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Four agronomically and genetically diverse spring barleys were used in a crossing program to study the inheritance of lysine in barley and to determine its possible association with certain agronomic and morphological characters. The cultivar Hiproly was used as the source of high protein and lysine. Characters measured included plant height, head type, awn type, lemma color, number of heads per plant, number of seeds per head, seed weight, and plant yield. Soluble protein content was determined by the Lowry colorimetric method. Lysine was analyzed by two methods: (1) enzyme hydrolysis and colorimetric determination with 2-chloro, 3, 5-dinitropyridine; (2) Udy dye-binding colorimetric method.

The F_1, F_2, and F_3 progenies were classified for the Hiproly endosperm character by histochemical techniques using Udy Orange G dye as a staining agent. Fractionation of the soluble proteins into the Mendal-Osborn fraction was used to determine the effects of the Hiproly gene on kernel proteins.

High lysine content in Hiproly was found to be inherited by one recessive gene. In addition several minor factors were indicated in all crosses. The recessive gene was associated with the recessive Hiproly endosperm gene with a recombination value of 22.45 percent. It is suggested that the gene designations for the two recessive genes be (lys) for the high lysine gene and (stb) for the starch binding endosperm gene.

High lysine was negatively associated with protein content; however, the association was not strong enough to limit selection for high lysine, high protein lines. No associations were found between lysine and any of the morphological or agronomic characters studied. It was concluded that it is possible to breed high yielding, high protein and lysine barleys using Hiproly as a source of high protein and lysine. Other parental lines can be selected to complement the Hiproly gene to obtain even better nutritional balance.

Chemical and Genetic Evaluation of High Lysine and Protein in Selected Barley Crosses

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In Dedication to Roy and Kay Berlin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (CONT)

LIST OF FIGURES

LIST OF APPENDIX PROCEDURES

LIST OF APPENDIX TABLES

CHEMICAL AND GENETIC EVALUATION OF HIGH LYSINE AND PROTEIN IN SELECTED BARLEY CROSSES

INTRODUCTION

The stresses of a rapidly increasing population on the available food supply make it essential that the nutritional value of foodstuffs be maximized. In the more developed countries there has been an increase of about 6 percent in protein intake since before World War II, whereas in developing countries it is estimated to have declined by about the same percentage. Protein rich foods are especially sparse and costly and protein malnutrition is a major health problem in these areas of the world. There is little chance that the developing countries will be able to apply the scientific and technical practices of modern agriculture rapidly enough to solve their food problems.

Cereal grains, which are the stable food of deprived areas, supply half of the total 80 million tons of protein available each year. An increase in the protein content of cereals from 10 to 11 percent would make an additional 4 million tons of protein available per year. An increase in the nutritional value of this protein would mean added gains. The nutritional potential of maize has been improved with the recognition

of genes for a higher lysine content. The success in improving protein quality of maize suggests the possibility that similar improvements in other cereal species can be obtained.

With the discovery of high lysine barley (Hiproly, C.I. 3947), by Munck et al. (41) the development of a barley variety that would yield high quality protein became a possibility.

This study was set up to determine the inheritance of lysine in Hiproly barley and to determine the potentialities of transferring genetic factors responsible for high lysine into high protein barley adapted to the Pacific Northwest.

The specific objectives of this study were: (1) to determine the inheritance of lysine content in Hiproly barley; (2) to determine the associations of high lysine with protein and yield; (3) to determine possible associations of high lysine with certain morphological markers; and (4) to determine the influence of different genetic backgrounds on the expression of the high lysine gene.

LITERATURE REVIEW

Cereals have long been known to be low in the essential amino acids lysine, methionine, threonine, and valine. Yet they supply half of the total protein consumed for food in the world and are the principal feed crops as well.

With the discovery of genetic factors responsible for high lysine corn, extensive work was begun on screening other cereal crops for improved protein lines (25, 33, 41). Corn, wheat, and barley have received the bulk of the attention. In 1968 Munck, Karlsson, Hagberg, and Eggum (41) in Sweden discovered in the world collection of barleys a high protein, high lysine barley, which they designated as 'Hiproly'. They reported that the Hiproly line was higher in lysine, threonine, valine, methionine, isoleucine, alanine, glycine, and aspartic acid while lower in the amino acids cystine, glutamic acid, and proline. With this discovery barley became a potentially valuable protein source for the human diet as well as a low cost livestock feed, particularly for nonruminant animals.

Other workers in Europe (18, 48) have since used induced mutations to obtain other strains of barley high in protein and lysine.

Presently the two primary uses of barley are for

malting and brewing, and for animal feed. The brewing industry is interested primarily in the carbohydrates and carbohydrases produced during germination to yield high amounts of extractable sugars, which nourish the yeast during the beer making processes. Proteins reduce the carbohydrate content of the seed and are the primary factor leading to turbidity and haze in the final product. Therefore high protein has been selected against in the development of malting cultivars. The hordein proteins have especially been selected against since it is known that they are the primary protein fraction related to the turbidity of the final product and the chief competitor of starch deposition in the endosperm (29).

Feed barleys are used primarily as a source of carbohydrates because total protein content is low and certain amino acids are limiting. With the increased lysine content now available through the Hiproly genotype, barley now promises to become more attractive particularly as a feed for monogastric animals. Studies have shown that lysine supplementation, in a barley diet, alone can increase the average daily gain of pigs from 23 to 65 percent and the nitrogen efficiency ratio from 22 to 46 percent, while decreasing the feed required per kilogram of weight gain 21 to 35 percent (17).

Barley compares favorably with corn, oats, and wheat as a feed grain (12). Eggum (16) shows barley to

have a total digestible protein content of 82 percent and a biological value of 71.8 percent. Biological value is expressed as a percentage of absorbed nitrogen retained by the animal. Wheat, corn, and rice have greater digestible protein contents but lower biological values as a percentage (Table 1). The concentrates, soybean and sunflower meal have high total digestible protein values, however low biological values. The greater concentration of proteins and balance of lysine in the concentrates make them a better protein source even though they are less efficient in releasing their useable proteins.

A significant variation in the protein and lysine content in wheat and barley has been reported by several workers (16, 25, 47). It is suggested that significant increases in protein content and quality can be made in all of the major cereal crops. The availability of screening techniques enabling the testing of large populations to find the natural variability that exists within the species are essential and may be used as the basis for renewed protein breeding programs (16, 36, 47).

Mechanical and chemical pretreatment have been used to help improve the quality of feed grains (9, 12, 37). Rolling, steeping, and pregermination increases the feed efficiency of barley as does enzyme pretreatment. Burnett (9) showed that poor growth of chicks fed

Table 1. Total digestible protein percent (TD), biological value percent (BV), nitrogen percent (N), and grams lysine per 16 grams nitrogen of cereal grains and protein-rich foodstuffs.a/

	TD%	BV%	N%	Lysine gm/16 gm N
Barley	82.0	71.8	1.40	3.69
Corn	87.6	58.1	1.38	2.73
Oats	84.1	70.4	1.61	4.03
Rice	101.1	65.5	1.11	3.49
Rye	77.0	77.7	1.41	3.67
Wheat	89.6	59.0	1.75	2.55
Cottonseed meal	79.9	66.1	6.44	4.00
Soymeal	90.7	62.0	7.39	5.98
Sunflower meal	91.9	70.7	6.13	3.50

a/ from Eggum (16) pages 132, 133

on bright barley was associated with the high viscosity of the barley, caused by the barley gums, in the small intestine. Water treatment and enzyme supplementation hydrolyzed the gums reducing the viscosity and permitting improved performance. The evidence points to beta-glucan as the material primarily responsible for this viscosity.

Alpha amylase content is positively associated with weight of gain in mice (37). Increased enzymatic

activity is thought to increase the energy and protein availability in the grain. The effects of alpha amylase are similar to those obtained by steeping and cooking of barley. High enzyme activity of the malting barleys may be an important factor in the nutritional utilization of the grain (9).

Seed Structure and Protein Patterns

Barley proteins can be separated into four soluble protein groups by the Mendal-Osborn fractionation process (29). The albumins are found primarily in the embryo and are soluble in water and dilute salt solutions, acids, and alkalies. The globulin fraction also found primarily in the embryo and scutellary proteins is insoluble in water but dissolves in dilute salt solution. The prolamins are soluble in 70 percent alcohol and comprise the major portion of the endosperm hordein proteins. The glutelin fraction, found primarily in the aleurone layer of the seed, is insoluble in water, salt solution, and alcohol, but is soluble in dilute alkalies.

During the germination process the hordein and glutelin fractions are extensively, though not completely, transformed into the salt soluble protein compounds (29).

Generally in a barley kernel the endosperm is low

in lysine while the embryo is relatively rich. The albumins are the best balanced of the soluble protein fractions for the amino acid lysine and are closely followed by the globulins, however these fractions represent a very small percent of the total protein content of the seed. The prolamins are by far the lowest in lysine content and comprise from 20 to 50 percent of the total protein of the seed (18, 24, 30, 40, 42, 47, 53). It is evident that the reduction of this group of proteins can increase the lysine content when considered as a percentage of protein. The glutelin fraction is intermediate between the albumin and prolamins in lysine content and comprise from 30 to 60 percent of the protein in the seed (30).

Increased lysine content of most cereals is related to the reduction in the prolamin fraction. Jimenez (24) reports that normal corn has twice as much protein in the alcohol soluble zein as the mutant opaque-2 and floury-2 lines and is almost three times as high as the double mutant.

He also reports that increases of 12 to 24 percent in the glutelins occur in the opaque-2 and floury-2 lines as opposed to the normal corn. The albumin fraction was larger by four times in the mutants than in the normal lines. However this fraction represents only a small percentage of the total protein of the seed, and does not

significantly increase the lysine content of the kernel. Similar differences were also observed in the globulins.

It is clear from the work of Jimenez (24) that most of the lysine present in all corn lines is contributed by the glutelins. The glutelin fraction is present in greater concentrations in the mutant and has a greater concentration of lysine when compared to the normal. The data showed that the main effect of the opaque-2 gene is to reduce the amount of zein synthesized and to increase the synthesis of the other three fractions. However, most of the effect, on a kernel basis, was due to increases in the glutelin fraction.

The zein content of the opaque-2 and floury-2 lines differ, suggesting that the high lysine content of the floury-2 line might have a different basis than the opaque-2 gene. The double mutant showed further reduction in the low lysine-zein fraction and continued increase in the other fractions giving higher lysine values (2, 24, 42, 53). Wolf, Khoo, and Seckinger (53), observed that the protein bodies of the opaque-2 endosperm were about one-twentieth the diameter of those found in the normal maize. The floury-2 endosperm seems to be devoid of any protein bodies.

In wheat and barley similar data are available (30, 37, 38, 40, 41). Munck (37) reported that preliminary studies with normal barley varieties showed that the

high protein lines were lower in the water soluble fractions and higher in the alcohol soluble proteins than low protein lines. Although Hiproly was high in protein content, it acted more like the low protein lines in this respect. The lysine content of the crude water soluble fraction was also found to be slightly higher in Hiproly than in the normal varieties at the same protein level. Very few changes were observed in the other protein fractions.

Munck, Karlsson, and Hagberg (40), have reported the amount of protein in the four soluble protein fractions to be: 13.6 percent in the water soluble fraction for Hiproly compared to 11.3 percent for Foma barley, 10.8 percent versus 11.1 percent in the K_2SO_4 fraction, 18.7 percent versus 21.2 percent in the alcohol fraction, and 40.9 percent versus 40.3 percent in the alkali fraction. The corresponding lysine values were 6.2 to 5.1, 6.3 to 6.7, 0.4 to 0.7, and 2.8 to 3.2 percent for Hiproly and Foma respectively.

Hiproly barley endosperm shows several distinctive characteristics (37, 38, 41). Hiproly is almost devoid of small starch granules and the large ones often are crumbled and misshapen. Most striking is the binding of the starch grains to the matrix protein. Munck (37) also reports a definite starch free subaleurone layer in the cross sections of the Hiproly seed.

The high lysine genes in corn, wheat, and barley seem to be relatively stable in different environments (2, 18, 20, 25, 37). However, protein content is greatly influenced by environmental fluctuations and nitrogen fertilizer (18, 25, 27, 34, 46, 51). Since nitrogen uptake and translocation from the foliage to the grain appears to be separate physiologic processes in wheat (25), and varieties differ for each, the levels of protein in the grain could be increased in a program designed to combine these two factors under high fertility conditions.

Inheritance

High lysine was found to be controlled by two recessive genes (opaque-2 and floury-2) in corn. With the identification of the Hiproly cultivar, it became essential to determine the genetics of high lysine in barley. Munck, Karlsson, Hagberg, and Eggum have shown in numerous studies that the high lysine character in barley is controlled by a single recessive gene (20, 37, 38, 40, 41).

Munck *et al.* (41) reported that the F_1 progeny from a Hiproly cross was lower in lysine than the Hiproly parent, and that the F_2 segregated in a 1:3.2 ratio. In further studies Munck (37) reported that in F_2 popula-

tions from four crosses, two crosses fit a 1:3 ratio for the Hiproly dye-binding capacity (D.B.C.) while two crosses differ significantly from the 1:3 ratio. F_1 seeds were low in dye-binding capacity (D.B.C.). F_4 material, which had been selected from F_2 families high in lysine, showed a significant association with two different levels of D.B.C. This was verified by amino acid analysis in which the high level of 4.4 grams of lysine differed from the medium level of 4.1 grams as compared to the normal level of 3.6 grams per 16 grams of nitrogen. All lysine levels were negatively associated with crude protein levels. Munck (37) hypothesized that additional genetic factors could interact with the recessive major gene influencing lysine synthesis in the seed. He reported the likelihood of detecting several minor genes for protein regulation in barley is strengthened by the reports of Vieuf and Doll in 1970 (37). Their material showed two intermediate classes between Hiproly and normal lines.

It seems possible to breed for high protein, high lysine lines in barley; however, there still seems to be the negative association between protein and lysine and protein and seed size in the Hiproly material (18, 20, 40, 41).

The Hiproly genotype seems to be associated with shriveled seed, flinty seed, small seed size, and bound

starch grain matrix protein complex as well as low yield (20, 37, 38, 40, 41).

Hagberg, Karlsson, and Munck (20) have shown in growth chamber studies that selection in the F_2 for grain yield seems to be effective. A large variation was found and it was possible to select high yielding families containing high lysine lines (20, 37). It was also possible to select individuals with high lysine and large plump seeds of both covered and naked types. They suggested that the shrunken seed character is inherited independently of lysine, as the frequency of the shrunken seed is just as high in the groups of normal protein lines as in the high lysine group. It thus seems possible to break the association between the favorable and unfavorable characters in the Hiproly type (19, 20, 38, 41).

Toft viuf (48) reports a positive correlation between the straw length and the protein content. Other workers (47) have found no association between dwarfing and lysine or protein content in barley.

Day and Dickson (10) reported an association between nitrogen percentage and the 2-row, 6-row character in linkage group I, which is on chromosome 2 (44). Manghers and Favret (32) reported a statistically significant negative correlation between nitrogen content per unit dry matter and average dry weight of seed. In

their cross, they hypothesized that seed weight was regulated by a single gene pair also located on chromosome 2.

Favret et al. (18) analyzed 20 F_2 plants for protein content, representing equivalent samples of higher and lower grain weights and of 2- and 6-row spikes and found a significant linear correlation between nitrogen content per seed and grain weight. They concluded that the same gene controls both characters, mainly grain weight and the capacity to synthesize protein.

Day, Down, and Frey (11), reported diastatic activity was associated with rough versus smooth awns in linkage group V, which is now listed as chromosome 7 (44), and was slightly associated with 2- to 6-row character in linkage group I, which is on chromosome 2 (44).

Nielsen and Frydenberg (43) report the linkage of alpha amylase and Orange Lemma on chromosome 6 to be .17 $\pm$ .03 for two crosses.

In Hiproly the adhesion of the matrix protein to the starch was closely linked with the high lysine character (37, 38, 40, 41). Either close linkage or pleotropism would account for the absolute association reported.

The discovery of high lysine corn stimulated much interest in improving protein quality and quantity

in the cereal grains. More recently with the discovery of the high lysine gene in Hiproly, new emphasis has been given to improve the protein quality and quantity in barley.

MATERIALS AND METHODS

Four agronomically and genetically diverse spring barleys were used in a crossing program to study the inheritance of protein and lysine in barley, and to determine their associations with certain morphological and agronomic characters. They were selected for genetic diversity in the morphological characters 2- versus 6-row, rough versus smooth awn, lax versus compact head, and naked versus covered seeds. The parents were Hiproly C.I. 3947, Orange Lemma (Experimental line), Wocus C.I. 8059, and OR 61-2141-9 an Oregon selection from a Hannchen/C.I. 3208-4 cross. Table 2 shows the characterization of the parental lines along with the mean values for plant height, yield components, and yield.

Hiproly is a mutant discovered in Sweden which has both high protein and high lysine content. It is, however, deficient in several agronomic traits including flinty, shriveled seed, small spikes, and uneven tillering pattern.

Orange Lemma, as the name implies, carries a marker for lemma color plus a factor for high alpha amylase activity. Both characters are associated with chromosome 6.

Wocus, is commercially grown as a 6-row feed barley in the Klamath Basin of Oregon. It has a compact

head, smooth awns, and has many good agronomic characteristics.

The cultivar OR 61-2141-9 was selected from the Oregon breeding program as a potentially high yielding, 2-row malting barley. It has good agronomic characteristics and yielding ability.

Crosses were made between these varieties and Hiproly in the greenhouse during the spring of 1969. Because of its extremely fragile spike, Hiproly was always used as the male parent. F_1 seed was planted in the greenhouse in the fall of 1969 and F_2 seed was harvested in December. The F_2 seed were bulked and F_2 plants and parents were grown in the greenhouse groundbeds during the spring and early summer of 1970. Entries, were grown in randomly placed 10-foot rows. Measurements for plant height were taken on the primary tiller. Classifications for the agronomic characters of 2-row, 6-row; rough or smooth awn; lax or compact head; orange or white lemma; and naked or covered seed were made on a single plant basis. Each head was numbered in relation to the primary culm. The seed was threshed by hand to minimize kernel damage and possible alteration of the protein content and quality. Number of seeds per head, weight per kernel, and plant yield were recorded. The crosses were then classified for the endosperm character under the microscope as described by Munck et al. (41). Those

F_3 seeds that were classified as having high lysine by this method were then tested for protein and lysine in the laboratory by chemical determinations.

Lowry protein determination was used to determine soluble protein (31). This procedure was used because the size of seed sample was inadequate to allow for Micro-Kjeldahl determinations on a single head basis along with lysine determinations. The Lowry method was tested on 48 lines of barley and compared to the Micro-Kjeldahl nitrogen determination. There was a significant correlation between the two tests and a paired T test indicated no significant difference existed between the two methods (Appendix page 76). It must be borne in mind, however, that the Lowry method is a determination of soluble protein only. This represents an average reduction of 2.69 milligrams of protein per 100 milligrams of ground seed as compared to the Micro-Kjeldahl (total nitrogen x 6.25) determination on 48 paired samples of barley. This will therefore inflate the lysine as a percent value of protein. However if this factor is taken into account the results of this study come very close to the determinations found by Munck et al. (41) as well as the determinations reported in the Biochemists' Handbook, edited by C. Long (30).

Lysine determinations were obtained by two different methods. The first was a colorimetric method

adapted from the corn-lysine work of CIMMYT reported by Villegas and Mertz (52). This method utilizes a papain enzymatic hydrolysis with the use of 2-chloro, 3, 5-dinitropyridine as a dye specific for lysine. The procedure seems to be very quantitative where protein is already in solution or a uniform particle size can be obtained. However, due to the hard flinty kernel resulting from those crosses involving Hiproly, it was not possible to obtain a uniform particle size with the methods of grinding that were available. Therefore on the parental material and the segregating populations involving Hiproly, this procedure did not prove adequate. However it was used on soluble protein portions for determining the amount of lysine in the modified Mendal-Osborn fractions of the protein. One modification was made in the Villegas and Mertz (52) procedure. The material was incubated at 32 C for 12 hours instead of the suggested 65 C. This was done because on a gluten standard the regression line obtained by the modified method was more closely correlated and parallel to the standard of free known amino acid lysine (Appendix page 75). This is most probably due to the fact that at 65 C and pH of 7.4 breakdown of the enzyme occurs in approximately two to three hours, while at 32 C more complete hydrolysis takes place.

The second method and the one used in this study

was an adaptation of the Udy dye-binding capacity method of determining basic amino acids (36). It was possible with the dye-binding capacity method to run the sample through a Mini-Mill with the dye solution, for four minutes, resulting in a uniform particle size among samples and allowing near complete dye binding with the basic amino acids. The correlation of this test to a known gluten protein was very high (r = .999). The test was repeatable within plus or minus 1 percent absorption on the Udy colorimeter scale. An outline of the procedure is found in the Appendix (page 70).

The dye concentration is critical in such an experiment. Concentrated dye contains 1.3 milligrams of dye particles per milliliter and large sample sizes are necessary to precipitate large enough quantities of dye to get separation on the colorimeter scale. In order to obtain a satisfactory separation of different levels of basic amino acids with reasonable degree of accuracy, it is necessary to determine the most desirable concentration of dye. Through dilution experiments it was found that a concentration of one part concentrated Udy dye plus two parts of distilled water contained the best concentration of dye particles for separating different basic amino acid compositions in a 100 milligram sample of barley. At this concentration even the lowest amount of dye precipitation could be read with accuracy on the colori-

meter scale. Greater concentrations of dye tended to decrease the sensitivity of the test requiring greater sample size to be used for similar accuracy. It was also necessary to correlate this test back to basic amino acids since in the standard gluten protein 22 percent of the basic amino acids is lysine whereas in normal barley lysine comprises approximately 32 percent of the basic amino acids (30). Therefore the calculations were made on total basic amino acids and lysine determined as a constant or 32.38 percent of the total basic amino acids. The calculations of the lysine and protein from the Udy, papain, and Lowry methods are found in the Appendix (page 77) along with the regression equations of the known standards for protein and lysine (page 76).

Sectioning and protein fractionation studies were conducted to determine how the 'hily' gene altered the protein structure and fractions of the seed. Both hand and freezing microtome sections were used with Udy dye as the differentiating agent. The protein fractionation studies were obtained by a modified Mendal-Osborn fractionation of the proteins into the water and salt, alcohol, and sodium hydroxide soluble fraction. For convenience the water and salt soluble fractions were combined since the water soluble fraction is only about 4 to 7 percent of the total protein in the seed (27) and adequate measurements could not be obtained of the very

small amount of lysine present. However, this fraction is known to have the best balance of lysine of the four protein fractions (30). The procedures followed for the protein fractionation study are outlined in the Appendix (page 73).

The data were tested by the use of simple linear correlation and regression analysis using a stepwise multiple regression correlation in order to determine the association between protein and lysine content. A similar analysis was used for the yield components along with the other morphological characters recorded. Linkage between the endosperm character of bound starch matrix protein and increased lysine content in the kernel was determined from those kernels classified for high lysine by the endosperm marker method (41). If two genes are involved, one controlling the endosperm character, and one controlling high lysine content, the class containing the homozygous recessive gene for the Hiproly endosperm character would contain half of the recombinants. By use of equation number seven from Allard (3) the proper equation can be derived for the coupling phase and the total recombination value calculated. Chi-square goodness of fit tests were used to test the segregation patterns as a guide for determining inheritance.

EXPERIMENTAL RESULTS AND DISCUSSION

The parents Hiproly, Orange Lemma, OR 61-2141-9, and Wocus were selected for their genetic diversity in the morphological characters 2- versus 6-row, rough versus smooth awn, lax versus compact head, and naked versus covered seeds, as well as for differences in yield and yield components. The characterization of the parental lines along with the mean values for plant height, yield components, and grain yield is given in Table 2. There was a significant difference between cultivars at the .01 level of significance for all traits.

In Table 3 the mean values for the traits plant height, culm number, number of seeds per culm, seed weight in grams, mg. protein/100 mg. of grain, lysine as a percent of protein, and grain yield is given on a sample of ten plants from the total population reported in Table 2. All values except number of culms and mg. protein/100 mg. of grain were significantly different at the .01 level of significance. Soluble protein content (mg./100 mg.) was significantly different between cultivars at the .05 level of significance while number of culms for the cultivars did not differ significantly. This was due to the fact that no plants with more than six heads were used thereby biassing the data for this trait.

Table 2. The morphological characters of the parental barley cultivars and the average values for plant height, culm number, seed weight, seed number/culm, and yield. a/

Cultivars	Row No.	Awn Type	Head Type	Seed Type	Lemma Color	Plant Height (cm)	No. Culms/ Plant	Seed Weight (gm)	No. Seeds/ Culm	Yield/ Plant (gm)
Hiproly (C.I. 3947)	2	rough	lax	naked	white	100.5	1.8	.0441	15.6	1.014
Orange Lemma	6	rough	lax	covered	orange	124.7	1.6	.0326	50.2	2.236
OR 61-2141-9	2	rough	lax	covered	white	100.9	3.3	.0544	19.9	1.227
Wocus (C.I. 8059)	6	smooth	compact	covered	white	116.6	1.3	.0592	27.7	1.920
						**	**	**	**	**

a/ F test of significant differences with 3 and 221 degrees of freedom, cultivars were significant at the .01 level of significance.

Table 3. Mean values of seven characters for four parental barley cultivars. a/

Cultivars	Plant Height (cm)	No. Culms/ Plant	No. Seeds/ Culm	Seed Weight (gm)	Soluble Protein mg/100 mg	Lysine % Protein b/	Yield/ Plant (gm)
Hiproly	94.8	2.1	14.4	.0453	8.824	6.98	1.153
Orange Lemma	131.1	2.2	57.0	.0331	8.293	5.26	3.539
OR 61-2141-9	102.7	2.7	20.0	.0531	8.269	4.91	2.237
Wocus	113.8	1.8	25.8	.0558	9.260	5.44	2.145
	**	NS	**	**	*	**	**

a/ F test of significant differences with 3 and 36 degrees of freedom, ** = .01 level of significance, * = .05 level of significance, NS = no significant difference.

b/ Lysine determined by Udy dye-binding method.

The lysine percent protein is based on soluble protein and shows Hiproly to be significantly higher than the other lines by 22.1 to 29.6 percent.

The average protein and lysine content of bulk samples of the parental lines on a Micro-Kjeldahl protein basis are comparable to data reported by several other workers (30, 40),(Table 4).

The soluble proteins were fractionated by a modified Mendal-Osborn procedure into the water plus salt soluble fraction (albumins plus globulins), the 70 percent ethanol soluble fraction (prolamins) and the .1 M NaOH fraction (glutelins),(Tables 5, 6, 7). It is evident that there was no difference in the amount of protein in the albumin and globulin fractions of the four cultivars (Table 5). There was no difference in the amount of protein in the alcohol soluble fraction of Hiproly and Wocus; however the high enzyme barleys, Orange Lemma and OR 61-2141-9 were markedly lower in total protein in this fraction (Table 5). This would be expected in OR 61-2141-9 since in malting lines vigorous selection is practiced for low total protein. They are especially low in the hordein fraction as it is this fraction that has the greatest effect both on total starch content of the kernel and beer quality from the standpoint of turbidity in the beer (29).

The Hiproly line had a greater amount of protein

Table 4. Micro-kjeldahl protein (% N x 6.25) and lysine as a percent of protein from bulked samples of four parental cultivars.

Cultivars	Total Protein mg/100 mg	Lysine % of Total Protein a/
Hiproly	11.40	3.86
Orange Lemma	9.70	2.00
OR 61-2141-9	9.50	3.20
Wocus	10.50	2.89

a/ lysine determined by papain hydrolysis and 2-chloro, 3, 5-dinitropyridine.

in its glutelin fraction when compared with the other parental lines. Unfractionated samples of grain showed no difference between cultivars for protein content. It is difficult to explain why the bulk samples were so low in protein compared to the protein fractions.

The total lysine content on a grain weight basis is given in Table 6 while lysine as a percent of protein in the soluble protein fraction is shown in Table 7. It is interesting to note that the Orange Lemma parent was quite high in lysine in the H_2O + salt soluble fraction compared with the other lines. Hiproly had an intermediate amount of lysine in this fraction. The increased lysine in the alcohol soluble fraction in the Hiproly

Table 5. Milligrams protein/100 milligrams grain, and relative percentage of protein distributed in the soluble protein fractions of four barley cultivars. a/

Cultivars	H_2O + Salt		Alcohol		NaOH		Whole Grain b/
	mg/100 mg	% Total Protein	mg/100 mg	% Total Protein	mg/100 mg	% Total Protein	mg/100 mg
Hiproly							
Kernel	2.40	21.12	2.19	19.26	6.78	59.63	7.49
Endosperm c/	1.71	14.91	2.35	20.49	7.41	64.60	8.06
Orange Lemma							
Kernel	2.14	22.18	1.23	12.75	6.28	65.08	8.83
Endosperm	1.72	18.78	1.07	11.68	6.37	69.54	8.32
OR 61-2141-9							
Kernel	2.19	23.13	1.36	14.36	5.92	62.51	6.36
Endosperm	1.78	19.41	1.21	13.20	6.18	67.39	7.89
Wocus							
Kernel	2.41	22.89	2.15	20.42	5.97	56.70	7.55
Endosperm	1.48	14.65	2.31	22.87	6.31	62.48	8.40

a/ Each value represents the mean of duplicate samples from two fractionations.

b/ Value from unfractionated ground grain or endosperm

c/ Composed of all seed parts except embryo

Table 6. Milligrams lysine/100 milligrams grain in the soluble protein fractions of four barley cultivars. a/

Cultivars	H_2O + Salt	Alcohol	NaOH	Whole Grain b/
Hiproly				
Kernel	.1454(2) c/	.0134(1)	.2424(1)	.2870(1)
Endosperm d/	.0877(4)	.0110(1)	.2290(2)	.3092(1)
Orange Lemma				
Kernel	.1795(1)	.0092(4)	.1696(4)	.1451(4)
Endosperm	.1838(1)	.0069(2)	.2028(3)	.1710(4)
OR 61-2141-9				
Kernel	.1276(3)	.0096(3)	.2217(2)	.2013(3)
Endosperm	.1060(3)	.0056(4)	.2613(1)	.1991(3)
Wocus				
Kernel	.1174(4)	.0111(2)	.1843(3)	.2194(2)
Endosperm	.1120(2)	.0062(3)	.1992(4)	.2229(2)

a/ Each value represents the mean of duplicate samples from two fractionations.

b/ Value from unfractionated ground grain or endosperm

c/ Rank

d/ Composed of all seed parts except embryo

Table 7. Milligrams lysine/100 milligrams protein in the soluble protein fractions of four barley cultivars.

Cultivars	H_2O+Salt	Alcohol	NaOH	Whole Grain [a]
Hiproly				
Kernel	6.06(2)[b]	.61(3)	3.58(2)	3.83(1)
Endosperm[c]	5.13(4)	.47(2)	3.09(4)	3.84(1)
Orange Lemma				
Kernel	8.39(1)	.75(1)	2.70(4)	1.64(4)
Endosperm	10.69(1)	.64(1)	3.18(2)	2.06(4)
OR 61-2141-9				
Kernel	5.83(3)	.71(2)	3.74(1)	3.17(2)
Endosperm	5.96(3)	.46(3)	4.23(1)	2.52(3)
Wocus				
Kernel	4.87(4)	.52(4)	3.08(3)	2.91(3)
Endosperm	7.57(2)	.27(4)	3.16(3)	2.65(2)

[a] Value from unfractionated ground grain or endosperm

[b] Rank

[c] Composed of all seed parts except embryo

line was primarily due to the increase in protein content in this line. The lower levels of lysine in the alcohol soluble fractions of the high enzyme barleys was due to lower level of proteins in this fraction giving a better balance of lysine in relation to the remaining proteins.

On a kernel basis the Hiproly genotype gave a greater total production of lysine per milligram of grain in the sodium hydroxide fraction compared with the other lines. When this is coupled with the higher protein content of this fraction, it is apparent that the NaOH fraction is the fraction primarily responsible for the increased lysine content of this cultivar.

It is also of interest to observe that the NaOH fraction of the endosperm of Orange Lemma and OR 61-2141-9 were higher in lysine content per milligram of endosperm as compared to the whole kernel. The OR 61-2141-9 line was especially high in this regard.

Computed biological values (C.V.B.) of the parental lines as a weighted estimate of the potential biological value of the parental lines for food or feed is given in Table 8 (24). When Hiproly was used as a base it was 23.4 to 48.0 percent better than the other parental lines.

It appears that several different approaches for increasing lysine content in the seed may be available: (1) reduced alcohol soluble protein (hordein) which is

Table 8. Biological value (BV) of protein fractions of four barley cultivars (milligrams protein/100 milligrams x percent lysine of protein).

Cultivars	H_2O + Salt	Alcohol	NaOH	Whole Grain a/
Hiproly				
Kernel	14.54(2) b/	1.34(1)	24.27(1)	28.69(1)
Endosperm c/	8.77(4)	1.10(1)	22.90(2)	30.95(1)
Orange Lemma				
Kernel	17.95(1)	.92(4)	16.96(4)	14.92(4)
Endosperm	18.39(1)	.68(2)	20.25(3)	17.14(4)
OR 61-2141-9				
Kernel	12.77(3)	.97(3)	22.14(2)	20.16(3)
Endosperm	10.60(3)	.55(4)	26.14(1)	19.88(3)
Wocus				
Kernel	11.74(4)	1.12(2)	18.39(3)	21.97(2)
Endosperm	11.20(2)	.62(3)	19.94(4)	22.26(2)

a/ Soluble protein (mg/100 mg) x percent lysine of soluble protein of unfractionated ground grain

b/ Rank

c/ Composed of all seed parts except embryo

low in lysine; (2) increased lysine content in water soluble proteins (albumins and globulins) by increasing embryo size or chemical composition; (3) increased lysine content in sodium hydroxide (glutelin) fraction in the embryo and scutellum and; (4) increased lysine content in sodium hydroxide fraction in the endosperm.

It is evident from these data that Hiproly has a higher lysine content in its protein than the other tested cultivars. It also has a slightly higher protein content. Crosses involving the Hiproly cultivar might be expected to increase protein and lysine content as well as grain yield. It is possible that lysine content might even be elevated over the Hiproly line since this genotype does not include all four of the above factors for increasing lysine in the kernel.

When the F_3 progeny are compared to the parental cultivars several interesting things become apparent. As is indicated in Table 9 the Hiproly genotype does not interfere with the transmission of the yield components from the three parental cultivars used in this study. Plant height, number of culms, number of seeds per culm, and seed weight all were significant among crosses at the .01 percent level of significance. However grain yield did not differ significantly. The Orange Lemma/ Hiproly cross had obtained the high seed number of the Orange Lemma parent to give it high yield. The

Table 9. Mean values of seven characters segregating in F_3 progenies representing three Hiproly crosses. a/

Crosses	Plant Height (cm)	No. Culms/ Plant	No. Seeds/ Culm	Seed Weight (gm)	Yield (gm)	Soluble Protein b/ mg/100 mg	% Lysine of protein b/
Orange Lemma/ Hiproly	117.97	1.68	33.74	.0439	2.0722	7.8989	6.5345
OR 61-2141-9/ Hiproly	106.35	2.00	20.39	.0555	1.9911	7.9118	6.0886
Wocus/ Hiproly	117.98	1.49	25.26	.0561	1.8300	8.2850	6.2880
	**	**	**	**	NS	NS	NS

a/ F test of significant differences with 2 and 600 degrees of freedom for first five characters and 2 and 150 degrees of freedom for protein and lysine, ** = .01 level of significance, NS = no significant difference.

b/ Only selected lines with the 'hily' gene for endosperm character were tested.

OR 61-2141-9/Hiproly cross had both the number of culms and seed size characters from the OR 61-2141-9 parent and the Wocus/Hiproly cross had obtained seed size and seed number from the Wocus parent. It is apparent that each parent has successfully passed its primary yield component into the F_3 progeny (Tables 2, 9).

The F_3 lines tested for protein and lysine content were those that were classified as Hiproly endosperm types by the method reported by Munck et al. (41). It is therefore expected that there should be no significant difference in these properties in the selected F_3 progeny.

The microscopic technique reported by Munck et al. (41) was used to follow high lysine in the F_1, F_2, and F_3 progenies. This character is quite distinctive. The Hiproly endosperm character is relatively free of symmetrical starch grains while those starch grains present are mostly uniform in size, misshapen, and associated with the matrix protein (Figure 1). For convenience this character will be referred to as the Hiproly endosperm character in this thesis. The normal parents show an abundance of free multisized starch grains with very little binding to the matrix protein (Figure 2).

The results of the segregation of the Hiproly endosperm character in the F_2 and F_3 progenies confirm

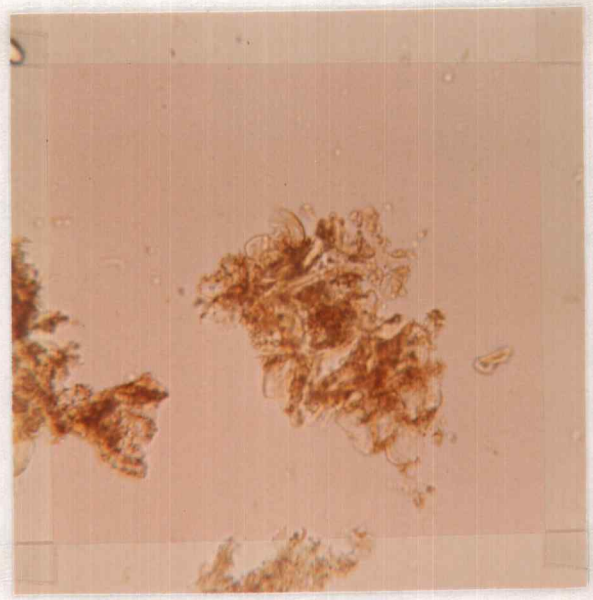

Figure 1. Endosperm preparation of Hiproly stained with Udy Orange G dye showing bound starch, matrix protein complex. Starch grains are large and misshapen.

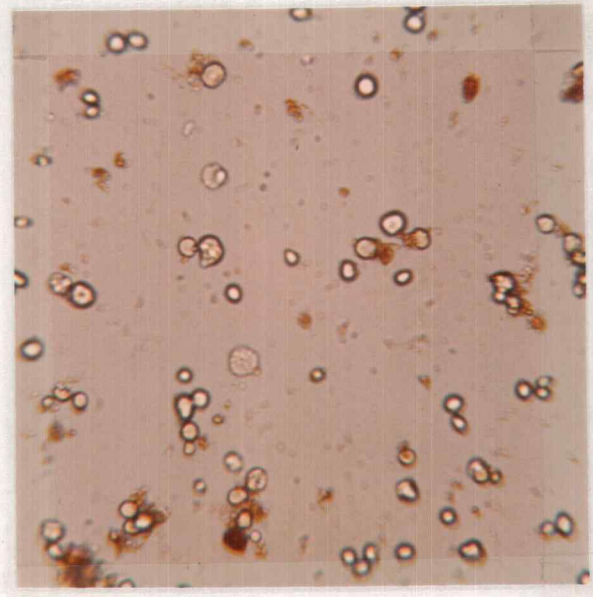

Figure 2. Endosperm preparation of Orange Lemma stained with Udy Orange G dye showing abundant free starch grains.

the reports of a single gene reported by Munck et al. (41). Segregation of the Hiproly endosperm character fit a 3:1 ratio in the F_2 and a 1:2:1 ratio in the F_3 of the Orange Lemma/Hiproly cross (Table 10). Similar results are seen for the crosses OR 61-2141-9/Hiproly and Wocus/Hiproly (Tables 11, 12).

Attempts were made to separate the endosperm character into four different classes representing a dosage effect in the 3n endosperm. The Hiproly type from the F_3 of the Orange Lemma/Hiproly cross with a homozygous recessive genotype is shown in Figure 3.

Typical endosperm type of the Orange Lemma parent with the suspected homozygous dominant genotype AAA, is shown in Figure 4. Figure 5 represents the class considered to be of the heterozygous genotype AAa. In this class there are abundant free starch grains as found in the normal parent along with the bound starch matrix protein complex characteristic of the Hiproly endosperm. The situation interpreted as being due to two doses of the Hiproly gene (Aaa) in the endosperm is shown in Figure 6. It is characterized by being almost a Hiproly type, however, there are considerably more free round starch grains than would be present in Hiproly. When F_2 progeny were classified into these classes they fit the appropriate 1:1:1:1 ratio. It was not felt that it was always possible to separate the Orange Lemma/Hiproly

Table 10. Segregation of hiproly endosperm character in F_2 and F_3 progenies of the Orange Lemma/ Hiproly cross.

F_2

Normal	High
33	11
3:1	P =>.995

F_3

Normal	Seg	High
45	96	47
1:2:1	P = .950 - .900	

Table 11. Segregation of hiproly endosperm character in F_2 and F_3 progenies of the OR 61-2141-9/ Hiproly cross.

F_2

Normal	High
31	9
3:1	P = .750 - .500

F_3

Normal	Seg	High
54	108	56
1:2:1	P = .975 - .950	

Table 12. Wocus/Hiproly segregation of endosperm character.

F_2			
Normal			High
66			22
3:1		P =>.995	
F_3			
Normal	Seg		High
49	104		50
1:2:1	P = .950 - .900		

cross into these four classes with accuracy for all protein levels. However it could be separated into three classes fitting a 2:1:1 ratio (Table 13).

The results of the chemical tests and endosperm characterization for the F_1 seeds and F_2 populations is given in Table 13. Differences in the protein and lysine found among crosses in the F_1, were not in agreement with those found among the parents. The cross OR 61-2141-9/Hiproly had the highest protein content and the cross Wocus/Hiproly had the lowest. If comparisons are made among parents, the Wocus/Hiproly cross might be expected to have the highest protein and lysine content and the OR 61-2141-9/Hiproly cross to have the lowest

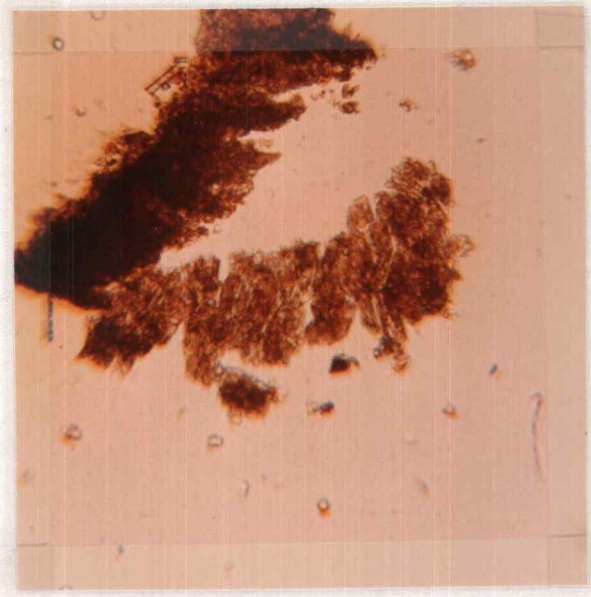

Figure 3. Endosperm preparation, stained with Udy Orange G dye, of Hiproly F_2 progeny of the cross Orange Lemma/Hiproly with genotype classified as aaa.

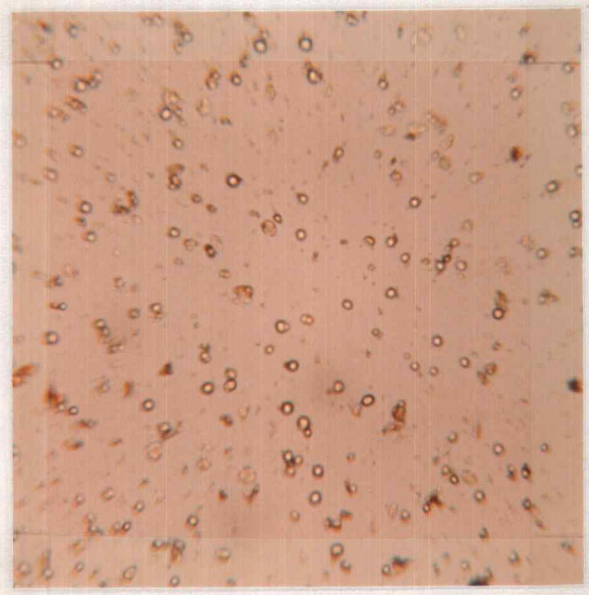

Figure 4. Endosperm preparation, stained with Udy Orange G dye, of low lysine segregates F_2 progeny of the cross Orange Lemma/Hiproly with genotype classified as AAA.

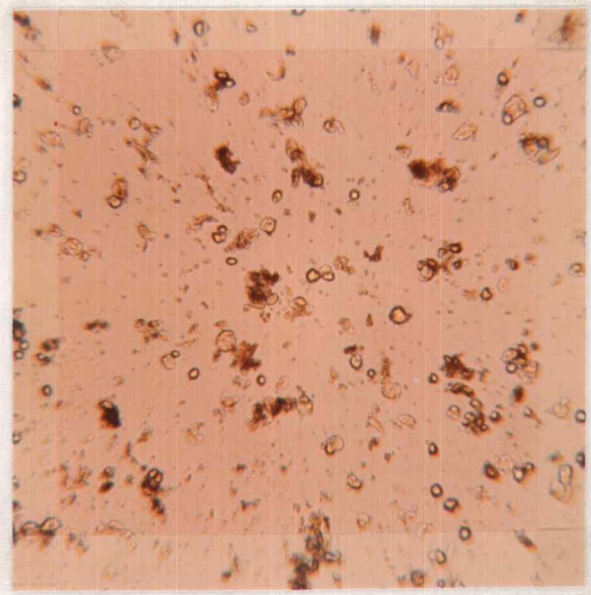

Figure 5. Endosperm preparation, stained with Udy Orange G dye, of segregating F_2 progeny for Hiproly gene of the cross Orange Lemma/Hiproly with genotype classified as AAa.

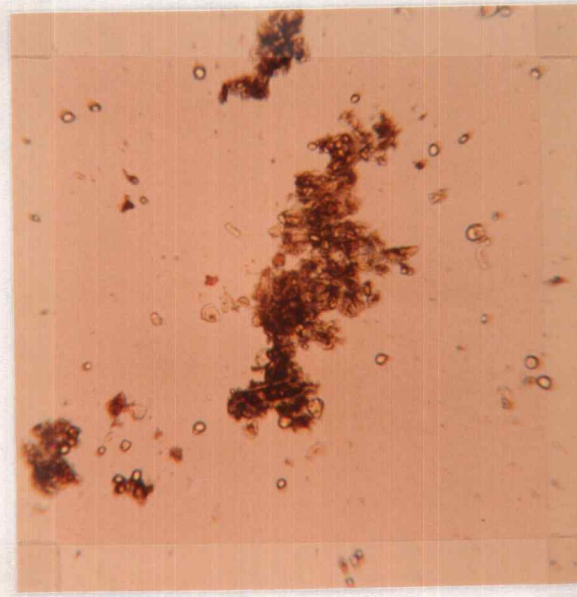

Figure 6. Endosperm preparation, stained with Udy Orange G dye, of segregating F_2 progeny for Hiproly gene in the cross Orange Lemma/ Hiproly genotype classified as Aaa.

Table 13. Endosperm character, protein content, and lysine percent protein for F_1 and F_2 progeny of three barley crosses.

	Endosperm Genotype	Soluble Protein mg/100 mg	Lysine % Protein
Parents			
Hiproly	aaa	8.82	6.98
Orange Lemma	AAA	8.29	5.26
OR 61-2141-9	AAA	8.27	4.91
Wocus	AAA	9.26	5.44
F_1			
Orange Lemma/ Hiproly	AAa	10.19	4.94
OR 61-2141-9/ Hiproly	AAa	11.34	5.16
Wocus/Hiproly	AAa	9.40	4.30
F_2			
Orange Lemma/ Hiproly	AAA	8.43	4.90
	AAa Aaa	8.00	5.37
	aaa	8.19	6.25
OR 61-2141-9/ Hiproly	AAA	10.01	4.70
	AAa	9.52	5.02
	Aaa	9.16	5.32
	aaa	8.25	6.20
Wocus/ Hiproly	AAA	9.04	5.30
	AAa	9.04	5.11
	Aaa	8.86	5.31
	aaa	9.10	5.53

protein content while maintaining a reasonable lysine content. The Orange Lemma/Hiproly cross was intermediate in the F_1, both in protein and lysine. The F_2 data for each cross, are similar to the F_1 data, except that the Orange Lemma and OR 61-2141-9 crosses have changed their relative positions. This may be due primarily to the protein levels since the Orange Lemma/Hiproly F_2 has a lower protein content than expected based on the parental and F_1 data. Also seen from Table 13 is the dosage effect of the 'hily' endosperm character on lysine expressed as a percent of soluble protein in the F_2. It is difficult to test this type of data for significant differences; however, there is a definite trend of increased lysine with each dose of the Hiproly gene, (a), in the endosperm with a relatively large jump when the homozygous condition is reached. This would indicate an additive effect of the gene and not recessiveness. However the F_1 data seem to indicate recessiveness since the lysine content of the F_1 is as low or lower than the low parent. Bates (4) reported a dosage effect for lysine in the endosperm of the opaque-2 and the floury-2 corn lines. Three doses of the wild (+++) gave 1.7 grams of lysine per 100 grams of endosperm protein. One dose of the opaque-2 gene increased this very slightly to 1.8 grams, a nonsignificant difference. Two doses however increased it to 2.3

grams, and three doses to 3.6 grams. The floury-2 gene acted similarly with the content of 1.8, 2.3, 3.0, and 3.6 for 0, 1, 2, and 3 doses respectfully. These data seem to indicate the same types of increases found by Bates (4) in the opaque-2 genotype. Munck et al. (41), however, indicate that in the F_1 where Hiproly was the female giving two doses of the gene, the lysine level was not increased over the parent and he labeled the gene as being recessive.

In the F_3, those lines carrying the Hiproly endosperm type, were selected for chemical analysis to determine the influence of the endosperm character on lysine content, that is, to determine if the gene for the endosperm character also increased lysine content. If the gene for these factors was pleotropic no low lysine lines should be found in the Hiproly endosperm group. If two independent genes were involved, three-quarters of the Hiproly endosperm lines should be low in lysine. Apparently two linked genes were involved because two classes, Hiproly endosperm-high lysine and Hiproly endosperm-low lysine, were recovered among the F_3 lines, but not in a ratio of 3:1. This suggests linkage.

Because the Hiproly endosperm character and high lysine were in the coupling phase in all three crosses, the equation:

$$c\ \frac{2(1-P)}{P(2-P)} + d\ \frac{-2}{1-P} = 0$$

was used to figure the recombination value (3). In the equation, c equals the number of Hiproly endosperm lines low in lysine, d equals the number of Hiproly endosperm lines high in lysine, and P equals the percent recombination. The linkage data are summarized in Tables 14 and 15. The percent recombination, 22.45 percent suggests that the association between the gene for the Hiproly endosperm character and the gene governing high lysine can be broken easily. The lower limit of the high lysine lines was determined by use of a 99 percent confidence interval for the mean of each set of Hiproly F_3 lines thereby avoiding biassing of the limits for any one cross. The cutoff points fit a natural break in the data of all three crosses. All lines with the exception of three that were low in lysine were also low in protein. This indicates that the low lysine value was not due to protein content alone.

Simple correlation coefficients and X^2 test of goodness of fit were used to determine associations of the high lysine gene with morphological and agronomic characters and protein. The Hiproly endosperm character was not associated with 2- or 6-row, smooth or rough awn, orange or white lemma, naked or covered seed, or lax or club head. There were significant negative correlations of the Hiproly endosperm character with seed weight and

Table 14. Summary table of recombination between endosperm character and high lysine on F_3 families by the maximum likelihood method.

p = .2245

ip = 2.5099

Cross	Score	Information	X^2	C	d	N
Orange Lemma/ Hiproly	+ 1.7189	471.8612	.0063	19	28	188
OR 61-2141-9/ Hiproly	+17.3285	547.1582	.5488	25	31	218
Wocus/ Hiproly	-19.0035	459.3117	.7862	15	30	183
	+ .0439	1478.3311	1.3413			

X^2p = .000001

p = .2245 + .00003 = .22453

% recombination = 22.45%

$$c\left[\frac{2(1-P)}{P(2-P)}\right] + d\left[\frac{-2}{1-P}\right] = 0$$

Equation derived from equation #7 Allard (3) for coupling phase 12:3:1 ratio.

If no linkage, c = 3, d = 1, and p = .5.

Table 15. Expected results 12:3:1 ratio if recombination value is 22.45 percent.

	AB 38.775	Ab 11.225	aB 11.225	ab 38.775
AB 38.775	1503.5006	435.2494	435.2494	1503.5006
Ab 11.225	435.2494	126.0006	126.0006	435.2494
aB 11.225	435.2494	126.0006	126.0006[a/]	435.2494[a/]
ab 38.775	1503.5006	435.2494	435.2494[a/]	1503.5006[b/]

a = 996.4994

b = 1503.5006

a+b = 2500.0000

10,000 - 2500 = 7500

Ratio	12	3	1	Total
	7500.00	996.4994	1503.5006	10,000
Expected	441.75	58.6644	88.5856	589
Observed	441.00	59.0000	89.0000	589

X^2 = .0013 + .0019 + .0019 = .0051

p $\geq$ .995 2 df

yield (Table 16), however, they are of no consequence to the plant breeder since they account for only about 4 percent of the population in the case of the seed weight and less than 3 percent of the population in the case of yield.

Protein showed significant negative correlations with plant height and seed weight (Table 17). However, again the percent of the population responsible for the association was quite low and of no significance to the plant breeder. Protein content showed a significant negative correlation with lysine as a percent of protein when the Hiproly endosperm F_3 progenies and parents were combined as well as in the F_3 progenies alone, the r values were, - .4368 and - .4488, respectively (Table 17). This represents only about 20 percent of the population responsible for the negative regression, however, selection for protein or lysine content alone may lead to the decrease of the other. It is therefore essential that selection be carried out for both traits. The association between protein content and yield was not significant.

The lysine content as a percent of protein was significantly associated with yield when tested with the parents and F_3 progenies with the Hiproly endosperm character. This was mostly due to the parents because the correlation was insignificant when the F_3 progenies

Table 16. Simple correlation coefficients of F_3 progenies of three crosses of barley for six agronomic characters. a/

	Plant Height (cm)	No. Seeds/ Head	Seed Weight (gm)	No. Culms/ Plant	Yield/ Plant (gm)
Endosperm Character	-.0115	-.0348	-.2161**	-.0984	-.1680*
Plant Height		+.4083**	+.1954**	+.0812	+.3977**
Seeds/ Head			-.4054**	-.0123	+.4956**
Seed Weight				+.1551**	+.1787**
Culms/ Plant					+.7160**

a/ n = 602, significance of r = .088* at .05 level, .115** at .01 level

Table 17. Simple correlation coefficients of parents and those F_3 progeny with the Hiproly endosperm character for seven factors. a/

	No. Culms/ Plant	Plant Height (cm)	No. Seeds/ Head	Seed Weight (gm)	Protein Content mg/100 mg	Lysine % Protein	Yield/ Plant (gm)
Culms/ Plant		-.0521	-.0539	+.0817	+.1147	-.2217**	+.6687**
Plant Height	-.0237		+.5264**	+.0737	-.2353**	+.0078	+.3785**
Seeds/ Head	-.1254	+.4574**		+.3670**	-.0760	-.0412	+.5162**
Seed Weight	+.1405	+.1687*	-.3102**		-.2017*	-.0875	+.1655*
Protein Content	+.0700	-.2622**	-.0616	-.2395**		-.4368**	-.0871
Lysine % Protein	-.0813	+.0596	+.0625	-.1388	-.4488**		-.2538**
Yield	+.6302**	+.4263**	+.4676**	+.3181**	-.1568	-.0912	

a/ TOP - Parents and F_3 progeny tested for lysine combined n = 195 significance, .05 = .159*, .01 = .208**
BOTTOM - F_3 progeny tested for lysine, n = 155, significance .05 = .159*, .01 = .208**

were tested alone (Table 17).

It is felt that these studies show that it is possible to breed high protein, high lysine barleys without the hindrance of linked agronomic and physiologic characteristics. Satisfactory techniques are available for screening large populations, using a limited seed source, for both protein and lysine content. Techniques are also available to test parental lines for differences in protein and lysine content of the protein groups thereby aiding in the selection of parents for a breeding program aimed at increasing digestible protein and lysine in barley.

CONCLUSIONS

It is evident from these and other studies (19, 20, 37, 38, 40, 41) that the high lysine content of Hiproly barley is simply inherited. Since it was possible to separate the F_2 kernels into four classes based on starch binding and lysine content (Table 14) a close association of the two genes for these two characters is indicated. The F_3 recessive class for the starch binding endosperm character could be classified into two lysine classes, not in the ratio of 3:1. These data can be explained if linkage is assumed with 22.45 percent recombination. It appears that one recessive major factor accounts for most of the variation observed in lysine for these crosses. High lysine is also negatively associated with protein content in the F_3 but the association is not so strong as to cause problems in developing high lysine, high protein cultivars. The association of the endosperm character with seed weight is significant; however, lysine content is not significantly associated with seed size. High lysine is not associated with 2- 6-row characters, rough or smooth awn, orange or white lemma, naked or covered seed, number of seeds per head, tillers, plant height or yield. It therefore seems possible to transfer the high lysine gene from Hiproly into high yielding high protein lines

without transmitting the undesirable agronomic characteristics of the Hiproly parent.

The high lysine gene does influence other interesting properties which may influence its successful use in improving barley proteins. Changes in structure of the kernel endosperm along with its flinty character may alter other qualities and digestibility factors not associated with total protein or lysine content. As reported by Munck (37) the Hiproly grain has a definite starch free layer adjacent to the aleurone compared with a normal type (Figures 7, 8). The influence of this layer on the total protein and lysine content of the kernel is not known. It may or may not have an effect on enzyme synthesis in the aleurone and subaleurone areas. Its influence on digestibility is not known, however it probably does reduce the starch content of the endosperm which is necessary for good malting quality. Since the sodium hydroxide soluble glutelins are comprised primarily of aleurone proteins and appear to have the greatest influence on the total lysine content of the kernel, this area of the kernel may be the area influenced most by the recessive high lysine gene.

It is evident from the literature (20, 37, 38, 40, 41) as well as from the results of this study that there is no one effect of the Hiproly genotype. Increases are found in protein and lysine content in all of the

Figure 7. Cross section of Wocus kernel stained with Udy Orange G dye showing aleurone layer and starchy endosperm.

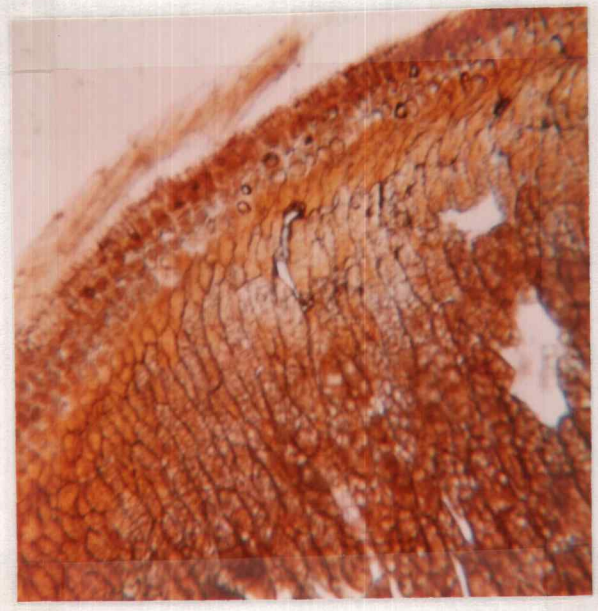

Figure 8. Cross section of Hiproly kernel stained with Udy Orange G dye showing aleurone layer, starch free subaleurone layer, and starchy endosperm.

soluble protein fractions in the kernel. It is also evident that protein and lysine content are not inherited in exactly the same manner in all Hiproly crosses. Wocus, which was consistently higher in protein and average in lysine, when crossed with Hiproly responded with poor transmissibility in the F_1 and F_2 populations for both factors. On the other hand, OR 61-2141-9, which was low in protein and lysine, responded with high transmissibility for both factors in the F_1 and F_2 populations when crossed with Hiproly. The complementation of the high enzyme activity, and the low hordein fraction of the OR 61-2141-9 malting line with the Hiproly gene seems to be the combination necessary to get maximum protein and lysine in the kernel. This is probably due to the fact that Hiproly increases the lysine content in the kernel by increasing the protein and lysine content of the water and salt soluble proteins of the embryo and the sodium hydroxide soluble proteins of the embryo and endosperm portions of the kernel. The OR 61-2141-9 selection complements this by reducing the low lysine alcohol soluble hordein proteins and increasing the lysine content of the endosperm part of the sodium hydroxide fraction as well as increasing the enzyme content of the grain. Orange Lemma has yet another effect. In this line both the amount of protein and lysine is increased in the water and salt soluble

proteins of the endosperm.

Munck (38) hypothesized the occurrence of minor genes for improved lysine content in barley. It is quite possible that minor genes do exist which influence lysine content in varying degrees. These data indicate that minor factors are having an effect in both the normal and Hiproly parents as well as the crosses. The fractionation studies show that in the parents the soluble protein fractions differed for both protein and lysine content. Differences are found in the F_1, F_2, and F_3 progenies for both protein and lysine. A dosage effect is indicated in the F_2, showing a greater jump in lysine content over the heterozygote when three doses of the recessive Hiproly endosperm are present. All of these indicate the presence of minor factors or other modifying influence. The primary effect of these influencing factors is in controlling the starch deposition in the endosperm and altering the protein content of the kernel.

It seems highly probable that many of the minor genes are located on the same linkage group with the lysine gene and the starch binding endosperm gene. This would explain why Munck and associates (41) could not differentiate between pleotropic effects of the 'hily' gene and possible linkage between the Hiproly endosperm gene and a separate locus for high lysine. In the present study, because of the method used to determine

limits on the F_3 Hiproly endosperm lines minimizing the influence of the minor genes, it was possible to show that two loci were involved in our crosses. These data are further substantiated by the reported significant association of seed weight with the 'hily' endosperm character while no association was found between seed weight and lysine content measured as a percent of the soluble protein content. The author would recommend that the two genes be designated as (lys) for the recessive gene for high lysine and (stb) for the recessive gene for the starch binding endosperm character.

With the development of rapid screening methods large populations can be handled and high quality feed and malting barleys developed. Screening of F_3 progenies for the recombinant classes high in lysine content but without the endosperm marker can be easily done by either of the methods used in this study. If the high lysine gene is coupled with the high enzyme activity and low hordein content of malting barleys it should be possible to breed a duel purpose barley. This would in effect increase the protein quantity and quality in those areas of the kernel not detrimental to malting quality while capitalizing on the reduction in the hordeins which are low in lysine. The high enzyme activity of the malting barleys would increase the lysine balance as well as improve the digestibility of the grain.

The greatest value of a barley variety with these characteristics would be to the livestock feed industry. However the possibility of improving the nutritional balance of those areas in the world now deficient in protein is a possibility.

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APPENDIX

Appendix Procedure I.[1/] Colorimetric determination of lysine by 2-chloro, 3, 5-dinitropyridine in papain hydrolyzed barley protein.

Reagents:

1. Papain solution - 4 mg. of papain per ml. of phosphate buffer (0.03 M) pH 7.4.

   40.3 ml. of 0.5 M KH_2PO_4.

   (70.99 g/liter the monobasic phosphate should be dried at 110 C for 1 to 2 hours prior to weighing).

   Mix with 30.0 ml. of 0.5 M NaOH then add water to 1 liter.

2. Bicarbonate buffer 0.6 M pH 9.0.

   43.21 ml. M $NaHCO_3$ (21 g/250 ml.; or 84 g/liter) and 3.41 ml. M NaOH to 1 liter.

   Keep solution under 20 C.

3. Borate buffer 0.05 M pH 9.0.

   6.184 g Boric acid H_3BO_3 and 7.456 g KCl to 1 liter (dry KCl at 160 C for several hours prior to weighing).

   50 ml. of above plus 20.8 ml. 0.1 M NaOH then add water to 100 ml.

4. Copper phosphate suspension

   Solution A. Dissolve 2.8 g of $CuCl_2 \cdot 2 H_2O$ in

[1/] Adapted from Villegas and Mertz (52)

100 ml. water.

Solution B. Dissolve 13.6 g of Na_3PO_4 . 12 H_2O in 200 ml. water.

Pour mixture A into B with swirling, centrifuge the mixture to bring out the precipitate and discard the supernatant. The pellet is then resuspended three times in 15 ml. of a borate buffer pH 9.0 and centrifuged after each suspension; after the third washing resuspend the pellet in 80 ml. of the borate buffer pH 9.0. The reagent is good for one week.

5. 2-chloro, 3, 5-dinitropyridine solution. Prepare daily a solution containing 30 mg. of 2-chloro, 3, 5-dinitropyridine per ml. of methanol.
6. 1.2 N HCl solution.
7. Stock solution of lysine.

   Dissolve 62.5 mg. of lysine monohydrochloride in 20 ml. of carbonate buffer (2500 µg lys/ml.).

8. Mixture of Amino Acids.

1.	Histidine	30 mg	9.	Alanine	30 mg
2.	Arginine	50 mg	10.	Cystine	20 mg
3.	Aspartic Acid	60 mg	11.	Valine	40 mg
4.	Threonine	30 mg	12.	Methionine	20 mg
5.	Serine	50 mg	13.	Isoleucine	30 mg
6.	Glutamic Acid	300 mg	14.	Leucine	80 mg
7.	Proline	80 mg	15.	Tyrosine	30 mg
8.	Glycine	40 mg	16.	Phenylalanine	40 mg

Weigh 100 mg. of this mixture and dissolve in 10 ml. of carbonate buffer.

Procedure.

1. Weigh 100 mg. of ground grain into a small bottle.
2. Add 5 ml. of papain solution. To wet all of the sample, shake at least twice in the first hour.
3. Incubate overnight. Keep at 32 C for 12 hours.
4. Shake and centrifuge at slow speed (900 g) for 10 minutes.
5. Take 1 ml. aliquot of the supernatant to a centrifuge tube.
6. Add 0.5 ml. bicarbonate buffer (pH 9.0) and 0.5 ml. of copper phosphate suspension. (prepare both water and papain blanks)
7. Shake 5 minutes and centrifuge at 5000 g for 10 minutes.
8. Remove 1 ml. of supernatant into 20 x 150 mm test tube.
9. Add 0.1 ml. of 2-chloro, 3, 5-dinitropyridine in methanol.
10. Shake for 2 hours at room temperature.
11. Add 5 ml. of 1.2 N HCl and shake well.
12. Add 5 ml. of ethyl acetate, mix well and draw the ethyl acetate phase off by vacuum. Do this three times.
13. Transfer remaining aqueous solution to cuvette and read in a spectophotometer at 390 mμ.
14. Calculate the lysine content in sample from free lysine standard curve or from gluten standard curve.

Preparation of lysine standard curve.

1. Use papain solution as the blank.
2. Dilute the stock solution of lysine (2500 µg/ml.) to 0, 250, 500, 750, and 1000 µg lys/ml. with bicarbonate buffer.
    a. 4 ml. lysine + 6 ml. carbonate = 1000 µg/ml.
    b. 3.75 ml. a + 1.25 ml. carbonate = 750 µg/ml.
    c. 2 ml. a + 2 ml. carbonate = 500 µg/ml.
    d. 1 ml. a + 3 ml. carbonate = 250 µg/ml.
    e. 4 ml. carbonate
3. Take 1 ml. from each dilution and add 4 ml. of papain solution.
4. Take 1 ml. of above into a centrifuge tube and add 0.5 ml. of the amino acid mixture and 0.5 ml. of copper phosphate suspension.
5. Continue from step No. 7 of papain colorimetric determination of lysine.

Preparation of lysine standard curve from papain hydrolyzed gluten.

1. Weigh 50, 40, 30, 20, 10, 5, 0 mg. gluten into small bottles.
2. Add 5 ml. of papain solution. To wet all sample, shake at least twice in the first hour.
3. Proceed from step No. 3 of colorimetric determination of lysine. The standard curve should parallel the pure lysine standard since pure gluten contains 1.42 percent of lysine by weight.

Appendix Procedure II. Lysine determination using Udy Orange G dye (disulfonic acid dye).

1. Weigh 100 mg. ground grain.
2. Add 15 ml. dilute Orange G dye (5 ml. concentrated dye, 1.3 mg. dye/ml., plus 10 ml. distilled water).
3. Run Sample through glass on glass homogenizer or Mini-Mill homogenizer to obtain uniform powdered particle size. Four minute time in Mini-Mill or 2 minutes in glass on glass homogenizer should be sufficient. Caution: constant and fine particle size is essential for accurate testing and repeatability.
4. Centrifuge at low speed (900 g) for 10 minutes.
5. Read supernatant in Udy colorimeter with a light filter for 470 mμ. Set colorimeter so standard Udy reference dye reads 20. This should then make dilute test dye read 32.
6. Compare to a standard curve obtained from known protein with known basic amino acid content.

This test was compared to a gluten protein standard with samples of 50, 40, 35, 30, 25, 22, 20, 18, 11, 8, and 5 mg. of protein per sample. The amount of basic amino acids and the amount of lysine was calculated from the Biochemists' Handbook, edited by Long, table page 994 (30).

Appendix Procedure III. Protein estimation with the Folin Ciocalteau reagent. 2/

Reagents:

A. Two percent Na_2CO_3 in 0.1 N NaOH; 20 g/l Na_2CO_3, 6.5 ml. concentrated NaOH/l.

B. 0.5 percent $CuSO_4 \cdot 5 H_2O$, 2.5 gm./500 ml., in 1 percent Na or K tartrate, 5 gm./500 ml.

C. Alkaline copper solution. Mix 50 ml. of reagent A with 1 ml. reagent B. Discard after 1 day.

D. Diluted Folin reagent. Dilute the Folin Ciocalteau reagent (below) to make it 1 N in acid. (Fischer's Phenol Reagent is 2 N, dilute 1:2)

Procedure.

1. 100 mg. sample ground fine.
2. Transfer to conical glass to glass homogenizer and homogenize at 1000 rpm for 2 minutes, or to Mini-Mill homogenizer at high speed for 10 minutes with a fineness of 3 on the scale, in extraction solution. 3/

   0.01 N NaOH + 0.4 N NaCl

   (0.04 g NaOH + 23.4 g NaCl/l)
3. Make up to 50 ml. and stir magnetically for 10 minutes.

2/ Adapted from Lowry et al. (31)

3/ Adapted from Trione (49)

4. Take 10 ml. of stirred sample and centrifuge at 5000 g for 15 minutes.
5. Put 0.4 ml. of the supernatant containing 5-100 µg protein into a 3 to 10 ml. test tube and add 2 ml. of reagent C.
6. Mix well and let stand 10 minutes or longer at room temperature.
7. Add 0.2 ml. of reagent D rapidly with <u>immediate</u> mixing.
8. Let color develop for 30 minutes or longer and read in a spectrophotometer.
    a. for 5-25 µg protein in final volumn read at 750 mµ.
    b. for 25-100 ug protein read at 500 mµ.
9. Calculate protein by comparison to a standard curve, developed with bovine albumin dissolved in salt buffer extraction solution.

Appendix Procedure IV. Procedure of protein fractionation into soluble protein groups.

1. Weigh out 1.50 gm. finely ground grain.
2. Wet with 20 ml. 0.4 N NaCl and homogenize for 15 minutes in a Mini-Mill.
3. Incubate the homogenate in refrigerator overnight.
4. Centrifuge at 5000 x g for 15 minutes and pour the supernatant into a 125 ml. flask.
5. Extract the residue two more times and combine the supernatant.
6. The supernatant is then evaporated to a final volume of 10 ml. This contains the water and salt soluble proteins (albumins and globulins).
7. Lowry protein and papain-lysine determinations are run on this sample with necessary dilutions.
8. The residue is then wet with 10 ml. 70 percent ethanol and allowed to incubate at room temperature overnight, shake well.
9. Centrifuge at 5000 x g for 15 minutes and pour supernatant into another 125 ml. flask.
10. Extract the residue two more times combining supernatant.
11. Evaporate supernatant to constant 10 ml. volume and run Lowry protein and papain lysine with necessary dilutions of solution. This fraction contains ethanol soluble proteins (prolamins).

12. Wet residue with 10 ml. of .1 M NaOH, shake well.
13. Incubate at room temperature overnight.
14. Centrifuge at 5000 x g for 15 minutes and pour supernatant into a third 125 ml. flask.
15. Extract the residue two more times and combine the supernatant.
16. Discard residue and precipitate protein in the NaOH solution with cold 20 percent trichloroacetic acid.
17. Wash precipitate with distilled water to remove the TCA until a pH of 5 to 6 is obtained.
18. Run Lowry protein and papain lysine on this fraction with necessary dilutions. This fraction contains 0.1 N NaOH soluble proteins (glutelins).

Appendix Table 1. Colorimetric determination of lysine in barley protein using enzymatic hydrolysis and 2-chloro, 3, 5-dinitropyridine.

Standard ug Lysine	Incubation at 35 C Y_1 % Transmission	Incubation at 65 C Y_2 % Transmission
85.00	59.00	39.66
56.66	68.66	47.00
42.50	76.66	41.66
28.33	83.33	56.00

Equation of Regression Line	Correlation Coefficient
Lysine monohydrochloride Standard	
$Y = 90.05 - .231520\ X$	- .9865
Barley flour standard incubated at 35 C	
$Y^1 = 94.28 - .422368\ X$	- .9969
Barley flour standard incubated at 65 C	
$Y^2 = 79.73 - .578860\ X$	- .8649

Appendix Table 2. Equations of regression lines of standards for techniques testing protein and lysine content in barley.

Protein (standard bovine albumin)	
X = ug protein	Y = % transmission Lowry
Y = 88.693762 - .514564 X	
correlation coefficient r = -.9936	
X = ug protein	Y = % transmission Udy dye
Y = - 21.033552 + .155388 X	
correlation coefficient r = +.9864	
X = % protein	Y = % transmission Lowry
Y = 88.693762 - 2.058257 X	
correlation coefficient r = -.9936	
X = % transmission Udy dye	Y = % transmission Lowry
Y = 161.958005 - 1.984317	
correlation coefficient r = -.9605	
Protein determination 48 barley samples	
X = Micro-Kjeldahl protein (% N x 6.25)	Y = Lowry protein
Y = 4.0942 + .391172 X	
correlation coefficient r = +.5635	
Paired t = .2294 (NS) 47 df	
Lysine (standard protein)	
Bovine albumin a/	
X = ug lysine	Y = % transmission Udy dye
Y = - 21.114313 + 1.385372 X	
correlation coefficient r = +.9870	
Wheat gluten a/	
X = mg basic amino acids	Y = % transmission Udy
Y = 29.794459 + 19.755170 X	
correlation coefficient r = +.9991	

a/ Lysine and basic amino acid content taken from Biochemists' Handbook, edited by Long (30) as a constant.

Appendix Table 3. Equations for calculating lysine and protein content from percent transmission for three colorimetric methods.

Calculation of Lysine from colorimeter reading with Udy dye.

$$\left[\frac{Y^{a/} - 29.7944}{17.7552}\right] .323759^{b/} = \text{milligrams lysine/100 mg.}$$

Calculation of protein from Lowry colorimetric reading

$$\frac{\left[\frac{Y - 88.693762}{.514564}\right] 125}{1000} = \text{milligrams protein/100 mg.}$$

Calculation of lysine from papain colorimetric readings

$$\frac{\frac{Y - 90.05}{.23152}}{1000} = \text{milligrams lysine/100 mg.}$$

Calculation of lysine as a percentage of soluble protein

$$\frac{\text{mg lysine}}{\text{mg protein}} = \text{percent of protein due to lysine}$$

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a/ Y is equal to percent transmission read from spectrophotometer.

b/ Portion of basic amino acids due to lysine, Biochemists' Handbook, C. Long editor (30).

Appendix Table 4. Confidence intervals on mean lysine content as a percent of soluble protein for 'hily' segregates of three F_3 progenies.

	Mean	99% Confidence Interval a/
Orange Lemma/ Hiproly	6.5345	6.09 - 6.98
OR 61-2141-9/ Hiproly	6.0786	5.70 - 6.45
Wocus/ Hiproly	6.2800	5.84 - 6.71

a/ $\bar{u} \pm \bar{x} - 2.576\ S\bar{x}$

Appendix Table 5. Means, variance, standard error of mean, high and low values and range of seven characters for four parental barley cultivars. a/

Cultivars		No. Culms/ Plant	Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Soluble Protein mg/100 mg	Lysine % Protein (gm)	Yield/ Plant (gm)
Hiproly	$\bar{X}$	2.100	94.800	14.400	.0453	8.824	6.980	1.153
	S^2	1.211	85.733	7.378	.0000	.151	.344	.451
	S.E.$\bar{X}$	.348	2.928	.859	.0010	.123	.142	.212
	High	4.000	108.000	20.000	.0507	9.280	7.820	2.348
	Low	1.000	80.000	10.000	.0401	8.250	5.890	.401
	Range	3.000	28.000	10.000	.0106	1.030	1.930	1.947
Orange Lemma	$\bar{X}$	2.200	131.100	57.000	.0331	8.293	5.260	3.539
	S^2	1.067	62.322	159.111	.0000	.452	.057	2.785
	S.E.$\bar{X}$	.327	2.496	3.989	.0017	.213	.075	.528
	High	4.000	139.000	74.000	.0402	9.520	5.580	5.642
	Low	1.000	113.000	38.000	.0206	7.330	4.900	.904
	Range	3.000	26.000	36.000	.0196	2.190	.680	4.738

Appendix Table 5. (cont)

Cultivars		No. Culms/ Plant	Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Soluble Protein mg/100 mg	Lysine % Protein (gm)	Yield/ Plant (gm)
OR 61-2141-9	$\bar{X}$	2.700	102.700	20.000	.0531	8.269	4.910	2.237
	S^2	2.230	98.233	4.222	.0000	.871	.157	1.029
	S.E.$\bar{X}$	0.473	3.134	.650	.0016	.295	.140	.321
	High	6.000	115.000	24.000	.0614	10.130	5.990	4.554
	Low	1.000	86.000	16.000	.0445	7.030	4.790	1.100
	Range	5.000	29.000	8.000	.0169	3.100	1.200	3.454
Wocus	$\bar{X}$	1.800	113.800	25.800	.0558	9.260	5.440	2.145
	S^2	.622	139.733	45.289	.0001	.629	.052	1.129
	S.E.$\bar{X}$	.249	3.738	2.128	.0041	.251	.072	.336
	High	3.000	127.000	43.000	.0738	10.370	5.790	3.781
	Low	1.000	92.000	18.000	.0364	8.120	5.090	.945
	Range	2.000	35.000	25.000	.0374	2.250	.700	2.836
		NS	**	**	**	*	**	**

a/ F test of significant differences with 3 and 36 degrees of freedom, ** = .01 level of significance, * = .05 level of significance, NS = no significant difference.

Appendix Table 6. Means, variance, standard error of mean, high and low values and range of agronomic characters and protein content of four parental barley cultivars and that portion of the three F_3 populations exhibiting the hiproly endosperm. a/

		Plant Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Soluble Protein mg/100 mg	Yield/ Plant (gm)
Hiproly	$\bar{X}$	100.830	16.000	.0457	8.906	1.220
	S^2	117.210	7.530	.0000	.498	.339
	S.E.$\bar{X}$	2.552	.647	.0007	.166	.137
	High	117.000	20.000	.0507	10.660	2.348
	Low	80.000	10.000	.0401	7.690	.401
	Range	37.000	10.000	.0106	2.970	1.947
Orange Lemma	$\bar{X}$	127.700	51.400	.0326	8.299	2.521
	S^2	85.480	149.830	.0001	.993	2.504
	S.E.$\bar{X}$	2.067	2.737	.0016	.223	.354
	High	139.000	74.000	.0467	10.780	5.642
	Low	104.000	35.000	.0162	6.530	.809
	Range	35.000	39.000	.0305	4.250	4.833

Appendix Table 6. (cont)

		Plant Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Soluble Protein mg/100 mg	Yield/ Plant (gm)
OR 61-2141-9	$\bar{X}$	101.158	19.890	.0539	8.004	2.816
	S^2	71.810	7.650	.0000	.683	1.701
	S.E.$\bar{X}$	1.944	.635	.0010	.190	.299
	High	115.000	25.000	.0614	10.130	5.592
	Low	86.000	13.000	.0445	6.610	.876
	Range	29.000	12.000	.0169	3.520	4.716
Wocus	$\bar{X}$	115.900	27.950	.0599	8.929	2.042
	S^2	122.940	46.160	.0002	.591	.577
	S.E.$\bar{X}$	2.479	1.519	.0027	.172	.170
	High	131.000	43.000	.0750	10.370	3.781
	Low	92.000	18.000	.0364	7.630	.945
	Range	39.000	25.000	.0386	2.740	2.836
Orange Lemma/ Hiproly	$\bar{X}$	116.620	31.120	.0403	7.899	1.361
	S^2	265.630	307.660	.0002	3.340	.596
	S.E.$\bar{X}$	2.377	2.559	.0018	.267	.113
	High	151.000	73.000	.0632	17.700	4.038
	Low	80.000	13.000	.0028	5.030	.205
	Range	71.000	60.000	.0604	12.670	3.833

Appendix Table 6. (cont)

		Plant Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Soluble Protein mg/100 mg	Yield/ Plant (gm)
OR 61-2141-9/ Hiproly	$\bar{X}$	104.530	19.500	.0501	7.912	1.512
	S^2	132.320	15.610	.0001	1.171	.783
	S.E.$\bar{X}$	1.485	.510	.0013	.140	.114
	High	135.000	29.000	.0742	11.650	4.370
	Low	77.000	8.000	.0324	5.880	.279
	Range	58.000	21.000	.0418	5.770	4.091
Wocus/ Hiproly	$\bar{X}$	115.570	28.370	.0508	8.285	1.724
	S^2	241.630	238.330	.0001	.714	1.069
	S.E.$\bar{X}$	2.292	2.276	.0013	.125	.153
	High	142.000	70.000	.0668	10.550	4.309
	Low	83.000	8.000	.0315	6.970	.361
	Range	59.000	62.000	.0353	3.580	3.948
					**	NS

a/ F test of significant differences with 6 and 223 degrees of freedom, ** = .01 level of significance, NS = no significant difference.

Appendix Table 7. Means, variance, standard error of mean, high and low values and range for five characters of three F_3 progenies. a/

Cross		No. Culms/ Plant	Plant Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Yield/ Plant (gm)
Orange Lemma/ Hiproly	$\bar{X}$	1.681	117.968	33.745	.0439	2.072
	S^2	.828	216.420	318.780	.0002	2.015
	S.E.$\bar{X}$	.066	1.073	1.302	.0009	.104
	High	6.000	155.000	75.000	.0928	8.367
	Low	1.000	80.000	10.000	.0028	.205
	Range	5.000	75.000	65.000	.0900	8.162
OR 61-2141-9/ Hiproly	$\bar{X}$	1.995	106.349	20.390	.0555	1.991
	S^2	.862	148.150	11.970	.0001	1.220
	S.E.$\bar{X}$	.063	.824	.234	.0007	.075
	High	5.000	144.000	29.000	.0925	5.964
	Low	1.000	64.000	8.000	.0258	.279
	Range	4.000	80.000	21.000	.0667	5.685

Appendix Table 7. (cont)

Cross		No. Culms/ Plant	Plant Height (cm)	No. Seeds/ Culm	Seed Weight (gm)	Yield/ Plant (gm)
Wocus/ Hiproly	$\bar{X}$	1.487	117.985	25.264	.0561	1.830
	S^2	.578	278.410	183.090	.0002	1.395
	S.E.$\bar{X}$	.054	1.189	.964	.0009	.084
	High	4.000	155.000	70.000	.0832	7.464
	Low	1.000	75.000	1.000	.0148	.022
	Range	3.000	80.000	69.000	.0684	7.442
		**	**	**	**	NS

a/ F test of significant differences with 2 and 600 degrees of freedom, ** = .01 level of significance, NS = no significant difference.

Appendix Table 8. Means, variance, standard error of mean, high and low values and range of five agronomic characters, protein milligrams/100 milligrams grain, and lysine percent of protein for that portion of the three F_3 populations exhibiting the hiproly endosperm. a/

Cross		No. Culms/ Plant	Plant Height (cm)	No. Seeds/ Head	Seed Weight (gm)	Soluble Protein mg/100 mg	Lysine % Protein	Yield/ Plant (gm)
Orange Lemma/ Hiproly	$\bar{X}$	1.319	116.617	31.106	.0403	7.899	6.535	1.361
	S^2	.439	265.630	307.660	.0002	3.340	1.389	.596
	$S.E.\bar{X}$	.097	2.377	2.559	.0018	.267	.172	.113
	High	4.000	151.000	73.000	.0632	17.700	10.170	4.038
	Low	1.000	80.000	13.000	.0028	5.030	4.940	.205
	Range	3.000	71.000	60.000	.0604	12.670	5.230	3.833
OR 61-2141-9/ Hiproly	$\bar{X}$	1.700	104.533	19.500	.0501	7.912	6.089	1.512
	S^2	.790	132.320	15.610	.0001	1.170	1.248	.783
	$S.E.\bar{X}$	.115	1.485	.510	.0013	.140	.143	.114
	High	5.000	135.000	29.000	.0742	11.650	8.420	4.370
	Low	1.000	77.000	8.000	.0324	5.880	3.820	.279
	Range	4.000	58.000	21.000	.0418	5.770	4.600	4.091

Appendix Table 8. (cont)

Cross		No. Culms/ Plant	Plant Height (cm)	No. Seeds/ Head	Seed Weight (gm)	Soluble Protein mg/100 mg	Lysine % Protein	Yield/ Plant (gm)
Wocus/ Hiproly	$\bar{X}$	1.457	115.565	28.370	.0508	8.285	6.288	1.724
	S^2	.743	241.630	238.330	.0001	.714	1.296	1.069
	S.E.$\bar{X}$	.127	2.292	2.276	.0013	.125	.168	.153
	High	4.000	142.000	70.000	.0668	10.550	8.070	4.309
	Low	1.000	83.000	8.000	.0315	6.970	3.500	.361
	Range	3.000	59.000	62.000	.0353	3.580	4.570	3.948
						NS	NS	NS

a/ F test of significant differences with 2 and 150 degrees of freedom, NS = no significant difference.