Agronomic, biochemical and genetic characterizations and adaptations of high lysine, shrunken endosperm mutants of barley (Hordeum vulgare L.)
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A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and Soil Science
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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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AGRONOMIC, BIOCHEMICAL AND GENETIC CHARACTERIZATIONS AND ADAPTATION OF HIGH LYSINE, SHRUNKEN ENDOSPERM MUTANTS OF BARLEY (*HORDEUM VULGARE* L.)

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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.

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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.