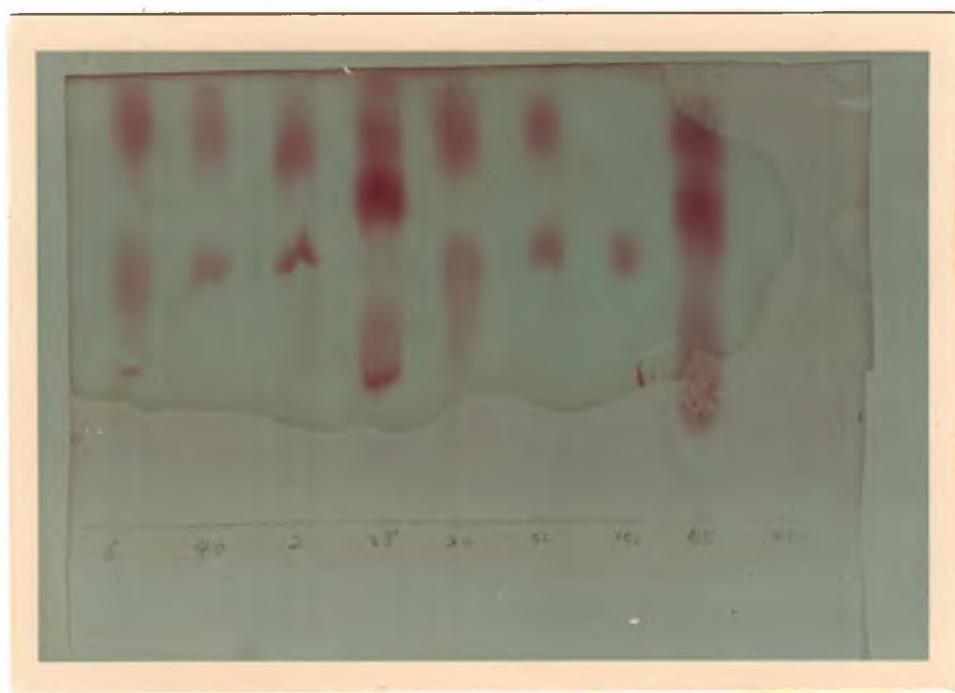
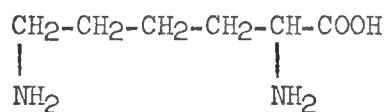


Figure 5. Separation of lysine on chromatogram after purification through column (numbers indicate sample number and "st" indicates standard solution).

Chemistry of lysine

Structure:



*l,α,ε* -diaminocaproic acid

*l,ε,ε* -diaminohexanoic acid

L-lysine is one of the eight indispensable amino acids for human beings and other higher animals. Lysine is considered essential because it must be included in the diet for optimal growth, or for the maintenance of nitrogen balance. Biological studies on lysine have been done in bacteria, fungi, etc.

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