Utilizing linkages of genetic male sterile and aleurone color genes in hybrid barley (Hordeum vulgare L.) systems
by Gregory David Kushnak

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and Soil Science
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Abstract:
The complementary genes for blue aleurone, B 11 and B12, were evaluated for use in preflowering selection for male sterility in barley (Hordeum vulqare L.). Male sterile (msgl0), located near the centromere of chromosome I, naked caryopsis (n) , and short awn (1k2) were linked to B12 with recombination values of 3.9 + 0.4%, 12.1 + 1.5%, and 21.5 + 1.8%, respectively. B12 was positioned on the short arm of chromosome 1. Recombination values for B11 with male steriles msg24v and msg25r were 11.2 _+ 1.3% and 5 = 4 + 0.6%, respectively, with the three loci positioned on the long arm of chromosome 4. A recessive blue aleurone color enhancer gene, en-Bl, which conditioned stable dark blue color in the presence of B11 and B12, was linked to msglO and B12 with recombination values of 29.5 + 3.0% and 30.1 + 3.0%, respectively. Evidence indicated a possible association of gamete selection factors with B11 and B12. Evidence indicated Blx was allelic to B11, and Bly non-allelic to B11 and B12. Blue aleurone, in the background homozygous genotype en-Bl n could be used as a preflowering male sterile selection tool in commercial hybrid barley production.

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ABSTRACT

The complementary genes for blue aleurone, *B11* and *B12*, were evaluated for use in preflowering selection for male sterility in barley (*Hordeum vulgare* L.). Male sterile (*msg10*), located near the centromere of chromosome 1, naked caryopsis (*n*), and short awn (*lk2*) were linked to *B12* with recombination values of 3.9 ± 0.4%, 12.1 ± 1.5%, and 21.5 ± 1.8%, respectively. *B12* was positioned on the short arm of chromosome 1. Recombination values for *B11* with male steriles *msg24v* and *msg25r* were 11.2 ± 1.3% and 5.4 ± 0.6%, respectively, with the three loci positioned on the long arm of chromosome 4. A recessive blue aleurone color enhancer gene, *en-B1*, which conditioned stable dark blue color in the presence of *B11* and *B12*, was linked to *msg10* and *B12* with recombination values of 29.5 ± 3.0% and 30.1 ± 3.0%, respectively. Evidence indicated a possible association of gamete selection factors with *B11* and *B12*. Evidence indicated *B1x* was allelic to *B11*, and *B1y* non-allelic to *B11* and *B12*. Blue aleurone, in the background homozygous genotype *en-B1 n* could be used as a preflowering male sterile selection tool in commercial hybrid barley production.

INTRODUCTION

Interest in hybrid barley (Hordeum vulgare L.) has been evident in recent years, not only as a method of commercializing barley breeding, but also from the standpoint of incorporating a large number of desirable genes into a single genotype. The assignment of approximately 20 known male sterile genes to loci (Hockett and Eslick, 1970; Hockett, 1972) and the detection of closely associated marker genes have made it possible to devise various genetic male sterile systems that would allow economic production of hybrid seed on a commercial scale (Wiebe, 1960; Wiebe and Ramage, 1970). In one system, Eslick (1970) proposed the use of the dominant blue aleurone gene B12 as a preflowering selective gene for the male sterile genes ms10 and ms14. He reported ms10 and ms14 to be very near the centromere of chromosome 1 and 7.5 $\pm$ 6.6 recombination units from naked caryopsis, n. Since B12 has been reported to be closely associated with n (Myler and Stanford, 1942; Wells, 1958), it was postulated that B12 would be quite close to ms10 and ms14. With the aleurone being 3N tissue, the blue factor would express xenia. If close linkage between B12, ms10, and ms14 exists, the male sterile phenotype could be determined prior to planting simply by observing seed color. Since the seed represents the same generation as the plant that it produces, selection for male sterility on the basis of seed color would be, in essence, prior to flowering; hence the term preflowering. It was also suggested that the blue aleurone factor B11 could be similarly employed with ms24v and

ms25r on chromosome 4.

The purpose of this study was to determine the feasibility of the two systems proposed by Eslick by establishing the necessary stocks and estimating the probable reliability of the systems and to investigate the possibility of establishing other blue aleurone hybrid systems based on blue aleurone sorting.

REVIEW OF LITERATURE

Gene Action and Number of Loci

Blue aleurone is quite common in some grass species including barley and wheat. In wheat, the character has been encountered in progeny from *Triticum* x *Agropyron* crosses and is conditioned by two complementary genes (Hurd, 1959; Kattermann, 1932).

In barley approximately 37% of the world collection and 41% of U. S. domestic selections are blue (Ward, 1962). Buckley (1930) originally reported the character to be due to a single dominant gene which was linked to the hooded factor *K* on chromosome 4 with a recombination value of 40.6%. The gene was designated as *Bl1*. Robertson, Deming and Koonce (1932), with considerably more data, placed *Bl1* 22.6 ± 0.8 recombination units from *K*. Later, Myler and Stanford (1942) found blue aleurone to be conditioned by two dominant complementary genes in a cross between the white seeded varieties Nepal and Goldfoil which gave blue F_1 seeds, and F_2 seeds in the proportion of 9 blue and 7 white. The second blue gene was designated as *Bl2*, and was shown to be linked to the naked caryopsis factor *n* on chromosome 1 with a recombination value of 9.9 ± 0.4%.

More recently, Wiebe (1972) showed evidence for at least one and possibly 2 additional blue aleurone genes. Homozygous blue lines were not established from more than 5000 blue kernels that originated from a heterozygous stock. Only white seeded lines and lines segregating 1:1 for blue and white seeds were obtained in subsequent generations.

Crosses between some of the white kerneled plants gave blue F_1 seeds, indicating two different white types and a complementary gene action for blue. He concluded that a very close translinkage between two dominant complementary factors was responsible, and designated them Blx and Bly.

Expression and Pigmentation

Various pigments influence the expression of blue aleurone in barley. Harlan (1914) found four color conditions existed due to the total absence or the different location and combination of anthocyanin and melanin-like pigments in the barley kernel as follows:

1. White or yellow seeded barley. (a) Hulled varieties without pigment in either the lemma, aleurone layer, or pericarp. (b) Hulless varieties without pigment in the aleurone layer or pericarp.
2. Blue seeded barley. (a) Hulled varieties with a blue aleurone layer showing through the lemma and pericarp. (b) Hulless varieties with a blue aleurone layer showing through a non-pigmented pericarp.
3. Purple seeded barley. (a) Hulled varieties with purple lemmas. (b) Hulless varieties with blue aleurone and red pericarp.
4. Black seeded barley. (a) Hulled varieties with black lemmas. (b) Hulless varieties with black pericarp.

The blue and purple conditions were due to one pigment, anthocyanin.

In an acid condition the anthocyanin appeared red, and in an alkaline condition, blue. Since the aleurone tissue is alkaline, and the pericarp acid (Reid and Wiebe, 1968), the presence of anthocyanin in the aleurone would result in blue seeds, and the presence of anthocyanin in the pericarp would result in red or violet seeds. When both tissues contain the anthocyanin, the seed would become purple. It was possible to change these colors with chemicals (Reid and Wiebe, 1968). When the pericarp over the blue aleurone layer was removed and a weak acid applied, a red color developed. Gudkov (1940) showed that color in unripe seeds could be developed with a 15% HCl solution in varieties that have color when ripe, but not in varieties that normally had yellow or white seeds when ripe; indicating the importance of the proper pH as well as the presence of anthocyanin.

The black seed color was due to a melanin-like pigment, and was unchanged by treatment with either an acid or an alkali (Harlan, 1914). Buckley (1930) states that black pericarp is always associated with black lemma and either the same gene is responsible for the coloring in both the lemma and the pericarp or that very close linkage between the two separate genes must exist. From the studies of Cottingham (1960), black completely covers the expression of blue aleurone. However, Kajanus and Berg (1924) reported that violet brown seed color was due to the presence of anthocyanins in the aleurone and a brown melanin-like pigment in the pericarp. This was probably a lighter shade of the

same pigment that causes black pericarp since Woodward (1941 and 1942) reported an allelomorphic series to exist for the black pericarp factor, giving various intensities from white to dark black.

The presence of other pigments in addition to anthocyanin can also alter seed color. Mullick et al (1958), analyzed the aleurone of certain blue and white varieties by paper chromatography to determine a biochemical basis for the inheritance of aleurone color. In all the varieties examined two leuco-anthocyanins, delphinidin and cyanidin, were found. In addition to these, two colored anthocyanins, B and C, were found only in the blue varieties. Robinson and Robinson (1933) and Bate-Smith (1954) studied the biosynthesis of anthocyanins and showed that cyanidin is the precursor to cyanidin-3-glucoside (anthocyanin C), and delphinidin is the precursor to delphinidin glycoside (anthocyanin B). It was therefore postulated that the two complementary genes control the steps required to develop the the anthocyanins from the two leuco-anthocyanins in the blue varieties. In addition to these pigments, it was noted that 'Trebi' contained a much greater quantity of flavonoid co-pigment than the other blue varieties, which modified the anthocyanin color in vivo. They also stated that many compounds other than anthocyanins appeared to contribute to color expression, and that orange or yellow flavonoid pigments in the pericarp may have altered the appearance of the blue shade.

In addition to color variations caused by the presence or lack of

certain pigments, various color intensities have been observed among normal blue aleurone lines of barley (Ward, 1962). Wiebe and Reid (1961) reported the blue color varied with variety and environment. It reached its highest expression when the plants were grown under irrigation in an arid climate and was best observed when the kernels were fully developed but before drying at maturity. In these areas, the separation of the varieties into dark blue, blue, and light blue classes was possible. In humid areas, the distinction between these shades of blue disappeared as the color faded out or was masked by stained hulls resulting from weathering. The lack of blue color under humid conditions was so complete in some cases that separations of it from white could not be made with certainty. Harlan (1914) developed a simple chemical test to determine the presence or absence of pigment in badly weathered seeds. When seeds with cut seed coats were submersed in a weak acid solution, a pink ring appeared in the region of the aleurone layer if the pigment was present.

Woodward (1941 and 1942) observed differences among barley varieties for color intensity of black lemma. He further noted that black was dominant to grey, and grey dominant to white.

Tuleen et al (1968) conducted a series of experiments leading to a possible explanation of foliage color variations among barley varieties. They treated barley which was homozygous for the yellow virescent gene yh with mutagenic agents and found 44 revertant types. The

revertant types represented varying degrees of green color intensity, including normal green. It was shown that the revertant types remained homozygous for the yellow virescent gene, and that reversion to green was due to the action of simply inherited suppressor genes. Allelism tests showed a minimum of 17 different suppressor loci represented among the 44 revertant types.

It is possible that a large contribution to genetic variation in color intensity is from quantitative effects. Background genotypes, such as those which influence the alkalinity or number of cell layers of the aleurone may have a large and direct influence. Kondo and Kasahara (1939) and Kondo and Takahashi (1938) using various phenol solutions were able to produce various intensities of brown in the pericarp depending on variety, temperature, and maturity of seed. Furthermore, if changing the pH from alkaline to acid changes the anthocyanin from blue to red, as Reid and Wiebe (1968) reported, it would seem reasonable that the pigment would express various shades of blue as it is converted to red.

Sawicki (1950) reported that the number of aleurone cell rows is a varietal characteristic, with Asiatic and African barley varieties exhibiting three and two rows of aleurone cells respectively. Wolf et al (1972) reported the aleurone is two to five cells thicker in primitive Coroico corn than in U. S. commercial hybrids, averaging 3.7 cell layers and 3.4 percent of the whole kernel weight. The aleurone

of the U. S. varieties was consistently one cell thick and made up less than two percent of the kernel weight. The increase in aleurone thickness brought about a higher crude protein content and a higher percentage of vitamins and essential amino acids since the aleurone was the site of B- vitamins and high quality protein. Thus, it may be possible that variations in thickness of barley aleurone may contribute to variations in seed color intensity by varying the total quantity of pigment.

Several investigations have shown aleurone pigment concentrations to vary among blue aleurone varieties of barley. In the study of Mullick et al (1958), total pigment extraction was much greater from the aleurone of 'Kwan' and Trebi than from 'Montcalm', with Kwan containing slightly more pigment than Trebi. Reid and Wiebe (1968) reported the amount of pigment in the kernel varied in red, purple, and blue seeded barley varieties. It was not stated whether the varieties under investigation varied for aleurone thickness, but it was likely that variation for pigment concentration was influenced by many physiological and morphological conditions of the plant.

Dosage Effect and Distorted Ratios

Gene dosage may have an influence on the expression of blue color and subsequently may alter segregation ratios. Knott (1958) noticed a distinct dosage effect where the F_1 seeds from crosses between blue and

white seeded wheat (Triticum aestivum) varieties were intermediate in color. When blue lines were used as the maternal parents, the hybrid seeds were clearly blue although not as dark as those of the blue parent. When the white lines were used as the maternal parent, the hybrid seeds were only slightly blue. Since the aleurone is triploid for any given genome and derives two of its chromosome complements from the maternal parent, the F_1 seeds from the blue maternal parent had a blue allele dosage two times greater than that of the F_1 seeds from the white maternal parent.

Hurd (1959), in studying segregations for blue aleurone in wheat under various environmental conditions, noted that when two or three dominant complementary alleles were present, the expression of blue occurred only under the most favorable conditions. Under all growing conditions, an endosperm with four or more dominant alleles was blue. Table 1 shows the 16 possible endosperm genotypes for the dihybrid cross and their respective phenotypes for sub-optimal growth conditions. Note that the genotypes consisting of the two complementary dominants with a dosage of two or three are listed as non-blue. Under optimum conditions, where moisture was not lacking, these genotypes were usually blue. Other studies have shown that, when adequate water is supplied to plants, the expression of blue color is more intense (Gortner and Gortner, 1949; Scott-Moncrieff, 1924). This is reasonable since it has been shown that plants become more acid with lack of

Table 1. Expected aleurone genotypes in the F_2 and their probable phenotypes when produced under sub-optimal growing conditions. 1/

Aleurone Genotype	Aleurone Phenotype
AABB/AB	Blue
AABB/Ab	Blue
AABB/aB	Blue
AABB/ab	Blue
AAbb/AB	Blue
AAbb/Ab	Non-blue
AAbb/aB	Non-blue
AAbb/ab	Non-blue
aaBB/AB	Blue
aaBB/Ab	Non-blue
aaBB/aB	Non-blue
aaBB/ab	Non-blue
aabb/AB	Non-blue
aabb/Ab	Non-blue
aabb/aB	Non-blue
aabb/ab	Non-blue

1/ From Hurd (1959)

moisture (Newton and Martin according to Hurd, 1959) and that the expression of blue requires a high pH (Harlan, 1914; Buckley, 1930; Reid and Wiebe, 1968). Robinson (1936) reported that low temperature, high light intensity, and excess oxygen also favor the appearance of pigments. Hurd (1959) observed a differential response to light and moisture between two crosses. In the first cross, abundant moisture appeared to be the most important factor for the expression of blue color. Full expression occurred under the low light intensity and high moisture conditions of the greenhouse in plants having two or more alleles of the complementary dominant genes, while in the field nursery blue color was only occasionally expressed with two complementary dominants. In the second cross, the expression of the blue color appeared to be more dependent on light intensity. When grown under field conditions, only two complementary dominants were required for complete expression whereas, under much reduced light in the greenhouse, four dominants were necessary.

With this apparent differential response of varieties and allelic dosages to light and moisture conditions, it would appear that, at least in wheat, phenotypic ratios deviating from the expected 9:7 or 3:1 could be expected to occur quite frequently.

Jain (1970) observed, in two barley composite crosses, aleurone color segregation ratios ranging from 4:1 to 1:4, with ratios of 3:1, 2:1, and 1:1 being the most prevalent. He postulated the deviating

ratios were caused by a gametophytic factor associated with one of the blue aleurone genes. However, he did not provide any evidence, other than the skewed ratios, that a gametophytic factor was actually the cause. Tabata (1957 and 1961) reported a gametophytic factor ga on chromosome 1 of barley which had a recombination value of 33.0 percent with the waxy endosperm gene, wx. Male gametes carrying the ga factor could fertilize eggs in a Ga ga stylar tissue only at frequencies of less than 9 percent. It was further noted that wx was 43.8 ± 1.3 recombination units from ac2 when ga was present, but independent from ac2 when ga was not present. It was proposed that the gene order could be either ga - wx - ac2 or wx - ga - ac2. The fairly close association of the B12 locus with ac2 (Eslick, 1970; Rahman, 1973) could possibly allow B12 segregations to be influenced by the presence of ga.

Loegering and Sears (1963) reported in wheat, a pollen sterilizing gene, where individual pollen grains carrying the factor were non-viable. The existence of such a gene in barley has not been reported.

Possible Associated Male Sterile Genes

Hockett and Eslick (1970) reported ms10, ms14, ms,,b, and ms,,e to be associated with chromosome 1, with ms10 and ms14 near the centromere, and ms,,b not near the centromere. In addition, ms6 and ms,,w were reported as possible associations with chromosome 1. Eslick

(unpub.) verified the association of ms,,w with chromosome 1, but showed ms6 to be near the centromere of chromosome 6. Hockett (1972) reported ms,,e and ms,,b were non-allelic with all the presently known male sterile genes and with each other, and were therefore designated as ms22e and ms23b, respectively. It was also determined that ms,,w is non-allelic to any of the other male sterile genes on chromosome 1 (Hockett, unpub.)

Jarvi and Eslick (1967), using genetic and translocation data, reported the association of ms24v with chromosome 4. In addition, Hockett and Eslick (1970) reported ms,,r to be associated with chromosome 4, with both ms24v and ms,,r near the centromere. It was noted however, that ms,,r showed approximately 0.7 percent selfing when grown in isolation at Bozeman, Montana. Hockett (1972) showed ms24v, ms,,ak, and ms,,an to be non-allelic to all of the other present male sterile genes, but allelic to one another. They were therefore designated as ms24ak and ms24an. The gene ms,,r was shown to be non-allelic to all present male sterile genes and was designated as ms25r (Hockett, 1974).

MATERIALS AND METHODS

The experiments were conducted mostly under irrigated field conditions on the Montana State University Agricultural Experiment Station near Bozeman, Montana, in 1970, 1971, and 1972. Some crosses and generations were advanced in the greenhouse at Bozeman and in the field near Mesa, Arizona, during the winters of 1970-71, 1971-72, and 1972-73.

Most of the seedstocks used in the inheritance and linkage studies were provided by Professor R. F. Eslick of Montana State University, Bozeman, Montana, and by Dr. E. A. Hockett, USDA, ARS, Montana State University, Bozeman, Montana, and are described in Table 2. Hulless early generation plants from the cross Sermo/7* Vantage segregating for aleurone color gave rise to the two white types b11 b11 B12 B12 and B11 B11 b12 b12, which were designated as 'Nuvan White-1' and 'Nuvan White-2' respectively. The hyphenated number following the name designates which blue aleurone locus is recessive in the respective stocks. Hulless homozygous blue plants from the Sermo/7* Vantage cross were designated as 'Nuvan Blue'. The name Nuvan is a contraction of "nude" and "Vantage". The hulless Sermo/7* Betzes stock was assigned the name Nubet, which is a contraction of "nude" and "Betzes". The short awned hulless Sermo/7* Compana stock and the long awned hulless Stamm/7* Compana stock were designated as Shonupana and Nupana respectively. The name Shonupana is a contraction of "short", "nude", and "Compana". The aleurone color genotypes of the stocks

Table 2. Seed stocks used in the inheritance studies and to obtain other necessary genotypes.

Stock Number 1/	Variety or Isogene	Aleurone Color	Homozygous Genotype 2/
	Sermo/7* Vantage	segregating	B11 b11 B12 b12 n En-b1 3/
	Sermo/7* Vantage (Nuvan Blue)	blue	B11 B12 n Lk2 En-B1
	Sermo/7* Vantage (Nuvan White-2)	white	B11 b12 n Lk2 En-B1
	Sermo/7* Vantage (Nuvan White-1)	white	b11 B12 n Lk2 En-B1
MT84366	Sermo/7* Betzes (Nubet)	white	b11 B12 n Lk2 En-B1
MT842148	Sermo/7* Compana (Shonupana)	white	b11 B12 n lk2 En-B1
MT24151	Stamm/7* Compana (Nupana)	white	b11 B12 n Lk2 En-B1
CI7776	Sermo	white	B11 b12 n lk2 En-B1
CI595	Nepal	white	B11 b12 n K En-B1
CI928	Goldfoil	white	b11 B12 N Lk2 En-B1
CI7149	Montcalm	blue	B11 B12 N Lk2 En-B1
CI2947	Manchuria	blue	B11 B12 N Lk2 En-B1
CI12167	Ubamer	dark blue	B11 B12 n Lk2 en-B1
MT1355	Mesa Dark Blue	dark blue	B11 B12 n Lk2 en-B1
MSS89	Betzes ms24v	white	b11 B12 N Lk2 En-B1
MSS86	Betzes ms25r	white	b11 B12 N Lk2 En-B1
MSS63	Unitan ms14	white	b11 B12 N Lk2 En-B1
MSS39	Titan ms1	blue	B11 B12 N Lk2 En-B1
MSS49	Trebi ms2	blue	B11 B12 N Lk2 En-B1
MSS58	Compana ms10	white	b11 B12 N Lk2 En-B1
MSS68	CI4961-1 ms19	blue	B11 B12 N Lk2 En-B1
	Blx blx Bly bly	segregating	B12 N Lk2 En-B1
	Blx Blx bly bly	white	B12 N Lk2 En-B1
	blx blx Bly Bly	white	B12 N Lk2 En-B1

Table 2. Continued

1/ Where MT designates Montana Stock numbers and MSS designates male sterile stock numbers.

2/ Where B11 and B12 are complementary dominants for blue aleurone; n is recessive for naked caryopsis; lk2 is recessive for short awn; K is dominant for hooded lemma; en-B1 is recessive for enhanced blue color intensity.

3/ Early generation material segregating for B11 and B12.

listed in Table 2 were determined by testcrossing to the known testers, Nepal and Goldfoil. Stocks carrying the blx and bly mutants were provided by Dr. G. A. Wiebe, USDA, Beltsville, Maryland; and are also listed in Table 2. Additional genotypes necessary for the studies conducted were derived from the stocks listed in Table 2, and are listed in Table 3. All gene symbols used in the tables or text are described in Appendix Table 1.

In crosses involving aleurone color, segregations for aleurone color occurred on the F_1 plant among the F_2 seeds. The general procedure was to sort the F_2 seeds into two or three color classes, depending upon whether the dark blue enhancing factor was segregating; and to count the seeds in each color class for determination of segregation ratios. Whenever possible, hulless parents were used in order to facilitate color sorting in the F_2. In situations where F_1 plants were of the covered caryopsis type, harvesting and color sorting were done during the dough stage of kernel development to minimize misclassifications for color. Plants read for aleurone color segregations were checked for semi-sterility. The monohybrid aleurone color ratios were examined on plants of B12 B12 B11 b11 or B12 b12 B11 B11 genotypes selected from progeny of the dihybrid crosses Unitan (B12 B12 b11 b11)/ Nepal (b12 b12 B11 B11), and Nupana (B12 B12 b11 b11)/ Nepal (b12 b12 B11 B11). Monohybrid data were also obtained from crosses of Nuvan Blue (B12 B12 B11 B11) with b12 b12 B11 B11 stocks derived from Nupana/Nepal

Table 3. Additional genetic stocks developed for use in linkage and inheritance studies.

Stock 1/	Source
En-B1 En-B1 B11 B11 B12 B12 Ms10 Ms10	Compana ms10/Nepal
En-B1 En-B1 B11 B11 B12 B12 ms10 ms10	Compana ms10/Nepal
En-B1 En-B1 B11 B11 b12 b12 ms10 ms10	Compana ms10/Nepal
En-B1 En-B1 B11 B11 B12 B12 ms14 ms14 N N	Unitan ms14/Nepal
en-B1 en-B1 B11 B11 b12 b12 Ms10 Ms10	Compana ms10/Nepal/2/Ubamer
en-B1 en-B1 B11 B11 b12 b12 ms10 ms10	Compana ms10/Nepal/2/Ubamer

1/ All stocks are homozygous for naked caryopsis (n) unless otherwise noted.

crosses. Testcrosses involving white aleurone plants from families segregating 2:1 for aleurone color were made to Betzes (B12 B12 b11 b11) and CI4961-1 (B12 B12 B11 B11). The dihybrid ratios were observed on F_1 plants from the dihybrid crosses Shonupana (B12 B12 b11 b11)/ Nuvan White-2 (b12 b12 B11 B11) and Shonupana (B12 B12 b11 b11)/ Nepal (b12 b12 B11 B11); and from early generation Sermo/7* Vantage plants segregating for aleurone color.

Reciprocal crosses among Nepal, Nupana, Shonupana, Nubet, Nuvan Blue, and a B11 B11 B12 B12 stock derived from Nepal/Shonupana crosses provided blue F_1 seed samples representing allelic dosages from 3 to 6 dominant alleles for the B11 and B12 loci. Blue F_1 seed samples representing an allelic dosage of 2 dominant aleurone alleles could not be obtained because of the unavailability of a b11 b11 b12 b12 stock, which is necessary to set up the genotype B11 b11 b11 B12 b12 b12. The F_1 seed samples were ranked according to color intensity on a scale of 1 to 4, from darkest to lightest blue. The color intensity scores were described in accordance to the R. H. S. Colour Chart (Royal Horticultural Society of London).

Blue seeds from Wiebe's B1x b1x B1y b1y stock were planted and the resulting plants crossed to male sterile plants of the blue aleurone stock Trebi ms2. At maturity, the B1x b1x B1y b1y parents were examined to make sure that they were segregating 1:1 for aleurone color. Approximately 230 F_1 seeds obtained from the cross were planted and the

resulting F_1 plants read for aleurone color segregations. Allelism tests of Blx and Bly were made by crossing Wiebe's white aleurone stocks blx blx Bly Bly and Blx Blx bly bly to Nupana (bl1 bl1 Bl2 Bl2), Nepal (Bl1 Bl1 bl2 bl2), and Ubamer (Bl1 Bl1 Bl2 Bl2). The F_1 and F_2 seeds were examined for aleurone color and segregation ratios.

One hundred one spring habit male sterile barley stocks were obtained from Dr. E. A. Hockett, ARS, USDA, Montana State University, Bozeman, Montana, and crossed to Bl1 Bl1 bl2 bl2, bl1 bl1 Bl2 Bl2, and Bl1 Bl1 Bl2 Bl2 testers. The F_1 plants were grown and classified for aleurone color. Nepal and CI4961-1 were used as the Bl1 Bl1 bl2 bl2 and Bl1 Bl1 Bl2 Bl2 testers, respectively; and Betzes, Goldfoil, Unitan, and Compana were used as the bl1 bl1 Bl2 Bl2 testers. The male sterile stocks, as reported by Hockett et al (1968), are listed in Table 4.

Twelve dark blue aleurone interchange point stocks and one dark blue aleurone male sterile stock, derived by backcrossing to Ubamer (CI12167), were obtained from Dr. G. A. Wiebe, USDA, Beltsville, Maryland, and are listed in Table 5. These along with Ubamer were crossed to white or normal blue aleurone stocks previously described in Tables 2 and 3. Reciprocal crosses involving Ubamer with two normal blue stocks were included. Crosses of normal blue with white aleurone lines, and normal blue with normal blue lines, were included as checks for normal blue color intensities. A Ubamer sib cross and a cross between

Table 4. Spring habit genetic male sterile stocks of barley reported by Hockett et al (1968).

Male Sterile Stock No.	C.I. No.	Variety or Strain	Male Sterile Gene
1	9556	Atlas 57	ms1
2	6250	Atsel	ms1
3	5105	Barbless	ms1
4	10284	Belownee	ms1
5	6398	Betzes	ms1
6	6398	Betzes	ms1
7	10852	Blanco Mariout	ms1
8	10084	Breuns Wisa	ms1
9	261	Club Mariout	ms1
10	5438	Compana	ms1
11	6011	Commander	ms1
12	5338	Comp. Cross Sel.	ms1
13	5366	Comp. Cross Sel.	ms1
14	2225	Deficiens	ms1
15	9562	Domen	ms1
16	7243	Gem	ms1
17	11758	Grande	ms1
18	531	Hannchen	ms1
19	8094	Heimdal	ms1
20	8097	Herta	ms1
21	1803	Hoodless Bdls.	ms1
22	8267	Ille de Re	ms1
23	10083	Ingrid	ms1
24	3236	Intro. (Russia)	ms1
25	3644	Intro. (Egypt)	ms1
26	5000	Intro. (Iraq)	ms1
27	5890	Intro. (Valki)	ms1
28	6225	Intro. (Turkey)	ms1
29	7847	Intro. (Turkey)	ms1
30	8576	Intro. (Turkey)	ms1
31	13636	Intro. (Turkey)	ms1
32	6969	Kindred	ms1
33	2330	Manchuria	ms1
34	1556	Minsturdi	ms1
35	7152	Ogalitsu	ms1
36	1077	Pandora	ms1
37	5401	Rojo	ms1

Table 4. Continued

Male Sterile Stock No.	C.I. No.	Variety or Strain	Male Sterile Gene
38	4343-1	Spiti	ms1
39	7055	Titan	ms1
40	936	Trebi	ms1
41	936	Trebi	ms1
42	10647	Trophy	ms1
43	7324	Vantage	ms1
44	8055	WS471	ms1
45	5438	Compana	ms2
46	8097	Herta	ms2
47	2330	Manchuria	ms2
48	7152	Ogalitsu	ms2
49	936	Trebi	ms2
50	936	Trebi	ms2
51	10072	Gateway (332)	ms3
52	7130	Freja	ms4
53	10114	Carlsberg II	ms5
54	9532	Heines Hanna	ms6
55	3351	Dekap	ms7
56	6398	Betzes	ms8
57	7324	Vantage	ms9
58	5438	Compana	ms10
59	2330	Manchuria	ms10
60	10072	Gateway (337)	ms11
61	10524	Svalof 50-109	ms12
62	10420	Haisa II	ms13
63	10421	Unitan	ms14
64	13446	Atlas x 2 Kindred	ms15
65	6398	Betzes	ms16
66	5438	Compana	ms17
67	5438	Compana	ms18
68	4961-1	Intro. (Russia)	ms19
69	11491	Ackermans MGZ	ms-a
70	11491	Ackermans MGZ	ms-a
71	6398	Betzes	ms-b
72	5438	Compana	ms-c
73	10861	Glacier x Compana	ms-e
74	9559	Piroline	ms-f
75	7243	Gem	ms-g