Shrunken endosperm mutants from barley, Hordeum vulgare L.
by Alvin John Jarvi

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree by
DOCTOR OF PHILOSOPHY in Genetics
Montana State University

Abstract:
Six spontaneous shrunken endosperm barley mutants were identified and described. All mutants were inherited as single recessive genes and assigned the symbols se through se6. Five of the mutants do not express xenia. The mutants varied in fertility,seed weight, and sieve size assortment. Cytological studies indicated that the greatest frequency of dividing endosperm nuclei were found in samples of the third to the seventh floret from the base of the spike from collections made 5-7 days after pollination at 1-3 p.m. One multiploid sporocyte plant was found and no mitotic abnormalities in endosperm tissue were observed. Four mutants were located on chromosome 1, one on chromosome 3, and one on chromosome 6.

Double crossovers in the interstitial segments of translocations is offered as an explanation of some ratios observed. The mutants may have potential as males or pre-flowering selective genes in hybrid barley systems.

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Approved:

P D Skaar
Head, Major Department

Robert G. Eslick
Co-Chairman, Examining Committee

G. Allan Taylor
Co-Chairman, Examining Committee

L. Loerch
Dean, Graduate Division

MONTANA STATE UNIVERSITY
Bozeman, Montana

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ABSTRACT

Six spontaneous shrunken endosperm barley mutants were identified and described. All mutants were inherited as single recessive genes and assigned the symbols se through se6. Five of the mutants do not express xenia. The mutants varied in fertility, seed weight, and sieve size assortment. Cytological studies indicated that the greatest frequency of dividing endosperm nuclei were found in samples of the third to the seventh floret from the base of the spike from collections made 5-7 days after pollination at 1-3 p.m. One multiploid sporocyte plant was found and no mitotic abnormalities in endosperm tissue were observed. Four mutants were located on chromosome 1, one on chromosome 3, and one on chromosome 6. Double crossovers in the interstitial segments of translocations is offered as an explanation of some ratios observed. The mutants may have potential as males or pre-flowering selective genes in hybrid barley systems.

INTRODUCTION

Very few qualitative factors affecting the endosperm have been described in barley (Hordeum vulgare L.). It is unusual that more of these factors have not been identified and studied in barley because of the ease with which endosperm characteristics expressing xenia can be handled. Endosperm mutants have played an important role in the basic studies of maize genetics. The characteristics most studied have been those which express xenia. In these cases F_2 segregation ratios can be obtained directly from the seed on F_1 ears.

The possible use of pre-flowering selective genes in hybrid barley systems suggested this study of six mutants influencing endosperm development. Gene action and linkage relationships are of prime importance in hybrid barley systems presently proposed.

The possible role of endosperm mutant types in barley hybrid systems was the objective of this study. Areas of investigation included the inheritance of the mutant genes, location of mutants on the barley genetic maps and the endosperm cytology of the mutant lines.

REVIEW OF LITERATURE

Weijer (1952) catalogued the existing genetic studies in maize and included the following endosperm characteristics: brittle endosperm, bt; defective endosperm, de; floury endosperm, fl; soft starch, n; mealy endosperm, me; opaque endosperm, o; reduced endosperm, re; shrunken endosperm, sh; sugary endosperm, su; and waxy endosperm, wx. All of the above mutant types are recessive and express xenia.

One plant characteristic influencing the endosperm phenotype in maize was reported by Mangelsdorf (1926). In a study of several xenia expressing defective endosperm types, Mangelsdorf included one plant character defective endosperm, dep1. Xenia was not expressed by dep1 which gave 3 normal : 1 defective endosperm plant segregations. Pollination of mutant plants with normal pollen resulted in defective endosperm F_1 seed and reciprocal crosses yielded normal F_1 seed. He concluded that the characteristic was dependent on the genotype of the mother plant, not of the developing seed.

A typical example of the xenia expressing endosperm characters in maize is the reduced endosperm genes re1 and re2 reported by Esther (1931). Both genes were single recessives and pollination of homozygous recessive plants with normal pollen and the reciprocal crosses resulted in normal F_1 seed. The F_2 seed, on the F_1 plant ear, segregated 3 normal : 1 reduced endosperm seed. There was a direct effect of the pollen on the trait being studied.

Harlan (1914) reported that the blue aleurone character in barley was due to an anthocyanin pigment. This is one of the few characters in barley which expresses xenia. Myler and Stanford (1942) demonstrated that two dominant complementary genes were involved in the expression of this character. For the blue aleurone to be expressed there must be at least one dominant allele present at each of the two loci. They found one gene to be in the linkage group that is now designated as part of chromosome 1 and the other on what is now designated as chromosome 4, Ramage, Burnham, and Hagberg (1961).

Two genes which influence the chemical composition of the starchy endosperm have been reported in barley. Nilan (1964) summarized the studies on the waxy endosperm character. One of the genes, wx, is a simply inherited recessive and expresses xenia for the trait. The mutant gene, wx, alters the composition of the starch by decreasing the amylose content from about 20% to nearly zero. Another gene, reported by Walker and Merritt (1969), approximately doubled the anylose content in the endosperm of the variety 'Glacier'. The mutant has been designated as ac38 and was inherited as a simple recessive. There was a dosage effect of the mutant gene and with increasing doses of the mutant gene there was a logarithmical increase in the amylose content. From the dosage effect it appears that this mutant gene expresses xenia.

Reid and Wiebe (1968) referred to a kernel type in barley in which the starch was replaced by a sugary liquid. As the seed matured it collapsed and the collapsed seed failed to germinate. Stocks of this mutant could be maintained as heterozygotes which expressed xenia. Harlan (1957) referred to a similar or possibly the same mutant type. Harlan and Pope (1925) discussed a similar nonheritable situation of "watery kernels" in which the seeds contained a sugary liquid. These seeds had a normal seed coat and embryo but no aleurone layer or starchy endosperm. The authors proposed that these may be cases of single fertilization in which only the embryo was fertilized. Another case of liquid endosperm was reported by Brown (1955) in Limnodea arkansana, which differs from the cases mentioned in barley. This type of endosperm remains as a liquid even under dry storage conditions where it contained about 26% water. Dore (1956), following Brown's observations, reported on an additional 17 genera in four species of grass having a similar liquid endosperm.

Robertson (1932) described a simply inherited recessive albino mutant (at2), which also influenced endosperm development in the barley variety 'Canada Thorpe'. Seed containing the mutant gene in the homozygous condition expressed an altered endosperm phenotype. The altered endosperm facilitated an accurate separation of the homozygous mutant seed prior to germination. Upon germination the

mutant seeds had a watery appearance compared to the white starchy appearance of normal seeds. The mutant seeds weighed 2.34 grams/100 compared to 4.65 grams/100 for the normal.

Harvey, Reinbergs, and Somaroo (1968) described a simply inherited recessive gene for female sterility derived from a colchicine-treated barley population. The character was a simply inherited recessive gene. Seed set on the female sterile plants ranged from 14-22% in a rough-awn, hairy-stigma genotype. The authors stated that the seed obtained on the sterile plants was small and could be removed mechanically from a mixture. They indicated the line may have potential as a pollen parent in hybrid barley in which the female sterile line could be mixed directly with the female parent. Nilan (1964) summarized quantitatively inherited factors influencing kernel weight.

Hakansson (1953) reported on endosperm development in 2x X 4x barley crosses and the reciprocal crosses. In the 2x X 4x crosses endosperm mitotic irregularities were common, especially the formation of giant endosperm nuclei. Small amounts of starch were deposited very late in development. In the reciprocal crosses, 4x X 2x, mitotic irregularities were rare and starch deposition began early. Brink and Cooper (1947) reviewed many studies similar to the one of Hakansson's. This review covered many species crosses and demonstrated

results similar to Hakansson's study. They indicated that the high chromosome number female X low chromosome number male crosses were more nearly in balance, with respect to ploidy level, between the endosperm, the embryo, and the maternal tissue, than were the reciprocal crosses. The high chromosome number female X low chromosome number male crosses generally resulted in fewer and plumper seeds than the reciprocals. The reciprocal seeds were badly shriveled and germinated poorly. The seed produced from the crosses between different ploidy levels and/or between species was generally smaller than normal. Ramage and Day (1960) reported that the frequency of trisomics produced from translocation heterozygotes is higher in the lighter seed portion. They pointed out that the frequency of the trisomics could be increased by the use of an aspirator or seed blower to separate the lighter seed.

The post fertilization period of 15 hours of barley was described by Pope (1937). The first endosperm division was within 6 hours after pollination and at 15 hours there were eight endosperm cells. Randolf (1936) followed the endosperm development in maize. At 3 days there were free endosperm nuclei with a definite tendency for the divisions to occur in unison. This tendency continued even after the endosperm was almost completely cellular. At first cell division activity was prevalent throughout the endosperm and later became localized in the

perpheral regions.

Clark and Copeland (1940) and Duncan and Ross (1950) used similar and quite simple techniques for fixing and preparing smear preparations of the dividing endosperm cells of maize. The fixing was accomplished with 3 parts 100% ethanol : 1 part acetic acid. The endosperm was smeared in a small drop of aceto-carmine and heated after the cover slip was in place. Clark and Copeland used the above method for studying abnormal endosperm division which gave rise to high rates of mosaic formations. Punnett (1953), using similar methods for fixing and staining, observed hexaploid endosperm cells in maize. It was postulated that these 6N=60 cells arose from two duplications during interphase followed by a single normal mitosis.

DESCRIPTION OF MUTANT LINES

Materials and Methods

The mutants involved in this study are characterized by a "thin" or "shrunken endosperm" phenotype. These mutants are designated as shrunken endosperm mutants and have been given the gene symbol "se".[1] All are natural occurring mutants in spring barley cultivars, Hordeum vulgare L. The mutants include 'Betzes' se and se2; 'Compana' se3, se4, and se6; and 'Sermo' x 'Glacier'[7] se5 which were collected and seed provided for this study by R. F. Eslick. A possible shrunken endosperm mutant in Betzes (se-x) was collected by the author.

Results and Discussion

General Comments. All of the numbered mutants are fairly easy to classify compared to the normal phenotypes. Comparisons of all mutant types, Betzes and Compana are presented in Table I. The mutants se, se2, se3 and se6 have normal fertility whereas se4 and se5 have significantly lower levels of fertility (Table I). Considerable variation exists in seed weight (Table I) and sieve size distribution (Figures 1, 2 and 3) among the various lines. When se, se2, se3, se4 and se5 were used as females, the F_1 seed (hybrid seed) was shrunken, but when these lines were used as a pollen source in crosses with normal types, the F_1 seed was normal. The F_1 plants and F_2 seed (seed produced on a F_1

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[1]Correspondence with T. Tsachiya, Colorado State University, Fort Collins, Colorado. Dates January 26, 1970.

plant) from the above crosses were of the normal phenotype and did not express xenia. The mutant se6 expresses xenia for the endosperm trait. F_1 seed from crosses using se6 either as a male or female with normal types does not express the shrunken characteristic. F_2 seed segregation of 375 normal seeds : 143 shrunken fit a 3 : 1 ratio at a probability of .10 - .25. This segregation was obtained from heads of se6 x normal F_1 plants expressing xenia. Plant segregations of the numbered mutants except se5 fit the hypothesis that each mutant is a single recessive gene (Table III). The hypothesis that se5 is a single recessive gene was rejected by the plant segregation reported in Table III, however, supported by the good fit to the independent Chi-squares with the unlinked translocations as tabulated in Table X.

Betzes se. Betzes shrunken endosperm-1 (se) was collected from a seed increase field at Aberdeen, Idaho in 1958. Fair stands of this mutant can be obtained under field conditions, but poor stands result from adverse conditions during emergence.

Betzes se2. Betzes shrunken endosperm-2 (se2) was collected from a commercial field of Betzes near Bozeman, Montana in 1965 as a shrunken endosperm mutant. The homozygous line has never produced a plant under field conditions. Plants can be obtained from the shrunken seeds by germinating the seed on blotters with a 10% sucrose solution. About 25% of the seeds germinated produce plants after transplanting the

seedlings to soil when the coleoptile has reached an inch in length. There is little or no starch deposited in the seed that develops on a homozygous mutant plant. Due to poor germination no F_1 plants were obtained from the hybrid seed produced when se2 was used as the female.

Compana se3. Compana shrunken endosperm-3 (se3) was collected from a commercial field of Compana near Bozeman, Montana in 1963 as a possible male sterile. The mutant se3 is phenotypically quite similar to Betzes se. Generally good stands of this mutant can be obtained under field conditions.

Compana se4. Compana shrunken endosperm-4 (se4) was collected from a commercial field of Compana near Bozeman, Montana in 1960 as a possible male sterile. It has a mean seed set of 51.2% which is significantly less than Compana (Table I). The sterile florets appear to start seed development but abort before they reach half the length of the lemma. The mutant se4 can be grown under field conditions but poor stands are obtained when less than optimum conditions prevail during germination and emergence.

Sermo X Glacier[7] se5. Sermo X Glacier[7] shrunken endosperm-5 (se5) was obtained from one of the backcross breeding programs at Bozeman, Montana in 1965. The mutant se5 has a reduced level of fertility with a mean seed set of 16.9% which is significantly less

than all of the other lines examined (Table I). Sieve size distribution of se5 and Glacier are compared in Figure 3 which indicates that se5 has a higher proportion of thinner seeds and a greater range in size than Glacier. It is difficult to classify se5 compared to normal types on seed size alone. The caryopsis of se5 generally extends beyond the lemma and palea more than the normal types. With the difference in caryopsis length and the high degree of female sterility it is possible to classify this mutant.

Compana se6. Compana shrunken endosperm-6 (se6) was collected from a commercial field of Compana near Bozeman, Montana in 1963 as a possible uniculm mutant. Compana se6 will grow equally as well as Compana under field conditions. No differences can be detected between se6 and Compana in development until the hard dough stage. At this stage, se6 develops a depression in the center of the lemma which becomes progressively more distinct with maturity. The mature endosperm of se6 appears much harder than Compana when cut with a knife but no qualitative tests were made. Segregation ratios can be separated into three classes due to the expression of xenia in the heterozygous plants (Table III).

Betzes se-x. Betzes shrunken endosperm-x (se-x) was collected as a shrunken mutant in 1969 at Tucson, Arizona. The original plant appeared to be similar to se. This line was planted at Bozeman in

1969 and did not appear to be a classifiable mutant. It was similar to Betzes in fertility and seed weight (Table I) and in-sieve size distribution (Figure 1). The F_1 seed, F_1 plants, and F_2 seed from crosses involving se-x did not appear to be abnormal in any way. This may be an example of material which must be screened to find heritable mutants or may be an environmentally sensitive mutant and possibly could be classified under a different environment.

TABLE I. Physical data on mutant lines.

Variety	Gene symbol assigned	Previous symbol	fertility[1/]	100 seed weight[1/]	Seed size distribution on 6/64 sieve	Seed size distribution thru 5/64 sieve
			%	gms	%	%
Betzes	---	---	98.1a	4.06bc	71	1
Betzes	se	th1	96.7a	1.34f	0	98
Betzes	se2	th2	96.8a	0.60g	0	100
Betzes	se-x	th-x	96.6a	3.82c	56	5
Compana	---	---	94.2a	5.68a	94	1
Compana	se3	th7	95.0a	1.88e	0	94
Compana	se4	th6	51.2b	2.13e	2	61
Compana	se6	th5	95.0a	4.26b	26	3
Sermo X Glacier[7]	se5	th8	16.9c	2.64d	36	17

[1/] Means with like letters are not significantly different from each other at the 1% probability level.

TABLE II. ANOVA for weight/100 seeds and fertility.

Source	DF	Mean Squares: Weight/100 seeds	Mean Squares: Fertility
Lines	8	62.11**	4132.14**
Error	36	0.08	8.69
Total	44		

** Significant at the 1% probability level.

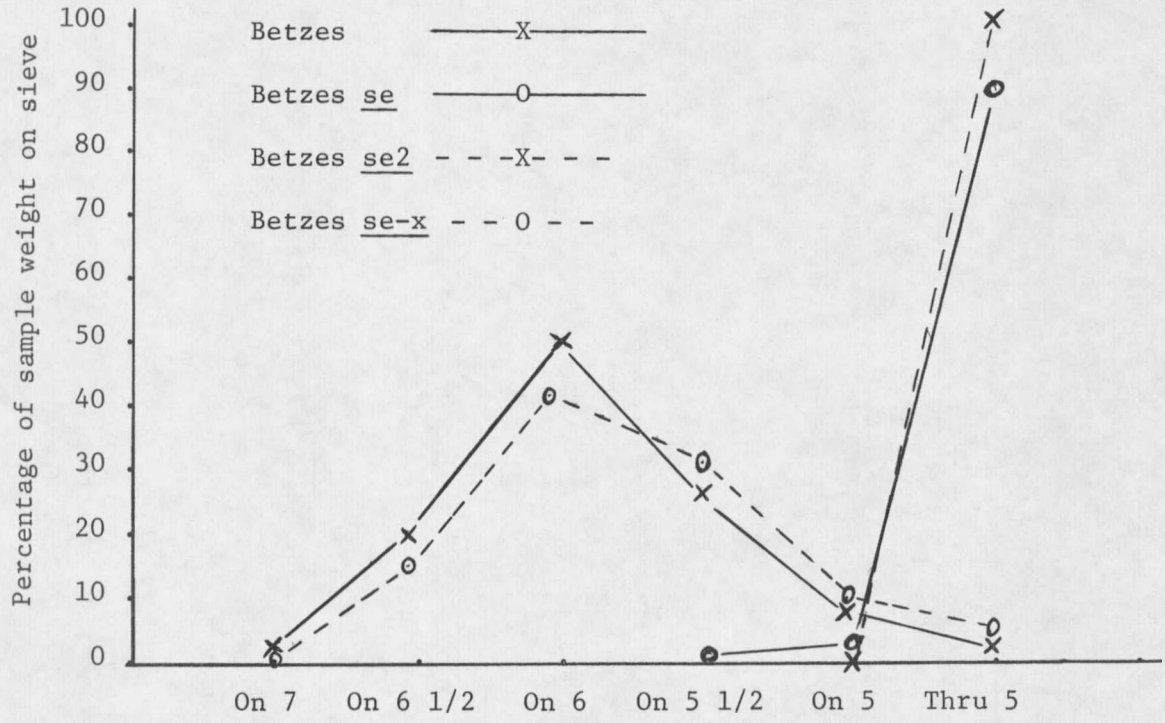

Figure 1. Sieve size distribution of Betzes and Betzes shrunken endosperm mutants.