

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 mutated 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 mutants of the *ms1* locus in chromosome arm 4BS (previously 4A α) 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 Collec-

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tion, Aberdeen, ID. We crossed male-sterile (*msms*) 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-carmin, 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 (*msms*) 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 *msms* 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 (*Msm*) 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 (*msms*) 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' + t'; *Msms*) were crossed as male to male-sterile (*msms*) 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.

Table 1. Segregation for male sterility in crosses of Cornerstone (*ms1cms1c*) with heterozygous-fertile plants (*Msms*) of four male-sterile mutants and goodness of fit to a monogenic ratio.

Male parent	No. of plants		Probability of fit to a 1:1 ratio
	Fertile	Sterile	
FS2	43	44	0.915
FS3	27	26	0.891
FS20	77	0	<0.001
FS24	23	24	0.884

RESULTS AND DISCUSSION

Allelism Tests

Because the allelism test was conducted by crossing male-sterile (*msms*) 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,

Table 2. Segregation for male sterility in the F₂ of Chris monosomic//FS20/Chris, in the BC₁ of FS20///Chris monosomic//FS20/Chris, and goodness of fit to monogenic ratios.

Chromosome	Pooled F ₂ †			Pooled BC ₁		
	Fert.	Ster.	3:1 Prob.	Fert.	Ster.	1:1 Prob.
1A	78	10	0.003	21	20	0.876
2A	30	6	0.248	11	6	0.225
3A	114	0	<0.001	(88	9)‡	<0.001
4A	69	8	0.003	19	17	0.739
5A	12	4	1.000	22	27	0.475
6A	31	7	0.349	13	16	0.577
7A	68	14	0.097	14	15	0.853
1B	62	13	0.125	29	21	0.258
2B	54	17	0.837	17	18	0.866
3B	61	9	0.019	19	19	1.000
4B	68	17	0.287	13	21	0.170
5B	60	11	0.064	20	21	0.876
6B	55	12	0.180	12	20	0.157
7B	51	13	0.386	14	13	0.847
1D	60	10	0.038	19	16	0.612
2D	42	10	0.337	21	19	0.752
3D	61	5	0.001	23	23	1.000
4D	57	13	0.214	21	18	0.631
5D	61	11	0.057	16	20	0.505
6D	47	13	0.551	22	24	0.768
7D	57	13	0.214	12	14	0.695

† The F₂ data are only from those F₁ plants used as parents of the backcrosses. Except for chromosome 3A, data from homozygous fertile families have been excluded.

‡ Includes 86 fertile plants with 42', 2 fertile plants with 41' and 9 sterile plants with 41'.

there was a deviation from the expected ratio towards an excess of fertile plants. The expression of chasmogamy can be influenced by environment, and because plants were classified as sterile only if they clearly expressed chasmogamy, a consistent deviation towards fertile plants was observed in the populations. However, the F₂ data still supported the conclusion that the mutant gene in FS20 was a hemizygous-effective gene on chromosome 3A. The population testing chromosome 3A was the only F₂ population where male sterility was not observed. This indicated that the three F₁ plants had the *Ms₋* genotype and, when selfed, produced homozygous fertile families. Segregation in the F₂ for the remaining 20 chromosomes indicated that the F₁ plants had the *Msms* genotype. All of the BC₁ populations except 3A fit monogenic ratios. These results eliminated all chromosomes except 3A as the location of the mutated gene in FS20.

As a final test of chromosome 3A, we examined chromosome number and segregation for male sterility in the BC₁ plants derived from crossing the three male-sterile monosomic 3A (*Ms₋*) plants to FS20 (*mms*). In this cross, all disomic plants should be male fertile (*Mms*) and all monosomic plants should be male sterile (*ms₋*). The BC₁ plants segregated 88 fertile: nine male sterile (Table 2) and this segregation did not fit the 1:1 ratio expected of a noncritical cross ($P < 0.001$). The population included 86 fertile-disomic plants, nine male-sterile monosomic plants, and two fertile-monosomic plants. The two anomalous fertile-monosomic BC₁ plants may have resulted from either misclassification or univalent shift. The Chris monosomics are known to have chromosomal instability that can lead to univalent shift (Carlson, 1982). We concluded that the results of the

backcrosses were consistent with the mutated gene being located in chromosome 3A.

Telosomic Analysis

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 $43.9 \pm 5.5\%$. 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

Table 3. Segregation for male sterility in a backcross population having the pedigree FS20//FS20/CS dt 3AL.†

Chromosome no.	No. of plants	
	Sterile	Fertile
42'	20	17
41' + t'	19	26
Other‡	14	7
Total	53	50

† Male-sterile (*mms*) plants of FS20 were used for crossing. Chinese Spring ditelosomic 3AL (CS dt3AL) had the *Mms* 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.

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