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Development and utility of cleaved amplified polymorphic sequences (CAPS) and restriction fragment length polymorphisms (RFLPs) linked to the *Fom-2* fusarium wilt resistance gene in melon (*Cucumis melo* L.)

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Abstract Fusarium wilt, caused by *Fusarium oxysporum* Schlecht f. sp. *melonis* Snyder & Hans, is a worldwide soil-borne disease of melon (*Cucumis melo* L.). Resistance to races 0 and 1 of Fusarium wilt is conditioned by the dominant gene *Fom-2*. To facilitate marker-assisted backcrossing with selection for Fusarium wilt resistance, we developed cleaved amplified polymorphic sequences (CAPS) and restriction fragment length polymorphisms (RFLP) markers by converting RAPD markers E07 (a 1.25-kb band) and G17 (a 1.05-kb band), respectively. The RAPD-PCR polymorphic fragments from the susceptible line 'Vedrantais' were cloned and sequenced in order to construct primers that would amplify only the target fragment. The derived primers, E07SCAR-1/E07SCAR-2 from E07 and G17SCAR-1/G17SCAR-2 from G17, yielded a single 1.25-kb fragment (designated SCE07) and a 1.05-kb fragment (designated SCG17) (the same as RAPD markers E07 and G17), respectively, from both resistant and susceptible melon lines, thus demonstrating locus-specific associated primers. Potential CAPS markers were first revealed by comparing sequence data between fragments amplified from resistant (PI 161375) and susceptible ('Vedrantais') lines and were then confirmed by electrophoresis of restriction endonuclease digestion products. Twelve restriction endonucleases were evaluated for their potential use as CAPS markers within the SCE07 fragment. Three (*Bcl*I, *Msp*I, and *Bss*SI) yielded ideal CAPS markers and were subsequently subjected to extensive testing using an additional 88 diverse melon cultigens, 93 and 119 F_2 individuals from crosses of 'Vedrantais' x PI 161375 and 'Ananas Yokneam'×MR-1 respectively, and 17 families from a backcross BC_1S_1 population derived from the breeding line 'MD8654' as a resistance source. *Bcl*I- and *Msp*I-CAPS are susceptible-linked markers, whereas the *Bss*SI-CAPS is a resistant-linked marker. The CAPS markers that resulted from double digestion by *Bcl*I and *Bss*SI are co-dominant. Results from *Bcl*I- and *Msp*I-CAPS showed over 90% accuracy in the melon cultigens, and nearly 100% accuracy in the F_2 individuals and BC_1S_1 families tested. This is the first report of PCR-based CAPS markers linked to resistance/susceptibility for Fusarium wilt in melon. The RFLP markers resulting from probing with a clone-derived 1.05-kb SCG17 PCR fragment showed 85% correct matches to the disease phenotype. Both the CAPS and RFLP markers were co-dominant, easier to score, and more accurate and consistent in predicting the melon phenotype than the RAPD markers from which they were derived.

Key words *Cucumis melo* · Molecular markers · RAPD · CAPS · RFLP · *Fusarium oxysporum* · Fusarium resistance · Marker-assisted selection (MAS)

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Introduction

Fusarium wilt of melon (*Cucumis melo* L.) is caused by *Fusarium oxysporum* Schlecht f. sp. *melonis* (Leach & Currence) W.C. Snyder & H.N. Hans. Since the first case of Fusarium wilt was reported in New York in 1930 (Chupp 1930a,b) 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 of *F. oxysporum* f. sp. *melonis* have been identified (0, 1, 2, and 1–2). In North America race 2 was essentially the only one known until 1985 when race 1 was isolated in Maryland, and subsequently in California and Ontario, Canada, in 1996 (Zitter 1997). Resistance to *F. oxysporum* f. sp. *melonis* races 0 and 1, and races 0 and 2 is controlled by two independent genes *Fom-2* and *Fom-1*, respectively (Risser and Mas 1965; Risser 1973; Risser et al. 1976; Robinson et al. 1976). In practice, control of Fusarium wilt depends primarily on resistant cultivars.

An immediate, direct, efficient, and practical use of linked markers is marker-assisted selection (MAS) in plant breeding programs. MAS provides significant advantages over traditional phenotypic screening alone, such as rapid and relatively inexpensive, pathogen unavailability, time and labor intensive pathogen inoculation, environmental limitation (off season), simultaneous screening for many diseases, etc. It would expedite the introgression of resistance genes against Fusarium wilt in the breeding process by rapid and efficient screening of large numbers of individuals in segregation generations (F_2, backcrosses) or germplasm lines. MAS also permits a reduced plant population size for effective selection, and greatly reduces the burden of inoculations with Fusarium-wilt pathogens. Perhaps most importantly, MAS allows simultaneous selection for multiple races or pathogens.

Randomly amplified polymorphic DNAs (RAPDs) have been widely used and are one of the most powerful and fastest ways for tagging resistant genes (Martin et al. 1991, 1993; Michelmore et al. 1991; Miklas et al. 1996; Paran et al. 1991, 1993; Reiter et al. 1992; Haley et al. 1993; Timmerman et al. 1994; Wechter et al. 1995; Baudracco-Arnas and Pitrat, 1996; Mayer et al. 1997). As noted below, however, they do have some disadvantages. Three RAPD markers (596, E07–1.25, and G17–1.05) linked to the *Fom-2* gene have been reported recently. Primer 596 yielded a 1.6-kb RAPD marker that was linked to the *Fom-2* gene only in the multi-disease resistant line MR-1 (Wechter et al. 1995). By using this primer under different PCR conditions, the 1.6-kb fragment was also detected in a few other resistant genotypes (Zheng and Wolff, manuscript in preparation). Primers E07 and G17 (see Table 1) were originally identified in the Fusarium-susceptible line 'Vedrantais' (Baudracco-Arnas and Pitrat, 1996). All three primers were evaluated and tested in diverse melon genotypes (Zheng and Wolff, manuscript in preparation). Primer 596 was highly genotype-specific. Although E07 and G17 were found to be very conservative across diverse melon genotypes, they were sometimes either inconsistent or difficult to score (Zheng and Wolff, manuscript in preparation), a characteristic of RAPD markers (Weeden et al. 1992; Staub et al. 1996a). Moreover, they are dominant and linked to the susceptible allele *Fom-2*$^+$.

Because of the disadvantages of RAPD markers, mentioned above, investigators have further characterized and converted the RAPD markers to more reliable and scorable markers (Staub et al. 1996b). The converted markers could be allele-specific associated primers (ASAPs) (Weeden et al. 1992; Gu et al. 1995; Yu et al. 1995; Mayer et al. 1997), sequence-characterized amplified regions (SCARs) (Paran and Michelmore 1993, Wechter and Dean 1998), or cleaved amplified polymorphic sequences (CAPSs) (Konieczny and Ausubel 1993; Jarvis et al. 1994).

RFLPs are one of the most common and earliest developed molecular markers used in genetic map construction, the tagging of disease resistance genes, and systemic and evolutionary studies (Burr et al. 1983; Beckmann and Soller 1988; Tanksley et al. 1989). In contrast to RAPD markers, co-dominant RFLP markers are more useful in marker-assisted selection. In addition, amplification of a RAPD of the same size across populations/species does not necessarily mean that the RAPD possesses the same sequence across the populations/species, unless proven by hybridization studies (Thormann et al. 1994) or bulked segregation analysis. RFLPs mapped in one population can, however, be used as probes for characterizing other populations within the same species (Staub et al. 1996b).

In this paper, we report our efforts in developing CAPS and RFLP markers from RAPD markers. The resulting CAPS and RFLP markers were evaluated in diverse melon cultigens, F_2 populations, and backcross families. The CAPS and RFLP markers reported here are more accurate and consistent, easier to score, and co-dominant compared to the RAPD markers from which they were derived.

Materials and methods

Genomic DNA

Healthy leaf tissues were harvested from melon seedlings grown in a greenhouse at the 3–5 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). The DNA samples of the BC_1S_1 families were extracted from bulked leaf tissue of 25 plants in each backcross family. A family segregating for *Fom-2* would then have the DNA sample from the pooled individuals and would be heterogeneous. For the F_2 population from 'Ananas Yokneam'×MR-1 used for the CAPS segregation study, DNAs were extracted by minipreparation procedures modified from Dellaporta et al. (1983). An additional 23 DNA samples of F_2 individuals from the same cross, received from R.A. Dean (Wechter et al. 1995), was also included in this study. DNA samples of at least 20 F_3 bulked plants from a 'Vedrantais'×PI 161375 cross were prepared according to Baudracco-Arnas (1995). Quantitative and qualitative analysis of DNAs was determined by a UV-VIS scanning spectrophotometer (UV-2101PC, Shimadzu). All DNA samples measured a ratio of A_{260}/A_{280} above 1.8.

Polymerase chain reaction (PCR)

The PCR conditions used for RAPD analyses in this study were modified from protocols employed by Baudracco-Arnas and Pitrat (1996) and Wechter et al. (1995) by decreasing the cycle number to 30 because the PCR products were used as inserts in cloning.

Table 1 Sequences of RAPD primers, and their derived LSAP primers, linked to the single dominant resistant gene (*Fom-2*) conferring resistance to Fusarium wilt caused by *F. oxysporum* f. sp. *melonis* races 0 and 1 in melon (*C. melo*)

Primer	Sequence (5′–3′)	Source (genotype/phenotype)	Fragment size (kb)
E07	AGATGCAGCC	'Vedrantais'/susceptible	1.25
E07SCAR-1	AGATGCAGCCCAAAATTA		
E07SCAR-2	AGATGCAGCCATCAAACT		
G17	ACGACCGACA	'Vedrantais'/susceptible	1.05
G17SCAR-1	ACGACCGACATATCATTG		
G17SCAR-2	ACGACCGATACTAAATAA		

Table 2 List of designations of plasmids constructed in this study

Clone[a]	Vector[b]	Insert[c]
pE07S	pCR2.1	1.25-kb/PCR/E07–1.25/Vedrantais
pSCE07S1	pCR2.1	1.25-kb/PCR/SCE07/Vedrantais
pSCE07S2	pCR2.1	1.25-kb/PCR/SCE07/Vedrantais
pSCE07S3	pGEM	1.25-kb/PCR/SCE07/Vedrantais
pSCE07R1	pCR2.1	1.25-kb/PCR/SCE07/PI 161375
pSCE07R2	pCR2.1	1.25-kb/PCR/SCE07/PI 161375
pSCE07R3	pGEM	1.25-kb/PCR/SCE07/PI 161375
pG17S	pCR2.1	1.05-kb/PCR/G17–1.05/Vedrantais
pSCG17S1	pCR2.1	1.05-kb/PCR/SCG17/Vedrantais
pSCG17S2	pCR2.1	1.05-kb/PCR/SCG17/Vedrantais
pSCG17R1	pCR2.1	1.05-kb/PCR/SCG17/PI 161375
pSCG17R2	pCR2.1	1.05-kb/PCR/SCG17/PI 161375

[a] S and R designate that the insert was the PCR product amplified from the susceptible and resistant parental lines 'Vedrantais' and PI 161375, respectively; 1, 2, and 3 designate the clones generated from independent experiments
[b] pCR2.1 and pGEM were vectors from Original TA Cloning Kit (Invitrogen Corp.) and Promega pGEM – T Easy Vector Systems (Promega Corp.), respectively
[c] The insert description was designated as insert size/PCR product/amplified by the RAPD primer or its derived SCAR primers/source of genomic DNA

Unless otherwise indicated, PCR parameter conditions for the derived SCAR primers (E07SCAR-1/E07SCAR-2 and G17SCAR-1/G17SCAR-2) (Table 1) were the same as for the RAPD primers. Derived primers were synthesized by Genosys Biotechologies, Inc, 1442 Lake Front Cir. Ste 185, The Woodlands, Tex. PCRs were run on a DNA Thermal Cycler 480 (Perkin-Elmer). Cycle parameters were 5 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 56°C, 2 min at 72°C, with a 10 min at 72°C extension at the end. PCR products were electrophoresed at 3–5 V/cm in 1.2% agarose (Sigma) gels in 1×TAE buffer and stained with 0.5 µg/µl of ethidium bromide. The following system of abbreviations was employed, with those for E07 serving as an example. E07 is the name of the primer used in the RAPD analysis, the corresponding amplified fragment linked with *Fom-2* is E07–1.25, the derived SCAR primers are E07SCAR-1/ E07SCAR-2, and the corresponding amplified fragment is SCE07. The same system of abbreviations is used for primer G17 and fragment G17–1.05, as well as primers G17SCAR-1/G17SCAR-2 and fragment SCG17.

Cloning and sequencing of the PCR fragments generated by RAPD primers or their derived SCAR primers

First the RAPD fragments E07–1.25 and G17–1.05, linked to the susceptible phenotype (Baudracco-Anas and Pitrat 1996; Zheng and Wolff, manuscript in preparation), were amplified from the susceptible line 'Vedrantais'. Then the fragments were cloned and sequenced (see below for details). The corresponding SCAR primers, E07SCAR-1/E07SCAR-2 and G17SCAR-1/G17SCAR-2, were derived from the sequence data of E07–1.25 and G10–1.05, respectively. Then, using the SCAR primers, the corresponding target fragments, SCE07 and SCG17, were amplified from both susceptible ('Vedrantais') and resistant (PI 161375) lines. For all these PCR fragments, whether they were amplified by RAPD primers or their derived SCAR primers from 'Vedrantais' or PI 161375, the cloning and sequencing procedures were as follows. After electrophoresis, of the PCR products, the target bands were cut out from the agarose gel. The DNA fragments were re-suspended in dH_2O by following either the Geneclean II Kit or the Spin Module, Bio 101 Inc.(La Jolla, Calif.), whereas PCR products (single fragment) amplified from derived primers were sometimes used directly for cloning. Either the Original TA Cloning Kit (Invitrogen Corp. San Diego, Calif.) or Promega pGEM – T Easy Vector Systems (Promega Corp. Madison, Wis.) were used and the manufacturer's protocols were followed for ligations and transformations. To identify correct clones, 4–6 putative clones were picked out and cultured in LB medium individually and subsequently, following plasmid preparation, *Eco*RI restriction endonuclease digestion, and gel electrophoresis was employed to check the inserts. The correct clone(s) showed a fragment that corresponded to their PCR product(s). The nucleotide sequences of the cloned fragments were determined by using either an automated DNA Sequencer Model 377, located at the DNA Sequencing/Synthesis Facility, Iowa State University, Ames, Iowa or the Sequenase 2.0 dideoxy chain termination method (United States Biochemicals, Cleveland, Ohio) which was conducted manually as detailed by the manufacturer using external T7 and SP6 primers. Internal primers were constructed based on former sequence data and synthesized by Genosys Biotechologies, Inc. For both the E07–1.25 and G17–1.05 RAPD fragments, amplified from the susceptible line 'Vedrantais', the cloning and sequencing experiments were conducted once because the only purpose was to determine the SCAR primer sequences. The cloning and sequencing experiments were conducted independently three times for SCE07 fragments and twice for SCG17 fragments amplified by SCAR primers SCE07SCAR-1/SCE07SCAR-2 and SCG17SCAR-1/SCG17SCAR-2, respectively, from either resistant line PI 161375 or susceptible line 'Vedrantais'. The details of the clone designation and the corresponding parental vector are listed in Table 2.

Identification of CAPS markers

All sequence editings, fragment assemblies, mappings, comparisons, and multiple sequence analyses were carried out by using GCG package Version 8.0 (Genetics Computer Group, Madison, Wis.). If an ambiguity was found, a third independent cloning and sequencing was performed and the consensus sequence obtained was used for further analysis. After sequence analysis revealed the potential CAPS sites (see Table 3), restriction endonuclease digestions of target fragments, generated by PCR from both resistant and susceptible DNA samples, were conducted. The overnight digestion products were electrophoresed in 1.0% agarose (Sigma) gels in 1×TAE buffer and stained with 0.5 µg/µl ethidium bromide.

Softwares

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

Southern blot and DNA hybridization

Approximately 10 µg of genomic DNA was used per enzymatic digestion in a 20-µl reaction vol. which included 1 µl of restriction endonuclease, 2 µl of 10× corresponding buffer, and 0.2 µl of 100×BSA. Digestions were carried out for 10 h at 37°C. Genomic DNAs of the two parental melon lines PI 161375 and 'Vedrantais', as well as MR-1 and 'Ananas Yokneam', were individually digested with each of 14 restriction endonucleases. For blots to be probed with the SCE07 fragment, the following enzymes were employed to digest the melon genomic DNA: *Bcl*I, *Eco*RI, *Eco*RV, *Xho*I, *Pst*I, *Nde*I, *Pac*I, *Hin*dIII, *Kpn*I, *Bgl*II, *Xba*I, *Bam*HI, *Sac*II, and *Bss*SI. The same enzymes were used for blots to be probed with the SCG17 fragment, except for *Bcl*I, *Pac*I and *Nde*I, which were replaced with *Cla*I, *Pvu*II and *Avr*II. The digestion products were electrophoresed in 0.8% agarose (Sigma) gels at 1 V/cm for 12 h in TAE buffer.

DNA blots were prepared as follows. After electrophoresis, gels were treated with 10 vol. 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 a Hybond-N^+ membrane (Amersham Life Science Inc., Arlington Heights, Ill.) for 2–3 h in an alkali-downward capillary blotting procedure modified from Koetsier et al. (1993).

Clone-derived PCR fragments SCE07 and SCG17 with the highest percentage similarity to the consensus sequence from clones pSCE07S2 and pSCG17R2 (Table 2), respectively, were employed as hybridization probes. To purify the inserts (i.e., fragments SCE07 and SCG17) 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. Then the DNA fragments were purified from the agarose gel and re-suspended in dH_2O by using either Geneclean II Kit or the Spin Module, Bio 101 Inc.(La Jolla, Calif.). Probe labelings, hybridizations, stringency washings, blockings and antibody incubations, and signal generations and detections were based on our optimized protocol (Zheng and Wolff 1999) and the recommended guidelines for the non-radioactive labeling and detection modules from the manufacturer (Amersham Life Science Inc., Arlington Heights, Ill.). Pre-hybridizations were carried out for at least 3 h at 60°C and then followed by overnight incubation in hybridization buffer (5×SSC, 0.1% SDS, and 5% dextran sulfate) at the same temperature. Membranes were washed with 2–5 ml/cm^2 of pre-warmed 1×SSC containing 0.1% SDS for 15 min at 60°C and then with 0.5×SSC under the same conditions. After removing the washing solution, blots were incubated with gentle agitation for 1 h at room temperature in 0.75–1.0 ml/cm^2 of 1:10 diluted liquid block agent in Buffer A (0.1 M Tris-HCl, 0.3 M NaCl, pH 9.5). Blots were further incubated in freshly prepared antibody solution (diluted anti-fluorescein-AP conjugate 1:5000 in buffer A containing 0.5% bovine serum albumin) with gentle agitation for 1 h at room temperature. After washing three times in buffer A containing 0.3% Tween 20, the blots were finally incubated in detection reagent (30 µl/cm^2) for 3–5 min in a plastic bag. The excess detection reagent was removed and the blot was exposed on Hyperfilm-MP for 5–60 min before developing the film.

Germplasm

After revealing the CAPS markers from the parental lines 'Vedrantais' and PI 161375 (see Fig. 1; Table 4, group A), they were evaluated in 88 melon cultigens (see Table 4, group B) or F_1 hybrids (see Table 4, group C) from diverse locations representing several melon types. Crosses between 'Vedrantais' (susceptible) and PI 161375 (resistant) were made to produce the F_1 generation, which was selfed to produce the F_2 population. Similarly, F_2 progenies between 'Ananas Yokneam' (susceptible) and MR-1 (resistant) were obtained and 96 F_2 individual plants were used for a segregation study of the CAPS markers in the F_2 population. Individual F_2 plants from both crosses were selfed and F_3 progenies were inoculated with Fusarium wilt. Homogeneous resistant or susceptible F_3s were chosen, corresponding to homozygous F_2 plants. In addition, 17 families from a backcrossing program (BC_1S_1) using breeding line 'MD8654' as a resistance source were also scored for the CAPS markers. Seeds for the BC_1S_1 families were provided by Dr. B. Mordshon (Asgrow Seed company). To evaluate the RFLP markers, the same list of melon cultigens and F_1 hybrids were employed.

Fungal culture maintenance, host inoculation, disease scoring for Fusarium wilt

Unless otherwise mentioned, the disease phenotypes of the melon cultigens and F_1 hybrids used in this study were determined as follows. To evaluate the Fusarium resistance of the parental lines 'Vedrantais'×PI 161375, and their F_2, F_3 progenies, as well as the resistant cultigens (except MR-1 and 'Vine Peach'), Fusarium isolate FOM 26 was used for root-dipping as 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 the susceptible plants died, whereas the resistance 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 (Zink and Thomas 1990; Zink 1992; Wechter et al. 1995; Pitrat et al, 1996). The disease phenotypes for all of the F_1 hybrids were determined by several seed companies or cited from seed catalog descriptions (Nunhems Seed Corp., Holland). Fusarium isolate sources, the inoculation procedure, and the disease reaction of the BC_1S_1 families were determined using a seedling tray root-dip procedure similar to Zink (1992). For the BC_1S_1 families, the disease reactions (%) were scored as the percent of infected seedlings present in a population that consisted of 25 individual plants in each family.

Results

Sequence comparisons between PCR fragments amplified from resistant (PI 161375) and susceptible ('Vedrantais') lines with RAPD primers E07 and G17, or their derived locus-specific primers E07SCAR-1/E07SCAR-2 and G17SCAR-1/G17SCAR-2.

Comparison of consensus sequences from three independent cloning and sequencing experiments of 1.25-kb PCR fragments amplified from genomic DNA of resistant (PI 161375) and susceptible ('Vedrantais') lines with primer E07 or its derivatives (E07SCAR-1and E07SCAR-

Table 3 Potential CAPS markers linked to the *Fom-2* gene in melon which confer resistance to Fusarium wilt caused by *F. oxysporum* f. sp. *melonis* races 0 and 1 in melon (*C. melo*)[a]

PCR fragment[a]	Restriction endonuclease	Recognition site	CAPS marker band (bp)		Conservation among genotypes
			Resistant (PI 161375)	Susceptible ('Vedrantais')	
SCE07	*Bcl*I	T´GATC_A		703, 541	Very conserved
(1244-bp)	*Msp*I	C´CG_G		656, 588	Very conserved
	*Bsp*GI	C´TGGA_C		990, 254	Not tested
	*Bstz*17I	GTA´TAC		969, 275	Some genotypes
	*Bss*SI	C´ACGA_G	873, 371		Conserved
	*Avr*II/*Sty*I	C´CwwG_G	1081, 163		Very conserved
	*Bse*MII	CTsAG	789, 289, 166	1078, 166	Not tested
SCG17	*Cje*I	AC6nTGG8n_6n´	629, 372, 34		Not tested
(1035-bp)	*Cje*PI	GA7nTGG7n_6n´	940, 62, 33	631, 276, 68, 33, 33	Not tested

[a] The PCR fragments can be amplified by using RAPD primers, or their derived primers, as indicated in the text. SCG17 fragments were different in size between amplification from the resistant line PI 161375 and the susceptible line Vedrantais. Not tested means that the enzymes were not commercially available

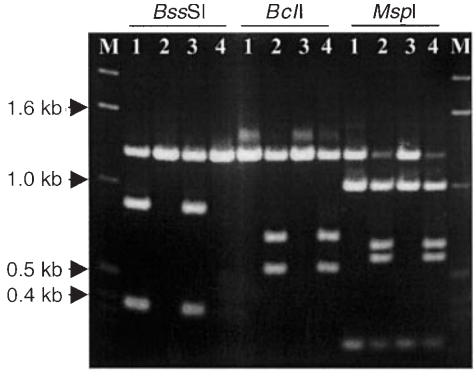

Fig. 1 Ethidium bromide-stained gel of cleaved amplified polymorphic sequences (CAPS) within SCE07 fragments digested with *Bss*SI, *Bcl*I, and *Msp*I, respectively. The SCE07 fragments were amplified by the polymerase chain reaction (PCR) using primers E07SCAR1/E07SCAR2 and genomic DNAs of melon lines resistant or susceptible to Fusarium wilt caused by *F. oxysporum* f. sp. *melonis* races 0 and 1. *Lanes 1, 2, 3, and 4* were digestion products of the 1.25-kb PCR fragment amplified from PI 161375, 'Vedrantais', MR-1, and 'Ananas Yokneam', respectively. Resistant lines PI 161375 and MR-1 had a *Bss*SI recognition site at 873-bp and resulted in additional bands of 873- and 371-bp after digestion. Susceptible lines 'Vedrantais' and 'Ananas Yokneam' had a *Bcl*I recognition site at 703-bp and resulted in additional bands of 703- and 541-bp after digestion. Susceptible lines also had an additional *Msp*I recognition site at 655-bp and resulted in additional bands of 655- and 588-bp after digestion, whereas resistant lines did not. *M* is a 1-kb DNA ladder from Gibco BRL, Life Technologies Incorporated

2) were performed with the GAP program of the GCG package. A total of 1244 nucleotide base pairs were compared. The sequence similarity among three clones from PI 161375 ranged from 97.1 to 99.4%. Among three clones from Vedrantais the similarity ranged from 98.7 to 99.5%. The highest sequence similarity, 99.4 and 99.5% respectively, occurred between the SCE07 fragments of independent clones for both resistance and susceptibility. The similarity of the consensus sequences of SCE07 from resistant and susceptibile clones was 98.4%. The similarity of SCG17 fragments amplified from resistant and susceptible genomic DNA was 98.3% and the sequence difference was mainly from a 'slippery' site due to a deletion at Δ982–987. Comparative searches of the non-redundant DNA databases accessible through the National Center for Biotechnology Information (Bethesda, M.D.) were performed using the BLAST algorithm. No significant matches were found for either fragment. Because the SCE07 fragments from the resistant line PI 161375 and the susceptible line 'Vedrantais' carried the resistant- and susceptible-linked CAPSs (see below), the consensus sequences for both fragments have been deposited in Genbank with the accession numbers AF039586 and AF039587, respectively. The consensus sequence of the SCG17 fragment, which resulted in RFLPs, from resistant line PI 161375, has also been deposited in Genbank with the accession number AF039588.

Identification of CAPS markers and a test of validity in diverse melon cultigens and F_1 hybrids

Potential CAPS markers were identified by computer analyses based on the sequence difference between fragments amplified from resistant line PI 161375 and susceptible line 'Vedrantais'. Some of the most promising potential CAPS markers and their recognition sites are shown in Table 3. Fragments SCE07 and SCG17 (amplified with E07SCAR-1/E07SCAR-2 and G17SCAR-1/G17SCAR-2 primers, respectively) from PI 161375 and 'Vedrantais' were digested with restriction endonucleases that corresponded to potential CAPS markers, according to computer analysis of the sequence data. The CAPS polymorphisms within the SCE07 fragment cut with *Bss*SI, *Bcl*I, or *Msp*I are shown in Fig. 1. *Bss*SI has one cutting site located at 371-bp which resulted in 873- and 371-bp fragments in resistant genotypes (PI 161375 and MR-1) but not in the susceptible ones ('Vedrantais' and 'Ananas Yokneam'). Conversely, the susceptible genotypes ('Vedrantais' and 'Ananas Yokneam') had one cutting site for *Bcl*I located at bp 703 which resulted in 703- and 541-bp fragments,

Table 4 Score of CAPS and RFLP markers in diverse melon (*C. melo*) cultigens and F_1 hybrids with different reactions to *F. oxysporum* f. sp. *melonis* races 0 and 1

Cultigens/F_1 hybrids	Source	Wilt reaction[a]	CAPS[b] -*Bcl*I / -*Msp*I	-*Bss*SI[c]	RFLP (*Bgl*II)[c]
Group A: parental lines					
PI 161375	Korea	R	–	+	AA
Vedrantais	France	S	+	–	BB
Group B: diverse cultigens					
Aodaisimouri	Japan	R	–	+	AA
Charentais Fom–2	France	R	–	ND	AA
Chenggam	Korea	R	–	+	AA
CM 17187	Extreme Orient	R	–	+	AA
Freeman's cucumber	Japan	R	–	ND	AA
Ginsen Makuwa	Japan	R	–	+	AA
Isabelle	France	R	–	+	AA
K 2005	China	R	–	ND	AA
Kanro Makuwa	Japan	R	–	+	AA
Kogane 9 Go Makuwa	Japan	R	–	+	AA
Kogane Sennari Makuwa	Japan	R	–	+	AA
LJ 34340	Extreme Orient	R	+	–	BB
LJ 90297	Extreme Orient	R	–	+	AA
LJ 90389	Extreme Orient	R	–	+	AA
Meshed	Iran	R	–	ND	AA
Miel Blanc	China	R	–	–	AA
MR1	88201—1040	R	–	+	AA
Nanbukin	China	R	–	+	AA
Nyumelon	Japan	R	–	ND	AA
Ogon 9	Japan	R	–	ND	AA
Ouzbeque 1	Japan	R	–	ND	AA
Perlicha 1.5	Guadeloupe	R	–	ND	AA
Persia 202	Iran	R	+	ND	AA
PI 17188	Extreme Orient	R	–	+	AA
PI 125915	Afghanistan	R	–	–	AA
PI 157084	China	R	–	ND	AA
PI 164723	India	R	–	–	AA
PI 223637	Iran	R	–	–	AA
Samarcande	USSR	R	–	+	AA
Semosouri Varamin	Iran	R	–	ND	AA
Shirouri Okyama	Japan	R	–	+	AA
Showa Kogane Nashi Makuwa	Japan	R	–	+	AA
Sisi	Iran	R	+	–	AA
Tokio Mammuth	Japan	R	–	+	AA
Vine Peach	Hollar	R	+/–	–	ND
Ananas Yokueam	Hollar/Wilhite	S	+	–	BB
Casaba Golden Beauty	Hollar	S	+	–	AA
Charentais	88201—1038	S	+	–	BB
Crenshaw	Hollar	S	+	–	AA
D21 1005	84302—1005	S	+	–	BB
D21 1014	84302—1014	S	+	–	ND
Delicious 51	Hollar	S	+	–	BB
Doublon	88201—1036	S	+	–	BB
Dulce	87401—1082	S	+	–	ND
Honey Dew Green Flesh	Hollar	S	+	–	BB
Honey Dew Orange Flesh	Hollar	S	+	–	BB
Iroquois	Hollar	S	+	–	AA
Israel Ogen	Wilhite	S	+	–	AA
Mondo	Nunhems	S	+	–	ND
Marygold	Hollar	S	+	–	AA
Perlita	87401—1081	S	+	–	ND
Perlita 45/21	83301—1036	S	+	–	ND
Persian	Hollar	S	+	+	AA
Santa Clause	Hollar	S	+	–	BB
SUNEX 7050	Sunseeds	S	+	–	ND
TAM Dew Improved	93201—1085	S	+	–	BB
TAM Mayan Sweet	87401—1083	S	+	–	AA
TAM Perlita 45	89FLD.ISO.BLK	S	+	–	ND
TAM Sun	90401—1030	S	+	–	ND
TAM Yellow Canary	87401—1084	S	+	–	AA
TAM Uvalde	89FLD.ISO.BLK	S	+	–	BB

Table 4 Continued

Cultigens/F_1 hybrids	Source	Wilt reaction[a]	CAPS[b] -*Bcl*I / -*Msp*I	-*Bss*SI[c]	RFLP (*Bgl*II)[c]
Topmark	Hollar	S	+	–	ND
UC Topmark	88201—1055	S	+	–	ND
Group C: F_1 hybrids					
Accent	Nunhems	R	–	–	BB
Aril	Nunhems	R	+	–	AA
Corin	Nunhems	R	+/–	–	AB
Daimiel	Nunhems	R	+	–	ND
Desio	Nunhems	R	–	–	AA
Galia	Nunhems	R	–	–	ND
Lutina	Nunhems	R	+/–	–	ND
Pandor	Nunhems	R	+	–	AA
Preco	Nunhems	R	+	+	AB
Solo	Nunhems	R	+	–	AA
Toledo	Nunhems	R	+	–	AA
Viva	Nunhems	R	–	–	ND
Athena	Rogers NK	S	+	–	ND
Castella	Nunhems	S	+	–	BB
Cruiser	Harris Moran	S	+	–	BB
Delada	Nunhems	S	+	–	AA
Deltex	Nunhems	S	+	–	BB
Fiola	Nunhems	S	+	–	ND
Honeybrew	Sakata	S	+	–	BB
Laguna	Asgrow	S	+	–	ND
Mission	Asgrow	S	+	–	BB
Moring Ice	Harris Moran	S	+	–	BB
Primo	Rogers NK	S	+	–	ND
Rocky Sweet	Hollar	S	+	–	BB
Spice	Hollar	S	+	–	BB

[a] R=Resistant, S=Susceptible
[b] + or –=presence or absence of the CAPS markers; *Bcl*I- and *Msp*I-CAPS are susceptible-linked markers located at 703- and 541-kb and 656- and 588-kb, respectively; *Bss*SI-CAPS is the resistant-linked marker located at 873- and 371-kb
[c] Data were from *Bgl*II digestion, AA=presence of a 5-kb fragment linked to resistance, BB=presence of a 3.5-kb fragment linked to susceptibility, and AB=presence of both the 5- and 3.5-kb bands and thus a heterozygous genotype; ND=not tested

whereas the resistant genotypes did not. Similarly, compared with the resistant genotypes, the susceptible genotypes had an additional cutting site for *Msp*I located at bp 656 which resulted in 656- and 588-bp fragments. To test these markers in melon lines other than the ones where they were originally identified, the 1.25-kb PCR fragments amplified from 88 other diverse melon cultigens (Table 4, group B) or F_1 hybrids (Table 4, group C), which included 47 resistant and 41 susceptible types, were cut with the restriction endonucleases listed above. Among the 47 resistant entries, 35 were either germplasm lines or cultivars and 12 were resistant F_1 hybrids. In the 41 susceptible entries, 28 were germplasm lines or cultivars and 13 were susceptible F_1 hybrids. The scoring of CAPS markers generated by *Bcl*I and *Msp*I resulted in 90% correct matches to the phenotype in 90 melon genotypes tested (Table 4). There were nine mismatches which included three cultigens (LJ 34340, 'Persia 202', and 'Sisi') and six F_1 hybrids ('Toledo', 'Daimiel', 'Aril', 'Solo', 'Pandor', and 'Preco'). In all cases these were resistant plants that showed the presence of the bands; there was no susceptible plant with an absence of the bands. The resistant linked *Bss*SI-CAPS was less conserved across different genotypes. It showed a 76% match to the phenotype out of 79 genotypes tested. Mismatches were of two kinds: 18 resistant without fragments, including seven cultigens (LJ 34340, PI 125915, PI 164723, PI 223637, 'Sisi', 'Miel blanc', and 'Vine Peach') and 11 F_1 hybrids ('Accent', 'Toledo', 'Viva', 'Corin', 'Lutina', 'Daimiel', 'Aril', 'Solo', 'Pandor', 'Desio', and 'Galia'), whereas fragments were found in only one susceptible line ('Persian').

Figure 1 also shows that digestions by *Bss*SI, *Bcl*I, or *Msp*I individually were not complete, which otherwise would have resulted in resistant- or susceptible-linked co-dominant CAPS from a single digestion. With the combination of *Bcl*I and *Bss*SI, however, co-dominant polymorphisms were available for the identification of homozygous-resistant (873- and 371-bp fragments), heterozygous resistant (873-, 703-, 541-, and 371-bp fragments), and homozygous-susceptible (703- and 541-bp fragments) F_2 individuals (Fig. 2). In addition, *Avr*II/*Sty*I, *Bstz*17I, and *Tsp*RI, together with *Acc*I, *Bbs*I, *Bsl*I, *Mnl*I, were also checked. The results obtained were less desirable as CAPS markers due to either lack of conservation across diverse genotypes or difficulty in scoring (data not shown). No data were available about the potential CAPS polymorphism for the SCG17 fragment (1.05-kb) amplified by G17SCAR-1/G17SCAR-2 be-

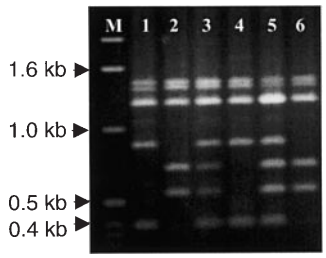

Fig. 2 Ethidium bromide-stained gel of cleaved amplified polymorphic sequences (CAPS) within SCE07 fragments resulting from double-digestion with *Bcl*I and *Bss*SI. The SCE07 fragments were amplified by the polymerase chain reaction (PCR) using primers E07SCAR1/ E07SCAR2 and genomic DNAs of parental lines MR-1 and 'Ananas Yokueam' and their F_1 and F_2 individuals homozygous resistant (RR), heterozygous resistant (RS), and susceptible (SS) to Fusarium wilt caused by *F. oxysporum* f. sp. *melonis* races 0 and 1. *Lanes 1 and 2* were MR-1 and 'Ananas Yokneam', respectively, which showed resistant-linked bands at 873- and 371-bp and susceptible-linked bands at 703- and 541-bp after digestion. *Lanes 3–6* were F_1 and F_2 RR, RS, and SS, respectively. Digestions were carried out at 37°C overnight. *M* is a 1-kb DNA ladder from Gibco BRL, Life Technologies Incorporated

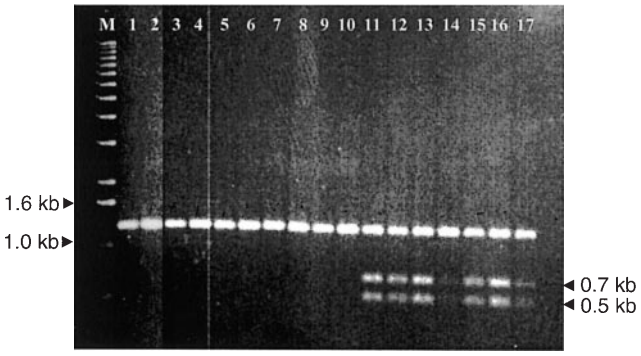

Fig. 3 Ethidium bromide-stained gel of cleaved amplified polymorphic sequences (CAPS) within SCE07 fragments resulting from double digestion with *Bcl*I and *Bss*SI. The SCE07 fragments were amplified by the polymerase chain reaction (PCR) using primers E07SCAR1/E07SCAR2 and genomic bulked DNAs of BC_1S_1 families (seeds provided by B. Mordshon from Asgrow seed company). The DNA sample for each family was extracted from bulked leaf tissues of 25 individual plants that contained a different percentage of infected plants to Fusarium wilt caused by *F. oxysporum* f. sp. *melonis* races 0 and 1. *Lanes 1–10* were bulks from plants in a family that showed no disease symptoms. *Lanes 11 and 12* were bulks of plants in a family that showed 100% disease. *Lanes 13–17* were bulks of plants in a family that showed different percentages of infected plants (30–94%). The SCE07 fragments from both the susceptible and the segregation family bulks had a *Bcl*I recognition site at 703-bp and resulted in two additional fragments of 703- and 541-bp after digestion, whereas resistant family bulks did not. Digestions were carried out at 50°C overnight. *M* is 1-kb DNA ladder from Gibco BRL, Life Technologies Incorporated

cause restriction endonuclease enzymes *Cje*I or *Cje*PI are not available commercially.

Score of CAPS markers in segregation populations

Ninety six DNA samples from F_2 populations from crosses between 'Ananas Yokneam'×MR-1, 93 F_2 individuals (46 homozygous resistant and 47 homozygous susceptible) from crosses between 'Vedrantais' x PI 161375, and 17 DNA samples from BC_1S_1 families using MD8645 as a resistance source were checked for CAPS polymorphisms within the SCE07 1.25-kb fragment. Results from *Bcl*I- and *Bss*SI-CAPS (double digestions) showed that among the 96 samples of the F_2 populations derived from the cross 'Ananas Yokneam'×MR-1, 19, 51, and 26 were scored as homozygous resistant (RR), heterozygous (RS), and homozygous susceptible (SS), respectively. The segregation of the CAPS markers *Bcl*I+*Bss*SI (19 RR:51 RS:26 SS) in the F_2 population fits the expected 1:2:1 ratio for a single gene model (χ^2=1.39, d.f.=2, *P*=0.50). Additionally, with the 23 F_2 individuals from a cross between 'Ananas Yokneam'×MR-1 received from other researchers (Wechter et al. 1996), all eight phenotype-susceptible samples were scored as susceptible, i.e., they contained two fragments 703- and 541-bp in size generated by *Bcl*I. Of the 15 phenotypically resistant samples, 11 were scored as heterozygous, i.e., they contained four fragments 873-, 703-, 541-, and 371-bp in size. The other four samples yielded fragments of 873- and 371-bp generated by *Bss*SI and thus were scored as homozygous resistant. The segregation of CAPS in this F_2 population (8 SS:11 RS:4RR) also closely fits the expected 1:2:1 ratio (χ2=1.435, df=2, *P*=0.49).

The CAPS markers scored for both *Bcl*I or *Msp*I, and *Bss*SI were 100% accurate in predicting the phenotypes of the 93 F_2 individual samples from the cross of 'Vedrantais'×PI 161375. The 46 homozygousresistant (RR) F_2 individuals showed one cutting site only for *Bss*SI and generated 873- and 371-bp fragments, whereas the 47 homozygous-susceptible (SS) F_2 individuals showed only one cutting site each for both *Bcl*I and *Msp*I and generated 703- and 541-bp, and 656- and 588-bp, fragments, respectively (data not shown).

Similarly, these CAPS polymorphisms were also able to precisely differentiate resistant bulked DNA samples (lanes 1–10) from susceptible bulked samples (lanes 11 and 12) and heterozygous-resistant bulked samples (lanes 13–17) of the 17 BC_1S_1 families, each consisting of 25 plants (Fig. 3). The 1.25-kb DNA fragments amplified from the resistant bulked tissues showed no cutting site for *Bcl*I, whereas the 1.25-kb DNA fragments from the susceptible and heterozygous-resistant bulks had one *Bcl*I recognition site at bp 703 that resulted in 703- and 541-bp fragments. However, no recognition site for *Bss*SI was evident in this backcrossing population which, assuming the resistance source 'MD8654', did not carry the resistant-linked *Bss*SI-CAPS marker.

Southern hybridization and RFLP markers

RFLPs were found after probing with the clone-derived 1.05-kb SCG17 fragment that originated from PCR-amplification of the genomic DNA of resistant line PI

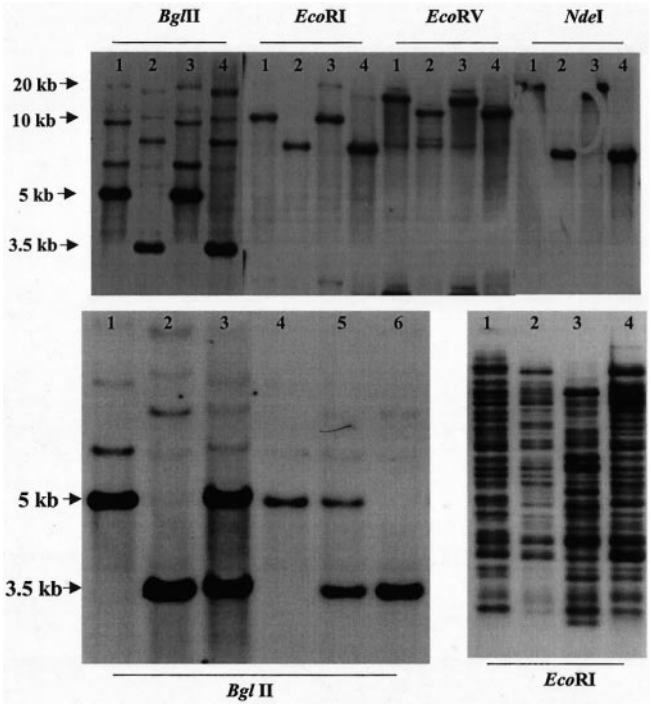

Fig. 4 Genomic Southern-hybridization analyses. *Top and lower-left panels* were probed with clone-generated fragments of 1.05-kb that were amplified by PCR using the derived primers G17SCAR1/G17SCAR2 and genomic DNA from resistant line PI 161375. *Top panel: lanes 1, 2, 3, and 4* were PI 161375, 'Vedrantais', MR-1, and 'Ananas Yokneam' genomic DNAs digested with *Bgl*II, *Eco*RI, *Eco*RV, and *Nde*I, respectively. *Lower-left panel* shows RFLP segregation resulting from the *Bgl*II digestion of genomic DNA. *Lanes 1–6* were parental lines PI 161375 and 'Vedrantais' and their F_1 and F_2 individuals of homozygous-resistant (RR), heterozygous-resistant (RS), and susceptible (SS) samples, respectively. *Lower-right panel* was probed with clone-generated 1.25-kb fragments that were amplified by PCR using the derived primers E07SCAR1/E07SCAR2 and genomic DNA of susceptible 'Vedrantais'. *Lanes 1–4* were PI 161375, 'Vedrantais', MR-1, and 'Ananas Yokneam' genomic DNAs digested with *Eco*RI. In all cases about 10 µg of genomic DNAs were used per digestion with the restriction enzymes indicated, separated in an 0.8% agarose gel, blotted onto a Hybond N^+ membrane (Amersham Life Science Inc., Arlington Heights, Ill.) and hybridized at 60°C with the fluorescein-labeled probes indicated above

161375 with primer G17SCAR-1/G17SCAR-2. Four (*Bgl*II, *Eco*RI, *Eco*RV, and *Nde*I) out of the fourteen restriction endonucleases tested were found to yield desirable and consistent polymorphisms in the two parental lines PI 161375 and 'Vedrantais', from which the RFLP markers were derived, and in MR-1 and 'Ananas Yokneam' (Fig. 4, top panel), as well as 20 other melon lines (data not shown). The RFLP polymorphisms generated from these four enzymes followed the same pattern, with the resistant co-dominant bands having a higher molecular weight than those of the susceptible. The resistant co-dominant fragments produced from digestion with *Bgl*II, *Eco*RI, *Eco*RV, and *Nde*I were in the size of 5-, 10-, 13-, and 20-kb, respectively, whereas the susceptible co-dominant fragments were of 3.5-, 7.5-, 11-, and 7.0-kb, respectively. The RFLPs resulting from *Bgl*II digestions of genomic DNA from F_1 and F_2 individuals (homozygous resistant RR, heterozygous resistant RS, and susceptible SS) from the cross of 'Ananas Yokneam'×MR-1 showed the expected profiles (Fig. 4, lower left panel). Results from scoring the RFLPs resulting from *Bgl*II digestion of genomic DNAs from diverse melon cultigens and F_1 individuals are shown in Table 4 (Groups B and C). Out of the 71 samples tested, 60 (85%) matched correctly with the phenotypes, which was slightly more accurate than the G17-RAPD marker (81%, Zheng and Wolff, manuscript in preparation) from which the RFLPs were derived. One resistant line (LJ 34340) and one F_1 hybrid ('Accent') had the fragments linked with the susceptible allele (3.5 kb), whereas eight susceptible lines ('Israel Ogen', 'Marygold', 'Persian', 'TAM Mayan Sweet', 'TAM Yellow Canary', 'Crenshaw', 'Casaba Golden Beauty', and 'Iroquois) and one F_1 hybrid ('Delada') had the fragments linked with the resistant allele (5 kb).

Southern hybridization revealed multiple copies of the SCE07 fragments located across the melon genome. A total of 14 enzymes either with or without cutting site(s) within the fragment were tested and every enzymatic digestion resulted in multiple hybridization bands (data not shown). The hybridization profiles obtained from *Eco*RI digestion of PI 161375, 'Vedrantais', MR-1, and 'Ananas Yokneam' are shown in the lower right panel of Fig. 4. These hybridization profiles could not result in RFLP markers; however, they could explain why the above sequence differences observed between independent cloning and sequencing experiments were greater than their theoretical rate due to the nature of PCR errors, and also why the CAPS enzymatic digestions were always incomplete (Figs. 1, 2, and 3).

Discussion

This is the first report of testing with CAPS and RFLP markers, and the demonstration of their linkage to resistance or susceptibility to Fusarium wilt in melon. The susceptible-linked *Bcl*I- and *Msp*I-CAPS markers were more conserved across the diverse melon genotypes examined than the resistant-linked *Bss*SI-CAPS marker, with 90% and 76% matches to the phenotypes, respectively. The *Bcl*I- and *Msp*I-CAPS markers also showed a 100% correct prediction in F_2 homozygous-resistant and susceptible individuals, as well as in BC_1S_1 families. Furthermore, both resistant and susceptible linked CAPS markers followed Mendelian single-gene segregation in the F_2 population. It should be pointed out that, as with the E07–1.25 RAPD marker (Baudracco-Arnas and Pitrat 1996; Zheng and Wolff, manuscript in preparation) from which the CAPS markers were derived, all the mismatches came from the phenotypically resistant plants being scored as susceptible. Among them, the majority (six out of nine mismatches) were resistant F_1 hybrids. One (LJ 34340) out of the other three genotypes was recently found to be segregating for Fusarium resistance in

a subsequent disease screening. A homozygous-resistant selection from LJ 34340 was obtained and will be tested later. Only two genotypes (2.2%) of the mismatches were not hybrids. This could either be due to heterozygosity for *Fom-2* or to recombination events between the markers and the gene. Baudracco-Arnas and Pitrat (1996) reported that SCE07 was 1.6±0.9 cM away from the resistance gene *Fom-2*, which would result in a 2–5% recombination frequency.

The RFLP marker was developed from the G17–1.05 fragment that is 4.5±1.5 cM away from the resistance gene *Fom-2* (Baudracco-Arnas and Pitrat, 1996). Theoretically a 6–12% recombination frequency will occur. Results from the RFLP analyses showed 85% correct matches, which is close to the theoretical expectation, and the RFLP is more accurate and easier to score than the G17–1.05 RAPD marker from which the RFLPs were derived. When comparing the results obtained with the G17–1.05 RAPD marker (Zheng and Wolff, manuscript in preparation) and the RFLP markers, the latter not only confirmed all samples that had been clearly scored by RAPD analyses, but also clarified the ambiguous results obtained by the RAPD analyses. Some of the ambiguities were F_1 resistant individuals that were scored as heterozygous by RFLP markers. Others, that had been difficult to score by RAPD analyses due to multiple fragments located in the marker position, were clearly scored as resistant. All the mismatches except one genotype ('Persian') were resistant, but were scored as susceptible. Among them most were either F_1 hybrids or else were heterozygous for *Fom-2* (such as LJ 34340).

The linkage between *Fom-2* and the CAPS or RFLP markers is surprisingly high. The land-race accessions are of different geographic origin (Table 4): Central Asia (Samarcande, Meshed, etc.), India (MR-1, PI 157084, etc.), Japan (Ginsen makuwa, etc.), and Korea (PI 161375, Chenggam). Resistant commercial cultivars (open-pollinated or F_1 hybrids) are derived from many backcrosses.

It was found throughout this study that digested products always contained the intact fragment (i.e., 1.25-kb, see Figs. 1, 2, and 3) besides the expected CAPS polymorphisms within the SCE07 fragments. This was especially the case when PCR products without any purification were used directly as enzyme-digestion substrates in CAPS tests. Even with fragments purified by the Geneclean II Kit or the Spin Module the overnight digestion product still contained the intact fragments. It seems that the intact fragment did not result from incomplete digestion due to the direct use of an unpurified PCR product which contained PCR buffer that altered the constitution of the digestion buffer. This phenomenon was found both in *Bss*SI, the resistant-linked CAPS, and *Bcl*I and *Msp*I, the susceptible-linked CAPS. Furthermore, fragments amplified from susceptible DNA samples partially shared the recognition site of enzymes that produced resistant-linked CAPS (such as *Avr*II/*Sty*I), and vice versa for enzymes that generated susceptible-linked CAPS (such as *Bstz*17I) (data not shown). These contradictions might be due to the multiple copies of the fragment located throughout the entire genome, which is indicated by the Southern DNA gel-blotting analyses (Fig. 4, lower right panel).

In spite of the complexity of the 1.25-kb SCE07 fragment, due to its multiple copies located throughout the genome, it was found that the *Bss*SI-CAPS, and *Bcl*I- and *Msp*I-CAPS, markers were always co-dominantly linked to resistance and susceptibility for Fusarium wilt. However, partial digestions by these enzymes would make it impossible to identify the heterozygous genotype (RS). This problem could be overcome by double digestions combining two enzymes each resistant- and susceptible-linked, for example *Bss*SI and *Bcl*I, or by simply comparing the data from two independent single digestions, as shown in this investigation. It would also be useful to further characterize the nature of the SCE07 fragments in the melon genome.

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 and difficul nature of RAPD markers is well documented (Weeden et al. 1992), and have already proven to be a problem in *Cucumis* (Staub et al. 1996a). The same situation occurred in our related work (Zheng and Wolff, manuscript in preparation) in using these three currently available RAPD markers linked to resistance/susceptibility to Fusarium wilt (Wechter et al. 1995; Baudracco-Arnas and Pitant 1996). In addition, the dominant characteristic of RAPDs makes them less desirable. Although CAPS and RFLP markers require additional work, or sometimes are procedurally more difficult after PCR amplification than RAPD markers, they are more accurate and easier to score than RAPDs, and are co-dominant. During the course of this study we have developed an optimized protocol for highly sensitive, rapid, and consistent application of genomic Southern hybridization for RFLP using a non-radioactive technique (Zheng and Wolff, 1999). In this protocol, consistent signals can be detected from 0.1 µg of genomic DNA per reaction and as many as 240 samples can be analyzed within 2 days. It is up to individual researchers to weigh the advantages and disadvantages of each marker in their own research programs.

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