Confirmation and Survey of RAPD and SCAR Markers for the Male-Sterile *ms-3* Gene in Melon

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ABSTRACT

Male sterility, controlled by a single recessive gene, is an important trait of melon (*Cucumis melo* L.). Both the randomly amplified polymorphic DNA (RAPD) marker OAM08.650 and the sequence characterized amplified region (SCAR) marker SOAM08.644 previously were reported to be closely linked to the male-sterile *ms-3* gene at a distance of 2.1 cM in an F_2 population from the melon cross of ms-3 x 'TAM Dulce'. However, the RAPD and SCAR markers for the *ms-3* gene were not confirmed in different populations to indicate their merit in breeding. The aim of this study was to confirm the linkage of the RAPD OAM08.650 and SCAR SOAM08.644 markers with the *ms-3* gene in an F_2 population derived from the melon cross of ms-3 with the different fertile cultivar Mission. The linked RAPD OAM08.650 and SCAR SOAM08.644 markers that displayed amplified DNA fragments in the male-sterile ms-3 parent, were confirmed in the F_2 population from the cross of ms-3 x 'Mission' to be consistently linked to the *ms-3* gene at a distance of 5.2 cM. These markers were also present in 22 heterozygous fertile F_1 plants having the *ms-3* gene. The RAPD and SCAR markers linked to the *ms-3* gene, confirmed and identified here, could be utilized for backcrossing of male sterility into elite melon cultivars and lines for use as parents for F_1 hybrid seed production.

RESUMEN

La esterilidad masculina, controlada por un gen único recesivo, es un carácter importante del melón (*Cucumis melo* L.). Previamente se reportó que tanto el marcador RAPD (randomly amplified polymorphic DNA) OAM08.650 como el marcador SCAR (sequence characterized amplified región) SOAM08.644 están ligados estrechamente al gen de esterilidad masculina *ms-3* a una distancia de 2.1 cM en una población F_2 de una cruza de melón ms-3 X 'TAM Dulce'. Sin embargo, los marcadores RAPD y SCAR para el gen ms-3 no fueron confirmados en diferentes poblaciones para indicar su importancia en mejoramiento. El objetivo de este estudio fue confirmar el ligamiento de los marcadores RAPD OAM08.650 y SCAR SOAM08.644 con el gen *ms-3* en una población F_2 derivada de una cruza de melón de ms-3 con el diferente cultivar fértil Mission. Se confirmó que los marcadores ligados RAPD OAM08.650 y SCAR SOAM08.644, que mostraron fragmentos de DNA amplificados en el progenitor masculino estéril ms-3, estuvieron constantemente ligados al gen *ms-3* a una distancia de 5.2 cM en la población F_2 de la cruza de ms-3 X Mission'. Estos marcadores también estuvieron presentes en 22 plantas F1 heterocigóticas fértiles que contuvieron al gen *ms-3*. Los marcadores RAPD y SCAR ligados al gen *ms-3*, confirmados e identificados aquí, podrían ser utilizados para retrocruza del gen de esterilidad masculina dentro de cultivares y lineas importantes de melón usadas como padres para la producción de semillas híbridas en la F_1 .

Additional Index Words: randomly amplified polymorphic DNA, sequence characterized amplified region, marker-assisted selection

Male sterility is a potentially stable and effective means to reduce high costs by generating larger quantities of hybrid seed with less labor and less contamination through insect and wind pollinations (McCreight and Elmstrom, 1984). Genic male sterility was reported to be controlled by a single recessive gene in four F_2 populations from different melon (*Cucumis melo* L.) crosses of the homozygous male-sterile ms-3 x homozygous male-fertile parents (McCreight and Elmstrom, 1984). They assigned the symbol *ms-3* for the single recessive gene controlling male sterility. Four other male-sterile phenotypes in melon have been reported (Bohn and Principe, 1964; Bohn and Whitaker, 1949; Lecouviour et al., 1990; Pitrat, 1991, 2002) to be controlled by different single recessive genes including *ms-1*, *ms-2*, *ms-4*, and *ms-5*. According to previous results obtained on the basis of allelism and linkage analyses (Lecouviour et al., 1990; McCreight and Elmstrom, 1984; Pitrat, 1991, 2002), the *ms-3* gene is known to be independent of the other four male-sterile genes.

Markers linked to the *ms-3* gene would be useful in transferring the male-sterile gene into elite melon cultivars and

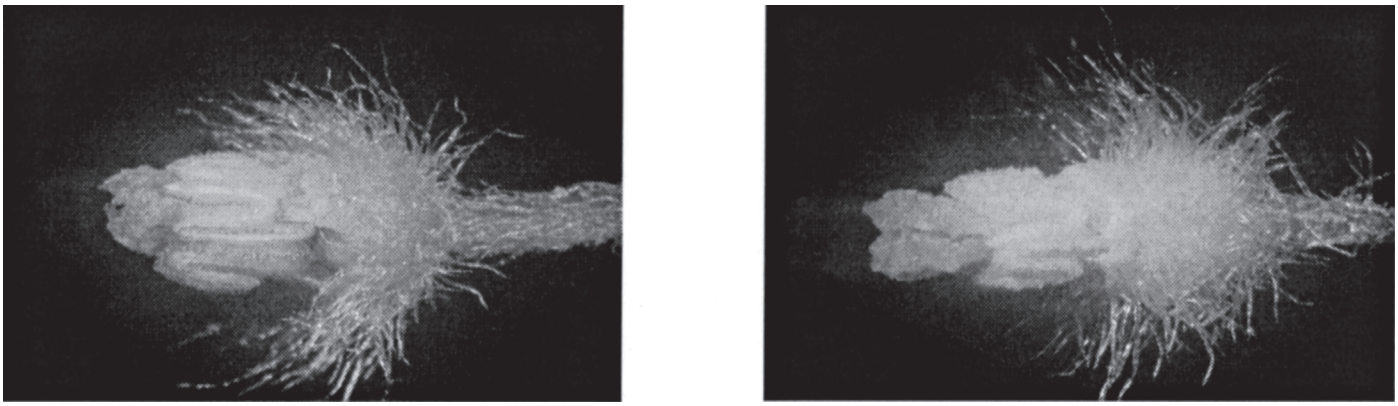

Fig. 1. (a) The male-sterile anther of ms-3 and (b) the male-fertile anther of 'TAM Dulce'.

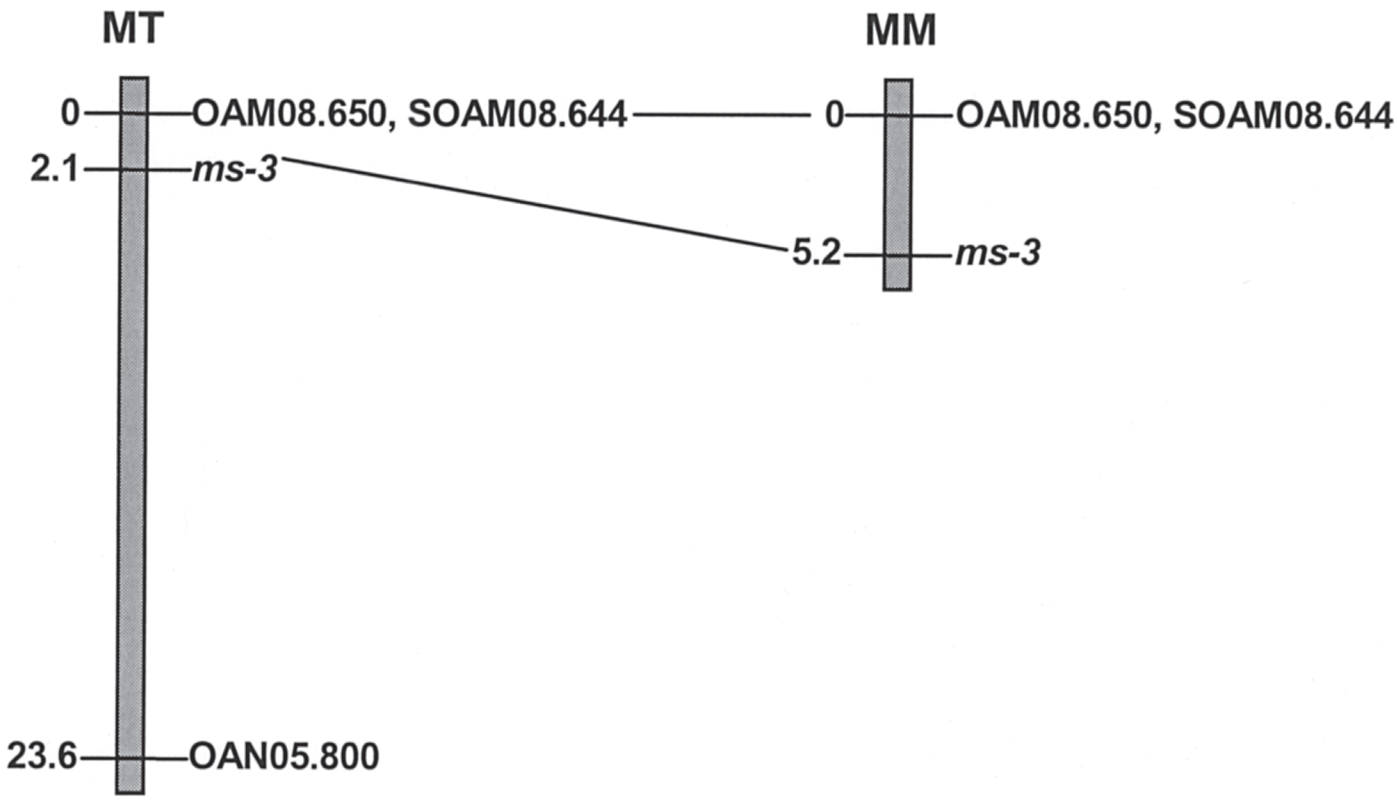

Fig. 2. Linkage group including the *ms-3* gene controlling male sterility and three RAPD and SCAR markers developed using an F_2 population of the melon cross ms-3 (male-sterile) x 'TAM Dulce' (male-fertile) (MT), and the *ms-3* gene and two RAPD and SCAR markers developed using an F_2 population of the melon cross ms-3 x 'Mission' (male-fertile) (MM). The gene and marker names are given on the right and the length in cM is indicated on the left of the linkage group. The RAPD marker OAM08.650, the SCAR marker SOAM08.644, and the male-sterile gene in the linkage group are connected between the MT and MM F_2 populations by lines.

breeding lines. Two randomly amplified polymorphic DNA (RAPD) markers OAM08.650 and OAN05.800 were previously identified to be linked to the *ms-3* gene by means of bulked segregant analysis in an F_2 population from the melon cross of ms-3 (male-sterile) x 'TAM Dulce' (male-fertile) (Park and Crosby, 2004). The RAPD marker OAM08.650 that displayed an amplified DNA fragment in the male-sterile bulk was closely linked to the *ms-3* gene at a distance of 2.1 cM. The sequence characterized amplified region (SCAR) marker SOAM08.644 was developed on the basis of the specific forward and reverse 20-mer primer pair designed from the sequence of the RAPD marker OAM08.650 (Park et al., 2003). The SCAR marker SOAM08.644 was also linked to the *ms-3* gene at 2.1 cM in the F_2 population from the cross of ms-3 x 'TAM Dulce'.

Park et al. (1999) emphasized the importance of confirming the marker-gene association in other populations before using molecular markers for marker-assisted selection in breeding programs. Therefore, the object of this study was to confirm the tight linkage of the RAPD OAM08.650 and SCAR SOAM08.644 markers with the *ms-3* gene in an F_2

population from the melon cross of ms-3 with the different fertile cultivar Mission. We then determined the presence or absence of these linked RAPD and SCAR markers in muskmelon, honeydew, casaba or ananas melon genotypes with or without the *ms-3* gene.

MATERIALS AND METHODS

Plant Material. One hundred ten F_2 plants from the melon cross of ms-3 x 'Mission' were planted in a greenhouse at the Texas Agricultural Research and Extension Center-Weslaco on

Table 1. Chi-square analyses for segregation of RAPD and SCAR fragments for two markers linked to the *ms-3* gene for male sterility in an F_2 population derived from the melon cross of ms-3 (male-sterile) x 'Mission'(male-fertile).

Marker			Number of F_2 plants				
Type	Name	Source	Presence	Absence	Ratio	X^2	*P*
RAPD	OAM08.650	ms-3	78	16	3:1	2.79	0.10
SCAR	SOAM08.644	ms-3	78	16	3:1	2.79	0.10

Table 2. Presence (+) or absence (-) of the RAPD marker OAM08.650 and the SCAR marker SOAM08.644 tightly linked to the *ms-3* gene in homozygous fertile (HF), heterozygous fertile (HeF), or homozygous sterile (HS) melon cultivars, breeding lines, and F_1 plants with or without the male-sterile allele.

Melon cultivar, breeding line, and F_1				RAPD and SCAR markers	
Entry	Type	Phenotype	Genotype	OAM08.650	SOAM08.644
ms-3	Muskmelon	Sterile	HS	+	+
'TAM Dulce'	Muskmelon	Fertile	HF	-	-
'TAM Uvalde'	Muskmelon	Fertile	HF	-	-
'TAM Perlita'	Muskmelon	Fertile	HF	-	-
'Mission'	Muskmelon	Fertile	HF	-	-
'Durango'	Muskmelon	Fertile	HF	-	-
'Primo'	Muskmelon	Fertile	HF	-	-
'Wescan'	Muskmelon	Fertile	HF	-	-
'Rio Gold'	Muskmelon	Fertile	HF	-	-
Breeding line 1405	Muskmelon	Fertile	HF	-	-
Breeding line 1405 gl	Muskmelon	Fertile	HF	-	-
Breeding line 1405 PMR	Muskmelon	Fertile	HF	-	-
Breeding line 1409	Muskmelon	Fertile	HF	-	-
'TAM Dew Improved (TDI)'	Honeydew	Fertile	HF	-	-
'TAM Mayan Sweet (TMS)'	Casaba	Fertile	HF	-	-
'Deltex'	Ananas	Fertile	HF	-	-
TGR1551	Agrestis	Fertile	HF	-	-
F_1 from ms-3 x 'TAM Dulce'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'TAM Uvalde'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'TAM Perlita'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'Mission'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'Durango'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'Primo'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'Wescan'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'Rio Gold'	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 1405	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 1405 gl	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 1405 PMR	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 1409	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x 'TDI'	Honeydew	Fertile	HeF	+	+
F_1 from ms-3 x 'TMS'	Casaba	Fertile	HeF	+	+
F_1 from ms-3 x 'Deltex'	Mixed	Fertile	HeF	+	+
F_1 from ms-3 x TGR1551	Mixed	Fertile	HeF	+	+
F_1 from ms-3 x MM-44	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x MM-46	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x MM-66	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x MM-67	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x MM-73	Muskmelon	Fertile	HeF	+	+
F_1 from ms-3 x MM-76	Muskmelon	Fertile	HeF	+	+

9 January 2002. The male-sterile ms-3 parent was originally noted in a single plant of PI 321005 selected from the cross of 'Georgia 47' x 'Smith's Perfect' (McCreight and Elmstrom, 1984). The male-fertile 'Mission' parent, a commercial muskmelon cultivar (Asgrow Vegetable Seeds, Oxnard, California), is resistant to powdery mildew (race 1) and sulfur. In addition, 39 melon cultivars, breeding lines, and F_1 plants were planted in a completely randomized design with five replications in the greenhouse on 10 September 2002. Male sterility and fertility were checked on all plants of F_2 and melon germplasm during flowering.

RAPD. Fully expanded leaves of 39 melon cultivars, breeding lines, and F_1 plants as well as 110 F_2 plants with their parents were collected at 21 days after planting. Total genomic DNA was extracted from the leaf tissue using the method of Skroch and Nienhuis (1995). Polymerase chain reactions (PCR) were performed on 96-well plates in a MJ Research thermalcycler (model PTC-0100; MJ Research, Waltham, Massachusetts). Protocols for PCR and the composition of the final volume of reactants were the same as those described by Skroch and Nienhuis (1995). A 100-base pair (bp) DNA ladder (Life Technologies, Grand Island, New York) was used to estimate the length of DNA markers. Two 10-mer primers (Operon Technologies, Alameda, California) that generated the RAPD markers OAM08.650 and OAN05.800 linked to the *ms-3* gene (Park and Crosby, 2004) were tested in the F_2 population derived from the cross between ms-3 and 'Mission' for confirming the RAPD marker-gene linkage. One primer that produced the most tightly linked RAPD marker to the *ms-3* gene was tested in 39 cultivars, breeding lines, and fertile F_1 plants for determining the presence or absence of the RAPD marker linked to the *ms-3* gene.

SCAR. The specific forward (5'-ACCACGAGTGTCGAGAAGAA-3') and reverse (5'-ACCACGAGTGAGGGATCTTC-3') 20-mer primer pair was tested in 39 cultivars, breeding lines, and fertile F_1 plants for determining the presence or absence of the SCAR marker as well as the F_2 population of the cross ms-3 x 'Mission' for confirming the SCAR marker-gene linkage. Protocols for PCR and the composition of the final volume of reactants were the same as those described by Rubio et al. (2001).

Linkage Analysis. To detect segregation distortion of markers, the F_2 population marker datum was tested for goodness-of-fit to a 3:1 ratio using the chi-square test. The linkage analysis of RAPD and SCAR markers with the *ms-3* locus for male sterility was performed on the data for F_2 plants of the cross ms-3 x 'Mission' using MAPMAKER version 3.0 (Lander et al., 1987). Map distances (centimorgan, cM) between ordered loci of marker and gene were calculated using recombination fractions and the Kosambi mapping function (Kosambi, 1944).

RESULTS AND DISCUSSION

Confirmation. Verification of the tight linkage of the RAPD OAM08.650 and SCAR SOAM08.644 markers with the *ms-3* gene identified in the F_2 population of the cross ms-3 (male-sterile) (Fig. 1a) x 'TAM Dulce' (male-fertile) (Fig. 1b) was needed in other populations to substantiate their merit in melon breeding. Of the three RAPD and SCAR markers linked to the *ms-3* gene developed as well as identified in the original F_2 population, OAM08.650 and SOAM08.644 were polymorphic between ms-3 and 'Mission'. These markers, tightly linked to the *ms-3* gene in the original F_2 population, were present in the ms-3 parent, and absent in the 'Mission' parent. A 3:1 goodness-of-fit ratio for band presence to band absence for each of the RAPD and SCAR markers was observed in the F_2 population of the cross ms-3 x 'Mission' (Table 1).

The linkage group containing the RAPD and SCAR markers as well as the *ms-3* gene developed using the F_2 population from the cross of ms-3 x 'Mission' is shown in Fig. 2. No recombination between the RAPD and SCAR markers was observed in the population, indicating that both were noted at the same marker locus. The RAPD OAM08.650 and SCAR SOAM08.644 markers were confirmed in the F_2 population from the cross of ms-3 with the fertile cultivar Mission to be consistently linked to the *ms-3* gene for male sterility at a distance of 5.2 cM. Different linkage distances were estimated between the gene and the markers in two F_2 populations. These differences may result from sampling variation in markers which can create variations in distances between the gene and the markers. Also, variable recombination frequencies and chromosomal differences can contribute to such observed discrepancies. The RAPD marker OAN05.800, very loosely linked to the *ms-3* gene detected in the original F_2 population, was not confirmed in this genetic population.

The andromonoecious gene (*a*) regulating stamen absence or stamen presence in female flowers was found to be unlinked to the *ms-3* gene in the F_2 population from the cross of ms-3 x 'Mission'. The lack of linkage between the *a* and the *ms-3* genes found here confirms the findings of Pitrat (1991 and 2002), who reported that the andromonoecious gene on linkage group 4 was not associated with the other four male-sterile genes as well as the *ms-3* gene.

Our confirmation of the marker-gene association in two different populations is an essential step prior to universal use of these linked markers in marker-assisted selection programs, as suggested by Park et al. (1999). They confirmed that RAPD markers and flower color were consistently associated with major genes affecting resistance to common bacterial blight in three different common bean backgrounds including recombinant inbred, backcross, and F_2 populations of different crosses. Therefore, the RAPD and SCAR markers linked to the *ms-3* gene identified and confirmed in two segregating populations here should be more reliable for backcrossing the gene into inbred lines than those evaluated in a single population.

Survey. We investigated the presence or absence of the markers OAM08.650 and SOAM08.644 linked to the recessive *ms-3* gene in 39 muskmelon, honeydew, casaba or ananas melon cultivars, breeding lines, and F_1 plants (Table 2). These melon germplasm with or without the sterility allele were observed to be male-fertile, except the *ms-3/ms-3* genotype. Of the fertile melon germplasm, 16 cultivars and lines lacking the sterile gene were homozygous fertile, whereas all F_1 plants having the recessive gene, derived from 22 different crosses of ms-3 with homozygous fertile cultivars and lines mainly

muskmelon types, were heterozygous fertile. The presence of the RAPD and SCAR markers was consistently associated with all heterozygous fertile F_1 plants carrying the *ms-3* gene. Furthermore, all homozygous fertile cultivars and lines without the gene lacked the RAPD and SCAR marker fragments linked to the *ms-3* gene. These results would be expected due to the tight linkage of the RAPD and SCAR markers with the *ms-3* gene. Therefore, the RAPD and SCAR markers could be used to expedite the transfer of this sterility gene into muskmelon, honeydew, casaba or ananas cultivars and lines using marker-assisted backcrossing.

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