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IDENTIFYING MAP LOCATION AND MARKERS LINKED TO THERMOSENSITIVE GENIC MALE STERILITY GENE IN 103S LINE

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ABSTRACT

The thermosensitive genic male sterile rice line, TGMS 103S, is popularly used in the rice hybrid seed production in North Viet Nam. It is well known as the mother line of national hybrid rice varieties, such as Vietlai 20 and Vietlai 24. Genes controlling sterility of the TGMS 103S were not determined. The objective of this study was to identify genes controlling f sterility of TGMS 103S by using DNA markers. Phenotypic and genetic analyses were conducted by using F_1 and F_2 population derived from TGMS103S and R20. Linkage groups and the order of markers were determined using MAPMAKER//EXP 3.0. The phenotypic and SSR data were combined for linkage analysis using the MAPMAKER program and a linkage map of specific chromosome region surrounding the *tgms* gene was constructed. The result showed that *tms*-103S of TGMS 103S was located on chromosome 2 with flanking SSR markers of *RM3294, RM6378, RM7575* and *RM71*. These markers could be used for marker-assisted selection/marker-assisted backcrossing to transfer *tms*-103S for developing female parent in two line hybrid rice breeding.

Keywords: Chromosome, hybrid rice, sterility, TGMS.

Xác định vị trí và chỉ thị phân tử liên kết với gen qui định tính bất dục đực trong dòng lúa TGMS 103S

TÓM TẮT

Dòng mẹ bất dục đực TGMS 103S sinh trưởng phát triển tốt trong điều kiện sản xuất hạt lai ở miền Bắc Việt Nam. Đây là dòng mẹ của một số giống lúa lai của Việt Nam như Việt Lai 20, Việt Lai 24. Gen điều khiển tính trạng bất dục đực của dòng TGMS 103S chưa được xác định. Do đó, nghiên cứu này sử dụng chỉ thị phân tử để xác định gen điều khiển tính trạng bất dục đực của dòng TGMS 103S. Thí nghiệm sử dụng phân tích kiểu hình và kiểu gen ở thế hệ F_1 và F_2 của cặp lai TGMS 103S với R20. Sử dụng phân tích liên kết bằng phần mềm MAPMARKER. Kết quả đã xác định gen *tms-103S* nằm trên nhiễm sắc thể số 2 và liên kết với các chỉ thị SSR *RM3294*, *RM6378*, *RM7575* và *RM71*. Những chỉ thị này có thể sử dụng trong chọn giống lúa lai cho chọn lọc MAS tính trạng bất dục đực.

Từ khoá: Bất dục, nhiễm sắc thể, lúa lai, TGMS.

1. INTRODUCTION

The application of thermo-sensitive genic male sterile (TGMS) lines has made a great contribution to hybrid rice production by providing the two-line hybrids in addition to commonly used three-line hybrids. Desirable TGMS lines have introduced for the hybrid - rice seed production. One of the successful achievements was the utilization of TGMS line 103S. This line is popularly used in the hybrid seed production under climate conditions of the northern part of Viet Nam. The first national hybrid rice variety Vietlai 20 was derived from the cross between TGMS 103S and male line R20. This variety showed good yield and short

growth duration, thus, it can be incorporated into the triple-cropping system. Another national hybrid rice variety Vietlai24 with TGMS 103S background had short grow duration and bacterial bright resistance

Up to date, nine *tgms* genes have been identified by scientists from China, Japan, IRRI, Viet Nam and India. The *tgms* genes, *tms1* on chromosome 8, *tms2* on chromosome 7, *tms3* on chromosome 6, *tms4-1* on chromosome 2, *tms5* on chromosome 2, *rtms1* on chromosome 10, *ms-h* on chromosome 9, *tms6* on chromosomes 5 and *tms8* on chromosome 11, were identified (Wang et al., 1995, Lopez et al., 2003, Subudhi et al., 1997, Dong et al., 2000, Wang et al., 2003, Jia et al., 2001, Koh et al., 1999, Lee et al., 2005, Appibhai et al., 2012). Genes controlling sterility of the TGMS 103S were not determined (Nguyen Van Hoan, 2002). The present paper reports, the chromosomal location of and SSR markers linked to male sterile gene in TGMS 103S line.

2. MATERIALS AND METHODS

2.1. Mapping population and phenotypic characterization

In order to identify PCR-based markers linked to the *tgms* gene in TGMS 103S, F1 and F_2 population were developed from cross between the thermo-sensitive genic male sterility line 103S (TGMS 103S) and R20 (male fertile line). The original F_2 population consisted of 96 individuals. The bulk segregant analysis (BSA) method was performed as reported in Michelmore et al., (1991). Two different DNA bulks were prepared by pooling equal amounts of DNA from five plants. In particular, one bulk included DNA from sterile plants and the other bulk was fertile plants. The PCR-based markers consisting of 768 simple sequence repeat (SSR) markers that cover all 12 rice chromosomes were used in BSA to detect the linkage between SSR marker and *tgms*. To reaffirm the BSA results, mapping of *tgms* gene was carried out.. Linkage analysis of the F_2 population was conducted and *tgms* gene was mapped by using r polymorphic SSR markers surrounding the *tgms* locus.

The parents, TGMS 103S and R20, F_1 and all the F_2 plants were sampled and evaluated for pollen fertility. The spikelets were sampled at anthesis from primary panicles and fixed in 70% ethanol solution. The pollen grains were stained with 1% iodine potassium iodide (I_2-KI) solution and observed under the microscope. The pollen grains were randomly scanned on each slide and classified as sterile or fertile based on their staining (Chaudhary et al., 1981). All round and dark brown-stained pollens were scored as normal fertile and irregular-shaped, yellowish or light brown colored pollen grains were scored as sterile.

2.2. DNA extraction and SSR analysis

Leaf materials for DNA extraction were collected and freeze-dried by vacuum desiccators. DNA was extracted from the ground tissues by the Cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modifications. The 15µl PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM primer, 1 unit of Taq polymerase and 5-10 µl of genomic DNA as template. The PCR amplification was performed using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The thermal cycle was programmed for the first denaturing step of 5 minutes at 95ºC, followed by 35 cycles, each of 95ºC (30 seconds), 55ºC (30 seconds), and 72ºC (30 seconds). The PCR products were resolved in 4% agarose gels by electrophoresis at 250V for 50 minutes in 0.5 x TBE buffer. The gels were stained with ethidium bromide and photographed under ultraviolet light.

The original sources and motifs for all the SSR markers used in this study are given in the Gramene database (www.gramene.org/microsa). The linkage map was constructed according to Temnykh et al., (2000) and McCouch et al., (1997). SSR analysis was performed by the procedure of Chen et al., (1997) with minor modifications.

2.3. Linkage analysis for *tgms*

Linkage groups and the order of markers were determined using MAPMAKER//EXP 3.0

(Lander et al., 1987). The Kosambi function was used to transform the recombination frequency to genetic distances (cM). The phenotypic and the SSR data were combined for linkage analysis using the MAPMAKER program and a linkage map of specific chromosome regions surrounding the *tgms* gene was constructed.

3. RESULTS AND DISCUSSIONS

3.1. Pollen fertilities in F_1, F_2 populations and its parents TGMS 103S and R20

Of the two parents, R20 showed normal pollen fertility while TGMS 103S showed complete pollen sterility in the autumn season.. The pollen fertility of 103S/R20F_1 was rather high in both spring and autumn seasons (88.1% and 91.2%, respectively) (Table 1).

Out of 96 F_2 plants analyzed, 70 plants were fertile. The Mendelian segregation pattern of fertile to sterile plants in these F_2 populations of 103S/R20 followed 3:1 ratio (χ^2= 0.0023). The results indicate that a single recessive nuclear gene controls the thermo-sensitive male sterility of TGMS 103S line. We tentatively named this gene as *tms*-103S.

3.2. Linkage map construction for *tgms* locus

Seven hundred sixty eight SSR primers were screened with two bulked DNA samples. Three of them, *RM154*, *RM211* and *RM71*, showed polymorphism between two bulks (Fig.3). Based on the linkage map (Temnykh et al., (2000); McCouch et al., 1997), these three markers are located on the chromosome 2 with

Table 1. Pollen fertility of the parental lines and F_1 in spring and autumn season

Parental line/F_1	Pollen fertility (%) (Mean ± SD)	
	Spring season	Autumn season
103S	78.4 ± 3.1	0.0 ± 0.0
R20	91.2 ± 2.2	86.0 ± 4.3
103S/R20F_1	88.1 ± 3.8	91.2 ± 2.9

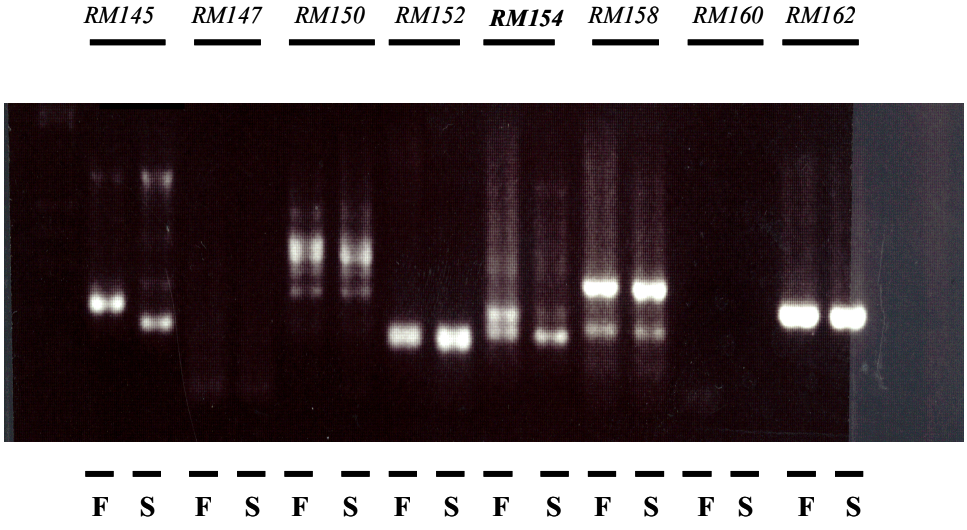

Fig. 1. Bulked segregant analysis of two bulks using SSR markers. The polymorphic result between two bulks of marker *RM154* showed the linkage of this marker with *tgms* locus

Note: F: fertile bulk, S: sterile bulk

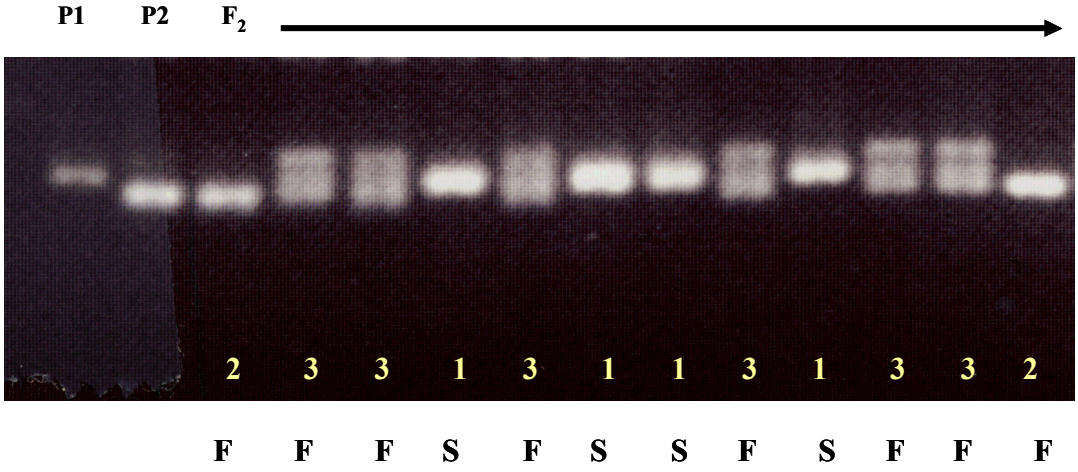

Fig. 2. F_2 individual mapping using a SSR marker *RM71* near *RM154* confirmed the result of bulked segregant analysis. Genotype notation: 1 as genotype of TGMS 103S (P1), 2 as genotype of R20 (P2) and 3 as genotype of heterozygous. Phenotypic score F as fertile plants and S as sterile plants

the PCR positions of 4.8cM for *RM154*, 10.0cM for *RM211* and 41.0cM for *RM71* (McCouch et al., 2002). To identify the chromosome location of *tms*-103S gene, more SSR markers on chromosome 2 were added to detect the polymorphism between two parents. The SSR markers *RM154, RM211, RM5529, RM3294, RM6378, RM7575* and *RM71* showed polymorphism between two parents and these markers were used in mapping analysis of the F_2 population consisting of of 96 individuals to construct the linkage map. The result of F_2 segregation population showed the linkage map of *tms*-103S and SSR markers *RM3294, RM6378, RM7575* and *RM71* (Figs.1 and 2). Using the BSA method the position of *tms*-103S of TGMS 103S was found on chromosome 2. The linkage map of *tms*-103S was constructed by using additional polymorphic SSR markers. The result showed that the flanking SSR markers of *tms*-103S, *RM3294, RM6378, RM7575* and *RM71*, could be used for MAS.

Up to now, nine *tgms* genes have been identified. The *tgms* genes, *tms1, tms2, tms3, tms4(t), tms5, rtms1, ms-h*, *tms6* and *tms8* were located on chromosomes 8, 7, 6, 2, 2, 10, 9, 5 and 11, respectively (Wang et al., 1995, Lopez et al., 2003, Subudhi et al., 1997, Dong et al., 2000, Wang et al., 2003, Jia et al., 2001, Koh et al., 1999, Lee et al., 2005, Appibhai et al., 2012).

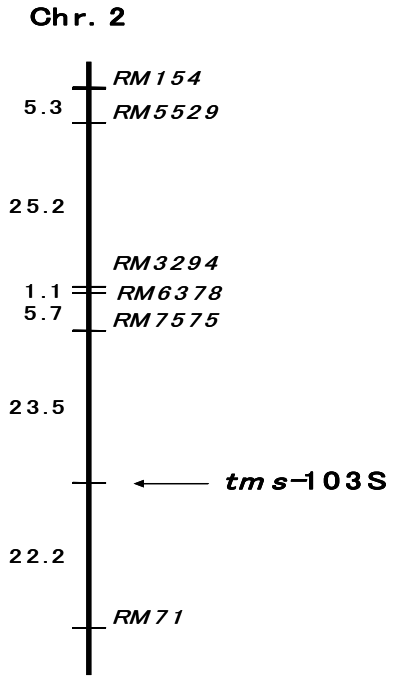

Fig. 3. Linkage map showing chromosomal location of *tms*-103S detected for pollen sterility on chromosome 2. SSR marker names are shown on the right of the chromosome with the distances (in cM) indicated on the left

The recessive *tms*103S of this study was identified on the short arm of chromosome 2. Dong et al., (2000) identified the SSR markers *RM27* linked to the *tms4(t)* on chromosome 2 based BSA of the F_2 population of the cross TGMS-VN ×CH1. Jia et al., (2001) used an F_2 population from the cross between AnnongS-1 and Nanjing11 to construct a genetic linkage map. A new TGMS gene, *tms5*, was identified on the short arm of the chromosome 2. It was found that *tms5* was located between markers *RM349* and *RM71* and it was closely linked to the marker *RM174*. The *tms5* was mapped between two SSR markers *RM279* and *RM492* with distances 19.0cM and 5.4cM, respectively (Wang et al., 2003). The position of *tms*-103S in the present study might be coinciding with *tms5* based on the SSR marker analysis. The question of whether *tms*-103S being allelic or closely linked to *tms5* merits further verification.

4. CONCLUSION

The bulk segregant analysis using simple sequence repeat (SSR) markers was able to identify the map position of *tms*-103S gene of TGMS 103S on chromosome 2. The flanking SSR markers of *tms*-103S gene, *RM3294, RM6378, RM7575* and *RM71*, were identified. The DNA markers tightly linked to TGMS gene (tms-103S) in TGMS 103S can be cost effectively used for marker-assisted selection of TGMS trait in breeding new TGMS lines in future.

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