Theor Appl Genet (2006) 113: 11–15
DOI 10.1007/s00122-006-0262-x

ORIGINAL PAPER

J. H. Tang · Z. Y. Fu · Y. M. Hu · J. S. Li
L. L. Sun · H. Q. Ji

Genetic analyses and mapping of a new thermo-sensitive genic male sterile gene in maize

Received: 30 November 2005 / Accepted: 7 March 2006 / Published online: 11 May 2006

Abstract The present study describes a novel thermo-sensitive genic male sterile (TGMS) line, Qiong68ms. To analyse the mode of fertility inheritance and tag the TGMS gene, a set of F_2, BC_1 and $F_{2:3}$ populations derived from a cross between Qiong68ms and K12 were evaluated for a period of 2 years. Classical genetic analyses and QTL mapping using the mean restoration percentage of the $F_{2:3}$ populations revealed that the fertility of Qiong68ms was likely to be governed by a single recessive gene, which was named *tms3*; the *tms3* gene was mapped to a location between SSR markers umc2129 and umc1041, at a distance of 3.7 cM form umc2129 and 1.5 cM form umc1041. The molecular markers tightly linked with *tms3* gene will aid in the transfer of the TGMS gene to various background inbred lines using the MAS method.

Introduction

Male sterility has been observed in a wide variety of plants, and is of clear importance for the utilization of heterosis in crops, especially cytoplasmic male sterility (CMS) and ecological male sterility (EMS). The first photoperiod-temperature sensitive male sterile line, Nongken58s, was identified in rice (Si and Deng 1986), and, subsequently, thermo-sensitive genic male sterile (TGMS) lines have been discovered in multiple cereal crops, including rice (Wang et al. 1995), maize (He et al. 1995) and wheat (Xing et al. 2003).

The discovery and identification of the TGMS germplasm has been vital for the "two-line" system of hybrid seed production. For example, in rice, seven TGMS genes, *tms1*, *tms2*, *tms3*, *tms4*, *tms5*, *ms-h* and *tms6(t)* have been identified and mapped onto chromosome 8, 7, 6, 2, 2, 9, and 3 respectively (Wang et al. 1995, 2004; Yamagushi et al. 1997; Subudhi et al. 1997; Dong et al. 2000; Jia et al. 2000; Wang 2001, 2003; Koh et al. 1999). In maize, only one TGMS line, Qiong-6ms, has been reported to date (He et al. 1995), and its fertility appears to be controlled by two recessive duplicated genes, *tms1* and *tms2* (Fu et al. 2004). Qiong68ms is a novel TGMS line discovered in maize that exhibits full sterility when sown in the summer in the Henan and Hainan province of China. In contrast, when planted in the spring in either of these regions or in the winter in Hainan province, it exhibits full restoration. In the present study, we evaluated the mode of fertility inheritance in Qiong68ms, and mapped the TGMS gene by using QTL mapping as well as qualitative gene tagging method.

Materials and methods

Plant materials

Qiong68ms was acquired through the spontaneous mutation of an elite inbred line designated as 414, which was selected from a synthetic population including the Chinese local germplasm, Tangsipingtou. The hybrid F_1, was derived from the crossing of Qiong68ms with K12, an inbred line used broadly in China. The F_1 generation was self-pollinated to produce the F_2 population, which was subsequently backcrossed with the TGMS line to produce the BC_1 population in the winter of 2002 in Hainan province (Sanya, China). From the derived lines, one set each of the F_2 and BC_1 populations was used for field evaluation in the Henan province

Communicated by R. Hagemann

J. H. Tang (✉) · Z. Y. Fu · Y. M. Hu · L. L. Sun · H. Q. Ji
College of Agronomy, Henan Agricultural University,
95 Wenhua Road, 450002 Zhengzhou, China
E-mail: Tangjihua1@163.com
Tel.: +86-371-63558127
Fax: +86-371-63558122

J. H. Tang · J. S. Li
National Maize Improvement Center of China,
China Agricultural University, 100094 Beijing, China

(Zhengzhou, China) in the summer of 2003. An additional F_2 population was self-pollinated to produce the $F_{2:3}$ populations in the winter of 2003 in the Hainan province. At that time, young leaves of the F_2 generation were harvested for DNA extraction.

Field evaluation

The field studies were carried out during the summer of 2003 and 2004, at the farm of Henan Agricultural University (Zhengzhou, E113°42′, N34°48′). To evaluate the mode of fertility inheritance of the TGMS line, two parent lines, the F_1 hybrid of Qiong68ms and K12, the F_2 and BC_1 population were investigated in the summer of 2003. The two parents and hybrid F_1 were sown in two plots respectively, each plot was 4 m in length and contained 15 plants. Seeds of the F_2 and BC_1 populations were sown using the single-seed planting method in 11 and 8 plots respectively. In the summer of 2004, a total of 210 $F_{2:3}$ families as well as two parent lines were evaluated; each $F_{2:3}$ family was sown in two plots containing a total of 30 plants. Field conditions consistent with those for normal maize production were employed.

The condition of the tassel for each plant in the field was evaluated daily and scored according to the condition of the protruding anthers. The degree of fertility was rated on a scale of 0–5 based on the percentage of exserted anthers: (0) no anthers exserted, (1) less than 5% anthers exserted, (2) 5–25% anthers exserted, (3) 25–50% anthers exserted, (4) 51–75% anthers exserted, (5) more than 75% anthers exserted. Simultaneously, the dehiscent condition of the anthers was scored using a scale of I–III, where: (I) no dehiscent anthers, (II) few dehiscent anthers; and (III) many normal dehiscent anthers. To characterize the fertility of each plant, the combined scores, 4/III and 5/III, were considered to be representative of a full restoration, whereas the combined scores, 0/I and 1/I, were considered to be representative of sterility (Ducick et al. 1961; Li et al. 1963). Additionally, the average of the restoration percentage for each $F_{2:3}$ family was calculated and used as input data for QTL mapping to assess fertility of the TGMS line.

Genetic linkage construction and gene mapping

In total, 550 pairs of SSR markers were selected from the maize genomic database (http://www.maizegdb.org) to screen the polymorphism between two parent lines Qiong68ms and K12. From these, 167 SSR markers had distinct polymorphisms between the two parents, which were amplified using the DNA from each individual of the F_2 population. The genetic linkage maps were constructed using the Mapmakers 3.0 program at a LOD threshold >3.0 (Lander et al. 1987).

Model 6 of the Zmapqtl module of QTL Cartographer 2.0 was used to identify potential QTLs (Zeng 1994), using scanning intervals of 2 cM between markers and putative QTLs with a windows size of 10 cM. The number of marker cofactors for a background control was determined using a forward–backward stepwise regression with five controlling markers. A genome-wide critical threshold value for the experiment wise type I error rate ($\alpha=0.05$) was set for each trait independently by 1,000 random permutations. The fine mapping of the TGMS gene was analysed with JOINMAP Version 3.0 (Stam 1993).

Results

Temperature-dependent restoration and sterility of the TGMS line, Qiong68ms

The fertility alteration temperature of the TGMS line was evaluated in the Zhengzhou location with 11 sowing stages from the spring to summer of 2003 and 2004 respectively, the interval time of two sowing stage was 7 days. Leafage in the fields was evaluated, fertility scores of each plant were recorded and temperature measurements were taken throughout the day (Tang et al. 2000); from these data a correlation analysis between sterility and temperature revealed that the fertility of Qiong68ms was directly influenced by the daily maximum temperature (data not show). The fertility alteration temperature was observed at 30–33°C when evaluating the spikelet differentiation in the staminate inflorescence. When the daily maximum temperature was higher than 33°C, plants exhibited complete sterility, and pollen was typically absent. By contrast, when the daily maximum temperature was below 30°C, plants exhibited complete restoration and displayed normal pollen (Fig. 1). Simultaneously, pollen grains were sterility when anthers were not exserted, and generally exhibited normal fertility when the anthers were exserted; therefore the fertility scores based upon the percentage of anther exsertion and dehiscent anthers were used for the further genetic analyses and gene mapping.

Genetic analyses

The TGMS line Qiong68ms exhibited complete sterility in the summer of 2003 in the Zhenzhou location, whereas the inbred line K12 expressed full restoration. The hybrid F_1 obtained from the crossing of Qiong68ms and K12 displayed a phenotype similar to the normal parent K12. The fertility scores for the F_2 and BC_1 populations are listed in Table 1. According to the criteria for restoration and sterility, there were 96 fertile plants and 41 sterile plants among the 155 F_2 individuals. The segregation ratio followed a 3:1 theoretical ratio predicted by a Chi-square test at a 0.05 significance level (Table 2). There were 68 fertile plants and 52 sterile plants among the 132 BC_1 individuals, and the segregation ratio followed a 1:1 Mendelian ratio predicted by Chi-square test at a significance level of $P<0.05$. Of the 210 $F_{2:3}$ families evaluated in the summer of 2004, a total of 42 families expressed full restoration, and 132 families exhibited

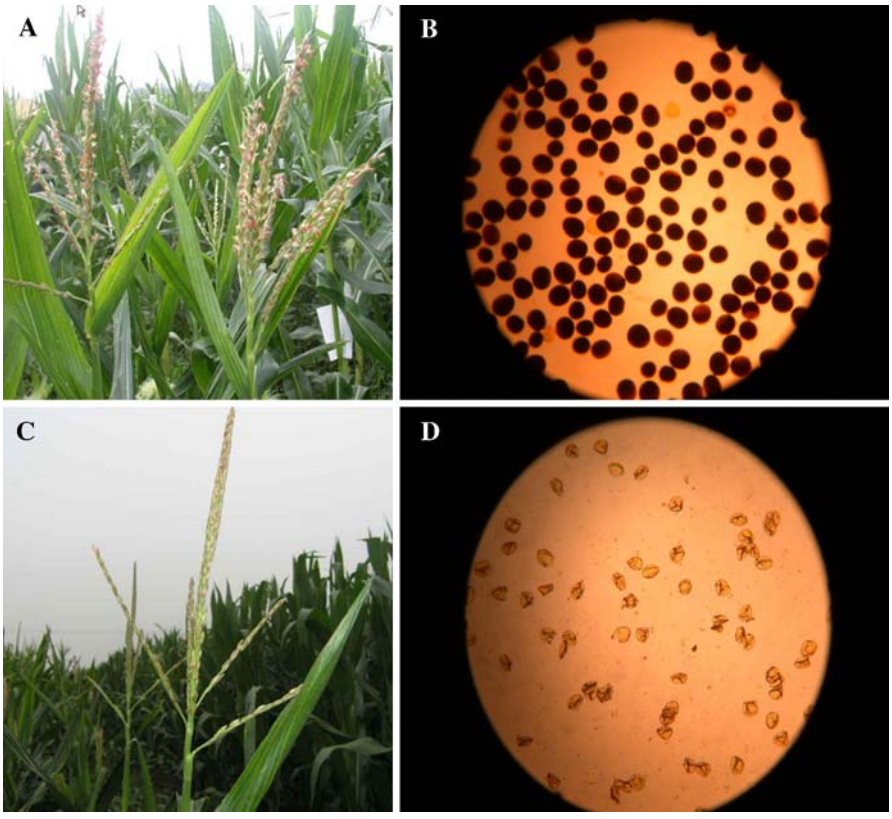

Fig. 1 The fertility performance of the tassel and pollen at the restoration and sterility stages. **a**, **b** The performances of tassels and pollens at the restoration stages; **c**, **d** the performances of tassels and pollens at the sterility stages

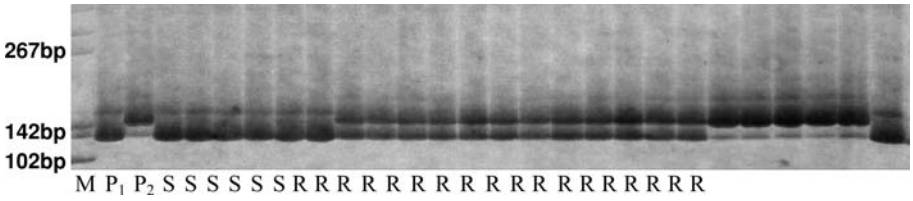

Fig. 2 The SSR products amplified by umc1042 in parental lines and some F_2 individuals. *M* Marker, P_1 Qiong68ms, P_2 K12; *S* some F_2 sterile individuals, *R* some F_2 restoring individuals

Table 1 The number of each fertility score for the F_2 and BC_1 populations which derived from the cross between Qiong68ms and K12

Populations	Total	The number of each fertility score							
		0/I	1/I	2/I	2/II	3/I	3/II	4/III	5/III
Qiong68ms	30	28	2						
K12	30								30
Qiong 68ms × K12	30	30							
(Qiong 68ms × K12)F_2	155	35	6	3	4	5	6	11	85
(Qiong 68ms × K12)BC_1	132	45	7	4	3	3	2	12	56

fertility segregation following a 3:1 theoretical ratio. Furthermore, 44 families represented full sterility, thus the theoretical segregation ratio of 1:2:1 obtained by Chi-square analysis was significant ($P<0.05$). These findings indicate that the fertility of the TGMS line is controlled by a recessive gene.

Genetic linkage maps construction and QTL analysis

The genetic linkage maps for the $F_{2:3}$ populations were constructed using 147 SSR markers, including 11 linkages, spanning a total of 2227.2 cM with an average spanning interval of 15.25 cM (data not show). Most of the SSR markers were consistent with the maize linkage maps of IBM (http://www. maizegdb.org).

The average restoration percentage for each $F_{2:3}$ family varied from 0 to 100%. Only one QTL was detected using the restoration percentage of the $F_{2:3}$ populations as the input data, accounting for 64% fertile genotypic variance. This QTL was localized to chromosome 2 between the SSR markers umc2402 and umc1042.

Fine mapping of the *tms3* gene

The results from the classical genetic analyses and QTL mapping of the entire genome demonstrated that fertility in the TGMS line was likely to be controlled by a single

Table 2 The fertility performance of the segregation populations evaluated in the field

Year	Populations	Total	Fertile	Sterile	Fertility segregating	Observed	Theoretical	$\chi 2$	Probability
			Plants/families	Plants/families	families	ratio	ratio		
2003	(Qiong68ms × K12)F_2	155	96	41		2.34:1	3:1	1.77	0.18
	(Qiong68ms × K12)BC_1	132	68	52		1.30:1	1:1	2.13	0.14
2004	(Qiong68ms × K12)$F_{2:3}$	210	42	44	120	1:1.05:2.86	1:1:2	5.62	0.06

Note: $\chi^2_{1,0.05} = 3.84$, $\chi^2_{2,0.05} = 5.99$

gene, which was named *tms3*. To fine-tag the gene responsible for the TGMS phenotype, the genotype of each F_2 individual was deduced by the fertility assessment of the $F_{2:3}$ populations. The amplified DNA patterns of umc1042 for representative F_2 individuals are shown in Fig. 2. Based on the genotype and marker results for the F_2 populations, the TGMS-associated gene *tms3* was localized to chromosome 2, and tightly linked with the SSR markers umc2402 and umc1042. The genetic distances between *tms3* and SSR markers umc2129 and umc1042 were 3.7 and 1.5 cM, respectively (Fig. 3).

Discussion

To date, only two TGMS lines have been described in the literature. The TGMS line Qiong-6ms (He et al. 1995, 1998a, b) is primarily influenced by temperature (Tang et al. 2000), and controlled by two duplicated recessive genes (Fu et al. 2004). The TGMS line Qiong68ms, described in the present study, exhibits fertility that is influenced by temperature and appears to be governed by a single recessive gene. When compared to Qiong-6ms, Qiong68ms demonstrates a simple inheritance pattern and a stable fertility alteration temperature, thus the discovery of the TGMS line Qiong68ms may facilitate the application of two-line systems in maize hybrid seed production. Furthermore, molecular makers which tightly linked with *tms3* can be used in TGMS line selection by means of the MAS method.

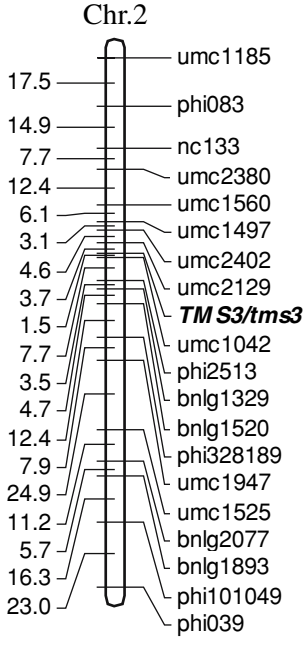

Fig. 3 The *Tms3*/*tms3* location on chromosome 2

The CMS system, also known as the three-line system, is the most widely used system for producing F_1 hybrids in rice, maize and rape. However, this technique is labour-intensive because the CMS lines require specific maintenance and restorer lines (Lopez and Virmani 2000). In contrast, the ecological genic male sterile lines present significant advantages not only as male-sterile lines but also as maintainer lines and a wide spectrum of restorer lines (Xing et al. 2003; Wang et al. 2003), and all of which lack an abnormal cytoplasm effect. In maize, the utilization of TGMS lines provides the additional advantage of avoiding the potential risk of leaf spot caused by some varieties of *Helminthosporium* that infect the special sterile cytoplasm in the three line seed production system (Hooker et al. 1970).

Maintaining 100% male sterility in TGMS lines is vital to the success of the two-line seed production system. However, various challenges have arisen, some of which encompass characteristics that are inherent to the TGMS lines, including sterile stability, fertility alteration and the number of controlling genes. Other challenges are ecological and more difficult to control, including fluctuations in temperature and day/night illumination; all of these factors can directly influence the fertility of the TGMS lines. To avoid risks posed by the above-named factors, the TGMS line should possess a lower critical temperature, and be controlled by a single gene. In addition, a suitable ecological environment should be identified for each TGMS line.

Acknowledgements This work was supported by the National High-technology Project of China.

References

Dong NV, Subdhi PK, Luong PN, Quang VD, Quy TD, Zheng HG, Wang B, Nguyen HT (2000) Molecular mapping of a rice gene conditioning thermo-sensitive genic male sterility using AFLP, RFLP and SSR techniques. Theor Appl Genet 100:727–734

Duvick DN, Snyder RJ, Anderson EC (1961) The chromosomal location of *Rf1*, a restorer gene for cytoplasmic pollen sterile maize. Genetics 46:1245–1252

Fu ZY, Zhao GY, Tang JH, Hu YM, He ZY, He J (2004) Comparison about traits related to fertility between two maize thermo-sensitive genic male sterile lines and primary mapping the genes for TGMS. Mol Plant Breed 2:633–636

He ZY, Li YB, Tan SY (1995) The discovery and preliminary study of the thermo-sensitive genic male-sterile maize. Crops (2):1–2

He ZY, Zhang YD, Tan SY (1998a) The application of RAPD in maize thermo-sensitive genic male sterility. Chin J Trop Crops 19(3):53–56

He ZY, Tan SY, Lin L (1998b) The ecological study of thermo-sensitive genic male sterility in maize. Chin Agri Sci Bull 15(5):4–10

Hooker AL, Smith DR, Lin SM, Beckett JB (1970) Reaction of corn seedlings with male-sterile cytoplasm to *Helminthosporium maydis*. Plant Disease Rep 54:708–712

Jia JH, Li CY, Qu XP, Wang Q, Deng QY, Weng ML, Yuan LP, Wang B (2000) Constructing a genetic linkage map and mapping a new thermo-sensitive genic male-sterile gene (*tms5*) in rice. In: Abstract of 1st National Plant Genome Meet China, Dalian, p 37

Koh HJ, Son YH, Heu MH, Lee HS, McCouch SR (1999) Molecular mapping of a new genic male-sterility gene causing chalky endosperm in rice (*Oryza sativa* L.). Euphytica 106:57–62

Lopez MT, Virmani SS (2000) Development of TGMS lines for developing two-line rice hybrids for the topics. Euphytica 114:211–215

Li JX, Dai JR, Xu QF (1963) Study on the inheritance of cytoplasmic male-sterile and fertility restoration in maize. Acta Agron Sin 2:339–362

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Etoh T (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Si MS, Deng JY (1986) The discovery, determination and utilization of the Hubei photo-sensitive genic male-sterile rice (*Oryza sativa* subsp. japonica). Acta Genet Sin 13:107–112

Subudhi PK, Bortatati RP, Virmani SS (1997) Molecular mapping of a thermo-sensitive genic male sterility in rice using bulked segregant analysis. Genome 40:188–194

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Tang JH, He ZY, Tan SY, Chen WC, Hu YM, Liu ZH, Li YL (2000) Study on the fertility conversion mechanism of the temperature-sensitive genic male-sterile line in maize. J Henan Agri Univ 34:4–6

Wang B (2001) Genetic and molecular study of photo-sensitive and thermo-sensitive male sterility in rice. In: Lu XG, Gu MH, Li CQ (eds) The theoretical and technical study of two-line hybrid rice. Science Press, Beijing, pp 39–56

Wang B, Xu WW, Wang JZ, Wu W, Zhang HG, Yang ZY, Ray JD, Nguyen HT (1995) Tagging and mapping the thermo-sensitive genic male-sterile gene in rice with molecular marker. Theor Appl Genet 91:1111–1114

Wang YG, Xing QH, Deng QY, Liang FS, Yuan LP, Weng ML, Wang B (2003) Fine mapping of the rice thermo-sensitive genic male-sterile gene tms5. Theor Appl Genet 107:917–921

Wang CH, Zhang P, Ma ZR, Zhang MY, Sun GC, Ling DH (2004) Development of a gentic marker linked to a new thermo-sensitive male sterile gene in rice (*Oryza sativa* L.). Euphytica 140:217–222

Xing QH, Ru ZG, Zhou CJ, Xue X, Liang CY, Yang DE, Jin DM, Wang B (2003) Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (*wtms1*) in wheat. Theor Appl Genet 107:1500–1504

Yamagushi Y, Ikeda R, Hirasawa H, Minami M, Ujihara P (1997) Linkage analysis of the thermo-sensitive genic male sterility gene *tms2* in rice (*Oryza sativa* L.). Breed Sci 47:371–377

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468