Chromosomal Location of Genetic Male Sterility Genes in Four Mutants of Hexaploid Wheat

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ABSTRACT

Few genetic male sterility (GMS) genes have been mapped in hexaploid wheat (*Triticum aestivum* L.). Our objective was to locate to chromosomes the GMS genes in mutants FS2, FS3, FS20, and FS24. We crossed each mutant to the Cornerstone male sterile, which has the *ms1c* allele, to determine allelic relationships. We crossed the FS20 mutant to ‘Chris’ monosomics, and observed segregation in the F2 and backcrosses to FS20. After observing the results, we made appropriate crosses to Chinese Spring (CS) ditelosomic lines to locate and map the genes to chromosome arms. The allelism test to Cornerstone indicated that the mutants FS2, FS3, and FS24 were allelic to *ms1*. In the monosomic analysis of the mutants FS20 gene, half of the monosomic 3A plants were male sterile. Therefore, the mutated gene in FS20 was located in chromosome 3A. This conclusion was confirmed by analyzing segregation ratios in backcross populations involving chromosome 3A. The FS20 mutant was crossed with CS ditelosomic 3AS and 3AL, and the monotelodisomic 3AS plants were male sterile. Therefore, the FS20 gene was in chromosome arm 3AL. A backcross of monotelodisomic 3AL plants to FS20 was used to map the mutated gene in FS20 relative to the centromere. The linkage chi-square test indicated that the FS20 gene was not linked to the centromere of chromosome 3A. The gene symbol *ms5* was assigned to the mutated gene in FS20, and gene symbols *ms1d, ms1e*, and *ms1f* were assigned to the mutations in FS2, FS3, and FS24, respectively.

Male sterility may be conditioned by either cytoplasmic specific or genetic (chromosomal) male sterility (GMS) genes. Cytoplasmic male sterility has been more extensively studied than GMS for hybrid wheat production. However, there have been proposals to use GMS for producing hybrid wheat (Driscoll, 1972; Trupp, 1971). In addition, GMS may be used in population improvement by facilitating crosses in recurrent selection schemes (Krishna Rao et al., 1990).

There have been many reports of GMS in the literature, but only four GMS loci have been located to wheat chromosomes. There are three known mutations of the *ms1* locus in chromosome arm 4BS (previously 4A) which are inherited as monogenic-recessive genes. These mutants were named Pugsley’s (*ms1a*; Suneson, 1962), Probus (*ms1b*; Fossati and Ingold, 1970), and Cornerstone (*ms1c*; Driscoll, 1977). Because the mutations in Probus and Cornerstone were radiation induced, these mutants are presumed to result from a terminal deletion of chromosome arm 4BS. The Pugsley’s mutant was isolated as a spontaneous mutant, and it likely has an intact 4BS arm. The location of the *ms1* gene has been physically mapped to a region comprising the distal 16% of the 4BS chromosome arm (Endo et al., 1991). The dominant male-sterile genes *Ms2* and *Ms3* are located in chromosome arms 4DS and 5AS, respectively (McIntosh et al., 1998). The *Ms4* gene is a dominant male-sterility gene that recently has been located by Maan and Kianian (2001) to chromosome arm 4DS.

Franckowiak et al. (1976) treated seeds of alloplasmic Chris (which has the cytoplasm of *T. tauschii* L.) with ethyl methanesulfonate in an attempt to induce mutations of a cytoplasmic male-sterility gene in chromosome 1D. All of the induced mutations proved to be cytoplasmic nonspecific (Sasakuma et al., 1978). Five of these mutants were of particular interest. The FS6 mutation was conditioned by a dominant gene subsequently assigned the gene symbol *Ms3*. The mutations in FS2, FS3, FS20, and FS24 were each inherited as monogenic-recessive genes. These four mutants had low levels of self-fertility in bagged spikes, and high levels of female fertility as indicated by seed set following hybridization. On the basis of segregation patterns in the F1 of intercrosses, Sasakuma (1978) concluded that mutations in FS2, FS3, and FS24 were allelic, but the FS20 mutant was nonallelic to the other mutations. Sasakuma et al. (1978) did not establish the chromosomal location of the mutant genes or determine the allelic relationship to *ms1*, which is the only mapped monogenic-recessive locus for GMS in wheat. Determining the chromosomal location of these genes is the first step in further genetic studies including gene cloning. The objective of our study was to determine the allelic relationship of FS2, FS3, FS20, and FS24 relative to *ms1* and to determine the chromosomal locations of these GMS genes.

MATERIALS AND METHODS

Mutant Stocks

Male sterile (*msms*) alloplasmic Chris plants carrying the FS2, FS3, FS20, and FS24 mutant genes were crossed to Chris (*MsMs*) to produce heterozygous male-fertile (*MsMs*) *F1* plants which were backcrossed to respective male-sterile plants of each mutant. The resulting populations segregated one heterozygous male fertile to one homozygous male sterile. Each mutant population was then maintained by mating male-sterile females with male-fertile sibs. Maintaining the populations in this fashion has the advantage that male-sterile plants occur at a 1:1 rather than a 3:1 ratio, and that all male-fertile plants in the population are known heterozygotes.

Allelism Tests

The Cornerstone (PI409014) mutant, which carries the *ms1c* allele, was obtained from the National Small Grains Collect-

Abbreviations: GMS, genetic male sterile; CS, Chinese Spring.
tion, Aberdeen, ID. We crossed male-sterile (mms) plants of Cornerstone with heterozygous male-fertile (Msms) plants of the FS mutants. The F1 plants were grown in the greenhouse and classified for anther extrusion and chasmogamy. The data were tested with chi-square analysis for fit to a 1:1 ratio.

**Cytogenetic Techniques**

Root-tips were excised, chilled for 24 h in 2°C tap water, fixed for 18 h in Farmers solution (3:1 95% ethanol/glacial acetic acid, v/v), hydrolyzed in 1 M HCl at 60°C for 10 min, stained in leuco-basic fuchsin for 3 h, and stored at 4°C in 70% (v/v) ethanol. Root tips were squashed in 1% (v/v) aceto-carmine, and cells were examined by brightfield microscopy to determine chromosome number.

**Monosomic Analysis**

Because the mutants were induced in alloplasmic Chris, we attempted to avoid any possible epistatic effects by using Chris rather than Chinese Spring (CS) monosomics to determine the chromosomal location of the sterility gene in FS20. Heterozygous male-fertile (Msms) plants of FS20 were crossed as males to the Chris monosomics. Chromosome number of the F1 plants was determined. Monosomic F1 plants were classified for male sterility by observing anther extrusion and chasmogamy in the primary tiller of each plant. Spikes of two or three secondary tillers on each male-sterile plant were bagged before anthesis to observe seed set. Monosomic F1 plants that were male fertile (therefore Msms for non-critical crosses and Ms_ for the critical cross) were crossed as male to male-sterile (mms) plants of FS20. The same male-fertile plants were self-pollinated to produce the F2 families. At least one bagged spike of each male-sterile plant of the FS mutant was left unpollinated to observe seed set and insure that the plant had the mms genotype.

We space-planted F2 families from each monosomic plant in the field. The planting rate was 10 seeds per meter of row. At flowering, each family was classified for male sterility by observing chasmogamous spikes. The number of male-sterile and male-fertile plants within families was counted and each family’s parental F1 plant was classified as either heterozygous (Msms) or homozygous dominant (MsMs) for the male-sterility gene. Following that classification, with the exception of the putative critical cross, the BC1 families tested were derived from F1 plants with the MsMs genotype. The BC1 families of noncritical crosses were planted in the greenhouse and the plants were classified for anther extrusion and chasmogamy at flowering. For the putative critical cross, root-tips of the BC1 plants were prepared, and the plants were classified for chromosome number and male sterility. Chi-square analysis was used to test the data for goodness of fit to monogenic ratios.

**Telosomic Analysis**

On the basis of the observed segregations in the greenhouse, additional appropriate crosses were made to CS telosomics (Chris telosomics were not available). Male-sterile (mms) plants of the FS20 mutant were crossed to CS double-ditelosomic 3A (dDt 3A), CS ditelosomic 3AS (Dt 3AS), and CS Dt 3AL. Finally, F1 plants that were monotelodisomic 3AL (41’ + 1’; Msms) were crossed as male to male-sterile (mms) plants of FS20 to map the position of the FS20 gene. Linkage chi-square was used to test the data for linkage of the 3A centromere and the mutated gene of FS20.

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**RESULTS AND DISCUSSION**

**Allelism Tests**

Because the allelism test was conducted by crossing male-sterile (mms) plants to heterozygous male-fertile (Msms) plants, segregation in a 1:1 ratio indicated an allelic relationship of the mutant genes with ms1c. Good fits to a 1:1 ratio were observed in crosses of FS2, FS3, and FS24 with the Cornerstone mutant (Table 1). We concluded that the mutations in FS2, FS3, and FS24 were located in chromosome 4B and allelic or closely linked to ms1. Male sterile plants were not observed in the cross to FS20. Therefore, this mutant represents an unmapped locus for male sterility.

**Monosomic Analysis**

Heterozygous plants of FS20 were crossed to the Chris monosomics, so only half of the F1 plants received the mutated gene. If the gene in FS20 is hemizygous effective, half of the monosomic F1 plants in the critical cross would have the ms_ genotype and be male sterile. All monosomic F1 plants were male fertile except in the cross of Chris monosomic 3A, where there were three male-fertile and five male-sterile monosomic F1 plants. This result indicated that sterility in FS20 was conditioned by a hemizygous-effective gene located in chromosome 3A.

Segregation for male sterility was observed in the F2 and BC1 for all chromosomes to confirm that the mutated gene in FS20 was located in chromosome 3A (Table 2). Except for chromosome 3A, the F2 data included only those families segregating for male sterility. In the case of chromosome 3A, larger population sizes and data from all families were included as this chromosome already had been putatively identified as the critical cross. The monosomic 5A-F2 population consisted of a small number of plants with a heading date similar to Chris and a larger number of late-heading plants. Chris monosomic 5A plants are very late in heading (Carlson, 1982). Because only those F2 plants with normal heading dates were classified, all of the 5A-F2 plants classified were likely disomic. For the 4B population, plants nullisomic for chromosome 4B would be expected to express sterility because of the absence of ms1. Because nullisomics occur at an average frequency of 3% (Sears, 1953), it is unlikely that more than four to five nullisomic plants could have occurred in the 4B population.

Monogenic segregation ratios were observed for 15 of the 21 F2 populations (Table 2). In addition to having an excess of populations that did not fit a 3:1 segregation ratio, in 14 of the 15 populations that did fit a 3:1 ratio,
To determine the chromosomal arm location of the mutated gene in FS20, male-sterile plants of FS20 were crossed as female to CS telosomics for chromosome 3A. We grew two F1 plants of the cross to CS dDt 3A and three F1 plants of the cross to CS Dt 3AL, and these plants were male fertile. There were nine monotelodisomic-3AS F1 plants, all of which were male sterile. Therefore, the mutated gene in FS20 was in chromosome-arm 3AL.

The results of mapping the mutated gene in FS20 are shown in Table 3. There were 103 plants classified for male sterility and chromosome number. Twenty-one of these plants had chromosome numbers other than 42 or 41 + t', and these plants were excluded from the analysis. Thirty-six of the remaining 82 plants were classified as recombinants, with an observed recombination frequency of 43.9 ± 5.5%. When the data were analyzed by linkage chi-square analysis, transmission of the telosome was found fit a 1:1 ratio (P = 0.377), and transmission of the sterility gene also fit a 1:1 ratio (P = 0.659). In analyzing both traits, the data did not differ significantly from a 1:1:1:1 ratio (P = 0.533) and the chi-square for linkage was not significant (P = 0.269). Therefore, we concluded that the mutated gene in FS20 was independent of the chromosome 3A centromere.

As CS Dt 3AS is fertile, 3AL was not suspected of having a gene conditioning male sterility (Sears and Sears, 1979). Therefore, locating the mutant FS20 gene to chromosome arm 3AL was somewhat surprising, and may indicate the presence of an additional gene(s) on 3AL that influences fertility. The wheat genome should contain many genes influencing sterility. In barley (Hordeum vulgare L.), there are 50 known GMS loci (Franckowiak, 1997). Each of wheat's genomes should have genes that are paralogous to the barley GMS genes. Because of the polyploid nature of wheat, mutations of these genes will be difficult to detect. Evidence for additional genes in Cornerstone that affect fertility (Barlow and Driscoll, 1981; Islam and Driscoll, 1984) supports a hypothesis that additional GMS genes with epistatic interactions may occur in Chris.

The conclusion that the mutation in FS20 was not allelic to the mutations in the other FS mutants was consistent with the results of Sasakuma et al. (1978). Our results provide additional information concerning

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<th>Chromosome no.</th>
<th>Sterile</th>
<th>Fertile</th>
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<tr>
<td>42'</td>
<td>20</td>
<td>17</td>
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<tr>
<td>41' + t'</td>
<td>19</td>
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† Male-sterile (msms) plants of FS20 were used for crossing. Chinese Spring ditelosomic 3AL (CS d3AL) had the MsMs genotype.
‡ Includes plants with 41', 40' + t', and 40' + 2t'.
the location of these mutant genes. We found that the mutation on FS20, which has been assigned the gene symbol ms5, was independent of the centromere in chromosome arm 3AL. Molecular mapping of this gene is needed to map more accurately its position in 3AL and to determine if it may be more suited for map-based cloning than some of the other male-sterility genes (Qi and Gill, 2001). The crosses to Cornerstone indicated that the mutated genes in FS2, FS3, and FS24 were located in the deleted segment of the Cornerstone mutant. While it is possible that there could be more than one gene for male sterility located in this segment, it is more likely that the genes in FS2, FS3, and FS24 are allelic to ms1. The gene symbols ms1d, ms1e, and ms1f are assigned to the mutations in FS2, FS3, and FS24, respectively.

REFERENCES


