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ORIGINAL PAPER

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Two recessive genes controlling thermophotoperiod-sensitive male sterility in wheat

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Abstract Male sterility of wheat-breeding line 337S (*Triticum aestivum* L.) is sensitive to both short day-length/low temperature and long day-length/high temperature. 337S was crossed with the common wheat variety, Huamai No. 8 and the F_1 was highly fertile. The F_2 population segregated in a 15:1 ratio for fertility/sterility in 243 individuals under long day-length/high-temperature. The two thermophotoperiod-responsive male sterile genes were mapped to chromosomes 5B and 2B using Simple Sequence Repeat (SSR) markers and bulked segregant analysis. Partial linkage maps around the sterility loci of chromosomes 2B and 5B were constructed using the 243 individuals in the F_2 population. One gene (*wptms1*) for male sterility was flanked by the SSR markers *Xgwm335* and *Xgwm371* at a genetic distance in chromosome 5B of 4.1 and 24.4 cM, respectively. The second gene (*wptms2*) was mapped between markers *Xgwm374* and *Xgwm120* at a genetic distance of 6.6 and 20.9 cM, respectively. The closest linked markers *Xgwm335* (*wptms1*) and *Xgwm374* (*wptms2*) explained 53 and 38% of phenotypic variation for the fertility. The SSR markers provide a useful tool to transfer the male sterile genes into elite wheat germplasm.

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Introduction

Utilization of heterosis has been an effective approach for increasing crop yield. Spontaneous male sterile mutants provide a practical way to produce the hybrid-seed for large-scale production. The discovery of a wild rice (*Oryza sativa L. f. spontanea*) cytoplasmic male sterile line resulted in a breakthrough for utilization of heterosis in a self-pollinated crop (Liao and Fu 1995). The three-line system (male sterile, maintainer, and restorer liner) is not only required to produce hybrid-seed for production, it is also required to generate male sterile lines for the next round of hybrid-seed production. The economic benefit of hybrid rice has been significantly reduced by the cost associated with hybrid-seed production. A second limitation for the cytoplasmic hybrid-production system is that there are limited numbers of fertility-restoring gene sources, which factor limits the screening for strong heterosis combinations. A second breakthrough for hybrid rice came from the discovery of a thermophotoperiod-sensitive male sterile line (Shi 1985), which significantly simplified the hybrid-seed production system. Because male sterility in that line was controlled by a recessive nuclear gene (Yang et al. 1992; Zhang et al. 1994; Wang et al. 2003), all rice varieties were able to restore fertility in the F_1, which provided a broad basis to screen for highly heterotic combinations.

Wheat is the most widely cultivated crop in the world. Breeding for hybrid wheat has encountered much more difficulty than for rice. Since Kihara (1951) obtained the first wheat cytoplasmic male sterile line, more than 70 wheat genetic male sterile lines such as T, K, V, D, A, P, and CMS have been identified (Zhang and Huang 1998; Liang and Wang 2003; Cao et al. 2004). Utilization of these male sterile lines for hybrid wheat has achieved limited success. The major limitations included negative alloplasmic and cytoplasmic effects (Yang 1983; Wu et al. 1995; Ikeguchi et al. 1999), limited numbers of restoring genes (Zhang 1992; Guan et al. 2001; Liu et al. 2002) and unstable male sterility (Murai et al. 1993; Liu et al. 1997;

Zhang and Huang 1998). Sasakuma and Ohtsuka (1979) reported the first long day-length sensitive D^2 type cytoplasmic male sterile line in wheat. Since then, long day-length, long day-length/high-temperature and short day-length/low-temperature sensitive male sterile lines have been identified in wheat (Tan et al. 1992; Wang 1996; Liang and Wang 2003). However, most of them are difficult to use for hybrid-wheat production due to their requirements for extreme day-length or temperature (Yang and He 1997; Luo et al. 1998; Ma and Shi 2002). Recently, we have identified a novel wheat male sterile line 337S, which showed a high degree of male sterility under both short day-length/low temperature and long day-length/high temperature. There are two sowing windows for this line to be used as a male sterile line. Under suitable sowing time, its self-fertility rate can reach over 50%, thus it can be self-maintained as a male sterile line. Our preliminary research found that the male sterility in 337S is governed by nuclear recessive genes (Guo et al. 2006).

The photoperiod–temperature sensitive male sterility results from the interaction of genetic factors and environment. The fertility/sterility varied with environments. In addition, the heterozygous genotype is fertile due to its recessive nature. Our preliminary research also showed that one or two genes control the male sterility under different environments (Guo et al. 2006). These factors make it difficult to select the male sterile genes based on the phenotype. The objective of this study was to map the two genes controlling the male sterility under long day-length/high temperature.

Materials and methods

Plant materials and field experiments

The wheat line 337S was first identified at the Shayang Agricultural Institute of Hubei Province as a temperature/day-length sensitive line (Rong et al. 1999). 337S was crossed with common wheat variety Huamai No.8 under natural environmental conditions in 2000. The mapping population consisted of 243 F_2 individual plants from that cross.

The F_2 seeds were planted in the Experimental Station of Huazhong Agricultural University, Wuhan of China (N30°32′ and E114°20′). Based on multiple years of sowing date trials, 337S showed a high degree of male sterility if sown before September 30 or after November 30, and it was partially fertile if sown in late October to early November (Guo et al. 2006). As wheat is an autumn-sown crop in Wuhan, an early sowing date exposes head development to relative low temperature/short day-length and a late sowing date results in head development under high temperature/long day-length. Seeds of two parental lines, F_1 and 243 F_2 were sown on November 30 in 2002. For each plant, the main head was bagged at the heading date to prevent cross-pollination. The bags were removed after 30 days and the fertility rate was calculated as the percentage of fertile spikelets. An individual was classified as male sterile if the self-fertility rate was less than 5% and as male fertile if the fertility rate was greater than 50% (Subudhi et al. 1997; Xing et al. 2003). Chi-square analysis was used to test the segregation ratio to fit a specific genetic model.

Construction of mapping population and extraction of genomic DNA

The leaf from each individual of the 243 F_2's was collected and frozen for DNA extraction. Total genomic DNA was extracted as described by Zhao and Lin (1998). Fifteen sterile and 15 fully fertile individuals were selected randomly from the population to construct two DNA pools. The leaf tissues were bulked equally for DNA extraction.

SSR analysis

The primers of SSR markers were synthesized based on the primer sequences reported by Roder et al. (1998) and by Timothy et al. (2002). PCR reactions were performed in a volume of 25 μl in a Biometra T3 thermocycler. The reaction mixture contained 250 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit Taq Polymerase, and 120 ng of template DNA. The amplification reactions were carried out using the following profile: 94°C for 4 min, then 40 cycles at 94°C for 1 min, 1 min at either 50, 55, or 60°C (depending on the individual primer set), 2 min at 72°C, with a final extension step of 5 min at 72°C (Roder et al. 1998). The PCR product was separated on a 3% agarose gel and stained by ethidium bromide.

Four-hundred and six SSR markers distributed in all 21 wheat chromosomes were used to screen for polymorphisms between the two parental lines. The polymorphic SSR markers were further selected to analyze the two DNA bulks for fertility and sterility. The markers found to be polymorphic between the two bulks were used to analyze the F_2 population. Linkage maps were constructed using the software MAPMAKER (Lander et al. 1987) and the genetic distance (centimorgan, cM) was derived using Kosambi function (Kosambi 1944; Xu and Zhu 1994). The two chromosomal linkage maps were constructed independently. The partial fertile individuals with one pair of recessive male sterile gene were excluded for linkage analysis. JMP4 software was used for other statistical analysis (SAS Institute).

Results

Genetic analysis of the male sterility

The parental line 337S was fully sterile while the F_1 and Huamai No. 8 were fully fertile under the experimental

conditions (data not shown). This demonstrated that the fertility was controlled by a recessive gene(s). The fertility rate varied from 0 to 97.9% and when plotted, a bimodal distribution was observed in the F_2 population (Fig. 1). There was no individual with a fertility rate between 2 and 50%. There are 18 sterile individuals in the F_2 population and the all of others are fertile. The ratio of sterile and fertile individuals was at 1:12.5 in the 243 F_2 individuals. Chi-square analysis showed that the observed segregation fits to a 1:15 segregation ratio ($\chi^2 = 0.3581$, $P = 0.05$), which suggested that the male sterility of 337S was controlled by two recessive genes under long –day-length and high temperature.

Polymorphism screening of the parents and gene pools

One-hundred and fifty-four SSR markers were polymorphic between the two parental varieties 337S and Huamai No. 8 from the 406 SSR markers tested. These polymorphic SSR markers were further used to screen for polymorphisms between the sterile and fertile DNA pools. Five SSR markers *Xgwm374, Xgwm16, Xgwm120, Xgwm148*, and *Xgwm335* from chromosomes 2B and 5B were polymorphic between the two DNA pools. The polymorphisms were further confirmed in the individuals used to construct the two gene pools. Thus, the two genes controlling male sterility in 337S were located on chromosomes 2B and 5B.

Genetic mapping of the genes for sterility

Four SSR markers from chromosome 2B and three SSR markers from chromosome 5B were used to analyze the individuals in the F_2 population. Partial linkage maps of chromosomes 2B and 5B were constructed based on the SSR marker and the phenotypic segregation data. One gene for the male sterility was mapped between the markers *Xgwm335* and *Xgwm371* at a genetic distance of 4.1 and 24.4 cM on chromosome 5B, respectively (Fig. 2). We designated this gene *wptms1*. The second gene was located on chromosome 2B, flanked by the markers *Xgwm374* and *Xgwm120* at a genetic distance of 6.6 and 20.9 cM, respectively. We named this gene *wptms2* (Fig. 3)

Genetic control of the sterility/fertility in 337S

The two genes controlling the male sterility in 337S were recessive, which was supported by the F_1 fertility data and our previous study (Guo et al. 2006). Using the closest linked SSR marker (*Xgwm335* and *Xgwm374*) as the individual's genotype, the F_2 population was classified into four genotypes, I: *WPTMS1_WPTMS2_*, II: *wptms1wptms1WPTMS2_*, III: *WPTMS1_wptms2wptms2*, and IV: *wptms1wptms1wptms2wptms2*. As there were 27 individuals missing the data pertinent for the least one-marker, only 216 F_2 individuals were used for this analysis. Figure 4 shows the fertility distribution with various genotypes. Average fertility rates for the four genotypes were 80.7, 58.2, 68.3, and 0%, respectively. As expected, the genotype I showed the highest fertility rate and genotypes II and III presented intermediate fertility rates. Four individuals in the genotypes I, II, and III expressed high percentages of sterility. We expected this due to marker-gene recombination. Step-regression analysis using the function Fit-Model by the JMP software showed that the SSR markers *Xgwm335* on chromosome 5B and *Xgwm374* on chromosome 2B explain 53 and 38% of the phenotypic variation of the fertility in the F_2 population. The gene on chromosome 5B had a slightly larger effect in controlling the fertility. When calculated as a two-gene model, the combined two markers accounted for 86% of the phenotypic variation. Thus, the two genes acted additively controlling the male sterility without significant epistatic effect.

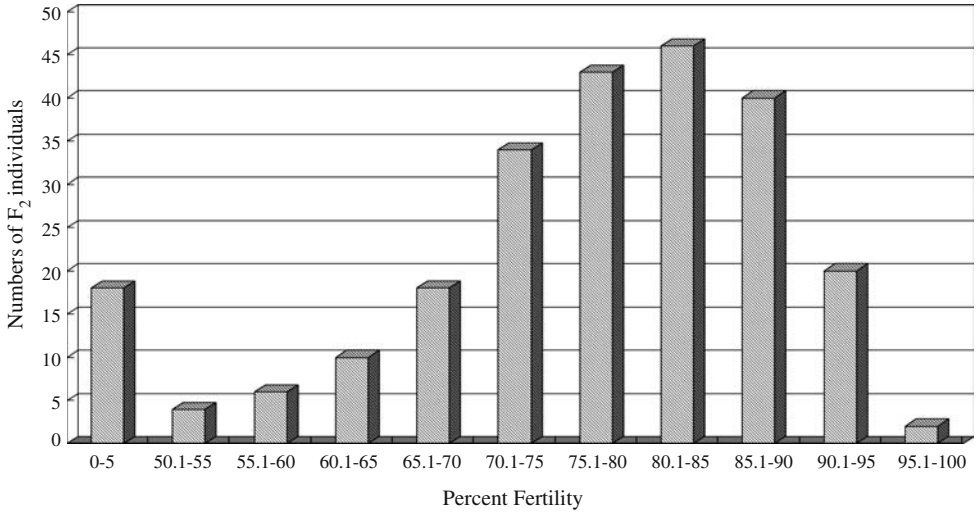

Fig. 1 Distribution of the fertility rate in the F_2 population of the cross 337S/Huamai No 8 grown under long day-length/high temperature

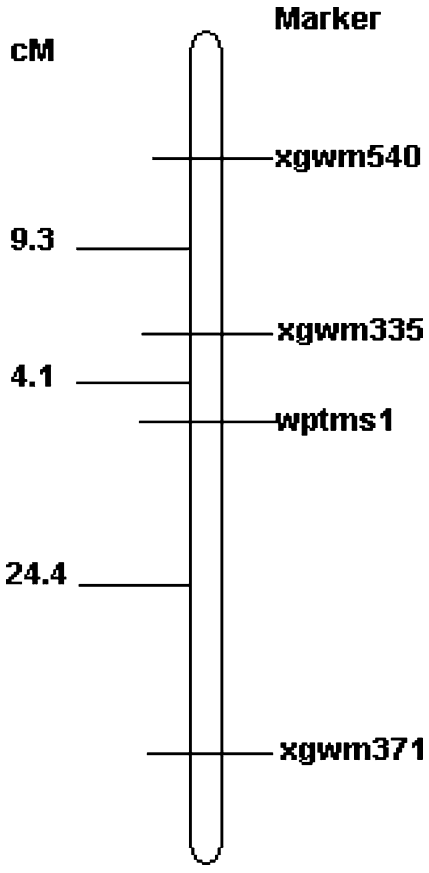

Fig. 2 Partial linkage map of chromosome 5B with the thermo-photoperiod-responsive male sterile gene *wptms1*

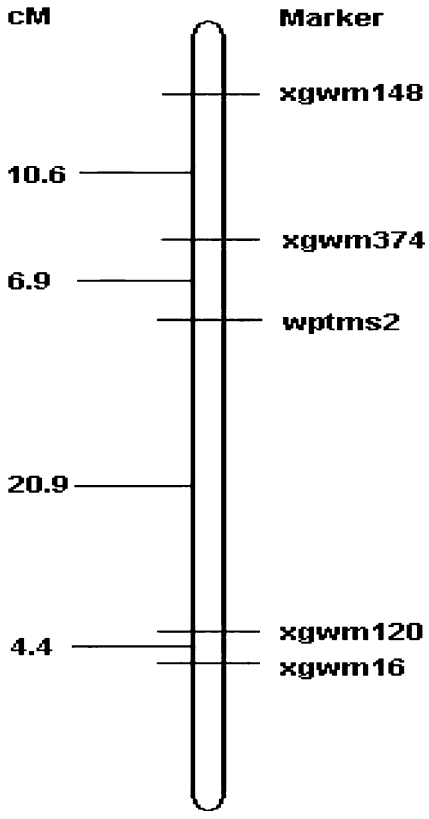

Fig. 3 Partial linkage map of chromosome 2B with the thermo-photoperiod- responsive male sterile gene *wptms2*

Discussion

Utilization of heterosis in wheat has only achieved limited success. One or more limiting factors of the known wheat male sterile lines have prevented them from being widely used for hybrid-seed production. These limiting factors have included negative alloplasmic and cytoplasmic effects (Huang 1990; Zhang 1992; Murai and Tsunewaki 1993; Zhang et al. 1996; Qiao et al. 2001), narrow fertility-restoring genes (Zhang and Huang 1998; Qiao et al. 2001), instability of male sterility (Yang 1983; Huang 1990; Zhang 1992; Murai and Tsunewaki 1993; Nonaka et al. 1993; Wang 1996), or strict requirements for temperature and day-length (Yang and He 1997; Luo et al. 1998). In our previous study, we reported a novel thermophotoperiod-sensitive male sterile line 337S (Guo et al. 2006). It is the first wheat male sterile-line sensitive to both low temperature/short day-length and high temperature/long day-length. This provides two time windows for it to be used as a male sterile line for hybrid-seed production. There is a wide sowing-date window for it to be used as a male sterile line or as a self-maintained line. The male sterility was controlled by recessive nuclear genes and no cytoplasmic effect was observed. A range of common wheat varieties carry fertility-restoring genes for this male sterile line, which provides a broad basis to screen hybrids for strong heterosis. Thus, the male sterile line 337S could be widely used for hybrid-wheat production (Guo et al. 2006). In the present study, we mapped two genes controlling the male sterility under long day-length/high temperature, which is consistent with our previous prediction based on the phenotypic data (Guo et al. 2006). The linked SSR markers provide a tool to transfer the male sterile genes into elite wheat genetic backgrounds using marker-assisted selection.

Our previous study also showed that a single gene controlled the male sterility in 337S under short day-length/low temperature (Guo et al. 2006). The SSR markers identified in this study may provide a quick method to test if one of the two genes controlling the male sterility under long day-length/high temperature also functions under short day-length/low temperature to induce male sterility.

Xing et al. (2003) has mapped a low-temperature-sensitive male sterile gene (*wtms1*) on chromosome 2B. That gene was also found to be linked with the same SSR marker *Xgwm374* as the male sterile gene *wptms2* in the present study. However, *wtms1* was 4.8 cM away from *Xgwm374* and proximal to the centromere while *wptms2* in the present study was 6.7 cM distal from the same SSR marker. As there were a limited numbers of markers mapped on chromosome 2B in the present study, more markers mapped to the chromosome 2B

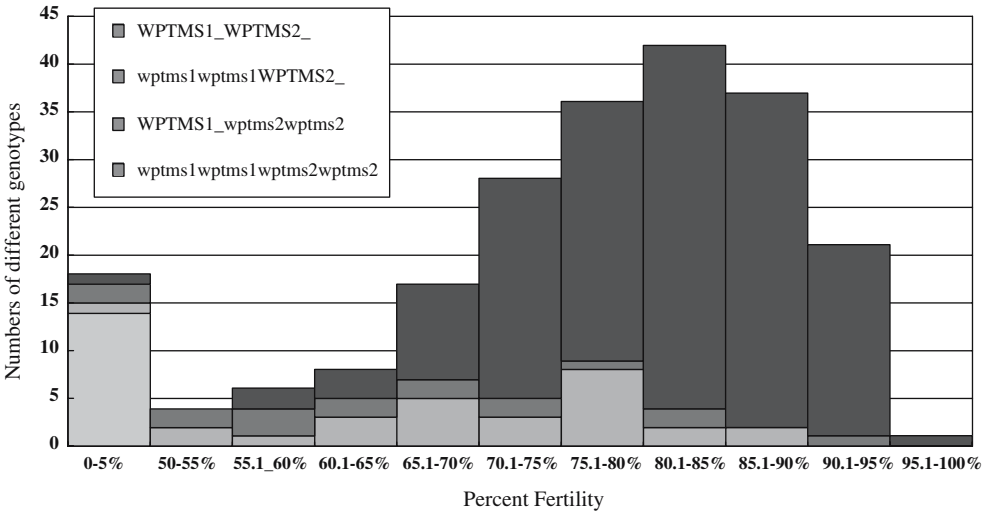

Fig. 4 Distributions of the fertility rates of the four genotypes for the two thermophotoperiod-responsive male sterile genes in the F_2 population from the cross 337S/Huamai No 8 grown under long day-length/high temperature

region would help in understanding the relationship between the two male sterile genes *wtms1* and *wptms2*.

Temperature—photoperiod-sensitive male sterility is the consequence of the interaction of genes and environments. It is not clear if the photoperiod or temperature plays the more important role in determining male sterility in 337S. Experiments with controlled day-length and temperature may help answer this question. In the present study, the gene *wptms1* on chromosome 5B played an important role in controlling fertility/sterility under long day-length/high temperature. If the two genes *wtms1* (Xing et al. 2003) and *wptms2* (present study) mapped on chromosome 2B are proved to be allelic, we predict that the male sterile gene in 337S under low temperature/short day-length should be on chromosome 2B, as the gene *wtms1* mapped by Xing et al. (2003) is a low-temperature-sensitive male sterile gene. If this is the case, the *wptms1* gene on chromosome 5B should be responsive to photoperiod changes. The SSR markers identified in the present study can be used to pick individuals with a single male sterile gene for testing the mechanism of each gene for male sterility.

The combination of SSR markers and bulked segregant analysis (BSA) may be one of the swiftest and the most effective methods in the mapping of wheat genes. But up until now, only a few wheat genes have been located by BSA in wheat due to its huge genome, poor polymorphism, and large numbers of repeat sequences. SSR markers enable BSA to become a more powerful and convenient tool for mapping genes in wheat.

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