Agronomic, biochemical and genetic characterizations and adaptations of high lysine, shrunken endosperm mutants of barley (Hordeum vulgare L.)
by Steven Edward Ullrich

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and Soil Science
Montana State University
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Abstract:
'Compana' sex1, seg3 and seg4, 'Betzes' seg1 (and seg2), 'Ingrid' seg6 and seg7, 'Glacier' seg5, 'High Amylose Glacier' amol and 'Hiproly' Iysl, spontaneous shrunken endosperm or low kernel weight mutants of barley (Hordeum vulgare L.), were compared to their normal isotypes for kernel weight, protein, and lysine contents. All mutants were lower in kernel weight and higher in seed protein (except amol) and lysine contents than respective normal isotypes indicating that eight new mutants should be added to the list of known "high lysine" mutants of barley.

'Bomi,' Risø mutants 7, 8, 9, 13 and 1508 and 'Carlsberg II', Risø mutants 29, 56 and 86, induced high lysine, shrunken endosperm and/or low kernel weight mutants of barley were also compared to their respective normal isotypes for kernel weight, protein and lysine contents in several varied Western U.S. intermountain environments. All mutants were lower in kernel weight and higher in lysine content, and six of the mutants were higher in protein content, with a relatively wide range of mutant-normal differences for each character.

The spontaneous mutants sex1, seg4, amol and perhaps Hiproly and most of the induced mutants are the most likely to maintain a positive mutant-normal difference in lysine percentage if normal kernel weights were achieved through breeding efforts.

Regression analysis was used to determine for the above mutants the effect of environment on the expression of the above mentioned kernel characters and eight other agronomic characters. Definite genotype x environment interactions were detected. The adaptation and stability of response of the various mutants for the various characters was quite variable. This analysis provided a basis for choosing environments in which to select with a given mutant for or against the various traits. Shrunken endosperm is best expressed in environments favorable for large seed in the normal isotypes. High lysine in the grain is expressed more or less equally in all environments.

The shrunken endosperm and high lysine traits of Risø 1508, and the shrunken endosperm trait of Risø 29 and 86 were found to be inherited as single recessive genes that express xenia, and Risø 29 and 86 were found to be allelic with sex1. These shrunken endosperm genes and those of several other Risø mutants and amol were assigned to chromosomes by trisomic analysis. Maternal inheritance effects for seed protein and lysine contents were indicated for Hiproly .

The association between shrunken endosperm and high lysine in barley was firmly established. Solutions of this problem for breeders is presented.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

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ABSTRACT

'Compana' sex1, seg3 and seg4, 'Betzes' seg1 (and seg2), 'Ingrid' seg6 and seg7, 'Glacier' seg5, 'High Amylose Glacier' amo1 and 'Hiproly' lys1, spontaneous shrunken endosperm or low kernel weight mutants of barley (Hordeum vulgare L.), were compared to their normal isotypes for kernel weight, protein, and lysine contents. All mutants were lower in kernel weight and higher in seed protein (except amo1) and lysine contents than respective normal isotypes indicating that eight new mutants should be added to the list of known "high lysine" mutants of barley.

'Bomi,' Risø mutants 7, 8, 9, 13 and 1508 and 'Carlsberg II', Risø mutants 29, 56 and 86, induced high lysine, shrunken endosperm and/or low kernel weight mutants of barley were also compared to their respective normal isotypes for kernel weight, protein and lysine contents in several varied Western U.S. intermountain environments. All mutants were lower in kernel weight and higher in lysine content, and six of the mutants were higher in protein content, with a relatively wide range of mutant-normal differences for each character.

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Regression analysis was used to determine for the above mutants the effect of environment on the expression of the above mentioned kernel characters and eight other agronomic characters. Definite genotype x environment interactions were detected. The adaptation and stability of response of the various mutants for the various characters was quite variable. This analysis provided a basis for choosing environments in which to select with a given mutant for or against the various traits. Shrunken endosperm is best expressed in environments favorable for large seed in the normal isotypes. High lysine in the grain is expressed more or less equally in all environments.

The shrunken endosperm and high lysine traits of Risø 1508, and the shrunken endosperm trait of Risø 29 and 86 were found to be inherited as single recessive genes that express xenia, and Risø 29 and 86 were found to be allelic with sex1. These shrunken endosperm genes and those of several other Risø mutants and amo1 were assigned to chromosomes by trisomic analysis. Maternal inheritance effects for seed protein and lysine contents were indicated for Hiproly .

The association between shrunken endosperm and high lysine in barley was firmly established. Solutions of this problem for breeders is presented.

Risø 1508 is probably the best mutant source of lysine in the grain, but low yield and current market conditions indicate it is not feasible to grow it commercially as a (swine) feed barley. Risø 7 and High Amylose Glacier seem to have potential in their present state as commercial varieties, particularly under dryland conditions in Montana.

INTRODUCTION

Barley (Hordeum vulgare L.) is an important cereal grain in Montana, the United States and the world. It is generally classified as a feed grain, but is used extensively for human food in a number of countries in the world, particularly developing countries in Asia and North Africa.

With the advent of high lysine barley came a renewed awareness and interest in cereal quality, especially protein quality. Nutritional superiority was quickly demonstrated for the "new" high lysine barley mutants. Development of commercial high lysine cultivars has been slow because shrunken endosperm or low kernel weight is associated with the high lysine property of barley.

The impetus for the research reported herein came from the requirement for basic information and understanding of high lysine barley mutants for effective progress in breeding barley cultivars with improved protein quality. The general objectives of this investigation were to 1) characterize a group of spontaneous and induced "shrunken endosperm" mutants of barley for kernel weight, and protein and lysine content, 2) determine and explain the effect of environment on several kernel and agronomic characters of these mutants, and 3) accumulate basic information on the genes involved such as gene action, chromosome location and allelic relationships.

LITERATURE REVIEW

The Occurrence of High Lysine Barley

The era of high lysine barley commenced in 1968 with the discovery of the genetically dependent high protein, high lysine cultivar 'Hiproly' (CI 3947) of Ethiopian origin (Munck et al., 1970). It was selected from the World Barley Collection by the dye-binding capacity (DBC) method of Mossberg (1969). Subsequently a number of induced high lysine mutants have been described including the series of Risø Demark mutants (Ingversen et al., 1973 and Doll et al., 1974) and notch mutants (Bansal, 1970). Verification of these various mutants as high lysine mutants has been by demonstrating an increased concentration of lysine in the seed protein compared with normal barley isolines by ion-exchange chromatography by automatic amino acid analysis (Spackman et al., 1958).

Interest in High Lysine Barley

One of the most significant combined contributions of biochemistry and animal physiology was the classical work by Osborne and Mendel (1914) which defined some of the most important amino acids that are essential for the growth and maintenance of monogastric animals. They demonstrated that lysine is essential and is the most limiting amino acid in most cereal grains including barley.

Nutrition studies with rats, mice, swine and/or poultry and Hiproly (Munck et al., 1970; Munck, 1972; Thomke and Widstroemer, 1975; Munck

and Wettstein, 1976; Newman and Eslick, 1976; and Newman et al., 1977), the Risø mutants (Doll et al., 1974; Køie et al., 1976; and Newman and Eslick, 1976) and the notch mutants (Balaravi et al., 1976 and Bansal et al., 1977) have overwhelmingly demonstrated the nutritional superiority of high lysine barley mutants over normal barley cultivars. Herein lies the ultimate justifacation for studying high lysine barley mutants.

At present in the Northwestern United States the primary protein feed supplements are soybean and cottonseed oil meals which have to be shipped from the Midwestern or Southern States. Partial or total replacement of these costly protein supplements in non-ruminant feed rations with high lysine barley would greatly benefit the economy of the Northwest. Newman et al. (1978) have presented evidence with swine to indicate this may be feasible.

Properties of High Lysine Barley Mutants

Genetic studies have revealed single recessive gene control of the high lysine property in barley (Munck et al., 1970; Doll, 1973 and 1976; Bansal, 1974; Karlsson, 1977; and Ullrich and Eslick, 1977).

The primary effect of these "high lysine" genes is not yet known, but for the mutants studied, either qualitative shifts within protein fractions (Ingversen and Køie, 1973 and El-Negoumy et al., 1977), quantitative shifts among protein fractions (Ingversen et al., 1973 and

Ingversen, 1975) or a combination of the two (Munck, 1972 and Køie et al., 1976) occur to effect an increased lysine content in the seed protein. In general, high lysine mutants compared with normal barley have a decreased low lysine prolamine fraction and increased higher lysine glutelin, albumin and/or globulin fractions accompanied with shifts within fractions (Køie et al., 1976).

Ultrastructural differences between high lysine and normal barley endosperm have been observed through transmission and scanning electron microscopy. Munck (1972) has shown a difference in the protein matrix-starch granule interphase between Hiproly and its sister-line 'Hiproly Normal' (CI 4362), and Ingversen (1975) has demonstrated a shift in the concentration of protein body components between 'Bomi', Risø mutant 1508 and Bomi . Additionally, Ingversen (1975) has provided information that equates protein body component change with low to high lysine protein fraction shifts.

High lysine barley mutants have an associated shrunken seed or reduced seed weight phenotype (Munck et al., 1970; Ingversen et al., 1973; Doll et al., 1974; Doll, 1976; and Balaravi et al., 1976). A number of spontaneously occurring barley mutants have been designated as shrunken endosperm (seg and sex) mutants (Jarvi and Eslick, 1975 and Ramage and Scheuring, 1976), which has led to the speculation of their seed lysine content. Eslick and Hockett (1976a) have stated that 'Betzes' segl was higher in seed lysine content than normal Betzes .

Effect of Environment on High Lysine Barley Mutants

The adaptation or the effect of environment on plants may be measured in a number of ways, for example, by the genotype x environment interaction in an analysis of variance. Regression analysis is another method.

In 1921 Mooers, perhaps, was the first to employ the regression analysis approach for determining environmental responses of corn. Since then, a number of variations of this approach have been described, including in recent years, those of Finlay and Wilkinson (1963) with barley, Eberhart and Russel (1966) with corn and Bilbro and Ray (1976) with cotton. In all of these attempts to grasp the genotype x environment interaction, the basic objective has been to identify superior genotypes for release and recommendation of varieties to be grown under specified environments. The Montana Agricultural Experiment Station breeders have been utilizing a regression analysis method for recommending varieties of small grains since 1969 (Hockett, 1970). Specific mutant characterization in relation to a normal isotype for environmental responses for given traits was first presented by Eslick and Hockett (1977) for two erectoides mutants of barley and Eslick and Ullrich (1977) for three high lysine mutants of barley.

Genetics of High Lysine Barley Mutants

Knowledge to date indicates that the inheritance of high lysine in barley is controlled by single recessive genes. This is true for all the mutants considered in this investigation that have been studied. The mode of inheritance of the high lysine trait of Hiproly was determined by Munck et al. (1970) and the gene was designated lys1. Karlsson (1976) provided evidence for the assigning of lys1 to chromosome 7 of barley. The gene action of high lysine for Risø mutants 7, 8, 9, 13, and 1508 was determined by Doll (1973 and 1976). For Risø 1508, Karlsson (1977) and Ullrich and Eslick (1977) found that the trait expresses xenia and they have presented initial evidence for the location of the high lysine gene on chromosome 7. Doll (1976) demonstrated for Risø 8 and 13 that shrunken endosperm and high lysine express xenia, and are inherited together as a single recessive gene(s).

Pomeranz et al. (1972) indicated that the high amylose mutant of the cultivar 'Glacier' had a higher seed lysine content than normal Glacier . The high amylose trait (40% amylose) of Glacier barley first described by Merritt (1967) was found to be inherited as a single recessive gene and to display dosage effects, but no maternal effects (Walker and Merritt, 1969). Eslick and Ullrich (1977) have suggested the gene be designated amo1.

The inheritance of six non-allelic spontaneous shrunken endosperm mutants (sex1 and seg1 - seg5) in barley and their potential for hybrid

barley production and as genetic markers were discussed by Jarvi and Eslick (1975). Ramage and Scheuring (1976) reported on the inheritance of seg6 and seg7.

SECTION I: LYSINE AND PROTEIN CHARACTERIZATION OF SHRUNKEN ENDOSPERM MUTANTS OF BARLEY

Introduction

Described high lysine barley mutants have an associated shrunken seed or reduced seed weight phenotype. This association suggested further investigation of the described shrunken endosperm mutants of barley. The objectives of these studies were to 1) describe the known spontaneous shrunken endosperm mutants in terms of kernel weight and lysine and protein contents, 2) better establish the expression of the genes involved or the magnitude of the mutant-normal differences in kernel weight and lysine and protein contents of several induced high lysine, shrunken endosperm mutants in a number of varied Western U. S. intermountain environments, and 3) determine if there are any obvious differences between the induced and the spontaneous shrunken endosperm mutants for the characters considered.

Materials and Methods

General

Seventeen mutants were involved in the studies. Paired comparisons were made over a range of environments between the mutants and their respective normal isotypes for kernel weight and protein and lysine contents. Each mutant-normal comparison involved a different location and/or year. The paired material was grown in various type

plots (single row, multiple row, yield nursery, etc.) at various seeding rates under dryland and irrigated conditions.

Kernel weight determinations were made on 30g samples of each line from each environment. Protein percentages were based on the macro Kjeldahl technique (N x 6.25). Lysine determinations were made by AAA Laboratories of Seattle, Washington using an Automatic Amino Acid Analyzer with standard hydrolysis times, and in our laboratory by a modified microbiological assay technique (Waters, 1976). All chemical analyses were performed on ground whole kernel samples. Statistical analyses included mean comparisons between mutant and normal isotypes by paired t-test for the various characters studied.

As an approach to explaining why shrunken endosperm mutants are higher in percent protein and lysine than normal barley, adjusted protein and lysine in the grain percentages were calculated on the basis of adjusting the mutant kernel weight to the normal kernel weight. The following simplified formula was used for this manipulation:

$$\text{adjusted mutant \% (protein or lysine)} = \frac{\text{observed mutant \% (protein or lysine)}}{\text{normal kernel wt./mutant kernel wt.}}$$

Study I: Characterization of Spontaneous Shrunken Endosperm Mutants

The spontaneous mutants in this study are characterized by a visible shrunken endosperm and/or a detectable low kernel weight phenotype. The mutants with descriptive references include Betzes *seg1*

(Jarvi and Eslick, 1975; Eslick and Hockett, 1976a); Betzes seg2; 'Compana' seg3 and seg4; Glacier seg5 (Jarvi and Eslick, 1975); 'Ingrid' seg6 and seg7 (Ramage and Scheuring, 1976); Compana sex1, originally reported as Compana se6 (Jarvi and Eslick, 1975) but subsequently redesignated as compana sex1 (Eslick and Hockett, 1976b); and Glacier amo1, high amylose (Meritt, 1967; Pomeranz et al., 1972). Hiproly compared with Hiproly Normal (CI 4362) was included in the study as a spontaneous mutant high lysine check. Seven randomly selected (from barley fields) spontaneous shrunken endosperm mutants were also analyzed for seed protein and lysine content. Material was grown from 1967-77 at various locations including Bozeman, Huntley, Sidney, Moccasin, Havre and Kalispell, Montana and Mesa and Marana, Arizona.

Betzes seg2 is usually propagated from the balanced tertiary trisomic because of the very small seed and difficulty of establishing field stands. Information based on the one or two samples available is included in the tables with no further comment.

Study II: Characterization of Induced Shrunken Endosperm Mutants

Eight chemical and radiation induced mutants were involved in this study, designated Risø mutant 7 (thermal neutrons), 8, 9, 13 (ethyl methane sulfonate) and 1508 (ethyleneimine) induced in the normal cultivar Bomi, and 56 (γ-rays), 29 and 86 (ethyl methane sulfonate) induced in the normal cultivar 'Carlsberg II'. Initial mutant and normal

isogenic seed stocks were obtained through the courtesy of the Agric. Res. Dept., Danish AES Risø Roskilde, Denmark.

This material was grown from 1974-77 at Bozeman, Huntley, Sidney, Moccasin, Fairfield, Havre, and Kalispell, Montana and Mesa, Arizona.

Results and Discussion

Study I: Characterization of Spontaneous Shrunken Endosperm Mutants

Kernel Weight and Protein Content--All mutants, in comparison with their normal isotypes, have lower kernel weights over the range of environments in which grown (Table 1-1). The average mutant kernel weights range from 38% (Ingrid *seg6*) to 95% (Glacier *amo1*) of normal.

All mutants except High Amylose Glacier have a significantly higher percentage of seed protein (Table 1-1). Significant mutant-normal mean differences range from 1.2% protein for Hiproly-Hiproly Normal to 3.7% protein for Glacier *seg5* - Glacier , or from 109% to 129% of normal, respectively. 'High Amylose Glacier', as an exception, does not have a visibly shrunken endosperm. Its lower kernel weight may be due to slightly smaller or less dense seed although this is not detectable with yield, test weight, percent plump and percent thin comparisons (Eslick and Ullrich, 1977 and Section II).

Lysine Content--The lysine analysis data obtained by microbiological assay (MBA) are presented in Table 1-2, and the data obtained with

Table 1-1. Kernel weight and protein comparisons of shrunken endosperm mutants and their normal isotypes.

Variety	Mutant gene	Kernel weight				Protein			
		No. of comparisons	Mutant isotype means (mg)	Normal isotype means (mg)	Differences	No. of comparisons	Mutant isotype means (%)	Normal isotype means (%)	Differences
Compana	sex 1	8	38.6	47.6	- 9.0**	14	17.9	14.4	3.5**
Compana	seg 3	8	26.2	46.9	-10.7**	12	16.6	14.4	2.2**
Compana	seg 4	9	37.8	47.8	-10.0**	10	16.5	14.2	2.3**
Betzes	seg 1	17	18.8	34.3	-15.5**	18	15.9	14.6	1.3**
Betzes	seg 2	1	6.0	40.6	-34.6	2	16.1	14.9	1.2
Ingrid	seg 6	3	15.1	39.9	-24.8**	7	14.5	12.6	1.9**
Ingrid	seg 7	8	26.8	36.7	- 9.9**	11	15.0	12.8	2.2**
Glacier	seg 5	7	32.6	45.9	-13.3**	10	16.3	12.6	3.7**
Glacier	amo 1	20	46.9	49.4	- 2.5**	21	12.6	12.5	0.1
Hiproly	lys 1	26	38.4	49.2	-10.8**	26	18.4	17.2	1.2**

** Significant at the 0.01 level.

Table 1-2. Microbiological assay lysine comparisons of shrunken endosperm mutants and their normal isotypes.

Variety	Mutant gene	No. of comparisons	Mean lysine in grain (%)			Mean lysine in protein (%)		
			Mutant isotype	Normal isotype	Difference	Mutant isotype	Normal isotype	Difference
Compana	sex 1	14	.598	.379	.219**	3.37	2.66	.71**
Compana	seg 3	12	.534	.391	.143**	3.40	2.76	.65**
Compana	seg 4	10	.624	.403	.221**	3.79	2.87	.91**
Betzes	seg 1	18	.518	.436	.082**	3.25	3.05	.20**
Betzes	seg 2	2	.704	.320	.384	4.38	2.18	2.20
Ingrid	seg 6	7	.514	.387	.127**	3.55	3.09	.46*
Ingrid	seg 7	11	.488	.407	.081**	3.25	3.20	.05
Glacier	seg 5	10	.473	.369	.104**	2.93	2.94	-.01
Glacier	amo 1	21	.430	.373	.057**	3.44	3.00	.44**
Hiproly	lys 1	26	.581	.448	.133**	3.15	2.61	.54**

*, ** Significant at the 0.05 and 0.01 levels, respectively.

the amino acid analyzer (AAA) are presented in Table 1-3.

When considering percent lysine in the grain determined by both MBA and AAA, all mutants have a higher lysine content than their normal isotypes. Microbiological assay average mutant values range from 115% (Glacier amo1) to 158% (Compana sex1) of normal while AAA average mutant values range from 109% (Glacier amo1) to 160% (Compana sex1) of normal.

Lysine content expressed as percent of the protein (g/16gN) equalizes differences in protein when making comparisons. All mutants compared to the normal have significantly higher percent lysine in the protein when determined by AAA except Glacier seg5 (Table 1-3) and when determined by MBA except Ingrid seg7 and Glacier seg5 (Table 1-2). Mutant values significantly different from normal ranged from 106% of normal for Betzes seg1 to 132% of normal for Compana seg4 by MBA analysis and 104% of normal for Ingrid seg7 to 136% of normal for Ingrid seg6 by AAA analysis. Similar lysine and protein results were obtained for two of the mutants considered here; Hiproly and Betzes seg1 by Ahokas (1977).

Seed size within a spike is quite variable for Glacier seg5 (Jarvi and Eslick, 1975). The presence of plump seed in the samples is a possible explanation for the non-significant difference between Glacier seg5 and Glacier in percent lysine in the protein. When sieve size fractions were obtained from bulk samples of Glacier seg5

Table 1-3. Amino acid analyzer lysine comparisons of shrunken endosperm mutants and their normal isotypes.

Variety	Mutant gene	No. of comparisons	Mean lysine in grain (%)			Mean lysine in protein (%)		
			Mutant isotype	Normal isotype	Difference	Mutant isotype	Normal isotype	Difference
Compana	sex 1	3	.729	.454	.275**	4.17	3.30	.87*
Compana	sex 3	3	.630	.454	.176**	4.10	3.30	.80*
Compana	seg 4	3	.701	.454	.247*	4.21	3.30	.91*
Betzes	seg 1	4	.587	.469	.118**	4.07	3.62	.45*
Ingrid	seg 6	3	.609	.408	.201**	4.50	3.32	1.17**
Ingrid	seg 7	4	.582	.464	.118*	4.08	3.93	.15*
Glacier	seg 5	3	.544	.427	.117*	3.58	3.47	.11
Glacier	amo 1	8	.481	.441	.040**	4.19	3.76	.43**
Hiproly	lys 1	16	.791	.542	.249**	4.35	3.23	1.12**

*, ** Significant at the 0.05 and 0.01 levels, respectively.

grown in three environments and protein and lysine contents determined, there were no significant differences among sieve size means (Table 1-4).

Table 1-4. Mean protein and lysine contents of sieve size fractions of Glacier seg5 and of random samples of Glacier and Glacier seg5 obtained from grain grown in three common environments.

Type of Sample	No. of Observations	Kjeldahl Protein (%)	MBA Lysine in Grain (%)	MBA Lysine in Protein (%)
on 2.8 x 19 mm sieve	2	14.8	.430	2.90
on 2.4 x 19 mm sieve	3	15.1	.433	2.88
on 2.2 x 19 mm sieve	3	15.8	.463	2.91
on 2.0 x 19 mm sieve	3	15.7	.460	2.94
thru 2.0 x 19 mm sieve	2	14.4	.432	3.02
Random Glacier	3	12.3	.370	3.00
Random seg5	3	15.2	.468	3.08
Differences (mutant-normal)		2.9*	.098*	.08

*Significant differences at 0.05 level were determined by pair t-test.

Plump seed may be obtained from homozygous 'Ingrid' seg7 plants in some environments (R. T. Ramage, personal communication). Some plots within the range of environments involved in this study also yielded plump seed. Microbiological assay analyses involved all environments in which seg7 was grown and, therefore, plump seed were analyzed and the data averaged with data from shrunken seed. When the obviously shrunken or smallest seed were selected and analyzed from bulk samples

of Ingrid seg7 from each environment in which it was grown, the mutant-normal mean difference in MBA percent lysine in the protein (-0.11%) was non-significant. On the other hand, only seg7 samples from environments that produced shrunken seed were analyzed by AAA and a significant difference between mutant and normal in percent lysine in the protein was obtained (Table 1-3). Comparing the obtained mutant-normal differences from common environments by the two methods of analysis, the MBA mean difference was still non-significant while the AAA mean difference was significant. The standard error was considerably higher for the MBA analysis.

General--It could be postulated that normal barley may have lower protein and lysine percentages in the grain than the mutant barley because of a starch dilution effect (Tables 1-1 and 1-2). This hypothesis can be tested, at least theoretically, by adjusting the mutant kernel weight upwards to the normal kernel weight and calculating adjusted protein and lysine in the grain percentages. The data presented in Table 1-5 represent this type of manipulation made possible by the fact that mutant-normal pairs are isogenic lines and the data are paired.

The mutants do not "behave" similarly when they are calculated as "plump." The adjusted mutant-normal difference in percent protein was non-significant for some mutants. The deposition of starch in these shrunken mutants may be restricted more than protein deposition during

Table 1-5. Theoretical comparisons of shrunken endosperm mutants adusted to normal kernel weights and their normal isotypes for mean Kjeldahl protein and MBA lysine contents.

Variety	Mutant gene	No. of comparisons	Protein (%)			Lysine in grain (%)		
			Adjusted mutant isotype+	Observed normal isotype	Difference	Adjusted mutant isotype	Observed normal isotype	Difference
Compana	sex 1	9	14.6	14.1	0.5	.568	.412	.156**
Compana	seg 3	8	9.5	13.9	-4.4**	.328	.415	-.087**
Compana	seg 4	9	13.2	14.1	-0.9	.518	.412	.106**
Betzes	seg 1	16	8.6	14.5	-5.9**	.281	.435	-.154**
Ingrid	seg 6	3	5.1	12.3	-7.2**	.184	.396	-.212**
Ingrid	seg 7	8	9.8	12.5	-2.7**	.310	.401	-.091*
Glacier	seg 5	6	10.6	11.9	-1.3	.327	.375	-.048**
Glacier	amo 1	20	12.0	12.5	-0.5	.402	.375	.027*
Hiproly	lys 1	26	14.4	17.2	-2.8*	.456	.448	.008

*, ** Significant at the 0.05 and 0.01 levels, respectively.

+ Adjusted mutant % = $\frac{\text{observed mutant \% (protein or lysine)}}{\text{normal kernel wt/mutant kernel wt.}}$.

during grain filling. With the remainder of the mutants, the adjusted percent protein of the mutants was significantly lower than normal. This perhaps indicates that protein as well as starch deposition is severely affected in the mutant kernel.

For those mutants in which protein accumulation in the kernel may be relatively little affected, it might be expected that lysine accumulation be little affected. This may be true for Compana sex1 and seg4 and Glacier amo1 as the mutant-normal difference in percent lysine in the grain is positive and significant whether the mutant is shrunken or calculated as "plump." These results might also indicate that a genetic deficiency in kernel plumpness increases lysine deposition in the kernel, as adding starch or weight to the kernel did not significantly dilute the lysine content. For Compana seg3, Betzes seg1 and Ingrid seg6 and seg7 in which protein deposition in the kernel appears to be restricted, lysine deposition also appears to be restricted as indicated by the significant negative adjusted mutant-normal difference.

If these theoretical considerations have validity, a practical application of the results would be to give the breeder a basis for selecting "high lysine" mutants, with which to work, from a relatively large number of possible candidates. It would appear that those mutants that maintain a significantly higher percent lysine in the grain with calculated added kernel weight or plumpness would be most desirable, e.g., sex1, seg4, amo1 and perhaps Hiproly .

It remains that the described shrunken endosperm or low kernel weight mutants of barley have associated high protein and high lysine characters with few possible exceptions. The data presented in this paper indicate that eight "new" mutants should be added to the list of known high lysine mutants.

These associations were also obtained from seven randomly selected "new" spontaneous shrunken endosperm mutants from segregating material and commercial barley fields (Table 1-6). From single paired comparisons, five of the mutants were higher in percent protein than appropriate normal checks, all were higher in lysine content expressed as percent of grain, and five were higher in percent lysine in the protein, indicating that "high lysine" mutants of this type can be relatively easily obtained by searching for shrunken endosperm mutants in barley fields.

Study II: Characterization of Induced Shrunken Endosperm Mutants

Kernel Weight--All mutants, in comparison with their normal isotypes, had lower kernel weights over the range of environments involved in this study (Table 1-7). The mean mutant kernel weights ranged from 72% (Risø 8) to 94% (Risø 7) of the normal isotypes. The magnitude of the mean kernel weight difference is relatively small for several of the mutant-normal pairs (Risø 7, 9, 29, 56, 86), particularly in comparison to the spontaneous shrunken endosperm mutants of barley grown

Table 1-6. Protein and lysine comparisons between randomly selected shrunken endosperm mutants and normal endosperm barley.

Variety		% Protein			% Lysine in Grain			% Lysine in Protein		
		Mutant	Normal	Diff.	Mutant	Normal	Diff.	Mutant	Normal	Diff.
Segregating Material	†	14.0	12.3	1.7	.600	.360	.240	4.28	2.93	1.35
"		14.8	16.0	-1.2	.490	.440	.050	3.31	2.75	0.56
"		18.0	12.5	5.5	.660	.460	.200	3.67	3.73	-0.06
"	†	15.5	12.3	3.2	.508	.360	.148	3.28	2.93	0.35
"		15.9	18.5	-2.6	.508	.436	.072	3.19	2.36	0.83
Klages	†	18.1	12.3	5.8	.526	.360	.166	2.91	2.93	-0.02
"	†	14.7	12.3	2.4	.544	.360	.184	3.70	2.93	0.77
Compana			11.7			.360			3.08	
Steptoe			11.6			.360			3.10	
Hiproly		17.9	16.0	1.9	.640	.414	.226	3.76	2.58	1.18

†'Betzes' was used as a normal check in lieu of a normal isotype.

Table 1-7. Kernel weight and protein comparisons of induced shrunken endosperm mutants and their normal isotypes.

	Mean kernel weight (mg)				Mean protein (%)			
Risø mutant	No. of comparisons	Mutant isotype	Normal isotype	Difference mutant-normal	No. of comparisons	Mutant isotype	Normal isotype	Difference mutant-normal
7	28	42.2	44.7	-2.5**	28	13.2	13.2	0.0
8	26	32.6	45.0	-12.4**	26	14.4	13.2	1.2**
9	26	39.6	45.1	-5.5**	28	13.2	13.2	0.0
13	27	35.6	45.0	-9.4**	28	15.0	13.2	1.8**
1508	44	34.3	43.4	-9.1**	47	13.8	13.2	0.6**
29	33	36.5	39.3	-2.8**	36	14.0	12.9	1.1**
56	25	35.9	39.7	-3.8**	28	14.7	12.9	1.8**
86	33	34.0	39.2	-5.2**	36	14.7	12.8	1.9**

**Significant at the 0.01 level.

in similar environments (Table 1-1). Doll (1976) detected no difference in kernel weight between Risø 7 and normal Bomi .

In the environments involved in this experiment Risø mutants 8, 13, 1508, 29, 56, and 86 displayed a shrunken kernel phenotype in which xenia was expressed. Risø mutants 7 and 9 produced kernels that were plump in appearance. Doll (1976) stated that Risø 9 produced shrivelled seed under Danish test conditions.

Protein Content--All mutants except Risø 7 and 9 had a significantly higher percentage of seed protein than the normal isotype (Table 1-7). For those mutant-normal pairs with significant differences, the relative mean mutant protein contents ranged from 104% (Risø 1508) to 115% (Risø 86) of the normal isotype. Risø 7 and 9, as exceptions, do not manifest a visibly shrunken endosperm as indicated above. These findings are similar to those for the spontaneous shrunken endosperm mutants (Table 1-1).

Lysine Content--By microbiological assay all mutants had a higher lysine content than their normal isotypes whether expressed as percent in the grain or in the protein (g/16g N)(Table 1-8). Mean mutant values ranged from 113% of normal for Risø 9 to 157% of normal for Risø 1508 for percent lysine in the grain and 113% of normal for Risø 7 to 149% of normal for Risø 1508 for percent lysine in the protein. In the environments considered in this study all mutants in general had a higher

Table 1-8. Microbiological assay lysine comparisons of induced shrunken endosperm mutants and their normal isotypes.

Risø mutant	No. of comparisons	Mean lysine in grain (%)			Mean lysine in protein (%)		
		Mutant isotype	Normal isotype	Difference mutant-normal	Mutant isotype	Normal isotype	Difference mutant-normal
7	28	.435	.384	.051**	3.30	2.93	.37**
8	26	.548	.386	.162**	3.80	2.92	.88**
9	28	.434	.384	.050**	3.32	2.93	.39**
13	28	.544	.384	.160**	3.68	2.93	.75**
1508	47	.607	.386	.221**	4.39	2.95	1.44**
29	36	.507	.391	.116**	3.66	3.07	.59**
56	28	.538	.379	.159**	3.69	2.97	.72**
86	36	.514	.390	.124**	3.56	3.06	.50**

**Significant at the 0.01 level.

lysine in the protein percentage relative to their normal isotypes compared with the findings of Ingversen et al. (1973), Doll et al. (1974) and Doll (1976).

With relatively fewer comparisons by amino acid analysis, (Table 1-9) Risø 9 was not significantly different from normal Bomi in percent lysine in the grain or in the protein, and Risø 56 was different from normal Carlsberg II in percent lysine in the protein at the p = 0.10 level only. All other mutants were significantly higher in percent lysine in the grain and in the protein than their normal isotypes.

Mutant means significantly different from normal ranged from 110% (Risø 7) to 148% (Risø 13) of the normal isotypes for percent lysine in the grain and 112% (Risø 7) to 140% (Risø 1508) for percent lysine in the protein by amino acid analysis (Table 1-9).

Considering shrunken endosperm mutants in general and comparing the induced Risø mutants with the spontaneous mutants (Section I, Study I) the relative lysine in the grain percentages are similar. The Risø mutants have a slightly higher relative percent lysine in the protein than the spontaneous mutants.

General--The results of this study, support previous findings that in barley there is usually a negative association between kernel lysine content and kernel weight or plumpness (Munck et al., 1970; Ingversen et al., 1973; Doll et al., 1974; Doll, 1976; Balaravi et al., 1976,

Table 1-9. Amino acid analyzer lysine comparisons of induced shrunken endosperm mutants and their normal isotypes.

Risø mutant	No. of comparisons	Mean lysine in grain (%)			Mean lysine in protein (%)		
		Mutant isotype	Normal isotype	Difference mutant-normal	Mutant isotype	Normal isotype	Difference mutant-normal
7	3	.502	.456	.046*	4.14	3.69	.45**
8	3	.613	.456	.157**	4.78	3.69	1.09**
9	3	.456	.456	.000	3.80	3.69	.11
13	3	.673	.456	.217*	4.88	3.69	1.19**
1508	7	.594	.428	.166**	5.05	3.62	1.43**
29	4	.571	.450	.121**	4.58	3.77	.81**
56	3	.601	.453	.148*	4.52	3.90	.62
86	5	.593	.441	.152**	4.47	3.70	.77**

*,**Significant at the 0.05 and 0.01 levels, respectively.

Section I, Study I and Section III, Study IV). In Section I, Study I it was postulated that a starch dilution effect could cause mutant-normal differences in protein and lysine content. This hypothesis may be tested as in Section I, Study I. Adjusted mutant-normal differences in percent protein were non-significant for Risø 29, 56 and 86 (Table 1-10). The deposition of starch in these mutants may be restricted more than protein deposition during grain filling. The adjusted percent protein values for the Bomi , Risø mutants were significantly lower than normal Bomi , indicating that protein as well as starch deposition is severely affected in the mutant kernel.

For all mutants, except Risø 8 and 9, the adjusted mutant percent lysine in the grain was significantly higher than their normal isotypes. This may indicate that a genetic deficiency in kernel plumpness or weight increases lysine deposition in the kernel, as adding starch or weight to the kernel did not eliminate a predicted high lysine content in a plump kernel.

It would appear that, if these theoretical considerations have predictive value, those mutants that maintain a significantly higher percent lysine in the grain with calculated added kernel weight should have the most potential in a breeding program for high lysine barley. It must be considered that these theoretical results do not indicate that the negative association between shrunken endosperm or low kernel weight and high lysine can necessarily be overcome using these mutants.

Table 1-10. Theoretical comparisons of induced shrunken endosperm mutants adjusted to normal kernel weights and their normal isotypes for Kjeldahl protein and microbiological assay of lysine in the grain.

		Mean protein (%)			Mean lysine in grain (%)		
Risø mutant	No. of comparisons	Adjusted mutant isotype	Observed normal isotype	Difference mutant-normal	Adjusted mutant isotype	Observed normal isotype	Difference mutant-normal
7	28	12.5	13.2	-0.7**	.412	.384	.028*
8	25	10.3	13.1	-2.8**	.388	.386	.002
9	26	11.6	13.2	-1.6**	.380	.387	-.007
13	27	11.8	13.1	-1.3**	.433	.385	.048**
1508	44	11.0	13.3	-2.3**	.481	.386	.095**
29	33	13.2	13.0	0.2	.482	.397	.085**
56	25	13.3	13.0	0.3	.494	.386	.108**
86	33	12.9	12.8	-0.1	.456	.397	.059**

*,**Significant at the 0.05 and 0.01 levels, respectively.

Summary

This study has determined that the designated spontaneous shrunken endosperm mutants of barley (seg1-7 and sex1) are low in kernel weight and high in grain protein and lysine. The high amylose mutant (amo1) of the cultivar Glacier was also found to be low in kernel weight and high in grain lysine but not protein. This adds eight "new" mutants to the list of barley high lysine mutants described in the literature.

The induced high lysine Risø mutants studied (Risø 7, 8, 9, 13, 1508, 29, 56, and 86) have a reduced kernel weight and in general have a higher grain lysine content relative to their normal isotypes in the environments considered, than has been previously reported in the literature. All mutants except Risø 7 and 9 had higher seed protein as well.

A similar spectrum of mutants occurred, whether they were spontaneous and visually selected or induced and chemically selected. Considering all the mutants involved in this study, whether they are designated as "high lysine" or "shrunken endosperm" appear to be a matter of choice. A cause and effect relationship has yet to be established.

By mathematically adjusting the mutant kernel weights to normal kernel weights and recalculating percent protein and lysine in the grain for both the spontaneous and induced mutants, a variety of results were obtained. The adjusted percent protein of the mutants was either the same or lower than their respective normal isotypes, but none remained higher. Overall, most mutants remained higher in adjusted percent

lysine in the grain but some were the same and some were lower than their respective normal isotypes. This indicates that perhaps most of the mutants would maintain a positive mutant-normal difference in lysine percentage, if normal kernel weights were achieved through breeding efforts.

SECTION II: EFFECT OF ENVIRONMENT ON BARLEY SHRUNKEN ENDOSPERM MUTANTS

Introduction

In evaluating new or mutant plant types isogenic analysis may play an essential role. Mutant-normal comparisons of means for selected characters are important in characterizing a mutant, but these indicate only average responses. Determining the adaptation and stability of a mutant over a range of environments or within certain environment groupings, is also desirable and critical to the total evaluation of the mutant. The effect of environment on a plant type is usually termed genotype x environment interaction. Regression analysis is one general approach to determining and explaining genotype x environment interactions.

This study encompassed the nine spontaneous and eight induced high lysine, shrunken endosperm mutants and their normal isotypes, which have been comprehensively described for kernel weight and lysine and protein contents in Section I. The objective of this study was to determine the effect of environment on several kernel and agronomic characters of these mutants using a regression analysis approach. Selection of a given trait of interest should be enhanced by knowledge of the kind of environments in which the trait is best expressed.

Materials and Methods

Seventeen shrunken endosperm or low kernel weight mutants and their normal variety isotypes were involved in this study; the same mutant-normal pairs as those considered in Section I, except the Betzes seg2 - Betzes pair is excluded. The same kernel weight, percent protein and percent lysine paired data, as in Section I, were utilized here, except they were used in the application of correlation and regression analyses. In addition, yield, test weight, percent plump, percent thin, plant height, heading date and percent lodging paired data were analysed. With the exception of percent plump and percent thin for some of the sex and seg mutants, the additional characters were measured in conjunction with Montana Intrastate and USDA Western Spring Barley Yield nurseries grown at Bozeman, Huntley, Sidney, Moccasin, Fairfield, Havre and Kalispell, Montana. A randomized complete block design with four replications was used in these trials with three or four row plots, 6 or 3 meters in length, respectively, and with a row spacing of 30 centimeters.

The central 1.44 m^2 of each plot was harvested for yield determinations. Test weights were determined with a standard Ohaus test weight measuring device. Percent plump (on 2.4 x 19 mm sieve) and percent thin (through 2.2 x 19 mm sieve) were determined with a timed sieve shaker device from 100 g random kernel samples from the seed harvested from each plot. Plant heights were the average of three measurements taken from the ground to the tip of the main culm (less the awns) in the

central 1.44 m^2 of each plot after the plants had fully headed. Heading date was determined when 50 percent of the plants in the plot had headed (at least 1/2 of the head emerged from boot of the main culm). Percent lodging was determined by the degree and prevalence of lodging at harvest time.

Paired data from 1966-1978 over a relatively wide range of Western environments were analyzed. Means were compared by paired t-test, and correlation and regression analyses were performed for each mutant-normal isotype pair for each character studied. The normal isotype was assigned the x-array and the mutant isotype the y-array. Regression coefficients (b_{yx}) were calculated with the recognition that the x (normal isotype) variable was not fixed. The assumption is made that for each x, the sample value of y is drawn from a normal population that has a mean, $\mu = \alpha + \beta_{x\,j}$ and a constant variance σ^2_{yx} (Snedecor and Cochran, 1967).

In addition to the statistics ($\bar{x}$ $\bar{y}$, r^2 and b_{yx}) derived from the data, graphs were constructed for each character studied utilizing the appropriate means and b_{yx} values. The named isotype (y-axis) was plotted against the normal isotype (x-axis). If the named isotype is the normal isotype, a unity line results with a slope and r equal to 1.0. If the named isotype is the mutant isotype, the resulting line is a linear regression line with a slope equal to the appropriate b_{yx}.

Results and Discussion

Interpretation of Regression Analysis

The unity line of the regression plots is interpreted as the response of the normal isotype and the regression line the response of the mutant isotype over the range of environments tested. The assumption is made that the x-axis represents a range of environments (toward the x,y axes coordinate are low protein, yield, plant height, etc. environments and toward the opposite end are high protein, yield, plant height, etc., environments).

The intercept of the mutant isotype mean with the normal isotype mean determines the relative position of the regression line. The slope of the regression line (b_{yx}) is interpreted as a measure of adaptation or how a mutant responds to changes in environmental conditions in comparison to a selected "check". A t-test is performed to determine if the calculated regression coefficient is significantly different from 1.0 or the unity line (Snedecor and Cochran, 1967). This allows interpretation of the mutant response relative to the response of the normal isotype. It allows for an answer to the question, "Does the mutant isotype respond differently than the normal isotype to changes in the environment as measured by the "check" or normal isotype?".

The magnitude of the coefficient of determination (r^2) indicates the percentage of the total variation that is accounted for by the

mutant-normal association. The greater the effect of variation in the environment on the response of either mutant (y) or normal (x), the lower the r^2 value. These variations would appear in an analysis of variance as a portion of the genotype x environment interaction, the remaining portion being represented by deviation of b_{yx} from a slope of 1.0. These components are not differentiated in the genotype x environment interaction term in an analysis of variance.

The coefficient of determination is also a measure of the closeness of fit of the mutant (y) values to the regression line. This may be equated with a measure of stability (Bilbro and Ray, 1976). Since mutant isotypes are compared to respective normal isotypes in this study, stability is defined by both r^2 and whether b_{yx} is different from a slope of 1.0. For a given character, a stable mutant is one that has a significant and high r^2 and a b_{yx} that is not different from a slope of 1.0. A stable mutant responds with a high degree of predictability and is consistently greater than, equal to, or less than the normal isotype for a given character in all environments tested.

When considering characters like yield, in which absolute values are measured and plotted, a b_{yx} of 1.0 indicates a constant mutant-normal relationship in absolute units, but this relationship in percentage terms changes along the length of the regression line. Conversely, when a b_{yx} is different from 1.0, the mutant-normal percentage relationship may be constant, while the absolute relationship varies.

If the regression coefficient is significantly different from 1.0, we may attribute this to an explainable difference associated with a significant genotype x environment interaction as might be observed in an analysis of variance table, whereas $1.0 - r^2$ could be interpreted as the non-associated portion of the genotype x environment interaction.

Single (non-replicated) paired comparisons can be used in this regression analysis approach, whereas, they could not be in an analysis of variance.

Kernel Weight

All mutants were lower in mean kernel weight than their normal isotypes. The environmental response of the mutants for kernel weight was variable (Table 2-1 and Figure 2-1). The slopes of the regression lines of sex1, seg5, amo1 and Risø 9 are not different from 1.0 indicating environmental responses for kernel weight similar to their respective normal isotypes over all environments tested. The r^2 values are relatively low, and for sex1 and seg5, r is non-significant, indicating a considerable amount of variation not associated with the responses of the normal isotypes.

The calculated b_{yx}'s for the remainder of the mutants are significantly different from a slope of 1.0 (Table 2-1), which means that for a given mutant-normal pair, kernel weight differences are greater or less depending upon environmental responses of the normal isotype. There may be environments where the mutant-normal means are different

Table 2-1. Kernel weight (mg) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx}†	r^2‡
Spontaneous mutants									
Compana	sex1	9	47.8	39.1-54.7	39.8	33.4-49.9	8.0**	0.42	.180
Compana	seg3	8	46.9	39.1-51.7	26.2	23.1-31.4	20.7**	-0.25**	.116
Compana	seg4	9	47.8	39.1-54.7	37.8	35.0-40.3	10.0**	0.15**	.157
Betzes	seg1	17	34.3	21.9-40.1	18.8	12.4-22.1	15.4**	-0.05**	.005
Ingrid	seg6	8	39.7	35.2-43.8	14.5	11.5-18.0	25.2**	0.54**	.617*
Ingrid	seg7	8	37.5	31.2-48.6	27.4	17.4-39.2	10.1*	-0.59*	.202
Glacier	seg5	7	45.9	40.4-51.4	32.6	26.9-35.9	13.3**	0.46	.368
Glacier	amo1	20	49.4	42.1-55.6	46.9	38.3-57.8	2.5**	0.77	.479**
Hiproly Norm	Hiproly lys1	26	49.2	39.9-57.0	38.4	32.9-45.1	10.8**	0.48**	.501**
Induced mutants									
Bomi	Risø 7	28	44.7	35.3-54.0	42.2	34.6-50.8	2.5**	0.67**	.680**
Bomi	Risø 8	26	45.0	36.1-54.0	32.6	25.0-42.9	12.4**	0.52**	.420**
Bomi	Risø 9	26	45.1	36.1-52.0	39.6	27.8-54.3	5.5**	0.85	.428**
Bomi	Risø 13	27	45.0	36.1-54.0	35.7	30.1-41.6	9.3**	0.47**	.698**
Bomi	Risø 1508	44	43.4	34.0-54.0	34.3	27.7-42.4	9.1**	0.46**	.711**
Carlsberg II	Risø 29	33	39.3	32.3-47.8	36.5	28.7-43.1	2.8**	0.67**	.706**
Carlsberg II	Risø 56	25	39.7	32.9-47.8	35.9	30.8-41.1	3.8**	0.55**	.419**
Carlsberg II	Risø 86	33	39.2	32.3-47.8	34.0	26.6-42.1	5.2**	0.56**	.326**

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

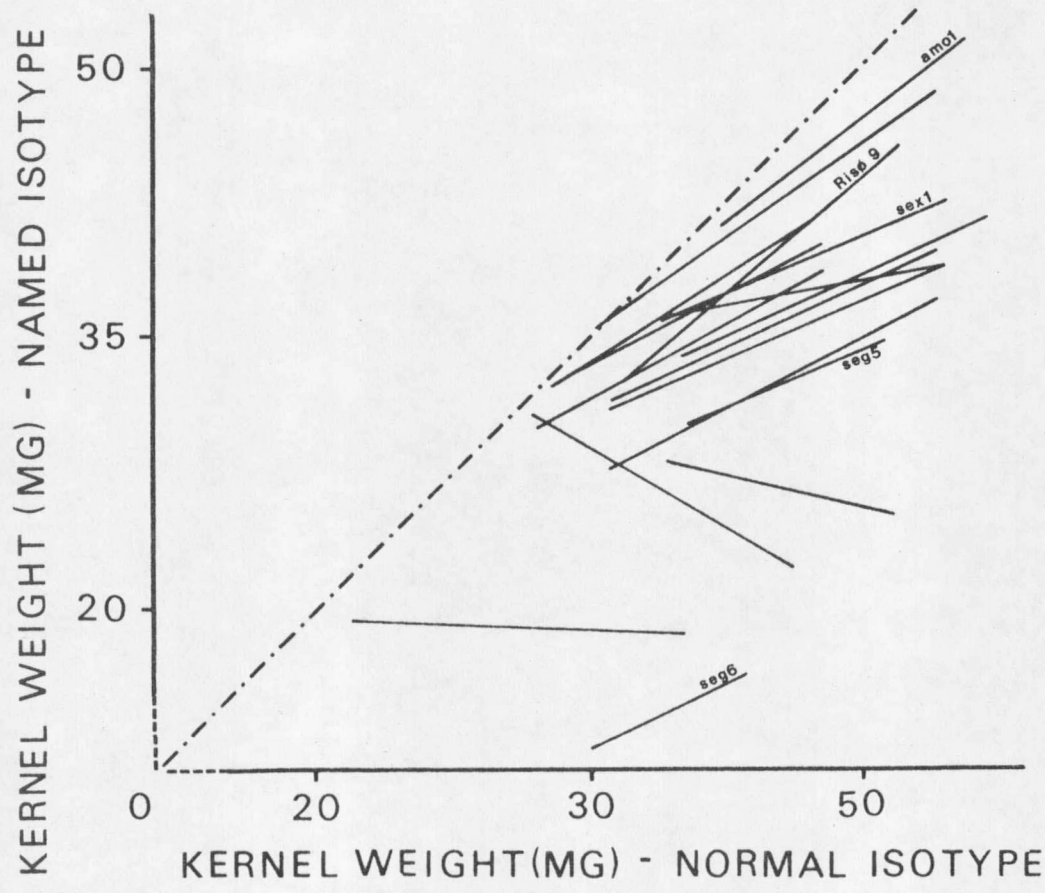


Figure 2-1. Kernel weight relationships between barley shrunken endosperm mutants and their respective normal isotypes.

and environments where the means are not different. For this grouping of mutants, in which the b_{yx} is different from 1.0, the mutant-normal differences diminish in the lower kernel weight environments as established by the normal isotype (Figure 2-1). Lower kernel weight environments probably represent conditions of stress.

If the regression line for Ingrid - Ingrid seg6 in Figure 2-1 is noted, and the mean difference and r^2 in Table 2-1 considered, in spite of the b_{yx} being different than 1.0, the conclusion, even with extrapolation, would be that in all environments seg6 would have a significantly lower kernel weight than Ingrid .

Considering Figure 2-1 for all mutant-normal pairs with a b_{yx} different than 1.0, the greatest mutant-normal differences are observed in the higher kernel weight environments. Selection for or against this trait would be most productive under conditions that favor large seed of the normal isotype, since this is where the shrunken trait is best expressed.

Percent Protein

All mutants except amol, Risø 7 and Risø 9 were higher in mean seed protein, percentage than their respective normal isotypes in the environments considered, while all correlations are significant and most r^2 values are relatively high (Table 2-2).

For those mutant-normal pairs with a b_{yx} not different from 1.0, the percent protein relationship between mutant and normal would not be

Table 2-2. Percent grain protein comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} †	r^2 ‡
Spontaneous mutants									
Compana	sex1	14	14.5	10.0-17.3	17.9	15.4-21.9	-3.4**	0.74	.517**
Compana	seg3	12	14.4	10.0-17.3	16.6	14.5-20.6	-2.2**	0.62**	.602**
Compana	seg4	10	14.2	10.0-17.3	16.5	14.9-19.0	-2.3**	0.49**	.642**
Betzes	seg1	18	14.6	12.3-18.6	15.9	12.8-18.6	-1.3**	0.65**	.768**
Ingrid	seg6	13	13.2	11.4-16.2	15.0	11.9-17.2	-1.8**	0.66	.518**
Ingrid	seg7	11	12.8	9.6-14.9	15.0	13.0-16.7	-2.2**	0.74	.810**
Glacier	seg5	10	12.6	10.2-14.3	16.3	11.6-20.7	-3.7**	1.66	.634**
Glacier	amo1	21	12.5	8.5-16.2	12.6	8.4-15.5	-0.1	1.03	.885**
Hiproly Norm.	Hiproly lys1	26	17.2	13.2-19.3	18.4	14.0-20.5	-1.2**	0.65**	.530**
Induced mutants									
Bomi	Risø 7	28	13.2	8.3-16.8	13.2	9.8-17.1	0.0	0.88*	.921**
Bomi	Risø 8	26	13.2	8.3-16.8	14.4	9.8-17.1	-1.2**	0.90	.862**
Bomi	Risø 9	28	13.2	8.3-16.8	13.2	8.9-17.4	0.0	0.75	.467**
Bomi	Risø 13	28	13.2	8.3-16.8	15.0	11.5-18.3	-1.8**	0.84*	.842**
Bomi	Risø 1508	47	13.2	8.3-16.8	13.8	9.3-16.8	-0.6**	0.84*	.874**
Carlsberg II	Risø 29	36	12.9	8.8-17.6	14.0	9.6-18.7	-1.1**	0.99	.923**
Carlsberg II	Risø 56	28	12.9	8.8-16.5	14.7	9.4-19.3	-1.8**	1.04	.741**
Carlsberg II	Risø 86	36	12.7	8.8-17.6	14.7	9.6-21.4	-2.0**	1.19	.798**

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

expected to change significantly from environment to environment for the environments evaluated. Mutant-normal pairs with a b_{yx} different from 1.0 have b_{yx} values less than one, which indicates that greater mutant-normal differences in percent protein would be expected in environments which favor low protein in the normal isotype (Table 2-2 and Figure 2-2). Further, if the mutant-normal pairs with significant mean differences were grown only in environments which favor high grain protein concentrations in the normal isotype, non-significant mean differences may be observed. If the Risø 7- Bomi pair is considered (mean difference = 0.0, b_{yx} = .88*, r^2 = .921**), a significant mean difference may occur if only very high or very low protein environments for 'Bomi' were evaluated (Figure 2-2). The central concept is that once a regression line with a b_{yx} different from 1.0 is established (particularly with a high r^2) for a range of environments, then the relationship between mutant and normal (significance of mean difference) may change dependent upon the environment or group of environments evaluated.

Several mutants are quite stable for percent protein, particularly amol, Risø 8 and 29. This is indicated by a b_{yx} not different from 1.0 and a high r^2. A predictable and consistant response relative to their normal isotypes would be expected from these mutants over the range of environments tested.

It might be expected that parallel responses would occur in these mutants for kernel weight and percent kernel protein. In general this

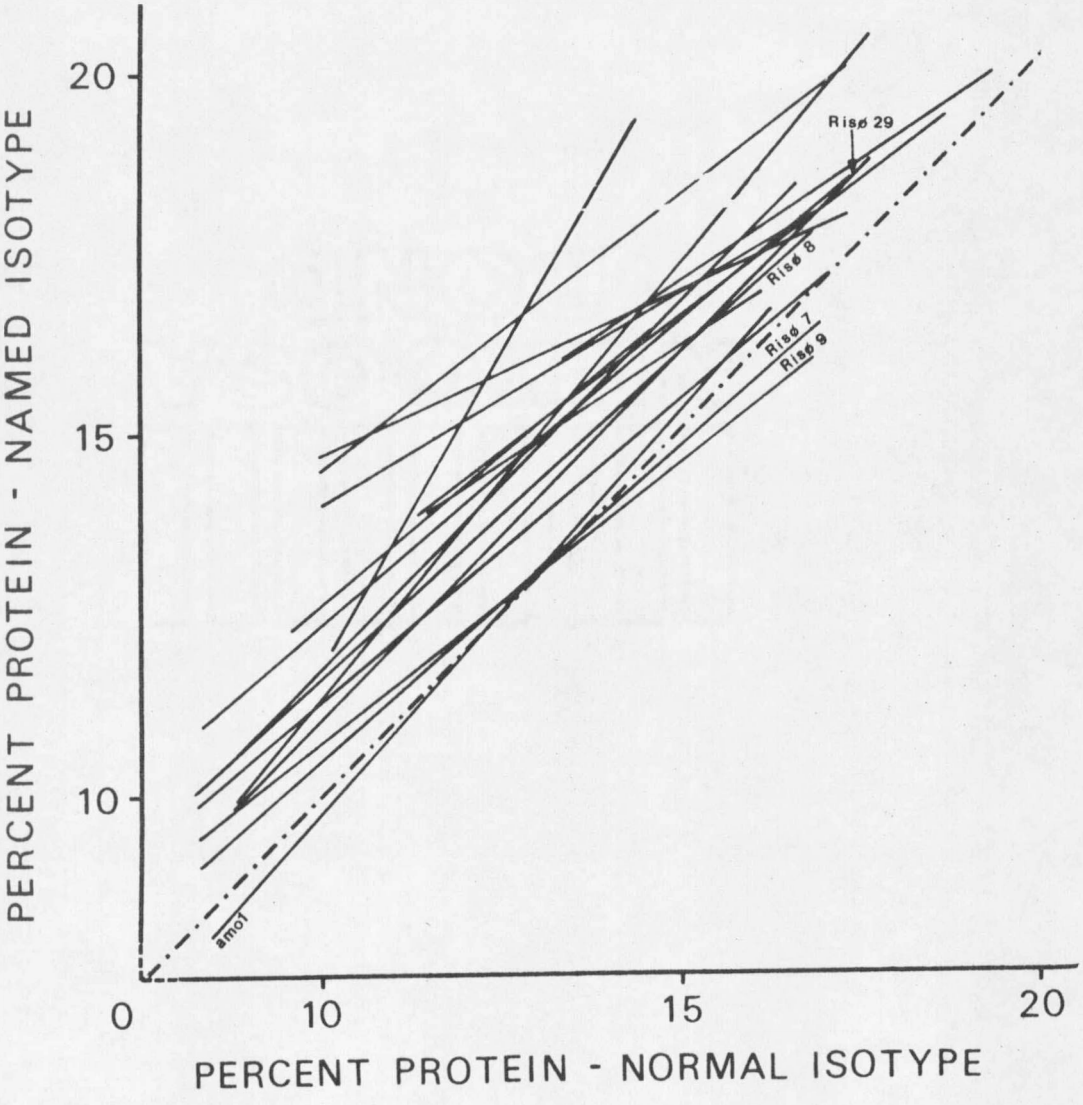


Figure 2-2. Percent protein relationships between barley shrunken endosperm mutants and their respective normal isotypes.

is the case. Low kernel weight in the mutants is best expressed in environments that favor large seed in the normal isotypes (Figure 2-1) and high percent protein in the mutants is best expressed in environments that favor low percent protein in the normal isotype (Figure 2-2), and in general, low kernel weight and high protein environments and high kernel weight and low protein environments are synonymous.

Percent Lysine in the Grain

The percent lysine in the grain mean differences are significant for all mutant-normal pairs and most correlations are significant, although the r^2 values are relatively low. Over all, the response of the mutants is quite uniform, indicated by all but two of the b_{yx} values being not different from 1.0 (Table 2-3).

The highly significant mean differences or the relationships between the mutant and normal means have positioned the regression lines well above the unity line (Figure 2-3). Considering the slopes of the lines, and even with extrapolation, it is concluded that selection for high lysine using percent lysine in the grain as the criterion could be done with these mutant sources in any environment. However, percent lysine in the grain can be strictly a function of percent protein in the grain.

Table 2-3. Percent lysine in the grain comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} †	r^2 ‡
Spontaneous mutants									
Compana	sex1	14	.379	.31-.49	.598	.41-.78	-.219**	2.08**	.747**
Compana	seg3	12	.391	.32-.49	.534	.37-.64	-.143**	1.62	.638**
Compana	seg4	10	.403	.32-.49	.624	.37-.72	-.221**	1.45	.364
Betzes	seg1	18	.436	.34-.53	.518	.40-.59	-.082**	0.34**	.213
Ingrid	seg6	13	.431	.39-.51	.565	.46-.72	-.134**	0.38	.057
Ingrid	seg7	11	.407	.34-.45	.488	.43-.62	-.081**	0.75	.176
Glacier	seg5	10	.369	.31-.47	.473	.37-.66	-.104**	1.85	.658**
Glacier	amo1	21	.373	.31-.52	.430	.33-.61	-.057**	1.16	.668**
Hiproly Norm.	Hiproly lys1	26	.448	.36-.52	.581	.42-.167	-.133**	0.99	.455**
Induced mutants									
Bomi	Risø 7	28	.384	.30-.52	.435	.30-.66	-.051**	1.11	.498**
Bomi	Risø 8	27	.386	.30-.52	.548	.33-.70	-.162**	1.13	.384**
Bomi	Risø 9	28	.384	.30-.52	.434	.33-.66	-.050**	0.73	.233**
Bomi	Risø 13	28	.384	.30-.52	.544	.33-.72	-.160**	1.28	.493**
Bomi	Risø 1508	47	.386	.30-.52	.607	.40-.80	-.221**	1.02	.247**
Carlsberg II	Risø 29	36	.391	.27-.55	.507	.35-.70	-.116**	0.87	.388**
Carlsberg II	Risø 56	28	.379	.27-.49	.538	.28-.77	-.159**	1.34	.461**
Carlsberg II	Risø 86	36	.390	.27-.55	.514	.33-.75	-.124**	0.66	.124*

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

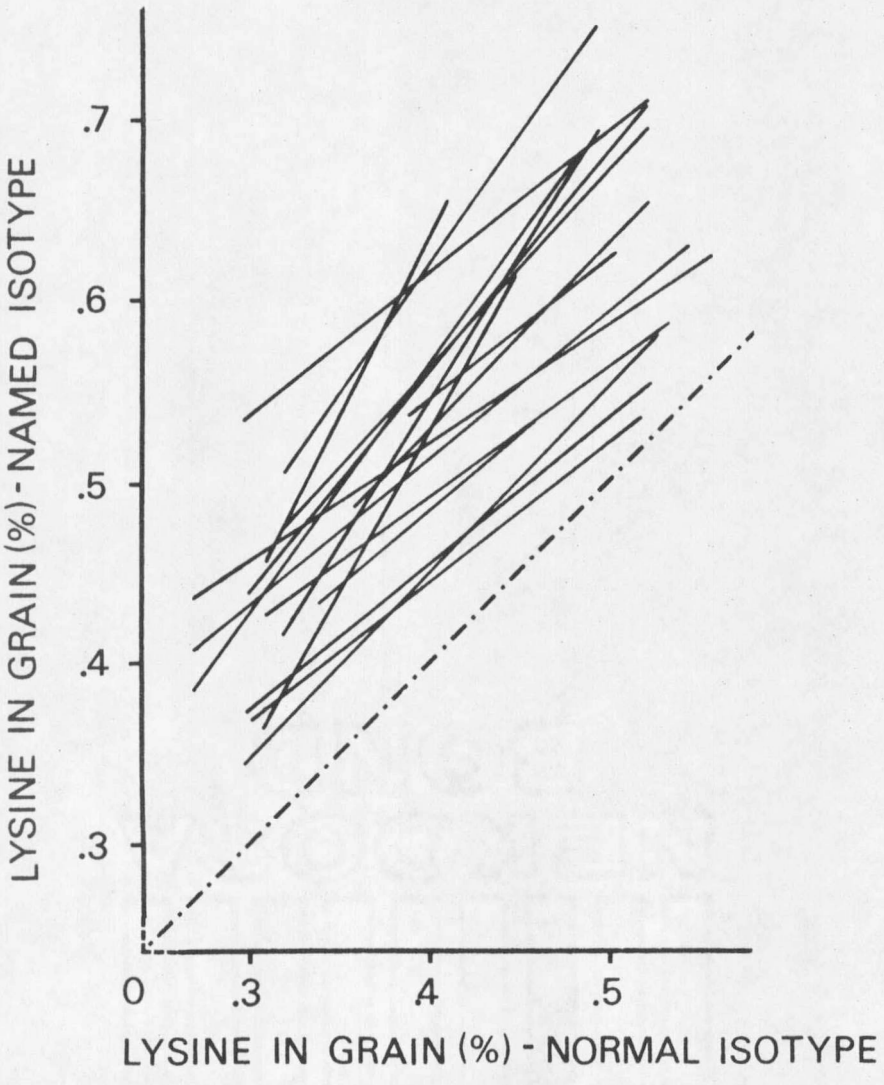


Figure 2-3. Percent lysine in the grain relationships between barley shrunken endosperm mutants and their respective normal isotypes.

Percent Lysine in the Protein

The responses of the mutants relative to their normal isotypes for lysine percentage in the protein (Table 2-4 and Figure 2-4) was considerably more variable than for lysine percentage in the grain (Table 2-3 and Figure 2-3).

Three basic response trends are observed for percent lysine in the protein (Table 2-4 and Figure 2-4). Those mutant-normal pairs that do not display a significant mean difference in percent lysine in the protein (seg5- Glacier and seg7- Ingrid) have significant correlations and b_{yx} values that are not different from 1.0. This indicates that non-significant mutant-normal difference in percent lysine in the protein is probable in any environment, and that the difference in lysine in the grain between mutant and normal is due to the shrunken kernel type only.

Another group of mutant-normal pairs have significant mean differences and b_{yx} values not different from 1.0. These mutants should maintain lysine in the protein percentages above their normal isotypes regardless of environmental conditions. Two of these mutants, sex1 and seg4 are particularly stable in their response relative to Compana , their normal isotype.

The third group of mutants, typified by Risø 1508, Risø 8 and Hiproly lys1 are higher in percent lysine in the protein than their respective normal isotypes, and the respective b_{yx} values are less than

Table 2-4. Percent lysine in the protein comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} ±	r^2 ‡
Spontaneous mutants									
Compana	**sex1**	**14**	2.66	2.03-3.52	3.37	2.33-4.67	-0.71**	1.40	.738**
Compana	seg3	12	2.76	2.12-3.52	3.40	2.20-5.10	-0.64**	1.25	.464*
Compana	seg4	10	2.87	2.12-3.52	3.79	2.34-4.56	-0.92**	1.41	.732**
Betzes	seg1	18	3.05	2.62-3.59	3.25	2.65-4.08	-0.20**	0.58*	.401**
Ingrid	seg6	13	3.29	2.76-3.73	3.75	3.16-4.56	-0.46**	0.53	.101
Ingrid	seg7	11	3.20	2.77-3.54	3.25	2.75-4.03	-0.05	1.22	.497*
Glacier	seg5	10	2.94	2.35-.345	2.93	2.10-3.59	0.01	0.95	.584*
Glacier	amo1	21	3.00	2.43-3.88	3.44	2.64-4.51	-0.44**	0.95	.498**
Hiproly Norm.	Hiproly lys1	26	2.61	2.18-3.09	3.15	2.53-3.46	-0.54**	0.14**	.016
Induced mutants									
Bomi	Risø 7	28	2.93	2.27-3.81	3.30	2.70-4.69	-0.37**	0.76	.318**
Bomi	Risø 8	26	2.92	2.27-3.81	3.80	3.19-4.65	-0.88**	0.34**	.121
Bomi	Risø 9	28	2.93	2.27-3.81	3.32	2.54-4.65	-0.39**	0.29**	.056
Bomi	Risø 13	28	2.93	2.27-3.81	3.68	2.76-4.86	-0.75**	0.66	.299**
Bomi	Risø 1508	47	2.95	2.27-4.31	4.39	3.23-5.43	-1.44**	0.22**	.037
Carlsberg II	Risø 29	36	3.07	2.35-4.01	3.66	2.90-4.59	-0.59**	0.45**	.160*
Carlsberg II	Risø 56	28	2.97	2.35-3.74	3.69	2.69-5.26	-0.72**	0.74	.238**
Carlsberg II	Risø 86	36	3.06	2.35-4.01	3.65	1.78-6.32	-0.50**	0.32*	.026

*,** Significant at the 0.05 and 0.01 levels, respectively.
± Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

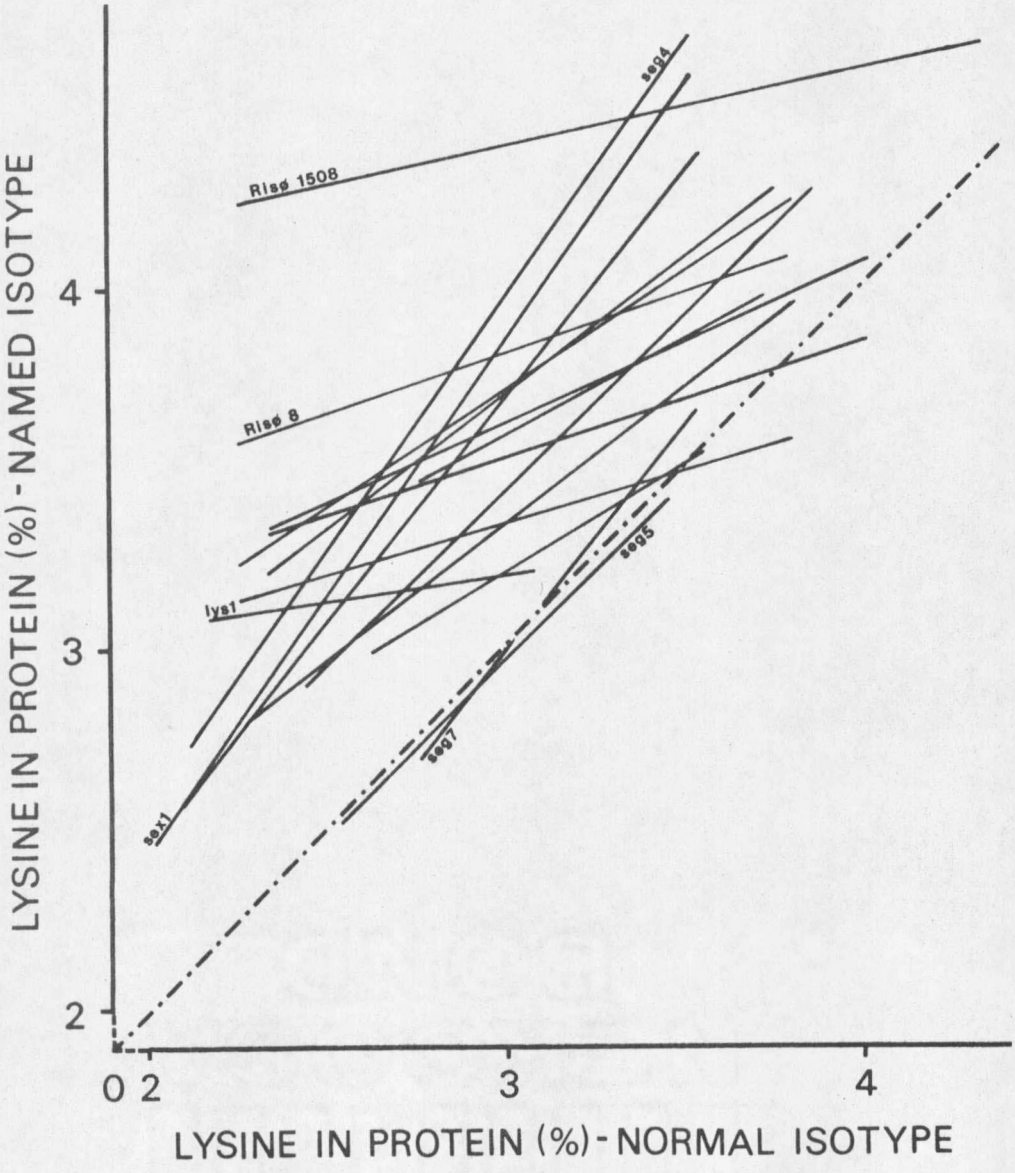


Figure 2-4. Percent lysine in the protein relationships between barley shrunken endosperm mutants and their respective normal isotypes.

1.0. For these mutants the trait is best expressed in environments conducive to low percent lysine in the protein of the normal isotypes. In spite of the low b_{yx} value of Risø 1508- Bomi , the position of the regression line is such that selection for high percent lysine in the protein should be successful in any of the environments evaluated. This conclusion cannot be made for some of the other mutants with b_{yx} values different from 1.0, e.g., Risø 9.

Yield

Risø 7 and 9 and amol were not different in yield from their normal isotypes (Table 2-5). This relationship should be stable over the range of environments evaluated for amol (b_{yx} not different from 1.0 and high r^2). There are environments in which Risø 7 and 9 could be significantly higher or lower in yield than Bomi (b_{yx} less than 1.0), but little association was determined between Risø 9 and Bomi indicated by a non-significant correlation and by a low r^2 (Table 2-5 and Figure 2-5).

The remainder of the mutants were lower in yield than their respective normal isotypes. For some of the mutant-normal pairs this relationship should be stable over the range of environments tested, e.g., Risø 29, (b_{yx} not different from 1.0 and high r^2), while for the others, the mutant-normal absolute difference significantly diminished in the lower yield environments for the normal isotype, e.g., Risø 86 (b_{yx} less than 1.0). Because of the position of the regression line,

Table 2-5. Yield (quintals/ha) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx}†	r^2‡
Spontaneous mutants									
Betzes	segl	9	32.8	11.5-49.2	10.1	4.9-31.9	22.7**	0.56*	.677**
Glacier	amol	8	34.3	16.1-45.3	33.6	13.3-43.9	0.7	0.82	.801**
Hiproly Norm.	Hiproly lysl	8	21.9	10.8-35.8	14.9	8.6-27.2	7.0**	0.75	.886**
Induced mutants									
Bomi	Risø 7	8	38.7	10.2-53.3	35.0	10.5-49.3	3.7	0.78*	.956**
Bomi	Risø 8	8	38.7	10.2-53.3	28.5	6.7-47.5	10.2**	0.69	.823**
Bomi	Risø 9	8	38.7	10.2-53.3	28.0	18.8-39.2	10.7	-0.14**	.137
Bomi	Risø 13	8	38.7	10.2-53.3	28.5	7.3-50.2	10.2**	0.73	.793**
Bomi	Risø 1508	25	36.6	10.2-60.6	29.3	8.3-52.7	7.3**	0.80*	.780**
Carlsberg II	Risø 29	16	34.8	10.1-56.8	30.6	7.9-53.6	4.2**	0.91	.935**
Carlsberg II	Risø 56	9	33.2	15.7-56.8	26.6	7.2-42.8	6.6**	0.83	.911**
Carlsberg II	Risø 86	16	34.8	10.1-56.8	26.6	6.0-43.8	8.2**	0.81*	.897**

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

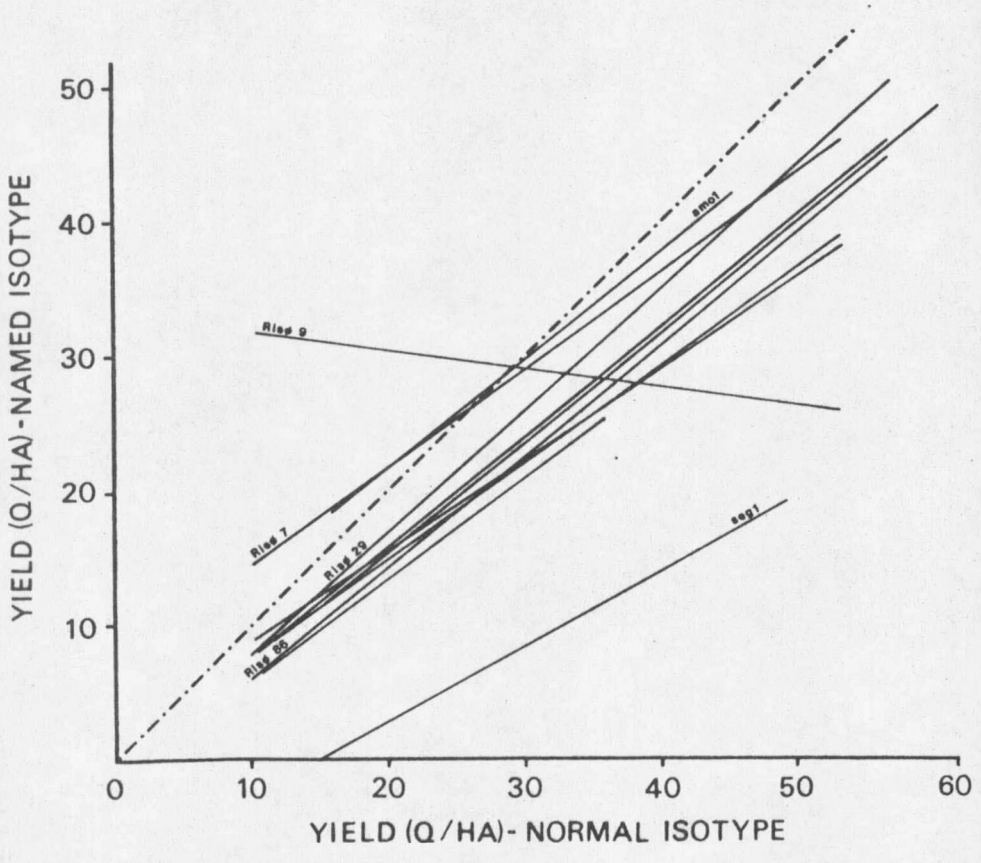


Figure 2-5. Yield relationships between barley shrunken endosperm mutants and their respective normal isotypes.

it is probable the significant seg1- Betzes yield difference would be maintained in all environments in spite of its less than 1.0 b_{yx} value (Table 2-5 and Figure 2-5).

An ultimate goal is to have available an acceptable yielding high lysine commercial feed variety for non-ruminant livestock, particularly in the Northwestern U.S., where costly protein supplements must be shipped in. Risø 1508 is the best mutant source of grain lysine (.607%, Table 2-3). Based on the lysine requirement for swine (.65% average, Natl. Res. Council, 1973) approximately 85% of the soybean oil meal (2.9% lysine, Church and Pond, 1974) could be saved in an average swine ration using Risø 1508 over normal Bomi . However, based on current average market values (barley - $5./45 kg, soybean oil meal - $7./23 kg), a grower would stand to lose approximately 6% economically, considering the 20% yield reduction of Risø 1508 compared to Bomi (calculated from the data in Table 2-5). These calculations are based on means, but the results are not altered appreciably over the range of environments evaluated. Based on these data Risø 1508 should be an excellent source of high lysine material for the breeder.

A mean mutant-normal yield difference was not detected for amo1 and Risø 7, and based on percent lysine in the grain expectations, about 20% of the soybean oil meal in a swine ration could be saved using either of these mutants over their normal isotypes. This translates into about a 3% dollar savings. At a predicted yield level of

18 quintals/ha (33.5 bu/a) for Risø 7 (b_{yx} = .78*, Figure 2-5) and amo1 (b_{yx} = .82), a 19% and 13% dollar savings, respectively, could be expected. This does not take into account the lysine digestibility of these barleys compared with that of soybean oil meal, but for normal barley it is less (Eggum, 1973). An 18 quintal/ha yield level is not unrealistic in Montana under dryland conditions, and further testing of Glacier amo1 and Bomi Risø 7 under these conditions is indicated.

Test Weight

Most mutants had a lower test weight than their respective normal isotypes, but a variety of environmental responses was observed (Table 2-6 and Figure 2-6). In general, there is considerable mutant variability that cannot be explained on the basis of the mutant-normal association, indicated by relatively low r^2 values.

The response of seg1 relative to normal Betzes is the most deviant (Figure 2-6) in comparison with the other mutant responses (large negative b_{yx}). Glacier , amo1 and perhaps Risø 7 are not different in test weight over the range of environments considered. The response of amo1 is considerably more stable than that of Risø 7.

The means of the remainder of the mutant-normal pairs differ significantly. This difference should be maintained by those mutant-normal pairs with a b_{yx} not different from 1.0. For those mutant-normal pairs with a b_{yx} different from 1.0, the mutant-normal difference

Table 2-6. Test weight (kg/hl) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} †	r^2 ‡
Spontaneous mutants									
Betzes	segl	8	63.2	59.2-67.7	42.4	30.3-55.7	20.9**	-2.47**	.512*
Glacier	amol	8	57.6	58.3-60.6	56.6	50.9-60.6	1.0	0.90	.647*
Hiproly Norm.	Hiproly lysl	8	71.4	67.7-77.5	66.1	58.2-71.6	5.3**	0.78	.652
Induced mutants									
Bomi	Risø 7	7	63.0	59.2-65.1	62.3	60.0-65.9	0.7	0.38	.197
Bomi	Risø 8	7	63.0	59.2-65.1	53.7	48.2-58.0	9.3*	0.73	.196
Bomi	Risø 9	7	63.0	59.2-65.1	56.4	49.9-61.0	6.6**	0.74	.170
Bomi	Risø 13	7	63.0	59.2-65.1	56.0	52.5-57.7	7.0**	-0.29*	.126
Bomi	Risø 1508	24	63.2	53.8-67.6	56.4	48.9-63.1	6.8**	0.33*	.075
Carlsberg II	Risø 29	15	62.1	50.8-67.1	57.3	48.5-62.4	4.8**	0.61*	.575**
Carlsberg II	Risø 56	8	62.4	60.0-65.1	56.7	53.0-62.4	5.7**	-0.35	.036
Carlsberg II	Risø 86	15	62.1	50.8-67.1	54.2	49.0-60.4	7.9**	0.40*	.175

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

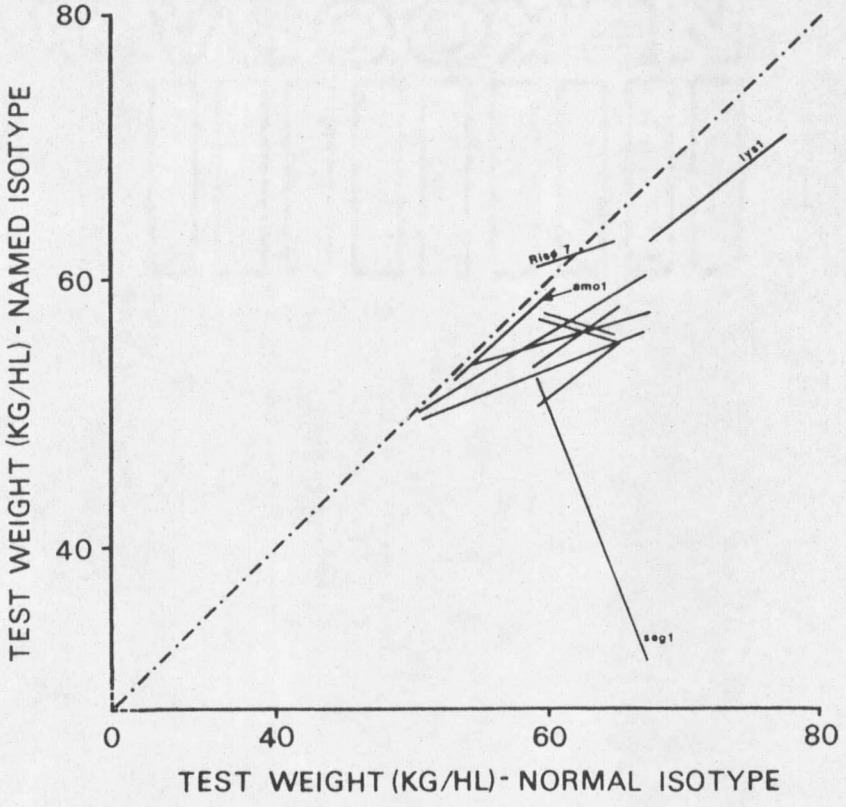


Figure 2-6. Test weight relationships between barley shrunken endosperm mutants and their respective normal isotypes.

diminishes in the lower kernel weight environments established by the normal isotypes.

The lofty position of the Hiproly - Hiproly Normal regression line is in part due to the fact that these cultivars are hulless.

Percent Plump and Percent Thin

Similar results were obtained for these two characters (Tables 2-7 and 2-8). The oppositeness of the traits is reflected in the means themselves, and the sameness of the environmental responses of the mutants relative to their normal isotypes had produced essentially mirror image regression lines or graphs of the two traits (Figures 2-7 and 2-8).

Risø 7 and amol were not different from Bomi and Glacier, respectively in percent plump and percent thin, and both responded in a relatively stable manner as their normal isotypes do, as indicated by the respective b_{yx} values being not different from 1.0 and relatively high r^2 values.

The rest of the mutant-normal mean differences for each mutant for each character are significant. For those mutant-normal pairs with a b_{yx} different from 1.0, the difference diminishes in low percent plump and high percent thin environments. Selection for or against these traits would be best accomplished under conditions that favor large seed in the normal isotype. This is in agreement with the conclusion previously made concerning kernel weight and the environments in which the shrunken character of these mutants is best expressed.

Table 2-7. Percent plump (on 2.4 x 19 mm sieve) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference x-y	b_{yx} †	r^2 ‡
Spontaneous mutants									
Compana	sex1	9	81.7	67.1-94.8	25.8	7.5-63.6	55.9**	0.49	.078
Compana	seg3	8	81.0	67.1-94.8	10.1	3.2-27.2	70.9**	-0.32**	.184
Compana	seg4	9	81.7	67.1-94.8	22.1	3.0-58.6	59.6**	0.32	.032
Betzes	seg1	19	46.8	0.0-74.3	3.9	0.0-31.6	42.9**	0.10**	.113
Ingrid	seg7	8	75.1	40.0-93.1	22.3	4.2-44.0	52.8*	0.54	.546*
Glacier	seg5	7	84.0	70.1-94.3	72.5	50.2-83.7	11.5*	0.46	.137
Glacier	amo1	20	86.3	59.8-97.1	84.3	58.7-97.2	2.0	0.89	.655**
Hiproly Norm.	Hiproly lys1	26	73.7	15.8-95.3	17.7	1.0-47.3	55.0**	0.25**	.141
Induced mutants									
Bomi	Risø 7	9	60.5	30.4-91.7	52.7	16.4-86.7	7.8	0.94	.519*
Bomi	Risø 8	9	60.5	30.4-91.7	16.2	2.1-29.3	44.3**	0.48**	.659**
Bomi	Risø 9	9	60.5	30.4-91.7	32.6	5.5-57.0	27.9**	0.53	.301
Bomi	Risø 13	9	60.5	30.4-91.7	16.7	1.5-61.7	43.8**	0.39	.188
Bomi	Risø 1508	24	62.0	15.2-95.0	36.7	1.6-88.7	25.3**	1.08	.766**
Carlsberg II	Risø 29	15	63.1	15.3-96.0	40.7	1.9-85.3	22.4**	1.07	.645**
Carlsberg II	Risø 56	9	55.0	15.3-89.7	26.6	1.7-63.4	28.4**	0.66	.350
Carlsberg II	Risø 86	15	63.1	15.3-96.0	33.0	1.3-86.0	30.1**	0.89	.653**

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

Table 2-8. Percent thin (through 2.2 x 19 mm sieve) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx}†	r^2‡
Spontaneous mutants									
Compana	sex1	9	7.4	2.6-15.4	37.5	11.2-80.1	-30.1**	0.68	.022
Compana	seg3	8	7.6	2.6-15.4	75.7	37.7-89.4	-68.1**	-1.57	.215
Compana	seg4	9	7.4	2.6-15.4	41.3	15.6-84.6	-33.9**	0.18	.001
Betzes	seg1	19	27.8	6.5-91.1	88.7	39.5-100.0	-60.9**	0.11**	.023
Ingrid	seg7	8	9.3	1.9-24.6	59.5	26.4-85.5	-50.2**	1.69	.380
Glacier	seg5	7	5.9	2.8-11.8	10.5	5.2-16.0	-4.7*	0.70	.214
Glacier	amo1	20	4.2	0.7-10.8	5.0	0.6-21.7	-0.8	1.09	.490**
Hiproly Norm.	Hiproly lys1	26	12.1	1.3-57.2	46.0	13.0-93.6	-33.9**	1.16	.416**
Induced mutants									
Bomi	Risø 7	9	16.4	3.3-39.5	22.0	4.1-54.5	-5.6	1.35	.772**
Bomi	Risø 8	9	16.4	3.3-39.5	55.9	21.5-88.2	-39.5**	1.99	.690**
Bomi	Risø 9	9	16.4	3.3-39.5	32.2	15.8-61.4	-15.8**	1.22	.548**
Bomi	Risø 13	9	16.4	3.3-39.5	59.4	13.6-94.2	-43.0**	1.72	.406
Bomi	Risø 1508	20	18.4	1.6-49.8	35.4	2.7-76.7	-17.0**	1.39	.613**
Carlsberg II	Risø 29	12	17.8	1.2-58.4	33.3	1.7-70.0	-15.5*	1.14	.415*
Carlsberg II	Risø 56	9	20.9	3.7-58.4	41.0	7.0-88.4	-20.1*	0.81	.227
Carlsberg II	Risø 86	12	17.8	1.2-58.4	42.1	1.7-88.9	-24.3**	1.20	.376*

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

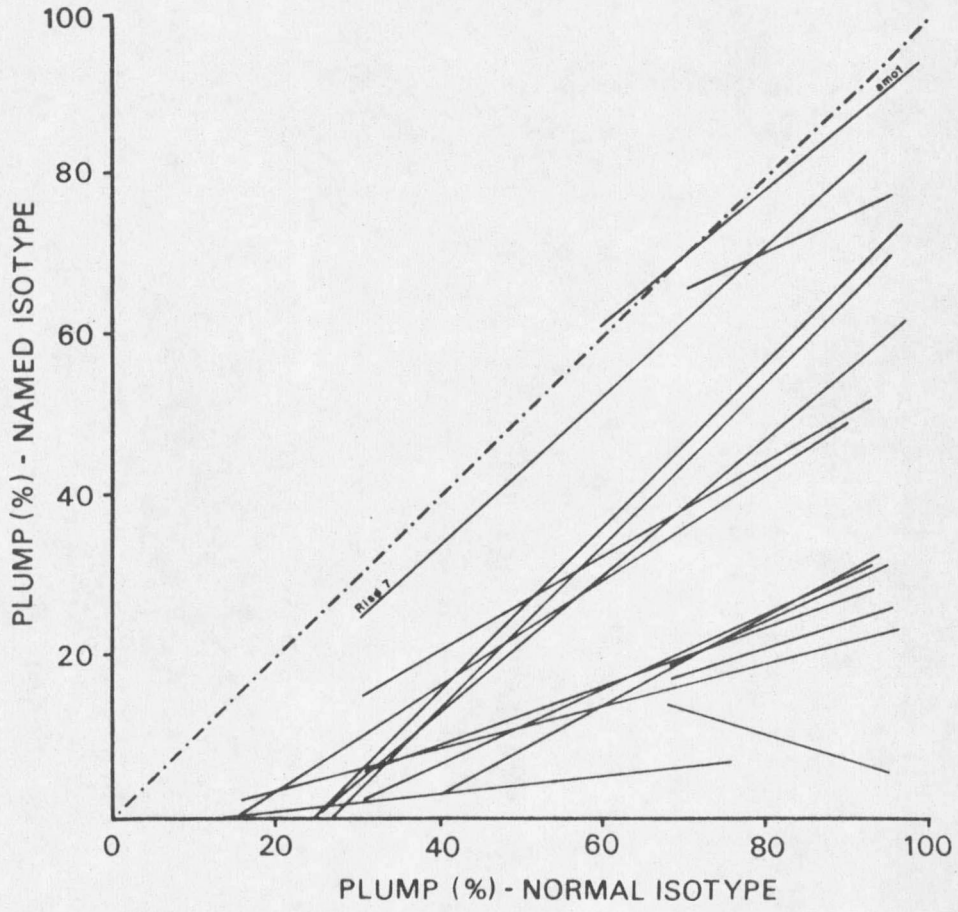


Figure 2-7. Percent plump (on 2.4 x 19 mm sieve) relationships between barley shrunken endosperm mutants and their respective normal isotypes.

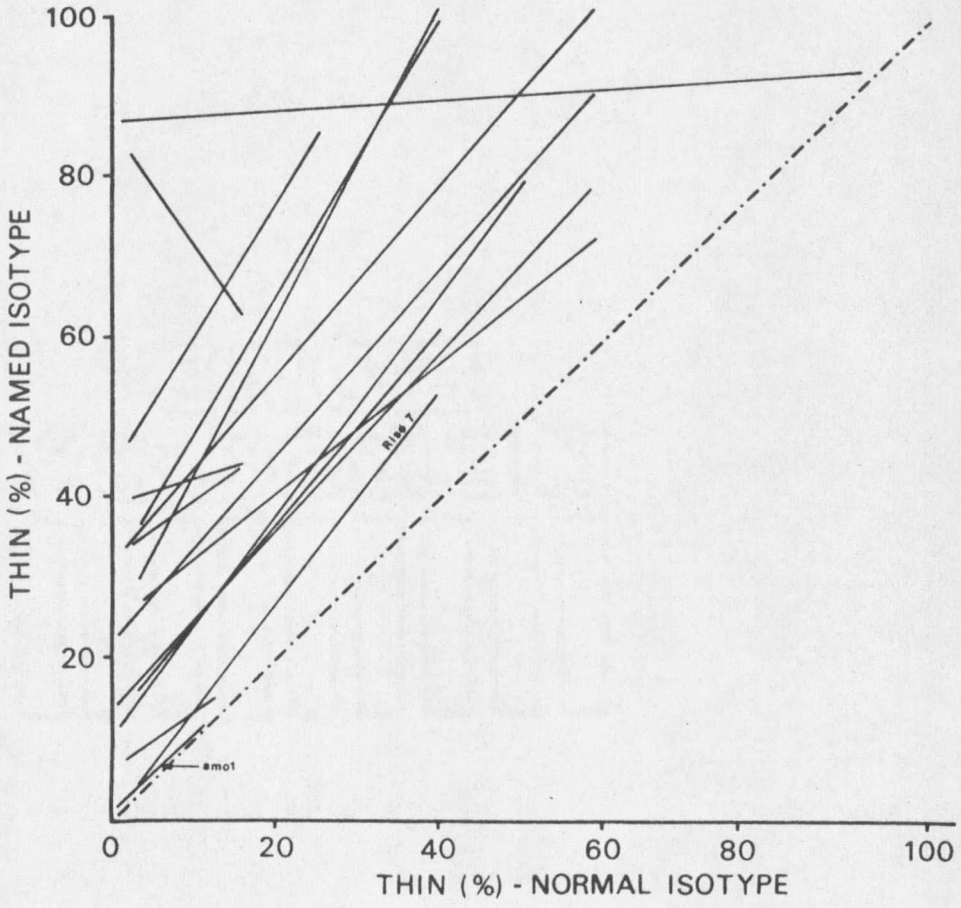


Figure 2-8. Percent thin (through 2.2 x 19 mm sieve) relationships between barley shrunken endosperm mutants and their respective normal isotypes.

Plant Height

Although some mutants were significantly shorter than their normal isotypes, the magnitude of the differences were not great (Table 2-9). This is further emphasized by the cluster of regression lines about the unity line (Figure 2-9). However, the mutant-normal pairs with significant mean plant height differences are stable in this relationship as indicated by b_{yx} values not different from 1.0 and high r^2 values. With regard to those mutants not different in plant height from their normal isotypes, the response of amo1, Risø 8 and 29 was stable relative to their normal isotypes, while that of the other mutants (seg1, Hiproly lys1 and Risø 86) was unstable in their response. There may be environments in which a significant mutant-normal difference would be obtained.

Differences in plant height could indicate vigor responses associated with differences in the relative weight of the seed planted.

Heading Date

Several mutants, on the average, head later than their respective normal isotypes, and of these Risø 7 and 8 are relatively stable in their response relative to their normal isotypes (Table 2-10 and Figure 2-10). Because the b_{yx} of the Risø 86 - Carlsberg II pair is different from 1.0, there may be environments in which the difference in their heading dates is non-significant. This may be the case for Hiproly lys1 also considering the b_{yx} and r^2 values.

Table 2-9. Plant height (cm) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} †	r^2 ‡†
Spontaneous mutants									
Betzes	segl	9	79.5	50.0-116.8	78.2	54.1-108.7	1.3	0.79*	.938**
Glacier	amol	7	72.2	40.6- 94.0	72.6	40.6-101.6	-0.4	1.12	.973**
Hiproly Norm.	Hiproly lysl	7	74.7	40.6-101.6	72.2	43.2- 96.5	2.5	0.90*	.992**
Induced mutants									
Bomi	Risø 7	7	80.9	66.0- 91.4	77.3	66.0- 91.4	3.6*	0.98	.878**
Bomi	Risø 8	7	80.8	66.0- 91.4	82.0	66.0- 94.0	-1.2	0.97	.844**
Bomi	Risø 9	7	80.9	66.0- 91.4	76.9	61.0- 86.4	4.0*	0.90	.878**
Bomi	Risø 13	7	80.9	68.2- 91.4	76.2	58.4- 88.9	4.7**	1.17	.976**
Bomi	Risø 1508	23	71.3	36.1- 99.1	68.8	38.1- 97.0	2.8**	0.97	.957**
Carlsberg II	Risø 29	15	68.1	43.2- 91.4	68.6	43.1- 91.4	-0.5	0.96	.974**
Carlsberg II	Risø 56	7	76.9	58.4- 94.0	70.0	55.9- 81.3	6.9**	0.76	.865**
Carlsberg II	Risø 86	16	69.7	43.2- 94.0	70.5	48.3- 91.4	-0.8	0.86*	.942**

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

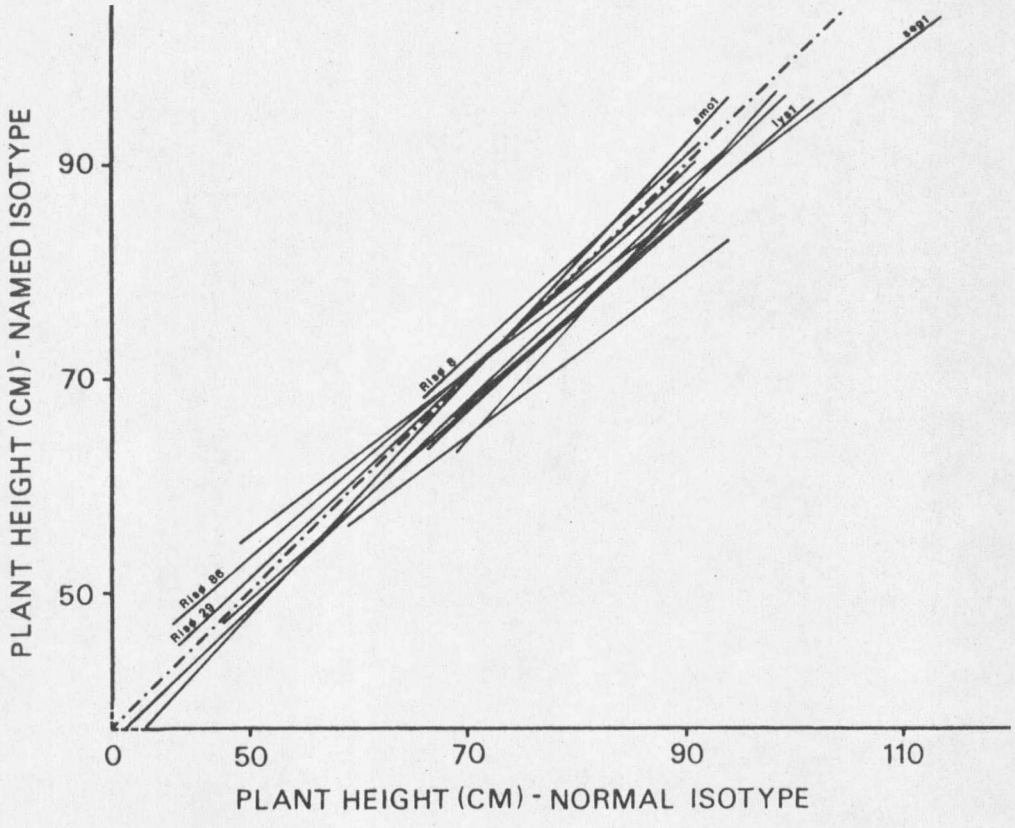


Figure 2-9. Plant height relationships between barley shrunken endosperm mutants and their respective normal isotypes.

Table 2-10. Heading date (days from Jan. 1) comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} †	r^2 ‡
Spontaneous mutants									
Betzes	segl	9	180.1	173.5-185.6	183.4	171.0-192.4	-1.8	1.71**	.998**
Glacier	amol	6	170.0	169.0-175.0	169.8	170.0-171.0	0.2	0.17**	.205
Hiproly Norm.	Hiproly lysl	6	175.5	175.0-179.0	178.5	175.0-182.2	-3.0*	0.38	.223
Induced mutants									
Bomi	Risø 7	7	181.8	168.0-204.0	183.1	170.0-205.0	-1.3**	0.98	.997**
Bomi	Risø 8	7	181.8	168.0-204.0	184.2	171.0-206.7	-2.4**	0.96	.986**
Bomi	Risø 9	7	181.8	168.0-204.0	183.8	171.0-207.0	-2.0	0.70	.510
Bomi	Risø 13	7	181.9	168.0-204.0	182.7	170.0-205.0	-0.8	0.95	.992**
Bomi	Risø 1508	19	183.5	159.0-210.0	184.1	160.0-207.0	-0.6	0.94*	.990**
Carlsberg II	Risø 29	12	187.4	170.0-206.0	189.8	173.0-206.0	-2.4	0.84	.881**
Carlsberg II	Risø 56	7	181.4	170.0-203.0	188.4	179.0-205.7	-7.0**	0.72	.852**
Carlsberg II	Risø 86	12	187.4	170.0-206.0	192.8	179.0-208.0	-5.4**	0.73*	.864**

*,** Significant at the 0.05 and the 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

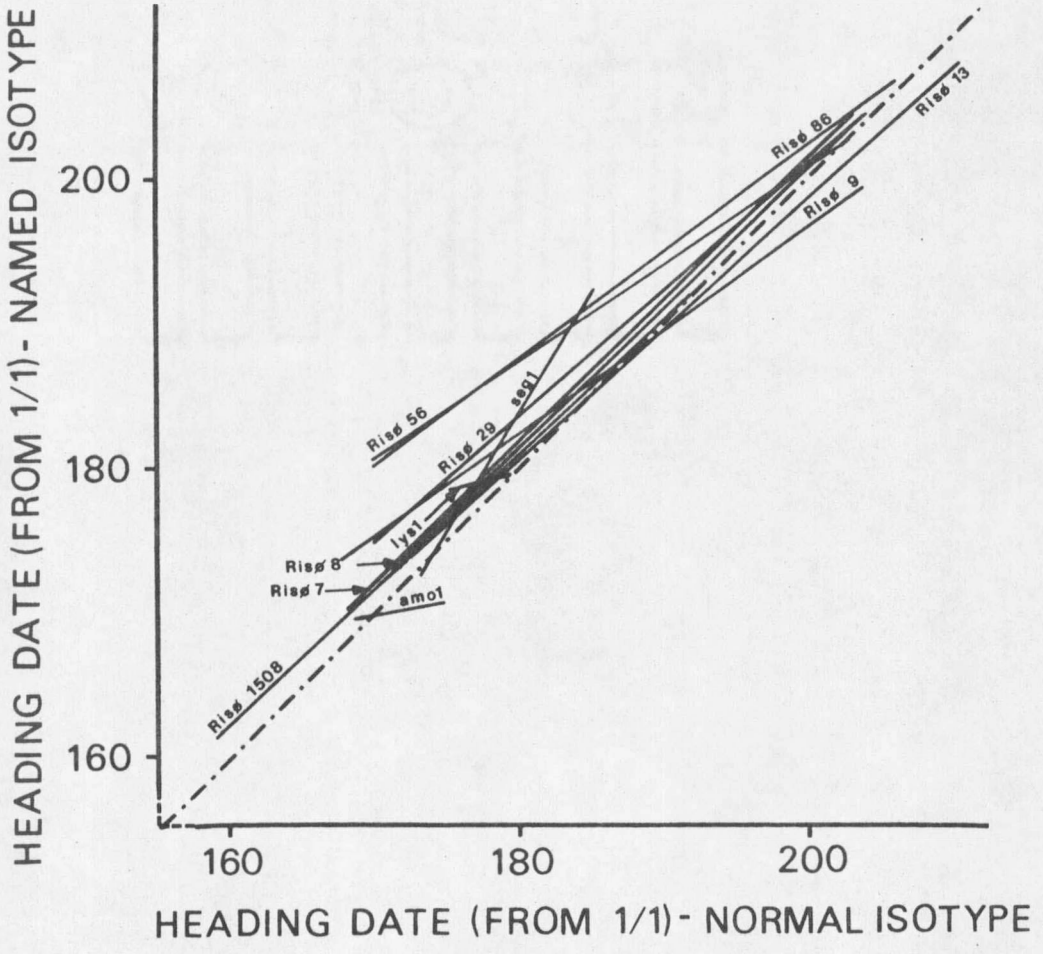


Figure 2-10. Heading date (days from Jan. 1) relationships between barley shrunken endosperm mutants and their respective normal isotypes.

The mean difference in heading date is not different for six of the mutant-normal pairs, three of which (segl- Betzes , amol- Glacier and Risø 1508- Bomi) could have significant differences in some environments (b_{yx} is different from 1.0). Risø 9, 13 and 29 should have the same relative heading date as their respective normal isotypes over the range of environments tested (b_{yx} not different from 1.0).

Percent Lodging

No significant mutant-normal differences in percent lodging were detected, but the magnitude of difference of several pairs was large (Table 2-11). The response of Risø 29 and 86 were comparatively stable relative to Carlsberg II , while Risø 1508 was unstable relative to Bomi . Overall, few comparisons were made, so no graph was prepared as few conclusions could be made.

Summary

Correlation and regression analysis was used to determine the environmental adaptation and stability of seventeen barley shrunken endosperm mutants relative to their normal isotypes for eleven chemical and physical kernel and agronomic characters. This was an extension of mutant characterization by paired mutant-normal mean comparisons. The respective mutant and normal means and, therefore, the mean differences depend upon the environments or groups of environments represented and most prevalent. Regression analysis allows an evaluation over a range

Table 2-11. Percent lodging comparisons between barley shrunken endosperm mutants and their normal isotypes.

Normal cultivar	Mutant	Number of comparisons	Normal isotype (x) mean	Normal isotype (x) range	Mutant isotype (y) mean	Mutant isotype (y) range	Difference $\bar{x}-\bar{y}$	b_{yx} †	r^2 ‡
Spontaneous mutants									
Hiproly Norm.	Hiproly lysl	2	46.0	3.0-90.0	25.0	0.0-50.0	21.0	0.54	1.000
Induced mutants									
Bomi	Risø 7	3	37.3	11.0-58.0	33.3	6.0-52.0	4.0	0.87	.748
Bomi	Risø 8	2	34.5	11.0-58.0	60.5	42.0-79.0	-26.0	-0.79	1.000
Bomi	Risø 9	3	37.3	11.0-58.0	78.3	45.0-95.0	-41.0	-0.25	.042
Bomi	Risø 13	2	34.5	11.0-58.0	55.0	39.0-76.0	-20.5	-0.89	1.000
Bomi	Risø 1508	9	54.2	11.0-80.0	52.0	3.0-80.0	2.2	0.16*	.034
Carlsberg II	Risø 29	7	56.1	11.0-80.0	51.6	10.0-80.0	4.5	0.96	.653*
Carlsberg II	Risø 56	3	58.0	32.0-72.0	42.7	16.0-87.0	15.3	1.08	.400
Carlsberg II	Risø 86	8	49.8	5.0-80.0	49.1	10.0-80.0	0.7	0.80	.791**

*,** Significant at the 0.05 and 0.01 levels, respectively.
† Significant differences from a slope of 1.0 are starred.
‡ Significant correlations of paired values are starred.

of environments, environment by environment. Assuming comparable ranges of environments and normal isotype responses, mutant-mutant comparisons can be made with more precision also.

The regression coefficient (b_{yx}) was interpreted as a measure of the adaptation of a mutant relative to its normal isotype for a given trait. The stability of the response was determined by considering the coefficient of determination (r^2) and regression coefficient. A stable mutant is defined as one with a b_{yx} not different from 1.0 and a high r^2. The responses of the various mutants for the various characters were quite variable, as was the stability of the responses.

It was concluded from interpreting the regression lines for the characters that describe shrunken endosperm (kernel weight, percent plump and percent thin) that this general trait is best expressed in environments that favor large seed in the normal isotypes. Selection for or against shrunken endosperm should be most productive in these environments, which are probably characterized in Montana by adequate moisture during the growing season. Parallel mutant responses were observed for percent protein.

With the mutants involved in this study selection could be made in any environment for high lysine using percent lysine in the grain as the criterion. If percent lysine in the protein is the criterion for high lysine, selection with some mutants could be made in any environment shile with others, environments favorable for low percent lysine in

the protein of the normal isotypes would be more productive if not required. Perhaps the "ideal" high lysine source for breeding purposes would be from a mutant with a maximum positive mutant-normal mean difference in percent lysine in the protein and a stable environmental response (b_{yx} and r^2 of 1.0). None of the mutants fit all of these criteria, but perhaps Risø 1508, Compana , sex1 and seg4 come the closest.

Most of the mutants were lower in yield and test weight than their respective normal isotypes. The environmental responses were variable. The mean differences and environmental responses were variable for plant height, heading date and percent lodging.

Risø 1508 is perhaps the best source of lysine in the grain, but considering its yield response compared to Bomi it is not economically feasible to grow it commercially as a (swine) feed barley under current market conditions. It may be feasible to grow the lower lysine, but higher yielding Risø 7 and amo1, particularly in lower yielding (dryland) environments.

SECTION III: INHERITANCE OF THE ASSOCIATED KERNEL CHARACTERS, HIGH LYSINE AND SHRUNKEN ENDOSPERM, OF BARLEY

Introduction

Several inheritance studies were conducted with some of the mutants considered heretofore. The overall objective of these genetic studies was to provide a basic understanding of the genes involved, which would allow a breeder of high lysine barley to better manipulate the genes for the production of improved barley germplasm and/or varieties.

Specifically, the studies involved determination of 1) allelic relationships, 2) chromosome location of genes, 3) mode of inheritance, and 4) high lysine-shrunken endosperm relationships.

Materials and Methods

Study I: Allelism Evidence for High Lysine, Shrunken Endosperm Xenia (sex) Mutants

Bomi , Risø mutants 13 and 1508 and Carlsberg II , Risø 29, and 86 express a shrunken endosperm xenia trait as well as high lysine in our environments. These mutants and Compana sex1 were intercrossed to determine allelism among the mutants for the associated shrunken endosperm xenia trait. The females for all crosses were genetic male

steriles. Plump:shrunken classification of F_2 seed on the F_1 plants was the major criterion for the sex allelism test.

As a second approach, twelve F_1 seed from all but one cross (Risø 13 x Risø 1508) were analyzed for lysine by microbiological assay technique in which half kernels are placed on agar plates which contain the bacterium Leuconostoc mesenteroides, which is lysine requiring, in a lysine bioassay medium (Difco). The radius of visible bacterial growth around the kernel is correlated with kernel lysine content. (Sands, Dull and Eslick, manuscript in preparation).

Study II: Chromosome Location of High Lysine Genes or Genes of Associated Traits

Bomi , Risø mutants 8, 13, and 1508, Carlsberg II , Risø 29, 56 and 86 display a shrunken endosperm phenotype that expresses xenia in our environments. Glacier amol displays a high amylose (40%) trait (Merritt, 1967). A high lysine trait is associated with a low kernel weight and the high amylose trait (Section I, Study I). The high amylose trait was followed in this study by an associated starch granule morphology trait as described by Walker and Merritt (1969). These mutants were crossed with a series of Betzes primary trisomics to assign the various traits of these mutants to chromosomes as described by Tsuchiya (1964).

The three F_2 endosperm genotypes (Sex Sex, Sex sex, sex sex) were sometimes difficult to differentiate in crossed progeny for some mutants

under some conditions. The degree of shriveling of the kernels varied somewhat with the environment. Where difficulty was experienced, observed (over all crosses), 3:1 and/or 1:2:1 ratios were tested by Chi-square analysis to try to sort out discrepencies.

Because of particular classification difficulties, lysine analyses by microbiological assay (Waters, 1976) were done on the trisomic x Risø 8 and 56 F_2 progeny to supplement the plump:shrunken classification data.

Study III: Protein and Lysine Maternal Inheritance Effects in 'Hiproly'

To determine if there are maternal effects in the inheritance of protein and/or lysine in Hiproly, reciprocal crosses were made between Hiproly and the "normal" barley variety Betzes. Twelve F_1 seed from each cross were screened for lysine content by the half kernel microbiological assay technique (Sands, Dull and Eslick, manuscript in preparation). The F_2 seed from four F_1 plants from each cross were analyzed for protein by the macro Kjeldahl technique (N x 6.25) and for lysine by a modified microbiological assay (Waters, 1976). Data were analyzed by among and within analysis of variance.

Study IV: Inheritance and Association of the Shrunken Endosperm and High Lysine Traits of Bomi, Risø 1508

Risø 1508 was crossed with 10 normal endosperm barley cultivars (Compana, Carlsberg II, Betzes, 'Heines Hanna', CI 4961, 'Hector', 'Steptow', 'Unitan', 'Piroline', and 'Erbet'). Inheritance was based on the expression of the mutant phenotype in the F_2 (seed on F_1 plants) and F_3 (seed on F_2 plants) generations.

Linkage studies involving known genes were used to assign the shrunken endosperm trait of Risø 1508 to a chromosome. Risø 1508 was crossed with stocks carrying genetic male sterile (msg) genes, one located near the centomere of each of the seven chromosomes of barley (Compana msg10, Compana msg2, Carlsberg II msg5, Betzes msg24, Betzes msg1, Compana msg1, Heines Hanna msg6 and CI 4961 msg19) (Table 3-13). Risø 1508 was also crossed with a Betzes derived stock of the short rachilla hair gene (s). All recombination estimates and standard errors were based on partial F_3 data (F_3 ratios for endosperm type, F_2 ratios for the other traits) and calculated by the maximum likelihood method (Allard, 1956).

The relationship between the shrunken endosperm and high lysine traits of Risø 1508 were determined by plotting frequency distributions of the lysine contents of F_3 progeny (F_3 seed from F_2 plants) from Risø 1508 crosses with 9 of the 10 normal endosperm cultivars listed

above, and identifying the endosperm genotypes on the histogram. Heterogeneity chi-square tests of the frequency distributions were also applied. Mean percent lysine data of the normal endosperm parents and F_3 progeny were compared by unpaired t-test, and the Risø 1508 parent lysine content values (single observations) were compared with the means of the other parents and progenies by a modified t-test (Sokal and Rohlf, 1969).

Lysine contents are expressed as percent in the grain and percent in the protein and were obtained by a modified microbiological assay technique (Waters, 1976). Protein contents upon which percent lysine in the protein values were based were determined by the Neotec Grain Quality Analyzer Model 1 with updated light source.

The material for these studies was grown in space-planted rows at Bozeman, Montana and Mesa, Arizona.

Results and Discussion

Study I: Allelism Evidence for High Lysine, Shrunken Endosperm Xenia (sex) Mutants

Risø 86 and Compana *sex1* and Risø 86 and Risø 29 were the only mutant sources found to be allelic, as no plump seed were found on F_1 plants (Table 3-1) or on F_2 plants from these crosses. It is concluded that the *sex* traits of Risø 29 and 86 are allelic with *sex1a* of Compana . This is consistant with trisomic analysis data (see Section III, Study II) which provided evidence for monofactoral recessive

Table 3-1. Segregation of F_2 seed from F_1 plants for shrunken endosperm from *sex* mutant crosses to determine allelisms.

Cross	Plump	Shrunken	Total	Ratio tested	χ^2	P level
Risø 13 x *sex1*	176	608	784	9:7	363	< .01
				1:1	238	< .01
				3:5	75.8	< .01
				1:3	2.721	.05-.10
Risø 13 x Risø 29	350	472	822	9:7	61.9	< .01
				1:1	18.0	< .01
				3:5	9.04	< .01
				1:3	135	< .01
Risø 13 x Risø 86	341	500	841	9:7	85.0	< .01
				1:1	30.0	< .01
				3:5	3.327	.05-.10
Risø 13 x Risø 1508	306	523	829	9:7	125.4	< .01
				1:1	56.8	< .01
				3:5	0.122	.50-.75
Risø 86 x *sex1*	0	all	-	-	-	-
Risø 86 x Risø 29	0	all	-	-	-	-
Risø 86 x Risø 1508	456	354	810	9:7	0.000	1.00
Risø 1508 x *sex1*	413	338	751	9:7	0.440	.50-.75
Risø 1508 x Risø 29	509	307	816	9:7	12.4	< .01
				1:1	50.0	< .01

inheritance and the assignment of the *sex* traits of Risø 29 and 86 to chromosome 6. Jarvi and Eslick (1975) using translocation breakpoint linkage information assigned Compana *sex1* to chromosome 6. It is proposed that the *sex* traits of Risø 86 and 29 be designated *sex1d* and *sex1e*, respectively, following the barley gene symbol nomenclature suggested by Eslick and Hockett (1976c).

The independent non-allelic genes in crosses with one another should segregate on F_1 heads in a 9:7, plump:shrunken, seed ratio, while linked non-allelic gene segregation should approach a 1:1 ratio, assuming monofactorial recessive gene action for each mutant. Single recessive gene action has been demonstrated for Compana sex1 (Jarvi and Eslick, 1975), Risø 1508 (Section III, Study IV), 29 and 86 (Section III, Study II) and for Risø 13 (Doll, 1976). Furthermore, evidence was provided for the location of the sex trait of Risø 13 on chromosome 6 and Risø 1508 on chromosome 7 (Section III, Study II and IV). A 9:7 or 1:1 F_2 seed progeny ratio was not found for some of the crosses (Table 3-1). For these crosses, in general, a deficiency of plump seed was observed. There are inconsistancies among crosses, particularly those involving Risø 13. No definite conclusions can be drawn and more investigation is needed, but perhaps endosperm dosage effects and/or epistatic interactions between genes is indicated. Minor or modifier genes in the various genetic backgrounds represented may also be involved.

Assuming that high lysine and shrunken endosperm are associated traits in the mutants under consideration (see Doll, 1976; Karlsson, 1977; and Ullrich and Eslick, 1977 and Section III, Study IV), lysine data in Table 3-2 generally support the sex allele test results. With allelism, the F_1 should be equal to, but not less than one or both parents. With non-allelism, the F_1 should be lower than one or both parents. In general, over the range of crosses made, the F_1's tended to

Table 3-2. Microbiological assay lysine index† data of F_1 seed from sex mutant crosses to determine allelisms.

Cross, P_1 x P_2	Mean radii of bacterial growth (mm)				
	P_1§	F_1	P_2	P_1-F_1	P_2-F_1
Risø 13 x sex1	9.9	7.9	10.5	2.0**	2.6*
Risø 13 x Risø 29	10.4	9.5	9.7	0.9+	0.2
Risø 13 x Risø 86	10.2	8.9	8.8	1.3**	-0.1
Risø 86 x sex1	9.5	11.7	11.6	-2.2**	-0.1
Risø 86 x Risø 29	10.2	9.8	9.2	0.4	-0.6
Risø 86 x Risø 1508	11.6	10.2	10.3	1.4+	0.1
Risø 1508 x sex1	11.2	9.1	11.2	2.1*	2.1*
Risø 1508 x Risø 29	10.6	9.2	10.3	1.4*	1.1

+, *, ** Significant at the 0.10, 0.05 and 0.01 levels, respectively, as determined by paired t-test.

† From half kernel microbiological assay technique.

§ P_1 analyses were on seed from the rows in which the female parents occurred, since the females were male sterile.

be higher in lysine than expected. This, as with the plump:shrunken endosperm ratios observed, may indicate epistatic relationships between mutant genes.

Additional information is needed to definitely establish the gene action involved in some of these crosses.

Study II: Chromosome Location of High Lysine Genes or Genes of Associated Traits

High Amylose (amo1)--Evidence for the location of amo1 on chromosome 2 is presented in Table 3-3. Additionally, the occurrence of homozygous recessive F_3 rows from F_2 trisomics from crosses with trisomics 3, 4 and 7 eliminates chromosomes 3, 4 and 7 from consideration. Trisomic 5 cross data are lacking, but Walker (1969) has indicated failure to detect linkage between amo1 and marker genes on chromosomes 4, 5 and 7. Linkage data from a relatively small population for amo1 and V indicated that the two traits were independent. Ramage and Eslick (1975) assigned the V locus approximately 24 recombination units from the centromere and msg2 on the long arm of chromosome 2. Additional information is needed to specifically locate amo1 on chromosome 2.

Table 3-3. F_2 segregation from Betzes trisomic x High Amylose Glacier (amo1) Crosses.

Trisomic crossed with amo1	F_2 plant classification (no.)			χ^2	P for fit to 3:1 ratio
	A--	a a a	Total		
1	21	4	25	1.080	.25-.50
2	57	8	86	5.584	< .05*
3	110	35	145	0.057	.75-.90
4	106	31	137	0.411	.50-.75
6	176	49	225	1.245	.25-.50
7	72	32	104	1.847	.10-.25

* reject the 3:1 hypothesis.

Heterogeniety χ^2 based on a 3:1 ratio = 8.215, $p < .01$. Heterogeniety χ^2 minus the trisomic 2 cross $\chi^2 = 2.631$, $p = .10-.25$.

Risø 1508 (sex3c)--Considering the data of Table 3-4 in total, the shrunken endosperm xenia trait of Risø 1508 would be assigned to chromosome 7. This conclusion is well supported by linkage data presented by Karlsson (1977) and in Section III, Study IV. Scheuring and Ramage (1976) assigned sex2b to an sex mutant on chromosome 7 that is, in effect, a zygotic lethal mutant. It is proposed that the sex trait of Risø 1508 be designated sex3c.

Risø 86 (sex1d) and Risø 29 (sex1e)--In Section III, Study I, evidence is presented that Risø 29 and 86 are allelic to each other and with Compana sex1a. Jarvi and Eslick (1975) assigned sex1 to chromosome 6, and Eslick and Reis (1976) further positioned it near the centromere of chromosome 6. The trisomic analysis data of Table 3-5 and 3-6 indicate that the shrunken endosperm loci of both Risø 29 and 86 are on chromosome 6. Additional support for this conclusion comes from the F_3 row data from F_2 trisomic plants presented in Table 3-7. No homozygous recessive (sex sex) F_3 rows are expected from F_2 plants that are trisomic for the chromosome on which the gene in question is located. The specific gene action (segregation) of the shrunken endosperm or high lysine characters of Risø 29 and 86 has not been reported. Since the sex traits are allelic with Compana sex1, monofactorial recessive gene action might be assumed. If the segregation of shrunken endosperm presented in Tables 3-5 and 3-6 (minus trisomic 6 cross data) are considered then single recessive gene action is concluded for the sex

Table 3-4. F_2 plant segregation from Betzes trisomic x Bomi, Risø 1508 (sex3c) crosses.

Trisomic crossed with Risø 1508	Classification (no.)					Ratio tested	χ^2	P level
	Sex–	Sex Sex	Sex sex	sex sex	Total			
2	–	2	5	0	7	observed	1.470	.25-.50
	–	2	5	0	7	1:2:1	2.429	.25-.50
	7	–	–	0	7	3:1	2.333	.10-.25
3	–	50	78	17	145	observed	0.650	.50-.75
	–	50	78	17	145	1:2:1	15.855	< .01
	128	–	–	17	145	3:1	13.629	< .01
4	–	57	118	39	214	observed	5.540	.05-.10
	–	57	118	39	214	1:2:1	5.290	.05-.10
	175	–	–	39	214	3:1	5.240	< .05
5	–	41	83	21	145	observed	1.925	.25-.50
	–	41	83	21	145	1:2:1	8.558	< .05
	124	–	–	21	145	3:	8.554	< .01
6	–	64	98	48	210	observed	13.761	< .01
	–	64	98	48	210	1:2:1	3.372	.10-.25
	162	–	–	48	210	3:1	0.514	.25-.50
7	–	117	153	23	293	observed	12.219	< .01
	–	117	153	23	293	1:2:1	60.890	< .01
	270	–	–	23	293	3:1	45.963	< .01

Table 3-5. F_2 plant segregation from Betzes trisomic x Carlsberg II, Risø 29 (sexle) crosses.

Trisomic crossed with Risø 29	Classification (no.)					Ratio tested	χ^2	P level
	Sex-	Sex Sex	Sex sex	sex sex	Total			
1	-	0	5	4	9	observed	5.050	.05-.10
	-	0	5	4	9	1:2:1	3.772	.10-.25
	5	-	-	4	9	3:1	1.870	.10-.25
2	-	69	141	62	272	observed	6.614	<.05
	-	69	141	62	272	1:2:1	0.728	.50-.75
	210	-	-	62	272	3:1	0.705	.25-.50
3	-	150	219	120	489	observed	1.372	.50-.75
	-	150	219	120	489	1:2:1	8.999	<.05
	369	-	-	120	489	3:1	0.055	.75-.90
4	-	48	87	41	176	observed	2.087	.25-.50
	-	48	87	41	176	1:2:1	0.580	.50-.75
	135	-	-	41	176	3:1	0.273	.50-.75
5	-	67	67	37	171	observed	4.002	.10-.25
	-	67	67	37	171	1:2:1	18.532	<.01
	134	-	-	37	171	3:1	1.031	.25-.50
6	-	93	70	23	186	observed	30.625	<.01
	-	93	70	23	186	1:2:1	64.064	<.01
	163	-	-	23	186	3:1	15.835	<.01
7	-	30	48	29	107	observed	1.635	.25-.50
	-	30	48	29	107	1:2:1	1.149	.50-.75
	78	-	-	29	107	3:1	1.955	.10-.25

Note: Heterogeniety χ^2 based on 1:2:1 ratio = 58.260, $p < .01$. Heterogeniety χ^2 without trisomic 6 cross data = 20.013, p = .02-.05. Heterogeniety χ^2 without trisomic 5 and 6 cross data = 10.097, p = .25-.50. χ^2 on totals without trisomic 5 and 6 cross data based on 1:2:1 ratio = 5.131, p = .05-.10. Heterogeniety χ^2 based on 3:1 ratio = 16.685, $p < .05$. Heterogeniety χ^2 without trisomic 6 cross data = 5.153, p = .25-.50. χ^2 on totals without trisomic 6 cross data based on 3:1 ratio = 0.736, p = .25-.50.

Table 3-6. F_2 plant segregation from Betzes trisomic x Carlsberg II, Risø 86 (sex1d) crosses.

Trisomic crossed with Risø 86	Classification (no.)				χ^2 for 1:2:1 ratio	P level
	Sex Sex	Sex sex	sex sex	Total		
2	17	26	9	52	2.462	.25-.50
3	42	93	53	188	1.299	.50-.75
4	30	37	23	90	3.923	.10-.25
5	47	66	36	149	3.564	.10-.25
6	34	30	10	74	18.215	< .01
7	23	67	22	122	4.34	.10-.25

Note: Heterogenity χ^2 based on 1:2:1 ratio = 27.895, $p < .01$. Heterogenity χ^2 without trisomic 6 cross data = 14.436, $p = .05 - .10$. χ^2 on totals without trisomic 6 cross data based on 1:2:1 ratio = 1.152, $p = .50-.75$.

Table 3-7. F_3 row segregation from F_2 trisomic plants from Betzes trisomic x Bomi and Carlsberg II Risø *sex* mutant crosses.

Trisomic crossed with Risø mutant	Number of rows					
	Risø 8			Risø 13			Risø 29		
	sex	*sex*	Total	*sex*	*sex*	Total	*sex*	*sex*	Total
3	-	-		2	21		-	-	
4	-	-		6	48		-	-	
5	0	22		4	33		2	30	
6	2	20		0	32		0	29	
	Number of rows					
	Risø 56			Risø 86			Risø 1508		
	sex	*sex*	Total	*sex*	*sex*	Total	*sex*	*sex*	Total
3	0	21		4	24		0	33	
4	-	-		3	25		-	-	
5	0	31		3	22		1	18	
6	0	8		0	13		1	16	

traits of Risø 29 and 86. Considering all the data available, it is proposed that the shrunken endosperm xenia trait of Risø 86 be designated sex1d and that of Risø 29 sex1e.

Risø 13 (sex4f)-- The trisomic analysis data of Risø 13, presented in Table 3-8, indicate that the sex locus be assigned to chromosome 6. This is supported by the F_3 row segregations given in Table 3-7. In Section III, Study I evidence is presented that the sex gene of Risø 13 is non-allelic with sex1 (of chromosome 6) of 'Compana , Risø 29 and Risø 86 as well as with sex3 of Risø 1508. Hence, it is proposed that the shrunken endosperm xenia character of Risø 13 be designated sex4f.

Risø 8 (sex5g)--The trisomic analysis data presented in Table 3-9 indicate that the sex locus of Risø 8 is associated with chromosome 5. The F_3 row segregation data of Table 3-7 support this conclusion. Since Doll (1976) has established the apparent absolute association between shrunken endosperm and high percent lysine in the protein for Risø 8, the lysine data in the histograms of Figures 3-1 and 3-2 also indicate that chromosome 5 (lack of sex sex progeny), and not chromosome 6, is the location of the sex gene of Risø 8. Since chromosome 5 is not the location of any of the other sex mutants described to date, it is proposedthat this gene be designated sex5g.

Table 3-8. F_2 plant segregation from Betzes trisomic x Bomi, Risø 13 (sex4f) crosses.

Trisomic crossed with Risø 13	Classification (no.)				Total	Ratio tested	χ^2	P level
	Sex—	Sex Sex	Sex sex	sex sex				
2	-	33	42	14	89	observed	2.666	.25-.50
	-	33	42	14	89	1:2:1	8.393	< .05
	75	-	-	14	89	3:1	4.079	< .05
3	-	41	79	45	165	observed	6.974	< .05
	-	41	79	45	165	1:2:1	0.491	.75-.90
	120	-	-	45	165	3:1	0.455	.50
4	-	37	104	34	175	observed	6.313	< .05
	-	37	104	34	175	1:2:1	6.325	< .05
	141	-	-	34	175	3:1	2.897	.05-.10
5	-	48	93	30	171	observed	0.693	.50-.75
	-	48	93	30	171	1:2:1	5.106	.05-.10
	141	-	-	30	171	3:1	5.071	< .05
6	-	44	32	7	83	observed	23.446	< .01
	-	44	32	7	83	1:2:1	37.337	< .01
	76	-	-	7	83	3:1	12.148	< .01
7	-	24	46	19	89	observed	0.374	.75-.90
	-	24	46	19	89	1:2:1	0.664	.50-.75
	70	-	-	19	89	3:1	0.633	.25-.50

Table 3-9. F_2 plant segregation from Betzes trisomic x Bomi, Risø 8 (sex5g) crosses.

Trisomic crossed with Risø 8	Classification (no.)					Ratio tested	χ^2	P level
	Sex—	Sex Sex	Sex sex	sex sex	Total			
2	–	45	52	18	115	observed	0.822	.50–.75
	–	45	52	18	115	1:2:1	13.731	< .01
	97	–	–	18	115	3:1	5.360	< .05
3	–	36	42	32	110	observed	11.149	< .01
	–	36	42	32	110	1:2:1	6.436	< .05
	78	–	–	32	110	3:1	0.981	.25–.50
4	–	33	44	8	85	observed	5.561	.05–1.0
	–	33	44	8	85	1:2:1	14.812	< .01
	71	–	–	8	85	3:1	11.016	< .01
5	–	44	25	2	71	observed	16.288	< .01
	–	44	25	2	71	1:2:1	55.901	< .01
	69	–	–	2	71	3:1	18.633	< .01
6	–	21	33	14	68	observed	3.216	.10–.25
	–	21	33	14	68	1:2:1	1.499	.25–.50
	54	–	–	14	68	3:1	0.705	.25–.50
7	–	35	15	15	65	observed	8.733	< .05
	–	35	15	15	65	1:2:1	31.154	< .05
	50	–	–	15	65	3:1	0.128	.50–.75

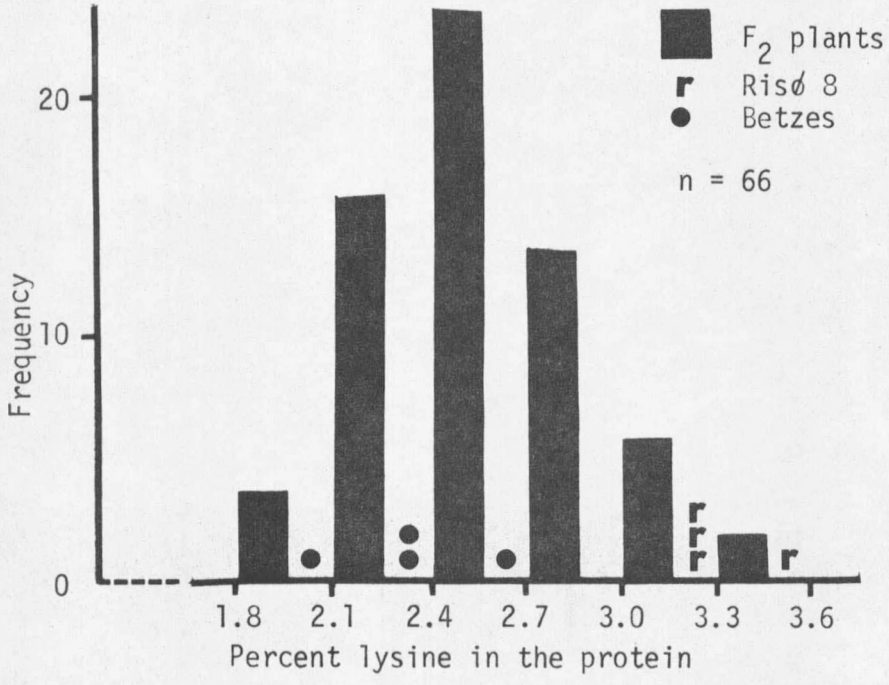


Figure 3-1. Frequency distribution of percent lysine in the protein of F_2 plants from Betzes trisomic 5 x Bomi, Risø 8 crosses.

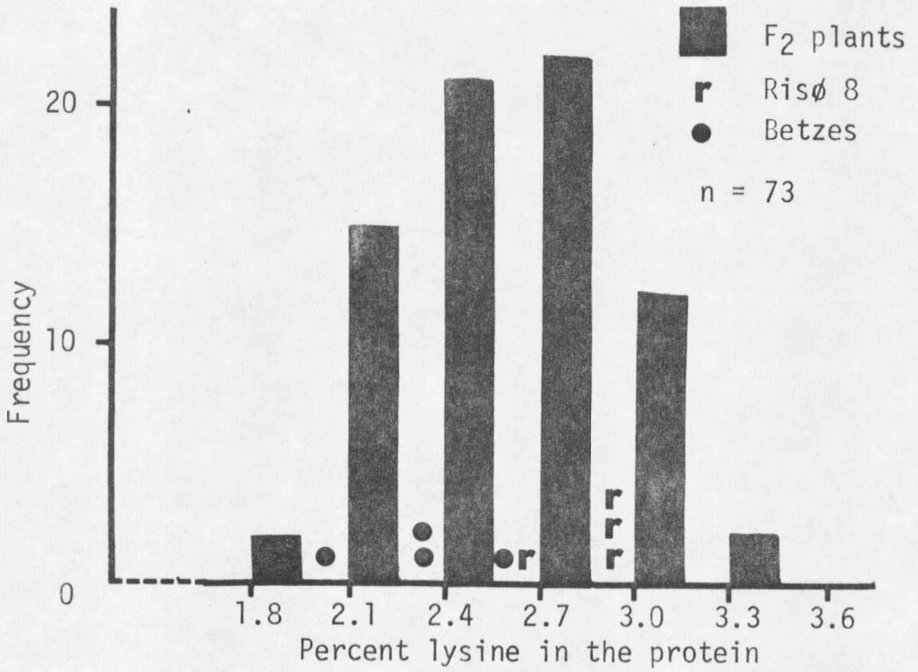


Figure 3-2. Frequency distribution of percent lysine in the protein of F_2 plants from Betzes trisomic 6 x Bomi, Risø 8 crosses.

Risø 56 (sex,h)-- The trisomic data available for Risø 56 is sketchy at best. The sex trait is difficult to read and the number of progeny from most crosses is small. Some conclusions may be drawn, if one assumes single recessive gene action and a shrunken endosperm-high lysine association. The best evidence from Table 3-10 indicates chromosome 4 is the location of the sex trait of Risø 56. The lysine data presented in Figures 3-3, 3-4 and 3-5 indicate chromosome 5 as the associated chromosome while probably eliminating chromosomes 3 and 6. The data for Risø 56 in Table 3-7 perhaps supports chromosome 5 as the gene location. Additional linkage investigation is certainly indicated with perhaps an emphasis on work with chromosome 4 and 5.

Additional linkage study is also indicated for the other sex mutants involved in this study particularly Risø8 and 13.

The chromosome location of a given gene of interest to the plant breeder has value in implementing gene transfer systems. In addition, a located gene may become a marker gene for subsequent genetic studies and breeding work.

Table 3-10. F_2 plant segregation from Betzes trisomic x Bomi, Risø 56 (sex,h) crosses.

Trisomic crossed with Risø 56	Classification (no.) Sex—	sex sex	Total	Ratio tested	χ^2	P level
1	3	2	5	observed	1.937	.10-.25
	–	–	–	3:1	0.600	.25-.50
2	25	5	30	observed	0.010	.90-.95
	–	–	–	3:1	1.111	.25-.50
3	43	8	51	observed	0.006	.90-.95
	–	–	–	3:1	2.360	.10-.25
4	167	27	194	observed	0.673	.25-.50
	–	–	–	3:1	12.708	< .01
5	15	4	19	observed	0.384	.50-.75
	–	–	–	3:1	0.157	.50-.75
6	16	1	17	observed	1.272	.25-.50
	–	–	–	3:1	3.313	.05-.10
7	7	6	13	observed	8.638	< .01
	–	–	–	3:1	3.103	.05-.10

Note: Heterogeniety χ^2_2 based on observed ratios = 12.920, $p < .05$. Hererogeniety χ^2 without trisomic 7 cross data = 4.083, $p = .50-.75$. Heterogeniety χ^2 based on 3:1 ratio = 9.483, $p = .10-.25$.

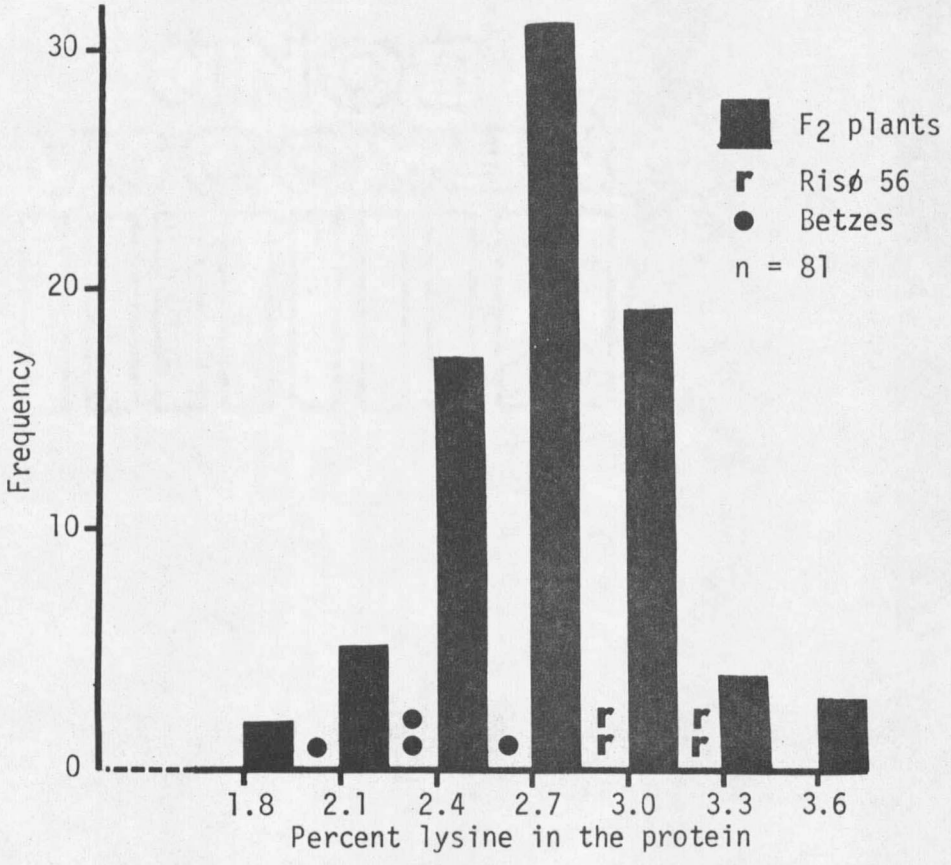


Figure 3-3. Frequency distribution of percent lysine in the protein of F_2 plants from Betzes trisomic 3 x Carlsberg II, Risø 56 crosses.

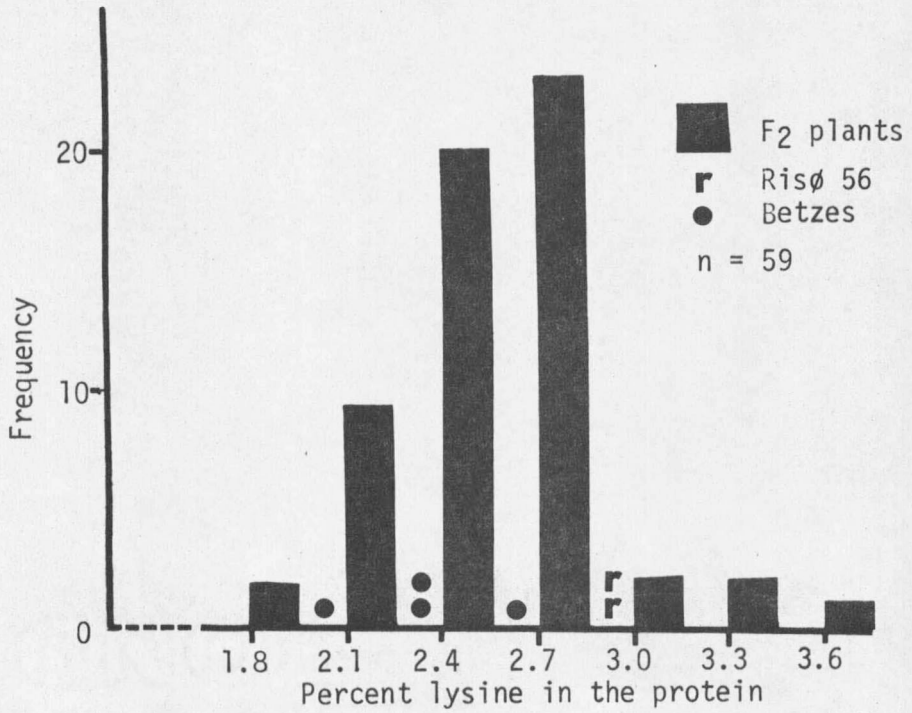


Figure 3-4. Frequency distribution of percent lysine in the protein of F_2 plants from Betzes trisomic 5 x Carlsberg II, Risø 56 crosses.

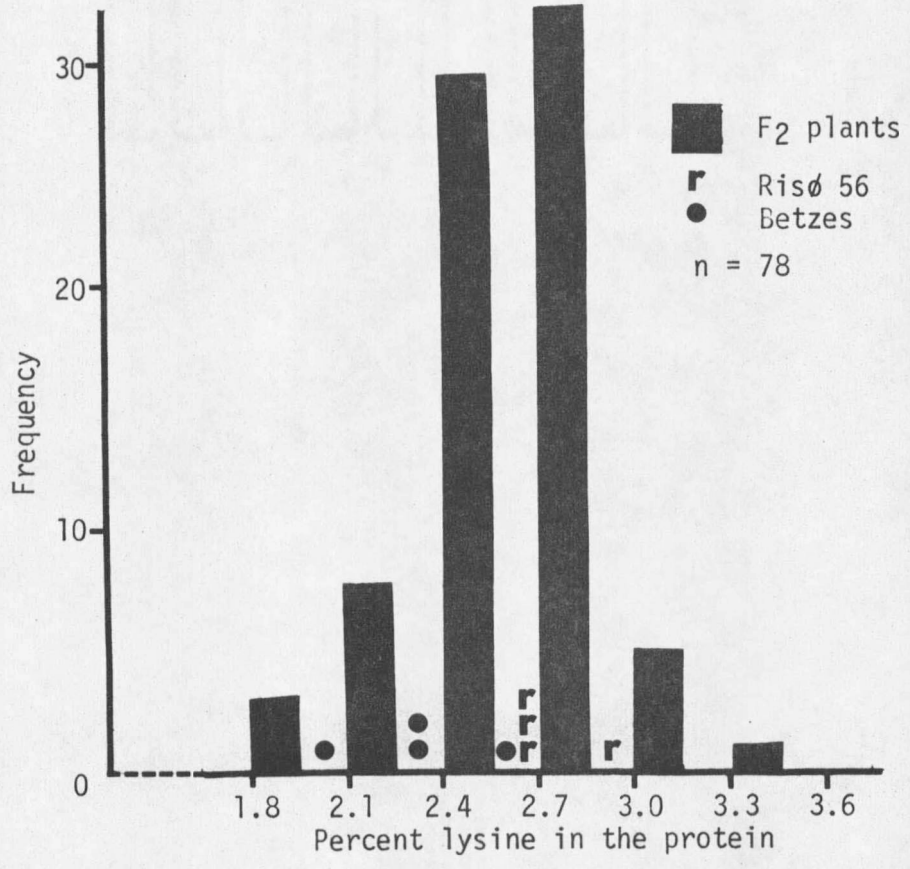


Figure 3-5. Frequency distribution of percent lysine in the protein of F_2 plants from Betzes trisomic 6 x Carlsberg II, Risø 56 crosses.

Study III: Protein and Lysine Maternal Inheritance Effects in Hiproly

The half kernel lysine evaluation indicated that the F_1 seed from the Hiproly x Betzes cross were higher in lysine than the Betzes x Hiproly F_1 seed (Table 3-11). Both percent protein and percent lysine were higher in the F_2 seed from the Hiproly x Betzes cross than in the Betzes x Hiproly cross.

These results provide evidence for maternal effects in the inheritance of protein and lysine in Hiproly. Endosperm dosage effects alone cannot account for the significant differences obtained in both the F_1 and F_2 seed generations. Indirect supportive evidence for these conclusions was presented by Peruanskii et al. (1976) in which they observed that Hiproly x normal variety progeny were higher in protein content than the progeny of reciprocal crosses.

According to S. O. S. Thomke (personal communication), Swedish investigators have found that an earlier than normal constriction of the vascular bundles entering the kernels occurs in Hiproly . This could explain the higher lysine content of F_1 seed harvested from Hiproly plants as contrasted to those F_1 seed harvested from Betzes plants. The difference in F_2 seed from reciprocal crosses cannot be explained on this basis. Cytoplasmic inheritance could explain the results of both types of progenies. The data suggests additional information is needed for Hiproly and other high lysine mutants.

Table 3-11. Mean protein and lysine data of F_1 and F_2 seed from reciprocal crosses between 'Hiproly' and 'Betzes'.

Character	Hiproly x Betzes	Betzes x Hiproly	Difference
F_1 lysine index, mm	6.2	5.1	1.1*
F_2 protein, %	14.6	12.9	1.7*
F_2 lysine in grain, %	.468	.380	.088*
F_2 lysine in protein, %	3.21	2.95	0.26*

*Significant at the 0.05 level.

For the breeder, knowledge of maternal inheritance effects for a given trait is critical, if maximum use of available genetic potential is to be realized. If cytoplasmic effects exist in a mutant of interest, then it should be used as the female parent in crosses with normal varieties to derive full benefit from the genetic transfer of the trait considered.

Study IV: Inheritance and Association of the Shrunken Endosperm and High Lysine Traits of Bomi, Risø 1508

Shrunken Endosperm Inheritance--The shrunken endosperm trait of Risø 1508 is recessive as indicated by acceptable fits to 3:1, 1:2:1 and combined ratios for most of the crosses (Table 3-12). Since it has shown xenia in all first crosses it was proposed that the trait be designated sex3c.

Table 3-12. The expression of shrunken endosperm in F_2 seed from F_1 plants and of F_2 plants from Bomi, Risø 1508 crosses.

Normal varieties used in crosses with Risø 1508	Material analyzed	Sex3—	Sex3 Sex3	Sex3 sex3	sex3 sex3	Total	X^2 for 3:1, 1:2:1 or combined ratios	P for fit to appropriate ratio
Compana	F_2 seed	613	–	–	182	795	1.88	.10–.25
	F_2 plants	–	56	138	65	259	1.74	.25–.50
	Combined					1054	3.62	.25–.50
Carlsberg II	F_2 seed	523	–	–	147	670	3.34	.05–.10
	F_2 plants	–	14	29	12	55	0.30	.75–.90
	Combined					725	3.64	.25–.50
Betzes	F_2 seed	503	–	–	137	640	4.41	<.05
	F_2 plants	–	77	126	61	264	2.48	.25–.50
	Combined					904	6.89	.05–.10
Heines Hanna	F_2 seed	445	–	–	103	548	11.25	<.01
	F_2 plants	–	17	20	5	42	6.95	<.05
	Combined					590	18.20	<.01
Hector	F_2 seed	575	–	–	101	676	36.48	<.01
	F_2 plants	–	37	83	25	142	5.03	.05–.10
	Combined					818	41.51	<.01
Steptoe	F_2 seed	401	–	–	101	502	6.38	<.05
	F_2 plants	–	22	67	23	112	4.33	.10–.25
	Combined					614	10.71	<.05
Unitan	F_2 seed	276	–	–	104	380	1.14	.25–.50
	F_2 plants	–	27	71	24	122	3.42	.10–.25
	Combined					502	4.56	.10–.25
Piroline	F_2 seed	310	–	–	101	411	0.04	.75–.90
	F_2 plants	–	24	100	25	149	17.47	<.01
	Combined					560	17.51	<.01
CI 4961	F_2 seed	404	–	–	104	508	5.55	<.05
Erbet	F_2 plants	–	24	40	14	78	2.61	.25–.50

Departure from 3:1 and 1:2:1 ratios may be noted in Table 3-12, and may be due to the presence of suppressor genes or modifiers for shrunken endosperm or differential survival of male gametes or pollen grains carrying the sex3 allele, as no obvious female sterility was noted. In all cases but one, rejection of the 3:1 or 1:2:1 hypotheses was due to deficiency of shrunken seed.

Shrunken Endosperm Linkage--Independence is indicated between sex3 and male sterile genes (msg) located on chromosomes 1 through 6 (Table 3-13). Nearly complete linkage is indicated between sex3 and msg19 which is located in chromosome 7 according to Eslick (1971) and Ullrich and Eslick (1977). Neither the coupled sex3 msg19 genotype from 105 F_3 progeny from shrunken seed nor the coupled Sex3 Msg19 genotype from 428 F_3 progeny from F_2 heterozygous plants were recovered.

Linkage was also indicated between the short rachilla hair gene, (s), and sex3. Partial F_3 linkage data show 28.6 $\pm$ 3.4 percent recombination of the two genes (Table 3-14). Karlsson (1977) has stated that these two characters are independent.

The short-haired rachilla gene, (s), is usually assigned to the long arm of chromosome 7 (Haus, 1977). Karlsson (1976) has indicated the high lysine gene, lys1, of Hiproly is probably 12 units distal to s in the same arm. Eslick (1971) and Ullrich and Eslick (1977) considered msg19 to be in the short arm near the centromere of chromosome 7. The results reported would indicate then, that the sex3 gene of Risø 1508

Table 3-13. Partial F_3 linkage data for male sterile genes (msg) x Bomi, Risø 1508 shrunken endosperm xenia mutant (sex3) crosses.

Cross, msg x Risø 1508, sex3	location of msg gene	Partial F_3 classification				% Recombination
		Msg - Sex3Sex3	Msg - Sex3sex3	Msg - sex3sex3	msg msg	
Compana msg10	1	10	36	8	12	Independent
Compana msg2	2	8	30	11	20	Independent
Carlsberg II msg5	3	14	31	12	24	Independent
Betzes msg24	4	14	27	13	17	Independent
Betzes msg1	5	18	30	9	24	Independent
Compana msg1	5	12	20	10	15	Independent
Heines Hanna msg6	6	17	21	5	21	Independent
CI 4961 msg19	7	0	33	12	19	0.0
CI 4961 msg19	7	--	--	105	0	0.0
CI 4961 msg19	7	0	428	--	--	0.0

Table 3-14. Partial F_3 linkage data for rachilla hair length (s) and Bomi Risø 1508 shrunken endosperm mutant (sex3), F_1 genotype S sex3/ s Sex3.

Genotype	Frequency[†]
S- Sex3 Sex3	31
S- Sex3 sex3	100
S- sex3 sex3	54
ss Sex3 Sex3	23
ss Sex3 sex3	23
ss sex3 sex3	2
Total	233
% Recombination and S.E.	28.6 ± 3.4

[†]The S-:ss ratio is 185:48 which fits a 3:1 ratio with $\chi^2 = 2.4048$, $p = .217$. The SexSex:Sexsex:sexsex ratio is 54:123:56 which fits a 1:2:1 ratio with $\chi^2 = 20.7596$, $p = .690$.

is probably distal to the centromere in the short arm of chromosome 7, which is in agreement with Karlsson (1977). This does not conflict with the findings of Muench et al. (1976) concerning the independence of Risø 1508's high lysine character and lys1 on chromosome 7. Trisomic analysis supports the location of sex3 on chromosome 7, also (see Section III, Study II).

Lysine Association with Shrunken Endosperm--The frequency distributions, Figures 3-6 and 3-7 suggest a discrete percent lysine distribution for each endosperm genotype, and this was confirmed by heterogeneity χ^2

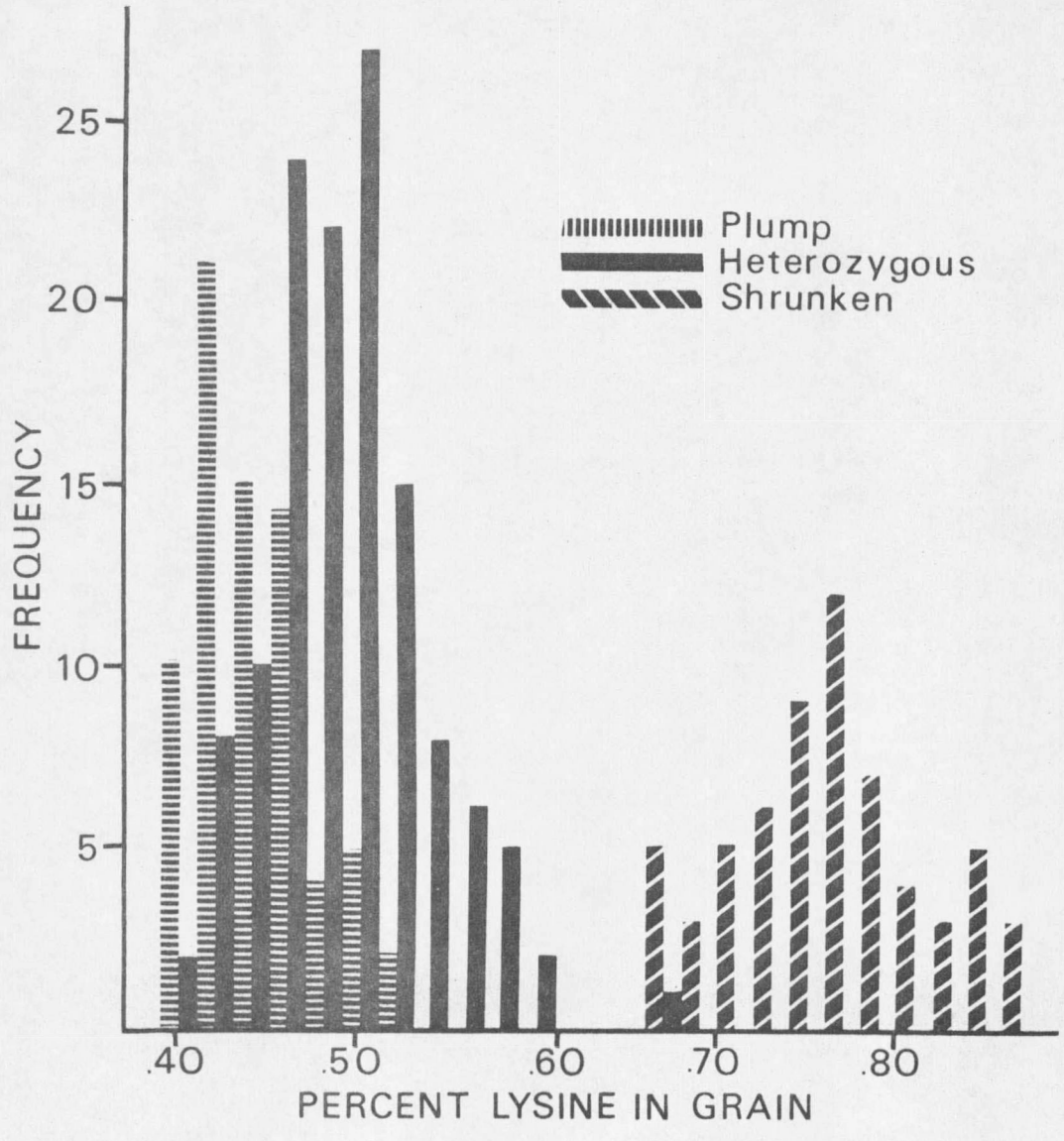


Figure 3-6. Frequency distribution of percent lysine in grain of Normal Variety x Risø Mutant No. 1508, F_3 Progeny.

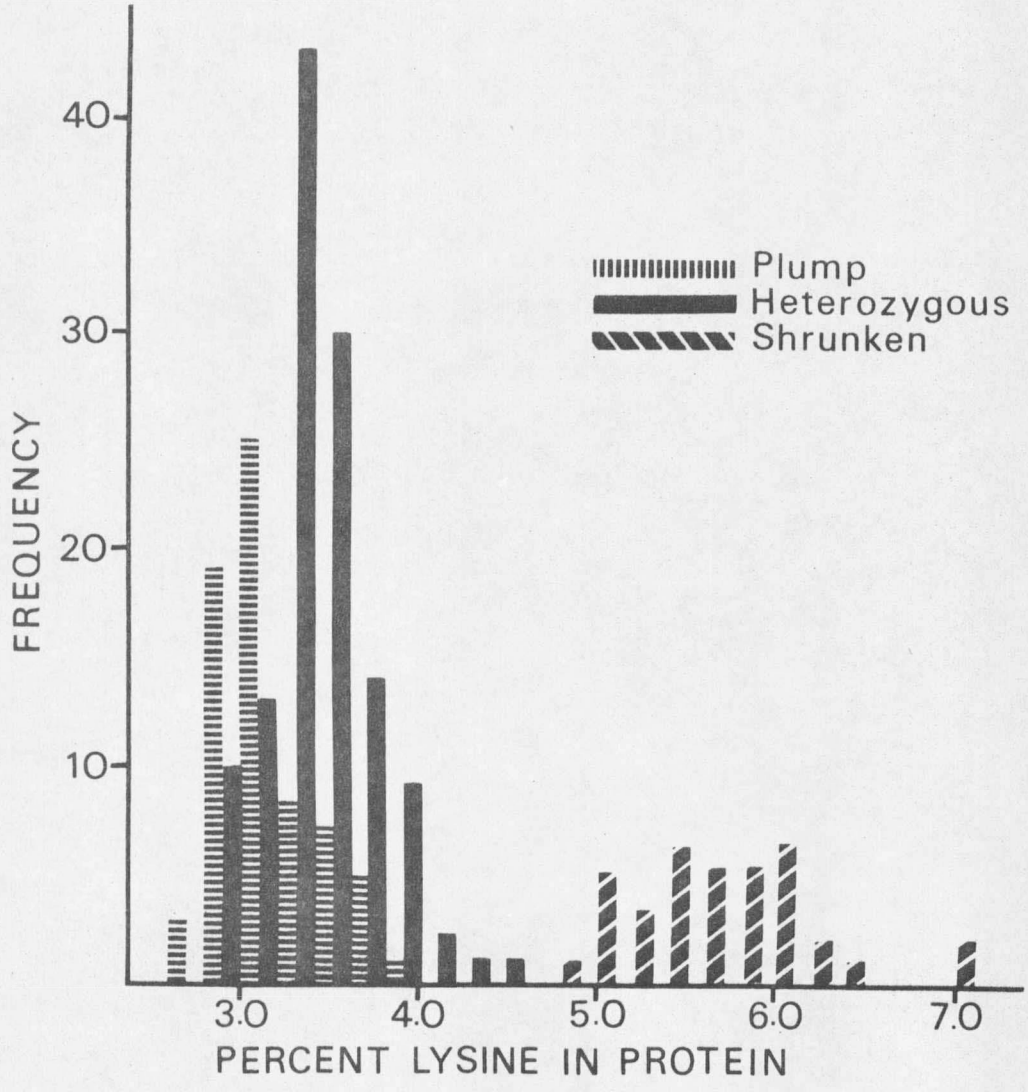


Figure 3-7. Frequency distribution of percent lysine in protein of Normal Variety x Risø Mutant No. 1508, F_3 Progeny.

tests of the frequency distributions of the plump and heterozygous classes represented in both figures. Probability of the same frequency was < .001. It may be noted in Figure 3-6 that a heterozygous individual is included in the shrunken endosperm distribution. Upon analysis of the progeny of this individual, no high lysine plump or heterozygous individuals were found, indicating a lysine analysis error for the heterozygous individual.

A close association between the high lysine character and the shrunken endosperm character is evident in Figures 3-6 and 3-7 and Table 3-15. The Risø 1508 parent and F_3 shrunken progeny were not different from each other in percent lysine in the barley or in the protein but both were higher than the other parents or progenies. The heterozygous F_3 progenies were intermediate in both characters. Plump parents were not different for percent lysine in the barley (Table 3-15).

The heterozygous F_3 progeny are higher in lysine than the plump F_3 progeny, .493 percent vs. .441 percent in the barley, respectively (Table 3-15). If xenia is assumed and adjustment for percent lysine in the barley made from the observed frequencies of plump and shrunken kernels (calculated from the data in Table 3-12) .79 and .21, respectively (e.g., .441 x .79 + .756 x .21 = .507), the observed .493 approximates the calculated theoretical .507.

It follows that the normal lysine characteristic, in agreement with Doll's report (1973), expressed complete dominance. In addition,

Table 3-15. Summary of F_3 lysine data for variety x Bomi, Risø 1508 crosses and parents represented in Figures 3-6 and 3-7.

Seed analyzed	Mean and confidence limits (p = .01)					
	Protein (%)	No. of observations	Lysine in protein (%)	No. of observations	Lysine in grain (%)	No. of observations
Risø 1508 parent	13.1	1	5.65	1	.740	1
F_3 shrunken	13.1±.4	41	5.76±.22	41	.756±.018	62
F_3 heterozygous	14.5±.3	124	3.41±.07	124	.493±.010	130
F_3 plump	14.2±.4	66	3.13±.08	66	.441±.010	71
Normal parents	13.4±.9	15	3.11±.11	15	.418±.023	15

the high lysine character also expresses xenia in the same manner as the shrunken endosperm character.

The two most likely explanations for the above results would be that one trait is a pleiotropic effect of the other or that they are two closely linked genes, one for shrunken endosperm, the other high lysine. My experience and that of others (Jenkins et al., 1977 and Karlsson, 1977) favors the former hypothesis, since no plump high lysine progeny have been found from Risø 1508 x normal variety crosses.

Summary

The sex traits of Risø 29 and 86 were found to be allelic with Compana sex1.

With trisomic analysis evidence, the shrunken endosperm xenia traits of Risø 86 (sex1d), 29 (sex1e) and 13 (sex4f) were assigned to chromosome 6; Risø 8 (sex5g) to chromosome 5; and Risø 56 (sex,h) possibly to chromosome 4 or 5 with more investigation required. The high amylose trait (amo1) of Glacier was assigned to chromosome 2.

Maternal inheritance effects for seed protein and lysine contents were indicated for the high protein, high lysine mutant, Hiproly .

The shrunken endosperm trait of Risø 1508 was found to express xenia and to be inherited as a single recessive gene. It was proposed the gene be designated sex3c. Marker gene linkage and trisomic analysis information indicated the gene be assigned to chromosome 7. The high lysine

trait was absolutely associated with the shrunken endosperm trait in these studies, indicating a pleiotropic or closely linked gene relationship.

SUMMARY AND CONCLUSIONS FROM ALL STUDIES WITH HIGH LYSINE MUTANTS OF BARLEY

By characterizing the spontaneous shrunken endosperm and/or low kernel weight mutants of barley (seg, sex and amol mutants) as being high in seed protein and lysine content, eight "new" mutants have been added to the list of barley high lysine mutants described in the literature.

Several Risø induced high lysine mutants were characterized as being low in kernel weight and high in seed protein and lysine content over a range of Western U.S. environments.

The spontaneous mutants were selected visually for shrunken endosperm and the induced mutants were selected chemically for high lysine. Both types of mutagenesis and both methods of selection produced a similar spectrum of mutants. A cause and effect relationship has yet to be established for the high lysine-shrunken endosperm phenomenon in barley.

A mathematical manipulation predicted the lysine levels of the various mutants on the basis of normal kernel weights. It appears that most of the mutants would maintain a high lysine grain content if a normal kernel weight were achieved through breeding efforts.

As an extension of the mutant characterizations for kernel weight and protein and lysine content, the effect of environment on the expression of these and eight other agronomic characters was determined. A regression analysis approach was used, which provided for visual as well

as a statistical insite into mutant-normal and mutant-mutant relationships. The adaptation of the various mutants for the various characters was quite variable, as was the stability of the responses. Adaptation was defined by the regression coefficient and stability by a combination of the coefficient of determination and the regression coefficient.

It was concluded that the characters that describe shrunken endosperm (kernel weight, percent plump and percent thin) are best expressed in environments that favor large seed in the normal isotypes. This provides a basis for selection for or against shrunken endosperm.

Selection for high lysine using these mutant sources could be done in any environment with percent lysine in the grain as the criterion. If percent lysine in the protein is the criterion, selection with some mutants could be done in any environment, but for others, selection in environments favorable for low percent lysine in the protein of the normal isotype would be most productive. Risø 1508, Compana sex1 and seg4 come the closest to fitting a model of the "ideal" high lysine mutant defined by adaptation and stability parameters for percent lysine in the protein.

Most of the mutants were lower in yield and test weight than their respective normal isotypes and environmental responses were variable. The mean differences and environmental responses were variable for plant height, heading date and percent lodging.

Risø 1508 is probably the best mutant source of lysine in the grain but considering its yield potential it is not economically feasible to grow it commercially as a feed barley under present market conditions. It may be feasible to grow commercially Glacier amo1 and/or Risø 7 particularly under lower yielding (dryland) conditions.

The shrunken endosperm traits of Risø 1508, 29 and 86 were found to be inherited as single recessive genes that express xenia, and Risø 29 and 86 were found to be allelic with Compana sex1. The absolute association between shrunken endosperm and high lysine was established in Risø 1508. The shrunken endosperm xenia expressing traits of these and several other Risø mutants (8, 13 and 56) and amo1 were assigned to chromosomes by trisomic analysis. Maternal inheritance effects for seed protein and lysine contents were indicated for Hiproly lys1. Additionally, inheritance, allelism and linkage information is indicated for some of the genes involved to support some of the data obtained in these studies.

These genetic studies and the characterization and adaptation investigations have helped provide information for the basic understanding of a group of high lysine mutants, which will allow the breeder of high lysine barley to better manipulate the genes involved for the production of improved barley germplasm and/or cultivars.

The association between high lysine and shrunken endosperm in barley appears to be quite general. The nature of this association is

not well understood. Linked genes or pleiotropy may be postulated. For commercial exploitation of this type of high lysine mutant, the detrimental association between high lysine and shrunken endosperm must be overcome. The following points are offered as suggestions for diminishing or overcoming the association, which gives direction to continuing research:

1. If linked genes are involved, select for plump-high lysine recombinants.
2. Breed for very large shrunken seed.
3. Search for or induce a suppressor gene for the shrunken endosperm trait that does not affect the high lysine trait.
4. Intercross shrunken endosperm-high lysine mutants and look for plump-high lysine types that result from epistasis.
5. Search for or induce and accumulate minor genes for high lysine in plump barley lines.
6. Isolate a new family of mutants that are high in lysine but plump.

Nutrition studies with high lysine barley mutants have indicated that the exploitation of high lysine mutants is warranted.

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