Development of a SCAR marker associated with Fusarium wilt resistance and the evidence of its segregation with the *Fom-2* gene in melon (*Cucumis melo* L.)

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ABSTRACT

Resistance to races 0 and 1 of Fusarium wilt, a worldwide soilborne disease of melon (*Cucumis melo* L.) caused by *Fusarium oxysporum* Schlecht f. sp. *melonis* Snyder & Hans, is conditioned by the dominant gene *Fom-2*. A RAPD fragment of 1.55 kb was amplified in resistant melon cultigens using a previously reported RAPD primer 596 and *Taq* polymerase that differs from that used by original investigators. Due to the inconsistent nature of RAPD markers, the SCAR (sequence characterized amplified region) primers (PI596B1 and PI596B2) that amplified a 1.4 kb DNA fragment were developed. The SCAR marker was tested for its segregation with the *Fom 2* gene by using bulked segregant analysis and evaluated for its application in diverse melon cultigens. Results showed that the 1.4 kb SCAR marker was amplified from 23 out of 36 (64 %) resistant genotypes but from none of the 31 susceptible genotypes tested. This suggests that it is more conserved across the diverse melon genotypes and potentially more useful than the previously reported RAPD and SCAR in marker-assisted selection for the introgression of the *Fom 2* gene into melon breeding lines.

RESUMEN

La resistencia a las razas 0 y 1 del hongo del suelo *Fusarium oxysporum* Schlecht f. sp. *melonis* Snyder & Hans agente causal del marchitamiento por Fusarium en melón (*Cucumis melo* L.), esta condicionada por el gen dominante *Fom*-2. Un fragmento de 1.55 kb obtenido mediante la técnica RAPD fue amplificado en cultivares de melón resistente usando el primer 596 de un RAPD previamente reportado y una Taq polimerasa diferente a la usada por los investigadores originales. Debido a la naturaleza inconsistente de los marcadores en RAPD, se desarrollaron los primers PI596B1 y PI596B2 para la técnica SCAR (regiones amplificadas de secuencias caracterizadas) que amplificaron un fragmento de DNA de 1.4 kb. Se evaluó la segregación de este marcador obtenido por SCAR con el gen *Fom 2* usando análisis de segregación en grupo y se evaluó su aplicación en diferentes cultivares de melones. Los resultados mostraron que el marcador de 1.4 kb se amplificó en 23 de 36 (64%) genotipos resistentes pero no se amplificó en ninguno de los 31 genotipos susceptibles evaluados. Lo anterior indica que el marcador esta más conservado a través de diversos genotipos de melones y que es potencialmente más útil que los marcadores obtenidos por RAPD y SCAR reportados previamente para la selección de marcadores asistidos para la introgresión del gene *Fom 2* en cultivares de melón.

Key Words Cucumis melo, molecular markers, RAPD, SCAR, Fusarium oxysporum, Fusarium resistance, marker-assisted selection (MAS)

Fusarium wilt of melon (*Cucumis melo* L.), caused by *Fusarium oxysporum* Schlecht f. sp. *melonis* (Leach & Currence,1938) W. C. Snyder & H. N. Hans, was first reported in New York in 1930 (Chupp, 1930a, Chupp, 1930b). Since then it has been found in many melon growing areas worldwide, including North America (Leach, 1933; Leach and Currence; 1938; Leary and Wibur, 1976; Martyn et al., 1987), Europe and Asia (Quiot et al., 1979; Sherf and Macnab, 1986), with reports as severe as 100 % of yield losses (Benoit, 1974; Sherf and Macnab, 1986). Four races have been identified as 0, 1, 2, and 1-2. In North America race 2 was essentially the only race known until 1985 when race 1 was isolated in Maryland, and then in California and Ontario, Canada in 1996 (Zitter, 1997). Race specific resistance genes *Fom-2* and *Fom-1* confer resistance to races 0 and 1, and races 0 and 2, respectively (Risser and Mas, 1965; Risser, 1973; Risser et al., 1976; Robinson et al., 1976). In some cases, resistance to races 0 and 2 is controlled by gene Fom-3 (Zink, 1991; Zink and Gubler, 1985). Use of resistant cultivars has been a major feasible strategy in the control of this disease.

An immediate, direct, efficient, and practical use of linked markers is marker-assisted-selection (MAS) in a plant breeding program. In order to develop a molecular marker for using in MAS, randomly amplified polymorphic DNAs (RAPD) have been used most widely and are one of the most powerful and fastest ways for tagging resistance genes (Baudracco-Arnas and Pitrat, 1996; Haley et al., 1993; Mayer et al., 1997; Michelmore et al., 1991; Miklas et al., 1996; Paran et al., 1991; Paran and Michelmore, 1993; Wechter et al., 1995). However, because of the inconsistent nature of the RAPD markers (Staub et al., 1996a; Weeden et al., 1992), they are often characterized and converted into more reliable and easier to score markers (Staub et al., 1996b). The converted markers could be allele-specific associated primers (ASAPs) (Gu et al., 1995; Mayer et al., 1997; Yu, et al., 1995), sequence characterized amplified regions (SCARs) (Paran and Michelmore, 1993), cleaved amplified polymorphic sequences (CAPSs) (Jarvis et al., 1994; Konieczyn and Ausubel, 1993; Zheng et al., 1999), or RFLP markers (Zheng et al., 1999).

Recently, several DNA markers linked to the resistance gene *Fom-2* have been reported which included RAPD (Baudracco-Arnas and Pitrat, 1996; Wechter et al., 1995), SCAR (Wechter et al., 1998), RFLP and CAPS (Zheng et al., 1999). However, these RAPD markers have the common feature of inconsistency (Baudracco-Arnas and Pitrat, 1996; Zheng and Wolff, 2000) as mentioned earlier. The application of the SCAR marker (Wechter et al., 1998) is limited because it was derived from a RAPD that was linked only with one breeding line 'MR-1' (Wechter, et al., 1995). The RFLP and CAPS (Zheng et al., 1999) are very conserved and consistent co-dominant markers, however, they require more difficult procedures. Therefore, a consistent SCAR marker with a high degree of conservation across the diverse melon genotypes could be useful in an MAS program to expedite the introgression of Fusarium wilt resistance genes through rapid and efficient screening of large germplasm collections and reducing the need for extensive inoculations with the Fusarium wilt pathogen.

Bulked segregant analysis creates distinct genotypic classes from a segregating population by pooling DNA of F_2 individuals with the same phenotype for a specific trait of interest. It has been used successfully in several cases in initial segregation studies that lead to linkage analyses (Michelmore et al., 1991; Wechter, et al., 1995). The bulked DNA pools can be prepared by either extracting DNA separately from each plant and then mixing them with an equal amounts (Michelmore et al., 1991) or pooling the equal weight of plant tissues and then extracting DNA from the pooled tissues (Wechter et al., 1995). In this paper, we report a SCAR marker derived from a newly identified RAPD marker that was revealed by using a previously reported RAPD primer (Wechter et al., 1995, Zheng et al., 1999). The resulting SCAR marker was tested in diverse melon cultigens and evaluated for its segregation with the resistance gene *Fom-2* by using the bulked segregant analysis.

MATERIALS AND METHODS

Germplasm. A total of 67 melon genotypes, including parental lines (Table 2, group A), resistant cultigens (Table 2, group B) and susceptible cultigens or F_1 (Table 2, group C) from diverse locations representing several melon classes were included. A cross between 'Vedrantais' (susceptible) and PI 161375 (resistant) was made to produce the F_1 generation, which was selfed to produce the F_2 population. Individual F_2 plants were selfed and F_3 progenies were inoculated with Fusarium wilt. Homogeneous resistant or susceptible F_3 were chosen, corresponding to homozygous F_2 plants.

Fungal culture maintenance, host inoculation, disease scoring for Fusarium wilt. Unless otherwise mentioned, the disease phenotypes of melon cultigens and F_1 hybrids used in this study were determined according to the following procedure. Disease responses of the parental lines 'Vedrantais' x PI 161375, and their F_2 and F_3 progenies, as well as the resistant cultigens (except MR-1 and 'Vine Peach') was determined by M. Pitrat using a Fusarium isolate FOM 26 (race 1) and root-dipping technique described by Risser and Mas (1965). Roots of 20 plantlets of each F_3 family were dipped in a conidial suspension before transplanting to sand. Two weeks after inoculation, susceptible plants died, whereas resistant ones remained green. For the resistant cultigens 'MR-1' and 'Vine Peach' and all of the susceptible cultigens, the disease phenotypes were cited from published screening experiments (Pitrat et al., 1996; Wechter et al., 1995; Zink, 1992; Zink and Thomas, 1990). The disease phenotypes for the F_1 hybrids were determined by several seed companies (Nunhems Seed Corp., Holland).

Genomic DNA. Healthy leaf tissues were harvested from melon seedlings grown in a greenhouse at the 3 to 5 leaf stage. Unless otherwise mentioned, genomic DNAs were extracted from either freshly harvested leaves frozen in liquid nitrogen or dehydrated leaves, following a modified procedure of Baudracco-Arnas (1995). In addition, 23 DNA samples of F_2 individuals derived from a cross between 'Ananas Yokneam'

Table 1. Sequences of RAPD primer 596 and its derived SCAR primers associated with or linked to the single dominant resistant gene (*Fom-2*) conferring resistance to Fusarium wilt caused by *Fusarium oxysporum* f. sp *melonis* races 0 and 1 in melon (*C.Melo*).

Primer	Sequence (5' (3')	Source (Genotype/Phenotype	Fragment size (kb)
596	CCCCTCGAAT	'MR-1'/Resistant	1.60
MUSKFOM I	TCGACCAGACGAAGTTCTTCGAGC		1.5
MUSKFOM II	GAACTAAGGTCACGTTTATCGATC		
596	CCCCTCGAAT	'PI 161375'/Resistant	1.55
PI596B1	GCAAAGGACCCAATCATC		1.4
PI596B2	GRGATTTTAAGTGGAGGC		

Table 2. Score of SCAR marker in diverse melon (*C. melo*) cultigens with different reactions to *Fusarium oxysporum* f. sp. *melonis* races 0 and 1.

Cultigens/F_1 hybrids		Source	Wilt Reaction[1]	SCAR marker[2]
Group A: Parental lines	PI 161375	Korea	R	+
	Vedrantais	France	S	–
Group B: Resistant cultigens	Aodaisimouri	Japan	R	–
	Charentais Fom-2	France	R	+
	Changgam	Korea	R	+
	CM 17188	Israel	R	–
	Freeman's cucumber	Japan	R	+
	Ginsen Makuwa	Japan	R	+
	Isabelle	France	R	+
	K 2005	China	R	+
	Kanro Makuwa	Japan	R	+
	Kogane 9 Go Makuwa	Japan	R	+
	Kogane Sennari Makuwa	Japan	R	+
	LJ 34340	TW Whitaker	R	+
	LJ 90389	TW Whitaker	R	+
	Meshed	Iran	R	–
	Miel Blanc	China	R	+
	MR1/PI 124111.I	India	R	+
	Nanbukin	China	R	–
	Nyumelon	Japan	R	+
	Ogon 9	Japan	R	+
	Ouzbeque 1	Japan	R	–
	Perlicha 1.5	Gualeloupe	R	+
	Persia 202	Iran	R	–
	PI 125915	Afghanistan	R	–
	PI 157083	China	R	+
	PI 157084	China	R	–
	PI 164723	India	R	+
	PI 223637	Iran	R	–
	PI 446928	Israel	R	+
	Samarcande	USSR	R	–
	Semosouri Varamin	Iran	R	–
	Shirouri Okyama	Japan	R	+
	Showa Kogane Nashi Makuwa	Japan	R	+
	Sisi	Iran	R	–
	Tokio Mammuth	Japan	R	–
	Vine Peach	Hollar	R	+
Group B: Susceptible cultigens	Ananas Yokueam	Hollar/Wilhite	S	–
	Casaba Golden Beauty	Hollar	S	–
	Charentais T	F. Zink	S	–
	Crenshaw	Hollar	S	–
	D21 1005	E. Cox	S	–
	D21 1014	E. Cox	S	–
	Delicious 51	Hollar	S	–
	Doublon	F. Zink	S	–
	Dulce	R.T. Correa	S	–
	Honey Dew Green Flesh	Hollar	S	–
	Honey Dew Orange Flesh	Hollar	S	–
	Iroquois	Hollar	S	–
	Israel Ogen	Wilhite	S	–
	Mondo	Nunhems	S	–
	Marygold	Hollar	S	–
	Perlita	R.T. Correa	S	–
	Perlita 45/21	R.T. Correa	S	–
	Persian	Hollar	S	–
	Santa Clause	Hollar	S	–
	SUNEX 7050	Sunseeds	S	–
	TAM Dew Improved	R.T. Correa	S	–
	TAM Mayan Sweet	R.T. Correa	S	–
	TAM Perlita 45	R.T. Correa	S	–
	TAM Sun	B. Scully	S	–
	TAM Yellow Canary	R.T. Correa	S	–
	TAM Uvalde	R.T. Correa	S	–
	Topmark	Hollar	S	–
	UC Topmark	U.C. Davis	S	–
	Mission (F_1)	Asgrow	S	–
	Morning Ice (F_1)	Harris Moran	S	–

[1]R = Resistant, S = Susceptible.

[2]+ or – = presence or absence of the SCAR markers.

received from R.A. Dean (Wechter et al., 1995) were also included in this study. Quantitative and qualitative analyses of DNAs were determined by a UV-VIS scanning spectrophotometer (UV-2101PC, Shimadzu). All DNA samples measured a ratio of A_{260}/A_{280} above 1.8.

Bulk segregant analysis. The bulk DNAs from both crosses 'Ananas Yokneam' x MR-1 and 'Vedrantais' x 'PI 161375' were prepared similar to the method of Michelmore et al. (1991). For the bulk DNAs from 'Ananas Yokneam' x MR-1, homozygous resistant bulk DNAs (referred to as resistant bulks) were prepared by mixing equal amounts of 4 individual F_2 homozygous resistant DNA samples. Likewise, the mixed resistant bulk DNAs contained equal amounts of 4 F_2 homozygous resistant individual DNA samples and 11 F_2 heterozygous resistant individual DNA samples. The susceptible bulk DNAs contained equal amounts of 8 F_2 susceptible individual DNA samples. For the bulk DNAs from 'Vedrantais' x PI 161375, the resistant bulk contained equal amounts of 46 F_2 homozygous resistant DNA samples, and the susceptible bulk DNAs contained equal amount of 47 F_2 susceptible DNA samples.

Polymerase Chain Reaction (PCR). The PCR conditions used for RAPD analyses in this study were modified from protocols used by Baudracco-Arnas and Pitrat (1996) and Wechter, et al. (1995) by decreasing the cycle number to 30 cycles because the PCR products were used as inserts in cloning. Derived SCAR primers were synthesized by Genosys Biotechologies, Inc, 1442 Lake Front Cir. Ste 185, The Woodlands, Texas. PCRs were run on a DNA Thermal Cycler 480 (Perkin-Elmer). Cycle parameters were 5 min at 95ºC, followed by 36 - 40 cycles of 1min at 95ºC, 1 min at 40ºC for RAPD primer or 64ºC for SCAR primers, 2 min at 72ºC, with a 10 min at 72ºC extension at the end. PCR products were electrophoresed at 3 - 5 V/cm in a 1.2 % agarose (Sigma) gels in 1 x TAE buffer and stained with 0.5 g/L ethidium bromide.

Cloning and sequencing. First, the RAPD fragments of 1.55 kb amplified from the resistant line 'PI 161375' were cloned and sequenced (see below for details). After electrophoresing PCR products, the target bands were cut out from agarose gel. The DNA fragments were resuspended in dH_2O by using either Geneclean II Kit or Spin Module, (Bio 101 Inc. La Jolla, CA), whereas PCR products (single fragment) amplified from derived primers were used directly for cloning sometimes. Either the Original TA Cloning Kit (Invitrogen Corp. San Diego, CA) or Promega pGEM — T Easy Vector Systems (Promega Corp. Madison, WI) was used and the manufacturer's protocols were followed for ligations and transformations. To identify correct clones, 4 - 6 putative clones were picked up and cultured in LB medium individually and subsequently following plasmid preparation, *Eco*RI restriction endonuclease digestion, and gel electrophoresis to check the inserts. The correct clone(s) showed a fragment that corresponded to their PCR products. The nucleotide sequences of the cloned fragments were determined by using an automated DNA Sequencer Model 377 located at the DNA Sequencing/Synthesis Facility, Iowa State University, Ames, IA. Then the SCAR primers were constructed based on former sequence data and synthesized by Genosys Biotechologies, Inc.

Software. GCG package version 8.0 (Genetics Computer Group, Madison, WI) and BLAST were used for sequence analyses and database comparative searches.

Southern blot and DNA hybridization. The PCR products were electrophoresed in 1.0 % agarose (Sigma) gels at 3 V/cm for 4 h in TAE buffer. DNA blots were prepared as follows. After electrophoresis, gels were treated with 10 volume of 0.25 N HCl for 10 -15 min and then with 0.4 M NaOH for 20 min with gentle shaking. DNAs were blotted onto Hybond-N^+ membranes (Amersham Life Science Inc., Arlington Heights, IL) for 2-3 h in an alkali-downward capillary blotting procedure (Zheng and Wolff, 1999) that was modified from Koetsier et al. (1993).

Clone-derived PCR fragment 1.55 kb which originated from PCR amplification from the resistant parental line 'PI 161375', was used as a hybridization probe. To purify the inserts to be used as probes, the plasmids containing corresponding inserts were digested with *Eco*RI. After electrophoresis of the digestion products, the corresponding bands were cut out from the agarose gel and were purified and resuspended in dH_2O by using either Geneclean II Kit or Spin Module, (Bio 101 Inc. La Jolla, CA). The non-radioactive labeling and detection system (Amersham Life Science Inc., Arlington Heights, IL) was used in probe labelings, hybridizations, stringency washings, blockings and antibody incubations, and signal generations and detection as modified from Zheng and Wolff (1999). The blots were exposed on Hyperfilm-MP for 5-60 min before developing the films.

RESULTS

Amplification of a 1.55 kb RAPD fragment from resistant melon cultigens. Fig. 1 shows the PCR profiles amplified from genomic DNAs of some melon cultigens (Table 2) by using the RAPD primer 596 (Wechter et al., 1995). The 1.55 kb RAPD fragment was found in 23 out of the total of 36 (64 %) resistant cultigens tested, including the parental line 'PI161375' (lane 1) and 'MR-1' (lane 3) in Fig.1, but in none of the 30 susceptible genotypes which included two F_1 varieties 'Mission' and 'Morning Ice' (Table 2).

Detection of the RAPD fragment in bulked DNA pools. To evaluate the segregation of the RAPD fragment with the resistance, the bulked DNAs from two segregating populations were used. Fig. 2 showed the 1.55 kb fragment was amplified from both homozygous and heterozygous resistant bulked DNA pools that derived from either the cross 'Vedrantais' x 'PI 161375' or 'Ananas Yokneam' x 'MR-1', but not from the susceptible bulked DNA pools accordingly.

DNA sequence of the RAPD fragment. Two independent clones that contained the 1.55 kb fragment amplified from the resistant parental line 'PI 161375' were used for sequencing the inserts. The consensus sequences were found after performing the computer analysis using the GAP program of the GCG package. Six hundred and five and 584 nucleotides from each end were sequenced using primers T7-1 and R-1, respectively. Comparative searches of the nonredundant DNA databases accessible through the National Center for Biotechnology Information (Bethesda, MD,

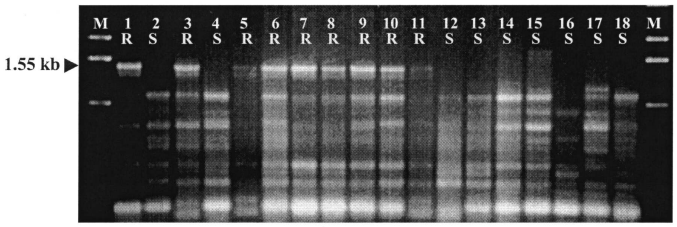

Fig. 1. Ethidium bromide-stained gel of PCR (polymerase chain reaction) products amplified by using RAPD primer 596 and genomic DNAs of melon lines resistant or susceptible to Fusarium wilt caused by *Fusarium oxysporum* f sp. melonis races 0 and 1. Lanes 1, 2, 3, and 4 were two pair of rarental lines 'PI 161375', 'Vedrantais', 'MR-1', and 'Ananas Yokneam', respectively. Lanes 12-18 were 'TAM Mayan Sweet', 'TAM Yellow Canary', 'Doublon', 'Charentais', 'TAM Uvalde', 'TAM Dew Improved', and 'Mission', respectively. The arrow indicated the 1.55 kb RAPD marker. M is 1 kb DNA ladder from Gibco BRL, Life Technologies, Inc.

http://www.ncbi.nlm.nih.gov) were performed using the BLAST algorithm. No significant matches were found.

Test of the SCAR primer in diverse melon cultigens. Using the same genomic DNA as in the RAPD - PCR, the expected 1.4 kb SCAR fragments were amplified not only from the same genotypes that showed the 1.55 kb RAPD fragments, but also from one other resistant genotype 'Ogon 9' (Fig. 3, panel A). None of the susceptible genotypes showed this SCAR fragment. The homology of these SCAR fragments was confirmed by DNA gel blotting analysis using the clone-derived 1.55 kb RAPD fragment from 'PI 161375' as probes (Fig. 3, panel B).

DISCUSSION

From a practical point of view, markers to be used in MAS need to be simple, fast, and cost-effective. RAPD markers fit these criteria best among the many markers described (Staub et al., 1996b). Unfortunately, the inconsistency of reproducibility of RAPD markers is well documented (Weeden et al., 1992), and has been a problem in *Cucumis* (Staub et al., 1996a). In particular, a similar situation occurred in our related work (Zheng and Wolff, 2000) using three currently available RAPD markers linked to resistance/susceptibility to Fusarium wilt (Baudracco-Arnas and Pitrat, 1996; Wechter et al., 1995). Ironically, with the same RAPD primer, the multiple bands of RAPD - PCR rendered a new RAPD marker using a *Taq* polymerase (i.e. Promega Inc., Madison, Wisconsin, USA) that differed from the one used by the original investigators (i.e. Perkin-Elmer Corp. Norwalk, CT, USA) (Wechter et al., 1995). For easy scoring and reliable result purposes, the RAPDs are usually converted to other PCR-based markers (Staub et al., 1996b). Among them, the SCAR marker is the simplest, fastest, and cheapest one. Therefore, we developed SCAR primers (PI596B1/PI596B2) that amplified a 1.4 kb fragment from the resistant cultigens. The newly developed SCAR

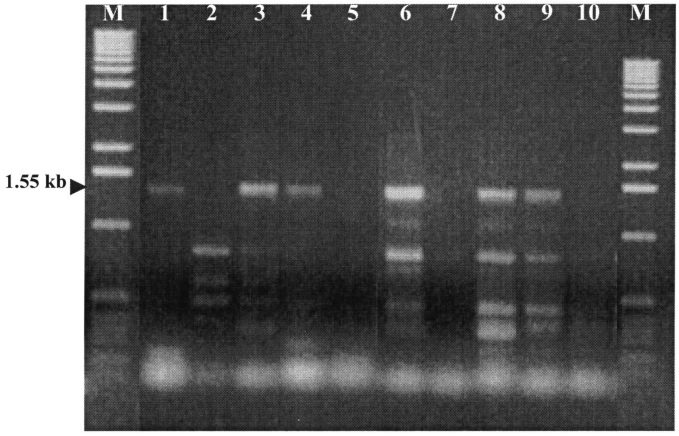

Fig. 2. Ethidium bromide-stained gel of PCR (polymerase chain reaction) products amplified by using RAPD primer 596 and either genomic DNAs fo the parental lines or the bulked DNAs of thier F2 populations resistant or susceptible to Fusarium wilt caused by Fusarium oxysporum f. sp. melonis races 0 and 1. Lanes 1-5 were parental lines PI 161375 and 'Vedrantais', their derived f2 homozygous and heterozygous resistant bulked DNAs and susceptible bulked DNAs, homozygous and heterozygous resistant bulked DNAs and susceptible bulked DNAs, respectively. The arrow indicated the 1.55 kb RAPD marker. M is 1 kb DNA ladder form Gibco BRL, Life Techonologies, Inc.

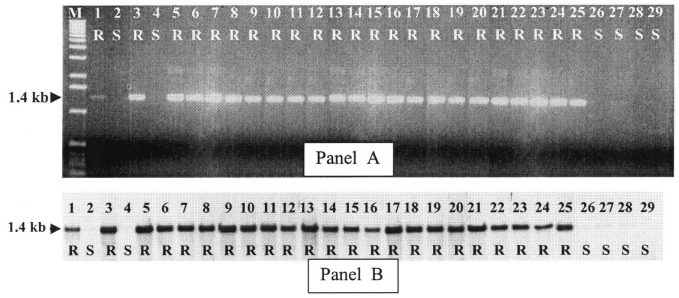

Fig. 3. Panel A: Ethidium bormide-stained gel of PCR (polymerase chain reaction) products amplified by using SCAR primers (PI596B1/pi596b2) derived from the RAPD marker and genomic DANs of the parental lines or other cultigens that were resistant and susceptible to Fusarium wilt caused by Fusarium oxysporum f. sp. melonis races 0 and 1. Lanes 1-4 were parental lines PI 161375, 'Vedrantais', MR-1, and 'Anannas Yokneam', respectively. Lanes 5-25 were resistant cultigens listed in Table 2. Lanes 26-29 were susceptible F1 ('Morning Ice') and cultigens 'Santa Clause', 'TAM Yellow Canary', and 'Doublon', respectively. The arrow indicated the 1.4 kb SCAR Marker. M is 1 kb DNA ladder from Gibco BRL, Life Technologies, Inc. Panel B: DNA gel blotting analysis of the above PCR products using fluorecein labeled clone-derived 1.55 kb RAPD fragment amplified from PI 161375 as probe. The PCR products were separated in 1.0% agarose gel and blotted on Hybond N+ membrane (Amersham Life Science, Inc., Arlington Heights, IL) and hybridized at 60ºC overnight.

marker was different from the previously reported (MUSKFOM I/MUSKFOM II) (Wechter et al., 1998) based on the BLAST sequence analysis and database comparative searches. More importantly, the newly developed SCAR marker (PI596B1/ PI596B2) was more conserved or prevalent across diverse melon cultigens than MUSKFOM I/MUSKFOM II. The former was able to amplify the expected 1.4 kb SCAR fragment from 23 out of 36 (64 %) resistant cultigens tested, whereas the latter amplified the expected 1.5 kb fragment from only a few genotypes (Wechter et al., 1998). The fact that the same size of DNA fragment amplified from different template sources across populations / species does not necessarily mean that they are exactly the same (Thormann et al., 1994). The homology of these SCAR fragments was confirmed by Southern hybridization analysis. For the preliminary screening with SCAR markers, Southern hybridization may not be necessary. Nevertheless, a complete optimized protocol for rapid and sensitive application of Southern hybridization by using fluorescein labeling and detection system was reported (Zheng and Wolff, 1999). Although the SCAR marker, like the RAPD marker, is dominant, making it less useful in scoring segregating populations, it could be very effective for screening large populations, such as germplasm collections, in the early stages of a breeding program because of its simplicity, rapidity, and cost-efficiency.

While bulked segregant analysis is an efficient and reliable way for identifying putative DNA markers segregating with a target trait, mapping the F_2 population is needed to confirm the linkage of the marker to the disease phenotype. Although the SCAR results were scored in and supported by diverse cultigens, mapping the marker in an F_2 population is indispensable for determining the genetic distance between the marker and the resistance gene.

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