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Efficient mapping of a female sterile gene in wheat (*Triticum aestivum* L.)

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Summary

Studies on inheritance of fertility are of great importance in wheat breeding. Although substantial progress has been achieved in molecular characterization of male sterility and fertility restoration recently, little effort has been devoted to female sterility. To identify the gene(s) controlling female sterility in wheat efficiently, an investigation was conducted for the seed setting ratio using a set of F_2 populations derived from the cross between a female sterile line XND126 and an elite cultivar Gaocheng 8901. Bulked segregation analysis (BSA) method and recessive class approach were adopted to screen for SSR markers potentially linked to female fertility gene loci in 2005. Out of 1080 SSRs in wheat genome, eight markers on chromosome 2D showed a clear difference between two disparate bulks and small recombination frequency values, suggesting a strong linkage signal to the sterility gene. Based on the candidate linked markers, partial linkage maps were constructed with Mapmaker 3.0 (EXP) instead of whole genome maps, and quantitative trait locus (QTL) mapping was implemented with software QTLNetwork 2.0. A major gene locus designated as *taf*1, was located on chromosome 2DS. The above result was confirmed by the analysis for 2007 data, and *taf*1 was identified on the same chromosome 2DS with a confidence interval of 2·4 cM, which could explain 44·99% of phenotypic variation. These results provided fundamental information for fine mapping studies and laid the groundwork for wheat fertility genetic studies.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the staple crops in the world. Genetic studies on fertility and reproduction are important in wheat breeding. Since the first report (Kihara, 1951) that announced a male sterile (MS) line in wheat, more than 70 kinds of wheat MS lines have been developed (Liang & Wang, 2003; Cao *et al*., 2004), such as T, K, V, D, A and P types of cytoplasmic male sterility (CMS) and genic male sterility (GMS). Several genes have been mapped (Tan *et al*., 1992; Wang, 1996; Rong *et al*., 1999; Xing *et al*., 2003; Guo *et al*., 2006) that control male sterility, including photo-thermo-sensitive male sterility. The MS line, generating a gynodioecy of plant species, can be used as pollen acceptor (female plant) in hybrid seed production of the selfing plants (Budar *et al*., 2003; Sodhi *et al*., 2006). On the other hand, the female sterile line, reflecting the evolution of a plant species from a gynodioecy to a dioecy, can be used as a pollenizer in a new hybrid seed production system in which both parents are mixedly sown to generate hybrid seeds (Brown & Bingham, 1984; Daskalov & Mikhailov, 1988; Sreiff, 1997). And this can also provide an opportunity to investigate sex phylogeny and evolution in higher plants (Maurice *et al*., 1994; Charlesworth & Wright, 2001; Delph, 2003).

Many female sterile materials have been found in various plants such as barley, rice, soybean, sugar beet, rapeseed, ramee and roselle (Jassem, 1971; Hanna & Powell, 1973; Ahokas, 1977; Ling *et al*.,

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1991; Vaidya, 1994). Female sterile lines in capsicum and clover have been used as pollinators in their hybrid seed production to shorten the parents' pollinating distance and to obtain higher hybrid seed yield (Brown & Bingham, 1984; Daskalov & Mikhailov, 1988). A female sterile variant in wheat was first reported in Hungary (Gotzov *et al*., 1979), of which the lower self-reproduction ability is a key issue that needs to be solved. Recently, a new female sterile line in wheat was found in China (Dou *et al*., 2001). The novel mutant possesses some important characteristics: female sterility (no seed setting on its spike) with a normal male fertility in the normal autumn sowing condition, and normal female and male fertilities (a good seed setting) in the late winter sowing condition (Dou *et al*., 2001). Obviously, these characteristics are helpful to solve the issue of self-reproduction.

Genetic mapping has been proven to be a powerful approach for elucidating the genetic architecture of fertility traits. The main objective of the study is to map the major gene(s) of female sterility. The polymorphic molecular markers potentially linked to the female sterile gene loci in wheat were first screened by bulked segregation analysis (BSA) and recessive class approach. Then, an interval mapping was performed based on the identified markers. The result obtained in the present study could provide basic information necessary for cloning the functional gene of female sterility, as well as for developing an elite female sterile line to promote hybrid wheat breeding by marker assisted selection.

2. Materials and methods

(i) *Plant materials and phenotyping*

A female sterile line XND126 and an elite cultivar Gaocheng 8901 with normal fertility were crossed for genetic analysis of female sterility. The parents, their F_1 and F_2 populations were planted at Huaian experimental station in the growth years 2004–2005 and 2006–2007 under the normal autumn sowing conditions. The sample sizes of the P_1, P_2, F_1 and F_2 were 10, 50, 20, 237 plants in 2005, and 10, 50, 20, 243 in 2007, respectively.

The seed setting ratio on fully pollinated spikes of each plant was evaluated to measure the female fertility, which is the ratio of the number of seed setting spikelets to the total number of available spikelets (Dou *et al*., 2001; Hou *et al*., 2006).

(ii) *Marker genotyping*

Genomic DNA was extracted individually from fresh leaf tissues of P_1, P_2, F_1 and F_2 plants, which were freeze-dried and powdered, according to cetyl trimethyl ammonium bromide (CTAB) method (Hoisington *et al*., 1994).

Simple sequence repeat (SSR) markers selected from reference maps were used in genotyping (Plaschke *et al*., 1996; Röder *et al*., 1998; Timothy *et al*., 2002; http://wheat.pw.usda.gov). A total of 1080 SSR markers distributed across 21 pairs of chromosomes of wheat were used in this experiment.

PCR amplification for the SSR makers was performed as follows: 10× Taq buffer, 2·0 mmol/l $MgCl_2$, 200 μmol/l dNTPs, 1·0 unit Taq DNA polymerase, 50 ng template DNA and 50 ng primer were mixed, which was then fixed to 10 μl with distilled water, and finally covered with one drop of mineral oil. The procedure of PCR was: 94 °C for 5 min, followed by 45 cycles of 1 min at 94 °C, 1 min at 50–60 °C (depending on the individual primer set), 2 min at 72 °C, and with a final extension step of 10 min at 72 °C (Röder *et al*., 1998). The amplification products were separated on the 8% PAGE gel with a standard size marker in the first lane of the gel at 180 V for about 2·0 h after pre-electrophoreses for 20 min. Then, the gel was removed from the apparatus and stained using the silver staining method (Xu *et al*., 2002).

(iii) *Screening for linked markers using BSA and recessive class approach*

To reduce the cost and increase efficiency, the polymorphisms under a combination of SSR primers were determined using the bulked extreme (Michelmore *et al*., 1991) and recessive class approach (Zhang *et al*., 1994). Two bulks were generated by pooling extreme F_2 individuals. The first bulk (bulk F) was constituted by equal amounts of DNA from six highly fertile plants, and the second bulk (bulk S) by equal amounts of DNA from six highly sterile plants. On the other hand, markers with different patterns between the two parents were also used to assay individuals in samples of extreme sterile plants from the F_2 populations. The recombination frequency (c value) between a marker locus and the female sterile locus was calculated by the maximum likelihood estimator (Allard, 1956): $c=(N_1+N_2/2)/N$, in which N is the total number of sterile plants surveyed, N_1 is the number of individuals homozygous for the SSR band from the fertile parent (recombinant homozygote) and N_2 is the number of plants heterozygous for the bands from the two parents. The variance was estimated by $V_c=c(1-c)/2N$.

(iv) *Linkage map construction and QTL mapping*

To map putative female sterile genes, the candidate markers (linked to the female sterile gene detected by BSA or recessive class approach) were further assayed through individual genotyping for the whole

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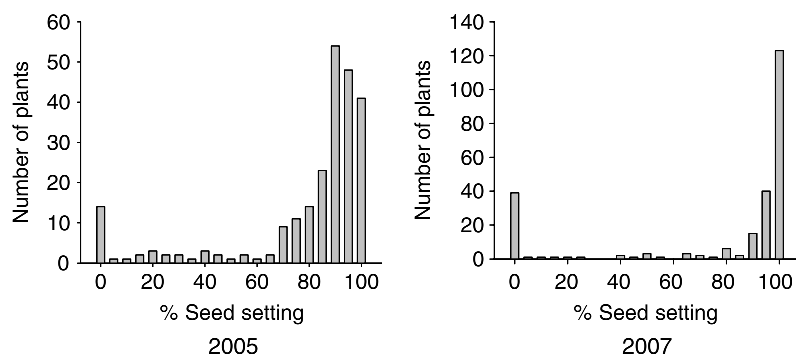

Fig. 1. Frequency distribution for seed setting ratio on fully pollinated spike in the F_2 derived from a cross Gaocheng 8901 × XND126 in 2004–2005, 2006–2007 growth season.

F_2 population. Using the molecular markers (SSR) data of the F_2 population, linkage groups were constructed by the MAPMAKER Version 3.0 (Lander *et al.*, 1987), where the Kosambi's map function (Kosambi, 1944) was adopted in calculating the genetic distances between the markers. A LOD score of 3·0 was used as the threshold for declaring the linkage between markers. To reduce the cost of experiment, the linkage map, covering the candidate region potentially harbouring female fertility gene, was constructed only by the polymorphic markers selected by the BSA or *c* value approach instead of whole genome.

For the data of F_2 population, a genetic model, including additive, dominant and epistatic effects of quantitative trait loci (QTLs) was employed in QTL mapping by the software QTLNetwork 2.0 (Yang *et al.*, 2007). The significance threshold for declaring the existence of a QTL was determined by 1000 permutation tests (Doerge & Churchill, 1996). An *F*-statistic based on Henderson method III was used in hypothesis test. The genetic effects of QTLs were estimated by the Markov Chain Monte Carlo (MCMC) algorithm via Gibbs sampling (Yang *et al.*, 2007). The data were reanalysed with the software WinQTLcart (Wang & Zeng, 2007) to verify the detected linkage.

3. Results

(i) *Frequency distribution of the female fertility in* F_2

The phenotypic data confirmed a significant difference in the fertility between two parents. XND126 and Gaocheng 8901 had a mean setting ratio of 0·0812 (±0·1016, SD) and 0·9397 (±0·0395, SD), respectively. The fertility of the F_1 population was more similar to that of the normal parent Gaocheng 8901. The 2005 and 2007 data showed a substantial variation in female fertility in the F_2 population (Fig. 1). The distribution of female fertility in the F_2 population was bimodal and skewed towards the normal parent (Gaocheng, 8901). These properties indicate that one or few major genes are probably responsible for the genetic variation of female fertility in the two parents (Hou *et al.*, 2006).

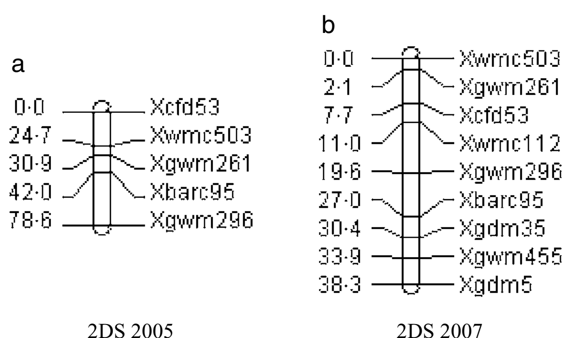

Fig. 2. Linkage groups on chromosome 2DS based on data 2005 and data 2007, the supposed gene locus might harbour based on BSA and *c* value approach.

(ii) *The chromosomal region likely to harbour the putative female sterile gene(s)*

We used 1080 SSR markers distributed across 21 pairs of chromosomes of wheat in 2005 and found that 70 SSR markers amplified polymorphic bands between two parental lines. To identify the positive SSR markers linked to the female sterile gene(s), the 70 polymorphic primer pairs were investigated by BSA. Eight primer pairs showed a clear difference in band pattern between two bulks, five primers on chromosome 2DS and the others on chromosome 2DL. To confirm the findings, the 70 polymorphic primer pairs were retested by 19 extreme sterile plants and the same five markers with a low *c* value were identified on the linkage group of chromosome 2DS (Kong, 2006). Based on the results by BSA, the linkage groups were constructed by the data of SSR markers of 237 F_2 plants, and five distinguished markers Xcfd53, Xwmc503, Xgwm261, Xbarc95 and Xgwm296 were identified to be in the same linkage group on chromosome 2DS (Fig. 2).

To increase the multi-locus mapping precision and the coverage of markers across the potential linkage

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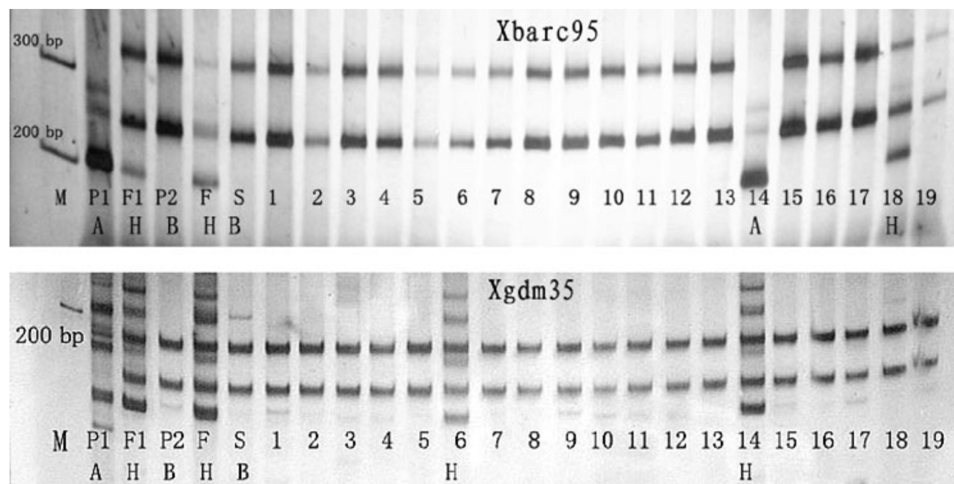

Fig. 3. The amplification pattern of the SSR marker Xbarc95 2005 and Xgdm35 2007 in parents, F_1, bulks and extreme sterile individuals of the F_2 population derived from a cross of Gaocheng 8901 (fertile, P_1) and XND126 (sterile, P_2). The samples in each lane are: M DNA marker; P_1, F_1, P_2, F fertile bulk, S sterile bulk, and 1–19 extreme sterile F_2 individuals. The different alphabetical letters A, B and H revealed the bands from P_1, P_2 and F_1, respectively; B type bands were not wrote on the pictures in 1–19 homology recessive sterile plants. The *c* value was 0·079 for Xbarc95 and also smaller value for Xgdm35.

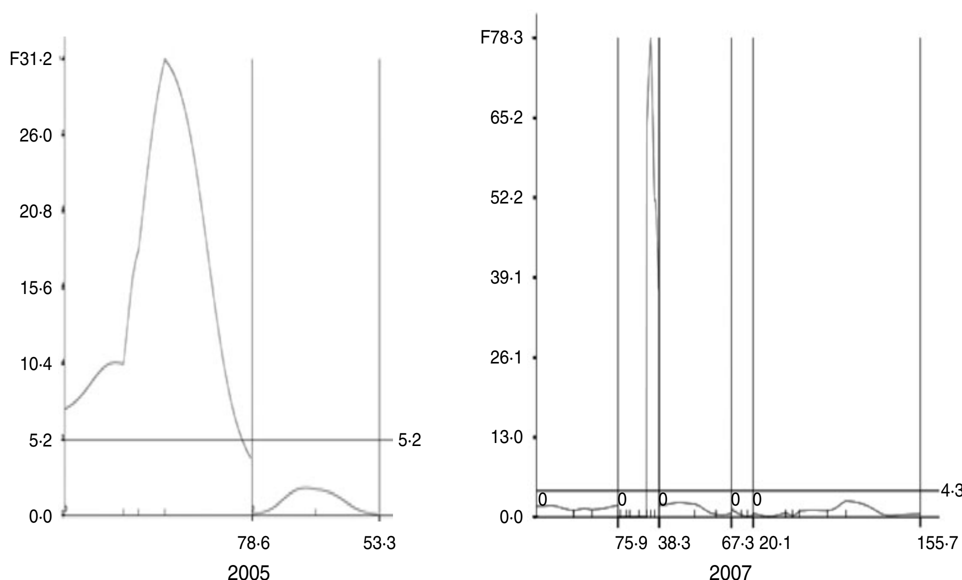

Fig. 4. The profile of *F*-value in the regions of the linkage groups 2DS and others, the peak point indicates the main QTL *taf*1 nearby 4th marker Xbarc95 in the result of 2005, and more closer 7th marker Xgdm35 in the result of 2007, X-axis is the genetic distance (cM) of the markers in turn on linkage maps.

region, in 2007, we investigated five markers identified in 2005 and additional SSR primers screened by the *c* value for 39 extreme sterile plants. Nine SSR markers Xwmc503, Xgwm261, Xcfd53, Xwmc112, Xgwm296, Xbarc95, Xgdm35, Xgwm455 and Xgdm5 of 243 F_2 plants were distinguished, a denser linkage group 2DS was developed (Fig. 2).

Figure 3 displayed the band pattern of markers Xbarc95 or Xgdm35. They had the smallest recombination frequencies (*c* value) 0·079 ± 0·0019 SD in 2005 and 0·141 ± 0·0015 SD in 2007 respectively in a recessive extreme class and two separate bulks comparing with their parents. It revealed that the female sterility gene might be tightly linked to these markers and resided in the linkage region.

(iii) *QTL mapping*

The QTL mapping results for the data of 2005 showed a steep peak over marker Xbarc95 (Fig. 4), indicating a major gene or QTL of female sterility is probably located in this region, with a confidence interval of

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Table 1. *The position, genetic main effect and contribution of QTL detected for the female sterile in wheat*

Year	Marker interval	Position (cM)	V(G)/V(P)	Additive effect (SE)	Dominant effect (SE)	Contribution h^2_A	Contribution H^2_D
2005	Xbarc95–Xgwm261, 29·0–46·6	36·6	0·2154	0·1615**, (0·0210)	0·1654**, (0·0314)	0·1292	0·0862
2007	Xgdm35–Xgwm455, 29·0–31·4	30·4	0·4499	0·2902**, (0·0177)	0·2748**, (0·0246)	0·2721	0·1399

** Significant at 1 % level.

17·6 cM. It was confirmed by the results of 2007 (Fig. 4), and the support interval of the detected QTL was narrowed down to 2·4 cM.

The detected QTL, denoted as ***T**riticuvum* ***a**estivum* **f**emale sterile (*taf*1) gene locus, was located on 2DS nearby marker Xbarc95, accounting for 21·54 % of the phenotypic variation according to 2005 data, and it displayed significant additive and dominant effects, explaining 12·92 and 8·62 % of the phenotypic variations, respectively (Table 1). In 2007 data, the QTL (*taf*1) was found more closely linked to marker Xgdm35 on the same chromosome 2DS, accounting for 44·99 % of the phenotypic variation; the additive and dominant effects explained 27·21 and 13·33 % of the phenotypic variations, respectively.

Consistent results were obtained by another QTL mapping software WinQTLcart (Wang & Zeng, 2007).

The convergent results from the BSA, the *c* value analysis of the recessive extreme approach and the QTL mapping by the QTLnetwork 2.0 for the data of 2005 and 2007 suggested that the major gene locus *taf*1 was located on the chromosome 2DS, controlling the remarkable genetic variation of female sterility in wheat.

4. Discussion

The XND126 is a new female sterile line in wheat, which could be self-reproduced. This study is the first report on female sterile gene mapping in wheat. Although it was reported that female fertility in wheat might be controlled by a few major genes and polygenes by traditional genetic analysis methods (Hou *et al.*, 2006), no further mapping studies were performed to elucidate the inheritance of female sterility. Two sets of F_2 samples from one cross in 2 years were used to map the gene(s) responsible for female fertility in the present experiment. The basic patterns of seed setting distribution standing for female fertility in the F_2 population were similar in 2005 and 2007, although the number of extreme fertile and sterile plants in 2007 was more than that in 2005 probably because of random sampling error from the F_2 population. Although F_2 is a temporary population, it is well known that F_2 is the best population for genetic effect analysis due to its plentiful genetic information involved including dominance, and its epistatic genetic effect. Thus, it was believed that the female sterile gene loci in wheat could be preliminarily mapped with the F_2 population.

For the cost-effective purpose, BSA and *c* value based approaches were adopted in the present study. The BSA screening strategy is helpful for quick selection of the markers much nearer to the major QTL(s) of fertility. When the BSA pools are small, it can be difficult to distinguish the distally linked markers from the background markers; indeed such a comparison-based approach between allele frequencies is subject to additional sources of error, including differential amplification, random variation in the amount of DNA contributed by individuals to a pool and measurement error (Chi *et al.*, 2009). Nevertheless, it is still efficient to identify closely linked markers. Michelmore *et al.* (1991) reported the bulked-extreme study that provided a fast and cost-effective means for identifying chromosomal regions likely to harbour the genes controlling genetic variations for a complex trait. On the other hand, the recessive class approach that requires more genotyping could provide complementary information on linkage and statistically more powerful when there is a larger distance between the marker and the gene of interest (Zhang *et al.*, 1994). Based on these considerations, *c* value and BSA data were used in 2005 to find candidate markers linked with female sterile locus, and only *c* value data was used to find new candidate markers in 2007.

With the locus information for the *c* values in 2007, a denser linkage group was constructed and a QTL was validated residing on 2DS consistent with the results in 2005. It seems that there was a larger genetic effect of the detected QTL in 2007, compared with the results in 2005. This might be because more markers on 2DS were used for linkage analysis in 2007 than in 2005. The estimated position of the female sterility gene locus *taf*1 was rather consistent in both years. For example, the female sterility gene locus *taf*1 was near to the marker Xbarc95 in both years, while the other markers nearer to locus *taf*1 were all additional

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SSR markers in 2007. Although it was usually believed that quantitative traits might significantly interact with environment and QTL mapping studies are needed to perform with the same population at different environments and time points (Jansen *et al*., 1995; Xing *et al*., 2001), our results indicated that QTLs detected in the 2 years were almost the same in the direction, suggesting that to some extent, the effect of QTL on wheat fertility is considerably stable across environments.

Only one major QTL was found in the present study. This may have been due to several reasons: first, in our study the QTL was detected on the basis of the linkage map constituted by only 28 polymorphic markers identified from BSA and *c* value-based approaches. This region only covered a very small region (357·3 cM) of the whole wheat genome. Another reason may have been the small sample size providing a limited resolution to identify QTL with lesser effects. Therefore, to gain more markers with low *c* value to cover larger range of genome and hunt other potential QTLs, future studies should be conducted with larger sample sizes containing sufficient numbers of chromosome crossovers.

From the analysis of different sets of F_2 samples in 2 years, we identified the same major gene locus *taf*1 on chromosome 2DS associated with female fertility. This locus was different from any other locus associated with male fertility found in wheat so far (Ma, 1995; Fu, 2002; Liu, 2002; Xing, 2003; Zhang, 2003; Cao, 2004; Li, 2005; Guo, 2006). Based on our results, female fertility might be an independent genetic system different from that of male fertility.

With the fast development of molecular biology techniques and bioinformatics, more studies have been focused on uncovering the genetic mechanism of quantitative traits. Many studies have elucidated that the genetic variation of a quantitative trait is generally regulated by a polygene network consisting of several major genes and many genes with minor effects. In summary, female fertility is a quantitative trait. Although the present study has identified a major QTL underlying the female fertility in wheat, it is necessary to conduct a follow-up study, such as fine mapping, to narrow down the QTL interval and identify more gene loci probably involved in epistasis and gene × environment interactions, for dissecting the genetic architecture of female fertility and understanding the phylogeny of sex diversity in higher plant evolution (Charlesworth & Wright, 2001).

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