Seed development in shrunken endosperm, high lysine mutants of barley (Hordeum vulgare L.)
by Christine Elizabeth Fastnaught

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:
'Betzes1 seg1, 'Ingrid' seg6 and seg7, 'Compana' seg3 and sex1a, 'Hiproly' lys1, 'Bomi' sex1f (Risθ 13), sex3c (Risθ 1508), and Risθ 8, and 'Carlsberg II' Risθ 56, shrunken endosperm, high lysine mutants of barley, were compared to their normal isotypes for moisture percentage, dry matter accumulation, alpha-amylase activity, and total and reducing sugar at 3 or 6 day intervals during seed development. All mutants had a lower mean kernel weight (averaged over 8 to 10 sampling dates) than normal. This occurred in the seg mutants (seg1, seg3, seg6, and seg7) because dry matter accumulation stopped 6 to 18 days earlier than normal, and in the sex mutants (all other mutants) because it proceeded at a slower rate than normal. Differences were not detected between mutants and normals in alpha-amylase activity because of a strong isotype x sampling date interaction, but a significant correlation was detected between peak activity at six days after anthesis and kernel weight at harvest in the six normal genotypes. Moisture percentage was lower than normal in seg3 and higher in sexla, sex1f, sex3c, and Risθ 8. Total sugar was lower than normal in all seg mutants, and higher in sex1a, sex1f, and sex3c. The correlation between total sugar and moisture explains the plumpness of the sex mutants during seed development. Reducing sugar was lower than normal in seg1 and seg3, and higher in sex1a and sex1f. The decrease in sugars in the seg mutants was proportional to the kernel weight decrease. The lower moisture, kernel weight, total and reducing sugars, and higher alpha-amylase activity of the seg mutants compared to the sex mutants may be related to a nutrient translocation problem.

Correlations among sampling dates for total sugar of all genotypes indicated that the ranking of genotypes for total sugar content was consistent from 12 days after anthesis to harvest. The mutant-normal difference in total sugar at harvest was highly correlated to the mean mutant-normal difference during seed development (r=,98**).

The above mutants and Carlsberg II sex1d (Risθ 86) were compared to their normal isotypes for total sugar content of harvest ripe seed grown in 7 to 13 environments. The results confirmed the differences observed during seed development. The allelic mutants, sex1a, sex1d, and sex1f, responded similarly relative to their normal isotypes.

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Christine Elizabeth Fastnaught

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Dec. 3, 1982
Date

Robert J. Eslick
Chairperson, Graduate Committee

Approved for the Major Department

12/3/82
Date

Dwane H. Miller
Head, Major Department

Approved for the College of Graduate Studies

12-6-82
Date

Michael D. Malone
Graduate Dean

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ABSTRACT

'Betzes' seg1, 'Ingrid' seg6 and seg7, 'Compana' seg3 and sex1a, 'Hiproly' lys1, 'Bomi' sex1f (Risø 13), sex3c (Risø 1508), and Risø 8, and 'Carlsberg II' Risø 56, shrunken endosperm, high lysine mutants of barley, were compared to their normal isotypes for moisture percentage, dry matter accumulation, alpha-amylase activity, and total and reducing sugar at 3 or 6 day intervals during seed development. All mutants had a lower mean kernel weight (averaged over 8 to 10 sampling dates) than normal. This occurred in the seg mutants (seg1, seg3, seg6, and seg7) because dry matter accumulation stopped 6 to 18 days earlier than normal, and in the sex mutants (all other mutants) because it proceeded at a slower rate than normal. Differences were not detected between mutants and normals in alpha-amylase activity because of a strong isotype x sampling date interaction, but a significant correlation was detected between peak activity at six days after anthesis and kernel weight at harvest in the six normal genotypes. Moisture percentage was lower than normal in seg3 and higher in sex1a, sex1f, sex3c, and Risø 8. Total sugar was lower than normal in all seg mutants, and higher in sex1a, sex1f, and sex3c. The correlation between total sugar and moisture explains the plumpness of the sex mutants during seed development. Reducing sugar was lower than normal in seg1 and seg3, and higher in sex1a and sex1f. The decrease in sugars in the seg mutants was proportional to the kernel weight decrease. The lower moisture, kernel weight, total and reducing sugars, and higher alpha-amylase activity of the seg mutants compared to the sex mutants may be related to a nutrient translocation problem.

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INTRODUCTION

The shrunken endosperm mutants of barley (Hordeum vulgare L.) have been of interest for two main reasons. The first involves an increase in the amino acid lysine, associated with the shrunken endosperm character, which increases the nutritional quality of such mutants. The quality of small grains is extremely important to the humans and livestock which rely almost solely upon them for food and feed. The increased lysine of the shrunken endosperm mutants is useful only if the lysine content of the mutants can be transferred to high yielding cultivars. Breeding programs around the world have approached this problem by attempting to break the association between the shrunken endosperm and the high lysine, i.e., increasing the plumpness of the mutant seed while maintaining the high lysine content. To date, complete success has not been reported.

A second reason for the interest in shrunken endosperm mutants is their potential usefulness in understanding the biochemical and developmental pathways of seed development. The actual mutant gene(s) function which results in a shrunken seed and high lysine content is unclear. Since a shrunken seed should contain less starch, an examination of starch precursors or degradation products along with the enzymes involved in such processes, may help explain the relationship between shrunken endosperm, high lysine, and the actual gene(s) function. A better understanding of these relationships should prove

useful to the breeder attempting to produce high yielding, high lysine cultivars.

The objective of this research was to compare starch associated characters (dry matter accumulation, alpha-amylase activity, total and reducing sugars) in shrunken endosperm, high lysine mutants and their normal isotypes during seed development.

LITERATURE REVIEW

Description of Mutants

Selection of shrunken endosperm, high lysine mutants has been based on both visual selection of the shrunken endosperm character and biochemical selection of the high lysine character. The mutants seg1, seg3, sex1a (Jarvi and Eslick, 1975), seg6, and seg7 (Ramage and Eslick, 1975), were selected on the basis of the shrunken endosperm character. These mutants were later classified as being high lysine (Eslick and Hockett, 1976b and Ullrich and Eslick, 1978c). The mutants 'Hiproly' (Munck et al., 1970), and Risø 8, 13, 56, 86, and 1508 (Doll et al., 1974), were selected on the basis of biochemical analysis for lysine, but all of them had an associated shrunken seed or reduced seed weight.

The shrunken endosperm and high lysine phenotype of all the mutants, except Risø 8, are inherited in a monofactotial, recessive manner (see Table 1 for authority on specific genes). The phenotype of Risø 8 is controlled by a dominant or semi-dominant gene (Jensen, 1979a). The shrunken endosperm (or reduced seed weight) and high lysine phenotype have not been separated in any of the mutants, indicating that either the two traits are controlled by the same gene and are pleiotropic (Oram et al., 1975; Ullrich and Eslick, 1978a; Jensen, 1979a; Nelson, 1979; and Olsen, 1980) or they are controlled by two tightly linked genes (Hagberg et al., 1970 and Eslick and

Table 1. Genetic and phenotypic classification of 11 shrunken endosperm, high lysine mutants of barley.

Normal Cultivar	Mutant Name	Type of Mutation	Type of Expression	Seed Phenotype	Chromosome Location	Authority
Betzes	seg1	spontaneous	non-xenia	thin	1	6, 8
Compana	seg3	spontaneous	non-xenia	thin	3	6, 9
Ingrid	seg6	spontaneous	non-xenia	thin	–	7, 11
Ingrid	seg7	spontaneous	non-xenia	thin	–	7, 11
Hiproly Normal	Hiproly	spontaneous	xenia	dorsal depression	7	1, 2
Compana	sex1a	spontaneous	xenia	dorsal depression	6	6, 10
Bomi	Risø 8	induced by ethyl methane sulfonate	xenia	dorsal depression	5	5, 14, 15, 19
Bomi	Risø 13	induced by ethyl methane sulfonate	xenia	dorsal depression	6	5, 14, 15, 18
Carlsberg II	Risø 56	induced by gamma-rays	xenia	dorsal depression	5	5, 14, 17
Carlsberg II	Risø 86	induced by ethyl methane sulfonate	xenia	dorsal depression	6	5, 14, 15
Bomi	Risø 1508	induced by ethyleneimine	xenia	dorsal depression	7	3, 4, 12, 13, 16

Authority

1. Munck et al., 1970
2. Karlsson, 1972
3. Doll, 1973
4. Ingversen et al., 1973
5. Doll et al., 1974
6. Jarvi and Eslick, 1975
7. Ramage and Eslick, 1975
8. Eslick, 1976a
9. Eslick, 1976b
10. Eslick and Hockett, 1976a
11. Ramage and Scheuring, 1976
12. Karlsson, 1977
13. Ullrich and Eslick, 1978a
14. Ullrich and Eslick, 1978b
15. Jensen, 1979a
16. Jensen, 1979b
17. Doll, 1980
18. Fastnaught et al., 1981
19. Jensen, 1981

Hockett, 1976b). The chromosome location of some of the mutant genes is found in Table 1. 'Compana', sex1a, 'Bomi', Risø 13, and 'Carlsberg II', Risø 86 are reported to be allelic (Jensen, 1979a and Fastnaught et al., 1981).

These mutants can be classified according to whether the inheritance of shrunken endosperm and high lysine exhibits xenia (Table 1). Those mutants which do not exhibit xenia (i.e., the F_2 segregates in a normal 3:1 ratio) have been classified as shrunken endosperm genetic (seg) mutants and those which do exhibit xenia (i.e., the F_2 segregates in a 1:2:1 ratio, with plump and shrunken seed produced on the same spike from the heterozygous Sex sex plant) have been classified as shrunken endosperm xenia (sex) mutants. The terms, seg and sex, have been used as gene symbols for those mutants visually selected for shrunken endosperm (Eslick and Hockett, 1976c).

To date, the general term, lys, has been used as a gene symbol for most of the mutants selected on the basis of high lysine (Jensen and Doll, 1979), regardless of whether or not they exhibited xenia. Based on the gene symbols suggested by Eslick and Hockett (1976c), the terms lyg and lyx would be used to symbolize the high lysine genes.

Confusion has arisen in recent years concerning the gene symbols for the shrunken endosperm, high lysine mutants. Some of the mutants have been assigned two symbols, seg or sex, and lys. One reason for doing so would be the assumption that the two characters are controlled by separate genes (Eslick and Hockett, 1976b). A second reason would be the point of view of the researcher. When selecting a mutant on the basis of a certain phenotype, a researcher would be

inclined to give that mutant a gene symbol related to that phenotype, regardless of other associated mutant characteristics (Ullrich and Eslick, 1978b and Jensen and Doll, 1979). Munck (1972b) suggested that the lys symbol should be revised once the basic gene action is understood. Jensen and Doll (1979) felt that was impractical. However, one of these mutants, Risø 56, was not assigned a gene symbol until recently when it was discovered that it caused a reduction in hordein-2 and was located at or near the previously described Hor locus. Thus, Risø 56 was assigned a completely different gene symbol, Hor2ca (Doll, 1980). The normal or dominant allele of this gene is found in Carlsberg II and designated Hor2Ca. A summary of the gene symbols assigned to seg1, seg3, seg6, seg7, sex1a, Hiproly, and Risø 8, 13, 56, 86, and 1508 are in Table 2. The symbols used in this manuscript are starred.

Mutant-normal comparisons of kernel weight, and protein and lysine content, were reported by Ullrich and Eslick (1978c and 1978e) over a range of Montana and Arizona environments. They indicated that all 11 mutants used in this study had significantly lower kernel weights (Table 3), significantly higher protein percentage (Table 3), significantly higher lysine in the grain percentage (Table 4), and significantly higher lysine in the protein percentage (Table 4) than their normal isotypes. Ullrich and Eslick (1978c and 1978e) postulated that a starch dilution effect could result in the higher protein and lysine contents of the smaller mutant seed. They tested this by adjusting the mutant kernel weight to the normal kernel weight and calculating adjusted percentages for protein and lysine in the grain

Table 2. Summary of gene symbols assigned to 11 of the shrunken endosperm, high lysine mutants of barley.

Symbol Proposed By	Mutant Name										
	seg1*	seg3*	seg6*	seg7*	Hiproly	sex1	Risø 8*	Risø 13	Risø 56*	Risø 86	Risø 1508
Munck, 1972a	–	–	–	–	lys1*	–	–	–	–	–	–
Eslick, 1976a	seg1a	–	–	–	–	–	–	–	–	–	–
1976b	–	seg3c	–	–	–	–	–	–	–	–	–
Eslick and Hockett, 1976a	–	–	–	–	–	sex1f	–	–	–	–	–
1976b	lys2b	–	–	–	–	–	–	–	–	–	–
Ramage and Scheuring, 1976	–	–	seg6g	seg7h	–	–	–	–	–	–	–
Ullrich and Eslick, 1978a	–	–	–	–	–	–	–	–	–	–	sex3c*
1978b	–	–	–	–	–	–	sex5g	sex4f	–	–	–
1978d	–	–	–	–	–	sex1a*	–	–	–	sex1d*	–
Jensen and Doll, 1979	–	–	–	–	–	lys5e	Lys4d	lys5f	–	lys5h	lys3a
Doll, 1980	–	–	–	–	–	–	–	–	Hor2ca	–	–
Fastnaught et al., 1981	–	–	–	–	–	–	–	sex1f*	–	–	–

*Mutant name or gene symbol used in this manuscript.

Table 3. Kernel weight and protein comparisons of 11 shrunken endosperm, high lysine mutants of barley and their normal isotypes. (Adapted, with permission, from Ullrich, 1978).

Normal Cultivar	Mutant Name	Mean Kernel Weight (mg)			Mean Protein (%)			
		No. of Comparisons	Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$	No. of Comparisons	Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$
Betzes	seg1	17	18.8	34.3	-15.5**	18	15.9	14.6	1.3**
Compana	seg3	8	26.2	46.9	-20.7**	12	16.6	14.4	2.2**
Ingrid	seg6	3	15.1	39.9	-24.8**	7	14.5	12.6	1.9**
Ingrid	seg7	8	26.8	36.7	-9.9**	11	15.0	12.8	2.2**
Hiproly Normal	Hiproly	26	38.4	49.2	-10.8**	26	18.4	17.2	1.2**
Compana	sex1	8	38.6	47.6	-9.0**	14	17.9	14.4	3.5**
Bomi	Risø 8	26	32.6	45.0	-12.4**	26	14.4	13.2	1.2**
Bomi	Risø 13	27	35.6	45.0	-9.4**	28	15.0	13.2	1.8**
Carlsberg II	Risø 56	25	35.9	39.7	-3.8**	28	14.7	12.9	1.8**
Carlsberg II	Risø 86	33	34.0	39.2	-5.2**	36	14.7	12.8	1.9**
Bomi	Risø 1508	44	34.3	43.4	-9.1**	47	13.8	13.2	0.6**

**Significant at the 0.01 level based on a paired t-test.

Table 4. Microbiological assay lysine comparisons of 11 shrunken endosperm, high lysine mutants of barley and their normal isotypes. (Adapted, with permission, from Ullrich, 1978).

Normal Cultivar	Mutant Name	No. of Comparisons	Mean Lysine in Grain (%)			Mean Lysine in Protein (%)		
			Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$	Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$
Betzes	seg1	18	0.518	0.436	0.082**	3.25	3.05	0.20**
Compana	seg3	12	0.534	0.391	0.143**	3.40	2.76	0.64**
Ingrid	seg6	7	0.514	0.387	0.127**	3.55	3.09	0.46*
Ingrid	seg7	11	0.488	0.407	0.081**	3.25	3.20	0.05
Hiproly Normal	Hiproly	26	0.581	0.448	0.133**	3.15	2.61	0.54**
Compana	sex1	14	0.598	0.379	0.219**	3.37	2.66	0.71**
Bomi	Risø 8	26	0.548	0.386	0.162**	3.80	2.92	0.88**
Bomi	Risø 13	28	0.544	0.384	0.160**	3.68	2.93	0.75**
Carlsberg II	Risø 56	28	0.538	0.379	0.159**	3.69	2.97	0.72**
Carlsberg II	Risø 86	36	0.514	0.390	0.124**	3.56	3.06	0.50**
Bomi	Risø 1508	47	0.607	0.386	0.221**	4.39	2.95	1.44**

*, **Significant at the 0.05 and 0.01 levels, respectively, based on a paired t-test.

using the following formula:

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

They suggested that when adjusted mutant-normal differences in protein percentage were nonsignificant (Table 5), starch deposition in the mutant may be restricted more than protein deposition. When the adjusted protein percentage of the mutant was significantly lower than the normal, protein as well as starch deposition may have been affected in the mutant. They observed that after the adjustment, seg mutants had a significantly lower lysine in the grain percentage than their normal isotypes, and sex mutants were either the same or significantly higher than their normal isotypes (Table 5).

Protein in Shrunken Endosperm, High Lysine Mutants

Greater than 50% of the protein found in normal barley seed is storage material having a high content of glutamine and proline which are easily mobilized and utilized during germination (Cameron-Mills et al., 1980). This storage protein, prolamin, is low in lysine and other amino acids essential for human and animal nutrition. Prolamin, or hordein as it is called in barley, is synthesized in the developing barley seed between two and five weeks after anthesis (Shewry et al., 1979) following a pattern similar to dry weight production. It is deposited primarily in protein bodies in the endosperm and can be separated into four components, A, B, C, and D hordein (Shewry et al., 1982). The composition of the two main components, C and B hordein (also called hordein-1 and hordein-2, respectively), is determined by