Identification of molecular markers linked to a new nuclear male-sterility gene *ms*$_7$ in sunflower (*Helianthus annuus* L.)

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ABSTRACT

Nuclear male sterility (NMS) is an important alternative system to the cytoplasm male sterility (CMS) in hybrid sunflower breeding programs because of its stable male sterility and the abundance of available restorers. NMS HA89-552 (*Helianthus annuus* L.) is a nuclear male-sterile mutant induced by mitomycin-C and streptomycin from inbred maintainer line HA89, which possesses a single recessive gene, *ms*$_7$, controlling male sterility. Molecular markers linked to the *ms*$_7$ gene were identified using a 93-plant F_2 population derived from the cross of NMS HA89-552 × RHA271 with simple sequence repeat (SSR), RFLP-derived sequence tagged site (STS), and target region amplification polymorphism (TRAP) markers. The *ms*$_7$ gene was mapped to linkage group 6 of the public SSR genetic map. Four SSR markers (ORS349, ORS608, ORS1229 and ORS483), two RFLP-STS markers (STS8C4, STS9C1) and two TRAP markers (Tg3r165a-220, Tg3r165a-185) were located near the *ms*$_7$ locus. The total genetic distance covered by the markers was 53.4 cM. SSR marker ORS608 and TRAP marker Tg3r165a-185 flanked the *ms*$_7$ gene, at distances of 2.6 cM and 4.7 cM, respectively. The markers linked with the *ms*$_7$ locus could be used to accelerate the breeding of the male-sterile line through marker-assisted selection.

Key words: molecular mapping – nuclear male sterility – sunflower

INTRODUCTION

Male sterility, including cytoplasmic male sterility (CMS) and nuclear male sterility (NMS), provides valuable tools for hybrid breeding programs. Four non-allelic NMS genes, designated *ms*$_6$, *ms*$_7$, *ms*$_8$ and *ms*$_9$, were identified from mutant HA89 after mitomycin-C and streptomycin treatment (Jan and Rutger, 1988; Jan, 1992a). The *ms*$_9$ gene of NMS HA89-360 has been mapped to linkage group 10 (Chen et al., 2006) of the public SSR genetic map (Tang et al., 2002; Yu et al., 2003), but *ms*$_7$ remains unmapped since the release of NMS HA89-552 (Jan, 1992b). The objective of the present research was to identify molecular markers linked to the *ms*$_7$ gene in NMS HA89-552.

MATERIALS AND METHODS

Ninety-three F_2 progenies from NMS HA89-552 × RHA271 were obtained by selfing individual F_1 plants. F_3 families from selfed male-fertile F_2 plants were grown in the field to differentiate heterozygous plants from homozygous F_2 plants. Male fertility for F_2 and F_3 individuals was visually scored at flowering. The Chi-square test was used to determine the segregation ratio of the male-fertile and male-sterile phenotype of the F_2 and F_3 progenies.

Genomic DNA was extracted from lyophilized leaf powder of the two parents and the F_1 and F_2 plants with the procedure reported by Rogers and Bendich (1985). Equal amounts of DNA from 12 male-sterile homozygous and 12 male-fertile homozygous F_2 individuals were pooled to form male-sterile and male-fertile bulks.

Two hundred and sixty-five ORS primer pairs were randomly chosen from 17 linkage groups of the public SSR genetic map (Tang et al., 2002; Yu et al., 2003) for screening the two bulks. After confirming that the *ms7* gene was located on linkage group 6 of the SSR genetic map, which cross-references to linkage group 15 of the RFLP map developed from a cDNA library (Jan et al., 1998), 15 single- or low-copy RFLP markers from linkage group 15 were chosen to design STS primers. Using expressed sequence tag (EST) database information, 224 TRAP markers were also generated to screen the male-

sterile and male-fertile bulks (Hu and Vick, 2003). The sequences of RFLP-STS and TRAP primers that produced polymorphic markers in the F_2 population are listed in Table 1.

PCR amplification for SSR and STS primers was performed according to Tang et al. (2002). The amplification products were separated on a 6.5% denaturing polyacrylamide gel at 60W for 2.0 h. Gel images were collected with a Typhoon 9410 variable mode imager (Molecular Dynamics Inc., CA, USA). TRAP analysis was conducted following the procedure of Chen et al. (2006).

Table 1. The sequences of TRAP and RFLP-STS primers producing polymorphic bands.

TRAP primers		Sequences (5'- 3')	STS primers	Sequences (5'- 3')
Fixed primer	Mir165a	GATCCGTCTATGCTTTT	STS8C4	GGGGATCATGAACAGTTTTA
				CCTTGGTTCCTTCAGACAC
Arbitrary primer	Ga3-800	TCATCTCAAACCATCTACAC	STS9C1	TGGCTTCACGTTTTAAAGTT
				GAATCGGACAAAACAAAAAC)

Linkage analysis was performed using MAPMAKER/EXP version 3.0b (Lander et al., 1987). Marker order was determined with a LOD threshold of 3.0, and map distances were estimated by the Kosambi function (Kosambi, 1944). The linkage map was produced using MapChart 2.0 (Voorips, 2002).

RESULTS

Inheritance of the ms7 gene in the F_2 population

The segregation of 78 male-fertile to 15 male-sterile plants in the F_2 population fit a 3:1 ratio (χ^2=3.89, 0.05<P<0.01), indicating a single recessive gene control of male-sterility. The F_3 progeny tests classified the 78 male-fertile F_2 plants into 23 homozygous male-fertile and 55 heterozygous male-fertile plants, further confirming that the segregation ratio best fit the 1:2:1 ratio for a single recessive gene control of male-sterility (χ^2=4.48, 0.2<P<0.1) (Table 2).

Table 2. Segregation of the *ms_7* male-sterility locus and eight markers in the F_2 population.

Traits or markers	Number of plants[1]	Observed number[2]				χ^2-value	
		AA	HH	BB	DD	1:3	1:2:1
ms_7	93	15	55	23			4.48
ORS1229	87			24	63	0.31	
ORS608	92			25	67	0.23	
ORS349	83	16	40	27			3.02
STS9C1	88	14	55	19			6.06*
STS8C4	92			24	68	0.06	
ORS483	92			28	64	1.45	
Tg3r165a-185	93			24	69	0.03	
Tg3r165a-220	93			21	72	0.29	

[1]For ORS1229, six plants were not scorable; for ORS608, STS8C4 and ORS483 one plant each was not scorable; for ORS349, ten plants were not scorable. For STS9C1, five plants were not scorable.
[2]Genotypes: AA, NMS HA89-552 (*msms*); HH, heterozygous (*Msms*); BB, RHA271 (*MsMs*); DD, not BB (*Msms*,*msms*).
*Significant at the 0.05 level of probability.

Molecular mapping of the ms_7 locus

The 265 pairs of SSR primers randomly chosen from 17 linkage groups (LG) of the public SSR genetic map (Tang et al., 2002; Yu et al., 2003) were screened for polymorphisms between the male-fertile and male-sterile bulks. Fourteen primers presented polymorphism between the two bulks. One SSR primer ORS349 on LG 6 showed a linkage with the *ms_7* gene in the F_2 population. Subsequently, seven ORS primers around ORS349 on LG 6 and 15 RFLP-derived STS primers from linkage groups 15 of the RFLP genetic map (Jan et al., 1998) were chosen to screen the F_2 population. Four ORS primers, ORS349, ORS608, ORS1229, ORS483, and two RFLP-STS primers, STS8C4 and STS9C1, were linked with the *ms_7* gene. ORS608 was present in all 15 homozygous male-sterile plants and 52 heterozygous male-fertile plants, but was absent in all 23 homozygous male-fertile plants and two heterozygous male-fertile plants (Fig. 1).

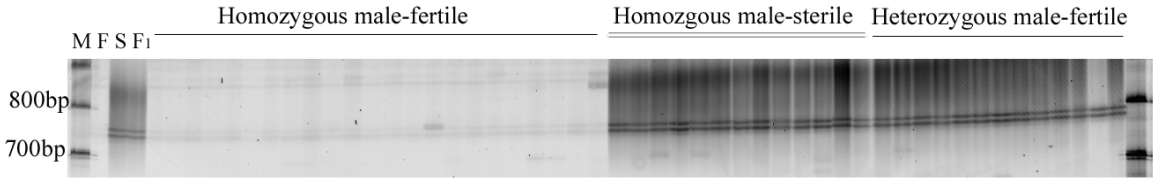

Fig. 1. PCR amplification of ORS608. The marker was present in all homozygous male-sterile and heterozygous male-fertile plants, and absent in all homozygous male-fertile plants. M, 100 bp DNA ladder (bp); F= bulk of male-fertile F_2 plants; S=bulk of male-sterile F_2 plants; F_1= F_1 hybrid.

To identify more markers linked to the *ms*$_7$ locus, 224 TRAP markers were used to screen the male-sterile and male-fertile bulks. Six polymorphic fragments from the primer combinations distinguished the two bulks, and two fragments (Tg3r165a-185 and Tg3r165a-220) were confirmed to link to male-sterile phenotypes. The segregation ratio of the two markers fit a 3:1 ratio, depending on their relationship with the *ms*$_7$ gene. Two polymorphic fragments were considered as candidate markers for the *ms*$_7$ gene.

Linkage analysis and map construction

A linkage map of the *ms*$_7$ gene region was constructed using MAPMAKER/EXP 3.0b with LOD>3.0. All markers could be placed on linkage group 6 around the *ms*$_7$ locus (Fig. 2). The total genetic distance covered by those markers was 53.4 cM. The NMS *ms*$_7$ gene was flanked by SSR marker ORS608 and TRAP markers Tg3r165a-185 at distances of 2.6, and 4.7 cM, respectively. Two RFLP-STS markers, STS9C1 and STS8C4, were 7.3 and 19.3 cM proximal, respectively, of the *ms*$_7$ locus. The locus order for the public SSR markers and the reference linkage maps (Tang et al., 2002; Yu et al., 2003) was identical. Therefore, it is concluded that the *ms*$_7$ gene is located on LG 6 of the public sunflower SSR genetic map.

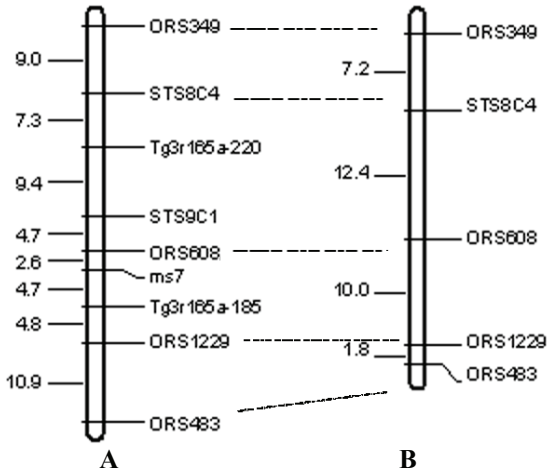

Fig. 2. A: The map position of the *ms*$_7$ gene on linkage group 6 in relation to four SSR markers, two STS markers, and two TRAP markers. B: A corresponding partial linkage map of the region surrounding ORS608 on linkage group 6 of the public sunflower SSR linkage map (Tang et al., 2002; Yu et al., 2003). Distances are shown in centiMorgan (cM).

DISCUSSION

The *ms*$_7$ gene was mapped to LG 6 on the public sunflower SSR genetic map, and was flanked by four SSR markers (ORS608, ORS1229, ORS349, ORS483), two RFLP-STS markers (STS9C1, STS8C4) and two TRAP markers (Tg3r165a-185, Tg3r165a-220). SSR marker ORS608 was the nearest to the *ms*$_7$ gene in LG 6. Two STS markers, STS9C1 and STS8C4, derived from the associated RFLP markers (Jan et al., 1998), were linked to the *ms*$_7$ gene in this study, indicating that it is feasible to convert RFLP markers into STS markers. The markers linked to the *ms*$_7$ gene provide a useful tool for easy identification of lines carrying the male-sterility allele when applying a marker-assisted selection technique.

The molecular mapping of NMS genes in sunflower was first reported by Pérez-Vich et al. (2005) for *ms*$_{10}$ and *ms*$_{11}$, which mapped to LG11 and LG8, respectively, on the public sunflower SSR linkage map. The *ms*$_9$ gene of NMS HA89-360 was mapped to LG 10 (Chen et al., 2006). Though NMS HA89-360 and NMS HA89-552 were derived from streptomycin-treated HA89 seed, the loci of the two NMS genes were different: the *ms*$_7$ gene of NMS89-552 was mapped to LG 6.

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