Balanced male sterile-lethal systems for hybrid barley production
by Mohammad Mokhlesur Rahman

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirement for the degree of
DOCTOR OF PHILOSOPHY in Crop and Soil Science
Montana State University


Abstract:
Linkage relationships between nine previously located lethal mutants and male sterile genes were determined. Nine lethal mutants not previously located were described. All nine mutants were inherited as monogenic recessives and temporary symbols, alb,,a through alb,,i were assigned. Linkage relationships between these mutants and identified male sterile, genes were determined and five mutants were assigned to the chromosomes. The effects of heterozygous and homozygous loci of 20 lethal mutants on yield and yield components were studied. Nine of the 20 population studied exhibited one locus heterosis (positive or negative advantages of Aa genotypes as compared to AA genotypes) for yield per plant or one or more of the components of yield.

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## ABSTRACT

Linkage relationships between nine previously located lethal mutants and male sterile genes were determined. Nine lethal mutants not previously located were described. All nine mutants were inherited as monogenic recessives and temporary symbols, alb,,a through alb,,i were assigned. Linkage relationships between these mutants and identified male sterile genes were determined and five mutants were assigned to the chromosomes. The effects of heterozygous and homozygous loci of 20 lethal mutants on yield and yield components were studied. Nine of the 20 population studied exhibited one locus heterosis (positive or negative advantages of Aa genotypes as compared to AA genotypes) for yield per plant or one or more of the components of yield.

# INTRODUCTION

The successful commercial production of hybrid barley, Hordeum vulgare L., depends upon a reliable source of male sterile individuals to serve as the female parents. Recent proposals for production of hybrid barley by Wiebe (1960), Ramage (1965) and Eslick (1970) utilizing genetic male sterility emphasize the desirability of locating many genes to facilitate such approaches. Each hybrid system requires a unique genetic mechanism, gene action, and linkage relationship.

The possible use of seedling lethality for elimination of undesired genotypes in the hybrid barley systems suggested this study.

The pusposes of this study were to determine the linkage relationship between located seedling lethal and male sterile genes, and to assign previously unlocated lethal genes to their respective chromosomes and determine their linkage relationship with male sterile genes.

Since several proposed systems result in $F_1$ commercial production with all or a portion of the plants heterozygous for seedling lethality available stocks were used to determine the effects of the heterozygous lethal condition on yield components and yield.

# REVIEW OF LITERATURE

## Male Sterility:

Male sterility in barley, Hordeum vulgare L., was first reported by Suneson in 1940, since then 335 different male sterile stocks of spring barley have been listed (Hockett et al., 1968; Hockett and Eslick, 1968, and Hockett, 1972). Twentythree have been determined to be non-allelic and the remaining need to be tested for allelism, except those that are derived through backcrossing.

Nuclear genes govern the genetic male sterility in tomato, Lycopersicon esculentum Mill., (Larson and Paur, 1948), sunflower, Helianthus annuus L., (Putt and Heiser, 1966), corn, Zea mays L., (Beadle, 1932), and wheat, Triticum aestivum L., (Athwal et al., 1967). Genetic male sterility in barley is controlled by single recessive nuclear genes, ms (Suneson, 1940; Hockett et al., 1968; and Hockett and Eslick, 1968). However, more complex cases of male sterility inheritance have been reported. Allison and Fisher (1964) reported that male sterility in cotton, Gossypium hirsutum L., was controlled by a single dominant gene, while in sunflower male sterility was governed by duplicate recessive genes (Putt and Heiser, 1966).

Cytoplasmic male sterility in different crops has been discussed by a number of investigators, but its existance in barley was first reported by Schooler in 1967. The behavior of this cytoplasmic male sterility was different from that of genetic male sterility and the fertility seemed to be controlled by two genes (Foster and Schooler,

1970); whereas the fertility restoration in genetic male sterility of barley is controlled by a dominant allele, Ms (Suneson, 1940; and Hockett and Eslick, 1968).

Hybrid Barley Systems:

Heterosis in corn, sugarbeet, Beta vulgaris L., sorghum, Sorghum vulgare Pers., and onion, Allium cepa L., has stimulated the development of hybrid barley. Since barley, by nature, is a highly self-pollinated species, time consuming hand emasculation precludes any possibility of commercial hybrid barley seed production without genetic or cytoplasmic sterility.

Barley researchers have proposed several schemes. The most important among them are: Utilization of stocks carrying informational gene or genes closely linked with natural or chemical roguing mechanisms for undesirable genotypes, and genetic and/or cytoplasmic male sterility systems for development of the desired populations.

Following the discovery of the DDT (Dichloro-dipheno-trichloro-ethane) resistance gene in barley (Hays, 1959), Wiebe (1960) proposed a scheme for hybrid barley using ms-ddt linkage which requires adequate isolation and one or more sprayings with DDT in the early seedling stage. Male sterility as well as DDT resistance are recessive and can be incorporated by back-crossing into a desired variety that could be used as the female in hybrid seed production. The parental genotype would be ms-ddt/Ms-Ddt, which upon selfing would produce 1 ms-ddt/ms-ddt, male sterile and resistant to DDT : 2 ms-ddt/Ms-Ddt, fertile and

susceptible to DDT : 1 Ms-Ddt/Ms-Ddt, fertile and susceptible to DDT. Application of DDT would kill all but the genotype ms-ddt/ms-ddt, resistant to DDT. Seeds on the male sterile plants would come from the pollen furnished by the unsprayed rows of the genotype, Ms-Ddt/ms-ddt.

To maintain the seed stock, seeds obtained from the male sterile plants, the genotypes of which would be Ms-Ddt/ms-ddt and ms-ddt/ms-ddt, would be sown in rows. Every alternate row would then be sprayed with DDT to obtain pure stands of male sterile plants and ensure enough pollen supply from the unsprayed rows. Seed harvested from the sprayed rows would be 1 ms-ddt/ms-ddt : 1 ms-ddt/Ms-Ddt. Repeating this cycle would ensure stock maintainance indefinately.

To produce hybrid seed, however, seeds from the sprayed rows would be planted in alternate rows or blocks with the desired male. The rows from the previously sprayed seeds would be sprayed again to obtain pure stands of male sterile plants, ms-ddt/ms-ddt. Seeds harvested from these sprayed rows would be hybrid. The closest linkage between a male sterile and ddt genes, so far as reported, was seven cross-over units between ddt and msl6 on chromosome 7 (Wiebe, 1964). Extensive hand roguing would be required to purify the female stock in the crossing block.

Ramage in 1963 first advocated the use of balanced tertiary trisomics (BTT) for hybrid barley seed production. The basic approach of this system was to establish a stock in which the recessive male sterile

allelle, ms would be carried on the normal diploid chromosome complement. The dominant allele, Ms would be carried on the translocated extra chromosome closely coupled with the interchange break-point (Ramage, 1965). Since the extra chromosome is usually not transmitted through pollen, BTT, upon selfing, would produce about 70 percent diploids, 30 percent BTT's and less than one percent primary trisomics.

The first commercial hybrid barley, 'Hembar', was developed utilizing the BTT system. Since this hybrid system lacks any genetic mechanism for the separation of diploids from trisomics or vice versa, hand roguing of diploids for parental stock maintenance is necessary and hand roguing or competition for removal of trisomics from diploid females in crossing blocks to produce pure diploid stand is relied upon.

To avoid hand roguing and to ensure sufficient pollen production by BTT's, Ramage (1965) suggested a modification of Wiebe's (1960) scheme, where two phytocides instead of one would be used. The dominant gene, A, would be resistant to phytocide "A". The recessive gene b would be resistant to phytocide "B". The dominant phytocide genes, A and B would be carried on the extra translocated chromosome of a BTT closely linked with a Ms gene. Trisomics would be resistant to phytocide "A" but susceptible to phytocide "B", while the diploids would be susceptible to phytocide "A" but resistant to phytocide "B".

To maintain the seed stock and produce the hybrid, two isolated blocks would be required. A field planted with trisomic seeds would be

sprayed with phytocide "A" to eliminate the diploids to maintain pure trisomic seed stock. The seeds from this sprayed trisomics would be planted in another block where application of phytocide "B" would ensure pure stands of the diploid. These diploids would serve as female parents for hybrid seed production.

An alternative method to this scheme would be to use sprayed trisomics as the male to pollinate an adjacent block planted with the seeds from the trisomic and sprayed with phytocide "B" to leave only male sterile diploids. The crossed seed harvested would serve as seed for diploid female seed for the hybrid seed production field without phytocide application. Vigorous trisomics as pollinators would be necessary.

To reduce the cost of production of hybrid seed Wiebe and Ramage (1970) suggested the use of an albino, a, gene in the BTT system as a sorting mechanism. This method is very similar to the system proposed by Ramage in 1965 except the phytocide is replaced by a lethal or albino gene, a.

This BTT with the seedling lethal albino, a, system requires establishment of a very close coupling of a male sterile allele, ms, and the recessive allele, a, of a seedling lethal or albino.

To produce this BTT-male sterile-albino stock, BTT-Ms-A*/ms-a/ms-a, an initial stock of ms-a/ms-A would be produced through selection from a cross between ms-A/ms-A and Ms-A/Ms-a. This ms-a/ms-A stock in turn

* Indicates coupling of Ms and A on the extra translocated chromosome.

would be crossed with a homozygous translocation where Ms and A are either closely linked with the break-point or on the interstitial segment. Through selfing and selection the BTT-Ms-A*/ms-a/ms-a genotype will be recovered. Selfing of the BTT-Ms-A*/ms-a/ms-a would produce 30 percent BTT, the parental type and 70 percent diploid. The diploid will die due to homozygous lethality. Assuming no crossing over repeated selfing of BTT in isolation would prepetuate this stock indefinitely.

In the second phase, the BTT-Ms-A*/ms-a/ms-a used as a male would be crossed onto the ms-A/ms-a genotype. Assuming no transmission of BTT-Ms-A* through the pollen, the homozygous albino seedlings would die leaving ms-A/ms-a which would be crossed with an appropriate male, Ms-A/Ms-A. Seeds harvested from this cross would be true hybrid seed for commercial production.

Eslick and Hockett (1967) suggested the use of a non-chromosomal aberation breeding method not requiring the use of translocations or trisomics. They proposed the use of male steriles in conjunction with dominant pre-flowering selective genes. Translocation data established ms10 and ms14 as being very close to the centromere of chromosome 1 (Eslick, 1970); there is no reported crossing over between these two male sterile genes, except in one case where in a very small population 2.0 ± 2.0 percent crossing over was observed (Eslick, Hockett and Kushnak, 1972). The general approach of this scheme was such that ms10 and ms14 would be in repulsion phase, ms10-Ms14/Ms10-ms14 to serve as

parental stock. Since there is practically no recombination between ms10 and ms14 the stock could be maintained indefinitely if grown in isolation.

To produce hybrid seed separation of homozygous male sterile seeds is needed. A pre-flowering selective gene, such as blue aleurone, B12 on chromosome 1 and B1 on chromosome 4 might be utilized (Eslick, 1970). These genes are complementary and at least one dominant allele at each locus is needed to express blue aleurone (Myler and Stanford, 1942). Eslick (1970) reported that ms10, ms14 and b12 were very close to the centromere of chromosome 1. To establish the system chromosome 4 would have the homozygous dominant alleles, B1 B1. Chromosome 1 would be heterozygous, B12 b12 and the parental genotype would be ms10-Ms14-B12/Ms10-ms14-b12. Upon selfing the segregation would be 1 ms10-Ms14-B12/ms10-Ms14-B12, male sterile and blue seeds: 2 ms10-Ms14-B12/Ms10-ms14-b12, fertile and blue seeds, : 1 Ms10-ms14-b12/Ms10-ms14-b12, male sterile and white seeds. In isolation with no recombination this stock would be self maintaining. About 25 percent of these seeds, i.e. white seeds, could be color sorted and utilized as female stock for hybrid seed production.

To separate the white seeds from the blue ones mechanically incorporation of the naked caryopsis, n, gene on chromosome 1 would be desirable, if not essential.

Alternately, Eslick (1970) also proposed the use of a balanced

male-sterile-recessive-lethal stock where one male sterile gene very closely linked with a lethal gene could be used. The parental genotype would be ms-A/Ms-a. One-fourth of the population, homozygous for fertility and lethality would die, leaving the heterozygous parental type and homozygous male sterile genotype. With no recombination, this stock would be self maintaining if grown in isolation.

For hybrid seed production, as for the balanced male sterile system, separation of the homozygous male sterile seeds from the parental type is necessary. This may be accomplished with any pre-flowering selective gene closely coupled with the male sterile and albino seedling lethal genes. The parental genotype would be ms-A-x/Ms-a-X*. In $F_2$ the segregation might be expected to be 1 ms-A-x/ms-A-x, male sterile, viable and short : 2 ms-A-x/Ms-a-X, fertile, viable and tall, : 1 Ms-a-X/Ms-a-X, fertile, lethal and tall. Homozygous fertile would be eliminated naturally due to homozygous lethality. The progeny of the parental type would be harvested in bulk and would maintain the stock by crossing or selfing in isolation. If the tall plants were cut previous to flowering only the short plant, ms-A-x/ms-A-x would remain to serve as female in the crossing block for hybrid seed production.

Other suggested systems have involved the use of male sterile genes with restorer cytoplasm (Hermsen, 1965; Hockett and Eslick, 1970; and Pfeifer, 1972) and restoration of fertility of genetic male steriles

* Hypothetical gene for plant height or other pre-flowering selective gene.

through the application of gibberellic acid (Kasembe, 1967).

Linkage:

Ten different translocations involving chromosome 1, 3, 4, 5, and 6 established male sterile, ms10 and male sterile, ms14 to be very close to the centromere of chromosome 1 (Eslick, 1970). Considering the double cross-overs from $F_3$ progeny rows Eslick, Rahman and Crowell (1971) gave a preliminary report that the gene sequence of male sterile, ms10, short awn, lk2, naked caryopsis, n, and albino seedling, $a_{c2}$ was lk2-n-ms10-$a_{c2}$ on chromosome 1; ms10 being very close to the centromere (Eslick, 1970). The three point recombination values confirmed the gene order: lk2-n-ms10 (Eslick, Hockett, and Kushnak, 1972). Using the recombination value between various genes belonging to linkage groups III and VII Takahashi and Hayashi (1958) summarized the gene orders of lax head, l, virescent seedling, $y_c$, brachytic br, lk2, n, & $a_{c2}$ in the sequence of lk2-n-l-$a_{c2}$-$y_c$-br, with the conclusion that these two linkage groups belonged to the same chromosome. This contention was supported by the translocation studies of Kramer, Veyl and Hanson (1954).

Results from crosses involving 24 translocations and 9 individual genes (Persson, 1969a,b) established the gene order to be br-$f_c$-$y_c$-$a_{c2}$; $a_{c2}$ being about 5 to 10 cross-over units from the centromere and the recombination value between $a_{c2}$ and n was about 11 percent. Tsuchiya (1972) utilizing telocentric chromosomes tentatively proposed the position of the centromere of chromosome 1 to be between $a_{c2}$ & n; $a_{c2}$

being on the short arm and n on the long arm (Persson, 1969b).

A brief summary of linkage values pertinent to this study are presented in Table 1.

Linkage was detected between Betzes male sterile, ms23b and se with recombination values of 11.3 ± 3.7 percent (Hockett unpublished data) and 6.6 ± 3.4 percent (Eslick unpublished data). Jarvi (1970) located ms23b on chromosome 1 and se was linked with ms23b. McProud (1971) observed ms23b to be independent of n and lk2.

Eslick (1970) observed no recombination between long awned outer glume, e, and male sterile, ms2, in T2-7d balanced tertiary trisomics; and complete linkage between ms2 and translocation break-points of T2-4a and T2-6a. However, when 13 translocations involving chromosome 2 were tested against ms2 nil to 2 percent recombination values between ms2 and e were observed (Ramage, 1966); this close linkage placed e and ms2 very close to the centromere of chromosome 2 (Ramage and Burnham, 1962). Since the recombination values between male sterile, ms3 and e was 4.6 ± 2.0 percent (Kasha and Walker, 1960) ms3 should be close to the centromere of chromosome 2. Robertson, Wiebe, and Shands (1955) proposed the gene ordering on chromosome 2 of orange seedling, or, chlorina plant color, f, virescent seedling, y, and virescent seedling, $y_x$, which was determined to be a multiple allele of y (Robertson, Wiebe, and Shands, 1955), as or-$y^{Yx}$-f-e. Subsequently, using the recombination percentages between various genes, Robertson et. al. (1965) and

Table 1. A brief summary of linkage data

| Character | Percent recombination | Authority |
|---|---|---|
| **Genes on Chromosome 1** | | |
| Normal vs. male sterile, Ms10 ms10 in relation to: | | |
| Covered vs. naked caryopsis, N n | 7.2 ± .4 | Eslick, Hockett and Kushnak, 1972 |
| Covered vs. naked caryopsis, N n | 12.7 ± 1.6 | Jarvi, 1970 |
| Long vs. short awn, Lk2 lk2 | 14.7 ± .7 | Eslick, Hockett and Kushnak, 1972 |
| Normal vs. albino seedling, $A_{c2}$ $a_{c2}$ in relation to: | | |
| Covered vs. naked caryopsis, N n | 27.2 ± 2.0 | Robertson, 1937 |
| Covered vs. naked caryopsis, N n | 11.3 ± 1.4 | Persson, 1969b |
| Covered vs. naked caryopsis, N n | 7.3 ± 1.6 | Takahashi and Hayashi, 1958 |
| Green vs. virescent seedling, Yc yc | 28.1 ± 1.6 | Haus, 1958 |
| Long vs. short awn, Lk2 lk2 | 36.1 ± | Takahashi and Hayashi, 1958 |
| Normal vs. waxy endosperm, Wx wx | 46.8 ± 2.3 | Tabata, 1957 |
| Normal vs. waxy endosperm, Wx wx | 43.8 ± 1.3 | Tabata, 1961 |
| Green vs. virescent seedling, Yc yc in relation to: | | |
| Long vs. short awn, Lk2 lk2 | 37.9 ± | Takahashi and Hayashi, 1958 |
| Covered vs. naked caryopsis, N n | 34.5 ± 4.1 | Persson, 1969b |
| Covered vs. naked caryopsis, N n | 31.5 ± 3.9 | Takahashi and Hayashi, 1958 |
| Long vs. short awn, Lk2 lk2 in relation to: | | |
| Covered vs. naked caryopsis, N n | 8.7 ± .6 | Takahashi and Hayashi, 1958 |
| Covered vs. naked caryopsis, N n | 7.9 ± .3 | Eslick, Hockett and Kushnak, 1972 |
| Covered vs. naked caryopsis, N n | 8.7 ± | Konishi, 1972 |
| Covered vs. naked caryopsis, N n | 8.8 ± | Takahashi et al., 1953 |

Table 1. Cont.

| Character | Percent recombination | Authority |
|---|---|---|
| Genes on Chromosome 2 | | |
| Normal vs. male sterile, Ms2 ms2 in relation to: | | |
| Normal vs. glossy sheath, Gs5 gs5 | 9.1 ± 0.7 | McProud, 1971 |
| Two row vs. six row, V v | 33.5 ± 2.1 | McProud, 1971 |
| Normal vs. long awned outer glume, E e | 23.0 ± 6.0 | Austenson, 1948 |
| Normal vs. chlorina, F f | 27.0 ± 6.9 | Austenson, 1948 |
| Normal vs. glossy sheath, Gs5 gs5 in relation to: | | |
| Two row vs. six row, V v | 32.0 ± 2.5 | Rasmusson and Lambert, 1965 |
| Two row vs. six row, V v | 24.2 ± 1.3 | Takahashi, Hayashi & Moriya, 1971 |
| Two row vs. six row, V v | 26.0 ± 1.4 | McProud, 1971 |
| Normal vs. long awned outer glume, E e in relation to: | | |
| Normal vs. white streak 4, Wst4 wst4 | 6.5 ± 1.0 | Takahashi and Moriya, 1969 |
| Two row vs. six row, V v | 31.0 ± 2.2 | Rasmusson and Lambert, 1965 |
| Two row vs. six row, V v | 22.9 ± 4.8 | Kasha and Burnham, 1965 |
| Two row vs. six row, V v | 26.5 ± 2.0 | Woodward, 1957 |
| Two row vs. six row, V v | 33.3 ± | Kasha and Walker, 1960 |
| Normal vs. glossy sheath, Gs5 gs5 | 2.5 ± 0.8 | Rasmusson and Lambert, 1965 |
| Normal vs. albino seedling, A2 a2 | 18.4 ± 1.2 | Robertson, 1967 |
| Normal vs. white streak 4, Wst4 wst4 | 6.5 ± 1.0 | Takahashi, Hayashi, & Moriya, 1971 |
| Normal vs chlorina, F f | 2.6 ± 0.4 | Robertson, et. al., 1944 |

Table 1. Cont.

| Character | Percent recombination | Authority |
|---|---|---|
| Genes on Chromosome 2 | | |
| Normal vs. orange seedling, Or or | | |
| in relation to: | | |
| Normal vs. chlorina, F f | 8.7 ± 0.4 | Robertson and Coleman, 1940 |
| Normal vs. long awned outer glume, E e | 14.8 ± 0.9 | Robertson, et. al., 1944 |
| Normal vs. virescant seedling, Y y | 13.3 ± 0.6 | Robertson and Coleman, 1940 |
| Normal vs. virescent seedling, Y y | 15.0 ± | Robertson and Coleman, 1942 |
| Normal vs. albino seedling, A2 a2 | 5.5 ± | Robertson, 1967 |
| Two row vs. six row, V v | | |
| in relation to: | | |
| Normal vs. white streak 4, Wst4 wst4 | 34.3 ± | Takahashi, Hayashi & Moriya, 1971 |
| Green vs. albino seedling, A2 a2 | | |
| in relation to: | | |
| Normal vs. chlorina seedling, F f | 13.0 ± 0.7 | Robertson, 1967 |
| Normal vs. long awned outer glume, E e | 18.3 ± 1.2 | Robertson, 1967 |
| Normal vs. chlorina seedling, F f | | |
| in relation to: | | |
| Normal vs. virescent seedling, Y y | 0.8 ± | Robertson and Coleman, 1940 |
| Position of genes | | |
| in relation to centromere: | | |
| Normal vs. orange seedling, Or or | 15.0 ±* | Ramage, 1966 |
| Normal vs. orange seedling, Or or | 13.0 ± 3.9* | Ramage, Burnham & Hagberg, 1961 |
| Normal vs. chlorina, F f | 8.0 ± 1.7* | Ramage, Burnham & Hagberg, 1961 |
| Two row vs. six row, V v | 28.0 ± 5.4* | Ramage, Burnham & Hagberg, 1961 |
| Two row vs. six row, V v | 28.0 ±* | Ramage, 1966 |

Table 1. Cont.

| Character | Percent recombination | Authority |
|---|---|---|
| **Genes on Chromosome 2** | | |
| Position of genes in relation to centromere: | | |
| Normal vs. long awned outer glume, E e | 10.7 ± 3.4* | Ramage, Burnham & Hagberg, 1961 |
| Normal vs. long awned outer glume, E e | 11.7 ± 2.6* | Persson, 1969a |
| Normal vs. male sterile, Ms2 ms2 | 2.0 ±* | Ramage, 1966 |
| **Genes on Chromosome 3** | | |
| Normal vs. Uzu dwarf, Uz uz in relation to: | | |
| Normal vs. Xantha seedling, Xs xs | 29.5 ± 3.9 | Kasha and Burnham, 1965 |
| Normal vs. Xantha seedling, Xs xs | 23.8 ± 3.5 | Kasha and Burnham, 1965 |
| Normal vs. Xantha seedling, Xs xs | 31.9 ± | Takahashi, 1972 |
| Normal vs. Xantha seedling, Xs xs | 29.3 ± | Takahashi, 1972 |
| Normal vs. Xantha seedling, Xs xs | 25.0 ± | Takahashi, 1972 |
| Green vs. albino seedling, Ac ac | 14.4 ± 1.0 | Takahashi and Hayashi, 1953 |
| Green vs. albino seedling, Ac ac | 10.7 ± | Takahashi and Yamamoto, 1951 |
| Green vs. albino seedling, Ac ac | 14.2 ± 3.2 | Takahashi and Hayashi, 1959 |
| Green vs. white seedling, An an | 19.0 ± 0.1 | Takahashi and Hayashi, 1958 |
| Green vs. white seedling, An an | 13.1 ± 2.1 | Kasha and Burnham, 1965 |
| Green vs. white seedling, An an in relation to: | | |
| Normal vs. xantha seedling, Xc xc | 9.0 ± | Robertson, 1929 & 1937 |
| Normal vs. xantha seedling, Xs xs | 15.5 ± 0.8 | Robertson, 1937 |
| Normal vs. xantha seedling, Xs xs | 16.0 ± | Kasha and Walker, 1960 |

Table 1. Cont.

| Character | Percent recombination | Authority |
|---|---|---|
| **Genes on Chromosome 3** | | |
| Green vs. white seedling, An an | | |
| in relation to: | | |
| Normal vs. xantha seedling, Xs xs | 15.0 ± 2.4 | Kasha and Burnham, 1965 |
| Position of genes | | |
| in relation to centromere: | | |
| Normal vs. uzu dwarf, Uz uz | 14.0 ± 3.0* | Ramage, Burnham & Hagberg, 1961 |
| Normal vs. uzu dwarf, Uz uz | 14.0 ± 1.9* | Ramage, Burnham & Hagberg, 1961 |
| Normal vs. uzu dwarf, Uz uz | 9.7 ± 2.2* | Persson, 1969b |
| Green vs. albino seedling, Ac ac | 3.0 ± 1.4* | Ramage, Burnham & Hagberg, 1961 |
| Green vs. albino seedling, Ac ac | 4.6 ± 1.0* | Persson, 1969b |
| Green vs. albino seedling, Ac ac | 5.7 ± 0.9* | Persson, 1969b |
| Green vs. white seedling, An an | 18.4 ± 3.6* | Persson, 1969a |
| Green vs. white seedling, An an | 12.3 ± 1.3* | Persson, 1969a |
| Green vs. white seedling, An an | 15.1 ± 2.1* | Persson, 1969a |
| Green vs. white seedling, An an | 17.0 ± 2.3* | Ramage, Burnham & Hagberg, 1961 |
| Green vs. white seedling, An an | 13.0 ± 1.5* | Ramage, Burnham & Hagberg, 1961 |
| Green vs. white seedling, An an | 12.0 ± 3.2* | Ramage, Burnham & Hagberg, 1961 |
| **Genes on Chromosome 7** | | |
| Rough vs. smooth awn, R r | | |
| in relation to: | | |
| Green vs. cream seedling, Cm cm | 2.3 ± 0.3 | Robertson, 1967 |
| Green vs. cream seedling, Cm cm | closely linked | Kramer and Blander, 1961 |
| Normal vs. fragile stem, Fs fs | 44.0 ± 3.3 | Ramage and Suneson, 1961 |
| Normal vs. male sterile, Ms11 ms11 | 17.3 ± 4.5 | Walker et. al., 1958 |
| Normal vs. variegated 3, Va3 va3 | 4.3 ± 0.9 | Walker et. al., 1963 |

Table 1. Cont.

| Character | Percent recombination | Authority |
|---|---|---|
| **Genes on Chromosome 7** | | |
| Long vs. short haired rachilla, S s in relation to: | | |
| Normal vs. variegated 3, Va3 va3 | 28.8 ± 8.3 | Walker et. al., 1963 |
| Green vs. cream seedling, Cm cm | 24.4 ± 0.3 | Robertson, 1967 |
| Rough vs. smooth awn, R r | 25.1 ± 1.6 | Takahashi and Hayashi, 1966 |
| Rough vs. smooth awn, R r | 18.2 ± 4.5 | Wells, 1958 |
| Rough vs. smooth awn, R r | 28.0 ± 2.2 | Ramage and Suneson, 1961 |
| Rough vs. smooth awn, R r | 28.0 ± | Yesuda and Takahashi, 1961 |
| Rough vs. smooth awn, R r | 26.5 ± 3.7 | Woodward, 1957 |
| Green vs. white strip, Wst2 wst2 | 15.6 ± | Takahashi and Moriya, 1964 |
| Normal vs. fragile stem, Fs fs | 24.0 ± 3.8 | Ramage and Suneson, 1961 |
| Normal vs. male sterile, Ms16 ms16 in relation to: | | |
| Resistance vs. susceptible to Ddt, Ddt ddt | 7.0 ± | Wiebe, 1964 |

* Translocation Data

Robertson (1967) suggested the gene sequence was rattail, rt-or-ms3 y-f-e-ms2. The recombination values between ms2 and f (Austenson, 1948) and between f and a2 (Robertson 1967) placed y between ms2 and a2.

Results with telocentric chromosomes suggested the possible gene order to be ms3-f-centromere-gs5-e-ms2-wst4-tr; ms3 and f on the short arm, while gs5, e, ms2, wst4 and tr were on the long arm (Tsuchiya, 1972).

Assignment of male steriles, ms5, msl6, msl9, ms23b and ms24v to the respective chromosome involved T1-6c, T1-7a, T2-4a, T2-6a, T3-5b, T3-7a and T4-5a translocation tester stocks (Hockett & Eslick, 1970). From the information based on $F_3$ family segregation ms5, msl6, msl9, ms23b and ms24v were assigned to chromosomes 3, 7, 7, 1, and 4, respectively. Male steriles, ms5, msl9 and ms24v were near the centromere while msl6 and ms23b were not. The location of msl6 agreed with Hayes and Rana (1966) who found msl6 to be linked with the ddt gene on chromosome 7.

From studies of three chlorophyll mutants Robertson (1929 and 1937) proposed the gene order of albino seedling $a_c$, xantha seedling, $x_c$ and white seedling, $a_n$ as $a_c$-$x_c$-$a_n$ on chromosome 3. Persson (1969a) confirmed this sequence; $a_n$ was calculated to be 15 to 20 cross-over units from the centromere, and $a_n$ and $x_c$ were placed on the long arm of chromosome 3.

Robertson (1967) located white streak, wst on chromosome 3, and

considering the recombination values between wst and $a_c$-$x_c$ - $a_n$ and xantha seedling, $x_s$, he proposed the gene order: $a_c$-$x_c$-wst-$a_n$-$x_s$. In order to locate albino lemma, al, Takahashi and Hayashi (1958) conducted a three-point linkage study which included uzu dwarf plant, uz. Their proposed gene sequence was, uz-$a_c$-al-$a_n$. Kasha and Walker (1958) suggested the gene order, including absent lower spikelet, als to be, als-uz-ys-$a_c$-$a_n$.

Jarvi and Eslick (1967) observed linkage between ms24v and translocation break-points of T2-4a and T4-5a, and independence with T1-6c, T1-7a, T2-6a, T3-5b, and T3-7c break-points. Using $F_3$ data from crosses between ms24v and translocation tester stocks, T1-6c, T1-7a, T2-6a, T3-5b and T3-7c ms24v was assigned to chromosome 4 and probably close to the centromere (Hockett and Eslick, 1970).

An $F_3$ linkage test showed that male sterile, msl, was on the short arm of chromosome 5 (Ramage 1961). Eighteen translocations involving chromosome 5 and four individual genes: third outer glume, trd, black lemma and pericarp, B, white seedling, $a_t$, and a male sterile, msl; positioned msl very close to the centromere of chromosome 5 (Ramage, 1961, and 1963); the proposed gene order in the short arm was trd-B-$a_t$-msl-centromere. The genes trd and B were about 15 recombination units apart and the genes B and $a_t$ were about 30 recombination units apart, while msl and $a_t$ showed independence (Ramage and Lehman, 1964). Contrary to Ramage (1963), Tsuchiya (1972) on the basis of telocentric trisomics suggested that B, trd and $a_t$ were on the long

arm instead of the short arm; and the gene order was: centromere-$a_t$-B-trd. Cream seedling, cm2 on chromosome 5 was observed to be linked with partially sterile ovule, os, with a recombination percentage of about 7 (Nilan and Moh, 1955).

Ramage and Burnham (1962) reported that orange lemma, o, and early heading, ec were close to the centromere of chromosome 6 having 0.5 ± 0.3 percent recombination (Ramage, 1962). Uniculm, uc2, was also determined to be very closely linked with o with a recombination percentage of 0.6 ± 0.1 (Shands, 1962); thus placing uc2 in the same region along with ec. Xantha seedling, $x_n$, was about 14 cross-over units away from o (Robertson, 1967).

Seven different translocations involving chromosome 7 placed male sterile, ms16 and ms19 on chromosome 7; ms19 being near to the centromere, while ms16 was distal (Hockett and Eslick, 1970). $F_3$ linkage data from crosses of 5 translocations involving chromosome 7 and 3 individual genes; fragile stem, fs, smooth awn, r, and short haired rachilla, s; fs showed 23.0 ± 1.2 percent recombination with the centromere (Ramage, 1963, and Ramage and Burnham, 1962); the gene order was fs-s-r. One linkage study involving white stripe, wst2, smooth awn, r, and short haired rachilla, s, suggested gene sequence to be wst2-s-r (Takahashi and Moriya, 1964.)

Kramer and Blander (1961) using T1-7a, T1-7b and T3-7a assigned cm to chromosome 7. This finding agrees with Robertson (1933) who placed

cm (his previous designation was $a_b$) in the linkage group V. The seedling mutant, cm was closely linked with smooth awn, r and the recombination value was 3.0 ± 2.0 percent (Kramer & Blander, 1961). The recombination percentages between cm and r; and between cm and short haired rachilla, s were 24.4 ± 0.3 and 2.3 ± 0.3, respectively, (Robertson, 1967).

Chlorophyll-deficient mutants:

The appearance of chlorophyll-deficient seedlings is common in self-pollinated corn (Wetz and Goodsell, 1929), in barley (Robertson, 1932) and in many other species. These chlorophyll-deficient genes, when homozygous may or may not cause death of seedlings. Assuming their detrimental effect on yield, plant breeders have been eliminating them from the population. A number of investigators attempted to determine the effects of these recessives upon yield when they occur in the heterozygous condition. Wetz and Goodsell (1929) found no definite relationship between the number of defects present and yield. Nevertheless, when the characters studied were divided into three classes: (seed defects, seedling chlorophyll defects and plant defects), the totals under each of these classes as well as the total for all the defects were negatively correlated with yield. Their experiment, however, was not designed to study the effects of any particular recessive in the heterozygous condition; but rather to show the general tendency of varieties with one recessive character to be more productive or less

productive than varieties with many recessive characters.

Robertson (1932) studied the effects of a single lethal gene in four barley varieties. He found no significant difference between the homozygous viable and heterozygous genotype for all the agronomic characters studied. However, when the lethality was associated with endosperm deficiency a significant difference in grain yield was observed.

In soybeans Wetz and Stewart (1927) and in sorghum, Karper (1930) observed differences between the homozygous and heterozygous genotypes, but the differences were not significant.

Mangelsdorf (1928) reported that homozygous (De De) corn plants were taller than heterozygous (De de) genotypes during early stages of growth; but the differences were overcome by the heterozygotes in the later stages of development.

Doll (1966) studied the effects of 49 chlorophyll-mutants in the two-rowed spring barley, Carlsberg II. Some of these mutants in heterozygous condition had little or no effect on the number of kernels per plant, while others showed noticable detrimental effects on the same character. Only one heterozygous chlorophyll mutant, 52-X, had significantly more kernesl per plant than the corresponding homozygous counterpart. This significant difference, however, was attributed to the interaction of another recessive mutant gene which was observed to be linked with the chlorophyll-mutant gene in the repulsion phase.

Contrary to the findings of Doll (1966), Robertson (1932), and others, Gustafsson, Nybom and von Wettstein (1950) observed superior performances of the heterozygotes of three spontaneously occurring chlorophyll mutants, albino-7, xantha-3 and albinoxantha in a pure line of the Golden vareity All three mutants in single heterozygous condition out-perform their homozygous counterparts when grown under higher plant density and heavy nitrogen fertilization. The dihybrid (albino-7 x xantha-3) heterozygotes exhibited similar performances under similar conditions. Similar results were also obtained by Gustafsson (1947). Robertson and Austin (1935) reported similar results except in one case where the double heterozygotes for $a_{c3}$ and $x_c$ were inferior to the homozygous counterparts. The two lethal mutants, $a_c$ and $x_c$ in single heterozygote condition as well as in double heterozygote condition was consistently superior for the characters studied.

## MATERIALS AND METHODS

Seed of all the mutants used in this experiment were provided by Prof. R. F. Eslick, Plant and Soil Science Department, Montana State University, Bozeman, Montana. The mutational events, assignment to chromosome when known, inheritance and the cultivar in which these mutants were originally found are described in Tables 2, 3, 4 and 5.

During the summer of 1968 each of these mutants, the male sterile tester series and an msl msl orange lemma (oo) stock were planted at the Agricultural Experiment Station, Bozeman. Only male sterile (ms/ms) plants were used as females. To avoid contamination, the male sterile spikes, to be used as females in crossing, were bagged with glassine bags when they were about to emerge from the boot. After complete emergence of these heads the bags were removed to make appropriate pollinations and rebagged immediately.

Each previously located heterozygote lethal, (Table 3) was crossed to male sterile (ms/ms) plants whose genetic male sterile gene had been previously assigned to a chromosome (Table 2). Each heterozygote lethal mutant not previously located (Table 4) was crossed onto each identified male sterile (ms/ms) and the orange lemma (o/o) stock (Table 2). In both cases three or four different males were used in making crosses to increase the probability of having at least one male heterozygous for the lethal gene. To identify crosses and parents each was marked with proper identification tags. At maturity crossed seeds and each male were harvested individually.

Table 2. Description of the spontaneous, monogenic, recessive male sterile genes and orange lemma gene used to determine the linkage with seedling lethal mutants.

| Gene symbol | Cultivar | Located on chromosome | Authority |
|---|---|---|---|
| ms10 ms10 | Compana | 1 | Eslick and Hockett, 1967; Hockett and Eslick, 1968. |
| ms14 ms14 | Unitan | 1 | Eslick and Hockett, 1967; Hockett and Eslick, 1968. |
| ms23b ms23b | Betzes | 1 | Hockett and Eslick, 1968; Hockett and Eslick, 1970. |
| ms2 ms2 | Trebi | 2 | Austenson, 1948; Hockett and Eslick, 1970; and Eslick, 1970. |
| ms5 ms5 | Carlsberg II | 3 | Hockett and Eslick, 1970. |
| ms24v ms24v | Betzes | 4 | Jarvi and Eslick, 1967. |
| ms1 ms1 | Betzes | 5 | Suneson, 1940; Ramage, Burnham and Hagberg, 1961. |
| Orange lemma (oo) | Titan | 6 | Myler and Stanford, 1942; and Ramage et. al., 1961. |
| ms16 ms16 | Betzes | 7 | Hockett and Eslick, 1968 & 1970. |
| ms19 ms19 | CI 4961 | 7 | Hockett and Eslick, 1968 & 1970. |

Table 3. Description of the previously located spontaneous, seedling lethal, monogenic recessive mutant genes used to determine their linkage with male sterile genes.

| Gene symbol | Cultivar | Located on Chromosome | Phenotype | Authority |
|---|---|---|---|---|
| $\underline{a_{c2}}$ $\underline{a_{c2}}$ | Coast II | 1 | Albino | Robertson and Deming, 1930, and Robertson, 1937. |
| $\underline{y_c}$ $\underline{y_c}$ | Coast III | 1 | Albino | Robertson and Demming, 1930; Robertson, Demming and Koonce, 1932. |
| a2 a2 | Coast | 2 | Albino | Robertson, 1967. |
| y y | Faust | 2 | Shows yellow colour | Robertson and Coleman, 1942. |
| $\underline{x_s}$ $\underline{x_s}$ | Smyrna | 3 | Yellow colour varies with temperature | Robertson, 1929. |
| $\underline{a_c}$ $\underline{a_c}$ | Colsses I | 3 | Albino | Robertson, 1929. |

Table 3. Cont.

| Gene symbol | Cultivar | Located on Chromosome | Phenotype | Authority |
|---|---|---|---|---|
| $\underline{a_t}$ $\underline{a_t}$ | Trebi | 5 | Albino | Robertson, 1929 |
| $\underline{cm_2}$ $\underline{cm_2}$ | Black-hulless | 5 | Pale yellow | Nilan and Moh, 1955. |
| cm cm | Black-hulless | 7 | Cream colour | Robertson, 1932; Kramer and Blander, 1961. |

Table 4. Description of spontaneous seedling lethal mutant genes not previously located.

| Gene symbol | Cultivar | Phenotype | Previous designation |
|---|---|---|---|
| alb,,a alb,,a | Erbet | White leaf with yellow tip. The yellow colour belonged to 3C RHS Yellow Colour chart.* | Erbet $x_{66}$ $x_{66}$ |
| alb,,b alb,,b | Carlsberg II | White | Carlsberg II $x_{66}$ $x_{66}$ |
| alb,,c alb,,c | Titan | White, seldom papyraceous at the margin | Titan $a_{48}$ $a_{48}$ |
| alb,,d alb,,d | Erbet | White leaf with yellow tip. The yellow colour belonged to the 3C RHS Yellow Group Chart.* | Early Betzes $x_{66}$ $x_{66}$ |
| alb,,e alb,,e | Titan | White | Titan $a_{65}$ $a_{65}$ |
| alb,,f alb,,f | Shabet | White | Shabet $a_{66}$ $a_{66}$ |
| alb,,g alb,,g | Titan | White, seldom with papyraceous margin. | Belonee short Titan aa |
| alb,,h alb,,h | Compana X Mars | White | T2-6a-aa |
| alb,,i alb,,i | Titan | Ivory white leaf with yellow green tip. The yellow green colour belonged to 144C of RHS Colour Chart.* | Shonufertoontan aa |

* R.H.S. Colour Chart. The Royal Horticultural Society, London.

Table 5. Determination of inheritance of the seedling lethal mutants not previously located.

| Lethal genes | Number of Progeny: Green (A-) | Albino (aa) | Total | Chi-square* | Probability* | Inheritance |
|---|---|---|---|---|---|---|
| alb,,a alb,,a | 280 | 99 | 379 | 0.25 | 0.75-0.50 | Monogenic |
| alb,,b alb,,b | 276 | 89 | 365 | 0.07 | 0.97-0.95 | Monogenic |
| alb,,c alb,,c | 304 | 88 | 392 | 1.36 | 0.25-0.10 | Monogenic |
| alb,,d alb,,d | 280 | 106 | 386 | 1.24 | 0.50-0.25 | Monogenic |
| alb,,e alb,,e | 276 | 86 | 362 | 0.29 | 0.75-0.55 | Monogenic |
| alb,,f alb,,f | 270 | 108 | 378 | 2.56 | 0.25-0.10 | Monogenic |
| alb,,g alb,,g | 293 | 90 | 383 | 0.44 | 0.75-0.50 | Monogenic |
| alb,,h alb,,h | 300 | 80 | 380 | 3.16 | 0.10-0.05 | Monogenic |
| alb,,i alb,,i | 248 | 77 | 325 | 0.29 | 0.75-0.50 | Monogenic |

* For goodness of fit 3 green:1 albino

During the winter of 1968 - 69 the males were checked for heterozygosity for the lethal genes in the greenhouse. To obtain seed for the $F_2$ generation, 20 $F_1$ seeds involving only those from heterozygous males were planted in the greenhouse in 1968-69. Each $F_1$ plant was harvested and checked for heterozygosity for the lethal gene.

Whenever possible 400 seeds from the segregating $F_2$ seed population were planted in rows 30 cm. apart in the field during the spring of 1969 at a rate of 25 seeds per three meter row. From these segregating rows, two sets of samples consisting of one spike from each $F_2$ plant were harvested. Male sterile plants were discarded.

Whenever a heterozygous male was not found to be involved in the crosses made in 1968, new crosses were made in 1969 following the same procedure as for 1968. The $F_1$ and $F_2$ seeds were handled in the same manner in 1970 as 1969.

During the summer of 1970 and 1971, $F_2$ spikes were planted in hills on 60 cm. centres and classified for $F_3$ phenotypes to obtain $F_2$ genotypes. The hills homozygous for Ms/Ms and Alb,,/Alb,, and the hills segregating for male sterility, ms/ms, but homozygous for Alb,,/Alb,, were marked with appropriate coloured flags as soon as they could be identified. Segregation data for lethal and male sterile genes, as well as for other pertinent markers, whenever possible, were recorded.

Suspected linkage between two genes was determined by Chi-square test. Significant deviations from the expected independent ratios, as

indicated by interaction Chi-square tests (Appendix Table 1) were used as indicators of linkage. The recombination percentages were calculated from the $F_3$ population data by the maximum likelihood method. This calculation was facilitated by the formulas and tables presented by Allard (1956).

To verify $F_3$ data, 50 seeds of possible cross-over types from fertile plants of Ms-A/Ms-a and Ms-A/ms-A hills were planted in two three meter rows 30 cm. apart in 1971 and 1972. Segregation for lethal and male sterile genes and other markers were recorded and the $F_3$ data corrected.

To evaluate the effects of heterozygous lethals on the yield of grain, the following characteristics of mature plants were measured. (1) number of spike bearing tillers, (2) Total number of seeds per plant and (3) weight of seeds per plant. A randomized complete block design with four replications of two row plots was used. The seeds heterozygous for the lethal mutants (Table 3 and 4) were planted in rows 30 cm. apart and spaced 30 cm. within the rows. Each row was three meters long.

To provide equal competition seedlings of a genetically marked genotype were transplanted where seedlings died due to lethality or failed to establish.

At maturity, each plant was harvested individually except the marked genotype that was planted later to provide equal competition.

Twenty seeds from each plant were sown in the greenhouse to differentiate the homozygous from heterozygous plants so that the effects of the heterozygous lethal condition could be evaluated.

Data were analyzed following the analysis of variance and chi-square methods of Steel and Torrie (1960). Homozygous and heterozygous plants for each plot were averaged and the two means considered as splits of variety plots.