Combining cytogenetics and mutagenesis to recover unusual mutants at specific loci in barley (Hordeum vulgare L.)
by Daniel Reid Biggerstaff

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
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Abstract:
Seed of translocation heterozygotes in barley (Hordeum vulgare L.) from crosses between male steriles and selected translocation homozygotes was treated with the chemical mutagen diethylsulphate. Translocations were selected to represent breakpoints in the 14 barley chromosome arms, with the breakpoints as far from the centromere as possible. The male sterile genes selected were located near the centromere of one chromosome involved in the translocation. Diethylsulphate was the mutagen of choice because it produces a high number of point mutations and relatively few chromosomal aberrations, is quite economical, and is easy to use.

M2 head rows were selected on the basis of aberrant ratios with respect to fertility. The expected ratio is 1 fertile:2 semisterile:1 male sterile; the aberrant ratio selected was 0:2:1, representing possible lethal translocation homozygotes. The frequency of recovered mutants in the interstitial segment was approximately 6%. Twenty-three lethal translocation homozygote mutants have been recovered. At least one breakpoint in each of the 14 barley chromosome arms, and all but six of the possible chromosome translocation combinations are represented.

Uses and advantages of lethal translocation homozygotes include: increasing the precision of linkage studies, maintaining recessive genes in heterozygous stocks, accomplishing gene transfers, and allelism testing with greater ease and confidence. Success in recovering mutations in specified regions of the barley chromosome, in coupling or repulsion, was much easier than anticipated. Methods such as this may offer one way of incorporating more of the available cytogenetic and genetic information into the art and science of plant breeding.

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Date December 4, 1981

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ABSTRACT

Seed of F_1 translocation heterozygotes in barley (Hordeum vulgare L.) from crosses between male steriles and selected translocation homozygotes was treated with the chemical mutagen diethylsulphate. Translocations were selected to represent breakpoints in the 14 barley chromosome arms, with the breakpoints as far from the centromere as possible. The male sterile genes selected were located near the centromere of one chromosome involved in the translocation. Diethylsulphate was the mutagen of choice because it produces a high number of point mutations and relatively few chromosomal aberrations, is quite economical, and is easy to use.

M_2 head rows were selected on the basis of aberrant ratios with respect to fertility. The expected ratio is 1 fertile:2 semisterile:1 male sterile; the aberrant ratio selected was 0:2:1, representing possible lethal translocation homozygotes. The frequency of recovered mutants in the interstitial segment was approximately 6%. Twenty-three lethal translocation homozygote mutants have been recovered. At least one breakpoint in each of the 14 barley chromosome arms, and all but six of the possible chromosome translocation combinations are represented.

Uses and advantages of lethal translocation homozygotes include: increasing the precision of linkage studies, maintaining recessive genes in heterozygous stocks, accomplishing gene transfers, and allelism testing with greater ease and confidence. Success in recovering mutations in specified regions of the barley chromosome, in coupling or repulsion, was much easier than anticipated. Methods such as this may offer one way of incorporating more of the available cytogenetic and genetic information into the art and science of plant breeding.

INTRODUCTION

Barley (Hordeum vulgare L.) is one of the first crops domesticated by man and is grown throughout the world as a major cereal crop. It is also used extensively for genetic studies, in mutagen experiments, and as a laboratory organism for physiologists, biochemists, and others. Because barley is an important commercial crop and is of great interest to scientists from many disciplines, any novel use of barley in a genetic study has far reaching implications.

The purpose of this study was to induce and recover mutant genes at specific loci. A translocation heterozygote having a lethal gene on the interstitial segment of the translocated chromosome, and a recessive male sterile gene near the centromere of the non-translocated chromosome, would provide barley breeders with a useful genetic tool. This lethal translocation homozygote would facilitate gene transfers, increase the speed and precision of linkage studies, and give breeders another way to maintain recessive genes in a known heterozygous condition. Success in this attempt would also serve to demonstrate the potential of combining mutagenesis and cytogenetics to obtain mutants at specific loci.

LITERATURE REVIEW

Genetic male sterility in barley was first reported by Suneson (1940). Since then 377 different male sterile mutants in spring barley have been reported and 33 have been determined to be non-allelic (Hockett, 1972; Hockett, et al., 1968; Hockett and Reid, 1981). Numerous workers have reported chromosomal location of these mutants (Eslick, 1976; Eslick, et al., 1974; Jarvi and Eslick, 1967; Ramage and Eslick, 1975; Tuleen, 1971). Eslick (1971), Hockett and Eslick (1971), and Roath and Hockett (1971) have presented additional information about origin, inheritance, pollen and anther morphology, and possible uses of genetic male sterile mutants.

Barley has been used extensively in studies of chemical mutagenesis (Constantin, 1975; Nilan, et al., 1963; Nilan, 1964). Probably more information is available on mutation induction in barley than in any other crop (Nilan, 1974). Diethylsulphate has been used successfully in barley mutation work because it induces a high number of point mutations with relatively few chromosomal aberrations (Constantin, 1975; Nilan, et al., 1963). Scholz (1971) presents a review of the utilization of induced mutations in barley.

A chromosomal aberration where terminal segments of nonhomologous chromosomes have exchanged positions is known as a chromosomal interchange, a reciprocal translocation, or simply a translocation.

Burnham (1962) and Ramage (1963) provide excellent discussions of the behavior and utilization of translocations. Zecevic (1975) presents an extensive literature review on barley translocations.

Following anaphase I, adjacent disjunction of the chromosomes involved in a translocation will result in spores that abort because of chromosomal duplications and deficiencies. The aborted spores serve as a phenotypic character, termed semisterility, by which the translocation heterozygote can be recognized (Ramage, 1963). When interstitial crossing over is followed by alternate disjunction, the crossover chromatids are in the spores that abort. In barley, which exhibits an excess of alternate disjunction, recovered crossovers are greatly reduced (Kramer and Blander, 1961).

The potential uses for the mutants being sought in this study were proposed by Eslick (1972). Initial work on this study was reported by Biggerstaff and Eslick (1978).

MATERIALS AND METHODS

Selected translocation homozygotes were crossed to genetic male steriles. Translocations were selected to represent breakpoints in the fourteen barley chromosome arms, with the breakpoints as far from the centromere as possible, as determined from the literature (Table 1). Seed of all the translocations used in this study were provided by Dr. R. T. Ramage, University of Arizona, Tucson, Arizona. Most of the genetic male sterile genes used are located near the centromere of one of the chromosomes involved in the reciprocal translocation (Table 2).

The F_1 progeny of these crosses were grown in a winter nursery at Mesa, Arizona, to obtain maximum seed increase. F_2 seed from each cross was treated with 0.015 M diethylsulfate using procedures developed at Washington State University, Pullman, Washington (Nilan, et al., 1963). This procedure is outlined in the Appendix.

Treated seed (M_1 seed, F_2 generation) were space planted in rows 30 cm apart to obtain populations of plants 5 to 8 cm apart within rows. M_2 seed was harvested from individual spikes taken at random in each population. M_2 head rows were planted as single, space planted rows 3 m in length, 30 cm apart.

At maturity each M_2 head row was examined for the absence of fully fertile plants. The expected ratio is 1 fertile (translocation homozygote):2 semisterile (translocation heterozygote):1 male sterile

Table 1. Description of translocations utilized to establish tester stocks.

Translocation Designation	Cultivar	Breakpoints†		Authority
T1-3e	Bonus	L	L	Persson (1970)
T1-4e	Bonus	L	S	Persson (1970)
T1-5f	Bonus	S	L	Ramage et al. (1961)
T1-6a	Mars	S	Sat	Ramage et al. (1961), Tuleen (1974)
T1-6e	Bonus	L	S?	Persson (1970), Nilan (1964)
T1-6j	Bonus	L	Sat	Persson (1970), Ramage (1971)
T1-7c	Mars	S?	Sat	Nilan (1964, Ramage and Suneson (1961)
T1-7i	Bonus, ert a	L	Sat	Ramage (1971)
T1-7k	Bonus, ert a	L	L	Persson (1970)
T2-3a	Gull	S?	S	Kasha and Burnham (1965)
T2-3c	Bonus	S?	L	Kasha and Burnham (1965)
T2-4a	Mars	-	-	
T2-4d	Bonus	S?	L	Ramage et al. (1961)
T2-5a	Bonus	L?	S?	Ramage et al. (1961)
T2-5e	Bonus	-	-	
T3-4b	Mars	-	S	Nilan (1964)
T3-4d	Bonus	-	-	
T4-5e	Bonus	L	L	Persson (1970), Ramage et al. (1961)
T4-7b	Bonus	S	Sat	Ramage et al. (1961)
T5-6b	Bonus	L	L	Hagbert et al. (1975)
T5-7g	Bonus	L	L	Tuleen (1974)
T6-7c	Bonus	S	S	Ramage et al. (1961), Ramage and Suneson (1961)
T3-7c+	Bonus	S	L	Kasha and Burnham (1965)
3-7d	Bonus	L	S	Kasha and Burnham (1965)

S = short arm; L = long arm; Sat = satellite; ? = probably in that arm

- = breakpoint not determined.

†Interchange chromosome with lower number listed first, higher number listed second.

Table 2. Description of genetic male steriles utilized to establish tester stocks.

Cultivar	CI No.	Symbol	Gene Location	Authority
Betzes	6398	msg1	near centromere of 5	Ramage (1966)
Compana	5438	msg2	near centromere of 2	Ramage (1966)
Carlsberg II	10114	msg5	near centromere of 3	Hockett and Eslick (1971)
Heines Hanna	9532	msg6	near centromere of 6	Eslick et al. (1974)
Compana	5438	msg10	near centromere of 1	Hockett and Eslick (1971), Eslick (1976)
Betzes	6398	msg14	near centromere of 1	Hockett and Eslick (1971), Eslick (1976)
Betzes	6398	msg16	on chromosome 7	Hockett and Eslick (1971)
Compana	5438	msg18	on chromosome 7	Ramage and Eslick (1975)
Intro.(Russia)	14393	msg19	near centromere of 7	Hockett and Eslick (1971)
Glacier/Compana	10861	msg22	on chromosome 1	Hockett (1972), Eslick (1976)
Betzes	6398	msg24	near centromere of 4	Jarvi and Eslick (1967)
Betzes	6398	msg25	near centromere of 4	Eslick (1971)
Betzes	6398	msg32	near centromere of 1	Eslick (1976)
Betzes	6398	msg,,bk	on chromosome 6	Eslick et al. (1974)
Betzes	6398	msg,,z	on chromosome 7	Eslick (1971)

genetic (normal homozygote)(Figure 1). Semisterile plants, usually four, from rows having no fertile plants were harvested individually. M_3 seed from these plants were planted as above. Each plant within a M_3 row was classified for spike fertility to confirm a 0 fertile:2 semisterile:1 male sterile genetic ratio (Figure 2). This sequence was continued through the M_5 generation for selected lines. Lines were selected for acceptable agronomic characteristics in addition to goodness of fit to a 0:2:1 ratio.

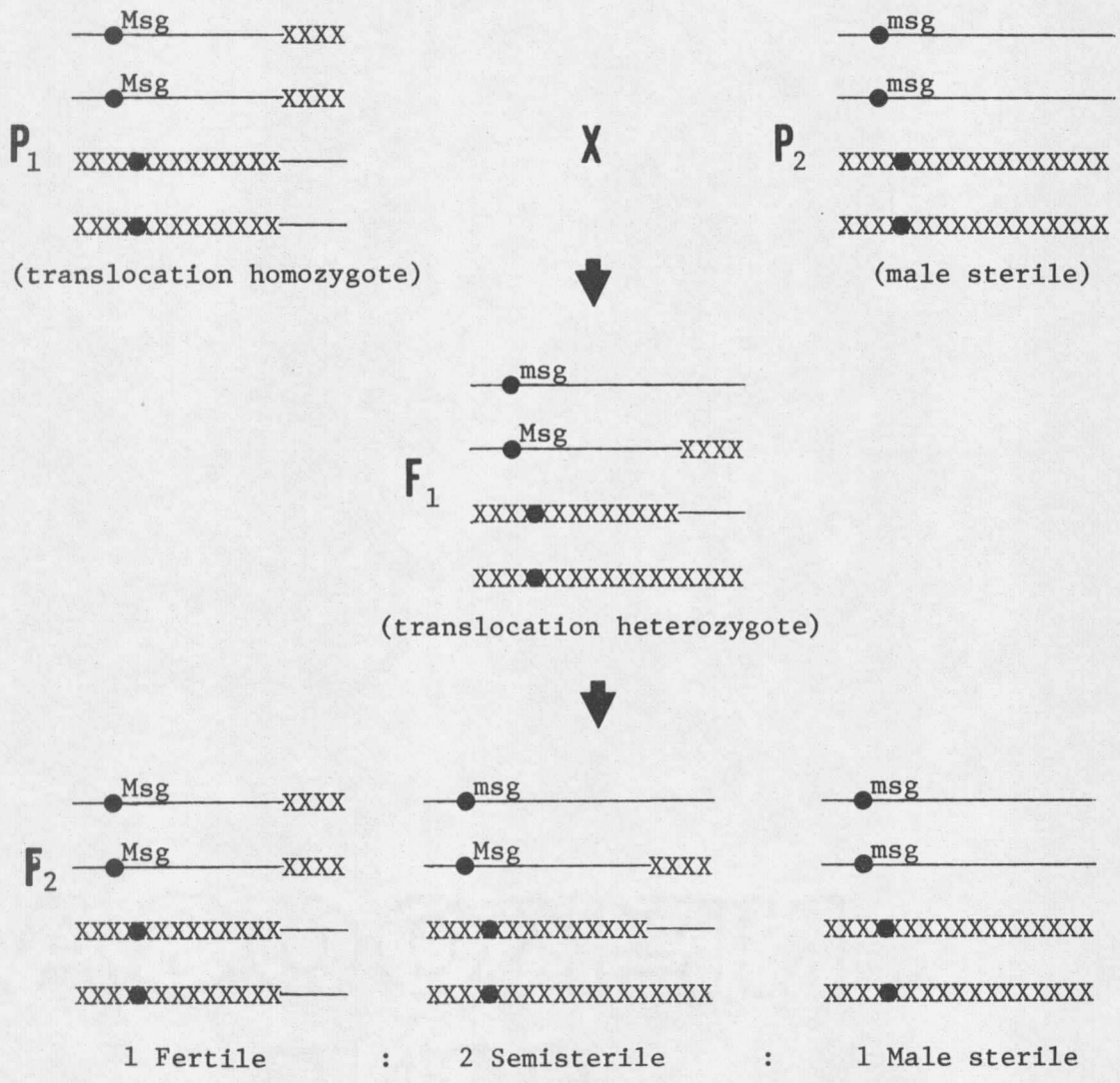

Figure 1. Diagramatic representation of the expected F_2 segregation (chromosomal and genetic) from a cross between a genetic male sterile and a translocation homozygote.

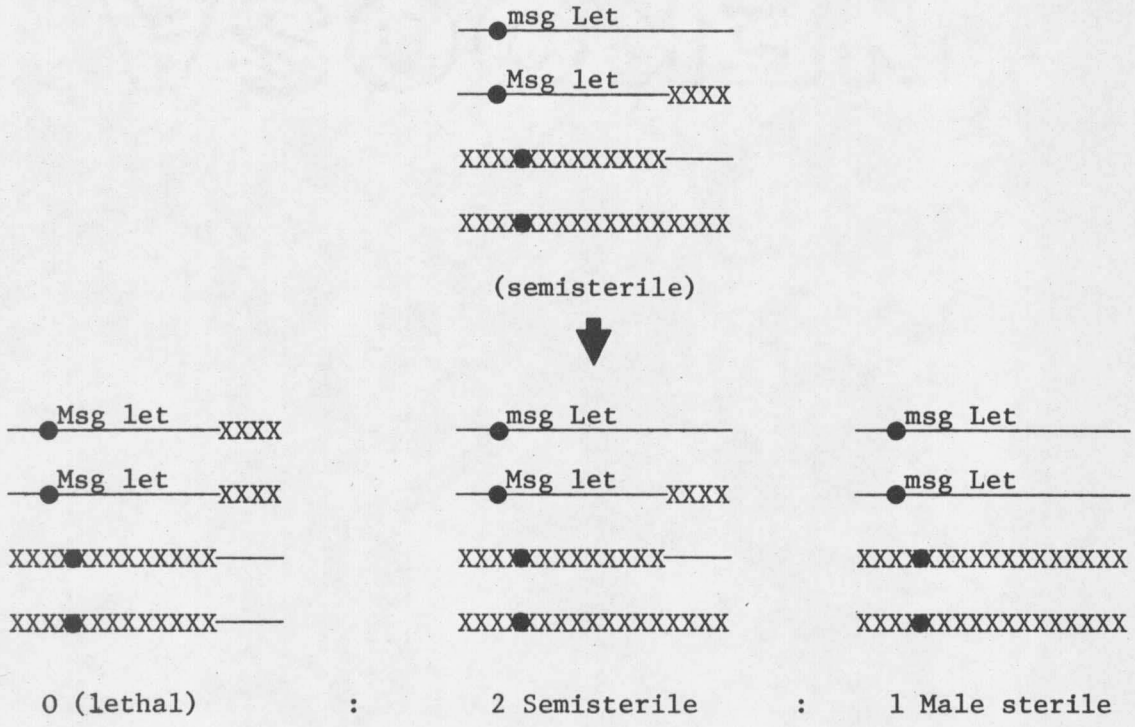

Figure 2. Diagramatic representation of the expected segregation (chromosomal and genetic) in the progeny of a translocation heterozygote having a recessive lethal gene in the interstitial segment, and a recessive male sterile gene proximal to the centromere on the normal chromosome. Msg and let represent hypothetical male sterile genetic and lethal genes, respectively.

RESULTS AND DISCUSSION

The results obtained in this study demonstrate that recessive lethal genes can be induced with diethylsulfate and recovered with the simple classification techniques outlined.

The number of head rows (or lines) selected and continued in each generation is given in Table 3. Out of 4,933 M_2 head rows, 858 appeared to be lacking the fertile (translocation homozygote) class. Most of the M_2 head rows were grown from single spikes on 2-row cultivars, thus the number of surviving plants per row was quite small. Therefore, some of the lines selected did not have fertiles by chance alone, and not due to homozygous recessive lethal genes. Approximately 70 percent of the selected M_3 and M_4 lines exhibited a 0:2:1 ratio with respect to spike fertility. Nearly 80 percent of the M_5 lines closely fit a 0:2:1 ratio. About 6 percent of the original M_2 head rows were found to fit a 0:2:1 ratio in the M_5 generation.

Use of a recessive marker gene in coupling with the translocation breakpoint is a refinement of the general procedure. The T1-7i and T1-7k translocation homozygotes used in this study were also homozygous for the ert-a dense ear locus. Ert-a has been reported to be very near the centromere of chromosome 1 (Persson, 1970), therefore, the absence of erectoides mutants in M_2 head rows was evidence of the lack of translocation homozygotes (fertiles).