Ries, Matthew Norman

HETEROPLOIDY AND CHROMOSOME INTERDEPENDENCE IN BARLEY (HORDEUM VULGARE): CYTOLOGICAL AND BREEDING BEHAVIOR OF AN EIGHT CHROMOSOME PAIRED BARLEY

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HETEROPLOIDY AND CHROMOSOME INTERDEPENDENCE IN BARLEY (*HORDEUM VULGARE*): CYTOLOGICAL AND BREEDING BEHAVIOR OF AN EIGHT CHROMOSOME PAIRED BARLEY

by

Matthew Norman Ries

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Matthew Norman Ries

entitled "HETEROPLOIDY AND CHROMOSOME INTERDEPENDENCE IN BARLEY (HORDEUM VULGARE): CYTOLOGICAL AND BREEDING BEHAVIOR OF AN EIGHT CHROMOSOME PAIRED BARLEY"

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

J E Endrizzi	Date Jan 25, 1982
M H Schonhorst	Date 25 Jan 82
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W P Bemis	Date Jan 25 1982
	Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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SIGNED: Matthew M. Ries

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To my wife Jody L. Ries and daughter Samantha. They offered the daily support which was very vital during the completion stages of this dissertation, I love you both.

This dissertation is dedicated to Dr. G. A. Wiebe (deceased), a pioneer of barley research. This dissertation research was stimulated by preliminary work conducted by him.

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ABSTRACT

The cytological and breeding behavior of barley with 8 pairs of chromosomes, of which 2 pairs are interdependent were evaluated. The 8II material originated from selfed progeny of a Balanced Tertiary Trisomic 57a msgl6. Chromosome interdependence was established after a naturally occurring reciprocal translocation between the normal chromosome 5 and the extra interchanged 57a chromosome. The interdependent chromosomes are fragment chromosomes.

A Male Sterile Facilitated Recurrent Selection Population (MSFRSP) of 8II plants was developed. Eight chromosome paired plants were crossed onto male sterile plants from barley Composite Cross XXXII. The F_1 plants from the 7II X 8II crosses carried a characteristic 15-chromosome cytotype. In the F_2 of this cross, 5 different cytotype classes of progeny were isolated by root-tip chromosome and microsporocyte analyses: 7II, F_1, 8II, tertiary trisomic where the extra chromosome is a fragment chromosome and a Unique-16 chromosome cytotype which consisted of 6 normal pairs, one pair of fragments, one normal chromosome 5 and one fragment chromosome from the other pair of fragments. The F_2 population was approximately 30% 7II, 50% F_1 and 20% 8II cytotype progeny.

Microsporocytes observed from F_2 cytotype plants indicated that the 7II and 8II progeny went through normal meiosis. The F_1 cytotypes produced functional gametes with 7 normal chromosomes, 8 chromosomes equivalent to gametes produced by 8II plants and gametes with 7 normal

chromosomes plus a fragment chromosome. The tertiary trisomic progeny produced functional gametes with 7 normal chromosomes and gametes with 7 normal chromosomes plus a fragment chromosome. The Unique-16 cytotype produced functional gametes with 7 normal chromosomes, 8 chromosomes equivalent to gametes produced by 8II plants, 7 chromosomes plus a fragment chromosome and 9-chromosome gametes with 6 normal chromosomes plus 3 fragment chromosomes, two of which are a pair.

Crosses and their reciprocals between 7II plants and plants of each F_2 cytotype indicated that the cytological stability of an 8II or 7II population would be disrupted if contaminated by pollen from 7II or 8II plants respectively. Growing the populations in physical isolation from each other is a must in order to maintain the cytotype of the population homozygous.

INTRODUCTION

Plant breeders, geneticists, cytogeneticists and genetic engineers feel that the duplication of specific chromosome segments may be beneficial to mankind. For example, the duplication of a gene for a specific plant disease resistance may increase the level of disease resistance within a population. Duplicating a gene for an enzyme in the barley seed, such as alpha amylase, may change the manner in which barley malt is produced. Duplicating a gene for an essential amino acid such as lysine in a grain may enhance its value as a food and feed. Through duplications, two members of a true multiple allelic series might be combined as a homozygote, whereas, ordinarily they could both be present only in the heterozygous condition.

Duplications of specific chromosome segments have been produced by crossing plants which were homozygous for two different interchanged chromosomes, and also by crossing plants which were homozygous for two different inverted chromosomes.

Cultivated food, feed and fiber crops may be diploids, autopolyploids or allopolyploids. Several cultivated crops expressing polyploidy are: potatoes (Solanum tuberosum), watermelon (Citrullus lanatus), sugar beets (Beta vulgaris), bread wheat (Triticum aestivum), oats (Avena sativa), alfalfa (Medicago sativa), and cotton (Gossypium hirsutum).

Polyploidy may not always be beneficial to plants. For instance, the doubling of the chromosome number of diploids to the

tetraploid level may have disproportionate effects on the physiological action of specific genes. One possible result may be male and/or female sterility.

Trisomic, monosomic, telosomic and nullisomic analysis have been instrumental in identifying linkage groups and assigning specific genes to linkage groups. Several genera where aneuploids have been employed for this use are: Nicotiana, Zea, Datura, Avena, Triticum, Gossypium, Lycopersicum and Hordeum.

Tertiary trisomics, another form of aneuploidy, provided the mechanism used by Dr. G. A. Wiebe to establish true breeding lines of barley with 8 pairs of chromosomes, as opposed to normal barley which carries 7 pairs of chromosomes. Two of the 8 pairs of chromosomes were pairs of translocated chromosomes that were described as being interdependent. Chromosomes 5 and 7 were involved in the chromosome interchanges which led to their eventual, naturally established, interdependence.

Twenty-one distinct interchange combinations are possible among the 7 pairs of barley chromosomes. The length of the exchanged segments from either chromosome involved in an interchange can be quite variable. This provides for an essentially unlimited number of interchanged chromosomes that may be used as Dr. Wiebe used T5-7a. Once plants have been established for various 8-chromosome-paired lines, they could be intercrossed to produce plants that carry 9 or more pairs of chromosomes.

The purpose of this dissertation research was to critically evaluate the mitotic and meiotic behavior of the 8-chromosome-paired

barley with two pairs of interdependent chromosomes, which is an example of heteroploidy and chromosome interdependence. Secondly, mitotic and meiotic chromosome behavior was evaluated for the progeny from crosses between 7-chromosome-paired plants and 8-chromosome-paired plants. The final aspect of the dissertation research was to develop a Male Sterile Facilitated Recurrent Selection Population (MSFRSP) of 8-chromosome-paired plants of which two of the pairs of chromosomes are interdependent.

REVIEW OF LITERATURE

Aneuploids have been used to produce duplications in both plants and animals. Beadle and Sturtevant (1935) discussed producing duplications of specific chromosome segments in Drosophila melanogaster by crossing individuals carrying specific chromosome inversions. Two types of inversion heterozygotes produced gametes carrying duplicated chromosome segments, due to crossing over during meiosis. In the first type, the two inversions have one breakpoint at the same locus (between the numbers 2 and 8 of Inverted Chromosome 1 and between the numbers 2 and 4 of Inverted Chromosome 2, see below) and the other break at a different locus (between the numbers 3 and 9 of Inverted Chromosome 1 and between numbers 3 and 5 of Inverted Chromosome 2, see below). Crossing over in the inverted region common to both chromosomes (hatch marked) during meiosis, results in one chromatid with a duplication for segment 5678.

Inverted Chromosome 1 1 2 8 7 6 5 4 3 9 10

Inverted Chromosome 2 1 2 4 3 5 6 7 8 9 10

In the second type of inversion heterozygote, the inversions overlap (see below), that is the breakpoints in the two inverted chromosomes are not at the same locus. Crossing over in the inverted region common to both chromosomes (hatch marked) results in one chromatid with a duplication for segment 3478. If the centromere is in regions 34 or 78, a dicentric chromosome will result from crossing over.

Inverted Chromosome 1 1 2 6 5 4 3 7 8 9 10

Inverted Chromosome 2 1 2 3 4 8 7 6 5 9 10

Duplicated chromosome segments have been produced by crossing individuals with selected interchanged chromosomes where the interchanges involve the same chromosomes. Gopinath and Burnham (1956) reported that Muller, in 1930, was not successful in producing duplications in Drosophila by utilizing this method. Blakeslee, Bergner and Avery (1936) used this method to produce phenotypically changed plants in Datura stramonium. They indicated that the phenotypic effects of the addition of blocks of genes without changing the chromosome number were similar to differences seen between species. Catcheside (1947) presented information on interchromosomal and intrachromosomal duplications in the progeny of a translocation heterozygote in Oenothera blandina. Catcheside accounted for these duplications by unequal crossing over.

Hagberg (1962) and Hagberg, Lehmann and Hagberg (1974) utilized the method of crossing plants with interchanges involving the same chromosomes to produce duplications in the barley genome. In the 1974 report, they were interested in duplicating segments of chromosome 5 which carried many of the known genes for resistance to powdery mildew. Chromosome 6 was used in the interchanges because the researchers were attempting to duplicate genes for high alpha amylase activity. Hagberg, Lehmann and Hagberg (1978) also reported on extensive work they had conducted utilizing chromosomes 6 and 7 of barley to produce duplications by crossing plants with interchanges involving the same chromosomes.

Kasha (1979) discussed producing duplications of Zea mays chromosomes by crossing plants from chromosome interchange stocks with interchanges in the same chromosomes. In this report, Kasha pointed out that this "Intercross Method" had been utilized in 1965 by Kasha and Burnham, to determine that chromosome pairing during meiosis of barley, was initiated at or near the chromosome ends.

Fragment chromosomes have been observed as an extra pair of chromosomes in a complement. Chromosome fragments have been inserted into an existing chromosome of a complement. Smith (1974) discussed two compensating fragment chromosomes in Triticum monococcum. The fragment chromosomes were initially isolated in progeny from an X-rayed dormant seed. The fragment chromosomes were determined to be an isochromosome and a telocentric chromosome.

Sears (1956) discussed the innovative transfer of rust resistance from Aegilops umbellulata to common wheat (Triticum aestivum). This was accomplished through a series of crosses and X-ray irradiation. Sears indicated that the cross T. aestivum (n=21; genomes A, B and D) X A. umbellulata (n=7; genome C) and its reciprocal cross produced only inviable seed. In order to produce viable seed, the cross had to be bridged with an amphiploid of T. dicoccoides (n=14; genomes A and B) X A. umbellulata.

Forty-seven seed were produced from the cross T. aestivum varity Chinese Spring X ((T. dicoccoides X A. umbellulata) X T. aestivum). Thirty-nine of the 47 seed germinated, 18 of the seedlings were classified as rust resistant. Two of the rust resistant

plants carried a chromosome complement of 20 pairs plus 3 univalents (7 chromosome pairs of the A genome, 7 chromosome pairs of the B genome, 6 chromosome pairs of the D genome plus a monosomic of the D genome and 2 Aegilops chromosomes).

One of the rust resistant plants with a chromosome complement of 20 pairs plus 3 univalents was crossed onto an emasculated female plant of the variety Chinese Spring. Seed from this cross produced a rust resistant seedling with a chromosome complement of 21 pairs plus a single Aegilops chromosome.

One hundred nineteen progeny were produced from the single rust resistant plant with a chromosome complement of 21 pairs plus a single Aegilops chromosome. One of the 119 progeny was rust resistant with a chromosome complement of 21 pairs plus an isochromosome for the long arm of the Aegilops chromosome.

Thirty-four plants with 21 chromosome pairs plus the isochromosome from the Aegilops chromosome were irradiated with x-rays. Six thousand ninety-one progeny were produced by the irradiated plants, 132 of these progeny were rust resistant. One of the rust resistant plants was shown cytologically to carry 42 chromosomes with one of the normal wheat chromosomes carrying an intercalary segment of the Aegilops chromosome, which carried genes conditioning for rust resistance.

Dyck and Rajhathy (1963) discussed the cytogenetics of a hexaploid oat, resistant to the crown rust fungus Puccinia coranata Cda. var. avenae Fraser and Led., with an extra pair of chromosomes.

The plants utilized by Dyck and Rajhathy were F_4 and F_5 progeny of a cross between an autotetraploid Avena strigosa (2n=28), line C.D. 3820, and two Avena sativa, (2n=42), varieties, Abegweit and Victory, reported by Zillinsky and Derick (1960). It was concluded that the extra pair of chromosomes, in these hexaploid oats, carried the dominant allele of the Pc-15 gene that conditioned resistance to races 264 and 294 of crown rust. A second gene, Pc-23, that conditioned resistance to race 264 of crown rust was also carried on the extra pair of chromosomes.

Dherawattana and Sadasaga (1973) reported that an oat line, designated X117, had a chromosome complement of 42 normal chromosomes plus a pair of fragment chromosomes. This line also carried the dominant allele of the Pc-15 gene that conferred resistance to races 264 and 290 of the crown rust fungus of oats. Line X117 originated from the cross C.I. 7232 X 'Burnett' 2X Clintland 3X 'Cherokee' 4X Clintland 5X C.I. 7555. All of the parental lines in the cross were hexaploid (2n=42) except C.I. 7232 (2n=28) which was a rust resistant derived tetraploid from the triploid hybrid between C.D. 4549 (Avena abyssinica) and C.D. 3820 (Avena strigosa). It was determined that the dominant allele of the Pc-15 gene, conferring resistance to races 264 and 290 of crown rust of oats was located on each of the fragment chromosomes.

Frost and Ising (1964) discussed two different fragment chromosomes of barley (Hordeum vulgare) which were pollen and ovule transmitted. Both fragment chromosomes originated from the selfed progeny of a spontaneous triploid barley plant. The fragment chromosomes were

designated fr_1 and fr_3 and both were shorter than normal barley chromosomes. Fragment chromosome fr_1 had a pronounced secondary constriction and a subterminal centromere. Fragment fr_3 was noted to have only the subterminal centromere. These researchers reported reduced ovule and pollen fertility of plants that carried the fragment chromosomes, compared to normal 7 chromosome paired barley. They also observed occasional multivalents during meiosis, in which the fragment chromosomes were involved.

In 1969, Tsuchiya reported the occurrence of a line of barley with 16 chromosomes, isolated from the progeny of a plant trisomic for chromosome 6, in which the extra chromosome had a pericentric inversion accompanied by a deficiency. The extra chromosomes were a pair of metacentric chromosomes. Circumstantial evidence suggested that the metacentric chromosomes arose either from crossover(s) among three chromosomes, six (two normal and one having an inversion-deficiency) or from a bridge-break-fusion cycle in the inverted chromosome. Tsuckiya reported no multivalent formation in his 16 chromosome barley line, but did report some precocious separation of chromosomes during meiosis. The chromosomes that displayed the precocious separation were assumed to be the pair of metacentric chromosomes. Tsuchiya also reported reduced ovule and pollen fertility in this barley line with 16 chromosomes.

Many times a complete chromosome rather than a specific chromosome segment is desired to be duplicated for a certain type of genetic or cytological analysis. Burnham (1962) cited the following researchers for reporting primary trisomics: Belling and Blakeslee, in 1924,

for primary trisomics of Datura; Beadle, in 1930, for maize (Zea mays); Beasley and Brown, in 1942, for cotton (Gossypium spp.); Poa and Li, in 1945, for wheat (Triticum vulgare); Lesley, in 1928, for tomato (Lycopersicon esculentum); Takagi, in 1935, for rye (Secale cereale); Sutton, in 1939, for peas (Pisum sativum); Emerson, in 1936, for Oenothera; Goodspeed and Avery, in 1939, for tobacco (Nicotiana sylvestris); and Ellis and Janick, in 1960, for spinach (Spinacia oleracea).

Primary trisomics were reported in cultivated barley (Hordeum vulgare) (Ramage, 1955) and in a wild species of barley (Hordeum spontaneum) (Tsuchiya, 1958). Rick and Notani (1961) concluded that the very old "Primitive" variety of tomato, Red Cherry, was more tolerant of aneuploidy than the highly selected horticultural varieties. This was not the case for the cultivated and wild species of barley reported by Ramage and Tsuchiya, respectively. Rick and Notani stated that according to Ramage the genotype of the plant was a more important factor to consider regarding the stability of aneuploids, rather than whether the plants were wild or domesticated.

Rick and Gill (1973) reported that variant extra-chromosomal types have been produced by tomato primary trisomics. Variant types reported were other primary trisomics, secondary trisomics, tertiary trisomics and a telotrisomic.

Polyploidy is a condition where an individual has more than two sets of chromosomes. In autopolyploids, each of the repeated sets of chromosomes is considered to be identical. In allopolyploids, the

two or more basic sets of chromosomes (genomes) are considered to be different from each other (Rieger, Michaelis and Green, 1976).

Allard (1960) stated many cultivated polyploid crop species, seedless bananas (Musa balbisiana) and seedless watermelons (Citrullus lanatus) are produced from triploids. Triploid sugar beets (Beta vulgaris) are commonly grown in Europe and in the United States. A large proportion of the apples (Malus pumila) in America and pears (Pyrus communis) in Europe are also triploids. Four of the major crop species are reported to be autotetraploids. These are alfalfa (Medicago sativa), potatoes (Solanum tuberosum), coffee (Coffea arabica) and peanuts (Arachis hypogaea). Other important cultivated allopolyploid crops are tobacco (Nicotiana tobacum), cotton (Gossypium hirsutum), three Brassica species, oil seed mustard (B. carinata), oilseed rape (B. juncea) and rutabaga (B. napus), wheat (Triticum aestivum) durum (Triticum durum) and oats (Avena sativa).

Wiebe, Ramage and Eslick (1974), and Ramage (1981), described an eight-chromosome-paired barley. Barley lines with 8 pairs of chromosomes originated from a cross between a normal 7-chromosome-paired plant that was homozygous for the genetic male sterile allele msg16 on chromosome 7, and an interchange homozygote T5-7a plant. Balanced tertiary trisomics (BTT 57a msg16) were isolated from the F_2 and later generations of this cross. Vigorous BTT's as well as some less vigorous true breeding lines were isolated from the progeny of BTT's. Cytological examination of the vigorous trisomics showed that the extra chromosome had fragmented to approximately one-third of its original length. The true breeding lines were found to have eight pairs of

chromosomes. The extra pair of chromosomes was a pair of fragments. The fragmentation had shortened the extra chromosome to a length which reduced the adverse effects of the extra chromosome and also permitted its transmission through the pollen. According to Ramage (1981), the 8II lines carried the recessive msg16 allele on both normal chromosomes 7.

Wiebe (1974) reported yield and other agronomic information for two different maturity classifications of 8 chromosome paired barley, "early maturity and late maturity." Yields were less than local check varieties, Unitan and Steptoe, but the 8 chromosome paired lines yielded up to 89% of the checks at some locations. According to Ramage (1981), this material was derived from crosses of 8II plants onto msg1 diploids from one of the components of Composite Cross XXXII (Ramage, Thompson and Eslick, 1976).

In 1974, when Wiebe and associates reported the development of the 8-chromosome-paired barley, they pointed out that the extra pair of chromosomes was a "short" pair. They attributed this reduction in length of the chromosomes to a "chopping" affect at both ends of the extra chromosomes. They proposed a genetic scheme, using two balanced lethal genes or "chopper stoppers", to assure that the extra chromosomes would not be totally eliminated.

Wiebe in 1975, discussed his 8-chromosome-paired barley with some encouraging information concerning the stability of the extra pair of chromosomes. Wiebe states; "In recent observations, we have observed a natural adjustment in the genome itself which stabilizes

plants at eight chromosome pairs. Our interpretation is that a reciprocal translocation occurred between normal chromosome 5 and the extra chromosome. This event makes the two translocated chromosomes interdependent and consequently the plant is stabilized at eight pairs. Here (the) normal chromosome 5 is missing and there are two pairs of shorter chromosomes differing somewhat in length." Now that the genome was stabilized at 8 chromosome pairs, with two pairs of interdependent chromosomes, it would be possible to develop a large stable population of 8-chromosome-paired plants with a wide germplasm base as well as carrying a male sterility gene to facilitate intercrossing.

Kasha and others (1975) reported on the production of 8 chromosome barley haploids from Wiebe's eight-chromosome-paired material. They utilized the initial 8-chromosome-paired lines, not the 8-chromosome-paired lines with two pairs of interdependent chromosomes. In their findings, rarely did they observe the small extra chromosome in association with the normal chromosomes 5 or 7 of which it is composed.

Fedak (1976a and 1976b) reported on the meiosis of 8-chromosome-paired barley from Wiebe, and on the meiosis of 8-chromosome barley haploids, respectively. Fedak commented that he had never observed the extra pair of chromosomes to be in paired associations with the normal chromosomes, in 8-chromosome-paired lines. Fedak, however, observed occasional associations, but never pairing, of the extra chromosome with normal chromosomes in 8-chromosome haploids.

Finch and Kasha (1976) confirmed the findings of Fedak concerning the pairing behavior of the 8-chromosome-paired barley and the 8-chromosome haploids. In addition, they stated that through personal communication with K. Noda, chromosome banding information indicated the extra chromosome has the centromere region of chromosome 5.

Fedak (1976c and 1977) restated his findings about meiotic chromosome pairing of the 8-chromosome-paired barley and the 8-chromosome barley haploids. From all of the information published to date, no haploids have been produced from the 8-chromosome-paired barley of which two pairs are interdependent. Also, to date, the only meiotic information reported concerning the 8-chromosome-paired barley of which two pairs are interdependent was by Wiebe at the Third International Barley Genetics Symposium in 1975.

Recently (Ries and Ramage, unpublished data) a new eight-chromosome-paired line was isolated from the selfed progeny of a tertiary trisomic, involving chromosomes 2 and 7. This was the way Wiebe isolated his eight-chromosome-paired barley. The extra pair of chromosomes in this eight-chromosome-paired line appeared to be sub-metacentric to nearly telocentric. The extra pair of chromosomes was observed paired with a pair of normal chromosomes at metaphase I of meiosis, forming a chain of four chromosomes.

Chromosome interdependence, from an irradiation induced reciprocal translocation, will try to be established between the extra chromosome pair and a normal pair of chromosomes. Once chromosome interdependence is established in the new eight chromosome paired

line, the two eight-chromosome-paired lines could be crossed to produce a nine-chromosome-paired barley.

MATERIALS AND METHODS

All of the experiments in this study were conducted in the cytology laboratory, in the greenhouse, in the field or required the use of a combination of these facilities.

The following modified acetocarmine squash technique was used for root tip chromosome analyses. Barley seeds were germinated at room temperature (20C). When the primary roots were approximately 2 cm in length, they were removed and placed into a vial containing a water saturated solution of alpha bromonaphthalene. After a period of two and one-half hours, the roots were transferred to a vial containing warm (55C) 1 N HCl for one minute to kill and hydrolyze the root tips. The root tips were then washed in water and stored in a vial filled with acetocarmine until they were analyzed.

Meiotic chromosome behavior was studied by analyzing microsporocytes at diakinesis or metaphase I with an acetocarmine squash technique. All material collected for microsporocyte analysis was killed and fixed in a solution of 3 parts 95% ethanol and 1 part glacial acetic acid.

Pollen viability was determined by staining mature pollen with a dilute solution of IKI_2. Pollen that stained blue was classified as viable, nonstaining pollen was classified as inviable.

Ovule fertility determinations were made by determining the percentage of seed-carrying florets present on a mature spike.

In the greenhouse, plants were grown in pots filled with a planting medium which was 4 parts topsoil, 1 part vermiculite, 1 part sand and 1 part peatmoss, on a volume basis. The plants were watered with ground water and a top dressing of 16-20-0, N-P-K, granular fertilizer was applied when needed.

In the field, the seed was sown into a soil classified as a 13 J Pima Loam. Seed produced from single plants and bulk populations of plants were both grown.

Initial research efforts were to verify, by root-tip-chromosome analyses the eight-chromosome-paired seed stocks. These stocks were used to produce eight-chromosome-paired plants.

Meiotic chromosome behavior of eight-chromosome-paired plants was studied to determine if meiosis was normal. Pollen viability and ovule fertility were also determined for the 8-chromosome-paired stocks.

The main objective of this research was to develop a wide germplasm base population of barley plants that had 8 pairs of chromosomes with two of the pairs being interdependent. To accomplish this objective, pollen produced by 8-chromosome-paired plants was used to pollinate male-sterile plants in barley Composite Cross XXXII. Composite Cross XXXII is a short, stiff-strawed, wide germplasm base population which has normal seven-chromosome-paired plants (Ramage, Thompson and Eslick, 1976). The F_1 was grown from which F_2 seed was harvested.

Root-tip-chromosome analyses were used to isolate 8-chromosome paired and other cytotype seedlings germinated from F_2 seed. These classified seedlings were transplanted to pots and grown in the

greenhouse so microsporocytes could be collected from them for analyses of meiotic chromosome behavior.

Microsporocyte analyses were also used to isolate 8-chromosome-paired and other cytotype plants. Microsporocytes were collected from individual, agronomically desirable plants, grown in a bulk F_2 population. The plants from which microsporocytes were collected were also classified as male fertile or male sterile. Seed was harvested from all fertile plants.

Another objective of this research, the determination of pollen and egg transmission of specific chromosomes, required making cross pollinations and their reciprocals between 7-chromosome-paired plants and all different cytotype plants isolated from the F_2 of the 7II X 8II cross. Seed was germinated from these crosses and reciprocal crosses for use in root-tip-chromosome analyses to determine which chromosomes were transmitted through the pollen and/or egg.

Seed produced on all plants classified as 8II from the 7II X 8II cross were used to develop the initial male sterile facilitated recurrent selection population for 8II plants. The population will be used as a source of potential varieties and as a germplasm source. Samples of the population will be treated with different mutagenic agents to try to induce favorable mutations in the extra genetic material carried in the extra pair of chromosomes.

RESULTS AND DISCUSSION

Introduction

A brief review of the origin of the 8 chromosome paired barley with two pairs of interdependent chromosomes will aid in the discussion of the results. Wiebe, Ramage and Eslick (1974) and Ramage (1981) described the origin of an 8-chromosome-paired barley as follows: Barley with 8 pairs of chromosomes originated from a cross between a normal 7-chromosome-paired plant that was homozygous for the genetic male-sterile allele, msg16 on chromosome 7, and an interchange homozygote T5-7a plant. Balanced-tertiary trisomics (BTT 57a msg16) were isolated from the F_2 and later generations of this cross. Vigorous BTT's as well as some less vigorous true-breeding lines were isolated from the progeny of BTT's. Cytological examination of the vigorous trisomics showed that the extra chromosome had fragmented to approximately one-third of its original length. The true breeding lines were found to have 8 pairs of chromosomes. The extra pair of chromosomes was a pair of fragments. The fragmentation had shortened the extra chromosome to a length that reduced the adverse effects of the extra chromosome and also permitted its transmission through the pollen. According to Ramage (1981) the 8-chromosome-paired lines carried the recessive msg16 allele on both normal chromosomes seven.

In 1975, Wiebe states, "In recent observations, we have observed a natural adjustment in the genome itself which stabilizes

plants at eight chromosome pairs. Our interpretation is that a reciprocal translocation occurred between normal chromosome 5 and the extra chromosome. This event makes the two translocated chromosomes interdependent and consequently the plant is stabilized at 8 pairs. Here normal chromosome 5 is missing and there are two pairs of shorter chromosomes differing somewhat in length."

The 8-chromosome-paired barley of which two pair are interdependent, from here on in the discussion, will be referred to simply as 8-chromosome-paired (8II). These plants had normal pairs of chromosomes 1, 2, 3, 4, 6 and 7 plus a pair of interchanged chromosomes five and a pair of double interchanged fragmented T57a chromosomes.

Verification of 8II Lines

Root-Tip Chromosome Analyses

Before using plants of the 8-chromosome-paired lines as pollen sources, in establishing the MSFRSP with 8-chromosome-paired plants, they were verified by root-tip-chromosome analyses. Root-tip chromosomes were analyzed from 87 germinated seed. All of the root-tip chromosome preparations were observed to carry 16 chromosomes. The seedlings were transplanted to the greenhouse and grown so microsporocytes could be collected from them.

Microsporocyte Analyses

Microsporocytes were collected and analyzed from plants from which root-tip chromosomes were analyzed. Microsporocytes were observed at diakinesis with 8 ring-bivalents, 7 ring-bivalents plus one

rod-bivalent or 6 ring-bivalents plus two rod-bivalents. The rod-bivalents were formed by one or both of the pairs of fragment chromosomes. Each fragment chromosome paired only with its respective homologue, never forming multivalents with any other chromosomes of the complement.

One possible explanation for the fragment chromosomes failing to consistently form ring-bivalents is as follows. Barley chromosomes were reported to initiate pairing of homologous chromosomes during meiosis at or near the ends of each chromosome arm (Kasha and Burnham, 1965). Linde-Laursen (1981 and 1975) and Noda and Kasha (1976) reported wide heterochromatic regions, from Giemsa C-banding experiments, adjacent to the centromeres of most barley chromosome arms. These heterochromatic regions may interfer with effective pairing of the short arms of the fragmented chromosomes of the 8-chromosome-paired barley lines.

Not observing any microsporocytes with multivalents is also supportive evidence that barley chromosomes initiate meiotic pairing at or near the ends of the chromosome arms. If pairing were initiated at an interstitial segment of the chromosome arm, one would expect to see some pairing of the fragment chromosome carrying a segment of chromosome 7, with the normal chromosomes 7 of the complement, thus forming multivalents. Pairing of one of the fragment pairs with the normal chromosome 7 was never observed.

The above evidence supports the idea that pairing initiates at or near the ends of the chromosome arms in barley. Once the pairing has been initiated, there are undoubtedly other areas of

pairing along the chromosome arm or it would take an infinite length of time to complete synapsis of a single chromosome. Microsporocytes divide much too rapidly to have only one pairing site on each chromosome arm.

Pollen Viability

Pollen viability ranged from 70.7 to 99.5% for 96 field-grown, 8-chromosome-paired plants (Table 1). From the complete group of 96 plants analyzed, only four plants, numbers 18, 62, 77 and 90 were determined to have pollen viabilities below 85%. The factor ascribed to the low pollen viability of these four plants was that the pollen was collected from flowers that were near the end of anthesis, instead of from flowers initiating anthesis. This conclusion was reached because all of the flowers except from plant numbers 18, 62, 77 and 90, were initiating anthesis.

One possible explanation for inviable pollen being isolated from flowers near the end of anthesis is as follows. The last pollen grains formed within the confines of the anther sac may be physically restricted from maturing. These immature pollen grains may be the last to be dispersed, or may never be dispersed under natural conditions. When anthers are cut and macerated for pollen viability determinations, the immature pollen may be teased from the anthers and are in the preparation. These pollen grains would not stain, therefore being classified as inviable.

Table 1. Pollen viability* determined for 96 field grown plants from Wiebe's original eight-chromosome-paired lines of which two pairs are interdependent.

Plant No.	% Viability	Plant No.	% Viability	Plant No.	% Viability	Plant No.	% Viability	Plant No.	% Viability	Plant No.	% Viability
1	90.4	17	90.7	33	96.3	49	96.5	65	95.4	81	96.2
2	94.3	18	73.0	34	97.4	50	97.0	66	97.5	82	97.8
3	93.5	19	92.1	35	93.5	51	97.6	67	97.8	83	97.3
4	97.3	20	97.1	36	97.5	52	95.5	68	98.3	84	96.9
5	96.4	21	96.3	37	94.3	53	95.3	69	97.2	85	96.2
6	94.2	22	95.7	38	94.2	54	97.3	70	95.0	86	97.7
7	97.4	23	97.5	39	96.4	55	98.6	71	96.7	87	99.3
8	97.1	24	98.0	40	94.8	56	96.2	72	97.3	88	98.2
9	97.8	25	97.6	41	97.0	57	99.5	73	92.0	89	97.4
10	91.3	26	98.0	42	98.9	58	97.1	74	97.8	90	70.7
11	95.6	27	87.5	43	94.5	59	98.7	75	97.7	91	95.5
12	95.6	28	96.8	44	98.2	60	97.9	76	95.1	92	92.1
13	98.8	29	95.0	45	96.6	61	97.7	77	81.1	93	96.2
14	97.4	30	96.8	46	89.4	62	78.0	78	87.1	94	97.6
15	91.4	31	95.8	47	96.5	63	98.9	79	96.3	95	98.5
16	96.9	32	94.5	48	97.7	64	96.1	80	93.1	96	96.2

*Pollen viability determined by staining pollen with IKI_2. Viable pollen stains blue, nonviable pollen does not stain.

Pollen viability was considered to be normal as compared to pollen produced from normal 7-chromosome-paired barley lines, with the exception of the four plants, numbers 18, 62, 77 and 90.

Ovule Fertility

Ovule fertility was determined to be essentially 100% for 288 spikes. These determinations were made from 3 spikes from each of the plants analyzed for pollen viability. There were one or two rudimentary florets at the base of each spike that did not contain seed. These florets were disregarded because this condition was consistent for all spikes counted, and is also a condition observed in most normal 7II barleys.

With all four factors considered, the root-tip-chromosome analyses, the microsporocyte analyses, the pollen viability determinations and the percentage of seed set, it was concluded that the 8-chromosome-paired lines would be excellent male parents for a 7II X 8II cross.

Establishing of an MSFRSP With 8 Pairs of Chromosomes

Production of F_2 Seed From 7II X 8II Crosses

The main objective of this research was to develop an MSFRSP of barley plants that had 8 pairs of chromosomes of which two pairs are interdependent. Genetic male-sterile plants in a Composite Cross XXXII population were crossed with verified 8II plants. The initial 7II X 8II crosses produced 10,162 seed. The number of crosses that

produced the F_1 seed was inadvertently not recorded. Information from previous years' crosses indicated that approximately 50 seed were produced from each cro-s. With this information it was estimated that between 200 and 250 crosses produced the F_1 seed.

All F_1 seed was bulked and planted as a population. All of the F_1 plants should have had a characteristic 15-chromosome cytotype. This cytotype will be referred to as the F_1 cytotype in further discussion.

One spike was harvested from each of 536 agronomically desirable F_1 plants. A bulk harvest of the F_1 population netted approximately 22.5 kg (50 lbs) of seed. This F_2 seed served as source material for the isolation of 8II plants for the MSFRSP.

Isolation of 8II Plants for the MSFRSP

Plants with 8 pairs of chromosomes were needed to establish the initial MSFRSP for plants with 8 pairs of chromosomes. These 8-chromosome-paired plants were isolated from the F_2 progeny of 7II X 8II crosses.

Root Tip Chromosome Analyses of F2 Progeny. Root tip chromosomes were analyzed from 638 germinated seed from F_2 spikes of 7II X 8II crosses. Five different cytotype classes were isolated: 171 plants had a 7II cytotype, 295 plants had the F_1 cytotype, 121 plants had an 8II cytotype, 22 plants had a tertiary trisomic cytotype and 29 plants had a Unique-16 chromosome cytotype.

Assuming that only genetically balanced pollen accomplishes fertilization and that balanced eggs as well as eggs with an extra

chromosome function, it was possible to illustrate the origin of the 5 different cytotype classes isolated from the F_2 of 7II X 8II crosses. There are 4 possible reciprocal interchanges between the fragment 57a chromosome and the normal chromosome 5, that could establish chromosome interdependence. Only one of the four possible reciprocal interchanges is illustrated. Any of the four combinations would have the same results.

Continuing with the above assumption, the F_1 cytotype produced four types of functional eggs and two types of functional pollen as illustrated in Figure 1. One type of egg carried 7 normal chromosomes. When 7-chromosome gametes were formed during meiosis, there were also 8-chromosome gametes formed, because the F_1 carried 15 chromosomes. The 8-chromosome gametes were equivalent to gametes produced by 8II plants, and were considered to be genetically balanced.

Two other types of eggs were also formed, those that carried 7 normal chromosomes plus one extra chromosome, the extra chromosome being one or the other of the fragment chromosomes. Both of these gametes carried a large duplication.

The two types of pollen that functioned carried either 7 normal chromosomes or 8 chromosomes, equivalent to the gametes produced by 8II plants. From Figure 1, it was determined that 5 different cytotype classes of progeny arose from the F_2 progeny of 7II X 8II crosses.

These 5 different cytotype classes were the normal 7II cytotype that had 7 pairs of normal chromosomes (Fig. 2), the F_1 cytotype that had 6 normal chromosome pairs, one normal chromosome five, and one chromosome from each of the pairs of fragment chromosomes (Fig. 3),

Figure 1. A schematic explanation of the cytotypes produced in the F_1 and F_2 of 7II X 8II crosses. Only normal chromosomes 5, interchanged chromosome 5 and interchanged fragment chromosome 57a are diagrammed, other chromosomes are represented by numbers.*

A. Initial 7II X 8II cross produced a 15 chromosome F_1.

7II Female		8II Male
1 2 3 4 .----o---- 6 7 1 2 3 4 .----o---- 6 7	X	1 2 3 4 .----o-xx -xx--o--+ 6 7 1 2 3 4 .----o-xx -xx--o--+ 6 7 Inter. 5 Inter. Frag. 57a

F_1 1 2 3 4 .----o---- .----o-xx -xx--o--+ 6 7
1 2 3 4 6 7

B. Self pollinating the F_1 resulted in 5 different cytotype F_2's.

eggs / pollen	.----o----	.----o-xx -xx--o--+	.----o---- .----o-xx	.----o---- -xx--o--+
.----o----	.----o---- .----o---- 7II	.----o---- .----o-xx -xx--o--+ F_1	.----o---- .----o---- .----o-xx Tertiary trisomic	.----o---- .----o---- -xx--o--+ Tertiary trisomic
.----o-xx -xx--o--+	.----o---- .----o-xx -xx--o--+ F_1	.----o-xx .----o-xx -xx--o--+ -xx--o--+ 8II	.----o---- .----o-xx .----o-xx -xx--o--+ Unique-16	.----o---- -xx--o--+ -xx--o--+ .----o-xx Unique-16

* x = terminal interstitial segment of chromosome 7 due to chromosome fragmentation of the 57a chromosome.
+ = terminal interstitial segment of chromosome 5 due to chromosome fragmentation of the 57a chromosome.

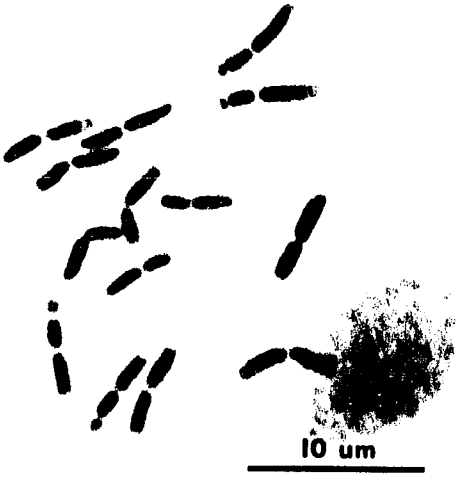

Figure 2. 7II cytotype, normal root-tip chromosomes.

Figure 3. F_1 cytotype, root-tip chromosomes, note normal chromosome 5 (upper arrow) and two fragment chromosomes, (other arrows).

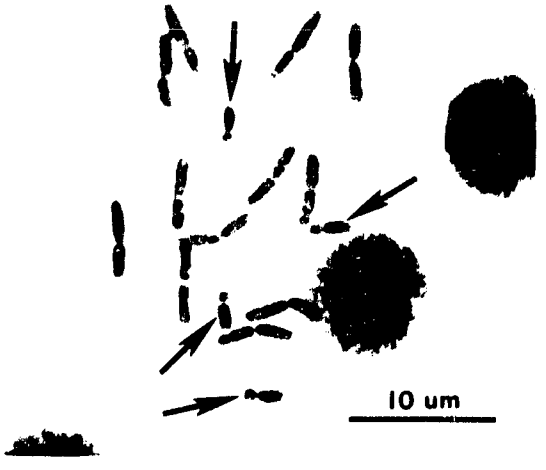

Figure 4. 8II cytotype, root-tip chromosomes, note two pairs of fragment chromosomes (upper and left arrows, bottom and right arrows).

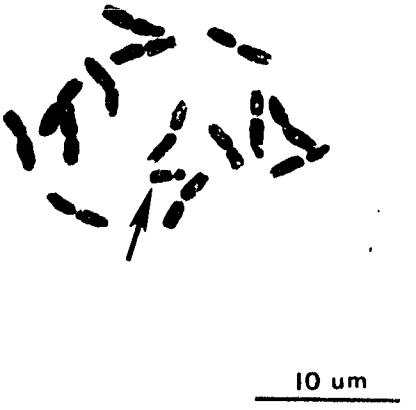

Figure 5. Tertiary-trisomic cytotype, root-tip chromosomes, note fragment chromosome (arrow).

the 8II cytotype that had 6 normal chromosome pairs plus two pairs of fragment chromosomes (Fig. 4), the tertiary trisomic cytotype that had 7 normal pairs of chromosomes plus one fragment chromosome (Fig. 5) and the Unique-16 cytotype that had 6 normal pairs of chromosomes, one pair of fragment chromosomes, one chromosome 5 and one fragment chromosome from the other pair of fragment chromosomes (Fig. 6).

Based on Figure 1, it was presumed that in the F_2 progeny the tertiary trisomic had two different chromosome complements depending upon which of the fragment chromosomes was extra. The Unique-16 cytotype plants also had two different chromosome complements, depending upon which fragment chromosome was present as a pair and which was present as a single chromosome. There was no distinction made between the two different complements of these two cytotypes when root tip chromosomes were analyzed.

The Initial MSFRSP of 8II Plants. Root tip chromosome analyses of the 638 germinated F_2 seed isolated 121 8II plants. Eighty-four rows were grown from seed of fertile 8II plants. These rows of plants constituted the initial MSFRSP of 8II plants. Fourteen of these rows segregated for male-fertile vs male-sterile plants. One hundred seventy-five intercrosses between rows produced 7953 F_1 seed. The F_1 seed was bulked, planted as a population, and harvested in bulk. A large F_2 population was planted in the fall of 1981 so the second cycle of recurrent selection and intercrossing could be made. This is the only population of barley, available to plant breeders, which has exploitable genetic variability in excess of what is present in a normal 7II barley population.

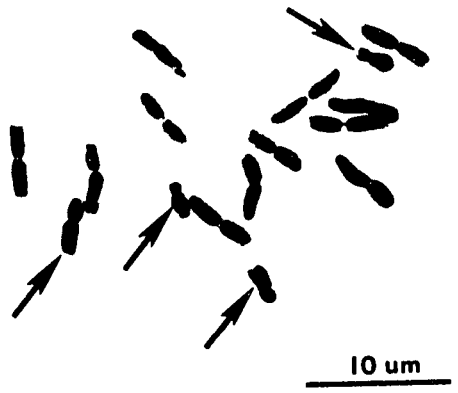

Figure 6. Unique-16 cytotype, root-tip chromosomes, note normal chromosome 5 (left arrow) and the three fragment chromosomes (other arrows).

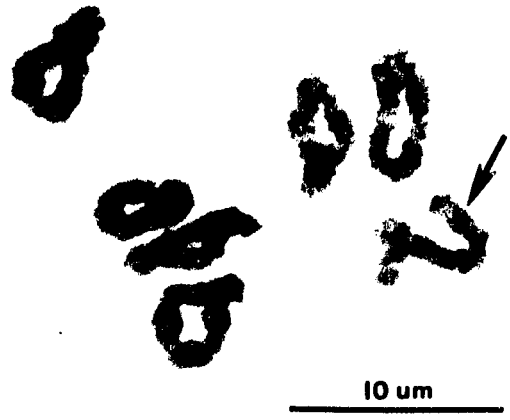

Figure 7. F_1 cytotype, diakinesis, 6II plus one chain-of-3 chromosomes (arrow).

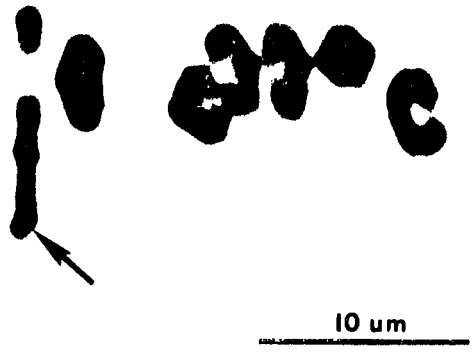

Figure 8. F_1 cytotype, metaphase I, 6II plus one straight chain-of-3 chromosomes (arrow).

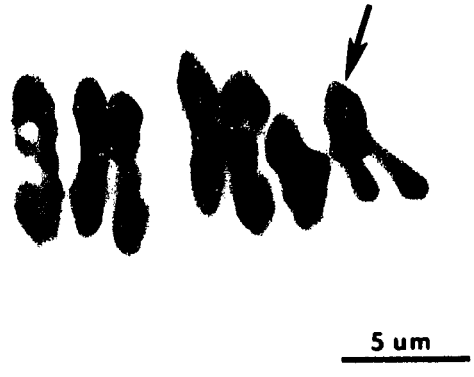

Figure 9. F_1 cytotype, metaphase I 6II plus one V-shaped chain-of-3 chromosomes (arrow).

Additional 8II plants were isolated by microsporocyte analyses of F_2 plants from 7II X 8II crosses. Microsporocyte analyses of 435 plants isolated 115 7II plants, 220 F_1 cytotype plants, 85 8II plants, 13 tertiary trisomic plants and 2 Unique-16 cytotype plants. Of the 85 8II plants, 81 were male fertile and 4 were male sterile. Seed was harvested from all of the fertile 8II plants. Progeny from these 8II plants will be crossed onto male sterile plants in the MSFRSP of 8II plants. Any additional 8II plants isolated by whatever means, from the F_2 of 7II X 8II crosses, will also be added to the MSFRSP of 8II plants.

Supportive Cytological Analyses for the MSFRSP of 8II Plants

Meiosis of the F_2 Cytotypes

Normal meiosis for the 8II plants was essential in order to develop an MSFRSP of 8-chromosome-paired plants. Analyses of the meiotic chromosome behavior of plants with the F_1 cytotype aided in determining the frequency of 8II plants and other cytotype plants from the F_2.

Microsporocyte Analyses of the 7II Cytotype. All microsporocytes produced by 7II cytotype plants were observed to be normal. Seven bivalents were observed in all microsporocytes and observations indicated that meiosis was normal.

Microsporocyte Analyses of the F_1 Cytotype. Microsporocytes collected from F_1 cytotype plants isolated from the F_2 of 7II X 8II crosses were analyzed. A total of 149 microsporocytes were observed

at diakinesis: 146 had 6 ring-bivalents plus one chain-of-3 chromosomes (Fig. 7). The chain-of-3 chromosomes was composed of a normal chromosome 5 with one of each of the fragment chromosomes attached to either end. Three microsporocytes were observed at diakinesis with 6 ring-bivalents, one unequal-bivalent and one univalent. The univalent was a fragment chromosome.

There is no way to determine the disjunction behavior of a specific chromosome configuration when they are at diakinesis. For instance, a microsporocyte observed at diakinesis with 6II plus one chain of 3 chromosomes may align at metaphase I as 6 ring bivalents and one straight chain-of-three or as 6 ring-bivalents and one V-shaped chain-of-3 chromosomes.

A total of 664 microsporocytes were observed at metaphase I: 223 had 6 ring-bivalents plus one straight chain-of-3 chromosomes (Fig. 8), 411 had 6 ring-bivalents plus a V-shaped chain-of-3 chromosomes (Fig. 9), and 30 had 6 ring-bivalents, one unequal-bivalent plus a univalent.

When the chromosomes of microsporocytes aligned as 6 ring-bivalents plus one straight chain-of-3 disjoined at anaphase I, there was a 2:1 chromosome distribution of the chain-of-3, to opposite poles of the dividing microsporocyte. The normal chromosome 5 and a fragment chromosome went to one pole and formed a gamete with an extra fragment chromosome, and the other fragment chromosome went to the opposite pole and formed a deficient gamete which is expected to abort. When the viable gametes were fertilized by normal 7-chromosome

or 8-chromosome pollen, only tertiary trisomic or Unique-16 cytotype progeny were produced.

Microsporocytes observed with 6 ring-bivalents plus a V-shaped chain-of-3 chromosomes would form gametes with 7 normal chromosomes or 8 chromosomes. The gametes with 8 chromosomes were equivalent to the gametes formed by 8II plants. When these eggs were fertilized by 7 or 8-chromosome pollen, 7II, F_1 and 8II cytotype progeny were produced.

A third arrangement of metaphase chromosomes was observed in microsporocytes produced by plants with the F_1 cytotype. This chromosome arrangement consisted of 6 ring-bivalents plus one unequal-bivalent plus one univalent. The unequal bivalent was made up of a normal chromosome 5 and a fragment chromosome. The univalent was a fragment chromosome. With the chromosomes so arranged, the univalent could migrate to either pole and would be included in one of the gametes, or would be lost at the first meiotic division. Both chromosomes of the unequal bivalent either go to the same pole, or they disjoin.

If the 6 ring-bivalents disjoined normally and the unequal-bivalent went to one pole and the univalent to the opposite pole, 50% of the gametes would be expected to abort. This occurs because 50% of the gametes would be expected to carry 7 normal chromosomes plus a fragment chromosome and 50% would be expected to be deficient for a large chromosome segment of the total complement. An equivalent situation occurs when the 6 ring-bivalents disjoin, the unequal-bivalent disjoins and the univalent is included with the normal chromosome 5.

If the 6 ring-bivalents disjoin normally and the unequal-bivalent plus the univalent go to the same pole, all gametes would be

expected to abort. This would be expected because of an excessive duplication in the gametes with 6 chromosomes plus the additional 3 chromosomes, and a drastic deficiency which occurs in the gametes with only 6 chromosomes.

If the 6 ring-bivalents disjoin normally, and the unequal-bivalent disjoins with the normal chromosome 5 going to the pole opposite of where the univalent is, normal 7-chromosomes and 8-chromosome gametes would be expected to form. All of these gametes should be viable.

If the univalent is lost during the first meiotic division, only 7-chromosome gametes or gametes with 7 chromosomes plus an extra fragment chromosome would be expected. Normal gametes would be expected when the unequal-bivalent disjoins. Gametes with 7 chromosomes plus an extra fragment chromosome would be expected when the unequal-bivalent does not disjoin.

Microsporocyte Analyses of the 8II Cytotype. Analyses of microsporocytes collected from plants with the 8II cytotype were very essential for the development of a breeding program for 8II barley. In order for the breeding population of 8II plants to be stable or to be utilized, meiosis has to be normal. Normal meiosis is 8:8 distribution of chromosomes during gamete formation.

Microsporocytes collected from 8II plants isolated from the F_2 of 7II X 8II crosses were analyzed. A total of 2233 microsporocytes were observed at diakinesis: 269 had 8 ring-bivalents (Fig. 10), 898 had 7 ring-bivalents plus one rod bivalent (Fig. 11) and 1066 had ring bivalents plus 2 rod bivalents (Fig. 12).

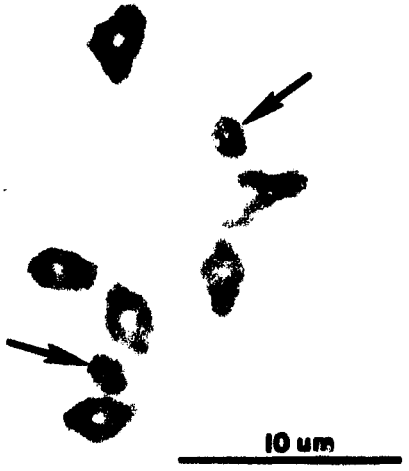

Figure 10. 8II cytotype, diakinesis, 8 ring-bivalents, note fragment ring-bivalents (arrows).

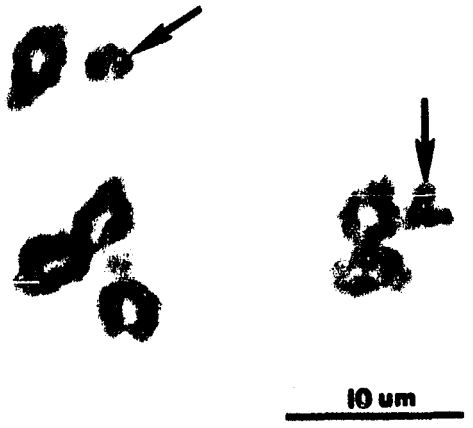

Figure 11. 8II cytotype, diakinesis, 6 normal ring-bivalents, one fragment ring-bivalent (left arrow) plus one fragment rod-bivalent (ring arrow).

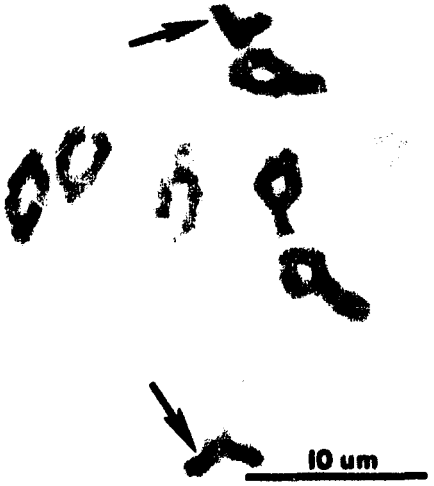

Figure 12. 8II cytotype, diakinesis, 6 normal ring-bivalents plus two fragment rod-bivalents (arrows).

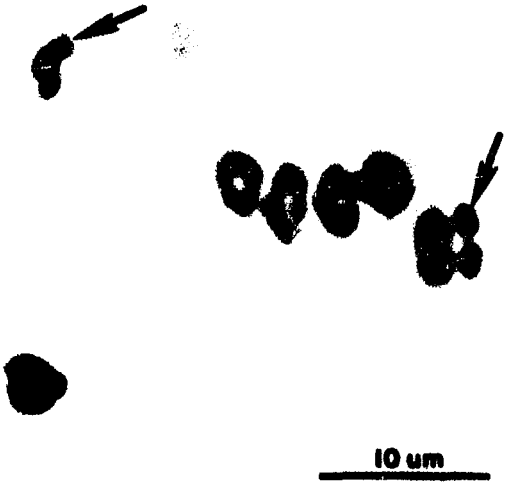

Figure 13. 8II cytotype, metaphase I, 6 normal ring-bivalents, one fragment ring-bivalent (right arrow) plus one fragment rod-bivalent (left arrow).

A total of 636 microsporocytes were observed at metaphase I: 2 had 8 ring-bivalents, 259 had 7 ring-bivalents plus one rod-bivalent (Fig. 13) and 375 had 6 ring-bivalents plus 2 rod-bivalents (Fig. 14). Only the fragment chromosomes were observed as rod-bivalents.

Meiosis was considered to be normal for plants with the 8II cytotype. Microsporocytes observed at diakinesis with 8 ring-bivalents were considered to actually have 7 ring-bivalents plus one rod bivalent. This conclusion came from the fact that essentially no microsporocytes were observed at metaphase I with 8 ring-bivalents. Apparently one pair of fragments almost always forms a rod-bivalent and the other forms a rod-bivalent more frequently than it does a ring-bivalent. The two microsporocytes reported with 8 ring-bivalents at metaphase I were considered artifacts of preparation. If they were not artifacts, there should have been a more frequent occurrence of microsporocytes observed at metaphase I with 8 ring-bivalents.

A possible explanation for the fragment chromosomes frequently forming rod bivalents was discussed under the verification of 8II lines.

Microsporocyte Analyses of the Tertiary Trisomic Cytotype. Microsporocytes collection from tertiary trisomic plants isolated from the F_2 of 7II X 8II crosses were analyzed. A total of 142 microsporocytes were observed at diakinesis: 69 had 7 ring-bivalents plus one univalent (Fig. 15) and 73 had 6 ring-bivalents plus one chain-of-3 chromosomes (Fig. 16). The chain-of-3 chromosomes in this case was composed of two normal chromosomes 5 with a fragment chromosome attached to one of the ends of one of the normal chromosomes 5.

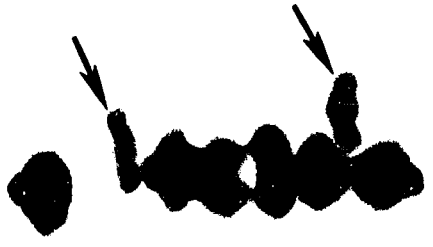

Figure 14. 8II cytotype, metaphase I, 6 normal ring-bivalents plus two fragment rod-bivalents (arrows).

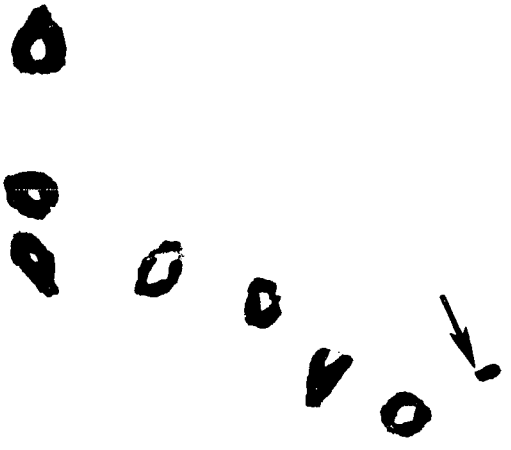

Figure 15. Tertiary-trisomic cytotype, diakinesis, 7II plus one fragment (arrow).

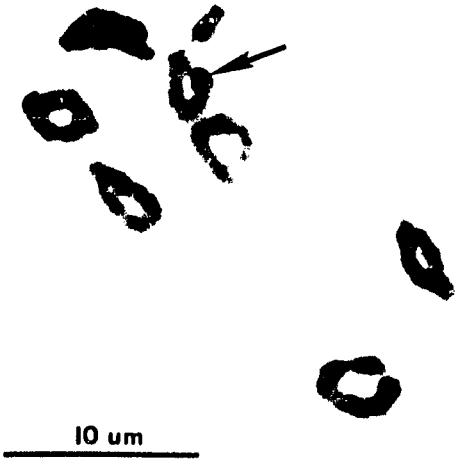

Figure 16. Tertiary-trisomic cytotype, diakinesis, 6II plus one chain-of-3 chromosomes (arrow).

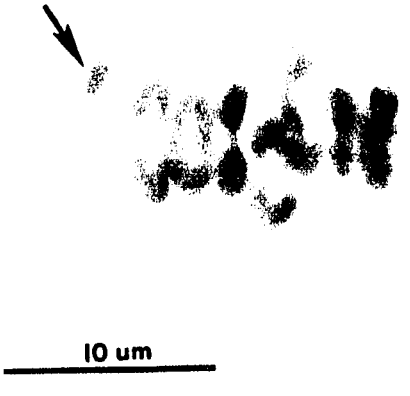

Figure 17. Tertiary trisomic cytotype, metaphase I, 7II plus one fragment (arrow).

A total of 290 microsporocytes were observed at metaphase I: 112 had 7 ring-bivalents plus a univalent (Fig. 17), 95 had 6 ring-bivalents plus one straight chain-of-3 chromosomes (Fig. 18) and 83 had 6 ring-bivalents plus one V-shaped chain-of-3 chromosomes (Fig. 19).

Two different gametes, those with 7 normal chromosomes and those with 7 normal chromosomes plus a fragment chromosome were expected to be formed from each of the chromosome arrangements observed.

Microsporocyte Analyses of the Unique-16 Cytotype. Microsporocytes collected from Unique-16 cytotype plants from the F_2 of 7II X 8II crosses were analyzed. A total of 22 microsporocytes were observed at diakinesis: 8 had 7 ring-bivalents plus an unequal-bivalent (Fig. 20), 11 had 6 ring-bivalents, one chain-of-3 chromosomes plus one univalent (Fig. 21) and 3 had 6 ring-bivalents plus a multivalent-of-4 chromosomes. The unequal-bivalent was composed of one normal chromosome 5 and a fragment chromosome. The chain-of-3 chromosomes was composed of a normal chromosome 5 with a fragment chromosome attached to each end. The multivalent of 4 chromosomes was composed of 3 fragment chromosomes and a normal chromosome 5. Two of the fragment chromosomes were a pair.

A total of 313 microsporocytes were observed at metaphase I: 6 had 7 ring-bivalents plus two univalents, 78 had 7 ring-bivalents plus one unequal-bivalent (Fig. 22), 143 had 6 ring-bivalents, one straight chain-of-3 chromosomes plus a univalent (Fig. 23), 44 had 6 ring bivalents, one V-shaped chain-of-3 chromosomes plus a univalent

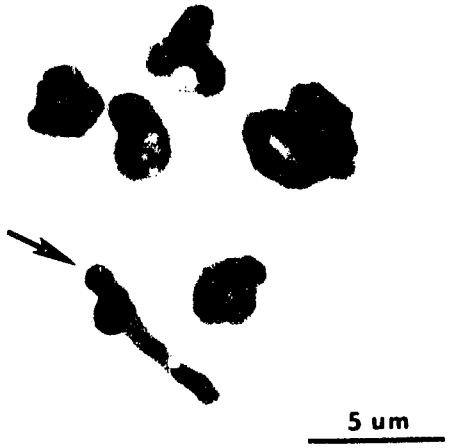

Figure 18. Tertiary-trisomic cytotype, early metaphase I, 6II plus one straight chain-of-3 chromosomes (arrow).

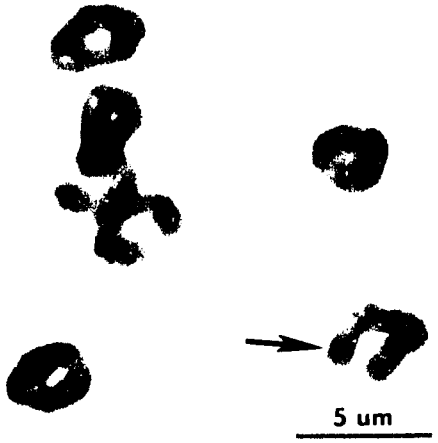

Figure 19. Tertiary trisomic cytotype, early metaphase I, 6II plus one V-shaped chain-of-3 chromosomes (arrow).

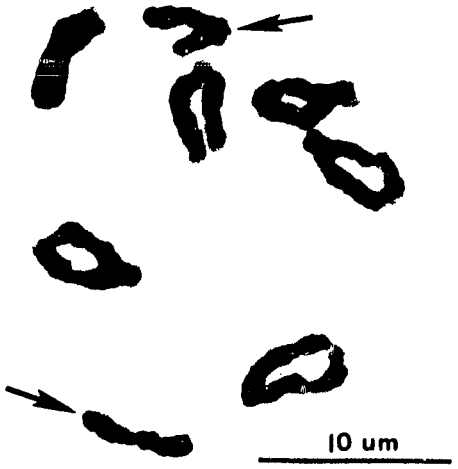

Figure 20. Unique-16 cytotype, diakinesis, 6II, one unequal pair (upper arrow) plus one fragment rod-bivalent (bottom arrow).

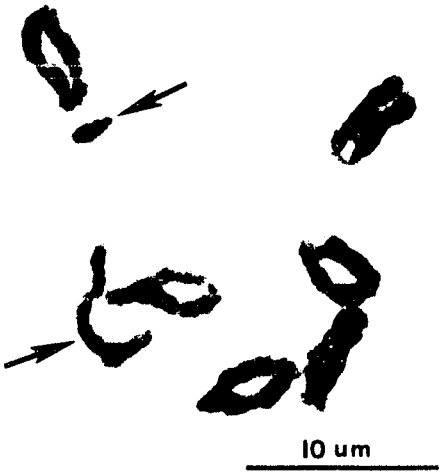

Figure 21. Unique-16 cytotype, diakinesis, 6II, one chain-of-3 (bottom arrow) plus a univalent (upper arrow).

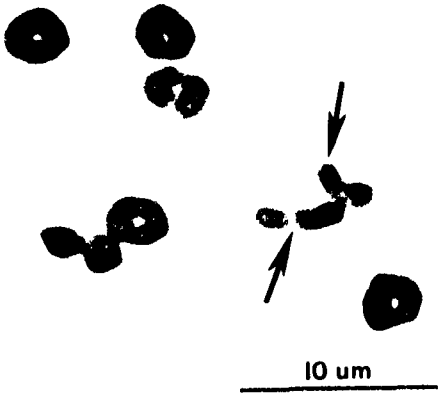

Figure 22. Unique-16 cytotype, early metaphase I, 6 ring-bivalents, one unequal-bivalent, (bottom arrow) and one fragment rod-bivalent (upper arrow).

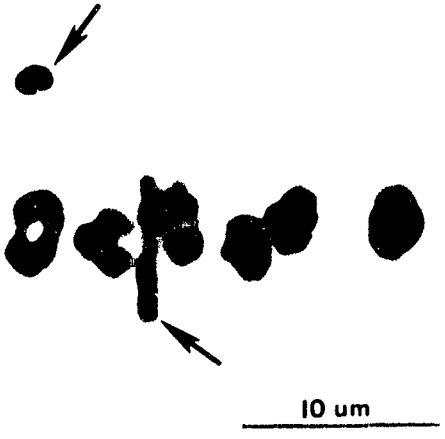

Figure 23. Unique-16 cytotype, metaphase I, 6 ring-bivalents, a straight chain-of-3 (bottom arrow) plus one fragment-univalent (upper arrow).

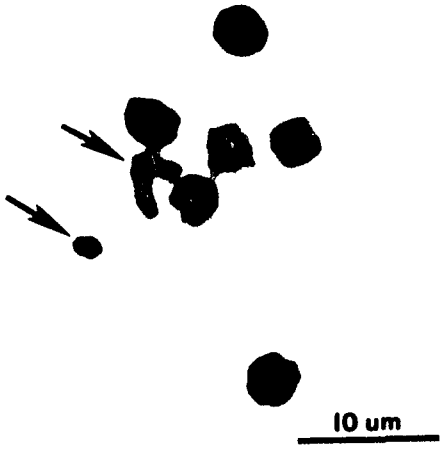

Figure 24. Unique-16 cytotype, early metaphase I, 6 ring-bivalents, a V-shaped chain-of-3 (upper arrow) plus one fragment-univalent (lower arrow).

Figure 25. Unique-16 cytotype, metaphase I, 6 ring-bivalents plus one multivalent-of-4 chromosomes (arrow).

(Fig. 24) and 42 had 6 ring-bivalents plus a multivalent-of-4 chromosomes (Fig. 25). Many times the microsporocytes reported with 7 ring-bivalents may actually have had 6 ring-bivalents and one rod-bivalent, because one of the pairs of chromosomes in this complement is a pair of fragments.

Microsporocytes observed at metaphase I with 7II plus two univalents were expected to produce gametes with 7, 8, or 9 chromosomes. Seven and 9-chromosome gametes were expected when the 7 pairs disjoined and the two univalents went to the same pole. The 7-chromosome gametes would be deficient and the 9-chromosome gametes would carry a large duplication, both are expected to abort.

Eight-chromosome gametes were expected when the 7 pairs disjoined normally and one of each of the univalents went to each pole. One of the gametes would carry 7 chromosomes plus a fragment chromosome, the other gamete would be equivalent to those formed by 8II plants. If both of the univalents were lost, no viable gametes would be expected to occur.

Microsporocytes observed with 7II plus one unequal pair would form the same types of gametes as those with 7II plus two univalents. The only difference is that the unequal-bivalent disjoins whereas in microsporocytes with 7II plus 2 univalents, the univalents migrate independently of each other.

In microsporocytes from Unique-16 cytotype plants the most frequently observed metaphase I configuration consisted of 6II, one chain-of-3 chromosomes plus a univalent. The chain-of-3 consisted of a normal chromosome 5 with a fragment chromosome attached to each

arm. The univalent was a fragment chromosome. The chain-of-3 chromosomes was observed as either a straight chain or a V-shaped chain.

If the 6 pairs of chromosomes disjoin normally, and the straight chain-of-3 undergoes 2:1 disjunction, gametes with 7, 8, or 9 chromosomes would be expected to occur. When the univalent accompanies the two chromosomes from a 2:1 disjunction, deficient 7-chromosome and duplicated 9-chromosome gametes would be expected to form. The 7-chromosome gametes would have 6 normal chromosomes plus one of the fragment chromosomes. The 9-chromosome gametes would have 7 normal chromosomes plus the 2 fragment chromosomes. Due to deficiencies and large duplications, the gametes would be expected to abort.

When the univalent accompanies the single chromosome from a 2:1 disjunction of the chain-of-3, all of the resulting gametes would carry 8 chromosomes and would be viable. Half of these gametes would carry 7 normal chromosomes plus one of the fragment chromosomes, and the other half would be equivalent to gametes formed by the 8II lines.

If the univalent is lost, the only viable gametes expected to occur would be those with 7 normal chromosomes plus a fragment chromosome. Seven-chromosome gametes carrying 6 normal chromosomes plus a fragment chromosome would be expected to abort.

A different group of gametes would be expected when the chromosomes in microsporocytes with 6II plus a V-shaped chain-of-3 plus a univalent disjoin. When the V-shaped chain-of-3 chromosomes disjoins, the fragment chromosomes go to one pole and the normal chromosome 5

goes to the opposite pole. The fate of the univalent determines the gamete's cytotype.

When the univalent accompanies the normal chromosome 5 from the chain-of-3, gametes with 7 normal chromosomes plus a fragment and 8-chromosome gametes equivalent to the gametes formed by the 8II lines would be expected. When the univalent accompanies the two fragments, normal 7-chromosome and 9-chromosome gametes occur. These 9-chromosome gametes may be functional because they are duplicated for less chromatin than the 9-chromosome gametes that carry 7 normal chromosomes plus two fragments.

If the univalent was lost, only normal 7-chromosome and 8-chromosome gametes are expected to occur. The 8-chromosome gametes are equivalent to the gametes formed by plants from 8II lines.

Microsporocytes observed with 6II plus a multivalent-of-4 chromosomes would form the same types of gametes as those expected from microsporocytes with 6II, a straight chain-of-3 plus a univalent. The types of gametes would be determined by where the multivalent disjoins.

Many of the gametes formed by plants with the Unique-16 cytotype abort. The gametes that accomplish fertilization are those with 7 normal chromosomes or those with 8 chromosomes equivalent to the gametes formed by 8II lines. Four different kinds of eggs are expected to survive, those with 7 normal chromosomes, those with 8 chromosomes equivalent to those produced by 8II lines, those with one extra fragment chromosome and those that carry 6 normal chromosomes plus 3 fragment chromosomes.

An overall total of 4447 microsporocytes from the F_2 progeny of 7II X 8II crosses were analyzed (Table 2). More than 2800 microsporocytes were analyzed from plants with the 8II cytotype. There was a concentration of analyses for the 8II cytotype in order to observe as many meiotic abnormalities as possible that may occur within this cytotype. It was concluded that the 8II cytotype had normal meiosis, and would not cause any foreseeable meiotic problems in an MSFRSP of 8II plants.

Chromosome Transmission in Crosses and Their Reciprocals Between 7II and F_2 Cytotype Plants

One major question about the MSFRSP of 8II plants was, what would be the fate of the population if contaminated with normal 7-chromosome pollen. The same question was asked concerning the contamination of a 7II population with 8-chromosome pollen. The information obtained from the crosses and their reciprocals between 7II plants and plants of each F_2 cytotype helped answer these questions.

7II X 7II Crosses. There were no 7II X 7II crosses made, because no pertinent information was expected. This cross represents selfed set seed on a 7II plant.

7II X 8II and 8II X 7II Crosses. Twenty-three seed produced from 7II X 8II crosses and 16 seed produced from 8II X 7II crosses were analyzed by root-tip chromosome analyses. All progeny were observed to have the F_1 cytotype indicating that the 8 chromosome pollen and eggs were functioning as expected.

Table 2. Chromosome configurations observed for the F_1, 8II, Tertiary-Trisomic and Unique-16 cytotypes at diakinesis and/or metaphase I.*

No. of Plants	Diakinesis			Metaphase I		
F_1 cytotype						
5	6II+ ---	6II+≠II +I		6II+---	6II+^	6II+≠II + I
	146	3		223	411	30
Total No. of Microsporocytes	149			664		
8II cytotype						
64	8II	7II+--	6II+2--	8II	7II+--	6II+2--
	269	898	1066	2	259	375
Total No. of Microsporocytes	2233			636		
Trisomic cytotype						
4	7II+I		6II+---	7II+I	6II+---	6II+^
	69		73	112	95	83
Total No. of Microsporocytes	142			290		
Unique 16 cytotype						
3	7II+ I≠II	6II+ --- +I	6II+ IV	7II+ I+I	7II+ I≠II	6II+ --- +I	6II+ ^+I	6II+ IV
	8	11	3	6	78	143	44	42
Total No. of Microsporocytes	22			313				

* --, ---, ^ = rod-bivalent, straight chain-of-3, V-shaped chain-of-3

7II X F_1 and F_1 X 7II Crosses. Fifty seed produced from 7II X F_1 crosses and 49 seed produced from F_1 X 7II crosses were analyzed by root-tip chromosome analyses. From 7II X F_1 crosses, 30 progeny had a 7II cytotype and 20 progeny had an F_1 cytotype. From F_1 X 7II crosses, 23 progeny had a 7II cytotype, 24 had the F_1 cytotype and 2 had a tertiary-trisomic cytotype.

Progeny from 7II X F_1 crosses indicated that plants with the F_1 cytotype produced two types of functional pollen. Sixty percent of the pollen carried 7 normal chromosomes and 40% of the pollen carried 8 chromosome equivalent to pollen produced from 8II plants. This information also indicated that pollen with 7 chromosomes plus a fragment, formed by microsporocytes with 6II plus a straight chain-of-three were not accomplishing fertilization.

In barley, it is commonly accepted that pollen carrying 7 chromosomes plus an extra chromosome is not as competitive as pollen with only 7 normal chromosomes. This cross also indicated that pollen with 7 normal chromosomes plus a fragment was less competitive than 8-chromosome pollen, that was equivalent to pollen produced from 8II plants. The 8-chromosome pollen carried a duplication, but it still accomplished fertilization 40% of the time when competing with normal 7-chromosome pollen.

Progeny from F_1 X 7II crosses indicated that an equal frequency of 7 and 8-chromosome eggs were formed. The 8-chromosome eggs were equivalent to eggs produced by 8II plants. The two trisomic progeny indicated that an occasional egg with 7 normal chromosomes plus a fragment were formed. This is in agreement with the

information obtained from microsporocytes with 6II plus a straight chain-of-three. This chromosome arrangement in a microsporocyte resulted in gametes with 7 normal chromosomes plus a fragment chromosome.

These two crosses provided direct evidence as to the frequency and type of gametes produced by the F_1 cytotype. With this information it was possible to determine the expected frequency of progeny with each cytotype in the F_2 of the initial 7II X 8II crosses.

7II X Tertiary Trisomic and Tertiary Trisomic X 7II Cross. Forty-eight seed produced from 7II X tertiary trisomic crosses and 43 seed produced from tertiary trisomic X 7II crosses were analyzed by root tip-chromosome analyses. From 7II X tertiary trisomic crosses, 47 progeny had the 7II cytotype and one progeny had a tertiary-trisomic cytotype. From tertiary trisomic X 7II crosses, 26 progeny had a 7II cytotype and 17 progeny had a tertiary-trisomic cytotype.

The one tertiary trisomic progeny from 7II X tertiary trisomic crosses indicated that occasionally a pollen grain with a duplication did accomplish fertilization, even when there was a predominance of normal 7 chromosome pollen present. The tertiary trisomic X 7II cross indicated that a much higher frequency, approximately 40% in this case, of the eggs with an extra chromosome functioned.

These results support the commonly accepted idea for barley that pollen with 7 normal chromosomes plus an extra chromosome accomplishes fertilization at a very low frequency, approximately 2% in this case. It must be kept in mind that this extra chromosome is a

fragment chromosome. The smaller the extra chromosome is, the more frequently it will be carried to the next generation through the pollen. These results also indicate that the eggs of barley commonly transmit extra chromosomes from one generation to the next.

7II X Unique-16 and Unique-16 X 7II Cross. Fifty seed produced from 7II X Unique-16 crosses and 34 seed produced from Unique-16 X 7II crosses were analyzed by root-tip chromosome analyses. From 7II X Unique-16 crosses, progeny with 3 different cytotypes were observed: 19 progeny had a 7II cytotype, 24 had the F_1 cytotype and 7 had a tertiary-trisomic cytotype. From Unique-16 X 7II crosses, progeny with four different cytotypes were observed: 6 had a 7II cytotype, 21 had the F_1 cytotype, 6 had a tertiary-trisomic cytotype and one had the Unique-16 cytotype.

Progeny from 7II X Unique-16 crosses indicated again that pollen with 7 chromosomes plus a fragment chromosome was accomplishing fertilization.

Progeny from Unique-16 X 7II crosses indicated that eggs with 7, 8 and 9 chromosomes did function. The 7-chromosome eggs carried 7 normal chromosomes. The 8-chromosome eggs were of two types, eggs with 7 normal chromosomes plus an extra fragment chromosome and 8-chromosome eggs equivalent to those formed by 8II plants. When eggs of these types were fertilized by normal 7-chromosome pollen, the resulting progeny were 7II, tertiary trisomic and the F_1 cytotype respectively.

One additional progeny was observed, a Unique-16 cytotype plant. The egg that was fertilized by normal 7-chromosome pollen to

produce this plant was carrying 9 chromosomes. The 9 chromosomes were 6 normals plus 3 fragments. The 9-chromosome eggs resulted from microsporocytes with 6II plus a V-shaped chain-of-three and a univalent. The univalent had to accompany the two fragments of the chain of three in order for the 9 chromosomes to end up in the same gamete.

The frequency of progeny with each cytotype from each cross is summarized in Table 3. It was concluded from these crosses that plants with the F_1 cytotype produced approximately 50% normal 7-chromosome eggs and approximately 50% 8-chromosome eggs. The 8-chromosome eggs were equivalent to eggs produced by 8II plants. These F_1 cytotype plants also produced approximately 60% normal 7-chromosome pollen and approximately 40% 8-chromosome pollen, the 8-chromosome pollen being equivalent to pollen produced by 8II plants.

The results of these crosses reemphasized the need of keeping 7II and 8II populations isolated from each other. Only 8 of the possible combinations of crosses between 7II and F_2 cytotype plants were evaluated. There are 15 additional combinations that could occur in a random-mating 8II population that was contaminated with pollen from 7II plants (Table 4). It cannot be emphasized enough that 7II and 8II populations must be grown in isolation from each other to maintain cytotype homozygosity.

Interrelationships Between F_2 Root-Tip and Microsporocyte Analyses and Chromosome Transmission Data

The frequency of 7II plants, F_1 cytotype plants and 8II plants from the F_2 of 7II X 8II crosses expressed continuity between the

Table 3. Frequency of cytotypes isolated by root tip chromosome analyses from crosses and their reciprocals between 7II and F_2 cytotype plants.

	Possible Cytotypes				
Cross	7II	F_1	8II	Tertiary Trisomic	Unique-16
7II X 8II	-	23	-	-	-
8II X 7II	-	16	-	-	-
7II X F_1	30	20	-	-	-
F_1 X 7II	23	24	-	2	-
7II X Tri	47	-	-	1	-
Tri X 7II	26	-	-	17	-
7II X Unique-16	19	24	-	7	-
Unique-16 X 7II	6	21	-	6	1

Table 4. A summary of all possible crosses and their reciprocals involving the different cytotype plants isolated from the F_2 of 7II X 8II crosses.

Female Parent Cytotypes	Male Parent Cytotypes				
	7II	F_1	8II	Tri	Unique 16
7II	Normal Barley	E*	E	E	E
F_1	E	NE*	NE	NE	NE
8II	E	NE	Normal 8II	NE	NE
Tri	E	NE	NE	NE	NE
Unique 16	E	NE	NE	NE	NE
	All Possible Progeny				

*E = evaluated, NE = not evaluated

root-tip and microsporocyte analyses. A population of 587 progeny, classified by root-tip chromosome analyses, showed that 29.13% of the population had the 7II cytotype, 50.26% had the F_1 cytotype and 20.61% had the 8II cytotype. A population of 420 progeny, classified by microsporocyte analyses, showed that 27.38% of the population had the 7II cytotype, 52.38% had the F_1 cytotype and 20.24% had the 8II cytotype. The frequencies of plants with the 7II, F_1 and 8II cytotypes, isolated by root-tip and microsporocyte analyses were essentially equivalent.

If only the frequency of either the 7II class or the 8II class were known, the expected frequency of the two unknown classes could be calculated. When the frequency of the 7II class, from root-tip chromosome analyses, was fixed at the observed frequency of 29.13%, the frequency of normal 7-chromosome gametes would be the square root of 29.13, which was 53.97%. Based on a total gamete frequency of 100%, this meant that 46.03% of the gametes carried the 8-chromosomes comparable to the 8-chromosome gametes produced by the 8II lines.

After calculating the gametic frequencies, it was determined that 49.68% of the population would be expected to be of the F_1 cytotype and that 21.19% would be of the 8II cytotype.

When the frequency of the 8II class, from root-tip chromosome analyses was fixed at the observed frequency of 20.61%, the theoretical frequency of the 7II and F_1 cytotype would be 29.81% and 49.58%, respectively.

Likewise, the above calculations were applied to the observed frequencies of 7II and 8II plants isolated by microsporocyte analyses.

When the 7II class was fixed at the observed frequency of 27.38%, the calculated F_1 cytotype frequency was 49.90% and the 8II cytotype frequency was 22.72%. When the 8II class was fixed at the observed frequency of 20.24%, the calculated F_1 cytotype frequency was 49.50% and the calculated 7II cytotype frequency was 30.26%.

The 7II X F_1 cross indicated that approximately 60% of the pollen carried 7 normal chromosomes and approximately 40% of the pollen carried a chromosome complement comparable to pollen produced from an 8II plant. The progeny from F_1 X 7II crosses indicated there was an equal frequency of normal 7-chromosome and 8-chromosome eggs produced. Knowing these gametic frequencies, it was calculated that approximately 30% of the F_2 population would have the 7II cytotype, 50% the F_1 cytotype and 20% the 8II cytotype. These frequencies were calculated from the direct evidence of the 7II X F_1 and F_1 X 7II crosses.

When all of the information, observed and calculated, was summarized (Table 5), it was concluded that the cytotype frequencies from the F_2 of 7II X 8II crosses were approximately 30% 7II, 50% F_1 and 20% 8II.

It was also concluded that a large MSFRSP of 8II plants could be developed by either using root-tip chromosome analyses or microsporocyte analyses. If the analyses were made from root-tip chromosomes, only the 8II plants would be saved and planted in isolation in the greenhouse. The first intercrosses between 8II plants to establish an MSFRSP could be made.

The first cycle of intercrossing could also be conducted in a field grown F_2 population from which 8II plants could be isolated by

Table 5. Summary of observed root-tip and microsporocyte analyses and calculated frequencies of 7II, F_1 and 8II cytotype progeny from the F_2 of the 7II X 8II crosses.

Source	Cytotype 7II	Cytotype F_1	Cytotype 8II
Observed frequency from root-tip chromosome analyses in the F_2 population of the 7II X 8II cross	29.13%	50.26%	20.61%
Observed frequency from microsporocyte chromosome analyses in the F_2 of the 7II X 8II cross	27.38	52.38	20.24
Expected frequency based on fixing the 7II cytotype at its observed root-tip analyses and calculating the F_1 and 8II cytotypes	29.13	49.68	21.19
Expected frequency based on fixing the 8II cytotype at its observed root-tip analyses and calculating the 7II and F_1 cytotypes	29.81	49.58	20.61
Expected frequency based on fixing the 7II cytotype at its observed microsporocyte frequency and calculating the F_1 and 8II	27.38	49.90	22.72
Expected frequency based on fixing the 8II cytotype at its observed microsporocyte frequency and calculating the 7II and F_1	30.26	49.50	20.24
Expected frequency based on pollen transmission of specific cytotype gametes from the 7II X F_1 cross.	30.00	50.00	20.00

microsporocyte analyses. The F_2 population also carries plants of the other 4 cytotype classes which would increase the chance of stray pollen contaminating the intercrosses.

Utilizing the expected gametic cytotypes shown in Figure 1 and the expected gametic frequency information from the 7II X F_1 cross, it was possible to determine the expected frequency of tertiary-trisomic and Unique-16 cytotype plants in the F_2 of 7II X 8II crosses. The two types of gametes with 7 normal chromosomes plus one fragment were assumed to occur at equal frequencies, because one is the reciprocal of the other. From the 7II X F_1 cross it was determined that approximately 60% of the pollen carried 7 normal chromosomes and 40% carried 8 chromosomes equivalent to pollen produced by 8II plants.

It was calculated that the tertiary-trisomic and Unique-16 cytotype portion of the F_2 population should contain 60% tertiary-trisomics and 40% Unique-16 cytotypes.

Using the root tip chromosome analyses information, 43.13% of the tertiary-trisomic and Unique-16 cytotype portion of the F_2 had a tertiary-trisomic cytotype and 56.86% a Unique 16 cytotype. This was the reverse of what was calculated based on chromosome transmission data. Using the microsporocyte analyses information, 86.66% of the population of these two cytotypes had a tertiary-trisomic cytotype and 13.34% had a Unique-16 cytotype.

The inconsistency between the two sets of observed data was accounted for by the fact that the root-tip chromosome analyses were from random F_2 seed from the 7II X 8II crosses, and microsporocytes were analyzed from agronomically desirable plants selected in a field

grown F_2 population from 7II X 8II crosses. In the greenhouse, the Unique-16 cytotype plants were generally weaker than the tertiary trisomic plants. This would indicate that they would not be selected as agronomically desirable plants in a field situation.

Considering the root-tip chromosome information from random seed, it is not known why the observed and expected frequencies of the tertiary-trisomic and Unique-16 cytotypes were in reverse order. The gametic frequencies from which the expected cytotype frequencies were calculated, were from extensive observations of both root-tip chromosomes and microsporocytes

Male Fertility vs Male Sterility in the F_2 of 7II X 8II Crosses

Genetic male sterility is the basis upon which an MSFRSP is established. It was known that in Composite Cross XXXII, there were plants homozygous recessive for the msg1 allele, on chromosome 5, plants homozygous recessive for the msg2 allele, on chromosome 2 and probably plants homozygous recessive for both alleles. Male-sterile plants from Composite Cross XXXII served as female parents for the initial 7II X 8II crosses.

Male sterility was also involved in the development of the 8II lines used as the male parent for the initial 7II X 8II crosses. The original 8II lines arose from the selfed progeny of a BTT 57a msg16. The msg16 allele is on chromosome 7. Also, in the development of the 8II lines, they were crossed onto male-sterile plants that carried the homozygous recessive msg1 allele.

At the time of the initial 7II X 8II crosses, the female plants from Composite Cross XXXII were either homozygous dominant, heterozygous or homozygous recessive for the msg 1 allele. If they were homozygous dominant or heterozygous for the msg1 allele, the msg2 allele would have been homozygous recessive. If the msg2 allele were homozygous dominant or heterozygous, the msg1 allele would have been homozygous recessive. There is a possibility that both of the male-sterile alleles were homozygous recessive. All female plants should have been homozygous dominant for the msg16 allele on chromosome 7.

Likewise, at the time of the initial 7II X 8II crosses, the male plants were 8II and were either homozygous dominant, heterozygous or homozygous recessive for the msg16 allele. They could carry the homozygous recessive allele and still be male fertile, because the dominant allele could be carried as a duplication on one of the pairs of fragment chromosomes. All of the 8II plants should be homozygous dominant for both the msg1 and msg2 alleles. All 8II plants carry the msg1 locus on both of the fragment chromosomes. The interchanged fragment 57a is homozygous dominant at the msg1 locus and the interchanged 5 could be either homozygous dominant, heterozygous or homozygous recessive at the msg1 locus.

One objective of the current research concerning male sterility was to determine if there were male sterile 8II plants. Male sterile 8II plants are necessary for the establishment of an MSFRSP of 8II plants.

From a population of 490 F_2 plants from 7II X 8II crosses, 355 were classified as male fertile and 135 were classified as male sterile. In the population, male-fertile plants vs male-sterile plants occurred in an approximate 3:1 ratio. This was concluded after fitting the observed data of 355:135 to an expected 3:1 ratio by a Chi-square test. A Chi-square value of 1.70 was calculated which corresponded to a probability value between .2 and .1 (Table 6).

Plants were classified as male fertile and male sterile in each of the F_2 cytotypes from 7II X 8II crosses. The male fertility vs male sterility was not observed in a 3:1 ratio within each cytotype. Seventy-three percent of the 7II plants were male sterile, 9.54% of the F_1 cytotype plants were male sterile and 4.70% of the 8II cytotype plants were male sterile (Table 7).

Ratios of male fertile plants and male sterile plants in the different cytotypes were obtained from 7II X 8II crosses where the 7II plants were male sterile. If the male-sterile parent was either homozygous recessive for msg1 or homozygous recessive for both msg1 and msg2, in the F_2, all 7II cytotype plants would be male sterile, all F_1 cytotype and 8II cytotype plants would be male fertile and tertiary trisomic and Unique 16 cytotype plants would occur in a ratio of 1 male fertile to 1 male sterile.

All of the 7II cytotype plants would be male sterile because the msg1 allele is carried on chromosome 5. The 7II cytotype plants from the F_2 of 7II X 8II cross carry two normal chromosomes 5, both of which carry the recessive msg1 allele. There is not a normal

Table 6. A Chi-square goodness of fit to a 3:1 ratio of male-fertile to male-sterile plants from a bulk F_2 population from 7II X 8II crosses.

Phenotype	Ratio	Observed Frequency	Expected Frequency	$\frac{(o - e)^2}{e}$
Fertile	3	355	367.5	.4251
Sterile	1	135	122.5	1.2755
Total		490	490	1.700 p=.2 to .1

Table 7. Frequency of male-fertile and male-sterile plants in each cytotype of plants from the F_2 of 7II X 8II crosses.

	Cytotypes					
Phenotype	7II	F_1	8II	Tri	Unique 16	N. D.*
Male Fertile	30	199	81	8	1	36
Male Sterile	85	21	4	5	1	19

*Cytotype could not be determined from microsporocyte analyses.

chromosome 5, carrying the dominant msg1 allele involved in the cross, subsequently all normal chromosomes 5 carry the recessive msg1 allele.

It was also determined that when a 7II X 8II cross was made utilizing 7II male sterile plants homozygous recessive for the msg2 allele only, all of the different cytotype plants in the F_2 of 7II X 8II crosses would occur in a 3:1 male fertile:male sterile ratio.

With the above determinations, plus knowing the frequency of male-sterile and male-fertile plants for each cytotype from 7II X 8II crosses (Table 7), it was concluded that approximately 65% of the 7II X 8II crosses were made onto 7II plants homozygous recessive for the msg1 allele, or homozygous recessive for both msg1 and msg2. The remaining 35% of the 7II X 8II crosses were made onto 7II plants homozygous recessive for the msg2 allele and homozygous dominant for the msg1 and msg16 alleles.

The theoretical F_2 results of making 65% of the 7II X 8II crosses onto plants homozygous recessive for the msg1 allele or homozygous recessive for both msg1 and msg2 and 35% of the crosses onto plants homozygous recessive for the msg2 allele only were: 73.75% of the 7II cytotype plants being male-sterile, 8.75% of the F_1 cytotype plants being male-sterile and 8.75% of the 8II cytotype plants being male-sterile. The actual F_2 results from the 7II X 8II crosses were 74% of the 7II plants being male-sterile, 9.54% of the F_1 cytotype plants being male-sterile and 4.80% of the 8II cytotype plants being male-sterile. The calculated and actual frequencies are essentially equivalent. The above calculations were based on the assumption that

both of the normal chromosomes 7 of the 8II lines carried the dominant msg16 allele.

This information indicates that within Composite Cross XXXII, approximately 65% of the male-sterile plants are either homozygous recessive for the msg1 allele only or homozygous recessive for both msg1 and msg2 and approximately 35% of the male-sterile plants are homozygous recessive for the msg2 allele only. This conclusion arises from the fact that random male-sterile plants from Composite Cross XXXII were used as female parents for the initial 7II X 8II crosses. It was also concluded that the MSFRSP of 8II plants will have to rely on the msg2 allele as its current source of male sterility. Any male-sterile allele that would not be dominant for fertility in the fragment chromosomes could be utilized.

SUMMARY

A population of barley plants with 8 pairs of chromosomes would provide additional genetic variability for barley breeders to exploit. Before developing an MSFRSP of 8II plants, it was essential to study the chromosome behavior of the 8II lines of which two pairs are interdependent. The two pairs of interdependent chromosomes are shorter than normal barley chromosomes.

Root-tip chromosome analyses of the 8-chromosome paired lines indicated all of the lines carried 16 chromosomes. Microsporocyte analyses of the 16-chromosome plants indicated that they carried 8 pairs of chromosomes. Many times the two pairs of fragment chromosomes were observed as rod-bivalents. Field-grown 8II plants indicated that pollen viability was normal. Seed set was determined to be essentially 100%.

In order to develop an MSFRSP of 8II plants, a segregating F_2 population from 7II X 8II crosses was produced. Male-sterile plants carrying either the msg1 allele or msg2 allele or carrying both alleles homozygous recessive were used as the female parents. Eight-chromosome-paired lines were crossed onto these females. These initial crosses produced 10,162 F_1 seed. The F_1 plants had a characteristic 15-chromosome cytotype.

From the F_2 of the 7II X 8II crosses, plants with 5 different cytotype classes were observed by root-tip chromosome analyses and by microsporocyte analyses. Plants with the 7II cytotype, the F_1

cytotype and the 8II cytotype constituted approximately 30, 50, and 20%, respectively, of the F_2 population from the 7II X 8II crosses. Two additional cytotypes were also observed, tertiary trisomics with 7 normal chromosome pairs plus one of the fragment chromosomes and Unique-16 chromosome cytotypes with 6 normal pairs of chromosomes plus a pair of fragment chromosomes, plus a normal chromosome 5 and one fragment chromosome from the other pair.

Normal meiosis for the 8II plants was essential in order to develop an MSFRSP of 8-chromosome-paired plants. Analyses of the meiotic chromosome behavior of plants with the F_1 cytotype aided in determining the frequency of 8II plants and other cytotype plants from the F_2. Meiosis of 7II cytotype plants was determined to be normal. The most frequently observed chromosome arrangement, at diakinesis and metaphase I, for the F_1 cytotype plants was 6II plus a chain-of-3 chromosomes. The chain-of-3 chromosomes was composed of one normal chromosome 5, and one of each of the fragment pairs of chromosomes.

Meiosis of 8II cytotype plants was determined to be normal, normal being 8:8 chromosome distribution at anaphase I.

Microsporocyte chromosome arrangements, at diakinesis and metaphase I of the tertiary trisomic cytotypes were observed to be 7II plus a univalent fragment, or 6II plus a V-shaped or straight chain-of-3 chromosomes.

The Unique-16 cytotype microsporocytes observed at metaphase I, showed 5 different chromosome arrangements: 7II plus 2 univalents,

7II plus an unequal-bivalent, 6II plus a V-shaped or straight chain-of-three chromosomes and a univalent or 6II plus a multivalent-of-4 chromosomes.

One major question about an MSFRSP of 8II plants was what would be the fate of the population if it were contaminated with normal 7 chromosome pollen. Cross and reciprocal cross pollinations designed to determine this, as well as to determine the transmission frequency of specific chromosomes for each cytotype were analyzed. These cross pollinations were between 7II plants and each of the different cytotype plants. From these crosses it was determined that plants with the 8II cytotype produced only 8-chromosome gametes. Plants with the F_1 cytotype produced approximately 60% 7-chromosome pollen and approximately 40% 8-chromosome pollen, equivalent to pollen produced by 8II plants. The F_1 cytotype plants produced approximately equal frequencies of normal 7-chromosome and 8-chromosome eggs. Occasionally an egg functioned that carried 7 normal chromosomes plus a fragment chromosome.

Plants with the tertiary-trisomic cytotype produced pollen with 7 normal chromosomes and pollen with 7 normal chromosomes plus a fragment chromosome. Essentially all pollen that accomplished fertilization was the 7 normal chromosome type. The tertiary trisomic cytotype plants produced eggs with 7 normal chromosomes and eggs with 7 normal chromosomes plus a fragment chromosome. Eggs with 7 normal chromosomes were formed approximately 60% of the time and eggs with 7 normal chromosomes plus a fragment chromosome were formed approximately 40% of the time. This was supportive of the commonly

accepted idea, that in barley, eggs carry extra chromosomes from one generation to the next more readily than does the pollen.

Plants with the Unique-16 cytotype produced functional pollen that carried 7 normal chromosomes, 8 chromosomes equivalent to pollen produced by 8II plants and pollen with 7 normal chromosomes plus a fragment chromosome. These plants produced functional eggs with 7 normal chromosomes, 8 chromosomes equivalent to eggs produced by 8II plants, eggs with 7 normal chromosomes plus a fragment chromosome, as well as an occasional egg with 6 normal chromosomes plus 3 fragment chromosomes.

Once the crosses and their reciprocals between 7II plants and plants of each of the F_2 cytotypes were analyzed, there was no question as to the fate of an 8II population that may be contaminated by pollen from 7II plants. The population would be totally disrupted cytologically. The same problem exists if pollen from 8II plants were to contaminate a population to 7II plants. Physical isolation of the two types of populations is very essential for maintaining their integrity.

Genetic male sterility is the basis which an MSFRSP is established. Male sterility in Composite Cross XXXII is due to plants being homozygous recessive for either the msg1 allele on chromosome 5, the msg2 allele on chromosome 2, or from both alleles being homozygous recessive in the same plant.

Male-sterility alleles msg1 and msg16 were involved in the isolation of the original 8II lines. There were 15 different genotype combinations for the alleles for the initial 7II X 8II crosses. From

an F_2 population of these crosses male fertility and male sterility were observed in approximately a 3:1 ratio. Plants within each cytotype group did not express the same 3:1 ratio for male fertility and male sterility.

It was determined that when 7II plants homozygous recessive for the msg1 allele, on chromosome 5, were used in the initial 7II X 8II crosses, all of the 7II F_2 progeny would be male sterile and all of the F_1 cytotype and 8II cytotype progeny would be male fertile. This resulted because the only chromosome 5 in the F_1 cytotype carries the recessive msg1 allele.

If the msg2 allele involved in the cross, the different cytotype F_2 progeny would occur in a 3:1 ratio for male-fertile and male-sterile plants.

If it is assumed that only plants homozygous recessive for the msg1, msg2 or homozygous recessive for both the msg1 and msg2 alleles were used from Composite Cross XXXII, and that the msg16 allele is homozygous dominant, it is possible to explain the deviation of male fertility vs male sterility in the different F_2 cytotype plants. With the above assumptions, it was determined that approximately 65% of the initial 7II X 8II crosses were onto plants homozygous recessive for the msg1 allele or homozygous recessive for only the msg 2 allele.

It was concluded that an MSFRSP of 8II plants could be developed for exploitation of the extra genetic material it carries. Once developed, the 8II population must be grown in physical isolation from any normal 7II plants.

This 8II population will rely upon the msg2 allele for its male sterility, but any msg allele not dominated by alleles on the duplicated chromosome material in the 8II plants could be utilized.

This 8II population of barley offers exploitable genetic material beyond that carried by normal 7II populations of barley. Once other 8II populations are developed, they could be intercrossed to develop barley populations with 9 or even more pairs of chromosomes. The genetic variability that could be generated in barley utilizing this approach is essentially unlimited.

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