Developing a Sequence Characterized Amplified Region (SCAR) Marker Linked to the Single Recessive Male-Sterile *ms-3* Gene in Melon

Soon O. Park and Kevin M. Crosby

Department of Horticultural Sciences, Texas Agricultural Research and Extension Center, Texas A&M University, Weslaco

ABSTRACT

Male sterility is an important trait of melon (*Cucumis melo* L.) for F_1 hybrid seed production. Sequence characterized amplified region (SCAR) markers have unique advantages over randomly amplified polymorphic DNA (RAPD) markers for use as a selection tool. The RAPD marker OAM08.650 previously was reported to be closely linked to the male sterile *ms-3* gene at 2.1 cM. However, a SCAR marker linked to the *ms-3* gene was not developed. The purpose was to convert the tightly linked RAPD marker OAM08.650 into a SCAR marker based on a specific forward and reverse primer pair. The exact length of the RAPD fragment OAM08.650 was 644 bp. The specific forward and reverse 20-mer primer pair was designed from the sequence of the RAPD marker OAM08.650. The sequence of the forward primer was 5'-ACCACGAGTGTCGAGAAGAA-3', while that of the reverse primer was 5'-ACCACGAGTGAGGGATCTTC-3'. The SCAR marker SOAM08.644 was developed on the basis of the specific primer pair. The SCAR marker SOAM08.644 was tightly linked to the *ms-3* gene at 2.1 cM on the linkage group. The SCAR marker linked to the *ms-3* gene at 2.1 cM on the linkage group. The SCAR marker linked to the *ms-3* gene at 2.1 cM on the linkage group. The SCAR marker linked to the *ms-3* gene at 2.1 cM on the linkage group.

RESUMEN

La esterilidad masculina es un carácter importante en el melón (*Cucumis melo* L.) en lo concerniente a la producción de semilla híbrida en la F₁. Los marcadores SCAR (Sequence characterized amplified región) tienen ventajas únicas sobre los marcadores RAPD (random amplified polymorphic DNA) cuando se usan como herramientas para selección. Anteriormente, se reportó que el marcador RAPD OAM08.650 estaba ligado estrechamente con el gen *ms-3* de esterilidad masculina a 2.1 cM. Sin embargo, no se desarrolló un marcador SCAR ligado al gen *ms-3*. El propósito de este estudio fue convertir al estrechamente ligado marcador RAPD OAM08.650 en un marcador SCAR en base a un par de iniciadores delantero y opuesto específicos. La longitud exacta del fragmento RAPD OAM08.650 fue de 644 bp. Los pares de iniciadores delantero y opuesto 20-mer específicos fueron diseñados a partir de la secuencia del marcador RAPD OAM08.650. La secuencia del iniciador delantero fue 5'-ACCACGAGTGTCGAGAAGAA-3', mientras que la del iniciador opuesto fue 5'ACCACGAGTGAGGGATCTTC-3'. El marcador SCAR SOAM08.644 fue diseñado en base al par específico de iniciadores. El marcador SCAR SOAM08.644 no mostró recombinación con el marcador RAPD OAM08.650 en una población F2 derivada de la cruza de ms-3 x 'TAM Dulce'. El marcador SCAR SOAM08.644 estuvo estrechamente ligado al gen *ms-3* a 2.1 cM en el grupo de ligamiento. El marcador SCAR ligado al gen *ms-3* desarrollado durante este trabajo podría ser de utilidad en la introducción de esterilidad masculina a cultivares de melón y lineas de mejoramiento fértiles para su uso como padres para la producción de semillas híbridas en la F1.

Additional Index Words: male sterility, randomly amplified polymorphic DNA (RAPD), marker-assisted backcrossing.

Five single recessive genes for male sterility including *ms*-1 to *ms*-5 have been identified in melon (Pitrat, 1991, 2002). Each of these possesses a unique phenotype (McCreight and Elmstrom, 1984; Pitrat, 1991, 2002). The *ms*-1 and *ms*-2 genes are difficult to detect in the greenhouse and field, whereas the *ms*-3 gene is easily detectable with the naked eye (McCreight

and Elmstrom, 1984). The *ms-4* and *ms-5* genes are easily observed due to male flower abortions at the bud stage (Lecouviour et al., 1990; Pitrat, 1991). No allelism was found between different male-sterile genes (Bohn and Principe, 1964; Lecouviour et al., 1990; McCreight and Elmstrom, 1984). These results were confirmed by Pitrat (1991), who reported

| Molecular marker | | | Number of F ₂ plants | | | | |
|------------------|------------|--------|---------------------------------|---------|-------|----------------|------|
| Туре | Name | Source | Presence | Absence | Ratio | \mathbf{X}^2 | Р |
| RAPD | OAM08.650 | ms-3 | 74 | 16 | 3:1 | 2.12 | 0.14 |
| SCAR | SOAM08.644 | ms-3 | 74 | 16 | 3:1 | 2.12 | 0.14 |
| RAPD | OAN05 800 | ms-3 | 72 | 18 | 3.1 | 0.95 | 0.33 |

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

that these sterility genes were located on five different linkage groups of the classical melon linkage map.

Introducing a male-sterile gene into fertile melon cultivars and inbred lines is a strategy recommended for facilitating F_1 hybrid seed production. Molecular markers linked to a malesterile gene would be useful in transferring the recessive allele into elite melon cultivars and inbred lines using backcrossing. Park and Crosby (2004) reported two randomly amplified polymorphic DNA (RAPD) markers linked to the *ms-3* gene for male sterility using bulked segregant analysis in an F_2 population from the melon cross of ms-3 (male-sterile) x 'TAM Dulce' (male-fertile). These markers displayed an amplified DNA fragment in the male-sterile bulk that was absent in the fertile bulk. The RAPD marker OAM08.650 was closely linked to the *ms-3* gene at a distance of 2.1 cM.

Merits of sequence characterized amplified region (SCAR) markers over RAPD have been reported and well discussed (Melotto et al., 1996; Paran and Michelmore, 1993). RAPD markers linked to genes for resistance to several diseases were converted into SCAR markers for use as a selection tool in horticultural crops (Haymes et al., 2000; Paran and Michelmore, 1993; Wechter et al., 1998). However, a SCAR marker linked to the *ms-3* gene for male sterility present in melon has not been reported. Thus, the goal was to convert the tightly linked RAPD marker OAM08.650 into a SCAR marker on the basis of a specific forward and reverse 20-mer primer pair.

MATERIALS AND METHODS

Plant Material. Ninety F₂ plants from the melon cross of ms-3 x 'TAM Dulce' were planted in a greenhouse at the Texas Agricultural Research and Extension Center (TAREC)-Weslaco, Texas A&M University on 20 October 2000. 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 *ms-3* gene was chosen over other male-sterile genes because in our preliminary study it could be easily phenotyped visually. The male-fertile 'TAM Dulce' parent, a Western Shipper muskmelon type, was developed at the TAREC-Weslaco (Kunkel, 1968). 'TAM Dulce' is resistant to powdery mildew (races 1 and 2), downy mildew, and Fusarium wilt (race 2). Male sterility and fertility were checked on all F₂ plants during flowering.

RAPD. Fully expanded leaves of 90 F_2 plants along 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 RAPD markers. The name of each RAPD marker is derived from an 'O' prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length (bp) of the marker (Park et al., 2004). Two 10-mer primers (Operon Technologies, Alameda, California) were tested in the F_2 population derived from the cross between ms-3 and 'TAM Dulce'.

SCAR. To develop a SCAR marker for the RAPD marker OAM08.650, the DNA fragment of the RAPD marker was excised and purified using the GENECLEAN II Kit (Q-BIO gene, Carlsbad, California). Insertion of the purified RAPD fragment into the pCR 2.1-TOPO and cloning of the transformed plasmid were conducted using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, California). The cloned plasmid was harvested using the GenElute Plasmid Miniprep Kit (Sigma, St. Louis, Missouri). The RAPD fragment was sequenced using the M13 reverse and forward primers at the DNA sequencing & synthesis facility of the Iowa State University Office of Biotechnology (Ames, Iowa). A specific forward and reverse 20-mer primer pair was designed on the basis of the forward and reverse sequences of the RAPD fragment. The forward and reverse primer pair was synthesized by Operon Technologies (Alameda, California). Polymerase chain reactions were performed on 96-well plates in the MJ Research thermalcycler. Protocols for PCR and the composition of the final volume of reactants were the same as those described by Rubio et al. (2001). The name of the SCAR marker is derived from a 'S' prefix for SCAR, the original RAPD marker name except the approximate length, and the exact marker length (Park et al., 2004). The specific forward and reverse 20-mer primer pair was tested in the F₂ population.

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 'TAM Dulce' 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

The RAPD marker OAM08.650 tightly linked to the *ms-3* gene for male sterility at a distance of 2.1 cM identified in the



Fig. 1. (a) RAPD marker OAM08.650 and (b) SCAR marker SOAM08.644 amplified with the specific primer pair designed from the sequence of the RAPD marker OAM08.650 expressing polymorphism between two DNA bulks from male-sterile and fertile F_2 plants, and between the male-sterile parent ms-3 and the male-fertile parent 'TAM Dulce'. 1=ms-3, 2='TAM Dulce', 3=DNA bulk from eight male-sterile F_2 plants, 4=DNA bulk from eight fertile F_2 plants, and 5=a 100-bp DNA marker ladder.

5'ACCACGAGTGTCGAGAAGAA GAGAGCCTAATTCCAGGATAAGAACATGTAAGCTTTCTCCACAAGGAATCCATGA GAGAGCCTAATTCCAGGATAAGAACATGTAAGCTTTCTTCCACAAGGAATCCATGA GATCGTTGATCCTTCATCAACAGGTAAACTGGATTGAAATTCCTCTTTTC CATTATCAATCTAACTCTAAGAATTTTGCCTTCATTGTGGATCTAAATCCAAATC AAAACAATCATCGCATTCAAGACATCCAGCGTATTGCTTTTGTGGATCTAAATCCAAATC AAAACAATCATCGCATTCAAGACATCAAGTAATGTTTTTGCCGATATAGCGTCGAAATCT GGTTTTCGGGCTTCGATGCTTTGAGTCTTAATTTTGCCGATATGCGCTCTCTGC CATGCCGGGTTTTGGATGCTTGGTTTGATCTTCAAGTCATGCCATCGCGCAATCG GGTTTTCGTGCAATGGTTTGATTTCGATACTCTCAAGGCATCTGGGCACTCTCGCC CATGTCTCTGCAATTGTTTGATTTCCAATTCTAAATTCGAAATTTAGAGAAAGCA GAACATTAATCTATCCTAATTTTGACTTCTAATTTCGATATTTGATTAATGTAACCT GAATCTTCCAAGCAAATTTGACTTCAATCGAATCTAAGTAATGCTGAAGCCA GAACATTCAACCTAACTTCGACTCCACGCTGATATCTAAGTAAATGCTGAAGTCTA GAAGATCCCTCACTCGGTGGT3'

Fig. 2. The full DNA sequence (644bp) of the tightly linked RAPD marker OAM08.650 to the *ms-3* gene controlling male sterility. The two underlined sequences above were the 20-mer sequences of the specific forward and reverse primers, respectively, used to develop the SCAR marker.

 F_2 population from the cross ms-3 x 'TAM Dulce' (Fig. 1a) (Park and Crosby, 2004) was converted into a SCAR marker on the basis of the specific forward and reverse 20-mer primer pair. The exact length of the RAPD fragment OAM08.650 was 644 bp based on the sequence data (Fig. 2). The sequence of the forward 20-mer primer was 5'-ACCACGAGTGTCGAGAAGAA-3', while that of the reverse 20-mer primer was 5'-ACCACGAGTGAGGGATCTTC-3'. The underlined sequences were the original 10-mer sequence of the OAM08 primer. Melting temperatures of the forward and reverse primers were 61°C and 63°C, respectively. We used 65°C as a consensus annealing temperature.

The marker SOAM08.644, the name of the SCAR marker amplified with the specific forward and reverse primer pair, is shown in Fig. 1b. The SCAR marker SOAM08.644 was present in the male-sterile parent ms-3 and the DNA bulk from male-sterile F_2 plants, whereas it was absent in the male-fertile

parent 'TAM Dulce' and the DNA bulk from male-fertile F2 plants. The SCAR marker fragment segregated in the F2 population of the cross ms-3 x 'TAM Dulce'. A goodness-of-fit to a 3:1 ratio for band presence to band absence for the SCAR marker SOAM08.644 was observed in the F₂ plants (Table 1). The integrated location of the male-sterile locus and the loci of markers including the SCAR marker is shown in Fig. 3. The SCAR marker SOAM08.644 showed no recombination with the RAPD marker OAM08.650 in the F₂ population, and thus, the SCAR and RAPD markers were observed at the same locus on the linkage group. The SCAR marker SOAM08.644 was also closely linked to the ms-3 gene at a distance of 2.1 Cm. These results confirm that the SCAR marker SOAM08.644 was derived from the RAPD marker OAM08.650. This is the first report on development of a SCAR marker linked to the ms-3 gene in melon.

No recombination between the RAPD and SCAR markers found here was the same as that reported previously in common bean by Park et al. (2004). However, Melotto et al. (1996) observed a few recombinations between the RAPD marker OW13.690 and the SCAR marker SW13. We observed the SCAR marker SOAM08.644 linked to the *ms-3* gene controlling male sterility to be dominant, whereas Adam-Blondon et al. (1994) noted a codominant SCAR marker linked to the *Are* gene for anthracnose resistance in bean.

Cultivars and inbred lines having the male-sterile gene can be utilized as parents to produce F_1 hybrid seeds. However, there is a major constraint in the use of male sterility for this purpose. Due to the single recessive nature of the *ms-3* gene, homozygous sterile plants can only be found in segregating populations. Twenty-five percent of an F_2 population are homozygous sterile



Fig. 3. 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). The gene and marker names are given on the right and the length in cM is indicated on the left of the linkage group.

plants, while 50% are sterile in a heterozygote backcross to a homozygous sterile plant. Homozygous and heterozygous fertile plants are indistinguishable, especially in populations developed by the backcross method for the transfer of the sterility gene into cultivars and lines. Progeny testing via self-pollination is a desirable way to identify heterozygous fertile plants. The use of markers tightly linked to the ms-3 gene, displaying an amplified DNA fragment in the male-sterile parent, is of special interest to breeders because it can be employed to easily distinguish between homozygous and heterozygous fertile plants in backcross populations without progeny testing. In a heterozygote backcross to a homozygous fertile plant the presence of the markers is associated with heterozygous fertile plants, whereas the absence of the markers is associated with homozygous fertile plants. Also, 50% of the plants (homozygous fertile) in the backcross and recombinant inbred populations, and 25% of the plants in an F₂ population can be discarded at early growth stages. This reduction of population size is crucial to breeders who are faced with rental costs or limitation of greenhouse space for screening plants. These RAPD and SCAR markers tightly linked to the ms-3 gene, identified and developed here, will allow the rapid transfer of the male-sterile gene into elite melon cultivars and breeding lines using marker-assisted backcrossing without progeny testing.

ACKNOWLEDGMENTS

We appreciate financial support from the South Texas Melon Committee. We also thank two research technicians Joe Molina and Hyun J. Kang, Texas Agricultural Research and Extension Center, for their assistance.

LITERATURE CITED

- Adam-Blondon, A.F., M. Sevignac, H. Bannerot, and M. Dron. 1994. SACR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor. Appl. Genet. 88:865-870.
- Bohn, G.W. and J.A. Principe. 1964. A second male-sterility gene in the muskmelon. J. Heredity 55:211-215.
- Haymes, K.M., W.E. Van de Weg, P. Arens. J.L. Maas, B. Vosman, and A.P.M. Den Nijs. 2000. Development of SCAR markers linked to a *Phytophthora fragariae* resistance gene and their assessment in European and North American strawberry genotypes. J. Amer. Soc. Hort. Sci. 125:330-339.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugenics 12:172-175.
- Kunkel, H.O. 1968. Dulce cantaloupe. Texas Agricultural Experiment Station bulletin L-760, Texas A&M Univ., College Station.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps with experimental and natural populations. Genomics 1:174-181.
- Lecouviour, M., M. Pitrat, and G. Risser. 1990. A fifth gene for male sterility in *Cucumis melo*. Cucurbit Genet. Coop. Rpt. 13:34-35.
- McCreight, J.D. and G.W. Elmstrom. 1984. A third muskmelon male-sterile gene. HortScience 19:268-270.
- Melotto, M., L. Afanador, and J.D. Kelly. 1996. Development of a SCAR marker linked to the *I* gene in common bean. Genome 39:1216-1219.
- Paran, I. and R.W. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85:985-993.
- Park, S.O. and K.M. Crosby. 2004. Identification of RAPD markers linked to the male-sterile *ms-3* gene in melon. Acta Hort. 637:243-249.
- Park, S.O., D.P. Coyne, J.R. Steadman, K.M. Crosby, and M.A. Brick. 2004. RAPD and SCAR markers linked to the *Ur-6* Andean gene controlling specific rust resistance in common bean. Crop Sci. 44:1799-1807.
- Pitrat, M. 1991. Linkage groups in *Cucumis melo* L. J. Heredity 82:406-411.
- Pitrat, M. 2002. 2002 gene list for melon. Cucurbit Genet. Coop. Rpt. 25:76-93.
- Rubio, L., Y. Abou-Jawdah, H.X. Lin, and B.W. Falk. 2001. Geographically distant isolates of the crinivirus *Cucurbit yellow stunting disorder virus* show very low genetic diversity in the coat protein gene. J. General Virology 82:929-933.
- Skroch, P.W. and J. Nienhuis. 1995. Qualitative and quantitative characterization of RAPD variation among snap bean genotypes. Theor. Appl. Genet. 91:1078-1085.
- Wechter, W.P., R.A. Dean, and C.E. Thomas. 1998. Development of sequence-specific primers that amplify a 1.5-kb DNA marker for race 1 Fusarium wilt resistance in *Cucumis melo* L. HortScience 33:291-292.