A molecular marker closely linked to the male sterile *Ms*2 gene in common wheat (*Triticum aestivum)*

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ABSTRACT

A male sterile wheat mutant named 'Taigu' was found in a wheat field in China in 1972. The male sterility is controlled by a single dominant gene that was referred to as *Ms*2. Recently this gene was linked to a dwarfing gene through crossing 'Taigu' with the short wheat 'Aibian 1' carrying the dwarfing gene *Rht-D1c*. The objective of this study was to develop molecular markers linked to the male sterility *Ms*2 gene in common wheat. One hundred and twenty two near isogenic lines were developed through backcrossing and sib inter-crossing, and used for the development of molecular markers. In the SSR analysis, after screening 48 pairs of SSR primers, a marker, *ms2-wmc617*, was identified closely linked to the male sterile *Ms*2 gene and mapped at the distal position of chromosome 4DS. The use of the molecular marker *ms2-wmc617* can facilitate recurrent selection in a wheat breeding program. In addition, the marker is at the same locus as the dwarfing gene *Rht-D1c*. Thus, the identification of the molecular marker, followed by the development of a fine genetic map of chromosome 4DS, could provide a firm foundation for cloning both *Ms*2 and *Rht-D1c* genes.

INTRODUCTION

In 1972 a male sterile wheat (*Triticum aestivum*) mutant was found in a wheat field in China by an agricultural technician (1). This mutant was called Taigu male sterile wheat and the dominant gene controlling it was labelled *Ta*1 (2). Using genome analyses and telosomic mapping, Liu and Deng (3) located the single dominant gene *Ta*1 (new nomenclature *Ms*2) on the short arm of chromosome 4D, 31.1 cM from the centromere. Liu (4) developed a dwarf male sterile wheat by crossing a Taigu male sterile plant with the dwarf wheat cultivar "Ai-Bian 1" that had the dominant dwarfing gene *Rht*10 (new nomenclature *Rht-D1c*) on chromosome 4D (5). The hybrid progeny involving Taigu male sterile wheat segregated 1 fertile plant: 1 male sterile plant. The male fertile F_1 plants were true breeding for male fertility, whereas outcrossed progeny of the male sterile F_1 plants again segregated 1 male fertile plant: 1 male sterile plant. The characteristics and genetic pattern of the male sterility were not affected by environmental conditions or genetic background (6). Because of its normal cytoplasm, wide open glumes and protruding stigma (favouring fertilization by foreign pollen), male sterile wheat is amenable to genetic and breeding studies. For example, it is a useful tool for backcrossing and recurrent selection in the development of improved quantitative characters, such as yield and disease resistance. If marker assisted selection is used at the seedling stage, the recurrent selection could be more efficient. Here we report a microsatellite marker closely linked to the male sterile *Ms*2 gene in wheat.

MATERIALS AND METHODS

Seed of the Chinese wheat cultivar 'Longmai 9' and a dwarf male sterile *Ms*2 "Ai-Bai" wheat in a 'Longmai 9' genetic background (four backcrosses with Longmai 9 as a recurrent parent) were provided by Fangpu Han at the Eastern Cereal and Oilseed Research Center, Ottawa, Canada in 2001. The dwarf male sterile *Ms*2 Ai-Bai wheat was developed originally by Liu (4). An additional backcross was made with the same recurrent parent 'Longmai 29' at Eastern Cereal and Oilseed Research Center, Ottawa, Canada in 2002. Five sib inter-crosses were made between male sterile plants and fertile plants in each generation segregating for fertile and sterile plants. Thus, one hundred and twenty two near isogenic lines (BC_5S_5) were developed through backcrossing and sib inter-crossing, and used for the development of molecular markers. The morphological characters among these 122 near isogenic lines were phenotypically similar, except for distinct differences in plant height. Marker analysis was the same as described by Somers et al. (7), with the initial screening of 48 pairs of primers for polymorphism. Four markers giving polymorphic patterns were used in QTL analysis.

RESULTS AND DISCUSSION

Fifty-four out of the 122 near isogenic lines were male sterile and sixty-eight were male fertile. The tall plants were male fertile, whereas the short plants were male sterile, indicating that the dominant dwarfing gene *Rht-D1c* and the male sterile *Ms*2 gene were closely linked. However, line T5-S5 109 had a tall, male sterile phenotype, revealing that a crossover had occurred between the *Ms*2 and *Rht-D1c* genes.

Four SSR markers: wmc617, wmc48, wmc89 and cfd23, on chromosome 4DS produced polymorphisms and were used to genotype the population. Microsatellite marker *WMC617* amplified products from multiple loci which are known to be mapped to chromosome 4AL, 4BL and 4DS (Somers et al., 2004). Specifically, a microsatellite amplification product of about 225 bp could be detected only in the dwarf, male sterile lines and amplified on alternate allele on tall, male fertile lines with one exception. Line T5-S5-109 was male sterile but showed the alternate allele at *WMC617* (228 bp). Due to the complex nature of PCR products amplified with *WMC617*, the alternate allele in tall, fertile lines overlaps and migrates to a similar position of a second locus co-amplified by this marker. The SSR marker was referred as to *MS2-WMS617*. A linkage map of chromosome 4DS was developed, based on the polymorphisms produced by these four markers (Fig.1). The marker *MS2-WMS617* was mapped at a distal position of chromosome 4DS, 1 cM away from the male sterile *Ms*2 gene and coincident with the *Rht-D1c* gene.

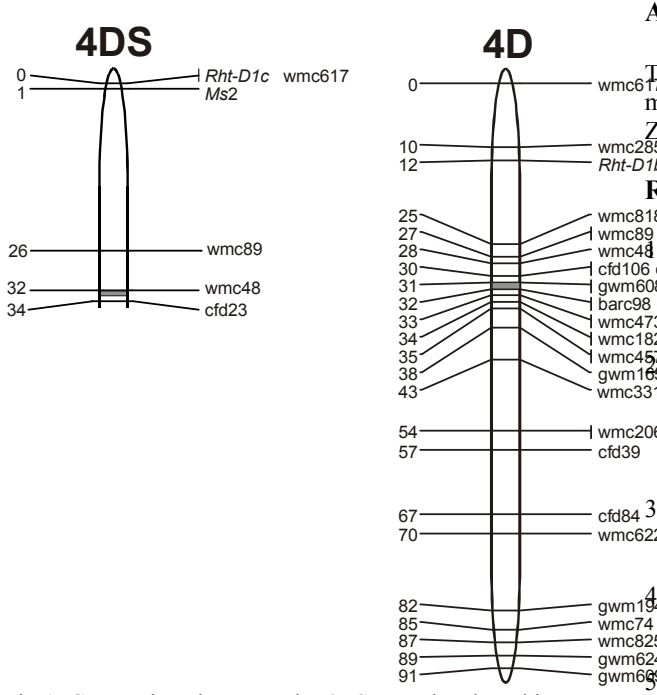

Fig.1. Comparison between the 4DS map developed in this study and the consensus 4D map (7).

Based on a telocentric mapping analysis, Izumi et al. (8) reported that the dwarf *Rht-D1c* gene is located on chromosome 4DS more than 50 cM from the centromere. Liu (9) repeated the genetic study by crossing Ai-Bian 1 carrying the dwarf gene *Rht-D1c* with 4DS ditelo of Chinese Spring and test-crossing the F_1 progeny with Chinese Spring. This latter study suggested that the *Rht-D1c* gene is located on chromosome 4DS 32 cM from the centromere. Results of the current study indicate that *Rht-D1c* is about 33 cM from the centromere of chromosome 4DS consistent with the consensus map of chromosome 4D (7). Liu (4) first established the linkage between *Ms*2 and *Rht-D1c,* by crossing Taigu male sterile carrying the male sterile *Ms*2 gene with the cultivar 'Ai-Bian 1' carrying the dominant dwarf *Rht-D1c* gene. The genetic distance between the two genes was 0.18 cM (9), based on a mapping population of 3917 plants. In the current study, the dwarf *Rht-D1c* gene was found to be located at about one cM from the male sterile *Ms*2. The results also showed that the dwarf *Rht-D1c* gene is at the same locus as marker *MS2-WMS 617*, suggesting that there is possibility to clone the dwarf *Rht-D1c* gene based on the marker sequence.

The male sterile *Ms*2 wheat can be used as a tool for recurrent selection to improve quantitative traits, such as resistance to scab in wheat. During recurrent selection, male fertile wheat plants in the male sterile (female) rows must be identified and removed before anthesis. Identification of the molecular marker *MS2-WMC617* closely linked to the male sterile *Ms*2 gene could facilitate recurrent selection in wheat by identifying male sterile plants at the seedling stage.

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REFERENCES

1. Gao, Z. L. 1987. The discovery of the Taigu genic male sterile wheat. *In* Taigu Genic Male-Sterile Wheat. Edited by J.Y. Deng. China Agricultural Scientech Press, Beijing. **pp** 5-6.
2. Deng, J.Y., and Gao, Z.L. 1987. Determination of a dominant male sterile gene and its prospect in practice. *In* Taigu Genic Male-Sterile Wheat. Edited by J.Y. Deng. China Agricultural Scientech Press, Beijing. pp. 7-21.
3. Liu, B.H., and Deng. J.Y. 1986. A dominant gene for male sterility in wheat. Plant Breeding, **97:** 204-209.
4. Liu, B.H. 1987. Isolation of a spontaneous chromosome translocation in common wheat. Plant Breeding, **98:** 266-267.
5. Börner, A., Röder, M., and Korzun, V. 1997. Comparative molecular mapping of GA insensitive *Rht* loci on chromosomes 4B and 4D of common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **95:** 1133-1137.
6. Zhang, Y.Z., and Ji, F.G. 1987. A study for stability of male sterility of Taigu male sterile wheat. Shanxi Agricultural Sciences, **6:** 5-8.
7. Somers, D.J., Isaac, P., and Edwards, K. 2004. A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **109:** 1105-1114.
8. Izumi, N., Sawada, S., and Sasakuma, T. 1983. Genetic analysis of dwarfness in *Tritcum aestivum* L., cv 'Ai-bian 1'. Seiken Ziho. **31:** 38-48.

9. Liu, B.H. 2001. Theory and methodology of crop improvement. China Agricultural Scientech Press, Beijing. Pp 404-409.