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
Montana State University
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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.

# UTILIZING LINKAGES OF GENETIC MALE STERILE AND ALEURONE COLOR GENES IN HYBRID BARLEY (*Hordeum vulgare* L.) SYSTEMS

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## ABSTRACT

The complementary genes for blue aleurone, Bl1 and Bl2, 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 Bl2 with recombination values of 3.9 ± 0.4%, 12.1 ± 1.5%, and 21.5 ± 1.8%, respectively. Bl2 was positioned on the short arm of chromosome 1. Recombination values for Bl1 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 Bl1 and Bl2, was linked to msg10 and Bl2 with recombination values of 29.5 ± 3.0% and 30.1 ± 3.0%, respectively. Evidence indicated a possible association of gamete selection factors with Bl1 and Bl2. Evidence indicated Blx was allelic to Bl1, and Bly non-allelic to Bl1 and Bl2. 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.

## 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 Bl2 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 Bl2 has been reported to be closely associated with n (Myler and Stanford, 1942; Wells, 1958), it was postulated that Bl2 would be quite close to ms10 and ms14. With the aleurone being 3N tissue, the blue factor would express xenia. If close linkage between Bl2, 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 Bl1 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 <u>yh</u> 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 bl1 bl1 Bl2 Bl2 and Bl1 Bl1 bl2 bl2, 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 | Bl1 bl1 Bl2 bl2 n En-bl 3/ |
| | Sermo/7* Vantage (Nuvan Blue) | blue | Bl1 Bl2 n Lk2 En-Bl |
| | Sermo/7* Vantage (Nuvan White-2) | white | Bl1 bl2 n Lk2 En-Bl |
| | Sermo/7* Vantage (Nuvan White-1) | white | bl1 Bl2 n Lk2 En-Bl |
| MT84366 | Sermo/7* Betzes (Nubet) | white | bl1 Bl2 n Lk2 En-Bl |
| MT842148 | Sermo/7* Compana (Shonupana) | white | bl1 Bl2 n lk2 En-Bl |
| MT24151 | Stamm/7* Compana (Nupana) | white | bl1 Bl2 n Lk2 En-Bl |
| CI7776 | Sermo | white | Bl1 bl2 n lk2 En-Bl |
| CI595 | Nepal | white | Bl1 bl2 n K En-Bl |
| CI928 | Goldfoil | white | bl1 Bl2 N Lk2 En-Bl |
| CI7149 | Montcalm | blue | Bl1 Bl2 N Lk2 En-Bl |
| CI2947 | Manchuria | blue | Bl1 Bl2 N Lk2 En-Bl |
| CI12167 | Ubamer | dark blue | Bl1 Bl2 n Lk2 en-Bl |
| MT1355 | Mesa Dark Blue | dark blue | Bl1 Bl2 n Lk2 en-Bl |
| MSS89 | Betzes ms24v | white | bl1 Bl2 N Lk2 En-Bl |
| MSS86 | Betzes ms25r | white | bl1 Bl2 N Lk2 En-Bl |
| MSS63 | Unitan ms14 | white | bl1 Bl2 N Lk2 En-Bl |
| MSS39 | Titan ms1 | blue | Bl1 Bl2 N Lk2 En-Bl |
| MSS49 | Trebi ms2 | blue | Bl1 Bl2 N Lk2 En-Bl |
| MSS58 | Compana ms10 | white | bl1 Bl2 N Lk2 En-Bl |
| MSS68 | CI4961-1 ms19 | blue | Bl1 Bl2 N Lk2 En-Bl |
| | Blx blx Bly bly | segregating | Bl2 N Lk2 En-Bl |
| | Blx Blx bly bly | white | Bl2 N Lk2 En-Bl |
| | blx blx Bly Bly | white | Bl2 N Lk2 En-Bl |

Table 2. Continued

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

2/ Where Bl1 and Bl2 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-Bl is recessive for enhanced blue color intensity.

3/ Early generation material segregating for Bl1 and Bl2.

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 Bl2 Bl2 Bl1 bl1 or Bl2 bl2 Bl1 Bl1 genotypes selected from progeny of the dihybrid crosses Unitan (Bl2 Bl2 bl1 bl1)/ Nepal (bl2 bl2 Bl1 Bl1), and Nupana (Bl2 Bl2 bl1 bl1)/ Nepal (bl2 bl2 Bl1 Bl1). Monohybrid data were also obtained from crosses of Nuvan Blue (Bl2 Bl2 Bl1 Bl1) with bl2 bl2 Bl1 Bl1 stocks derived from Nupana/Nepal

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

| Stock 1/ | Source |
|---|---|
| En-Bl En-Bl Bl1 Bl1 Bl2 Bl2 Ms10 Ms10 | Compana ms10/Nepal |
| En-Bl En-Bl Bl1 Bl1 Bl2 Bl2 ms10 ms10 | Compana ms10/Nepal |
| En-Bl En-Bl Bl1 Bl1 bl2 bl2 ms10 ms10 | Compana ms10/Nepal |
| En-Bl En-Bl Bl1 Bl1 Bl2 Bl2 ms14 ms14 N N | Unitan ms14/Nepal |
| en-Bl en-Bl Bl1 Bl1 bl2 bl2 Ms10 Ms10 | Compana ms10/Nepal/2/Ubamer |
| en-Bl en-Bl Bl1 Bl1 bl2 bl2 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 (Bl2 Bl2 bl1 bl1) and CI4961-1 (Bl2 Bl2 Bl1 Bl1). The dihybrid ratios were observed on $F_1$ plants from the dihybrid crosses Shonupana (Bl2 Bl2 bl1 bl1)/ Nuvan White-2 (bl2 bl2 Bl1 Bl1) and Shonupana (Bl2 Bl2 bl1 bl1)/ Nepal (bl2 bl2 Bl1 Bl1); and from early generation Sermo/7* Vantage plants segregating for aleurone color.

Reciprocal crosses among Nepal, Nupana, Shonupana, Nubet, Nuvan Blue, and a Bl1 Bl1 Bl2 Bl2 stock derived from Nepal/Shonupana crosses provided blue $F_1$ seed samples representing allelic dosages from 3 to 6 dominant alleles for the Bl1 and Bl2 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 bl1 bl1 bl2 bl2 stock, which is necessary to set up the genotype Bl1 bl1 bl1 Bl2 bl2 bl2. 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 Blx blx Bly bly stock were planted and the resulting plants crossed to male sterile plants of the blue aleurone stock Trebi ms2. At maturity, the Blx blx Bly bly 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 | Ile 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 |

Table 4. Continued

| Male Sterile Stock No. | C.I. No. | Variety or Strain | Male Sterile Gene |
|---|---|---|---|
| 76 | 13637 | Heines Hanna x Bonus | ms-h |
| 77 | 13638 | 80 TT 25 | ms-i |
| 78 | 13639 | Betzes x Domen | ms-j |
| 79 | 2256 | Lion x Manchuria | ms-k |
| 80 | 4894 | Intro. (China) | ms-l |
| 81 | 7015 | Mars | ms-m |
| 82 | 6171 | Trebi x CI 5434 | ms-n |
| 83 | 4666 | Oderbrucker | ms-o |
| 84 | 9559 | Piroline | ms-p |
| 85 | 13640 | Midwest Bulk | ms-q |
| 86 | 6398 | Betzes | ms-r |
| 87 | 11501 | Schweigers Erika | ms-s |
| 88 | 10421 | Unitan | ms-u |
| 89 | 6398 | Betzes | ms-v |
| 90 | 6398 | Betzes | ms-w |
| 91 | 6398 | Betzes | ms-x |
| 92 | 6398 | Betzes | ms-y |
| 93 | 6398 | Betzes | ms-z |
| 94 | 10072 | Gateway (336) | ms-ab |
| 95 | 11638 | Conquest | ms-ac |
| 96 | 531 | Hannchen | ms-ad |
| 97 | 10088 | Firlbecks III | ms-ae |
| 98 | 5438 | Compana | ms-ag |
| 99 | 13641 | HB 421/78 | ms-ah |
| 100 | 6398 | Betzes | ms-ai |
| 101 | 6398 | Betzes | ms-aj |

Table 5. Dark blue aleurone male sterile and translocation stocks obtained from Dr. G. A. Wiebe for use in the Ubamer Dark Blue inheritance study.

| Stock 1/ |
| --- |
| Unitan ms26u/4 * Ubamer |
| Bonus T1-4a/2 * Ubamer |
| Bonus T1-4i/2 * Ubamer |
| Bonus T1-4j/2 * Ubamer |
| Bonus T1-6i/2 * Ubamer |
| Bonus T2-6k/2 * Ubamer |
| Bonus T2-7b/2 * Ubamer |
| Bonus T2-7h/2 * Ubamer |
| Mars T3-5b/2 * Ubamer |
| Bonus T3-5c/2 * Ubamer |
| Bonus T3-7j/2 * Ubamer |
| Bonus T4-5d/2 * Ubamer |
| "A" T5-7a/2 * Ubamer |

1/ All stocks are hulless.

Ubamer and Mesa Dark Blue (MT 1355) were used as checks for dark blue color intensities. The color intensities of the $F_1$ seeds were classified as either dark or normal blue, based on comparisons with the checks. Dark and normal blue were described as 103A and 104B respectively of the R. H. S. Colour Chart (Royal Horticultural Society of London). The $F_1$ seeds were planted and $F_2$ aleurone color ratios obtained from the $F_1$ plants. From the crosses between Ubamer (*en-Bl en-Bl Bl1 Bl1 Bl2 Bl2 Ms10 Ms10*) and the stock *En-Bl En-Bl Bl1 Bl1 bl2 bl2 ms10 ms10*, the dark blue, normal blue, and white $F_2$ seeds were sorted and planted in separate plots. The number of male sterile and fertile plants occurring in each color class was recorded for linkage information. A total of twenty-nine male sterile plants from both white aleurone plots were selected at random and pollinated with Ubamer or Nuvan Blue. The frequencies of crosses producing all normal blue, all dark blue, or both normal and dark blue $F_1$ seeds were recorded. The $F_1$ seeds were planted and classified for aleurone color intensity genotype by examining ratios of $F_2$ color classes. The total frequency of *en-Bl* among the white aleurone and male sterile classes in the $F_2$ of the original *En-Bl En-Bl Bl1 Bl1 bl2 bl2 ms10 ms10* x Ubamer cross were estimated from the frequency of *en-Bl* observed in the sample of 29 white aleurone male sterile plants when testcrossed to Ubamer and Nuvan Blue. The data were used in the estimation of recombination values for *en-Bl* vs *ms10* and *en-Bl* vs *bl2*.

Stocks of the appropriate genotypes were crossed to obtain linkage data for genes or interchange points on chromosomes 1 and 4. The crosses, along with the respective genes under study, are listed in Table 6. Where aleurone color was involved, $F_2$ seeds were planted in separate plots according to color for $F_2$ classification of genes for other characters. Fertile $F_2$ plants in each plot were checked for aleurone color in order that misclassifications could be corrected. Whenever possible, at least 300 seeds from the segregating $F_2$ seed population for a given linkage test were planted in order to keep standard errors at a minimum for the recombination values (Allard, 1956). Seeds were planted in rows 30 cm. apart in the field at a rate of 16 seeds per 3 meter row in order that misclassifications could be easily detected. From these segregating rows, one spike from each $F_2$ plant was harvested for $F_3$ classification. Since this was done during the summer of 1972, no $F_3$ data were available and therefore, the linkages reported here were based on $F_2$ data.

Recombination values and their standard errors were calculated from the $F_2$ data by the maximum likelihood method. These calculations were facilitated by a computor program, developed for use with this thesis, using the formulas presented by Allard (1956) and Hanson and Kramer (1950). A chi-square for fit to the calculated recombination value was computed for each set of data to support the relation between a given set of data and the recombination value assigned to it. When-

Table 6. Heterozygotes from crosses used to estimate recombination values for genes on chromosomes 1 and 4.

| Cross No. | Cross | Chromosome Under Study 1/ |
|---|---|---|
| 1 | b12 ms10/Nuvan Blue B12 Ms10 | 1 |
| 2 | b12 ms10/Nuvan Blue B12 Ms10 | 1 |
| 3 | b12 ms10 En-B1/Ubamer B12 Ms10 en-B1 | 1 |
| 4 | b12 ms10 En-B1/Ubamer B12 Ms10 en-B1 | 1 |
| 5 | b12 ms10 En-B1/Ubamer B12 Ms10 en-B1 | 1 |
| 6 | B12 ms10/Nepal b12 Ms10 | 1 |
| 7 | Montcalm B12 N/Nepal b12 n | 1 |
| 8 | Manchuria B12 N/Nepal b12 n | 1 |
| 9 | Sermo b12 n lk2/B12 N Lk2 | 1 |
| 10 | B12 ms14 N/Nepal b12 Ms14 n | 1 |
| 11 | B12 ms14 N/Nepal b12 Ms14 n | 1 |
| 12 | Sermo b12 lk2/Nuvan Blue B12 Lk2 | 1 |
| 13 | Sermo b12 lk2/Nuvan Blue B12 Lk2 | 1 |
| 14 | Betzes b11 ms24v/Ubamer B11 Ms24v | 4 |
| 15 | Betzes b11 ms24v/Montcalm B11 Ms24v | 4 |
| 16 | Betzes b11 ms25r/Montcalm B11 Ms25r | 4 |
| 17 | Betzes b11 ms25r/Ubamer B11 Ms25r | 4 |
| 18 | Betzes Erti27 ms24v/Bonus erti27 Ms24v | 4 |

Table 6. Continued

| Cross No. | Cross | Chromosome Under Study 1/ |
|---|---|---|
| 19 | Betzes Erti27 ms24v/Bonus erti27 Ms24v | 4 |
| 20 | Betzes Erti27 ms24v/Bonus erti27 Ms24v | 4 |
| 21 | Betzes Erti27 ms24v/Bonus erti27 Ms24v | 4 |
| 22 | Betzes ms24v k/Nepal Ms24v K | 4 |
| 23 | Betzes ms24v k/Nepal Ms24v K | 4 |
| 24 | Betzes ms24v k/Nepal Ms24v K | 4 |
| 25 | Betzes ms24v/Bonus Ms24v T2-4d | 4 |
| 26 | Betzes ms24v/Mars Ms24v T4-5a | 4 |
| 27 | Betzes ms24v/Mars Ms24v T4-5b | 4 |
| 28 | Betzes ms24v/Bonus Ms24v T5-5c | 4 |
| 29 | Betzes ms25r/Mars Ms25r T4-5a | 4 |
| 30 | Betzes i/Mars I T2-4a | 4 |
| 31 | Betzes i/Mars I T4-5a | 4 |

1/ All parents used for chromosome 1 aleurone color study are homozygous for Bl1 on chromosome 4; all parents used for chromosome 4 aleurone color study are homozygous for Bl2 on chromosome 1.

ever more than one set of data was used to estimate a recombination value, a heterogeneity chi-square was calculated to determine if all data sets in a given linkage study were consistent for the combined recombination value.

## RESULTS AND DISCUSSION

### Ratios

Segregation ratios for aleurone color were difficult to read with accuracy on plants with a covered caryopsis. As a consequence, ratios reported herein were taken from crosses involving hulless parents unless otherwise noted. From crosses between blue and white parents, only monohybrid ratios were obtained (Tables 7 and 8). This is in agreement with the earliest reports that there was only a single gene difference between blue and white aleurone. The 2:1 ratios observed in some families (Table 8) are in agreement with the report of Jain (1970) suggesting that some form of gamete selection was associated with one of the blue aleurone loci in some populations. The 2:1 ratios suggested that the homozygous blue class was missing, leaving the heterozygous blue and homozygous white classes at expected frequencies of 0.67 and 0.33, respectively. To explore this possibility, blue seeds from plants segregating 2:1 for blue and white aleurone color were progeny tested for genotype. One would expect roughly 33 percent of the plants grown from the blue seeds to be homozygous blue if the gametes did not undergo differential selection. The progeny test showed only 4 out of 296 plants, or 1.4 percent, to be homozygous blue (Table 9); a near complete absence of the homozygous blue class. The 4 homozygous blue plants could have been the result of outcrosses with normal <u>Bl1</u> <u>Bl1</u> <u>Bl2</u> <u>Bl2</u> plants; or, if gamete selection was conditioned by a specific gametophytic factor, from crossovers between the gametophytic factor and the

Table 7. Monogenic segregations approaching the expected 3:1 ratio observed on $F_1$ and $F_2$ plants of the genotype Bl1 Bl1 Bl2 bl2 or Bl1 bl1 Bl2 Bl2.

| | Unitan/ Nepal 1/ | Nupana/ Nepal 1/ | bl2 bl2 Bl Bl/ Nuvan Blue 2/ | All Families |
|---|---|---|---|---|
| No. of families | 2 | 27 | 5 | 34 |
| No. of blue seeds | 211 | 3416 | 1675 | 5272 |
| No. of white seeds | 73 | 1169 | 520 | 1762 |
| Chi-square for a 3:1 ratio | 0.074 | 0.601 | 2.008 | 0.009 |
| P for 3:1 chi-square | .90-.75 | .50-.25 | .25-.10 | .95-.90 |
| Heterogeneity chi-square | 1.416 | 16.442 | 2.930 | 23.462 |
| P= | .25-.10 | .95-.90 | .75-.50 | .90-.75 |

1/ Selected $F_2$ plants from the cross Bl2 Bl2 bl1 bl1/bl2 bl2 Bl1 Bl1.
2/ $F_1$ plants.

Table 8. Observed $F_2$ segregation ratios that did not approach the expected 3:1 ratio on plants of the genotype Bl1 Bl1 Bl2 bl2 or Bl1 bl1 Bl2 Bl2.

| | Unitan/ Nepal 1/ | Nupana/ Nepal 1/ | bl2 bl2 Bl Bl/ Nuvan Blue 2/ | All Families |
|---|---|---|---|---|
| No. of families | 3 | 15 | 1 | 19 |
| No. of blue seeds | 347 | 1548 | 338 | 2233 |
| No. of white seeds | 169 | 741 | 152 | 1062 |
| Chi-square for a 2:1 ratio | 0.078 | 0.954 | 1.180 | 1.807 |
| P for 2:1 chi-square | .90-.75 | .50-.25 | .50-.25 | .25-.10 |
| Heterogeneity chi-square | 1.011 | 5.023 | -- | 6.459 |
| P= | .50-.25 | .99-.975 | -- | .995-.99 |

1/ Selected $F_2$ plants from the cross Bl2 Bl2 bl1 bl1/bl2 bl2 Bl1 Bl1.
2/ $F_1$ plants.

Table 9. Progeny tests of blue seeds taken from plants that segregated 2:1 for blue and white aleurone color.

| | |
|---|---|
| No. of families | 14 |
| No. of plants segregating aleurone color | 292 |
| No. of plants with homozygous blue aleurone | 4 1/ |

1/ From 3 of the 14 families

associated blue aleurone locus. It would also be possible that a small percentage of gametes carrying the gametophytic factor would function, as was the case in the report of Tabata (1961).

If the deficiency of the homozygous blue class in the 2:1 ratios was caused by one specific gamete selection factor, the factor is likely to be associated with either one or the other of the blue aleurone loci under study. Subsequently, one would expect all of the plants segregating 2:1 for aleurone color to be of the same blue aleurone genotype; namely all Bl1 Bl1 Bl2 bl2, or all Bl1 bl1 Bl2 Bl2. To verify this, the white seeds from the plants segregating 2:1 for aleurone color were planted and the resulting plants testcrossed to Betzes (bl1 bl1 Bl2 Bl2) and CI4961 (Bl1 Bl1 Bl2 Bl2). When crossed to Betzes, all of the families produced only segregating $F_2$ seed samples for aleurone color (Table 10). This would indicate the white aleurone plants tested were of the genotype Bl1 Bl1 bl2 bl2, and the 2:1 ratios deficient in the Bl2 Bl2 class rather than the Bl1 Bl1 class. When the families were crossed with CI4961-1, all $F_2$ seed samples were segregating for aleurone color (Table 10) indicating that no color misclassification had occurred in sorting white seeds for the testcrosses.

From the data presented in Tables 8, 9, and 10, I concluded the 2:1 ratios encountered consisted of the classes Bl1 Bl1 Bl2 bl2 and Bl1 Bl1 bl2 bl2, with Bl1 Bl1 Bl2 Bl2 missing or occurring at a very low frequency. The data also provide evidence that some type of gamete

Table 10. Aleurone color observed on $F_1$ plants from testcrosses of stocks of the genotype bl1 bl1 Bl2 Bl2 and Bl1 Bl1 Bl2 Bl2 with plants grown from white seeds obtained from families that segregated 2:1 for aleurone color.

| Family that segregated 2:1 for aleurone color involved in testcross | Betzes bl1 Bl2/ white from 2:1, no. of $F_1$'s and color | C.I. 4961-1 Bl1 Bl2/ white from 2:1, no. of $F_1$'s and color |
|---|---|---|
| 1 | 18 seg. | 20 seg. |
| 2 | 13 seg. | 20 seg. |
| 3 | 20 seg. | 18 seg. |
| 4 | 13 seg. | 20 seg. |
| 5 | 18 seg. | 19 seg. |
| 6 | 17 seg. | 13 seg. |
| 7 | 18 seg. | 14 seg. |
| 8 | 20 seg. | 20 seg. |
| 9 | 16 seg. | 20 seg. |
| 10 | 18 seg. | 16 seg. |

selection factor is associated with the dominant B12 allele in the stocks giving rise to 2:1 ratios. Evidence for the occurance of a gamete selection factor in the B12 region of chromosome 1 was also noted in crosses between waxy endosperm (wx) fertile stocks and non-waxy male sterile (ms10) stocks, where a deficiency of waxy endosperm fertiles occurred in the $F_2$ (Eslick, unpub.).

The type of selection on male B12 gametes in this study was probably not like that conditioned by the gametophytic factor ga reported by Tabata (1957 and 1961), where gametes carrying ga would not function on stylar tissue of the genotype Ga ga. If that was the case here, and Tabata's ga factor was tightly linked to B12, a 1;1 ratio would have occurred rather than a 2:1 (Table 11). However, a 2:1 ratio could be obtained if ga were linked to B12 with a recombination value of 34 percent (Table 12). The recombination values reported by Tabata for ga vs waxy endosperm (wx) and waxy endosperm vs albino seedling (ac2) would place ga approximately 22 recombination units from B12 (Figure 3). The recombination value of 33.0 percent for ga vs wx reported by Tabata is somewhat arbitrary, and could have a range to allow ga to be positioned 34 recombination units from B12. However, if this were so, the progeny test data presented in Table 9 would have included approximately 17 percent homozygous blue types rather than 1.4 percent (Table 12). Therefore, if the 2:1 ratios were caused by gamete selection, the selective action was probably one which inhibited the entry of male gametes

Table 11. Expected genotypes and frequencies in the $F_2$ from the hypothetical cross B12 B12 ga ga x b12 b12 Ga Ga, where B12 is completely linked to the gametophytic factor ga.

| Type and functional frequency of female gamete 1/ | Type and Functional Frequency of Male Gamete 1/ | | Total |
|---|---|---|---|
| | B12 ga | b12 Ga | |
| | 0 | 1.0 | |
| B12 ga<br>0.5 | B12 B12 ga ga<br>0 | B12 b12 Ga ga<br>0.5 | 0.5 |
| b12 Ga<br>0.5 | B12 b12 Ga ga<br>0 | b12 b12 Ga Ga<br>0.5 | 0.5 |
| Total | 0 | 1.0 | 1.0 |

1/ Where function of female gametes is not influenced by the gametophytic factor, and male gametes carrying the recessive ga do not compete with gametes carrying the dominant Ga on stylar tissue of the genotype Ga ga.

Table 12. Expected genotypes and frequencies in the $F_2$ from the hypothetical cross B12 B12 ga ga x b12 b12 Ga Ga, where B12 is linked to the gametophytic factor ga with a recombination value of 34 percent.

| Type and Functional Frequency of Parental and Crossover Female Gametes 1/ | Type and Functional Frequency of Parental Male Gamete 1/ | | Type and Functional Frequency of Crossover Male Gamete 1/ | | Total |
|---|---|---|---|---|---|
| | B12 ga<br>0 | b12 Ga<br>0.66 | B12 Ga<br>0.34 | b12 ga<br>0 | |
| B12 ga<br>0.33 | B12 B12 ga ga<br>0 | B12 b12 Ga ga<br>.2178 | B12 B12 Ga ga<br>.1122 | B12 b12 ga ga<br>0 | 0.33 |
| b12 Ga<br>0.33 | B12 b12 Ga ga<br>0 | b12 b12 Ga Ga<br>.2178 | B12 b12 Ga Ga<br>.1122 | b12 b12 Ga ga<br>0 | 0.33 |
| B12 Ga<br>0.17 | B12 B12 Ga ga<br>0 | B12 b12 Ga Ga<br>.1122 | B12 B12 Ga Ga<br>.0578 | B12 b12 Ga ga<br>0 | 0.17 |
| b12 ga<br>0.17 | B12 b12 ga ga<br>0 | b12 b12 Ga ga<br>.1122 | B12 b12 Ga ga<br>.0578 | b12 b12 ga ga<br>0 | 0.17 |
| Total | 0 | 0.66 | 0.34 | 0 | 1.0 |

Total B12 B12 types 0.17.
Total B12 b12 types 0.50.
Total b12 b12 types 0.33.

1/ Where the function of female gametes is not influenced by the gametophytic factor, and male gametes carrying the recessive ga do not compete with gametes carrying the dominant Ga on stylar tissue of the genotype Ga ga.

carrying a recessive gamete selection factor (proposed as gsf for illustration) into egg cells also carrying the recessive selection factor (Table 13). This situation would allow female gametes carrying the selection factor to be fertilized almost exclusively by male gametes carrying the normal dominant allele. When all of the male gametes carry the recessive factor, as in the case where Bl2 Bl2 gsf gsf is selfed, no competition would occur and fertilization would take place. Although this type of gamete selection has not been reported in barley, it does not provide a possible explanation for the 2:1 ratios encountered here. However, no conclusion can be made from the data obtained so far as to the real cause of the 2:1 ratio.

$F_2$ data for the dihybrid heterozygote Bl1 bl1 Bl2 bl2 are presented in Tables 14 and 15. The data presented in Table 14 fit the expected 9:7 ratio (.75>P>.5) which is in agreement with the report of Myler and Stanford (1942) that two complementary dominant genes condition the expression of blue aleurone. The data of Table 15 approached a 27:37 ratio (.25>P>.10), which indicated that three complementary genes segregated. The possibility that the data represent a 1:1 ratio is remote (.005>P). However, if the data do represent a 1:1 ratio, it is possible that the gamete selection factor suspected of being associated with Bl2 was present in the crosses listed in Table 15. Assuming the gene action proposed in Table 13 was correct, a dihybrid cross for the blue aleurone loci would produce a 1:1 ratio when the gamete

Table 13. Expected $F_2$ genotypes and frequencies from the hypothetical cross B12 B12 gsf gsf x b12 b12 Gsf Gsf, where B12 is completely linked to gsf and male gametes carrying the recessive gsf cannot compete with male gametes carrying the dominant Gsf to fertilize eggs carrying the recessive gsf. 1/

| Type and frequency of female gamete | Type and frequency of male gamete | | Total |
|---|---|---|---|
| | B12 gsf<br>0.5 | b12 Gsf<br>0.5 | |
| B12 gsf<br>0.5 | B12 B12 gsf gsf<br>0 | B12 b12 Gsf gsf<br>0.333 | 0.333 |
| b12 Gsf<br>0.5 | B12 b12 Gsf gsf<br>0.333 | b12 b12 Gsf Gsf<br>0.333 | 0.666 |
| Total | 0.333 | 0.666 | 1.0 |

1/ Where gsf is a hypothetical recessive gamete selection factor.

Table 14. Dihybrid $F_2$ segregation ratios that approach the expected 9:7 ratio observed on plants of the genotype Bl1 bl1 Bl2 bl2.

| | Shonupana/ Nuvan White-2 | Sermo/7*Vantage (Bl1 bl1 Bl2 bl2) | All families |
|---|---|---|---|
| No. of families | 4 | 2 | 6 |
| No. of blue seeds | 778 | 130 | 908 |
| No. of white seeds | 596 | 91 | 687 |
| Chi-square for a 9:7 ratio | 0.075 | 0.592 | 0.294 |
| P for 9:7 chi-square | .90-.75 | .50-.25 | .75-.50 |
| Chi-square for a 1:1 ratio | 24.105 | 6.882 | 30.621 |
| P for 1:1 chi-square | <.005 | .01-.005 | <.005 |
| Heterogeneity chi-square | 5.758 | 0.211 | 6.339 |
| P = | .25-.10 | .75-.50 | .50-.25 |

Table 15. Observed $F_2$ segregation ratios that did not approach the expected 9:7 ratio on plants of the genotype B11 b11 B12 b12.

| | Shonupana/ Nepal | Shonupana/ Nuvan White-2 | All families |
|---|---|---|---|
| No. of families | 4 | 5 | 9 |
| No. of blue seeds | 418 | 272 | 690 |
| No. of white seeds | 542 | 347 | 889 |
| Chi-square for a 27:37 ratio | 0.770 | 0.823 | 1.485 |
| P for 27:37 chi-square | .50-.25 | .50-.25 | .25-.10 |
| Chi-square for a 1:1 ratio | 16.017 | 9.087 | 25.080 |
| P for 1:1 chi-square | <.005 | <.005 | <.005 |
| Heterogeneity chi-square | 15.066 | 0.667 | 15.841 |
| P = | .005 | .975-.95 | .05-.025 |

selection factor is present (Table 16). It is also possible that gamete selection factors were associated with both Bl1 and Bl2 in the crosses presented in Table 15. In this case, one could expect an $F_2$ ratio of 4:5 (or 28.4:35.6) (Table 17), which is a reasonable approach to a 27:37 ratio.

If gamete selection factors are associated with either of the blue aleurone loci, they would be very useful in the hybrid system as a means of eliminating the homozygous blue, fertile types. The source of the factors suspected of giving rise to the skewed ratios encountered in this study could be one or several varieties. The crosses that produced 2:1 ratios involved the same varieties as those that produced 3:1 ratios (Table 18). This suggested that the variety carrying the gamete selection factor was also heterogeneous for the factor. All of the crosses listed in Table 18 that produced monohybrid ratios involved Nepal or Nepal derived types, suggesting that Nepal is the source of the gamete selection factor. This would require crossovers producing the necessary Bl2 gsf gametes since the aleurone color genotype of Nepal is Bl1 Bl1 bl2 bl2. The parental type gametes bl2 gsf should occur more frequently, and would therefore give rise to an abundance of families showing $F_2$ segregation ratios that are deficient in the white class. Although $F_2$ ratios deficient for the white class were not observed from the crosses listed in Table 18, the situation did occur in another series of crosses involving mostly Nepal or Nepal derived types with Ubamer

Table 16. Expected genotypes and frequencies upon selfing the heterozygote B11 b11 B12 b12, where B12 is closely associated with a gamete selection factor and male gametes carrying the selection factor do not readily fertilize female gametes carrying the selection factor.

| Genotype | Frequency | Occurence and Color |
|---|---|---|
| B11 B11 B12 B12 | 1 | not present |
| B11 B11 B12 b12 | 2 | blue |
| B11 B11 b12 b12 | 1 | white |
| B11 b11 B12 B12 | 2 | not present |
| B11 b11 B12 b12 | 4 | blue |
| B11 b11 b12 b12 | 2 | white |
| b11 b11 B12 B12 | 1 | not present |
| b11 b11 B12 b12 | 2 | white |
| b11 b11 b12 b12 | 1 | white |

Table 17. Expected genotypes and frequencies based on the assumptions that both Bl1 and Bl2 are closely associated with gametophytic factors and male gametes carrying the selection factors do not readily fertilize female gametes carrying the selection factors.

| Genotype | Frequency | Occurence and color |
|---|---|---|
| Bl1 Bl1 Bl2 Bl2 | 1 | not present |
| Bl1 Bl1 Bl2 bl2 | 2 | not present |
| Bl1 Bl1 bl2 bl2 | 1 | not present |
| Bl1 bl1 Bl2 Bl2 | 2 | not present |
| Bl1 bl1 Bl2 bl2 | 4 | blue |
| Bl1 bl1 bl2 bl2 | 2 | white |
| bl1 bl1 Bl2 Bl2 | 1 | not present |
| bl1 bl1 Bl2 bl2 | 2 | white |
| bl1 bl1 bl2 bl2 | 1 | white |

Table 18. Types of ratios obtained from the crosses used in the aleurone color segregation ratio study.

| Line or Cross | Ratio Obtained | | | | Source of Bl2 |
|---|---|---|---|---|---|
| | 3:1 | 2:1 | 9:7 | 27:37 | |
| Unitan ms14/Nepal ($F_3$ individuals) | x | x | | | Unitan |
| Nupana ms10/Nepal ($F_3$ individuals) | x | x | | | Compana |
| bl2 bl2 Bl1 Bl1/Nuvan Blue 1/ | x | x | | | Vantage |
| Shonupana/Nuvan White-2 | | | x | x | Compana |
| Sermo/7*Vantage (Bl1 bl1 Bl2 bl2) | | | x | | Vantage |
| Shonupana/Nepal | | | | x | Compana |
| Bl1 Bl1 Bl2 Bl2 ms14 ms14/Nepal 2/ | | x | | | Unitan |

1/ bl2 bl2 Bl1 Bl1 stock derived from Compana/Nepal cross.
2/ $F_2$ ratio presented in Table 36.

(Table 19). However, the deficiency of the white class for the ratios presented in Table 19 is not of the same magnitude as the deficiency of the homozygous blue class discussed previously for the 2:1 ratios. If the same type of gamete selection and association with the Bl2 locus has occurred in both cases, one would expect a near complete elimination of the $F_2$ white class from the crosses listed in Table 19. It is more likely that the two situations are caused by separate factors, which may or may not act in an additive manner. Other possible sources for the alleged gamete selection factor are Unitan and Nupana, since all of the crosses listed in Table 18 that produced skewed ratios involved either Unitan or Compana derived types.

Another possible explanation for the 27:37 ratios is the interaction among dosage effect, background genotype, and environmental stress as reported for blue aleurone color in wheat by Hurd (1959). The endosperm tissues of grass seeds are triploid for a given genome. In the process of double fertilization, one sperm nucleus unites with the egg nucleus to initiate the embryo, while the other sperm nucleus fuses with the two polar nuclei to form the primary, 3N, endosperm nucleus. Genotypes of endosperm characters are usually expressed as fractions, AABB/ab, where the numerator represents the female gamete and the denominator represents the male. In a plant with the genotype Bl1 bl1 Bl2 bl2, two to six doses of the dominant alleles are possible in any selected blue $F_2$ seed. It is possible that a certain level of gene

Table 19. Observed $F_2$ aleurone color segregation ratios from the heterozygote Bl1 Bl1 Bl2 bl2.

| Cross | No. of Families | No. of Blue Seeds | No. of White Seeds | Chi-Square for 3:1 | P |
|---|---|---|---|---|---|
| ms26u/4*Ubamer/2/Nepal | 6 | 420 | 106 | 6.595 | .025-.01 |
| Sermo/Ubamer | | 514 | 125 | 10.078 | <.005 |
| bl2 ms10 Bl1/Ubamer 1/ | 22 | 3838 | 959 | 64.173 | <.005 |
| Total | 31 | 4772 | 1190 | 80.779 | .005 |

1/ bl2 bl2 ms10 ms10 Bl1 Bl1 stocks derived from Nupana ms10/Nepal crosses.

dosage is required to express blue color in certain plants grown under environmental stress. This would result in an increase in the number of white seeds in proportion to the number of blue. The observations recorded in Table 20 indicate a slight dosage effect, which is in agreement with the report of dosage effect for aleurone color in wheat by Knott (1958).

In summary, 3:1 and 9:7 aleurone color ratios were obtained from monohybrid and dihybrid crosses, respectively. In addition, 2:1 and 27:37 ratios were obtained from monohybrid and dihybrid crosses, respectively. Progeny tests and testcrosses indicated the 2:1 ratios were highly deficient for the homozygous blue class, B12 B12. This suggested a very close association of a gamete selection factor with B12 in the stocks giving rise to 2:1 ratios. The gametophytic factor, ga, reported by Tabata (1961) was considered as a possible cause. However, it was noted that the selective action of ga would result in 1:1 ratios, rather than 2:1 ratios, for genes closely associated with it. It was also noted that the recombination value given for ga vs waxy endosperm, wx, would not place ga close enough to B12 to provide the near complete elimination of the homozygous blue class. The alleged gamete selection factor was designated as gsf for illustrative purposes. The selective action of gsf was postulated as to inhibit the union of male and female gametes which carry the factor. It was illustrated that this type of selective action would cause a 2:1 ratio to occur.

Table 20. Color intensities observed on seeds of varying known allelic dosages for Bl1 and Bl2.

| Cross or Variety | Year of Experiment | Aleurone Genotype of F1 Seed | No. of Dominant Alleles | Color Intensity Score 2/ |
|---|---|---|---|---|
| Nuvan Blue (Bl1 Bl2) | 1971 | Bl1 Bl1 Bl1 Bl2 Bl2 Bl2 | 6 | 1 |
| Bl1 Bl2/Nepal (Bl1 bl2) | " | Bl1 Bl1 Bl1 Bl2 Bl2 bl2 | 5 | 2 |
| " / " | " | " | 5 | 2 |
| " /Nupana (bl1 Bl2) | " | Bl1 Bl1 bl1 Bl2 Bl2 Bl2 | 5 | 2 |
| " /Shonupana (bl1 Bl2) | " | " | 5 | 2 |
| Nepal (Bl1 bl2)/Bl1 Bl2 | " | Bl1 Bl1 Bl1 Bl2 bl2 bl2 | 4 | 4 |
| Shonupana (bl1 Bl2)/Bl1 Bl2 | " | Bl1 bl1 bl1 Bl2 Bl2 Bl2 | 4 | 4 |
| Nepal (Bl1 bl2)/Shonupana (bl1 Bl2) | " | Bl1 Bl1 bl1 Bl2 bl2 bl2 | 3 | 4 |
| Shonupana (bl1 Bl2)/Nepal (Bl1 bl2) | " | Bl1 bl1 bl1 Bl2 Bl2 bl2 | 3 | 4 |
| Nuvan Blue (Bl1 Bl2) | 1972 | Bl1 Bl1 Bl1 Bl2 Bl2 Bl2 | 6 | 1 |
| Nuvan Blue/Nubet (bl1 Bl2) | " | Bl1 Bl1 bl1 Bl2 Bl2 Bl2 | 5 | 3 |
| Nubet (bl1 Bl2)/Nuvan Blue | " | Bl1 bl1 bl1 Bl2 Bl2 Bl2 | 4 | 3 |

1/ All crosses within a given year were made on the same day. The Nuvan Blue variety was clipped and bagged in efforts to reduce bias between it and the crosses.

2/ From darkest to lightest, 1=104B, 2=104C, 3=104D, and 4=100D of the R.H.S. Colour Chart (Royal Horticultural Society, London).

Although the 27:37 ratios could have occurred as a result of the presence of a third segregating blue aleurone gene, gamete selection factors associated with one or both Bl1 and Bl2 were considered as possible causes. Hypothetical data was used to illustrate that 1:1 and approximate 27:37 ratios could be obtained from the dihybrid, Bl1 bl1 Bl2 bl2, when gamete selection factors were closely associated with one and both aleurone color genes, respectively. Considering that a 1:1 ratio is similar to a 27:37 ratio, there is a remote possibility that gsf could have caused the 27:37 ratios to occur from the dihybrid aleurone color crosses.

The possible sources of the gamete selection factor included Unitan, Compana, Vantage, and Nepal. Nepal was considered the least likely because it was homozygous for the recessive bl2 allele, and the alleged gamete selection factor was postulated as being associated with the dominant Bl2 allele. Both 3:1 and 2:1 ratios were obtained from crosses with derived types of Unitan, Compana, and Vantage. It was therefore suggested that gsf was common to all three stocks, and that the stocks were heterogeneous for gsf.

The three way interaction among gene dosage, background genotype, and the environment was considered as another possible cause of the skewed ratios. Testcrosses producing varying allelic dosages for Bl1 and Bl2 indicated a slight dosage effect. However, the dosage effect was not of sufficient magnitude to allow kernels carrying the minimum

complement of Bl1 and Bl2 to be mistaken for white under the conditions of the experiment.

No conclusions were made as to the cause of the distorted ratios. The data presented favored the gamete selection factor hypothesis, although mutator or suppressor genes could have been used to explain the situation. Further study is necessary to ascertain the specific cause of the distorted ratios.

## Blx and Bly

A linkage test was conducted for Blx vs Bly in efforts to substantiate the contention of Wiebe (1972) that a close translinkage exists between Blx and Bly. If his contention is true, the repulsion phase Blx blx Bly bly stock should produce gametes which are almost exclusively Blx bly and blx Bly. The crossover gametes Blx Bly and blx bly would occur at a very low frequency. Therefore, if Wiebe's Blx blx Bly bly stock was crossed to a Blx Blx Bly Bly tester stock, the $F_1$ genotypes should be predominantly Blx Blx Bly bly and Blx blx Bly Bly. $F_1$ plants of the genotypes Blx Blx Bly Bly and Blx blx Bly bly would result from the union of crossover gametes with the Blx Bly gametes of the tester stock, and would be non-existent if linkange were complete.

For the linkage test, a sample of Blx blx Bly bly plants were crossed to the blue aleurone stock, Trebi ms2. It was assumed that a blue aleurone variety would be homozygous dominant for all known and

unknown complementary blue genes. Therefore, Trebi was designated as Blx Blx Bly Bly Bl1 Bl1 Bl2 Bl2. Out of 218 $F_1$ plants examined, no Blx Blx Bly Bly types were observed (Table 21). The other crossover type, coupling phase Blx blx Bly bly, could not be distinguished from the non-crossover Blx Blx Bly bly and Blx blx Bly Bly types because at high linkage intensities, all three types would show 3:1 segregation ratios for aleurone color. Therefore, it was not known whether any Blx blx Bly bly types were among the 218 plants examined. However, there was an indication that the frequency of blx bly gametes was, at the most, very low. If blx and bly were not tightly linked, and the Blx Bly gametes were suppressed by gamete selection factors like those discussed in the previous section, the testcross $F_1$ plants from blx bly gametes would have had aleurone color segregation ratios approaching a 9:7. All of the 218 $F_1$ plants examined segregated 3:1 for aleurone color. Therefore, it was assumed that the frequency of blx bly gametes giving rise to $F_1$ plants was low or zero, as was the case for the Blx Bly gametes (Table 21). This assumption allowed the estimated recombination value of 0.0 $\pm$ 0.7% for Blx vs Bly to be calculated.

The Blx Blx bly bly and blx blx Bly Bly white types, which originated from Wiebe's Blx blx Bly bly stock, produced 3:1 aleurone color ratios in the $F_2$ when crossed with the blue variety Ubamer (Table 22). This indicated that the Blx Blx bly bly and blx blx Bly Bly stocks were recessive for only one blue aleurone gene each, and are homozygous

Table 21. Genotypic ratio for the testcross Trebi (Bl2 Bl2 Bl1 Bl1 Bl3y Bl3y) x the repulsion phase heterozygote Bl2 Bl2 Bl1x bl1x Bl3y bl3y.

| Genotype | Expected with Independence | Expected with Complete Linkage | Observed |
|---|---|---|---|
| Bl1 Bl1x Bl3y bl3y 1/ | .25 | .5 | 218 3/ |
| Bl1 bl1x Bl3y Bl3y 1/ | .25 | .5 | |
| Bl1 Bl1x Bl3y Bl3y 2/ | .25 | 0 | 0 |
| Bl1 bl1x Bl3y bl3y 2/ | .25 | 0 | 0 4/ |
| Total | 1 | 1 | 218 |

Recombination value for Bl1x vs Bl3y = 0.0 ± 0.7%.

1/ From parental gametes.
2/ From crossover gametes.
3/ All plants segregated 3:1 for aleurone color.
4/ It was assumed that the coupling phase Bl1 bl1x Bl3y bl3y genotype did not occur.

Table 22. Allelism tests of *blx* and *bly* vs. *bl1* and *bl2*.

| Cross and Homozygous Genotype of Parents | Expected $F_1$ Seed Color 1/ | Observed Number and Color of $F_1$ Seed | Observed $F_2$ Seed Color Segregation |
|---|---|---|---|
| Nupana (*Bl2 bl1 Bl3y*)/*Bl2 Bl1x bl3y* | blue | 11 blue | 1:1 |
| Nupana (*Bl2 bl1 Bl3y*)/*Bl2 bl1x Bl3y* | white | 12 light blue or white 2/ | white |
| Nepal (*bl2 Bl1 Bl3y*)/*Bl2 Bl1x bl3y* | blue | 18 blue | 9:7 |
| Nepal (*bl2 Bl1 Bl3y*)/*Bl2 bl1x Bl3y* | blue | 15 blue | 9:7 |
| Ubamer (*Bl2 Bl1 Bl3y*)/*Bl2 Bl1x bl3y* | blue | 9 blue | 3:1 |
| Ubamer (*Bl2 Bl1 Bl3y*)/*Bl2 bl1x Bl3y* | blue | 18 blue | 3:1 |

1/ Expected when *Bl1x* is allelic to *Bl1*, and *Bl3y* non-allelic to either *Bl1* or *Bl2*.
2/ These were probably white since no color segregation occurred in the $F_2$.

dominant for the remaining known and unknown complementary blue aleurone genes.

Nupana (bl1 bl1 Bl2 Bl2)and Nepal (Bl1 Bl1 bl2 bl2), when crossed to blue varieties in this study, have always produced monohybrid ratios for blue vs white aleurone in the $F_2$. It was therefore assumed that Nupana and Nepal were homozygous dominant for all known and unknowm complementary blue aleurone genes except bl1 and bl2, respectively. Therefore, a cross between Nepal and the blx blx Bly Bly stock producing all blue seeds in the $F_1$ and a 9:7 ratio in the $F_2$ would indicate Blx and Bl2 to be non-allelic, independent, and complementary. If the blx blx Bly Bly stock were then crossed to Nupana, and only white $F_1$ and $F_2$ seeds were produced, it could be concluded that blx is allelic to bl1. The blx blx Bly Bly stock could then be designated as bl1x bl1x Bl2 Bl2 Bly Bly. If the Blx Blx bly bly stock were similarly tested with Nepal and Nupana, but showed bly not to be allelic to either bl1 or bl2, then bly could be designated as bl3y. Subsequently, the blx blx Bly Bly and Blx Blx bly bly stocks could be designated as bl1x bl1x Bl2 Bl2 Bl3y Bl3y and Bl1x Bl1x bl2 Bl2 bl3y bl3y respectively. The data presented in Table 22 indicates this to be so. The Bl1x Bl1x Bl2 Bl2 bl3y bl3y stock produced $F_1$ seed which were all blue, and $F_2$ segregations that approached a 9:7 ratio when crossed with either Nupana or Nepal. The bl1x bl1x Bl2 Bl2 Bl3y Bl3y stock, when crossed to Nupana, produced $F_1$ seeds that appeared to be white or very

light blue and $F_2$ seeds which were all white. The non-occurence of blue among the $F_2$ seeds indicates that the $F_1$ seeds were also white, and suggests that blx is allelic to bl1. Therefore, Blx and Bly were designated as Bl1x and Bl3y, respectively.

Further indication that Bl1x is allelic to Bl1 and closely linked to Bl3y is shown by the apparent difference in ratios among the Nupana and Nepal crosses that produced aleurone color segregations (Table 22). If Bl1x and Bl3y were closely linked, one would expect a 1:1 ratio in the $F_2$ for the Nupana x Bl1x Bl1x Bl2 Bl2 bl3y bl3y cross. The heterozygote configuration of this cross can be depicted as

$$\frac{Bl2}{Bl2} \quad \frac{blx \;\; Bl3y}{Bl1x \;\; bl3y} ,$$

where Bl2 does not segregate, and the repulsion phase Bl1x blx Bly bl3y segregates 1 blx blx Bl3y Bl3y (white) : 2 Bl1x blx Bl3y bl3y (blue) : 1 Bl1x Bl1x bl3y bl3y (white). In the case of the Nepal x Bl1x Bl1x Bl2 Bl2 bl3y bl3y cross, where the heterozygote would be

$$\frac{bl2}{Bl2} \quad \frac{Blx \;\; Bl3y}{Bl1x \;\; bl3y} ,$$

bl2 and bl3y would assort independently and result in a 9:7 ratio. Similarly, a 9:7 ratio would be obtained from the Nepal x bl1x bl1x Bl2 Bl2 Bl3y Bl3y cross. Among the $F_2$ segregations observed on plants from the Nupana x Bl1x Bl1x Bl2 Bl2 bl3y bl3y cross, the white kernels consistently occurred at a slightly higher frequency than the blue kernels. Among the plants examined from the Nepal crosses, the frequencies of

white kernels were consistently lower than for blue. Approximately 70 seeds were examined on each plant, which probably constituted samples too small to distinguish between 1:1 and 9:7 ratios with reliability. However, the observations showed a trend in favor of 1:1 $F_2$ ratios for the Nupana x Bl1x Bl1x Bl2 Bl2 bl3y bl3y cross, and 9:7 $F_2$ ratios for the Nepal crosses.

The data presented in Tables 21 and 22 support the contentions that Bl1x is allelic to Bl1 and closely linked to Bl3y. However, it was desired that more testcrosses be made before conclusions be made regarding the allelism of Blx to Bl1.

## Classification of Male Sterile Stocks For Bl1 and Bl2

The spring habit collection of male sterile stocks reported by Hockett et al (1968) were tested for aleurone color genotype at the Bl1 and Bl2 loci in order that use of these stocks in future aleurone color studies or hybrid systems would be facilitated. The number and aleurone color of $F_1$ plants obtained from the testcrosses involving the male sterile stocks are presented in Table 23. All successful crosses with Nepal (bl2 bl2 Bl1 Bl1) produced segregating seed colors on the $F_1$ plants, indicating the respective male sterile stocks were dominant for Bl2. Most of these same male sterile stocks, when crossed to the tester stocks Bl2 Bl2 bl1 bl1, produced only white $F_2$ seeds, which indicated that the respective stocks were recessive at the Bl1 locus. The exceptions were

Table 23. Aleurone color observed on $F_1$ plants from testcrosses of the spring collection of male sterile stocks with aleurone color testers.

| Male Sterile Stock Number | Number of $F_1$'s and Color from Tester | | | bl1 B12 Tester | Homozygous Genotype of MS Stock Tested |
|---|---|---|---|---|---|
| | CI4961-1 | Nepal | | | |
| | B11 B12 | B11 b12 | b11 B12 | | |
| 1 | 9 seg | | 4 wht | Betzes | b11 |
| 2 | 5 seg | | 10 wht | Betzes | b11 |
| 3 | 10 seg | | 10 wht | Betzes | b11 |
| 4 | 8 seg | | 19 seg | Betzes | B11 |
| 5 | | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 6 | 4 seg | | 4 wht | Betzes | b11 |
| 7 | 10 seg | | 10 seg | Betzes | B11 |
| 8 | | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 9 | 15 seg | | 15 wht | Betzes | b11 |
| 10 | 15 seg | 5 seg | 10 wht | Betzes | b11 B12 |
| 11 | 8 seg | | 9 wht | Betzes | b11 |
| 12 | 4 seg | | 14 seg | Betzes | B11 |
| 13 | 10 seg | | 15 seg | Betzes | B11 |
| 14 | 10 seg | | 10 seg | Betzes | B11 |
| 15 | 2 seg | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 16 | 10 seg | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 17 | 2 seg | | 4 seg | Betzes | B11 |
| 18 | | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 19 | | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 20 | | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 21 | 13 seg | | 18 seg | Betzes | B11 |
| 22 | 10 seg | | 8 seg | Betzes | B11 |
| 23 | | 5 seg | 5 wht | Goldfoil | b11 B12 |
| 24 | 10 seg | | 15 wht | Betzes | b11 |
| 25 | 6 seg | | 6 seg | Betzes | B11 |
| 26 | 10 seg | | 17 seg | Betzes | B11 |
| 27 | | | 15 seg | Betzes | B11 |
| 28 | 10 seg | | 10 wht | Betzes | b11 |
| 29 | | | 10 seg | Betzes | B11 |
| 30 | 13 seg | | 17 wht | Betzes | b11 |
| 31 | 10 seg | | 7 wht | Betzes | b11 |
| 32 | 10 seg | | 9 seg | Betzes | B11 |

Table 23. Continued.

| Male Sterile Stock Number | Number of $F_1$'s and Color from Tester: CI4961-1 Bl1 Bl2 | Nepal Bl1 bl2 | bl1 Bl2 | bl1 Bl2 Tester | Homozygous Genotype of MS Stock Tested |
|---|---|---|---|---|---|
| 33 | 10 blue | 8 seg | 10 seg | Betzes | Bl1 Bl2 |
| 34 | 10 seg | | 10 wht | Betzes | bl1 |
| 35 | | | 10 seg | Betzes | Bl1 |
| 36 | 3 seg | | 2 seg | Betzes | Bl1 |
| 37 | 16 seg | | 9 wht | Betzes | bl1 |
| 38 | 10 seg | | 20 seg | Betzes | Bl1 |
| 39 | | 5 seg | 10 seg | Betzes | Bl1 Bl2 |
| 40 | 2 blue | 4 seg | 10 seg | Betzes | Bl1 Bl2 |
| 41 | 6 blue | 8 seg | 15 seg | Betzes | Bl1 Bl2 |
| 42 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 43 | | 10 seg | 10 wht | Goldfoil | bl1 Bl2 |
| 44 | 3 seg | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 45 | | 5 seg | 4 wht | Betzes | bl1 Bl2 |
| 46 | 5 seg | | 3 wht | Betzes | bl1 |
| 47 | 17 blue | 9 seg | 10 seg | Betzes | Bl1 Bl2 |
| 48 | 6 seg | | 10 wht | Betzes | bl1 |
| 49 | 5 blue | | 10 seg | Betzes | Bl1 Bl2 |
| 50 | | | 10 seg | Betzes | Bl1 Bl2 |
| 51 | | | 17 wht | Betzes | bl1 |
| 52 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 53 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 54 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 55 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 56 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 57 | 10 seg | | 10 wht | Betzes | bl1 |
| 58 | | 12 seg | 6 wht | Goldfoil | bl1 Bl2 |
| 59 | 8 blue | 8 seg | 3 seg | Compana | Bl1 Bl2 |
| 60 | | 5 seg | 4 wht | Betzes | bl1 Bl2 |
| 61 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 62 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 63 | | 9 seg | 5 wht | Compana | bl1 Bl2 |
| 64 | 6 seg | | | | |
| 65 | 19 seg | | 14 wht | Betzes | bl1 |
| 66 | 8 seg | | 10 wht | Betzes | bl1 |

Table 23. Continued.

| Male Sterile Stock Number | Number of $F_1$'s and Color from Tester: CI2961-1 Bl1 Bl2 | Nepal Bl1 bl2 | bl1 Bl2 | bl1 Bl2 Tester | Homozygous Genotype of MS Stock Tested |
|---|---|---|---|---|---|
| 67 | 10 seg | | 9 wht | Betzes | bl1 |
| 68 | 10 blue | 12 seg | 10 seg | Betzes | Bl1 Bl2 |
| 69 | 10 seg | | 20 wht | Betzes | bl1 |
| 70 | 10 seg | | 10 wht | Betzes | bl1 |
| 71 | 8 seg | | 4 wht | Unitan | bl1 |
| 72 | 8 seg | | 3 wht | Betzes | bl1 |
| 73 | | 5 seg | 5 wht | Goldfoil | bl1 Bl2 |
| 74 | | | | | |
| 75 | 12 seg | | 17 wht | Betzes | bl1 |
| 76 | 10 seg | | 18 wht | Betzes | bl1 |
| 77 | 8 seg | | 7 seg | Betzes | Bl1 |
| 78 | | | 14 wht | Betzes | bl1 |
| 79 | 10 seg | | 10 wht | Betzes | bl1 |
| 80 | 20 seg | | 21 wht | Betzes | bl1 |
| 81 | 10 seg | | 15 seg | Betzes | Bl1 |
| 82 | 10 seg | | 7 seg | Betzes | Bl1 |
| 83 | 9 seg | | 10 wht | Betzes | bl1 |
| 84 | 4 seg | | 7 wht | Betzes | bl1 |
| 85 | | | 12 seg | Betzes | Bl1 |
| 86 | 7 seg | | 6 wht | Betzes | bl1 |
| 87 | 15 seg | | 4 wht | Betzes | bl1 |
| 88 | 10 seg | | 5 wht | Goldfoil | bl1 |
| 89 | 10 seg | | 3 wht | Betzes | bl1 |
| 90 | 5 seg | | 7 wht | Betzes | bl1 |
| 91 | 4 seg | | 10 wht | Betzes | bl1 |
| 92 | 9 seg | | 13 wht | Betzes | bl1 |
| 93 | 10 seg | | 19 wht | Betzes | bl1 |
| 94 | | | 10 seg | Betzes | Bl1 |
| 95 | | 10 seg | 3 seg | Betzes | Bl1 Bl2 |
| 96 | | 3 seg | 3 wht | Betzes | bl1 Bl2 |
| 97 | 10 seg | | 10 wht | Betzes | bl1 |
| 98 | | | | | |
| 99 | 10 seg | | 10 wht | Betzes | bl1 |
| 100 | 10 seg | | 10 wht | Betzes | bl1 |
| 101 | | | | | |

Manchuria ms1, Manchuria ms2, Manchuria ms10, Titan ms1, Trebi ms1, CI4961-1 ms19, and Conquest ms,,ac. These varieties are blue seeded types which would be expected to produce $F_2$ color segregations when crossed to either of the white types. Although Titan was reported as a white aleurone type by the Malting Barley Improvement Association (1957), blue types which could not be distinguished morphologically from white types have been common in this variety (Dubbs, 1958). It is possible that the Titan male sterile stock was derived, at least in part, from blue aleurone Titan plants.

The white aleurone male sterile stocks from which successful crosses were obtained with the bl1 bl1 Bl2 Bl2 testers, but not with Nepal, were classified only for the Bl1 locus. All male sterile stocks with blue aleurone were classified as Bl1 Bl1 Bl2 Bl2.

The data obtained from successful crosses with the tester stock CI4961-1 were used to verify that a given male sterile stock was blue or white aleurone. Crosses that produced all blue $F_2$ seeds indicated that the respective male sterile stocks were blue aleurone types. Crosses that produced $F_2$ aleurone color segregations indicated that the respective male sterile stocks were white aleurone types. Efforts were also made to determine the actual segregation ratios from the CI4961-1 crosses in order to detect the possible occurrence of other white aleurone loci among the male sterile stocks. For example, a white aleurone variety of the genotype Bl2 Bl2 bl1 Bl1 producing a 9:7 aleurone color

ratio when crossed to CI4961-1 (Bl2 Bl2 Bl1 Bl1) would indicate the existence of a third and independent complementary blue aleurone gene. Unfortunately, CI4961-1 was not a very good tester. The $F_1$ plants produced from this parent generally expressed blue aleurone colors that were too weak to allow accurate color sorting through the adherent hulls of the $F_2$ seeds.

A simpler method of detecting blue aleurone loci other than Bl1 and Bl2 would employ a hulless male sterile tester stock of the genotype bl1 bl1 bl2 bl2. Any white aleurone line producing blue $F_1$ seeds, when crossed to the bl1 bl1 bl2 bl2 tester, would be recessive for a blue aleurone gene other than Bl1 and Bl2. The bl1 bl1 bl2 bl2 stock could be obtained from the dihybrid Bl1 bl1 Bl2 bl2. The white seeds from the dihybrid would be grown and the resulting plants testcrossed to Bl1 Bl1 bl2 bl2 and bl1 bl1 Bl2 Bl2 tester stocks. Plants producing only white $F_1$ seeds, when crossed to both tester stocks, would be of the double recessive genotype, bl1 bl1 bl2 bl2. The double recessive types would be expected to occur one out of seven times, and should therefore be easy to obtain. Efforts were made to obtain the double recessive during the course of this study. However, the stock used for the Bl1 Bl1 bl2 bl2 tester was later discovered not to be of the genotype Bl1 Bl1 bl2 bl2.

### Inheritance of Ubamer Dark Blue

A very dark shade of blue aleurone, equivalent to 103A of the

R.H.S. Colour Chart (Royal Horticultural Society, London), was discovered in two lines, Ubamer (CI12167) and Mesa Dark Blue (MT1355). The dark blue has two qualities necessary for the employment of blue aleurone as a sorting device. One, it is very dark, which minimizes the chance of misclassifications when sorting blue and white kernels. Second, the trait appears to be highly heritable and stable. Ubamer when grown in close proximity to normal blue lines has always expressed the dark blue phenotype, whereas the normal blue lines have never expressed the dark blue phenotype, and in some cases failed to exhibit any detectable blue due to fading or lack of pigment development.

From the $F_1$ data presented in Table 24, it may be concluded that dark blue is recessive to normal blue. Ubamer or dark blue lines derived from Ubamer were crossed with white or normal blue lines. In all crosses, the color intensity of the $F_1$ seed was normal blue. Normal blue lines crossed to white or normal blue lines were included for comparison, and produced $F_1$ seeds of the same color intensity as $F_1$ seeds from the Ubamer crosses. The Ubamer/Ubamer sib cross was included as a further check, showing that the crossing procedure did not decrease the color intensity of $F_1$ seeds.

Also included in Table 24 are results of a cross between the two dark blue lines, Mesa Dark Blue and Ubamer. The $F_1$ seeds are dark and of the same intensity as the parents, indicating that Mesa Dark Blue and Ubamer carry the same recessive factor for dark blue. The reciprocal

Table 24. Color intensity of $F_1$ seed from crosses involving Ubamer, or dark blue lines derived from Ubamer, as one of the parents.

| Cross | No. of Crosses | Color Intensity of $F_1$ Seed |
|---|---|---|
| Ubamer * 4/ms26u/2/white Nepal | 2 | Normal Blue |
| Normal Blue/Ubamer | 2 | " |
| White ms10/Ubamer | 2 | " |
| White ms14/Ubamer | 4 | " |
| White Betzes ms24v/2/Betzes/Ubamer | 4 | " |
| Shonupana/2*Ubamer/2/Ubamer | 1 | " |
| Blue Manchuria/2/Ubamer/Manchuria | 1 | " |
| Blue Titan/Ubamer | 1 | " |
| Normal Blue/2/Bonus T1-4a/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T1-4i/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T1-4j/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T1-6i/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T2-6k/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T2-7b/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T2-7h/2*Ubamer | 1 | " |
| Normal Blue/2/Mars T3-5b/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T3-5c/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T3-7j/2*Ubamer | 1 | " |
| Normal Blue/2/Bonus T4-5d/2*Ubamer | 1 | " |
| Normal Blue/2/A T5-7a/2*Ubamer | 1 | " |
| White ms10/Nuvan Blue 1/ | 4 | " |
| White ms14/Nuvan Blue 1/ | 4 | " |
| Nuvan Blue/Nuvan Blue 1/ | 1 | " |
| Ubamer/Ubamer | 1 | Dark Blue |
| Mesa Dark Blue/Ubamer | 2 | " |
| Ubamer/Normal Blue 2/ | 2 | Normal Blue |
| Normal Blue/Ubamer 2/ | 2 | " |
| Ubamer/Nuvan Blue 3/ | 2 | " |
| Nuvan Blue/Ubamer 3/ | 2 | " |

1/ These crosses are included for comparison.
2/ 3/ Reciprocal crosses.

crosses between Ubamer and normal blue lines produced $F_1$ seed of the same intensity (Table 24), indicating no cytoplasmic influence. Ubamer dark blue is being transferred successfully into several named varieties and translocation stocks (Wiebe, pers. comm.), indicating the factor to be simply inherited and not quantitative.

If dark blue is conditioned by a single recessive gene, a cross between dark blue and normal blue should yield an $F_2$ ratio of 3 normal blue to 1 dark blue. Although the chi-square for goodness of fit to a 3:1 ratio indicates a poor fit (.01>P>.005), the observed values of 3169 normal blue and 953 dark blue as given in Table 25 provide a reasonable approach to the expected 3:1 ratio of 3092:1030; further indicating that dark blue is probably conditioned by a single recessive chromosomal gene.

It could then be hypothesized that the dark blue gene is allelic and recessive to one of the known dominant blue genes, <u>Bl2</u> or <u>Bl1</u>. Such a hypothesis was rejected on the basis of the results presented in Tables 26 and 27. Listed in Table 26 are $F_2$ segregation ratios from crosses of Ubamer by white lines of the genotype <u>bl2</u> <u>bl2</u> <u>Bl1</u> <u>Bl1</u>. Assuming Ubamer to be <u>Bl2'</u> <u>Bl2'</u> <u>Bl1</u> <u>Bl1</u>, where <u>Bl2'</u> is the hypothetical dark allele and recessive to <u>Bl2</u>, one would expect only dark blue and white seeds in the $F_2$ since only <u>Bl2'</u> and <u>bl2</u> would be segregating. The data in Table 26 includes normal blue as well as dark blue classes. The allelism hypothesis is thus narrowed to the <u>Bl1</u> locus on chromosome 4,

Table 25. Observed $F_2$ segregation ratios for crosses between Ubamer and normal blue, *en-Bl Bl2 Bl1* / *En-Bl Bl2 Bl1*.

| | Ubamer / normal blue | Ubamer / Nuvan Blue | All families |
|---|---|---|---|
| No. of families | 20 | 6 | 26 |
| No. of normal blue seeds 1/ | 2531 | 638 | 3169 |
| No. of dark blue seeds 2/ | 777 | 176 | 953 |
| Chi-square for a 3:1 ratio | 4.029 | 4.954 | 7.770 |
| P for 3:1 ratio | .05-.025 | .05-.025 | .01-.005 |
| Heterogeneity chi-square | 13.844 | 1.393 | 16.450 |
| P= | .90-.75 | .95-.90 | .95-.90 |

1/ 104B of the R.H.S. Colour Chart (Royal Horticultural Society, London).
2/ 103A of the R.H.S. Colour Chart (Royal Horticultural Society, London).

Table 26. Observed $F_2$ segregations for the heterozygote en-Bl Bl2 Bl1/ En-Bl bl2 Bl1.

| Parentage and Bl plot number | No. of Dark Blue Seeds 1/ | No. of Normal Blue Seeds 2/ | No. of White Seeds | Chi-Square for 3:9:4 | P |
|---|---|---|---|---|---|
| ms26u/4*Ubamer/2/Nepal | | | | | |
| 15-1 | 18 | 60 | 24 | 0.275 | .90-.75 |
| 15-2 | 18 | 46 | 23 | 0.424 | .90-.75 |
| 15-3 | 26 | 71 | 23 | 2.359 | .50-.25 |
| 16-1 | 20 | 50 | 13 | 4.396 | .25-.10 |
| 16-2 | 23 | 54 | 17 | 3.457 | .25-.10 |
| 16-3 | 9 | 25 | 6 | 2.178 | .50-.25 |
| Sermo/Ubamer | | | | | |
| 190-1 | 36 | 74 | 27 | 5.794 | .10-.05 |
| 192-2 | 50 | 140 | 54 | 1.253 | .75-.50 |
| 190-3 | 75 | 139 | 44 | 21.421 | .005 |
| bl2 ms10 Bl/Ubamer | | | | | |
| 99-1 | 58 | 169 | 45 | 10.413 | .01-.005 |
| 99-2 | 67 | 190 | 54 | 9.846 | .01-.005 |
| 99-3 | 48 | 119 | 39 | 5.391 | .10-.05 |
| 100-1 | 55 | 172 | 63 | 1.748 | .50-.25 |
| 100-2 | 39 | 78 | 35 | 4.763 | .10-.05 |
| 100-3 | 50 | 90 | 30 | 14.307 | .005 |
| 102-1 | 86 | 200 | 92 | 4.042 | .25-.10 |
| 102-2 | 85 | 150 | 67 | 17.496 | .005 |
| 2 | 139 | 294 | 123 | 14.549 | .005 |
| 3 | 182 | 351 | 139 | 31.823 | .005 |
| 5 | 126 | 267 | 95 | 19.186 | .005 |
| 7 | 72 | 169 | 39 | 21.811 | .005 |
| 19-1 | 27 | 55 | 13 | 9.653 | .01-.005 |
| 19-2 | 19 | 47 | 20 | 0.655 | .75-.50 |
| 19-3 | 14 | 33 | 12 | 1.295 | .75-.50 |
| 19-4 | 20 | 52 | 13 | 4.604 | .25-.10 |
| 19-5 | 14 | 37 | 14 | 0.586 | .75-.50 |
| 19-6 | 24 | 46 | 13 | 7.483 | .025-.01 |
| 19-7 | 15 | 36 | 14 | 0.968 | .75-.50 |
| 19-8 | 18 | 22 | 5 | 14.734 | .005 |
| 20-5 | 20 | 33 | 12 | 6.462 | .05-.025 |

Table 26. Continued.

| Parentage and Bl plot number | No. of Dark Blue Seeds 1/ | No. of Normal Blue Seeds 2/ | No. of White Seeds | Chi-Square for 3:9:4 | P |
|---|---|---|---|---|---|
| 20-6 | 11 | 39 | 22 | 1.407 | .50-.25 |
| Total | 1464 | 3308 | 1190 | 108.376 | .005 |

Heterogeneity chi-square 76.406 (.10>P>.05).

1/ 103A of the R.H.S. Colour Chart (Royal Horticultural Society, London).
2/ 104B of the R.H.S. Colour Chart (Royal Horticultural Society, London).

Table 27. Observed $F_2$ segregations for the heterozygote en-Bl Bl2 Bl1/En-Bl Bl2 bl1.

| Parentage and Bl Plot Number | No. of Dark Blue Seeds 1/ | No. of Normal Blue Seeds 2/ | No. of White Seeds | Chi-Square 3:9:4 | P |
|---|---|---|---|---|---|
| Nupana/Ubamer | | | | | |
| 47-3 | 31 | 49 | 29 | 8.039 | .025-.01 |
| 47-4 | 35 | 56 | 28 | 9.109 | .025-.01 |
| 49-1 | 19 | 48 | 27 | 1.078 | .75-.5 |
| Shonupana/Ubamer | | | | | |
| 665 | 62 | 146 | 78 | 3.273 | .25-.10 |
| 41-1 | 33 | 77 | 31 | 2.207 | .50-.25 |
| 41-3 | 34 | 94 | 40 | 0.296 | .90-.75 |
| Betzes/Ubamer | | | | | |
| 165 | 39 | 67 | 44 | 8.907 | .025-.01 |
| 17 | 56 | 144 | 80 | 2.819 | .25-.10 |
| Total | 360 | 725 | 337 | 21.797 | .005 |

Heterogeneity chi-square 13.931 (.50>p>.25).

1/ 103A of the R.H.S. Colour Chart (Royal Horticultural Society, London).
2/ 104B of the R.H.S. Colour Chart (Royal Horticultural Society, London).

designating dark blue as Bl1'. Crossing Ubamer Bl2 Bl2 Bl1' Bl1' to a white line of the genotype Bl2 Bl2 bl1 bl1 should produce only dark blue and white seeds in the $F_2$ as before. But again, normal blue seeds are found in the $F_2$ (Table 27), indicating the dark blue gene to be non-allelic to Bl1.

Assuming that dark blue is conditioned by a single recessive gene (hereafter referred to as en-Bl) requiring the presence of the complementary dominants for expression, a 3:9:4 ratio for dark blue, normal blue, and white seeds respectively should have occurred for the crosses presented in Tables 26 and 27. However, neither set of data fits the 3:9:4 ratio very closely ($P < .005$). The data presented in Table 26 was obtained from material that had very good color development. The near 2:1 ratio for normal blue vs dark blue suggests linkage between en-Bl and Bl2. At complete linkage, the selfed repulsion phase heterozygote en-Bl Bl2/En-Bl bl2 would give a ratio of 1 dark blue : 2 normal blue : 1 white. However, this does not appear to be the only source if deviation from the expected 3:9:4 ratio. Even at complete linkage between en-Bl and Bl2, one would still expect a 3:1 ratio for total blues vs white. In the data presented in Table 26, there is a considerable deficiency in the white class. This could again be an occurrence of a gamete selection factor. In this case, the selection factor would have to be associated with the recessive allele bl2, rather than the dominant allele Bl2. If the factor that caused the deficiency of $F_2$ white

kernels from these crosses was the same as that which caused the elimination of the <u>Bl2</u> <u>Bl2</u> class in the case of the 2:1 ratios discussed previously, one would expect the white class to be almost completely eliminated. It is therefore possible that either a separate gamete selection factor is involved here, or there is a series of the factors on chromosome 1 which could have an additive effect.

The combined data from the crosses listed in Table 27 approached a 3:1 ratio for total blues vs white, which indicated that <u>Bl1</u> on chromosome 4 segregated normally. The 2:1 ratio for normal blue vs dark blue suggests the association of a gamete selection factor with the dominant allele <u>En-Bl</u>. Most of the crosses listed in Table 27 involved Compana derived types, as was the case for the 2:1 ratios of blue vs white aleurone discussed earlier. If the selection factor is the same as that proposed as being associated with <u>Bl2</u>, then <u>En-Bl</u> should be linked to <u>Bl2</u> on chromosome 1. The Betzes/Ubamer cross in Table 27, along with the other crosses that produced skewed ratios in this study and that of Jain's (1970), support the contention that some form of gamete selection is commonly associated with the <u>Bl2</u> locus in more than a few varieties.

The testcross data presented in Table 28 provides further evidence that dark blue is conditioned by a recessive gene which is non-allelic to <u>Bl2</u> and <u>Bl1</u> and linked to <u>Bl2</u>. The data was obtained by testcrossing the $F_2$ white male sterile progeny of the cross <u>En-Bl</u> <u>bl2</u> <u>ms10</u> <u>Bl1</u>/<u>en-Bl</u>

Table 28. Observed aleurone color of $F_1$ and $F_2$ seeds from testcrosses of Nuvan Blue and Ubamer with the white male sterile progeny from the heterozygote En-Bl bl2 ms10 Bl1/en-Bl Bl2 Ms10 Bl1.

| 1972 Bl Parentage | No. of Normal Blue $F_1$ Seeds 1/ | No. of Dark Blue $F_1$ Seeds 2/ | $F_2$ from Normal Blue Seeds, No. of Plants Segregating | | $F_2$ from Dark Blue Seeds, No. of Plants Segregating | Genotype of White Male Sterile Tested |
|---|---|---|---|---|---|---|
| | | | D:N:W 3/ | N:W | D:W | |
| (En-Bl bl2 ms10 Bl1/ en-Bl Bl2 Ms10 Bl1/ 2/Nuvan Blue) | | | | | | |
| 124-1 x 365 | 11 | 0 | 4 | 4 | 0 | En-Bl en-Bl |
| 124-2 x 365 | 16 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| 124-3 x 365 | 18 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| 124-4 x 365 | 13 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| 124-5 x 365 | 19 | 0 | 5 | 4 | 0 | En-Bl en-Bl |
| 124-6 x 365 | 12 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| 124-7 x 365 | 4 | 0 | 3 | 1 | 0 | En-Bl en-Bl |
| 124-8 x 365 | 12 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| 124-9 x 365 | 14 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| 124-10 x 365 | 17 | 0 | 0 | 8 | 0 | En-Bl En-Bl |
| 124-12 x 365 | 17 | 0 | 3 | 5 | 0 | En-Bl en-Bl |
| 124-13 x 365 | 12 | 0 | 0 | 9 | 0 | En-Bl En-Bl |
| (En-Bl bl2 ms10 Bl1/ en-Bl Bl2 Ms10 Bl1/ 2/Ubamer) | | | | | | |
| 116-1 x 364 | 14 | 0 | - | - | - | En-Bl En-Bl |
| 116-5 x 364 | 9 | 0 | 9 | 0 | 0 | En-Bl En-Bl |

Table 28. Continued.

| 1972 Bl Parentage | No. of Normal Blue $F_1$ Seeds 1/ | No. of Dark Blue $F_1$ Seeds 2/ | $F_2$ from Normal Blue Seeds, No. of Plants Segregating | | $F_2$ from Dark Blue Seeds, No. of Plants Segregating | Genotype of White Male Sterile Tested |
|---|---|---|---|---|---|---|
| | | | D:N:W 3/ | N:W | D:W | |
| 116-7 x 364 | 3 | 0 | 3 | 0 | 0 | En-Bl En-Bl |
| 116-9 x 364 | 3 | 0 | 3 | 0 | 0 | En-Bl En-Bl |
| 116-11 x 364 | 9 | 0 | 9 | 0 | 0 | En-Bl En-Bl |
| 116-13 x 364 | 11 | 0 | 9 | 0 | 0 | En-Bl En-Bl |
| 116-14 x 364 | 11 | 0 | 9 | 0 | 0 | En-Bl En-Bl |
| 116-18 x 364 | 16 | 0 | 9 | 0 | 0 | En-Bl En-Bl |
| 116-3 x 364 | 0 | 7 | 0 | 0 | 7 | en-Bl en-Bl |
| 116-2 x 364 | 0 | 11 | 0 | 0 | 9 | en-Bl en-Bl |
| 116-15 x 364 | 0 | 12 | 0 | 0 | 9 | en-Bl en-Bl |
| 116-6 x 364 | 8 | 10 | 4 | 0 | 5 | En-Bl en-Bl |
| 116-8 x 364 | 1 | 2 | 1 | 0 | 0 | En-Bl en-Bl |
| 116-10 x 364 | 7 | 10 | 4 | 0 | 5 | En-Bl en-Bl |
| 116-12 x 364 | 2 | 6 | 2 | 0 | 6 | En-Bl en-Bl |
| 116-13 x 364 | 9 | 6 | 9 | 0 | 0 | En-Bl en-Bl |
| 116-17 x 364 | 15 | 26 | 4 | 0 | 5 | En-Bl en-Bl |

1/ 104B of the R.H.S. Colour Chart (Royal Horticultural Society, London).
2/ 103A of the R.H.S. Colour Chart (Royal Horticultural Society, London).
3/ D = dark blue, N = normal blue, W = white.

Bl2 Ms10 Bl1 with Nuvan Blue (En-Bl En-Bl Bl2 Bl2 Bl1 Bl1) and Ubamer (en-Bl en-Bl Bl2 Bl2 Bl1 Bl1). If the hypothesis is true, the testcrosses with Nuvan Blue should yield only normal blue $F_1$ seed, since the dominant allele En-Bl from Nuvan Blue would cover the expression of dark blue on white females carrying the en-Bl allele. The testcrosses with Ubamer should produce dark blue seeds on the females carrying the en-Bl allele. The testcross data of Table 28 shows this to be true. Ubamer testcrosses producing only normal blue seeds indicate the respective white females to be of the genotype En-Bl En-Bl bl2 bl2, and crosses producing both intensities of blue indicate the respective white females to be of the genotype En-Bl en-Bl bl2 bl2. The testcrosses producing only dark seeds indicate the genotype of the white females to be en-Bl en-Bl bl2 bl2. Since all three of the expected genotypes were present, it is unlikely that en-Bl is allelic to Bl2. However, the frequency of the genotypes among the white plants did not fit an expected 1:2:1 ratio. The observed frequency, where the genotypes of the testcross $F_1$ seeds were determined by $F_2$ color segregations (Table 28), was 16 En-Bl En-Bl bl2 bl2, 10 En-Bl en-Bl bl2 bl2, and 3 en-Bl en-Bl bl2 bl2, indicating linkage between en-Bl and bl2. If linkage is present between en-Bl and bl2, the observed ratio would be expected since the original cross, En-Bl bl2 ms10/en-Bl Bl2 Ms10, was in repulsion phase for bl2 and en-Bl, and in coupling phase for bl2 and ms10. Since only white male sterile plants were used in the testcrosses, selection

in favor of the dominant allele (En-Bl) would be expected; thus giving an excess of the En-Bl En-Bl bl2 bl2 class.

All of the available $F_2$ and testcross data from the En-Bl bl2 ms10/en-Bl Bl2 Ms10 crosses were then used to estimate recombination values between en-Bl and bl2, and en-Bl and ms10. The combined recombination value for en-Bl vs ms10 was 29.5 ± 3.0% (Table 29). The heterogeneity chi-square of 0.620 with 1 degree of freedom indicated that the combined estimate applied to both families (.5>P>.25).

The combined recombination value for en-Bl vs bl2 was 30.1 ± 3.0% (Table 30). This being similar to the recombination value for en-Bl vs ms10 indicates ms10 and bl2 to be closely associated. The heterogeneity chi-square of 0.215 with 1 degree of freedom indicated the combined estimate applied to both families (.75>P>.5).

The data from the Ubamer crosses presented in Table 28 were verified by planting the testcross seed in the greenhouse. The dark blue seeds produced plants that segregated dark blue and white only (Table 28). The $F_2$ ratios of dark blue to white seeds from the greenhouse progeny tests are listed in Table 31 and show a good fit to a 3:1 (.75>P>.5), suggesting the genotype of the dark blue testcross seeds to be en-Bl en-Bl Bl2 bl2 as expected. All of the normal blue testcross seeds from the Ubamer crosses listed in Table 28 produced plants which segregated for dark blue, normal blue, and white, suggesting the genotype of the normal blue testcross seed to be En-Bl en-Bl Bl2 bl2 as expected.

Table 29. Segregation ratios for crosses involving normal blue and dark blue aleurone (En-Bl en-Bl) and fertile and male sterile (Ms10 ms10), En-Bl bl2 ms10/en-Bl Bl2 Ms10 Bl1/Bl1.

| Cross No. 1/ | 3 | 4 | Total |
|---|---|---|---|
| Phase 2/ | R | R | |
| En-Bl ___ MS10 ___ | 218 | 256 | 474 |
| En-Bl ___ ms10 ms10 | 81 4/ | 82 4/ | 163 |
| en-Bl en-Bl Ms10 ___ | 119 | 124 | 243 |
| en-Bl en-Bl ms10 ms10 | 12 4/ | 7 4/ | 19 |
| Total | 430 | 469 | 899 |
| Chi-square 3/ | 0.228 | 0.409 | |

Recombination value for en-Bl vs ms10 = 29.5 ± 3.0%.
Heterogeneity chi-square = 0.620 (.5>P>.25).

1/ See Table 6.
2/ R = repulsion.
3/ For approximate goodness of fit to 29.5 recombination ratios.
4/ Apportionment to these two classes based on testcrosses of 29 plants.

Table 30. Segregation ratios for crosses involving normal blue and dark blue aleurone (En-Bl en-Bl) and blue and white aleurone (Bl2 bl2), En-Bl bl2 ms10/en-Bl Bl2 Ms10 Bl1/Bl1.

| Cross No. 1/ | 3 | 4 | Total |
|---|---|---|---|
| Phase 2/ | R | R | |
| En-Bl ___ Bl2 ___ | 216 | 258 | 474 |
| En-Bl ___ bl2 bl2 | 83 4/ | 80 4/ | 163 |
| en-Bl en-Bl Bl2 ___ | 119 | 123 | 242 |
| en-Bl en-Bl bl2 bl2 | 12 4/ | 8 4/ | 20 |
| Total | 430 | 469 | 899 |
| Chi-square 3/ | 0.157 | 0.066 | |

Recombination value for en-Bl vs bl2 = 30.1 ± 3.0% (approximate).
Heterogeneity chi-square = 0.215 (.75>P>.50).

1/ See Table 6.
2/ R = repulsion.
3/ For approximate goodness of fit to 30.1% recombination ratios.
4/ Apportionment to these two classes based on testcrosses of 29 plants.

Table 31. $F_2$ segregation ratios observed on plants of the genotype en-B1 B12 B11/en-B1 b12 B11.

| | en-B1 en-B1 b12 b12 ms10 ms10/ Ubamer |
|---|---|
| No. of plants | 21 |
| No. of dark blue seeds 1/ | 1042 |
| No. of white seeds | 333 |
| Chi-square for a 3:1 ratio | 0.448 |
| P for 3:1 chi-square | .75-.50 |
| Heterogeneity chi-square | 20.263 |
| P= | .50-.25 |

1/ 103A of R.H.S. Colour Chart (Royal Horticultural Society, London).

The Nuvan Blue testcross seed were also planted for verification (Table 28). Families consisting only of plants which segregated normal blue and white originated from En-Bl En-Bl bl2 bl2 females, which produced En-Bl En-Bl Bl2 bl2 testcross seed when crossed with Nuvan Blue. Families consisting only of plants segregating dark blue, normal blue, and white originated from en-Bl en-Bl bl2 bl2 females, which produced En-Bl en-Bl Bl2 bl2 testcross seed when crossed to Nuvan Blue. Families with both types of plants originated from En-Bl en-Bl bl2 bl2 females; which, when crossed to Nuvan Blue, produced both En-Bl En-Bl Bl2 bl2 and En-Bl en-Bl Bl2 bl2 testcross seed.

In conclusion, Ubamer dark blue aleurone was conditioned by a single recessive gene on chromosome 1, and was non-allelic to the two known blue genes, Bl1 and Bl2. The occurrence of the genotypes En-Bl En-Bl, En-Bl en-Bl, and en-Bl en-Bl among white plants of the genotype bl2 bl2 Bl1 Bl1 indicates that the presence of Bl2 and Bl1 are necessary for the expression of dark blue. Therefore, the dark blue factor could be designated as an enhancer to the complementary dominants. One could also look at the dominant allele En-Bl as being a suppressor to Bl1 and Bl2, as in the case of the chlorophyll mutant suppressors reported by Tuleen et al (1968). In this study, the enhancer designation was used. According to the gene symbol nomenclature rules recommended by the barley genetics committee of the American Barley Research Workers (Ramage, 1972), the genetic symbols for recessive enhancers are designated by the

symbol en followed by a hyphen and the symbol of the allele affected. Therefore, the gene symbol for Ubamer dark blue will be proposed as en-Bl.

It is further concluded that en-Bl can be easily incorporated into blue and white hybrid parents because of its low degree of association with bl2 and ms10, and non-association with bl1. The recombination values for en-Bl vs bl2 and ms10 were estimated as 30.1 ± 3.0% and 29.5 ± 3.0%, respectively.

## Linkages on Chromosome 1

Data and recombination values for en-Bl vs Bl2 and ms10 were reported in the section dealing with Ubamer dark blue. Recombination values for blue aleurone (Bl2) vs male sterile (ms10), male sterile (ms14), naked caryopsis (n), and short awn (lk2); and for short awn (lk2) vs naked caryopsis (n) were estimated from the data presented in Tables 32 through 36. Recombination values for Bl2 vs ms22e, ms23b, and ms,,w are not available since development of the appropriate blue gene constitution for these male sterile stocks was not far enough along to make the necessary crosses for linkage determination. In most cases, the ratios presented in Tables 32 through 36 deviated from the expected 3:1 when considering a single locus at a time, further supporting the contention that a gamete selection factor occurred in the Bl2 region of chromosome 1.

The combined estimate for Bl2 vs ms10 was 3.9 ± 0.4% (Table 32).

Table 32. Segregation ratios for crosses involving fertile and male sterile (Ms10 ms10) and blue and white aleurone (Bl2 bl2) on chromosome 1.

| Cross No. 1/ | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Phase 2/ | C | C | C | C | C | R |
| Ms10 ___ Bl2 ___ | 104 | 280 | 328 | 508 | 374 | 114 |
| Ms10 ___ bl2 bl2 | 7 | 13 | 9 | 2 | 6 | 56 |
| ms10 ms10 Bl2 ___ | 5 | 10 | 7 | 12 | 7 | 48 |
| ms10 ms10 bl2 bl2 | 65 | 72 | 86 | 123 | 82 | 0 |
| Total | 181 | 375 | 430 | 645 | 469 | 218 |
| Chi-square 3/ | 1.709 | 5.429 | .004 | 3.961 | .891 | .066 |

Recombination value for ms10 vs bl2 = 3.9 ± 0.4%.
Heterogeneity chi-square = 12.010 (.05>P>.025).
1/ See Table 6.
2/ C = coupling, R = repulsion.
3/ For approximate goodness of fit to 3.9% recombination ratios.

Although the heterogeneity chi-square is significant (.05>P>.025), four out of six of the families fit the calculated recombination value with non-significant chi-square values ranging from 0.004 to 1.709 (.95>P> .10). When separate recombination values were calculated for each individual family, a recombination value was estimated by placing B12 within the limits of ±2 standard errors of each value (Figure 1). The new estimate was the same as the combined estimate. The only standard error that did not include the combined estimate was that for data set 4. Since all of the remaining data sets included the combined estimate within two standard errors, the value of 3.9 ± 0.4% was considered the best estimate.

The recombination value for B12 vs N was estimated to be 12.1 ± 1.5% (Table 33). This is in good agreement with the recombination value for B12 vs N reported by Myler and Stanford (1942) which was 9.9 ± 0.4%. However, the heterogeneity chi-square was significant (.01>P> .005), which indicated that separate recombination values should be calculated for each family. With the exception of cross number 7, estimation of the recombination value by fitting a line within the limits of ±2 standard errors coincided with the combined estimate (Figure 2). Thus, the combined estimate of 12.1 ± 1.5% was considered the best estimate.

The combined estimate for B12 vs lk2 was calculated to be 21.5 ± 1.8% (Table 34). The heterogeneity chi-square of 0.166 (.95>P>.90)

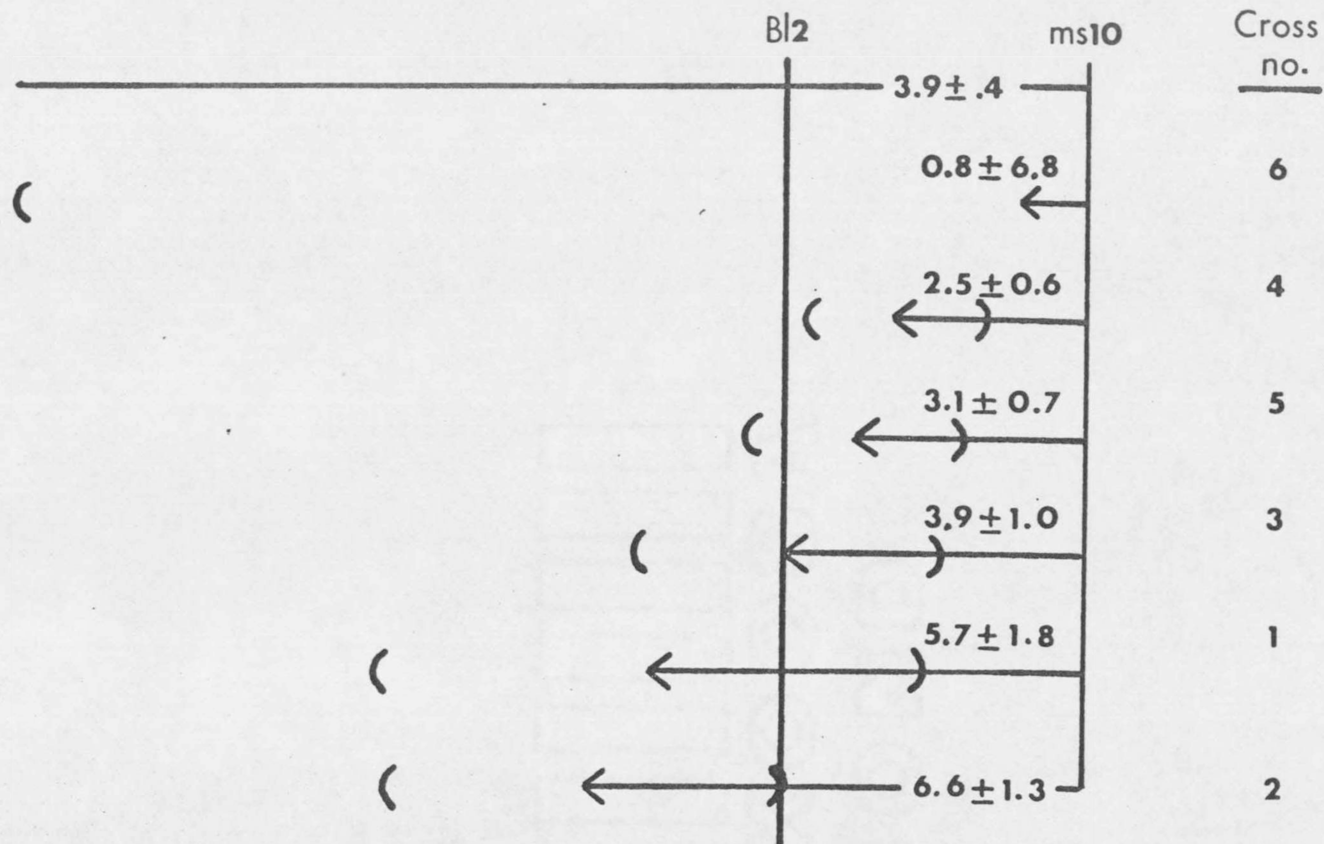


Figure 1. Linkage map representing the 6 recombination values obtained for Bl2 vs. ms10.

( ) ± 2 standard errors.

Table 33. Segregation ratios for crosses involving covered and naked caryopsis (N n) and blue and white aleurone (Bl2 bl2), n bl2/N Bl2.

| Cross No. 1/ | 7 | 8 | 9 | 10 | 11 | |
|---|---|---|---|---|---|---|
| Phase 2/ | C | C | C | C | C | Total |
| N ___ Bl2 ___ | 57 | 63 | 199 | 34 | 63 | 416 |
| N ___ bl2 bl2 | 1 | 4 | 14 | 12 | 13 | 44 |
| n n Bl2 ___ | 0 | 3 | 11 | 2 | 4 | 20 |
| n n bl2 bl2 | 16 | 13 | 36 | 16 | 21 | 102 |
| Total | 74 | 83 | 260 | 64 | 101 | 582 |
| Chi-square 3/ | 6.966 | 0.333 | 0.084 | 5.017 | 2.433 | |

Recombination value for n vs bl2 = 12.1 ± 1.5%.
Heterogeneity chi-square = 14.831 (.01>P>.005).
1/ See Table 6.
2/ C = coupling.
3/ For approximate goodness of fit to 12.1% recombination ratios.

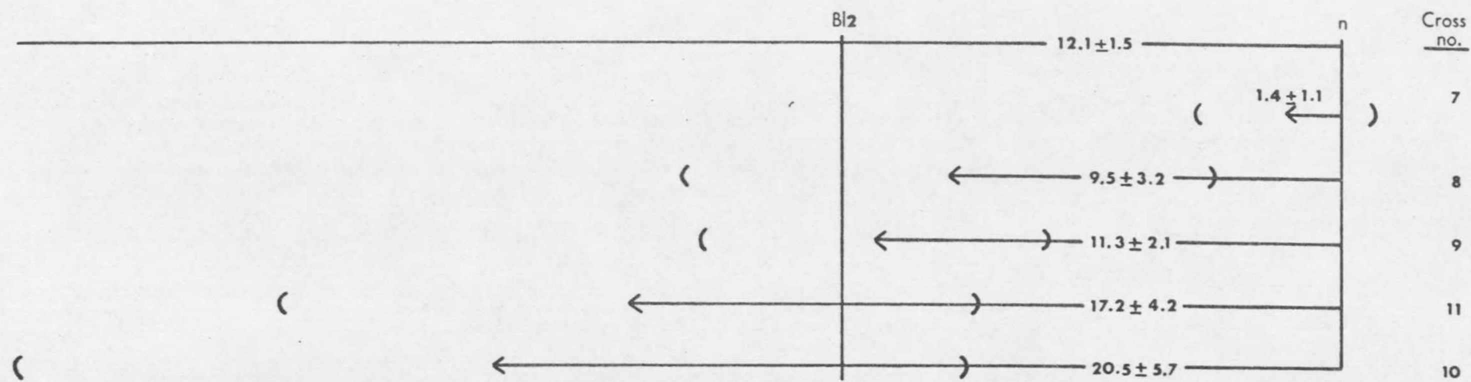




Figure 2. Linkage map representing the 5 recombination values obtained for Bl2 vs. n.

( ) ± 2 standard errors.

Table 34. Segregation ratios for crosses involving long and short awn (Lk2 lk2) and blue and white aleurone (Bl2 bl2), lk2 bl2/ Lk2 Bl2.

| Cross No. 1/ | 12 | 13 | 9 | Total |
|---|---|---|---|---|
| Phase 2/ | C | C | C | |
| Lk2 __ Bl2 __ | 59 | 212 | 187 | 458 |
| Lk2 __ bl2 bl2 | 10 | 30 | 23 | 63 |
| lk2 lk2 Bl2 __ | 8 | 38 | 23 | 69 |
| lk2 lk2 bl2 bl2 | 18 | 59 | 27 | 104 |
| Total | 95 | 339 | 260 | 694 |
| Chi-square 3/ | .054 | .078 | .160 | |

Recombination value for lk2 vs bl2 = 21.5 ± 1.8%.
Heterogeneity chi-square = 0.166 (.95>P>.90).
1/ See Table 6.
2/ C = coupling.
3/ For approximate goodness of fit to 21.5% recombination ratios.

indicates that the combined recombination value applies to all three families.

Data from a single cross were used to estimate the linkage intensity for lk2 vs N, giving a recombination value of 10.4 ± 2.1% (Table 35). Eslick, Hockett, and Kushnak (1972), with considerably more data, estimated the recombination value for lk2 vs N to be 7.9 ± 0.32%. This is in agreement with the 10.4 ± 2.1% value when the ranges of the standard errors are considered.

Eslick, Hockett, and Kushnak (1972) reported the recombination value for ms10 vs ms14 to be 2.0 ± 2.0% which would place Bl2 in close proximity to ms14 as well as ms10. Data representing 101 plants from a single repulsion phase cross were used to estimate the linkage intensity for ms14 vs bl2, giving a recombination value of 22.8 ± 9.4% (Table 36). The estimate, minus 2 standard errors, would place ms14 4 map units from Bl2. Since the standard error is relatively high, the recombination value for ms10 vs bl2 would provide a better estimate for positioning bl2 on chromosome 1.

It was further reported by Eslick, Hockett, and Kushnak (1972) that the recombination values for lk2 vs ms10, and n vs ms10 were estimated to be 14.7 ± 0.71% and 7.2 ± 0.41%, respectively. With these recombination values plus those mentioned above, it is possible to estimate the probable location of Bl2 in relation to lk2, N, ms10, and ms14. Eslick (1970) concluded, from crosses involving ten different

Table 35. Segregation ratio for the cross involving long and short awn (Lk2 lk2) and covered and naked caryopsis (N n), lk2 n/ Lk2 N.

| Cross No. 1/ | 9 |
|---|---|
| Phase 2/ | C |
| Lk2 ___ N ___ | 200 |
| Lk2 ___ n n | 10 |
| lk2 lk2 N ___ | 13 |
| lk2 lk2 n n | 37 |
| Total | 260 |
| Chi-square 3/ | .106 |

Recombination value for lk2 vs n = 10.4 ± 2.1%.

1/ See Table 6.
2/ C = coupling.
3/ For approximate goodness of fit to a 10.4% recombination ratio.

Table 36. Segregation ratio for the cross involving fertile and male sterile (Ms14 ms14) and blue and white aleurone (B12 b12), Ms14 b12/ms14 B12.

| Cross No. 1/ | 11 |
|---|---|
| Phase 2/ | R |
| Ms14 ___ B12 ___ | 37 |
| Ms14 ___ b12 b12 | 31 |
| ms14 ms14 B12 ___ | 30 |
| ms14 ms14 b12 b12 | 3 |
| Total | 101 |

Recombination value for ms14 vs b12 = 22.8 ± 9.4%.
1/ See Table 6.
2/ R = repulsion.

translocations, that ms10 and ms14 were very near the centromere of chromosome 1. It was later established from double crossover and three-point linkage tests, that the gene order was lk2 - N - ms10 with ms14 very near ms10 (Eslick, Rahman, Crowell, 1971; Eslick, Hockett, and Kushnak, 1972). Tsuchiya (1972), using telocentrics, established N to be on the long arm of chromosome 1, which would also put lk2 on the long arm.

The recombination values for Bl2 vs ms10, n, and lk2 suggest the probable gene order to be lk2 - N - ms10 - Bl2, with Bl2 on the short arm (Figure 3). Rahman (1973) reported the albino seedling mutant $a_{c2}$, to be on the short arm of chromosome 1; 10.7 ± 1.0 units from ms10, and 6.3 ± 1.5 units from ms14. This would place Bl2 approximately 3 to 6 units from $a_{c2}$, indicating the possibility of using $a_{c2}$ as a balanced lethal in conjunction with Bl2 in the male sterile sorting scheme.

## Linkages on Chromosome 4

The recombination value for blue aleurone (Bl1) vs male sterile (ms24v) was estimated to be 11.2 ± 1.3% (Table 37). The heterogeneity chi-square of 1.777 (.25>P>.10) indicates the combined recombination value to apply to both families. Both families segregated 3:1 for blue vs white aleurone, which indicated that Bl1 was segregating normally.

The data presented in Table 38 were used to estimate the recombination value for blue aleurone (Bl1) vs male sterile (ms25r). The

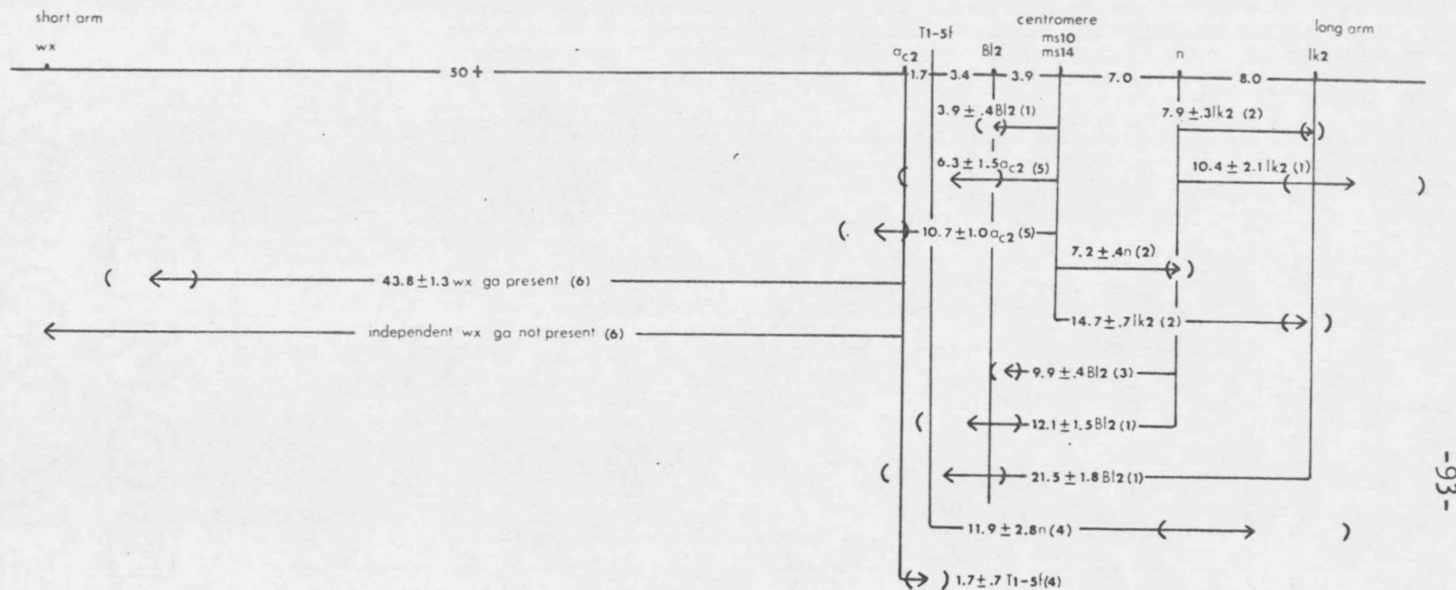


Figure 3. Proposed linkage map of chromosome 1.

(1) Data contributed by this paper.
(2) Eslick, Hockett, and Kushnak (1972).
(3) Myler and Stanford (1942).
(4) Persson (1969a and 1969b).
(5) Rahman (1973).
(6) Tabata (1961).
( ) ± 2 standard errors.

Table 37. Segregation ratios for crosses involving fertile and male sterile (Ms24v ms24v) and blue and white aleurone (Bl1 bl1).

| Cross No. 1/ <br> Phase 2/ | 14 <br> C | 15 <br> C | Total |
|---|---|---|---|
| Ms24v ___ Bl1 ___ | 162 | 325 | 487 |
| Ms24v ___ bl1 bl1 | 13 | 27 | 40 |
| ms24v ms24v Bl1 ___ | 18 | 17 | 35 |
| ms24v ms24v bl1 bl1 | 48 | 94 | 142 |
| Total | 241 | 463 | 704 |
| Chi-square 3/ | 1.437 | .381 | |

Recombination value for ms24v vs bl1 = 11.2 ± 1.3%
Heterogeneity chi-square = 1.777 (.25>P>.10)
1/ See Table 6.
2/ C = coupling.
3/ For approximate goodness of fit to 11.2% recombination ratios.

Table 38. Segregation ratios for crosses involving fertile and male sterile (Ms25r ms25r) and blue and white aleurone (Bl1 bl1).

| Cross No. 1/ | 16 | 17 | Total |
|---|---|---|---|
| Phase 2/ | C | C | |
| Ms25r ___ Bl1 ___ | 182 | 587 | 769 |
| Ms25r ___ bl1 bl1 | 2 | 56 | 58 |
| ms25r ms25r Bl1 ___ | 11 | 24 | 35 |
| ms25r ms25r bl1 bl1 | 58 | 510 | 568 |
| Total | 253 | 1177 | 1430 |
| Chi-square 3/ | .003 | .479 | |

Recombination value for ms25r vs bl1 = 5.4 ± 0.6%
Heterogeneity chi-square = 0.016 (.90>P>.75)
1/ See Table 6.
2/ C = coupling.
3/ For approximate goodness of fit to 5.4% recombination ratios.

combined estimate was 5.4 ± 0.6% with a heterogeneity chi-square of 0.016 (.90>P>.75). The 3:1 aleurone color ratio for the family grown from cross number 16 indicated that Bl1 segregated normally. The blue and white seeds from cross number 17 were sampled until approximately an equal amount of each was obtained for the linkage study. Therefore, the actual segregation ratio for blue vs white $F_2$ seeds was not known. However, this form of sampling does not affect the recombination value when maximum likelihood equations are used. Both sets of data presented in Table 38 would give similar recombination values, as indicated by the low heterogeneity chi-square.

Since both male sterile genes are fairly close to Bl1, the gene order and respective distances from the centromere were estimated to further evaluate the possibility of employing ms24v and ms25r with bl1 in the male sterile sorting scheme. This was achieved by using all available linkage information relating to ms24v, ms25r, and Bl1 to establish the chromosome map. Whenever possible, genes were positioned by working out from a starting point in steps in order to minimize bias due to double crossing over. An example of this method of mapping is presented in Figure 4a. The interchange points T2-4a, T2-4d, T3-4a, T4-5a, T4-5b, and T4-5c have been established on the short arm; with Bl1 on the long arm (Hanson, 1952; Persson, 1969a; Persson, 1969b). Consequently, recombination values between these breakpoints and ms24v, ms25r, and Bl1 will allow positioning of these genes relative to the centromere

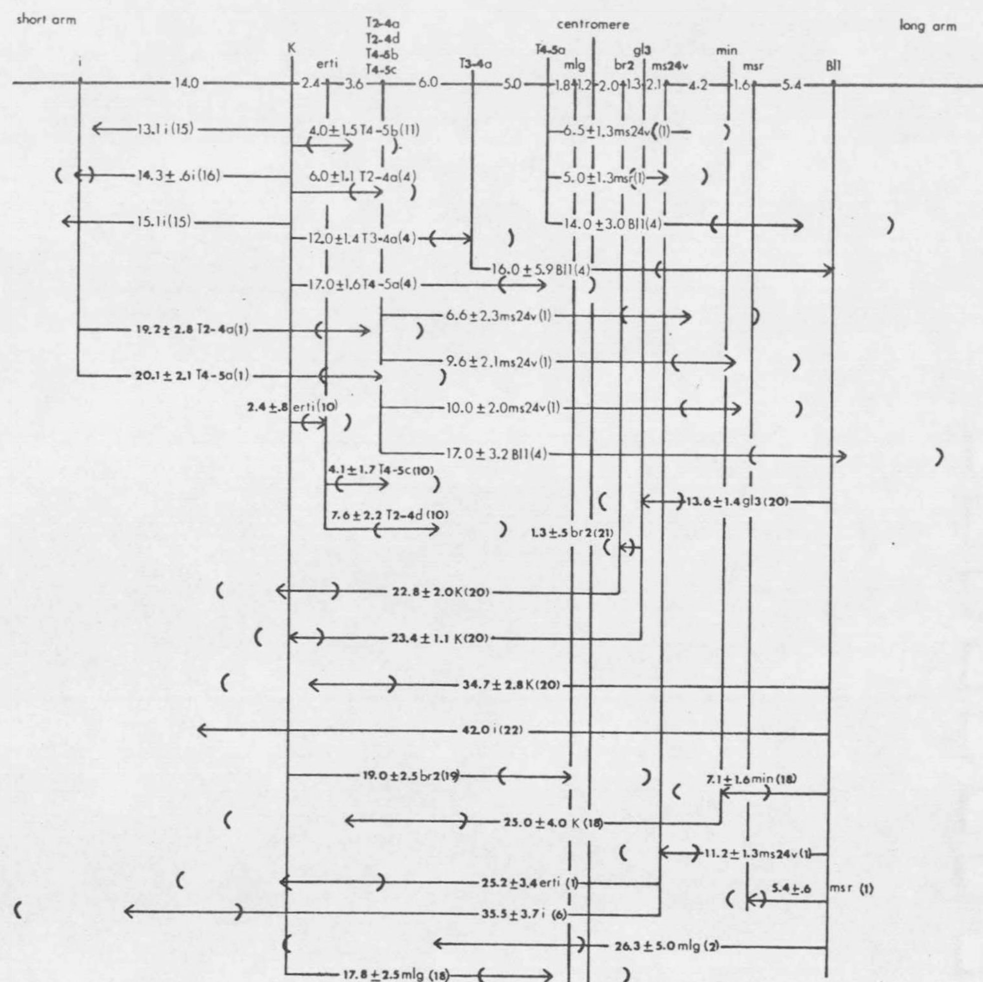


Figure 4a. Positioning ms24v, ms25r 1/, Bl1, and K on chromosome 4.

( ) numbers in parentheses refer to authority, listed in Appendix Table 2.

( ) ± 2 standard errors.

1/ ms25r represented by msr in figure.

(Kramer and Blander, 1961; Ramage, 1963). Presented in Table 39 are data and recombination values for ms24v vs the interchange points T2-4d, T4-5a, T4-5b, and T4-5c. By fitting a line within two standard errors of each estimate, the position of ms24v is tentatively placed 7 units from the centromere on the long arm. Since Bl1 was previously established on the long arm (Hanson, 1952; Persson, 1969a and 1969b), the recombination values for ms24v vs Bl1 and the short arm breakpoints did not allow ms24v to be positioned on the short arm. Similarly, the recombination values for ms25r vs T4-5a (Table 40), and Bl1 vs T4-5a, T2-4a, and T3-4a (Hanson, 1952) allow the positioning of ms25r and Bl1. The gene positions are then shifted slightly within the limits of their standard errors to allow the best overall fit when the recombination values for Bl1 vs ms24v and ms25r are included. With the positions of ms24v, ms25r, and Bl1 established, it is possible to use them as starting points to position other genes on the chromosome. Working from Bl1 for example, the position of the hooded lemma gene K is established stepwise from the recombination values for Bl1 to gl3, gl3 to br2, and br2 to K. Notice that the total distance between Bl1 and K is greater when it is estimated indirectly through gl3 and br2 than when estimated directly from Bl1. The latter distance is likely to be an under estimate since the frequency of double crossovers would be higher. Therefore, working in steps would provide a more accurate estimate of distance between genes that are not closely associated.

Table 39. Segregation ratios for crosses involving fertile and male sterile (Ms24v ms24v) and the interchange points T2-4d, T4-5a, T4-5b, and T4-5c on chromosome 4.

| | Interchange Point 1/ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | T2-4d | | T4-5a | | T4-5b | | T4-5c | |
| | SS | N | SS | N | SS | N | SS | N |
| Ms24v Ms24v | 8 | 27 | 13 | 42 | 6 | 23 | 1 | 19 |
| Ms24v ms24v | 47 | 7 | 136 | 7 | 59 | 11 | 23 | 6 |
| Total | 55 | 34 | 149 | 49 | 65 | 34 | 24 | 25 |
| Chi-square 2/ | 1.179 | 1.744 | 0.923 | 1.877 | 0.004 | 0.136 | 0.294 | 0.011 |

Recombination values for ms24v vs T2-4d, T4-5a, T4-5b, and T4-5c are 9.6 ± 2.1%, 6.5 ± 1.3%, 10.0 ± 2.0%, and 6.6 ± 2.3% respectively.
Heterogeneity chi-squares for crosses with T2-4d, Tr-5a, T4-5b, and T4-5c are 2.819 (.30>P>.20), 2.738 (.30>P>.20), 0.085 (.98>P>.95), and 0.271 (.90>P>.80), respectively.

1/ SS and N designate semi-sterile and normal classes, respectively.

2/ For approximate goodness of fit to the respective recombination values.

Table 40. Segregation ratios for crosses involving fertile and male sterile (Ms25r ms25r) and the interchange point T4-5a on chromosome 4.

| Genotype | Semi-Sterile | Normal |
|---|---|---|
| Ms25r Ms25r | 4 | 41 |
| Ms25r ms25r | 48 | 7 |
| Total | 52 | 48 |
| Chi-Square 1/ | 1.183 | 0.073 |

Recombination value for ms25r vs T4-5a = 5.0 ± 1.3%.
Heterogeneity chi-square = 1.152 (.70>P>.50).
1/ For approximate goodness of fit to 5% recombination ratios.

The map distances for nonintermedium i vs T2-4a and T4-5a from Tables 41 and 42 are included to confirm the report of Hanson (1952) that these interchange points are on the short arm. The remaining map distances presented in Figure 4a support the positioning of K in relation to Bl1. The position of K is then shifted slightly within the limits of 2 of its standard errors to give the best overall fit. In shifting the various gene positions in Figure 4a to provide a good overall fit, it was not necessary to shift any position beyond 3 standard errors of its estimate. However, discrepancies arise when all of the available Bl1 vs K linkage information is used. Figure 4b shows that the various recombination values reported for Bl1 vs K range from 21 to 44 units, with an average of 30.6 units. Since all are direct estimates, one could assume they are not as reliable as those used in Figure 4a where the total distance was estimated in shorter sections. Further more, one could expect such a range of values when different environments, genotypes, population sizes, and methods of data analysis are considered.

It is also possible that there are two positions for K as a result of chromosome inversions. Note for example, the map distances for ms24v vs k and erti in Figure 4b. With the report of a recombination value for K vs erti of 2.4 ± 0.8% (Persson, 1969a), one would expect the recombination values for ms24v vs K and erti to be similar. The recombination values for ms24v vs K and erti were estimated from different

Table 41. Segregation ratios for crosses involving infertile intermedium and nonintermedium (I i) and the interchange point T2-4a on chromosome 4.

| Genotype | Semi-sterile | Normal |
|---|---|---|
| I I | 11 | 22 |
| I i | 29 | 12 |
| Total | 40 | 34 |
| Chi-square 1/ | 1.810 | 2.418 |

Recombination value for I vs T2-4a = 19.2 ± 2.8%.
Heterogeneity chi-square = 4.132 (.20>P>.10).
1/ For approximate goodness of fit to 19.2% recombination ratios.

Table 42. Segregation ratios for crosses involving infertile intermedium and nonintermedium (I i) and the interchange point T4-5a on chromosome 4.

| Genotype | Semi-sterile | Normal |
|---|---|---|
| I I | 29 | 33 |
| I i | 79 | 14 |
| Total | 108 | 47 |
| Chi-square 1/ | 3.670 | 7.144 |

Recombination value for I vs T4-5a = 20.1 ± 2.1%.
Heterogeneity chi-square = 10.758 (.01>P>.005).
1/ For approximate goodness of fit to 20.1% recombination ratios.

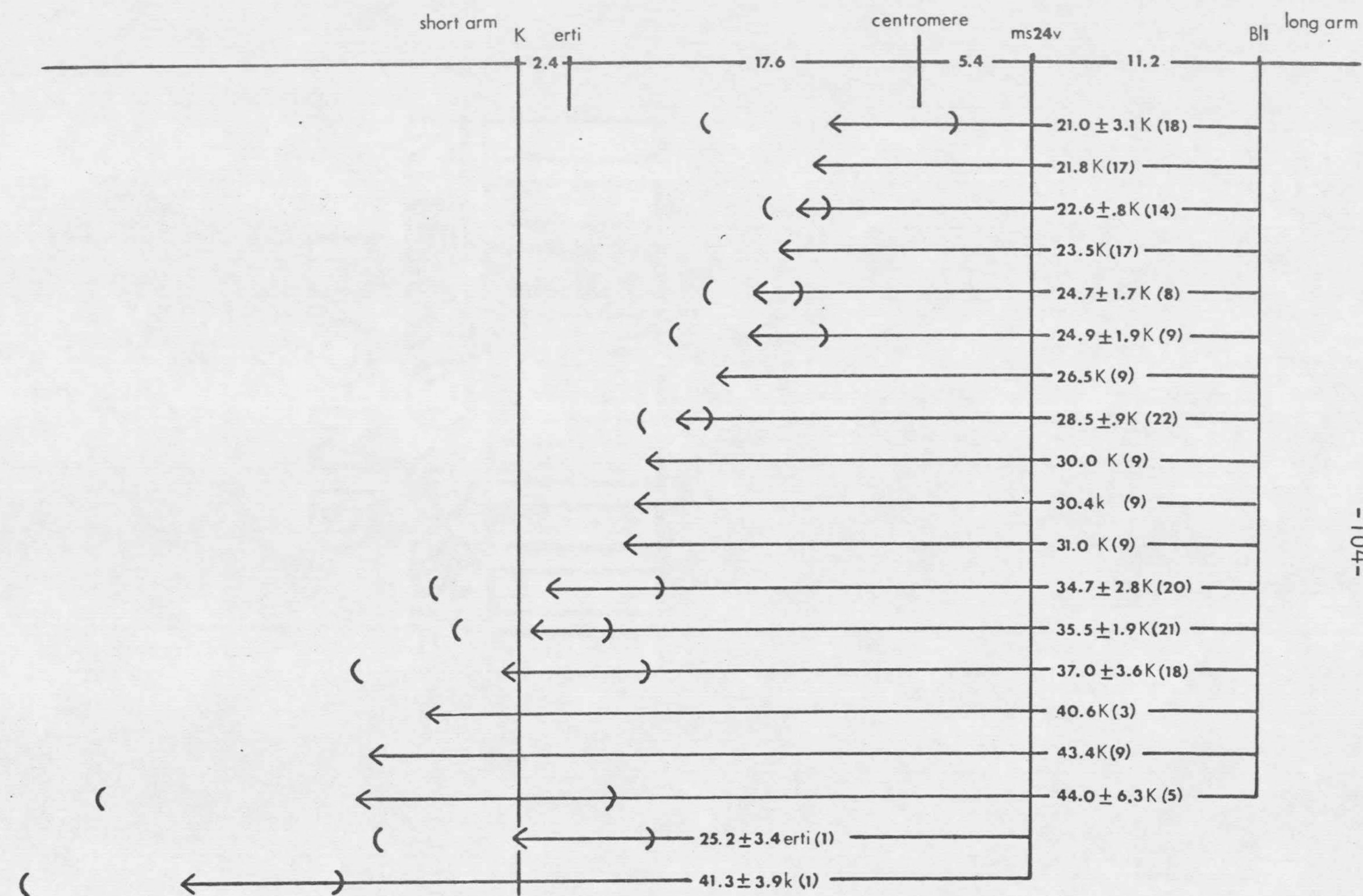


Figure 4b. Recombination values for K vs. Bl1 and ms24v, and ms24v vs. erti.

( ) numbers in parentheses refer to authority, listed in Appendix Table 2.
( ) $\pm$ 2 standard errors.

tests (Tables 43 and 44). It is possible that one of the tests involved a parent carrying an inversion for chromosome 4, although no semi-sterility was detected.

Figure 4c shows some further discrepancies. Note that the limits of ± two standard errors for several of the estimates for K vs gl and Bl1 vs gl must be exceeded in order to place gl between K and Bl1; and similarly for zbc. The larger values for K vs gl and K vs zbc are likely to be closer to the correct value since Tsuchiya (1971a and 1972), in using telotrisomics, established gl (or gl2) and zbc on the long arm. Furthermore, the recombination value for gl vs the T4-5a breakpoint has been reported to be 4.0 ± 0.9% (Ramage, Burnham, and Hagberg, 1961) which would put gl on the long arm approximately 4 units from the centromere and within the range of most of the K vs gl estimates. Thus, the Bl1 vs gl and Bl1 vs zbc estimates are probably incorrect. This is not unlikely since blue aleurone is often easily misclassified, which could result in erroneous linkage values. Furthermore, some workers report recombination values involving blue aleurone where both blue aleurone genes are segregating in the material. Linkage values based on such data can be highly misleading since 9:7 ratios provide almost no information in a linkage trial (Allard, 1956).

Considering the majority of the present chromosome 4 information, the positioning of Bl1, ms25r, ms24v, and K presented in Figure 4a seems to provide the best fit. The maps in Figures 4a, b, and c are summarized

Table 43. Segregation ratios for crosses involving fertile and male sterile (Ms24v ms24v) and normal and compact spike (Erti erti) on chromosome 4.

| Cross No. 1/ | 18 | 19 | 20 | 21 | Total |
|---|---|---|---|---|---|
| Phase 2/ | R | R | R | R | |
| Ms24v Ms24v Erti Erti | 0 | 1 | 2 | 2 | 5 |
| Ms24v Ms24v Erti erti | 8 | 5 | 6 | 6 | 25 |
| Ms24v Ms24v erti erti | 6 | 6 | 8 | 8 | 28 |
| Ms24v ms24v Erti Erti | 6 | 2 | 5 | 5 | 18 |
| Ms24v ms24v Erti erti | 22 | 22 | 23 | 21 | 88 |
| Ms24v ms24v erti erti | 5 | 12 | 4 | 3 | 24 |
| Total | 47 | 48 | 48 | 45 | 188 |
| Chi-square 3/ | .017 | .154 | .056 | .041 | |

Recombination value for ms24v vs erti = 25.2 ± 3.4%.
Heterogeneity chi-square = 0.262 (.975>P>.95).
1/ See Table 6.
2/ R = repulsion.
3/ For approximate goodness of fit to 25.2% recombination ratios.

Table 44. Segregation ratios for crosses involving fertile and male sterile (Ms24v ms24v) and hooded and non-hooded lemma (K k) on chromosome 4.

| Cross No. 1/ | 22 | 23 | 24 | Total |
|---|---|---|---|---|
| Phase 2/ | C | C | C | |
| Ms24v ___ K ___ | 62 | 53 | 53 | 168 |
| Ms24v ___ k k | 14 | 16 | 22 | 52 |
| ms24v ms24v K ___ | 20 | 21 | 14 | 55 |
| ms24v ms24v k k | 6 | 8 | 16 | 30 |
| Total | 102 | 98 | 105 | 305 |
| Chi-square 3/ | .403 | .735 | 1.169 | |

Recombination value for ms24v vs K = 41.3 ± 3.9%.
Heterogeneity chi-square = 2.259 (.5>P>.25).
1/ See Table 6.
2/ C = coupling.
3/ For approximate goodness of fit to 41.3% recombination ratios.

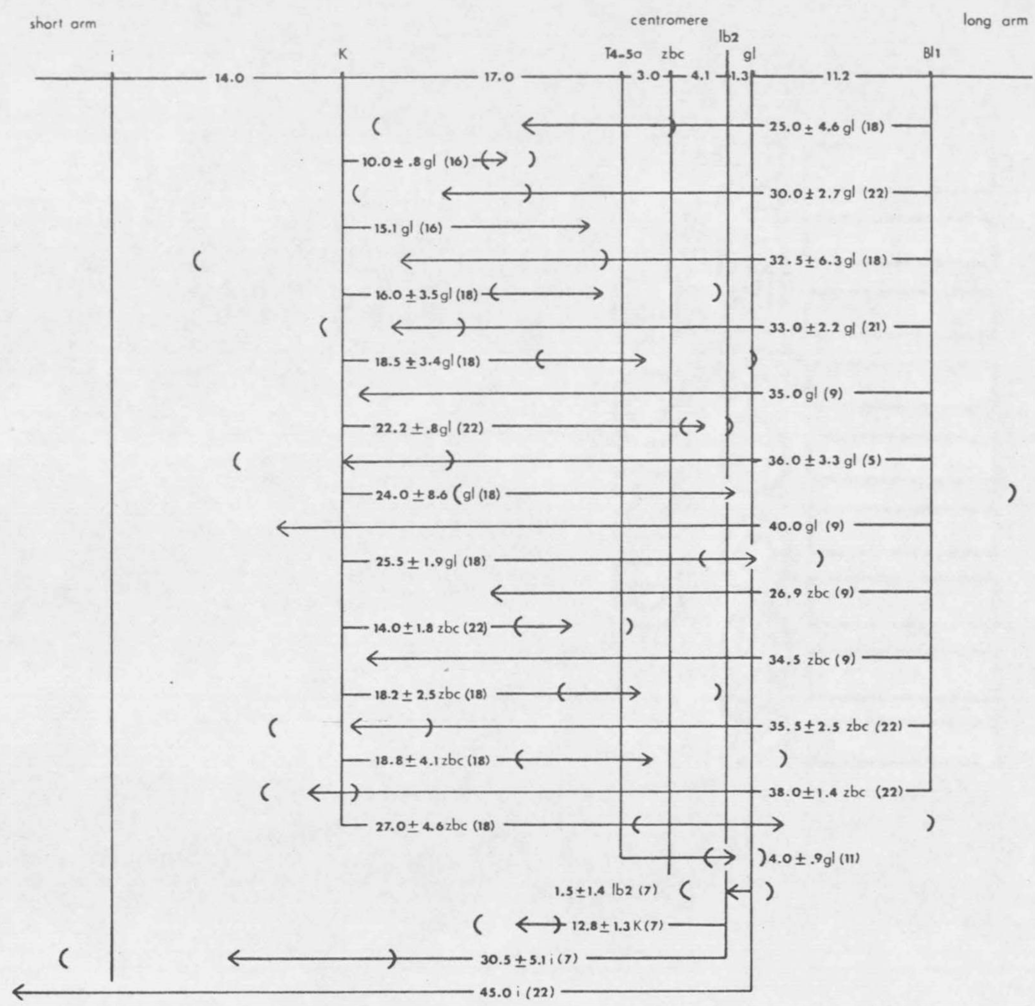


Figure 4c. Recombination values for gl and zbc vs. K and Bl1.
( ) numbers in parentheses refer to authority, listed in Appendix Table 2.
( ) ± 2 standard errors.

in Figure 5. Haus (1972), in using most of the available chromosome 4 linkage information, reported an up to date gene order for chromosome 4 which is in agreement with the summary map presented in Figure 5. In addition, Ramage and Burnham (1962) stated that the centromere of chromosome 4 was in the general region of gl, which is also in agreement with the map presented in Figure 5.

Although ms24v is not tightly linked to Bl1, the proposed positions of the two genes, along with ms25r, are close enough to the centromere to allow the use of an interchange point on or distal to Bl1. The interchange point would act to suppress or eliminate entirely the occurrence of crossover gametes (Hanson and Kramer, 1949). Since the homolog carrying the recessive alleles ms24v, ms25r, and bl1 does not carry the interchange point, only normal chromosomes would be present in the male sterile plants and ultimately in the commercial hybrid.

## Hybrid System Proposals

From the foregoing study, it can be concluded that the linkage intensities for bl2 vs ms10 and ms14 on chromosome 1 and possibly bl1 vs ms24v and ms25r on chromosome 4 are high enough to employ blue aleurone as a preflowering selection tool for male sterility in hybrid seed production. Since production of hybrid seed for commercial use would be on a large scale, it is necessary to employ a system requiring a minimal amount of seed sorting. If, for example, Montana's 704,000 hectares of

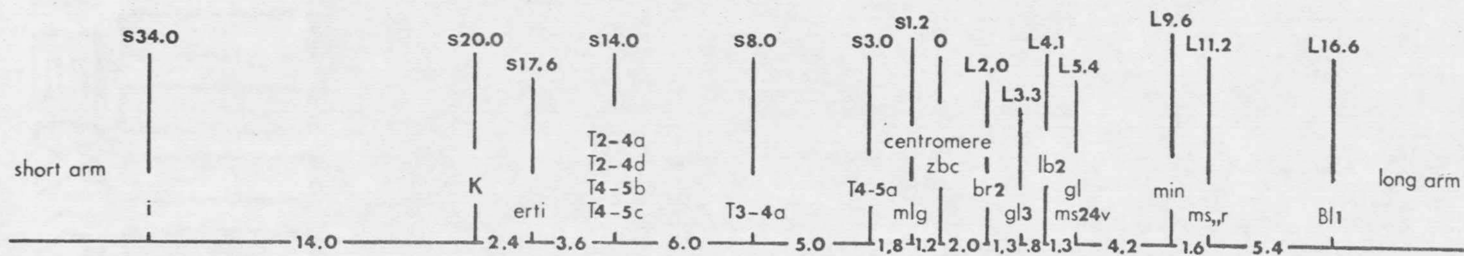


Figure 5. Proposed linkage map of chromosome 4.

irrigated and dryland barley production fields were seeded to hybrid barley, some 40,188,544 kg of hybrid seed would be needed; which would require a hybrid seed production field of about 9960 hectares, assuming a 4036 kg/ha (75 bu/a) yield. This in turn would require approximately 536,700 kg of white male sterile seed to be sorted if the simplest and least efficient system was used. With more efficient systems, the amount of white male sterile seeds needed to be sorted would be reduced to only about 7170 kg.

The very simplest self-maintaining stock would involve the genotype configuration

$$\frac{Bl1}{Bl1} \quad \frac{en\text{-}Bl \quad n \quad Ms14 \quad ms10 \quad bl2}{en\text{-}Bl \quad n \quad ms14 \quad Ms10 \quad Bl2} .$$

In this case en-Bl in the homozygous condition allows only dark blue and white seeds to be produced; therefore, minimizing misclassifications in color sorting. The presence of the naked caryopsis factor n further enhances color sorting. Upon selfing, the stock would produce $F_2$ seed in the proportion of 3 dark blue to 1 white; and when planted back in isolation would be self maintaining regardless of the crossed seed set on the male steriles in the population. When male sterile plants for hybrid seed production are needed, the white seed would be sorted out and planted to produce a pure population of male sterile plants. Since only 25 percent of the seed would be white, a considerable amount of blue seeds would have to be separated out in obtaining the white male sterile seeds. This could be improved by going through an additional

cycle where the white male steriles (Ms14 ms10 b12/Ms14 ms10 b12) are pollinated by the heterozygous stock (Ms14 ms10 b12/ms14 Ms10 B12). This would result in a blue-white segregation ratio of 1:1 for the final sorting process. However, in this system, all sorted seed is used directly for the hybrid seed production field; making the system very inefficient.

A much more efficient system involves the use of two separate stocks where initially, the male steriles are produced by the self-maintaining stock

$$\frac{\text{Bl1} \quad \text{en-Bl n ms14 ms10 b12 } A_{c2}}{\text{Bl1} \quad \text{en-Bl n Ms14 Ms10 B12 } a_{c2}} .$$

The presence of the albino seedling mutant $a_{c2}$ eliminates the homozygous blue, male fertile genotypes from the stock maintenance process. The white male steriles produced from this stock are crossed with the self-maintaining balanced male sterile stock

$$\frac{\text{Bl1} \quad \text{N} \quad \text{Ms14 ms10 b12 } A_{c2}}{\text{Bl1} \quad \text{N} \quad \text{ms14 Ms10 b12 } A_{c2}} ,$$

resulting only in the male sterile genotypes n ms14 ms10 b12/N Ms14 ms10 b12 and n ms14 ms10 b12/N ms14 Ms10 b12. Thus, the required 536,700 kg of male sterile seeds can be obtained by initially sorting a total of only 7170 kg of white male sterile seeds. The covered caryopsis factor is introduced in the second cycle of male sterile seed production in order that the commercial hybrid seed will be covered. If a hulless commercial hybrid is desired, the naked caryopsis factor would

be incorporated into all seed stocks used in the hybrid seed production scheme.

One difficulty with the foregoing system is the possibility of crossovers occurring between ms10, ms14, bl2, and $A_{c2}$ in the initial stock, and between ms10 and ms14 in the second stock. Crossing over in the initial stock can be suppressed or eliminated entirely by incorporating a translocation breakpoint on or distal to the $a_{c2}$ locus on the en-Bl n Ms14 Ms10 Bl2 $a_{c2}$ homolog (Hanson and Kramer, 1949). This would result in normal diploid male steriles since the en-Bl n ms14 ms10 bl2 $A_{c2}$ homolog does not carry the breakpoint. The difficulty with setting up this scheme however, is finding a suitable breakpoint in the necessary genotype. A good portion of the world collection of translocation stocks carry the dominant alleles Ms10, Ms14, Bl2, and $A_{c2}$. Although this is advantageous for the Ms14, Ms10, and Bl2 arrangement, it poses a problem for incorporating $a_{c2}$ into the interstitial segment. This could be very difficult; and therefore, it may be easier to induce the necessary breakpoint in a Ms10 Ms14 Bl2 $a_{c2}$ genotype.

Crossing over in the Ms14 ms10/ms14 Ms10 stock must be suppressed by some other means than translocation breakpoints since both gametes are ultimately present in the commercial hybrid. One method would be to balance a lethal or male sterile gene on each side of ms10 and ms14. It is possible that ms22e and ms,,w may be used for at least one or both of the necessary genes, provided they are found to be closely associated

with ms10 and ms14. Assume for example, the genotype configuration

ms22e Ms14 ms10 Ms,,w
Ms22e ms14 Ms10 ms,,w .

Any crossover gamete would require fertilization with another specific type of single or multiple crossover gamete in order to allow complementation of all male sterile alleles with their respective male fertile alleles. The probability of this occurring would be considerably lower than for the case where only two male sterile genes were employed. For the same reason, the occurrence of fertiles would be considerably decreased in the male sterile population of the hybrid seed production field.

Another hybrid system that could be used would involve the use of a balanced tertiary trisomic which would not require the albino lethal $a_{c2}$ for stock maintenance. Thus, the problem of inserting $a_{c2}$ into the interstitial segment would no longer be of concern. The system is similar to the previous one except the genotype configuration of the initial male sterile producing stock for chromosome 1 is

B11 en-B1 N(ms22e)ms14ms10 b12
B11 en-B1 n(ms22e)ms14ms10 b12 .
en-B1 n(Ms22e)Ms14Ms10 $B12_{xxxxx}$

Upon selfing, the trisomic produces $F_2$ seed in the proportion of 0.70 white male sterile normal diploids to 0.30 blue fertile balanced tertiary trisomics, with less than one percent white primary trisomics (Ramage, 1965). Since the efficiency of producing white male sterile

seeds is increased from 0.25 to 0.70, the total quantity of seeds necessary to pass through the sorting machine is reduced from 28,680 kg to 10,242 kg for the Montana hybrid seed example. Although the success of this system depends much on the vigor of the balanced tertiary trisomic, the problem may not be of any great concern if the trisomics were grown in a pure stand without competition from diploids. Assuming that the trisomics could be managed to yield 2691 kg/ha (50 bu/a), approximately 5.3 hectares of trisomics would have to be grown to produce the 2170 kg of first cycle male sterile seed. As before, the second cycle male sterile seed would be produced by crossing the white male steriles from the trisomics with the balanced male sterile stock

N    ms22e Ms14 ms10 Ms,,w .
N    Ms22e ms14 Ms10 ms,,w

Although the two previous systems discussed are quite efficient in production of male sterile plants, the development of the initial stocks could be a cumbersome task. The following system has the advantage of being very simple to set up and yet maintains the efficiency of male sterile production. The system involves ms10 as the only male sterile gene and requires that only one stock be developed and maintained. The stock is a balanced tertiary trisomic of the genotype configuration

B11    en-B1 n ms10 b12
B11    en-B1 n ms10 b12 ,
       en-B1 n Ms10 $B12_{xxxx}$

and serves to produce the first cycle male sterile seed along with pollen carrying the male sterile gene for second cycle male sterile seed production. Some progress has been made toward setting up this system. The genotype en-Bl n ms10 bl2/en-Bl n ms10 bl2 is available from the material that provided the data in Table 38. In addition, the alleles en-Bl, n, Ms10, and Bl2 are present in a large proportion of the world collection of interchange points as a result of backcrossing the interchange points to Ubamer (Weibe, pers. comm.). Therefore, it is possible that the homolog

$$\underline{\text{en-Bl n Ms10 Bl2}}_{xxxx}$$

may also be available. If so, the system could be set up immediately and backcrossed into the desired parent. From linkage values reported in the literature, the most likely interchange point to bracket Bl2 appears to be Tl-5f. Persson (1969a and 1969b) reported recombination values for Tl-5f vs $a_{c2}$ and n to be 1.7 ± 0.72% and 11.9 ± 2.8%, respectively. This would place the Tl-5f interchange point distal to Bl2 on the short arm since both $a_{c2}$ and n are approximately 8 recombination units from the centromere, with $a_{c2}$ on the short arm and n on the long (Figure 1). In addition, Tl-5f is carried in the variety Bonus (Bl2 Bl2 bl1 bl1) which means that the dominant allele Bl2 would already be present in the interstitial segment. Therefore, if further testing verifies that Tl-5f brackets Bl2 and can further produce a reasonably good trisomic, it would appear that the latter system could be established in

relatively short time. However, obtaining adequate vigor in the trisomic may be a difficult task. Tsuchiya (1971b) points out that telotrisomics for the long arm of chromosome 1 are very weak, but telotrisomics for the short arm are similar in vigor to the diploids. The balanced tertiary stock used in the hybrid system would carry an extra long arm for chromosome 1, which would result in a weak plant. Selection of vigorous plants would tend to result in selection for telo-trisomic plants, where the long arm of the translocated chromosome 1 is eliminated through fragmentation. Tsuchiya (1971c) reported that telocentric chromosomes are transmitted through the pollen. This would result in a breakdown of the hybrid system, as male gametes carrying B12 and Ms10 would contaminate the male sterile seed production process.

An alternate approach would be to use a diploid system involving a translocated chromosome, where the genotype configuration of the initial male sterile seed producing stock would be

B11 ________ b12 ms10 ms14 .

B11 xxxxxxxB12 Ms10 Ms14

The homozygous blue types would be eliminated periodically by roughing the fertile types from the blue population. The semi-sterile types would serve to perpetuate the male sterile seed producing stock.

The foregoing systems could similarly be employed with ms24v, ms25r, and B11 on chromosome 4. In addition, a balanced B11x b11x B13y b13y Ms24v ms24v Ms25r ms25r system could possibly be used. If B11x is

allelic to Bl1 and closely linked to Bl3y as indicated in this study, one could assume the linkage intensities of ms24v and ms25r with bl1x and bl3y to be the same as with bl1. The self-maintaining stock

$$\frac{ms24v\ Ms25r\ Bl1x\ bl3y}{Ms24v\ ms25r\ bl1x\ Bl3y}$$

would provide white aleurone male steriles at a frequency of 0.5. No additional crossing cycles would be necessary to increase the male sterile seed. One difficulty with this system is that an interchange point cannot be used to suppress crossing over between the male sterile and blue aleurone genes. Since the homologs have an equal chance of ending up in the male sterile plant, an interchange point on one of the homologs would result in half of the hybrids being heterozygous for the interchange point and semi-sterile. Another problem with any chromosome 4 system involving ms25r is the partial fertility expressed by ms25r under certain conditions (Hockett and Eslick, 1970). This would probably limit chromosome 4 systems to the use of only ms24v if environments allowing an expression of complete sterility by ms25r could not be feasibly used.

In summary, bl2, ms10, and ms14 on chromosome 1 are closely enough associated to employ in hybrid seed production schemes. Furthermore, since n is on the opposite arm from Bl2, and since en-Bl is not closely associated with ms10 and Bl2, the incorporation of n and en-Bl into a known translocation stock could be achieved quite easily. Of the systems proposed, the diploid bl2 ms10 ms14/Bl2 Ms10 Ms14 with an inter-

change point bracketing Bl2 appears to be the most reliable and easy to maintain. The possibilities for obtaining the appropriate interchange point in the desired genotype are fairly good, since the world collection of chromosome translocations are in the fourth back cross to the genotype en-Bl en-Bl n n Bl2 Bl2 Bl1 Bl1. Of these, T1-5f very likely has the desired interchange point position because of its close linkage to ac2 (Persson, 1969a and 1969b). Although the balanced tertiary chromosome 1 trisomic systems are very efficient in male sterile seed production, the vigor and maintenance of the trisomic plants are questionable on the basis of Tsuchiya's (1971b and 1971c) report.

Bl1, ms24v, and ms25r on chromosome 4 could be used in a similar fashion as the chromosome 1 systems. However, the self fertility encountered with ms25r may require chromosome 4 systems to be abandoned.

Regardless of the systems used, the presence of the recessive blue aleurone enhancer in the homozygous condition would be mandatory in stocks producing blue and white seed color segregations in order to eliminate the occurrence of normal blue color intensities. The recessive naked caryopsis gene n would also be employed to enhance color sorting.

## SUMMARY AND CONCLUSION

Linkage intensities for the two complementary blue aleurone genes, Bl1 and Bl2, with three male sterile genes and two marker genes were estimated in order to determine the feasibility of employing blue aleurone as a preflowering selection device for male sterility in hybrid barley systems.

Blue aleurone, Bl2, male steriles, ms10 and ms14, short awn, lk2, and naked caryopsis, n, were previously assigned to chromosome 1, with ms10 and ms14 very closely linked and near the centromere. This study has shown the recombination values for Bl2 vs ms10, n, and lk2 to be 3.9 ± 0.4%, 12.1 ± 1.5%, and 21.5 ± 1.8% respectively. The present study confirms the previously reported associations between ms10, lk2, and n, and suggests the gene order to be lk2 - n - ms10 - ms14 - bl2 - $a_{c2}$, where the association of $a_{c2}$ with ms10, ms14, n, and lk2 was previously reported. From the close association between bl2 and ms10, it was concluded that blue aleurone could be employed for male sterile selection by coupling the white allele, bl2, with the male sterile alleles, ms10, and ms14, in a heterozygous stock.

Blue aleurone, Bl1 and male steriles, ms24v and ms25r were previously assigned to chromosome 4. In the present study, the recombination values for Bl1 vs ms24v and ms25r were estimated as 11.2 ± 1.3% and 5.4 ± 0.6% respectively. Previously reported recombination values for Bl1, ms24v, and ms25r vs various marker genes and interchange points were used to estimate the positions of Bl1, ms24v, and ms25r relative to the

centromere. The gene order was suggested as ms24v - ms25r - Bl1, with distances from the centromere of 5.4, 11.2, and 16.6 units, respectively, on the long arm. From previous reports that ms25r exhibits some degree of self fertility, it was suggested that the use of chromosome 4 systems should not include ms25r.

Various hybrid systems were discussed. It was suggested that the linkage intensities among the blue aleurone and male sterile genes be increased by utilizing chromosome interchange points on the homologs carrying the dominant blue aleurone and male fertile alleles. The use of balanced tertiary trisomics was not advised due to previous reports that fragmented chromosomes occurring in trisomics can be transmitted through the pollen. This would result in the occurrence of fertile plants in the male sterile population.

In this study, the inheritance of the environmentally insensitive, very dark shade of blue of the variety Ubamer (C.I.12167) was determined. The character was conditioned by a single recessive color enhancer gene in the presence of Bl1 and Bl2, and was estimated to be 29.5 ± 3.0 recombination units from ms10 on chromosome 1. The symbolization of the enhancer was proposed as en-Bl. It was concluded that the incorporation of en-Bl and n into hybrid system seed stocks was necessary for accurate color sorting.

Most of the world collection of spring habit male sterile stocks were classified for blue gene complement to provide information for

future investigations regarding the associations among various male sterile and blue aleurone genes.

Evidence was presented for at least one, and possibly two additional blue aleurone genes, Blx and Bly. Limited data suggested Blx to be allelic to Bl1, with Bly closely linked. It was concluded that further testing was necessary to verify the allelism of Blx to Bl1.

Monohybrid crosses for aleurone color exhibited both 3:1 and 2:1 ratios in the $F_2$. From the results of testcrosses and progeny tests of families exhibiting 2:1 ratios, the association of a gamete selection factor with Bl2 was suggested. Dihybrid crosses, Bl1 bl1 Bl2 bl2, exhibited both 9:7 and 27:37 ratios in the $F_2$. Although the possibility of three segregating genes was not ruled out, it was suggested that the 27:37 ratios could have occurred as a result of the association of gamete selection factors with one or both Bl1 and Bl2. It was also suggested that certain dosages of the blue aleurone alleles would not express blue color under environmental stress, and would, therefore, result in an increased frequency of white seeds in the $F_2$.

## LITERATURE CITED


Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:253-279.

Bate-Smith, E. C. 1954. Leuco-anthocyanins. 1. Detection and identification of anthocyanins from leuco-anthocyanins in plant tissue. Biochem. J. 58:122-125.

Briggs, F. N., and E. H. Stanford. 1943. Linkage relations of the Goldfoil factor for resistance to mildew in barley. J. Agr. Res. 66:1-5.

Buckley, G. F. H. 1930. Inheritance in barley with special reference to the color of caryopsis and lemma. Sci. Agr. 10:460-492.

Cottingham, C. 1960. Inheritance of resistance to covered smut in several hybrids of barley. Dissertation Abstr. 20:3484.

Dubbs, A. L. 1958. Natural selection for blue and white aleurone color in barley. M. S. Thesis, Montana State University. pp. 42 typed.

Eslick, R. F. 1970. Balanced male sterile and dominant preflowering selective genes for use in hybrid seed production. Barley Genet. II. Second Int. Barley Genet. Symp. 1970. II:292-296.

______________, E. A. Hockett, and G. D. Kushnak. 1972. Recombination of four genes on chromosome 1. Barley Genet. Newsletter 2:123-126.

______________, M. M. Rahman, and C. W. Crowell. 1971. Gene ordering near the centromere of chromosome 1. Barley Genet. Newsletter 1:20-21.

Gortner, R. A., and W. A. Gortner. 1949. Outlines of biochemistry. 3rd ed. John Wiley and Sons, Inc., New York, N. Y.

Gudkov, A. N. 1940. Determination of color of barley seeds by chemical methods. Breeding and Seed Growing 6:32 (Russian). Plant Breed. Abstr. Vol XI, No. 3. 707.

Hanson, W. D. 1952. An interpretation of the observed amount of recombination in interchange heterozygotes in barley. Genetics 37:90-100.

______________, and H. H. Kramer. 1949. The genetic analysis of two chromosome interchanges in barley from $F_2$ data. Genetics 34:687-700.

______________, and ______________. 1950. The determination of linkage intensities from $F_2$ and $F_3$ genetic data involving chromosomal interchanges in barley. Genetics 35:559-569.

Harlan, H. 1914. Some distinctions in our cultivated barleys with reference to their use in plant breeding. USDA Bul. 137:1-38. (cited by Wiggans, R. G. 1921. A classification of the cultivated varieties of barley. Cornell Univ. Agr. Exp. Sta., Mem. 46:365-456.)

Haus, T. E. 1972. Coordinators report: chromosome 4. Barley Genet. Newsletter 2:132.

______________, and T. Tsuchiya. 1972. Allelic relationships among glossy seedling mutants. Barley Genet. Newsletter 2:79-80.

Hockett, E. A. 1972. Coordinators report on the genetic male sterile collection. Barley Genet. Newsletter 2:139-144.

______________. 1974. Coordinators report on the genetic male sterile collection. Barley Genet. Newsletter (in press).

______________, and R. F. Eslick. 1970. Genetic male sterile genes useful in hybrid barley production. Barley Genet. II. Proc. Second Int. Barley Genet. Symp. 1970. II:298-307.

______________, ______________, D. A. Reid, and G. A. Wiebe. 1968. Genetic male sterility in barley. II. Available spring and winter stocks. Crop Sci. 8:754-755.

Hurd, E. A. 1959. Inheritance of blue kernel color in wheat. Can. J. Plant Sci. 39:1-8.

Immer, F. R., and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Jain, S. K. 1970. Gene pools, variation and selection. Barley Genet. II. Proc. Second Int. Barley Genet. Symp. 1970. II:422-429.

Jarvi, A. J., and R. F. Eslick. 1967. A male sterile gene on chromosome 4. Barley Newsletter 11:17.

Kajanus, B., and S. O. Berg. 1924. Kreuzungstudien an Gerste. Hereditas 5:287-296. (cited by Smith, L. 1951. Cytology and genetics of barley. Bot. Rev. 17:135).

Kasha, K. F. and G. W. R. Walker. 1960. Several recent barley mutants and their linkages. Can. J. Genet. Cytol. 2:397-415.

Kattermann, G. 1932. Farbzenien bei Weizenkreuzungen und das erhliche verhalten blaugfärbter aleuronschicht bei der verwendeten neuartigen weizenrasse im allgemeinen. A. für Züchtung. Reihe A. Pflanzenzücht 17:413-446. (cited by Hurd, E. A. 1959. Inheritance of blue kernel color in wheat. Can. J. Plant Sci. 39:1-8).

Knott, D. R. 1958. The inheritance in wheat of a blue endosperm color derived from Agropyron elongatum. Can. J. Bot. 36:571-574.

Kondo, M., and Y. Kasahara. 1939. Variety distinction of wheat and barley by means of phenol coloration. Crop Sci. Soc. Japan, Proc. 11:230-252. (cited by Smith, L. 1951. Cytology and genetics of barley. Bot. Rev. 17:135).

_____________, and R. Takahashi. 1938. Feststellung der sortenechtheit des saatgutes des weizens durch phenolfärbung. Ber. Ohara Inst. Landw. Fors. 8:211-221. (cited by Smith, L. 1951. Cytology and genetics of barley. Bot. Rev. 17:135).

Kramer, H. H., and B. A. S. Blander. 1961. Orienting linkage maps of chromosomes of barley. Crop Sci. 1:339-342.

Loegering, W. Q., and E. R. Sears. 1963. Distorted inheritance of stem-rust resistance of Timstein wheat caused by a pollen killing gene. Can. J. Genet. Cytol. 5:65-72.

Malting Barley Improvement Association. 1957. Titan-supplement to the Barley Variety Dictionary. MBIA, Milwaukee, Wisc.

Mullick, D. B., D. G. Faris, V. C. Brink, and R. M. Acheson. 1958. Anthocyanins and anthocynidins of the barley pericarp and aleurone tissues. Can. J. Plant Sci. 38:445-456.

Myler, J. L., and E. H. Stanford. 1942. Color inheritance in barley. J. Amer. Soc. Agron. 34:427-436.

Nilan, R. A. 1964. Cytology and genetics of barley, 1951-1962. Monog. Sup. 3. Res. Studies. pp. 278. Wash. State Univ., Pullman.

Persson, G. 1969a. An attempt to find suitable genetic markers for dense ear loci in barley. I. Hereditas 63:25-96.

______________. 1969b. An attempt to find suitable genetic markers for dense ear loci in barley. II. Hereditas 63:1-28.

Rahman, M. M. 1973. Balanced male sterile-lethal systems for hybrid barley production. Ph. D. Thesis Montana State Univ. pp. 90 typed.

Ramage, R. T. 1963. Chromosome aberrations and their use in genetics and breeding. Barley Genet. I. Proc. First Int. Barley Genet. Symp. 1963. I:99-115.

______________. 1965. Balanced tertiary trisomics for use in hybrid seed production. Crop Sci. 5:177-178.

______________. 1972. Report from the barley genetics committee of the American Barley Research Workers' conference. Barley Genet. Newsletter 2:11-15.

______________, and C. R. Burnham. 1962. Centromere position in the linkage maps in barley. Barley Newsletter 6:51.

______________, ______________, and A. Hagberg. 1961. A summary of translocation studies in barley. Crop Sci. 1:277-279.

Reid, D. A., and G. A. Wiebe. 1968. Taxonomy, botany, classification, and world collection in Barley: origin, botany, culture, winter-hardiness, genetics, utilization, pests. Agr. Handbook No. 338. USDA, ARS. pp. 75-76.

Robertson, D. W. 1963. Summary of linkage studies in barley, 1959-1963. Fifth Barley Improvement Abstracts.

______________. 1966. Summary of linkage studies in barley, 1963-1966. Barley Newsletter 10:50-65.

______________. 1970. Recent information of linkage and chromosome mapping. Barley Genet. Symp. II:220-242.

______________, G. W. Deming, and D. Koonce. 1932. Inheritance in barley. J. Agr. Res. 44:445-466.

______________, G. A. Wiebe, and F. R. Immer. 1941. A summary of linkage studies in barley. J. Amer. Soc. Agron. 33:47-64.

______________, ______________, and R. G. Shands. 1947. A summary of linkage studies in barley: supplement I, 1940-1946. J. Amer. Soc. Agron. 39:464-473.

______________, ______________, and ______________. 1955. A summary of linkage studies in barley: supplement II, 1947-1953. Agron. J. 47: 418-425.

______________, ______________, ______________, and A. Hagberg. 1965. A summary of linkage studies in cultivated barley, Hordeum species: supplement III, 1954-1963. Crop Sci. 5:33-43.

Robinson, G. M., and R. Robinson. 1933. A survey of anthocyanins. Biochem. J. 27:206.

Robinson, R. 1936. Formation of anthocyanin in plants. Nature 137:172.

Royal Horticultural Society. R. H. S. Colour Chart of the Royal Horticultural Society, London.

Sawicki, J. 1950. Studies on the structure of the aleurone layer in varieties of the cultivated barley _Hordeum sativum_ Jess. Bul. Acad. Polon. Sci. Lettres. Ser. B. 1:101-148. (cited by Nilan, R. A. 1964. Cytology and genetics of barley, 1951-1962. Monog. Sup. 3. Res. Studies. Wash. State Univ., Pullman. p. 134).

Scott-Moncrieff, R. 1924. The nature and inheritance of flower color. Sci. Hort. 6:124-132.

Smith, L. 1951. Cytology and genetics of barley. Botan. Rev. 17:1-51.

Tabata, M. 1957. Ga factor in linkage group III. Barley Newsletter 1:48-49.

______________. 1961. Studies of a gametophytic factor in barley. Plant Breeding Abstr. 32:3337 (1962).

Takahashi, R., J. Hayashi, and I. Moriya. 1961. Linkage studies. Barley Newsletter 5:42.

______________, ______________, and ______________. 1971. Linkage studies in barley. Barley Genet. Newsletter. 1:51-58.

Tsuchiya, T. 1971a. Telotrisomic analysis of 14 marker genes on chromosome 1, 2, and 4. Barley Genet. Newsletter 1:61-62.

______________. 1971b. Screening for primary trisomics in the progenies of telotrisomics. Barley Genet. Newsletter 1:73.

______________. 1971c. Male transmission of telocentric chromosomes in four telotrisomics. Barley Genet. Newsletter 1:60-61.

______________. 1972. Cytogenetics of telotrisomics in barley. Barley Genet. Newsletter 2:93-98.

Tuleen, N. A., L. A. Snyder, R. S. Caldecott, and V. S. Hiatt. 1968. Genetic investigation of suppressors of a chloroplast mutation in _Hordeum vulgare_. Genetics 59:45-55.

Ward, D. J. 1962. Some evolutionary aspects of certain morphological characters in a world collection of barleys. USDA Tech. Bul. No. 1276. pp. 112.

Wells, S. A. 1958. Inheritance of reaction to _Ustilago hordei_ (Pers.) Lagerh. in cultivated barley. Can. J. Plant Sci. 38:45-60.

Wheatley, G. W. 1956. Linkage relationships in group IV in barley. Plant Breeding Abstr. 26:2329.

Wiebe, G. A. 1960. A proposal for hybrid barley. Agron. J. 52:181-182.

______________. 1972. Blue aleurone caused by complementary genes in very close translinkage. Barley Genet. Newsletter 2:109.

______________, and D. A. Reid. 1961. Classification of barley varieties grown in the U. S. and Canada in 1958. USDA Tech. Bul. No. 1224. pp. 234.

______________, and R. T. Ramage. 1970. Hybrid barley. Barley Genet. II. Proc. Second Int. Barley Genet. Symp. 1970. II:287-291.

Wolf, M. J., H. C. Cutler, M. S. Zuber, and U. Khoo. 1972. Maize with multilayer aleurone of high protein content. Crop Sci. 12:440-442.

Woodward, R. W. 1941. Inheritance of a melanin-like pigment in the glumes and caryopsis of barley. J. Agr. Res. 63:21-28.

______________. 1942. Linkage relationships between the allelomorphic series B, $B^{mb}$, $B^{g}$, and _At at_ factors in barley. J. Amer. Soc. Agron. 34:659-661.

______________. 1957. Linkages in barley. Agron. J. 49:28-32.

# APPENDIX

Appendix Table 1. Alphabetical list of barley genes mentioned in the text, tables, and figures. 1/

| Gene Symbol | Character | Chromosome | Authority |
|---|---|---|---|
| Ac2 ac2 | Normal vs. albino seedling | 1 | Ramage, Burnham, and Hagberg (1961) |
| Bl1 bl1 | Blue vs. white aleurone | 4 | Buckley (1930) |
| Bl2 bl2 | Blue vs. white aleurone | 1 | Myler and Stanford (1942) |
| Blx blx (Bl1x bl1x) | Blue vs. white aleurone | 4 | Wiebe (1972) |
| Bly bly (Bl3y bl3y) | Blue vs. white aleurone | 4 | Wiebe (1972) |
| Br2 br2 | Normal vs. brachytic spike | 4 | Takahashi, Hayashi, and Moriya (1971) |
| En-Bl en-Bl 1/ | Normal vs. dark blue aleurone | 1 | |
| Erti27 erti27 | Normal vs. erectoides spike | 4 | Persson (1969a) |
| Gsf gsf 1/ | Gamete selection factor (hypothetical) | 1 | |
| Ga ga | Gametophytic factor | 1 | Tabata (1957, 1961) |
| Gl gl (Gl2 gl2) | Normal vs. glossy seedling | 4 | Haus and Tsuchiya (1972) |
| I i | Fertile vs. infertile intermedium | 4 | Woodward (1957) |
| K k | Hooded vs. non-hooded lemma | 4 | Woodward (1957) |
| Lb2 lb2 | Normal vs. long weak basal rachis internode | 4 | Kasha and Walker (1960) |
| Lk2 lk2 | Long vs. short awn | 1 | Smith (1951) |
| Min min | Normal vs. minute | 4 | Nilan (1964) |
| Mlg mlg | Resistance vs. susceptability to race 3 of E. graminis hordei | 4 | Smith (1951) |
| Ms2 ms2 | Fertile vs. male sterile | 2 | Smith (1951) |
| Ms6 ms6 | Fertile vs. male sterile | 6 | Robertson (1963) |
| Ms10 ms10 | Fertile vs. male sterile | 1 | Robertson (1963) |

Appendix Table 1. Continued.

| Gene Symbol | Character | Chromosome | Authority |
|---|---|---|---|
| Ms14 ms14 | Fertile vs. male sterile | 1 | Hockett et al (1968) |
| Ms22e ms22e | Fertile vs. male sterile | 1 | Hockett (1972) |
| Ms23b ms23b | Fertile vs. male sterile | 1 | Hockett (1972) |
| Ms24v ms24v | Fertile vs. male sterile | 4 | Hockett (1972) |
| Ms24ak ms24ak | Fertile vs. male sterile | 4 | Hockett (1972) |
| Ms24an ms24an | Fertile vs. male sterile | 4 | Hockett (1972) |
| Ms25r ms25r | Fertile vs. male sterile | 4 | Hockett (1972, 1974) |
| Ms26u ms26u | Fertile vs. male sterile | | Hockett (1974) |
| Ms,,w ms,,w | Fertile vs. male sterile | 1 | Hockett (1972) |
| N n | Covered vs. naked caryopsis | 1 | Ramage, Burnham, and Hagberg (1961) |
| Wx wx | Normal vs. waxy endosperm | 1 | Tabata (1957, 1961) |
| Yh yh | Normal vs. yellow head | 4 | Nilan (1964) |
| Zbc zbc | Normal vs. zebra stripe | 4 | Robertson et al (1965) |

1/ Symbol contributed by this study.

Appendix Table 2. Authorities cited for recombination values presented in Figures 4a, b, and c.

| Citation Number | Authority |
|---|---|
| 1 | Data contributed by this paper |
| 2 | Briggs and Stanford (1943) |
| 3 | Buckley (1930) |
| 4 | Hanson (1952) |
| 5 | Immer and Henderson (1943) |
| 6 | Jarvi and Eslick (1967) |
| 7 | Kasha and Walker (1960) |
| 8 | Myler and Stanford (1942) |
| 9 | Nilan (1964) |
| 10 | Persson (1969a; 1969b) |
| 11 | Ramage, Burnham, and Hagberg (1961) |
| 12 | Robertson (1966) |
| 13 | Robertson (1970) |
| 14 | Robertson, Deming, and Koonce (1932) |
| 15 | Robertson, Wiebe, and Immer (1941) |
| 16 | Robertson, Wiebe, and Shands (1947) |
| 17 | Robertson, Wiebe, and Shands (1955) |
| 18 | Robertson et al (1965) |
| 19 | Takahashi, Hayashi, and Moriya (1961) |
| 20 | Takahashi, Hayashi, and Moriya (1971) |
| 21 | Wheatley (1956) |
| 22 | Woodward (1957) |

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