Molecular mapping of a new induced gene for nuclear male sterility in sunflower (*Helianthus annuus* L.)

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ABSTRACT

Nuclear male sterility (NMS) is a valuable breeding tool for producing sunflower hybrids without any tedious emasculation. A new NMS line, NMS HA89-872, induced by mitomycin C and streptomycin carries a single recessive male-sterile gene ms_6. The objectives of this study were to identify molecular markers linked to the male sterility gene, and to map the *ms* locus on the sunflower genome. An F_2 population of 88 plants was obtained from a cross between the nuclear male-sterile mutant NMS HA89-872 (*msms*) and the male-fertile line RHA271 (*MsMs*). More than 230 pairs of primers, including 225 SSR primers and 9 STS primers were assayed. Nine SSR and three STS markers were polymorphic between male-fertile and male-sterile bulks, and were used to screen the mapping population. Seven SSR (ORS31, ORS294, ORS495, ORS519, ORS885, ORS807, ORS996) and two RFLP-derived STS (STS5C1 and STS11D3) markers were identified to be linked with ms_6. The ms_6 locus was flanked by ORS 807 and ORS996 at a distance of 7.2 and 18.5 cM, respectively, on linkage group 16 of the sunflower SSR genetic map.

Key Words: nuclear male sterility – Sequence Tagged Site (STS) – Simple Sequence Repeat (SSR) – sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important worldwide oil crops based on the utilization of the heterosis phenomenon. The male sterility represents the malfunction of plants to produce functional anthers, pollen, or male gametes (Kaul, 1988), while the gynoecium is functional. The male sterility can appear spontaneously or chemically induced via mutations in nuclear and/or cytoplasmic genes. As a result, it leads to the production of nuclear or genic male sterility (NMS or GMS) and cytoplasmic male sterility (CMS), respectively. Genetic control of NMS can be determined by several types of gene actions (Chaudhury, 1993; Horner and Palmer, 1995). However, the most common genetic control of nuclear male sterility is monogenic (Gundaev, 1971; Leclercq, 1966). About 10 Romanian nuclear male sterility lines were established which were under the control of five NMS genes, designated ms_1-ms_5 (Vrânceanu, 1970).

Four additional NMS lines were obtained from HA89 treated with mitomycin C and streptomycin. The resulting NMS lines were designed as ms_6-ms_9 (Jan and Rutger, 1988; Jan, 1992). The ms_9 gene was mapped to linkage group 10 of the sunflower SSR genetic map by Chen et al. (2006). NMS genes in lines B11A3 and P21 were designated ms_{10} and ms_{11} (Jan, 1992), and mapped to linkage group 11 and 8, respectively, by Pérez-Vich et al. (2005). However, other NMS genes have not been mapped. The objectives of this study were to identify molecular markers linked to ms_6 in NMS HA89-872, and to map the ms_6 locus onto the sunflower linkage group.

MATERIALS AND METHODS

Plant material

The line NMS HA89-872 induced by chemical mutagenesis (Jan and Rutger, 1988) and RHA271 were used as parents in this study. NMS HA89-872 is homozygous for the recessive alleles ms_6ms_6 controlling nuclear male sterility (Jan, 1992). RHA271 is a fertility restorer line for the cmsPET1 cytoplasm. An F_2 mapping population of 88 plants was developed from crossing NMS HA89-872 and RHA271. F_3 families were produced by self-pollination of male-fertile F_2 plants, and scored as male-fertile or male-sterile to

distinguish hetero- and homozygous F_2 progeny. The segregation of male-fertile to male-sterile genotypes of F_2 progeny was tested by Chi-square goodness of fit.

DNA isolation

Genomic DNA was isolated from lyophilized leaf powders of each individual. DNA extraction was performed in CTAB buffer, following standard steps of DNA purification. Bulk segregant analysis (Michelmore et al., 1991) was performed by pooling an equal quantity of DNA from 10 homozygous male-fertile and 10 homozygous male-sterile F_2 plants.

Primer design

In total**,** 225 SSR primers selected from the Compositae species database (compositdb.ucdavis.edu) were used for the *ms₆* gene screening. Sequence Tagged Site (STS) primers were designed based on the associated RFLP sequences (Jan et al., 1998), using Primer3 software (http://frodo.wi.mit.edu). The sequences of two STS primers which produced polymorphic markers are listed in Table 1.

Table 1. STS primers used in genetic mapping of *ms₆* locus.

Locus	Primer	
	Forward (5'→3')	Reverse(5'→3')
STS5C1	GGATTTCCGAAAACAGTACA	TTGTTGTAAGCCTGGAGAGT
STS11D3	AAAAACATTTGTCCCATTTG	CAAAAGGACATGTGAAAAGC

PCR amplification and data analysis

For SSR analysis, about 20 ng genomic DNA was used as a template in a 15 µl reaction volume per PCR reaction and the products were amplified following the Touchdown PCR profile (Tang et al., 2002) with slight modifications. Electrophoresis was performed in 6.5% polyacrylamide gel at 60 w for 2.2 h. The images were obtained using a Typhoon 9410 variable mode imager (Molecular Dynamics Inc., CA, USA) and ImageQuant software (GE Healthcare). The scoring codes were 1 for present, 0 for absent, and a dash "-" for missing.

A genetic linkage map was constructed using the Kosambi function (Kosambi, 1944) of Mapmaker/Exp version 3.0b (Lander et al., 1987). Map distances in centimorgans (cM) were evaluated and the linkage map was drawn with MapChart 2.0 (Voorips, 2002).

RESULTS

Inheritance of the ms₆ gene

The analysis of F_2 progeny phenotype allowed scoring the male-sterile/male-fertile plants, with 65 classified as male-fertile and 23 male-sterile. Chi-square analysis of F_2 population revealed a good fit to 3 fertile: 1 sterile ratio (χ^2 =0.06, 0.80<P<0.90), indicating that male sterility was conditioned by a single recessive gene. The $F_{2:3}$ progeny test separated the F_2 progeny into 23 homozygous male-fertile plants, 42 heterozygous male-fertile plants, and 23 male-sterile plants, which correspond to the segregation ratio of 1:2:1 (χ^2 = 0.18, 0.90 < P<0.95).

Molecular mapping of the ms₆ gene

Two hundred and twenty-five SSR primers randomly chosen from 17 linkage groups of the public SSR genetic map (Tang et al., 2002) were used to screen the male-sterile and male-fertile bulks. Nine SSR markers were shown to be polymorphic between the two bulks (Table 2), and seven of them, ORS31, ORS294, ORS495, ORS519, ORS807, ORS885, ORS996, were linked to male-sterile allele *ms₆* on linkage group 16. The segregation ratios of these markers fit a 3:1 ratio, with small deviations (χ^2 =0.06-2.18, 0.1< P <0.8). (Table 3)

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Table 2. Genetic polymorphism of molecular markers between male-fertile and male-sterile bulks.

	SSR markers	STS markers
No. of primers	225	9
No. of Polymorphic primers	9	3
No. of Polymorphic primers linked to *ms₆*	7	2
Percentage of polymorphic primers (%)	4.1	33.3

Table 3. Chi-square test for the segregation ratio of the male sterility/fertility trait, SSR markers, and STS markers in the F_2 population of NMS HA89-872 × RHA271.

	Number of plants	Type of inheritance[1]					Ratio fit	χ^2-value	Probability ($>\chi^2$)
		AA	HH	BB	CC	DD			
ms$_6$	88	23	42	23			1:2:1	0.18	0.91
STS5C1	88	17	46	25			1:2:1	1.64	0.44
ORS996	88	24			64		3:1	0.24	0.62
STS11D3	88	28			60		3:1	2.18	0.14
ORS31	74	22			52		3:1	0.88	0.35
ORS294	88	28			60		3:1	2.18	0.14
ORS495	87	27			60		3:1	1.69	0.19
ORS885	88	21			67		3:1	0.06	0.81
ORS519	88	29			59		3:1	2.97	0.08
ORS807	84			25		59	3:1	1.02	0.31

[1]Genotypes: AA = NMS 89-872 (*msms*); HH = heterozygous (*Msms*); BB = RHA271 (*MsMs*); CC = not AA (*MsMs* or *Msms*); DD = not BB (*Msms* or *msms*).

After confirming the location of *ms*$_6$ to linkage group 16, nine STS primers were designed based on the sequences of RFLP markers from linkage group 3 (Jan et al., 1998), which cross-referenced with linkage group 16 of the SSR genetic map (Yu et al., 2003), to screen the F_2 population. Two STS primers, STS5C1 and STS11D3, were polymorphic and linked to *ms*$_6$ male sterility gene (Table 3). STS5C1 was represented by 25 homozygous male-fertile, 46 heterozygous male-fertile plants, 17 homozygous male-sterile plants (Fig. 1), with a ratio of 1:2:1 (χ^2 =1.64, 0.4< P <0.5). Segregation of STS11D3 fit to 3:1 ratio, indicating a recessive male sterile inheritance. Based on 8 SSR markers and 2 STS markers linked with *ms*$_6$ gene, the map of the *ms*$_6$ gene spanned within a region of 69.2 cM, and the *ms*$_6$ locus was flanked by ORS 807 and ORS996 at the distance of 7.2 and 18.5 cM, respectively (Fig. 2).

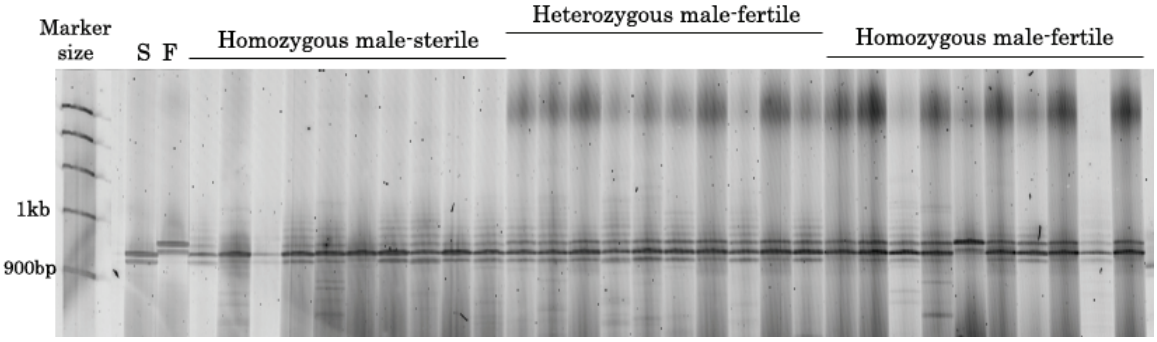

Fig. 1. The banding pattern of the marker STS5C1 for two bulks and homozygous male-sterile, heterozygous male-fertile and homozygous male-fertile plants in the F_2 generation. F=male-fertile bulk; S=male-sterile bulk.

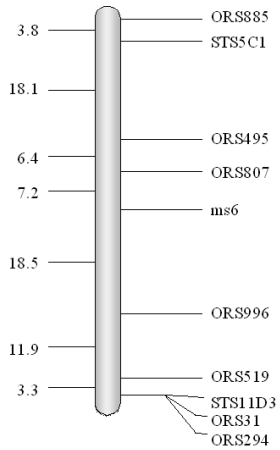

Fig. 2. Mapping of the *ms*$_6$ gene on LG 16. Distances are shown in centiMorgans (cM).

DISCUSSION

Pollen development in higher plants is a complex process (McCormick, 1993). The abortion of anther development results in the appearance of male-sterile plants. Molecular markers linked with *ms* locus can offer the quick and precise detection of genotype bearing a male-sterility allele.

In this study, different marker systems, including SSR and RFLP-derived STS, were used for mapping the *ms* locus in sunflower. Of 234 markers, nine proved to be associated with the male- sterility gene. The ms_6 gene was mapped to linkage group 16 and it was flanked by SSR markers ORS807 and ORS996. A total of two STS markers based on RFLP sequences were effectively mapped and integrated into the previously described linkage maps (Jan et al., 1998; Yu et al., 2003). The locus order for the SSR and STS markers were similar to the reference maps (Jan et al., 1998; Yu et al., 2003). These SSR and STS markers with co-dominant and dominant gene actions would be useful for marker-assisted selection in breeding programs.

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