Genetic analysis and molecular markers associated with multi-gynoecia (*Mg*) gene in Trigrain wheat

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Wang, Z., Xu, D., Ji, J., Wang, J., Wang, M., Ling, H., Sun, G. and Li, J. 2009. **Genetic analysis and molecular markers associated with multi-gynoecia (*Mg*) gene in Trigrain wheat**. Can. J. Plant Sci. **89**: 845–850. Trigrain wheat normally produces up to three gynoecia in a single floret and forms three close-set grains. The gene conferring the multi grain phenotype was earlier designated *Mg*, the multiple gynoecia gene. Different genetic patterns controlling this trait have been reported. In the present work we studied the inheritance of the three grains trait and identified simple sequence repeats (SSR) markers linked to the *Mg* gene. Segregation analysis in the cross IGDB-TW (trigrain wheat)/Chinese Spring confirmed that a single dominant gene controlled the three grains trait. An allelism test showed that the same gene controlled the trigrain trait in line Trigrain-Yin 1. A total of 339 microsatellite markers were tested for polymorphism by bulked segregation analysis (BSA) in an F_2 population. Six microsatellite markers, *Xcfd233*, *Xgdm6*, *Xgdm87*, *Xgwm311*, *Xgwm349* and *Xgwm539*, on chromosome 2DL, were linked to *Mg*. Using the CS 2D deletion lines, *Mg* gene was localized to the distal region of chromosome 2DL. The microsatellite markers identified in this study have the potential for further mapping and map-based cloning of the gene.

Key words: Simple sequence repeats, physical mapping, trigrain wheat

Wang, Z., Xu, D., Ji, J., Wang, J., Wang, M., Ling, H., Sun, G. et Li, J. 2009. **Analyse génétique et marqueurs moléculaires du gène codant la multi-gynoécie (*Mg*) chez le blé Trigrain**. Can. J. Plant Sci. **89**: 845–850. Normalement, le blé Trigrain produit jusqu'à trois gynoécies par fleuron, ce qui donne trois grains collés l'un à l'autre. Le gène responsable de ce phénotype a été désigné *Mg*, pour gène des pistils multiples. Plusieurs processus génétiques semblent réguler ce caractère. Les auteurs se sont penchés sur l'hérédité du caractère « trois grains » et ont identifié les marqueurs SSR associés au gène *Mg*. L'analyse de ségrégation de l'hybride IGDB-TW (blé Trigrain)/blé de printemps chinois confirme qu'un seul gène dominant commande le caractère du grain triple. Un test d'allélisme indique que le même gène commande le caractère trigrain de la lignée Trigrain-Yin 1. En tout, les auteurs ont testé 339 microsatellites pouvant servir de marqueur par analyse de ségrégation massive d'une population de la F_2. Six microsatellites (*Xcfd233, Xgdm6, Xgdm87, Xgwm311, Xgwm349* et *Xgwm539*) du chromosome 2DL sont associés au gène *Mg*. Grâce aux lignées délétantes CS 2D, les auteurs ont situé ce gène dans la région distale du chromosome 2DL. Les microsatellites identifiés dans le cadre de cette étude pourraient servir à une cartographie plus poussée du gène et à son clonage éventuel.

Mots clés: SSR, cartographie physique, blé trigrain

Trigrain wheat was first reported in 1983 and initially recommended as a novel type of wheat because each floret normally had three gynoecia, which consequently formed three close-set grains in a back-to-back manner after pollination (Fig. 1) (Chen et al. 1983; Tong and Tong 1984). The gene controlling three grains traits was named as multi-ovary gene (multi-gynoecia gene in this study).

The obvious difference between trigrain wheat and normal cultivated wheat is that the florets have a lemma, a palea, two lodicules, three stamens, and three gynoecia. Trigrain wheat is also different from the multi-pistil phenotype resulting from pistillody. In multi-pistil structured wheat, the floral components have a lemma, a palea, two lodicules, a pistil and three pistil-like structures instead of stamens (Murai et al. 2002). In addition, the three-gain trait is highly heritable. Because of its distinct floral structure and fertility, trigrain wheat has potential to increase yield and the reproductive index of hybrid wheat, and to provide genetic material for the study of floral development (Ng and Yanofsky 2000). Therefore, it was of interest to study the inheritance of the three grains trait and to locate the gene in the wheat genome.

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Abbreviations: **SSR**, simple sequence repeats

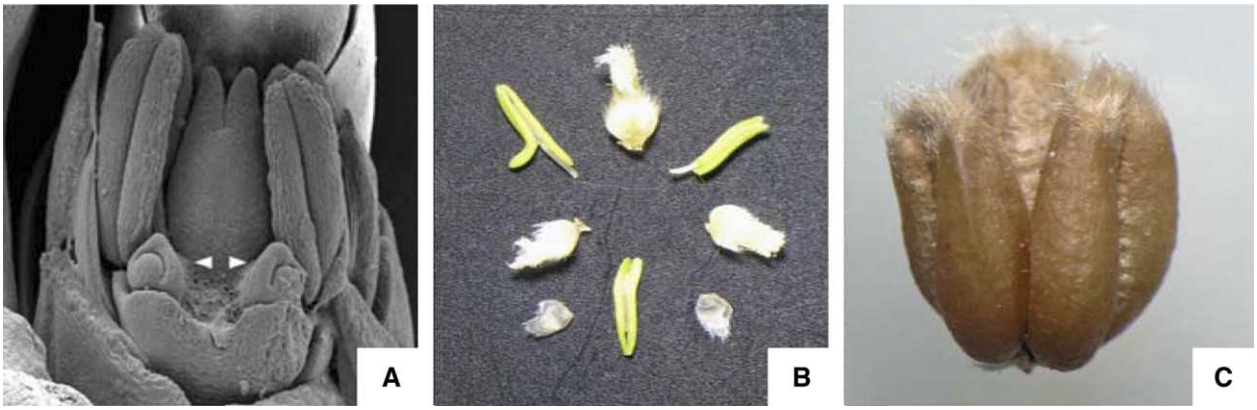

Fig. 1. The two additional gynoecia (A), the floret components without palea and lemma (B), and the three close-set grains (C) of the Trigrainwheat line IGDB-TW.

Since the discovery of trigrain wheat, research has focused on the differentiation and development of floral components. It is clear that the process of floral development in trigrain wheat is similar to that in cultivated common wheat, but the two additional gynoecia initiate after emergence of the first one (Wang et al. 1987; Wang and Ding 1990). Recently, various lines of trigrain wheat with significant morphological variation were developed, and used for genetic studies (Wu et al. 2000; Ma et al. 2000, 2006; Peng 2003). The three grains trait was controlled by a single dominant gene, either with (Wu et al. 2000), or without cytoplasmic effects (Zhi et al. 2002; Peng 2003; Ma et al. 2006), and by recessive genes (Shen et al. 1992; Ma et al. 2000). Genes controlling the trigrain trait were located on chromosomes 5DS, 6BS, and 6B by monosomic and ditelosomic analyses (Shen et al. 1992; Ma et al. 2000). Wang et al. (2005) identified one random amplified polymorphic DNA (RAPD) marker linked to multi- gynoecia gene.

In recent years, molecular markers, such as RAPD, restriction fragment length polymorphism (RFLP), microsatellite or simple sequence repeats (SSR), and amplified fragment length polymorphisms (AFLP) have been employed for genetic mapping in wheat (Williams et al. 1990; Nelson et al. 1995; Vos et al. 1995; Röder et al. 1998). SSR are more informative than other markers, not only because of co-dominance and high levels of polymorphism, but also because they are locus-specific (Powell et al. 1996; Röder et al. 1998; Gupta et al. 2002). High density microsatellite maps of hexaploid common wheat and its diploid progenitors were constructed, and many microsatellite markers were physically assigned to wheat chromosomes using wheat aneuploids and deletion stocks (Endo and Gill 1996; Pestsova et al. 2000; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005). This has provided a wealth of information on the genetic and physical positions of genes, including many conferring morphological and agronomic attributes such as genes for disease and pest resistances (e.g., Adhikari et al. 2004; Zhu et al. 2004).

A trigrain wheat line was developed by our research group. The objectives of the present study were: (1) to characterize the genetic control of the three grains trait in this line, (2) to deter mine allelism of the trait in two different wheat lines, and (3) to identify microsatellite markers linked to the *Mg* gene, and to physically map the gene.

MATERIALS AND METHODS

Plant Materials

Triticum aestivum L.'Chinese Spring' (CS) and two different lines of trigrain wheat, IGDB-TW and Trigrain-Yin1, were used for this study. CS and IGDB-TW, which may have originated from a cultivar in Gansu Province, are maintained at the Centre for Agricultural Resource Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang. Trigrain-Yin1, which originated in Gansu Province, was kindly provided by Dr. J. Wu, University of Northwest Science and Technology of Agriculture and Forestry, Yangling, Shaanxi.

For genetic analysis, reciprocal crosses between CS and IGDB-TW were studied in the field at Shijiazhuang from 2002 to 2004.

IGDB-TW and Trigrain-Yin1 were also inter-crossed and F_1 plants were backcrossed with CS. Sixteen Chinese Spring 2D deletion lines (Table 1), including eleven 2DL deletion lines and five 2DS deletion lines (kindly provided by Dr. T. R. Endo, Kyoto University, Japan) were used to deter mine the locations of markers.

Phenotype Evaluation

Ten spikes from each of the parents, F_1 and plants in the segregating generations were investigated for the multi grain phenotype at two growth stages. The first stage was at the late panicle differentiation stage in March or April, and the second stage was during the flowering and seed-filling periods in May or June. Plants having two or three gynoecia and grains were scored as multi-grained, and plants with one gynoecium and grain were

Table 1. The name, order of Chinese Spring 2D deletion lines listed based on fraction length

Strain name	Deletion line name	Fraction length	Strain name	Deletion line name	Fraction length
LPGKU1092	2DL-06	0.94	LPGKU1093	2DL-07	0.12
LPGKU1095	2DL-09	0.76	LPGKU1089	2DL-02	0.09
LPGKU1097	2DL-11	0.66	LPGKU1103	2DS-05	0.47
LPGKU1094	2DL-08	0.58	LPGKU1102	2DS-04	0.41
LPGKU1088	2DL-01	0.49	LPGKU1101	2DS-03	0.36
LPGKU1090	2DL-03	0.49	LPGKU1099	2DS-01	0.33
LPGKU1096	2DL-10	0.47	LPGKU1100	2DS-02	0.00
LPGKU1091	2DL-04	0.26			

scored as single-grained. Chi-squared statistics were used to test the differences between the observed data and expected segregation ratios.

DNA Extraction

Young leaves were harvested from the parents and F_2 plants of CS/IGDB-TW 3 wk after sowing. The leaves were lyophilized in liquid nitrogen and stored at –80°C. Genomic DNA was isolated using the CTAB method. The precipitated DNA were air-dried and dissolved in 50 μL TE. DNA concentrations were standardized for SSR analysis.

PCR Amplification and Electrophoresis

Microsatellite PCR were performed in 20 μL reaction solutions containing 20 ng of genomic DNA, 250 nM of each primer, 200 μM of dNTPs, 150 μM of $MgCl_2$, and 1 U of *rTaq* DNA polymerase (Toyobo Co., LTD). PCR amplifications were conducted according to Röder et al. (1998). Forty-five PCR cycles were performed in a GeneAmp PCR System 9700 (Applied Biosystems), with each cycle consisting of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. The first cycle was preceded by a 3 min denaturation at 95°C, and the last cycle was followed by a 10 min final extension at 72°C before cooling at 4°C. PCR products were separated by electrophoresis in 6% bis-acrylamide gels for 2.5 h. Gels were stained with ethidium bromide and DNA fragments were recorded with a Typhoon^{TM} 9410 (GE Healthcare) instrument.

SSR-BSA Analysis

Bulked segregation analysis (BSA) was used to identify microsatellite markers putatively linked to the multi-gynoecia gene. Based on the phenotypic data of the F_2 mapping population, two bulk DNA samples were prepared by pooling equal amounts of DNA from 10 multi-grained plants and 10 single-grained plants; these pools and the parental DNAs were used to screen polymorphic microsatellite markers. A total of 339 microsatellite primer pairs, comprising 153 BARC primer pairs, 173 GWM primer pairs, 8 WMC primer pairs, and 5 GDM primer pairs, were screened. Based on the results of testing, 15 F2 plants, including 5 multi-grain homozygotes, 5 mono-grain homozygotes and 5 heterozygotes were chosen to test for association between markers and the *Mg* gene.

Physical Mapping of the Multi-grain Gene

Genomic DNA from Chinese Spring, IGDN-TW and 16 CS 2D deletion lines were amplified with primers putatively located in chromosome 2D. PCR conditions were similar to those described above.

RESULTS

Inheritance of the Multi-grain Trait

F_1 plants from the reciprocal crosses between Trigrain Wheat and CS showed the multi-grain trait, indicating dominance of the trait. F_2 families derived from two F_1 individuals segregated 3 multi-grained:1 single grained ratio (Table 2), indicating that a single gene was involved.

Allelic Relationship Between Two Trigrain Wheat Lines

F_1 plants from the cross IGDB-TW/Trigrain Yin-1 were multi grained. All 92 F_2 and 211 BC_1F_1 individuals were multi grained, suggesting a common allele controlling the multi-grained phenotype.

Microsatellite Markers Linked to *Mg*

Among 339 microsatellite markers screened on DNA from GDB-TW, CS, and the contrasting multi-grained and single-grained DNA pools, 23 (6.8%) produced polymorphic DNA fragments contrasting the multi-grained and single grain pools. These primer pairs were then used to characterize 15 F_2 individuals to test putative associations with the multi-grained allele. Six primer pairs, CFD233, GDM6, GDM87, GWM311, GWM349, and GWM539, generated specific DNA fragments that showed co-dominant patterns. All microsatellite markers

Table 2. Segregation of multi-grain trait (MG) and single grain (SG) phenotypes in the reciprocal cross of IGDB-TW and Chinese Spring

Cross	No. of MG plants	No. of SG plants	χ^2 (3:1)
IGDBTW (♀)/ CS (♂)	124	34	1.03
CS (♀)/IGDB-TW(♂)	107	28	1.31
Pooled the data	231	62	2.30

The value for significance at $P = 0.05$ and df = 1 is 3.84.

amplified more than one polymorphic product. For example, the microsatellite markers *Xcfd233* and *Xgwm349* amplified as five specific fragments from the DNA of CS, the single-grained DNA pool and the five single-grained F_2 plants, and five fragments from the GDB-TW, the multi-grained DNA pool, and five multi-grained F_2 plants, respectively (Fig. 2). Published map data showed that all six markers were located on the long arm of chromosome 2D (Röder et al. 1998; Pestsova et al. 2000; Somers et al. 2004). Our results thus indicated that *Mg* gene was also likely located on 2DL.

Physical Location of the Multi-grain Gene

In order to physically map *Mg*, eleven CS 2DL deletion lines, and five 2DS deletion lines along with CS and GDB-TW, were used to locate six microsatellite markers (Table 3). All six primer pairs amplified the same banding pattern in CS and almost all the 2D short arm deletion lines, but amplified no, or only nonspecific, banding patterns in the other 2DL deletion lines with longer deletions. Two microsatellite primers, GWM539 and CFD233, also amplified the same bands in 2DL-9 or 2DL-6 lines indicating that they retained more of the long arm than the other deletion lines. The results in Table 3 therefore indicate that *xgdm6*, *Xgwm349*, *Xgwm311* and *Xgdm87* are distal to *Xgwm539* and *Xcfd233*, and consistent with consensus genetic maps placing the genes at positions 78, 98, 98, 98, 141.7 and 156cM from the distal end of the short arm [obtained from GrainGenes, Sourdille et al. (2004)]. The bin map locations for those markers are 2DL3-0.49 (*Xcfd233*), 2DL3-0.49 (*Xgwm539*), 2DL3-0.49-1.00 (*Xgwm349*), 2DL9-0.76-1.00 (*Xgwm311*), 2DL9-0.76-1.00 (*Xgdm6*) and 2DL9-0.76-1.00 (*Xgdm87*). Our results indicate that six microsatellite markers were located on the distal

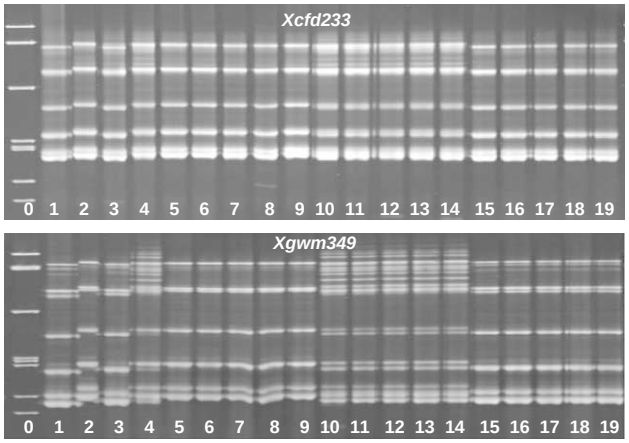

Fig. 2. DNA amplification products obtained with SSR primers, *Xcfd233* and *Xgwm349*. Lane 0: φX174 DNA-Hae III Digest DNA marker, 1: Chinese Spring, 2: trigrain wheat IGDB-TW, 3: single-grain DNA pool, 4: multi-grain DNA pool, 5-14: multi-grain F_2 plants (5-9: homozygous genotype, 10-14: heterozygous genotype), 15-19: single-grain F_2 plants.

Table 3. The physical location of six SSR markers on Chinese Spring, Trigrain wheat, and CS 2D deletion lines

	CS	TW	2DL-06	2DL-09	2DL-11	2DL-08	2DL-01	2DL-03	2DL-10	2DL-04	2DL-07	2DL-02	2DS-05	2DS-04	2DS-03	2DS-01	2DS-02
Xcfd233	A	B	A	–	–	–	–	–	–	–	–	–	A	A	A	A	A
Xgwm539	A	B	A	A	–	–	–	–	–	–	–	–	A	A	A	A	A
Xgwm349	A	B	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A
Xgwm311	A	B	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A
Mg																	
Xgdm6	A	B	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A
Xgdm87	A	B	–	–	–	–	–	–	–	–	–	–	A	–	A	A	A

A, B indicate the banding pattern of Chinese Spring and Trigrain wheat; – indicates no or products.

region of 2D (Fig. 3), and suggesting the *Mg* gene is also located at this region.

DISCUSSION

Inheritance of the Three Grain Trait

The three grained trait was first reported to be controlled by a single dominant gene by Chen et al. (1983). Using different genetic resources Tong and Tong (1984) reported two recessive genes, *mo1* and *mo2*, located on chromosomes 5D and 6B. The former source was thought to be a mutant of common wheat Ganmai 8 (Chen et al. 1983; Peng. 2003), whereas the latter was selected from a segregating population of Ailiduo/Luoyangqing. Zhi et al. (2002), Peng (2003) and Wu et al. (2000) reported that the three-grained trait was controlled by a single dominant gene. On the other hand, Ma et al. (2000) found the three-grained trait to be conditioned by both dominant and recessive genes in three different trigrain lines, and that the cytoplasms of *Ae. kotschyi* and *Ae. ventricosa* suppressed the three grained phenotype in F_1 hybrids. Ma et al. (2006) further confirmed a single dominant gene in the trigrain line DuoII. Our study supports reports that the three grained trait is controlled by the single dominant gene *Mg*.

Molecular Markers for the *Mg* Gene

A RAPD marker was putatively linked to the *Mg* (Wang et al. 2005). In this study, six microsatellite markers appeared to be associated with the *Mg* allele, which could be used as candidate molecular markers to map the *Mg* gene. But, the lower percentage of polymorphism in two studies suggests a close genetic background between common wheat and trigrain wheat. To indentify more markers linked to the *Mg* gene, it seems that other molecular markers, such as AFLP, RFLP, EST, need to be employed.

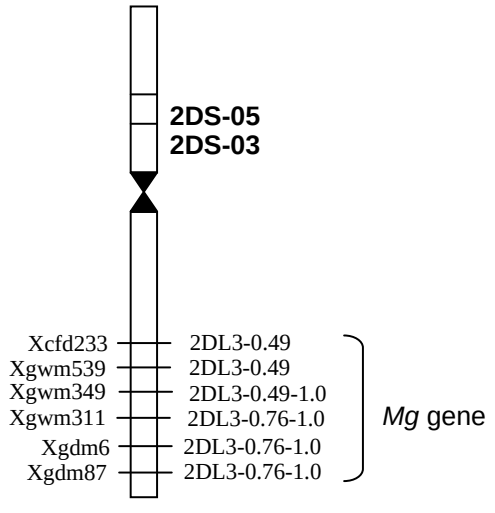

Fig. 3. The physical location of the *Mg* gene was referred to the 2D deletion bin map. The numbers in right side indicated the order of deletion bin for each marker.

Genes conferring the trigrain phenotype were located on 6BS and 5DS using wheat monosomic and ditelosomic lines (Shen et al. 1992). Our result suggest a single gene, presumably, *Mg*, located on chromosome 2DL. The *Mg* gene locus may be same to the *Pis1* locus reported by Peng et al. (2008). However, since we cannot obtain the Trigrain line used by Peng et al (2008), it is impossible to perform the allelic test between these two trigrain lines. The six SSR markers were physically mapped to the distal part of 2DL, with *Xgwm539* and *Xcfd233* the most proximally located. However, the order of the two genes as indicated by the deletion lines was not consistent with the consensus map of Sourdille et al. (2004).

The *Mg* Gene in Plant Flower Development

The early significance of the trigrain phenotype was a belief that it might increase wheat yields and enhance the application of hybrid wheat. Indeed, hybrids between cultivated wheat and trigrain wheat displayed yield improvement (Wu et al. 2000; Ma et al. 2002). It is more likely, however, that trigrain or multi-gynoecia lines will have a role in studying genes regulating floral development (Ng and Yanofsky 2000; Thomas 2001; Nagasawa et al. 2003; Hama et al. 2004).

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