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Development of Sequence-specific Primers that Amplify a 1.5-kb DNA Marker for Race 1 Fusarium Wilt Resistance in *Cucumis melo* L.

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Abstract. **Two 24-mer primers, MUSKFOM I and MUSKFOM II, were developed that amplify a 1.5-kb DNA fragment in race 1 Fusarium wilt resistant muskmelon (*Cucumis melo* L.), but not in race 1 susceptible germplasm tested. Three race 1 resistant cultivars and two race 1 resistant breeding lines as well as eight race 1 susceptible lines were analyzed using the two sequence-specific primers in the polymerase chain reaction. These primers should prove valuable for nondestructive determination of *Fom 2* gene introgression in breeding programs.**

Cucumis melo L. groups Cantalupensis and Inodorus include cantaloupe, crenshaw, cassaba, and honeydew melons and are collectively referred to as muskmelon (Whitaker and Davis, 1962). Nearly 1.5 million t of muskmelon accounted for nearly $500 million in agricultural sales in the United States in 1995 [U.S. Dept. of Agriculture (USDA), 1996]. Worldwide, >15 million t of these melons were produced in the same year (Food and Agriculture Organization of the United Nations, 1996). Fusarium wilt, caused by *Fusarium oxysporum f. sp. melonis*, is a devastating disease of muskmelon occurring throughout North America, Europe, and Asia (Benoit, 1974; Mas et al., 1981).

We previously reported (Wechter et al., 1995) a random amplified polymorphic DNA (RAPD) marker in the multidisease-resistant breeding line MR-1 that is tightly linked to the *Fom 2* gene, and controls resistance to *F. oxysporum f. sp. melonis* races 0 and 1 (Zink and Thomas, 1990). The 10-mer primer 596 ($5'$CCCCTCGAAT$3'$) from the Univ. of British Columbia was shown to amplify a 1.6-kb fragment using DNA from resistant plant material derived from MR-1, but not using DNA from other race 1 resistant melon germplasm tested. Herein we show that sequence-specific primers amplify a specific fragment in MR-1 and other race 1 resistant melon germplasms.

Since first reported (Wechter et al., 1995), the 1.6-kb RAPD fragment has been further characterized in order to develop specific primers to simplify fragment determination in a breeding program utilizing MR-1. The 1.6-kb amplification product was isolated from a 1% agarose gel using Geneclean II (Bio 101, LaJolla, Calif.), ligated into the pGEM-T PCR vector (Promega, Madison, Wis.), and then transformed into JM109 *E. coli* cells. The entire nucleotide sequence of the 1.6-kb fragment was determined using the PRIZM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer, Norwalk, Conn.) and has been deposited into Genbank accession number AF005262. Utilizing these data, two 24-mer primers, MUSKFOM I ($5'$TCGACCAGACGAAGTTCTTCGAGC$3'$) and MUSKFOM II ($5'$GAACTAAGGTCACGTTTATCGATC$3'$), internal to the original 10-mer primer sites in the 1.6-kb fragment were designed. Each primer has between 40% to 60% G-C content and was determined to be free of fold-back and self-annealing characteristics. The use of a pair of longer primers in conjunction with more stringent (higher) annealing temperatures generates what are referred to as sequence characterized amplification regions (SCARs) (Paran and Michelmore, 1993). Such SCARs eliminate or greatly reduce the amplification of nonassociated bands common with a 10-mer RAPD primer, thus making gel scoring a less ambiguous operation.

The primer pair and the following PCR protocol were used to amplify a predicted 1.5-kb fragment in DNA from race 1 resistant germplasm. To thoroughly examine this pair of primers, bulked samples of both heterozygous and homozygous resistant, bulked susceptible muskmelon, F_2 individuals derived from MR-1, as well as other melon lines unrelated to MR-1 were evaluated. Amplification was carried out in 25-μL reaction mixtures containing 10 ng target DNA, 8.375 μL dH_2O, 2.5 μL Perkin-Elmer 10X buffer II, 4 μL $MgCl_2$ (25 mM stock), 0.5 μL each Perkin-Elmer dATP, dCTP, dGTP, and dTTP (10 mM stock), 0.125 μL Perkin-Elmer AmpliTaq Polymerase, 2 μL nonacetylated BSA (New England Biolabs, Beverly, Maine), and 2.5 μL each of the 24-mer primers (10 mM stock). Thermal cycling conditions were 96 °C for 5 min followed by 37 cycles of 94 °C for 1 min, 68 °C for 1 min, and 72 °C for 2 min, with a final 5 min 72 °C extension. Melon growth conditions, *Fusarium* inoculation protocol, DNA isolation from plants, and DNA bulking procedures were performed as described in a previous paper (Wechter et al., 1995).

As expected, the 1.5-kb SCAR fragment was visible in MR-1 resistant material and absent from all susceptible lines (Fig. 1; Panel A). However, to our surprise, a product of the same size was amplified using DNA from the other resistant lines (Fig. 1; Panel A; lanes 3, 10, and 11). To determine if these were homologous products, Southern hybridization was performed (Sambrook et al., 1989) using ^{32}P αdCTP labeled 1.5-kb PCR fragment from MR-1 as a molecular probe (Multiprime DNA Labelling Kit; Amersham International, Amersham, U.K.). After overnight hybridization at 65 °C, the membrane was washed under high stringency conditions (0.1× SSC/0.1% SDS at 65 °C), and then exposed to autoradiograph film for 30 min. All 1.5-kb amplification products hybridized to the MR-1 fragment, strongly indicating they were homologous products (Fig. 1; Panel B).

For comparison purposes, amplification products were produced by the 10-mer primer 596 using the same DNA templates as in Fig. 1, Panel A, which failed to produce the 1.6-kb product in resistant lines other than MR-1 (Fig. 1; Panel C). A base difference in one of the 10-mer RAPD primer DNA binding sites between MR-1 and the other resistant lines may explain this fortuitous result. The binding site in other resistant lines may be sufficiently different (changed by as little as a single base) such that the 10-mer primer cannot bind and no product can accumulate. The design of the sequence-specific primer pair internal to these regions circumvented this problem. DNA from an advanced resistant breeding line, PI161375, provided by David Wolff of Texas A&M Univ., also produced the 1.5-kb amplification product using the sequence-specific primer pair (Fig. 2).

Few muskmelon lines have been reported to be resistant to race 1 Fusarium wilt and only the above five lines have been obtained and tested by our group as reported here. The origin of the introgressed *Fom 2* gene in the resistant lines cannot be verified with com-

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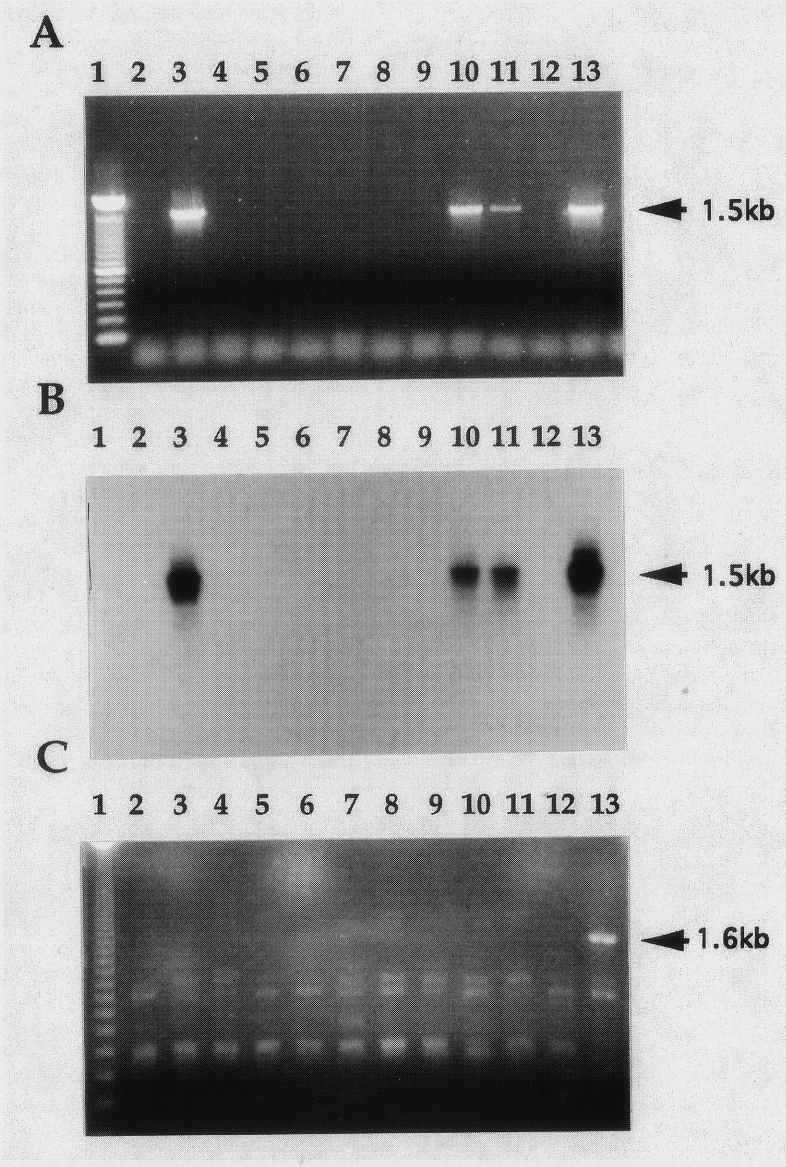

Fig. 1. (**Panel A**) Amplification products from both race 1 Fusarium wilt resistant and susceptible germplasms using sequence-specific primers, MUSKFOM I and MUSKFOM II; (**Panel B**) autoradiograph of the above gel using a [α-^{32}P] dCTP labeled 1.5-kb amplification product from MR-1 as the probe; (**Panel C**) amplification products of the above germplasms using the decamer primer, 596. The lanes are labeled as follows: 1 = 123-bp marker ladder, 2 = 'Sweet Supreme' (S), 3 = 'Opera' (R), 4 = 'Hy-mark' (S), 5 = 'Charentais T' (S), 6 = 'Supermarket' (S), 7 = 'Dorado' (S), 8 = 'Topmark' (S), 9 = PMR-45 (S), 10 = CM17-187 (R), 11 = 'Sierra' (R), 12 = 'Ananas Yoknum' (S), and 13 = MR-1(R). The letters S and R indicate susceptible or resistant germplasm.

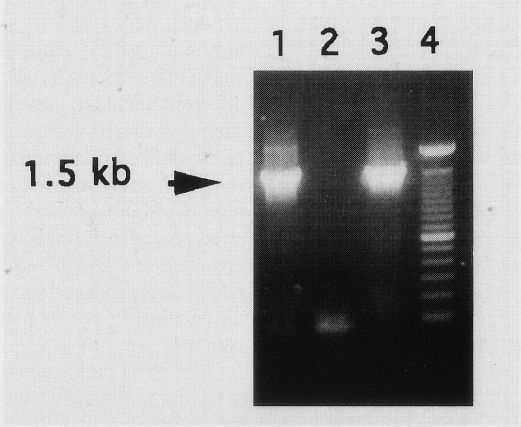

Fig. 2. Amplification products of a race 1 Fusarium wilt resistant advanced breeding line PI161375 (lane 1), the susceptible line 'Ananas Yoknum' (lane 2), and MR-1 (lane 3) using the MUSKFOM I and MUSKFOM II primers. Lane 4 is a 100-bp marker ladder.

plete certainty because the information is proprietary. However, suppliers of this germplasm, USDA and Peto Seed Co., do not think this resistance was derived from MR-1. The ability of these specific primers to produce an amplification product linked with resistance in lines other than those derived from MR-1 indicates their potential in a marker-assisted selection program for race 1 Fusarium wilt resistance in *C. melo*. These primers allow for an accurate, easily discernible, nondestructive assay that can greatly reduce screening time for race 1 resistant introgression while saving valuable germplasm lost to current screening techniques.

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