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# Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (*wtms1*) in wheat

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**Abstract** A thermo-sensitive genic male-sterile (TGMS) wheat line (*Triticum aestivum* L.) BNY-S was obtained from the spontaneous mutant of BNY-F. Its fertility was decided by the temperature during the differentiation stage of the spikelets. BNY-S was completely sterile when the temperature was lower than 10°C during the differentiation stage of the spikelets, but fertile when the temperature was higher than 10°C. Genetic analysis indicated that the sterility of BNY-S was controlled by a single recessive gene, which was named as *wtms1*. An $F_2$ population, consisting of 3,000 individuals from the cross between BNY-S and Lankao 52-24, was used for genetic analysis and statistical analysis of the TGMS and, out of them, 158 sterile and 93 fertile extremes were present for molecular tagging and mapping of the *wtms1* gene. SSR (simple sequence repeat) and AFLP (amplified fragment length polymorphism) techniques combined with BSA (bulked segregant analysis) were used to screen markers linked to the target gene. As a result, *wtms1* was preliminarily mapped on chromosome 2B according to SSR analysis. In AFLP analysis, 14 polymorphic AFLP loci were identified with a linkage relation to the *wtms1* gene. Then linkage analysis using the $F_2$ population showed that three of them, E: AAG/M: $CTA_{163}$, E: AGG/M: $CTC_{220}$ and E: ACA/M: $CTA_{160}$, were linked to the *wtms1* gene relatively close to a genetic distance of 6.9 cM, 6.9 cM and 13.9 cM, respectively. Finally, the *wtms1* gene was mapped between the SSR marker *Xgwm 374* and the AFLP marker E: AAG/M: $CTA_{163}$ with the distance of 4.8 cM and 6.9 cM, respectively. A partial linkage map was constructed according the SSR and AFLP data.

**Keywords** Wheat · TGMS gene · Genetic analysis · Molecular mapping

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## Introduction

Wheat (*Triticum aestivum* L. em. Thell.) is one of the most important food crops in the world. It is reported that between 1966 and 1990, the population of densely populated and low-income countries grew by 1.8-fold while food production more than doubled. Such a rapid increase in the volume of world food production was mainly due to the development of high yielding varieties of wheat and rice (Khush 1999). However, during the last 10 years, the growth rate of the world population has exceeded the rate of growth in grain production. It is predicted that the world population will exceed 8 billion by 2025. To meet these global food demands, grain production will need to increase 50% by 2025 (Khush 1999). This is a great challenge facing the crop breeders. It is well known that hybrid rice has made a great contribution for increasing rice production, and the discovery and application of thermo-sensitive genic male-sterile (TGMS) rice has simplified the procedure of rice hybrid seed production because TGMS lines can be used not only as male-sterile lines but also as maintainer lines. Up to now, TGMS lines of rice have made a great contribution both in theoretical research and hybrid rice seed production. It has been expected that wheat also can be hybrid-like rice, and to make a great progress in aiming this goal (Zhang et al. 1998). Wheat breeders all over the world have been making a great effort to achieve this goal.

The photoperiod-temperature sensitive genic male-sterile (PTGMS) line and the thermo-photoperiod sensi-

tive genic male-sterile (TPGMS) line in wheat have already been reported in the last few years (Wang et al. 1998; Cao et al. 2002). But no photoperiod-sensitive genic male-sterile (PGMS) line or the TGMS line was reported up to now. In this study, a stable TGMS line BNY-S, i.e. a spontaneous mutant of BNY-F, was first used in the theoretical research of wheat male sterility. Its fertility is decided by the temperature during the differentiation stage of the spikelets. The TGMS wheat line, BNY-S, will be useful in hybrid wheat production in the near future. It can be used for hybrid seed production in a large area by adjusting its sowing time. The utilization of the wheat TGMS line will facilitate both theoretical research and hybrid seed production in wheat. This paper reports the genetic analysis of the TGMS trait, molecular tagging and the mapping of the first TGMS gene (*wtms1*) in wheat by using molecular-marker techniques combined with bulked segregant analysis (BSA), aiming to improve the understanding of the wheat male sterility and heterosis.

## Materials and methods

### Plant materials and fertility characterization

BNY-S, obtained from the spontaneous mutant of BNY-F, shows complete male sterility when the temperature is lower than 10°C during the differentiation stage of the spikelets, but fertile or partially fertile when the temperature is higher than 10°C. An $F_2$ segregation population was developed from the progeny of the cross between BNY-S and Lankao52–24, and then was used in genetic analysis and mapping of the *wtms1* gene.

A few seedlings of every $F_2$ individuals were harvested for DNA extraction after the peak tillering stage. At the same time, the seed setting rate of 3–4 panicles per plant were tested to confirm their fertility by bagging the spikelets of the $F_2$ individuals prior to anthesis to prevent cross-pollination. Bags were removed 30 days after anthesis. Filled and unfilled spikelets were counted and the fertility was measured as filled grains were divided by the total number of grains (Subudhi et al. 1997). This fertility characterization was performed at the Henan Vocational-Technical Teachers' College in 2002. The $F_2$ population from the cross BNY-S and Lankao52–24 consisted of 3,000 individuals. Out of the 3,000 $F_2$ plants, 251 (158 sterile and 93 fertile extreme plants) were selected and used for molecular analysis (Zhang et al. 1994).

### DNA preparation

Genomic DNA from each $F_2$ individual, along with both parents, was isolated from the young leaves, which were freeze-dried and ground to powder following the cetyl trimethyl ammonium bromide (CTAB)-method described by Hoisington et al. (1994). The concentration of each sample was estimated by comparing the band intensity with that of lambda DNA of known concentrations, after 0.8% agarose-gel electrophoresis with ethidium-bromide staining. Fifteen individual DNAs of the sterile extreme were mixed with an equal amount to form a sterile bulk ($B_S$), and 15 individual DNAs of the fertile extreme were mixed with an equal amount to form a fertile bulk ($B_F$) (Michelmore et al. 1991).

In order to confirm the linkage relationship, the potential bands linked to the w*tms1* gene detected by BSA analysis were further assayed with a large number of $F_2$ plants.

### SSR analysis

The basic procedure of the SSR reaction was as follows: 1 × reaction buffer, 2.0 mM $Mg^{2+}$, 0.1 mM of each dNTP, 2.0 units *Taq* DNA polymerase, 50 ng template DNA and 30 ng primer, then fixing to 20 µl with distilled water, and finally covered with one drop of mineral oil. The amplification reactions were carried out using the following profile: 94°C for 4 min, then 35 cycles were performed for 1 min at 94°C, 1 min at either 50°C, 55°C 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 (Röder et al. 1998). The amplification products with a standard size marker in the first lane of the gel were separated on the 6% PAGE sequencing gel at 100 W for about 1.0 h after pre-electrophoreses for 20–30 min. Then the gel was removed from the apparatus and stained using the silver-staining method (Xu et al. 2002).

### AFLP analysis

AFLP analysis was performed following the Gibco-BRL AFLP Analysis System Kit (Life Technologies Incorporated, Gaithersburg, Massachusetts) with minor modifications. DNA (500 ng) was double-digested with *Eco*RI and *Mse*I. The digested DNA fragments were ligated with *Eco*RI and *Mse*I adaptors, respectively. A pre-amplification was carried out with primer pair *Eco*RI+A and *Mse*I+C, and the PCR products were diluted in a ratio of 1: 20 with TE buffer and then used as a template for the selective amplification. Selective amplifications using [$\gamma$-$^{33}$P] ATP-labeled *Eco*RI primers were conducted and the products were separated on 6% PAGE sequencing gel at 100 W for 2.5 h after pre-electrophoreses for 30 min. The gel was removed, dried and then exposed to X-ray film at −70°C for 5–7 days until the film was adequately exposed.

### Linkage analysis

Based on the SSR and AFLP data, linkage analysis was performed with MAPMAKER Version 3.0 (Lander et al. 1987). Recombination fractions were converted into genetic map distance (cM) using the Kosambi mapping function (Kosambi 1944).

## Results

### Segregation analysis of the TGMS trait gene in the $F_2$ generation

The results from field characterization showed that the fertility difference between the parents was obvious. BNY-S was sterile when the temperature is lower than 10°C during the spikelet differentiation stage, but Lankao 52-24 was fertile. The progeny $F_1$ from the cross between BNY-S and Lankao52-24 showed the same phenotype of the normal parent of Lankao52-24. In the $F_2$ generation, trait segregation occurred. The sterile plants exhibited apparent differences in phenotype from that of the fertile plants during the anthesis stage, with the gluma of sterile individuals opening and the stigma exposured. After the fertility confirmation, 773 individuals of the $F_2$ population were considered as sterile, and 2,227 individuals were identified as fertile ones. The segregation ratio in the $F_2$ population followed a 3:1 ratio. These results indicated that the sterility in BNY-S was controlled by a recessive gene, which was named as *wtms1*.

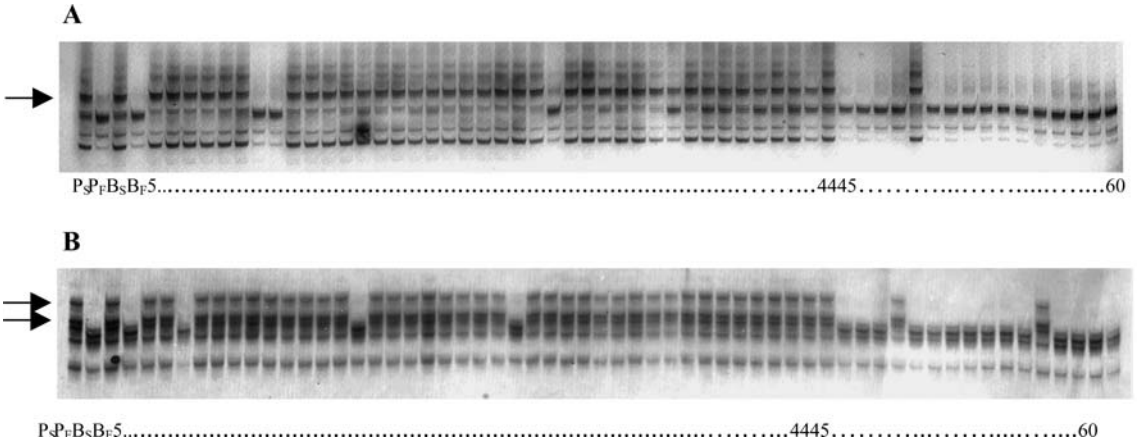


**Fig. 1A, B** The amplification pattern of the SSR markers *Xgwm374* (**A**) and *Xgwm191* (**B**) in parents, bulks and a part of individuals of the $F_2$ segregant population. The samples in each lane are: $P_S$ sterile parent BNY-S; $P_F$ fertile parent Lankao 52–24; $B_S$ sterile bulk; $B_F$ fertile bulk; *5–44* $F_2$ sterile individuals; *45–60* $F_2$ fertile individuals. *Arrows* indicate the specific amplification products linked to the *wtms1* gene

## Screening SSR markers linked to the *wtms1* gene

A total of 297 SSR primers based on the published data (Plaschke et al. 1996; Röder et al. 1998; Timothy et al. 2002) were screened among the two parents and the two bulks. Forty one percent primers amplified polymorphism between the parental lines, but only five primers (*Xgwm374*, *Xgwm191*, *Xgwm120*, *Xgwm148* and *Xgwm630*) amplified identical polymorphism between the two parents and the two bulks. These polymorphic products amplified by the five primers might link to the *wtms1* gene. In order to confirm the linkage relationship, a large number of the $F_2$ individuals were tested using these five SSR markers. As a result, amplification products of *Xgwm374*, *Xgwm191*, *Xgwm120*, *Xgwm148* and *Xgwm630* were identified to be linked to the *wtms1* gene with a genetic distance of 4.8 cM, 6.0 cM, 19.8 cM, 24.0 cM and 29.0 cM, respectively. The amplified patterns of *Xgwm374* and *Xgwm191* were shown in Fig. 1A and in Fig. 1B as examples, respectively. Because all of the five linked markers were mapped on chromosome 2B from the SSR map of Röder et al. (1998), therefore the *wtms1* gene should also locate on chromosome 2B.

## Screening AFLP markers linked to the *wtms1* gene

In order to find markers more closely linked to the *wtms1* gene, AFLP was subsequently performed. Two parents and two bulks were amplified using 64 AFLP primer combinations. As Fig. 2 showed there were about 80–110 bands amplified in wheat in each 3+3 primer combination. Among the bulked segregant analysis of all the employed primer combinations, 14 polymorphic AFLP bands showed potential linkage relationship with the *wtms1* gene, and out of them nine were from the sterile plants and five from the fertile ones. In some primer combinations, there were two different-size bands having polymorphism between the parents and the bulks. Then a large number of $F_2$ progeny was conducted to confirm the linkage relationship between the AFLP products and the

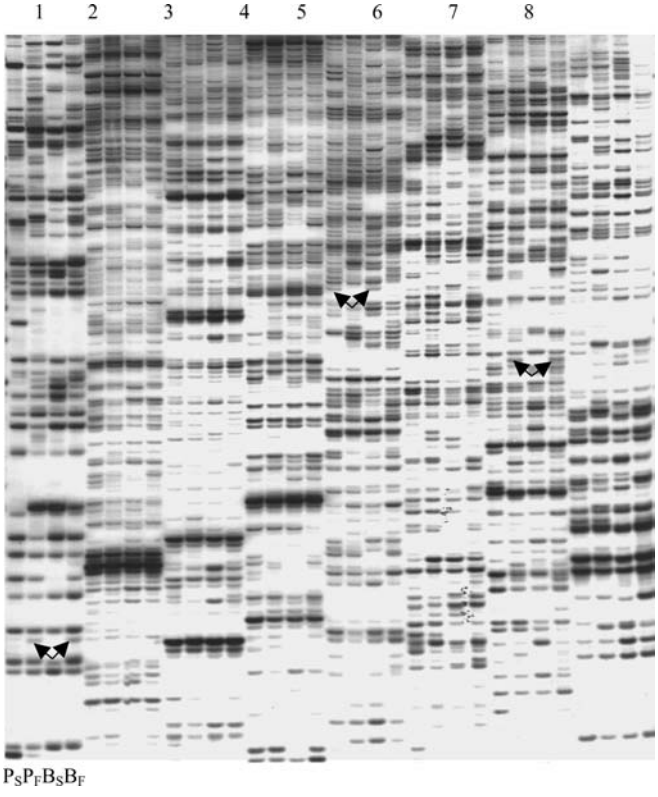


**Fig. 2** BSA analysis of the *wtms1* gene using different AFLP primer combinations. Primer combinations from left to right are: *1* E: ACG/M: CTA; *2* E: AAC/M: CAA; *3* E: AAC/M: CAC; *4* E: AAC/M: CAG; *5* E: AAG/M: CTA; *6* E: AAG/M: CTG; *7* E: ACA/M: CTA; *8* E: AGG/M: CAC, respectively. The samples are: $P_S$ sterile parent BNY-S; $P_F$ fertile parent Lankao 52–24; $B_S$ sterile bulk; $B_F$ fertile bulk. *Arrows* indicate the polymorphic bands

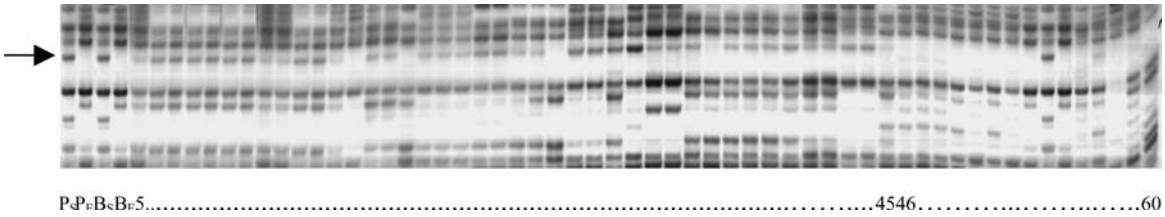


**Fig. 3** The amplification result of AFLP marker E: AAG/M: CTA in parents, bulks and a part of the individuals of the $F_2$ segregant population. The samples in each lane are: $P_S$ sterile parent BNY-S; $P_F$ fertile parent; Lankao 52–24; $B_S$ sterile bulk; 5–24 $F_2$ sterile individuals; 46–60 $F_2$ fertile individuals. The *arrow* indicates the relevant polymorphic amplification fragments

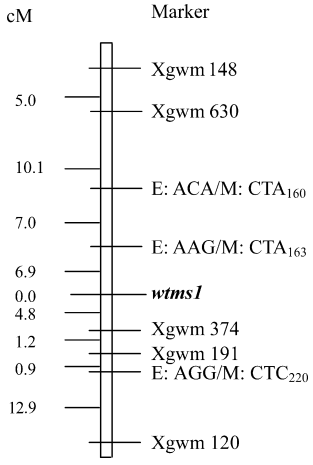


**Fig. 4** Map showing the *wtms1* gene and its linkage markers on chromosome 2B

target gene. The results suggested that three AFLP products (E: AAG/M: $CTA_{163}$, E: AGG/M: $CTC_{220}$ and E: ACA/M: $CTA_{160}$) were linked to the w*tms1* gene with a genetic distance of 6.9 cM, 6.9 cM and 13.9 cM, respectively; while the recombinant values of the other polymorphic bands were over 18%. A partial result of the progeny analysis using the primer combination E: AAG/M: CTA was shown in Fig. 3.

### A local linkage map of the *wtms1* gene-encompassing region

The phenotype, SSR and AFLP data were combined for linkage analysis. According to the results, a local genetic linkage map of the *wtms1* gene-encompassing region on chromosome 2B was constructed (Fig. 4). The *wtms1* gene was localized between the SSR marker *Xgwm 374* and the AFLP marker E: AAG/M: $CTA_{163}$ with the distance of 4.8 cM and 6.9 cM, respectively.

## Discussion

Compared to the cytoplasmic male sterility (CMS), the genic male-sterility (GMS) system has significant advantages being used not only as male-sterile lines but also as maintainer lines, the wide spectrum of restorers and without an abnormal cytoplasm effect. In the past few years, several rice TGMS genes in different TGMS lines have been discovered and studied (Wang et al. 1995; Subudhi et al. 1997; Yamagushi et al. 1997; Koh et al. 1999; Dong et al. 2000). Rice TGMS lines have made a great contribution to the hybrid rice production of the two-line system. However, theoretical research and practical application of hybrid wheat far lags behind that of rice. The major reason is that no suitable sterile line can be used in theoretical research and practice utilization. Up to now, the reported sterile lines in wheat is either the photoperiod-temperature sensitive male sterility (PTGMS) or the thermo-photoperiod sensitive genic male sterility (TPGMS) (Wang et al. 1998; Cao et al. 2002), in which the two factors, photoperiod and temperature, are simultaneously involved, making their theoretical research more complex. While the TGMS line and the PGMS line will make the theoretical research simple and easy, it will get the correct result. This is the first report on wheat TGMS genetic analysis and gene mapping. The discovery and application of BNY-S will facilitate the theoretical research and the application of the two-line system in hybrid wheat production. On the one hand, BNY-S is completely sterile when the temperature is lower than 10°C during the differentiation stage of the spikelets, but is fertile when the temperature is higher than 10°C. Therefore, the breeders can produce hybrid wheat seeds through adjusting the sowing time in the autumn within a large wheat production area. On the other hand, the stigmas of BNY-S are exposed during anthesis, indicating it is easy to accept pollen from the fertile plants, which is the most important factor in producing wheat hybrid seeds with high quality and high yield.

The use of the SSR combined with BSA analysis is probably one of the faster and easiest strategies to locate and map genes in wheat. Until now only a few genes have been tagged by BSA in wheat, essentially because of a lack of polymorphism, the large genome size and the presence of many repeated sequences (Chantret et al. 2000). The recent development of markers, such as SSR or AFLP, gives the tagging of gene with BSA a better chance to succeed. Furthermore, SSRs have the significant advantage of being genome-specific (Röder et al. 1995). Having one or two SSR markers linked to a gene means that it can be immediately assigned to a location (Chantret et al. 2000). The results reported in this paper

also clearly demonstrated that the SSR and AFLP techniques are powerful, reliable, stable and a rapid assay in molecular marker screening, and gene tagging and mapping (Jia et al. 2001; Lima et al. 2002); and that the simultaneous use of SSR and BSA or AFLP and BSA reduced the risk of false positives.

Cao et al. (2002, 2003) have reported their research results of the PTGMS in wheat line BAU3338. They indicated that the PTGMS phenotype was controlled by polygenes, which included the main effect loci and modified loci, and that the gene expression was significantly different between the sterile and fertile conditions during the fertility transformation phase. The wheat PTGMS line they used has two different traits of photoperiod-sensitivity and thermo-sensitivity, so the gene loci controlling these two traits should be different. In this research, the TGMS line, but not the PTGMS or the TPGMS, BNY-S was selected and used. The genetic analysis of the TGMS gene in BNY-S showed that the sterile phenotype was controlled by a single recessive gene, liking all of the TGMS genes being reported in rice (Wang et al. 1995; Subudhi et al. 1997; Yamagushi et al. 1997; Koh et al. 1999; Dong et al. 2000; Jia et al. 2001). From the mapping results described above, the *wtms1* gene was primarily mapped on chromosome 2B between the SSR marker *Xgwm 374* and the AFLP marker E: AAG/M: $CTA_{163}$, with a genetic distance of 4.8 cM and 6.9 cM, respectively. Fine mapping and cloning of the *wtms1* gene in wheat will promote the molecular study of the thermo-photoperiod sensitive genic male sterility. However, there is still much more to do to understand the genetics of the TGMS gene in wheat. Currently, we are developing a permanent population with more extreme individuals to perform the fine mapping of the *wtms1* gene.

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