

Development of a molecular method for rapid differentiation of watermelon lines resistant to *Fusarium oxysporum* f. sp. *niveum*

Ying-Hong LIN¹, Kan-Shu CHEN², Tsung-Dao LIOU², Jenn-Wen HUANG^{1,*}, and Pi-Fang Linda CHANG^{1,*}

¹Department of Plant Pathology, National Chung Hsing University, Taichung City 402, Taiwan

²Fengshan Tropical Horticultural Experiment Branch, Agricultural Research Institute, Council of Agriculture, Fengshan, Kaohsiung Hsien 830, Taiwan

(Received August 14, 2008; Accepted December 18, 2008)

ABSTRACT. The vascular wilt disease of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (Fon) is one of the major factors limiting watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) production worldwide. Breeding highly resistant lines is one of the best ways to control the disease. In order to accelerate the breeding programs, a reliable PCR (polymerase chain reaction) technique was developed to rapidly differentiate the disease-resistant watermelons. The G05-SCAR (sequence characterized amplified region) primer set GsF5/GsR5, derived from the OP-G05 random primer-amplified fragment sequence, produced an 898 bp DNA fragment which was specific to watermelon lines resistant or tolerant to Fon. With optimized PCR assay, the molecular method could easily differentiate Fon-resistant and -susceptible hybrid watermelon populations.

Keywords: Breeding programs; *Citrullus lanatus*; *Fusarium oxysporum* f. sp. *niveum*; Fusarium wilt; Molecular detection; Molecular marker; PCR; RAPD; SCAR.

Abbreviations: Fon, *Fusarium oxysporum* f. sp. *niveum*; PCR, polymerase chain reaction; SCAR, sequence characterized amplified region; RAPD, random amplified polymorphic DNA; BSA, bulked segregate analysis; gDNA: genomic DNA.

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) is the second largest vegetable crop in the world, with a cultivation area of 3,785,475 ha and a yield of 100,602,405 tons in 2006 (FAO, 2006). The area dedicated to watermelon in Taiwan (12,403 ha) is reported to be the largest of any vegetable (COA, 2006). Fusarium wilt is one of the most severe watermelon diseases and limits its production worldwide (Zhang et al., 2005). It is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *niveum* (E. F. Smith) Snyder & Hansen (Fon). Fon can survive in soil as a saprophyte for many years (Notz et al., 2002). Therefore, watermelons should not be replanted in locations with infected soil. No known chemicals can control this disease safely, economically, and effectively (Forsyth et al., 2006) because Fon can

transform itself into thick-walled chlamydospores, highly resistant to chemical fumigation (Shi et al., 1991). One of the best ways to manage Fusarium wilt disease at present is based on breeding resistant lines (Diener and Ausubel, 2005). Planting resistant cultivars will not only increase the quality and yield of watermelon, it will reduce the use of chemical fungicides. The commercial cultivars of watermelon, however, usually do not resist Fusarium wilt (Chen et al., 2003b). A molecular differentiating method would help to prescreen watermelons resistant to Fon. In order to accelerate breeding programs, this investigation therefore focuses on development of such a method to rapidly identify watermelons with Fusarium wilt resistance.

Random amplified polymorphic DNA (RAPD; originally designed by Williams et al., 1990) was used to construct a linkage map of Fon resistance in hybrid populations of watermelon (Hawkins and Dane, 2001). It has also been used to identify markers linked to Fusarium wilt resistance in watermelon (Xu et al., 2000) and muskmelon (Wechter et al., 1995; 1998). RAPD has been described as a powerful molecular tool for marker

*Corresponding authors: E-mail: pfchang@nchu.edu.tw, Phone: +886-4-2284-0780 ext. 335, 330, Fax: +886-4-2286-0442 (Pi-Fang Linda Chang); E-mail: jwhuang@nchu.edu.tw, Phone: +886-4-2284-0780 ext. 351, Fax: +886-4-2285-1676 (Jenn-Wen Huang).

screening (Juchum et al., 2007; Ngezahayo et al., 2007). This technique relies on differential amplification of DNA fragments using PCR with arbitrary oligonucleotide primers, usually 10 nucleotides (nt)-long or even shorter. DNA polymorphisms of RAPD result from chromosomal divergence in the amplification regions that shift primer binding. However, the polymorphisms may not be reproducible because the short primers are easily mismatched to amplify other non-target DNA sequences due to the low annealing temperature and small variation in PCR conditions. Subsequently, a more reliable and specific PCR-based tagging technique known as sequence characterized amplified region (SCAR) was described by Paran and Michelmore in 1993. SCAR primers are longer primers extending from the 3' ends of the original random primer sites based on the sequence of the polymorphic RAPD marker the random primer amplified. The SCAR primers will bind to the original RAPD marker and specifically generate the SCAR marker to increase the reproducibility (Clercq et al., 2007). Unlike RAPD, SCAR is effective and sensitive (Dias et al., 2007), and its high specificity allows direct tagging of a single locus of a genome as an easily determining DNA band by DNA agarose gel electrophoresis. Therefore, SCAR markers are commonly used as genetic markers by plant breeders to accelerate breeding programs (Huaracha et al., 2004). In addition, the bulked segregate analysis (BSA) approach (Michelmore et al., 1991) was applied to SCAR PCR analysis. The method was developed to rapidly identify markers linked to any specific genomic loci or genes. It provides a rapid method of distinguishing resistant hybrid populations. This approach is a highly efficient identification tool to screen molecular markers, especially when dealing with a single dominant gene (Liebenberg and Pretorius, 2004).

In this study, we developed a novel molecular tool based on SCAR PCR for the rapid differentiation of Fon-resistant watermelons. The developed tool will assist in breeding watermelon against *Fusarium* wilt.

MATERIALS AND METHODS

Plant materials

Two watermelon (*Citrullus lanatus*) lines, Sugar Baby (SB, highly susceptible to Fon-H0103) and JSB (bred from SB, highly resistant to Fon-H0103) (Chen et al., 2003b), were used for screening the RAPD markers related to *Fusarium* wilt resistance in this study. The hybrid progenies of F_1 and F_2 populations in both crossed directions (SB \times JSB and JSB \times SB) were used for an inoculation test. In addition, twelve commercial susceptible cultivars provided by Known-You Seed Co., Ltd. (Kaohsiung, Taiwan) and seven resistant lines were used for a specificity test of SCAR PCR. All the cultivars and lines used are genetically different from JSB and SB with distinct horticultural traits, i.e. growth period, peel color, strip pattern, fresh color, and size of the fruits.

Inoculation test

Watermelon plantlets, with one true leaf, of SB, JSB, and F_1 and F_2 hybrids were inoculated with spore suspension (10^5 spores mL^{-1}) of Fon-H0103 isolate and then grown for 1-2 weeks at 28/25°C (day/night temperature) and 60-70% relative humidity.

The Fon-infected watermelon plantlets showing symptoms of leaf wilting were sampled, scored as susceptible, and used for plating experiment on Nash-PCNB plate (1.5% peptone, 2% agar, 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% pentachloronitrobenzene, 0.03% streptomycin, and 0.1% neomycin) (Nash and Snyder, 1962) to confirm successful inoculation.

DNA bulks preparation and isolation

Genomic DNA (gDNA) was extracted according to Dellaporta et al. (1983) with minor modifications. Fresh young cotyledons (0.3 g) before inoculation were cut from plantlets, quickly frozen in liquid nitrogen, and ground to fine powders using mortar and pestle. DNA was extracted with 5 mL modified TNE buffer (100 mM Tris-HCl, pH 8.0; 50 mM Na_2EDTA , pH 8.0; 50 mM NaCl; 8 μM β -mercaptoethanol; 1% SDS and 10 $\mu\text{g/mL}$ RNase) and incubated at 65°C for 30 min. A 0.33 \times volume of 5 M potassium acetate was added, mixed, and centrifuged at 20,000 $\times g$ for 5 min. The supernatant was transferred into a fresh tube and mixed with an equal volume of isopropanol to precipitate crude DNA. The samples were incubated at -20°C for 20 min and centrifuged at 4°C, 20,000 $\times g$ for 20 min. The DNA pellet was resuspended in 200 μL dH_2O , and an equal volume of chloroform/isoamyl alcohol (24:1; V/V) was added and mixed thoroughly. After centrifugation at 4°C, 20,000 $\times g$ for 5 min, the upper aqueous phase was transferred to a fresh tube, and 0.1 \times volume of 3 M sodium acetate (pH 5.2) and 2.5 \times volume of absolute ethanol were added. After centrifugation at 4°C, 20,000 $\times g$ for 5 min, the supernatant was decanted, and the DNA pellet was washed with 300 μL 75% ethanol, allowed to air dry, and finally dissolved in 1 \times TE buffer (10 mM Tris-HCl, pH 8; 0.1 mM EDTA) for further analysis.

Bulked DNA samples can be generated from a segregating crossing population. Each bulk contains individuals identical for a particular trait (Michelmore et al., 1991). Two pools of DNA are obtained from two genetically identical populations except for the trait of interest. For DNA bulk preparation, DNA samples were extracted separately from each hybrid. According to the inoculation results, five individual DNA bulks containing resistant F_1 (ten hybrids as one bulk), two crossed directions of resistant F_2 (for a total of 40 hybrids, 7 SB \times JSB as one bulk, and 33 JSB \times SB as the other bulk), and two crossed directions of susceptible F_2 (for a total of 17 hybrids, 3 SB \times JSB as one bulk, and 14 JSB \times SB as the other bulk) were individually pooled. Equal amounts of DNA from individual plants were pooled as corresponding DNA bulks according to their resistance or

susceptibility to Fon. These five DNA bulks were diluted to equal concentrations ($10 \text{ ng } \mu\text{L}^{-1}$) for further SCAR PCR analysis.

Primer design and PCR amplification

In order to obtain DNA markers that could specifically differentiate phenotypic characteristics of Fon resistance, more than 300 random decamers were tested in DNA samples of JSB and SB on a PCR assay. For RAPD analysis, a $50 \text{ } \mu\text{L}$ reaction mixture for PCR contained 50 ng gDNA, $1\times$ reaction buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 2.5 mM MgCl_2), 0.1 mM of each dNTP, 0.8 μM random decamer primer (Operon Technologies Inc., Alameda, CA, USA), and 2.5 unit *Taq* DNA polymerase (MDBio, Inc., Taipei, Taiwan). The parameters for RAPD were first denatured at 94°C for 90 s, followed by 40 cycles of denaturing at 94°C for 30 s, annealing at 42°C for 30 s, and polymerizing at 72°C for 90 s, and then a final extension at 72°C for 10 min. For specific PCR, 0.4 μM primers (GsF-/GsR- or RPS10F/RPS10R primers) and 50 ng gDNA were added in a $50 \text{ } \mu\text{L}$ reaction containing $1\times$ reaction mixture as mentioned above. The amplification protocol involved denaturing at 94°C for 90 s, followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 57°C (for primer sets GsF-/GsR-) or 62°C (for RPS10F/RPS10R primers) for 30 s, and polymerizing at 72°C for 50 s, and then a final extension at 72°C for 10 min. The high-fidelity PCR was performed using the *PfuTurbo*[®] DNA polymerase (Stratagene, La Jolla, CA, USA) with primer set GsF5/GsR5 for the bulked DNA samples of Figure 3. PCR products were subjected to electrophoresis in 1.5% agarose gels, and the DNA bands were observed on UV box (312 nm, Vilber Lourmat, Eberhardzell, Deutschland) after ethidium bromide staining.

RESULTS

Screening of RAPD markers associated with *Fusarium* wilt resistance

An RAPD fragment related to JSB was amplified by the random primer OP-G05, 5'-CTGAGACGGA (Figure 1). This PCR fragment (designed as JSB-OPG05₈₉₈) was present in all the resistant F_1 populations (data not shown). We further cloned and sequenced this marker. The nucleotide (nt) sequence (accession number: EU543439) confirmed that this RAPD fragment was 898 bp long and had the original primer sequence (OP-G05) at both ends. In addition, other nine polymorphic RAPD markers such as OPY16₇₄₀, UBC25₃₂₀, UBC38₃₅₀, UBC38₅₀₀, UBC38₁₁₅₀, UBC52₉₈₀, UBC53₇₅₀, UBC53₁₀₀₀, and UBC53₁₄₀₀ could be used for differentiating JSB from SB (Table 1). However, most of them, except for OPY16₇₄₀, did not give good reproducibility.

Specificity and SCAR PCR amplification

In order to develop a molecular differentiating system for Fon resistance, eight G-SCAR primers (4 GsF- and 4

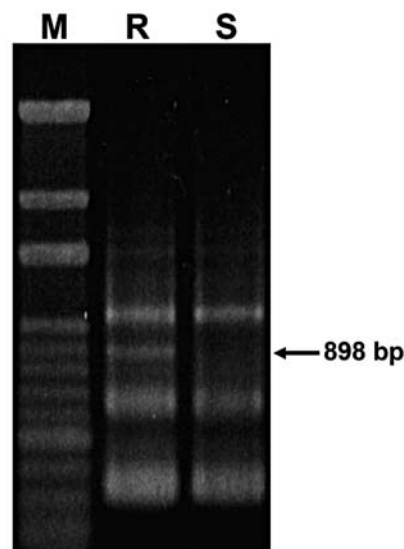


Figure 1. Fingerprinting of random amplified polymorphic DNA (RAPD) in JSB and SB lines. Genomic DNA (gDNA) of JSB (R, Fon-resistant) and SB (S, Fon-susceptible) were used as templates for amplification by using random primer OP-G05. The position of 898-bp DNA band specific to JSB line was indicated on the right. M = molecular markers of Gen-100 DNA ladder (GeneMark Technology Co., Ltd., Tainan, Taiwan).

GsR-) based on the sequence of JSB-OPG05₈₉₈ were used for differentiating JSB from SB. These primers contain the original primer sequence (OP-G05, 5'-CTGAGACGGA, 10 nt) plus the internal 4 to 7 nt (Table 1). The 14 to 15 nt-long G-SCAR primers (GsF4/GsR4 and GsF5/GsR5) were able to amplify this JSB-OPG05₈₉₈ marker only from gDNA of JSB but not from gDNA of SB (Figure 2). The results indicate that primer set GsF5/GsR5 generated stronger DNA polymorphic signal and could be used to differentiate JSB from SB. Using longer primer sets such as GsF6/GsR6 and GsF7/GsR7, however, could not differentiate JSB from SB since faint DNA bands with similar sizes also appeared in the SB samples. In order to determine the specificity of our PCR differentiation system using primer set GsF5/GsR5 against the gDNA of the progeny populations of JSB and SB, DNA bulks were individually pooled from each Fon-resistant or -susceptible hybrid. The results showed that this primer set was able to easily distinguish between the DNA bulks of Fon-resistant and -susceptible hybrid populations (Figure 3).

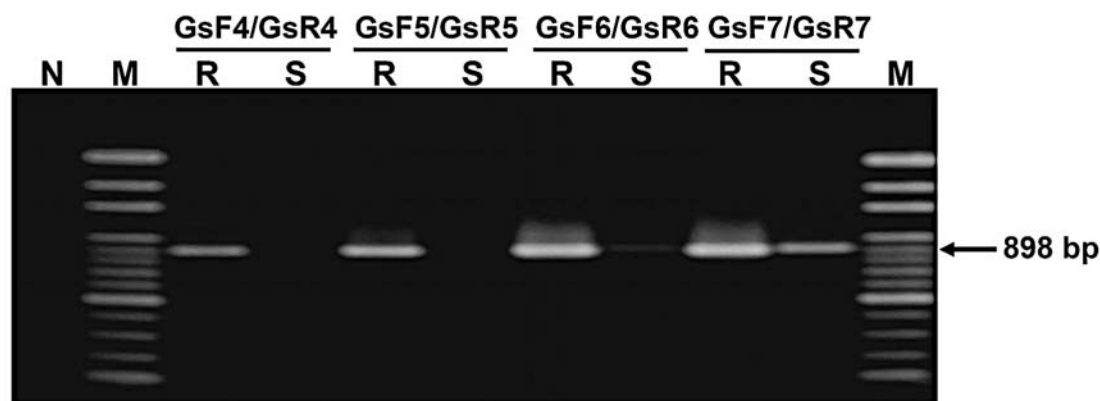
SCAR PCR analysis of twelve Fon-susceptible cultivars and seven Fon-resistant lines

In order to determine if the SCAR primer set GsF5/R5 is beneficial to the screening of Fon-resistant lines, gDNA samples extracted from twelve Fon-susceptible cultivars and seven Fon-resistant lines were subjected to PCR assay with the SCAR primer set GsF5/GsR5. This primer set did not generate a corresponding OPG05₈₉₈ band in the gDNA of any of the 12 Fon-susceptible cultivars (Figure 4). In addition, the OPG05₈₉₈ marker was present in the

Table 1. PCR primers used in this study and the corresponding PCR results showing polymorphisms to Fon-resistant JSB and Fon-susceptible SB.

Primer name	Sequence (5′-3′)	Showing polymorphisms		Size (bp)
		JSB (R) ^a	SB (S) ^b	
Random primer				
OP-G05	CTGAGACGGA	+ ^c	- ^d	898
OP-Y16	GGGCCAATGT	+	-	740
UBC25	ACAGGGCTCA	-	+	320
UBC38	CCGGGGAAAA	+	-	350
		-	+	500
		+	-	1150
UBC52	TTCCCGGAGC	+	-	980
UBC53	CTCCCTGAGC	+	-	750
		+	-	1000
		+	-	1400
G-SCAR primer				
GsF4/GsR4	CTGAGACGGAGCAA/	+	-	898
	CTGAGACGGAGTGT			
GsF5/GsR5	CTGAGACGGAGCAAA/	+	-	898
	CTGAGACGGAGTGTT			
GsF6/GsR6	CTGAGACGGAGCAAAA/	+	M ^c	898
	CTGAGACGGAGTGTTT			
GsF7/GsR7	CTGAGACGGAGCAAAAC/	+	+	898
	CTGAGACGGAGTGTTTC			
RPS10F/RPS10R	AGGCTCACCTAAAAGAAGG/	+	+	486
	GGTCAACACAAGGATCTTACT			

^aR: resistant; ^bS: susceptible; ^c+: distinct fragments present; ^d-: distinct fragments absent; ^eM: faint fragment present.

**Figure 2.** Ethidium bromide-stained patterns of SCAR PCR products using genomic DNA of JSB (R, Fon-resistant) and SB (S, Fon-susceptible) lines as templates. In each PCR, 0.4 μ M SCAR (GsF-/GsR-) primers and 50 ng gDNA were added in a 50 μ L reaction containing 1 \times reaction mixture with *Taq* DNA polymerase as described in Materials and Methods. The location of corresponding OPG05₈₉₈ marker is indicated on the right. N, negative control using sterile dH₂O as the PCR template. M, molecular markers of Gen-100 DNA ladder (GeneMark Tech. Co., Ltd., Tainan, Taiwan).

corresponding SCAR PCR products of all the DNA samples of Fon-resistant lines (Figure 5).

DISCUSSION

The main method of protecting plants from *F. oxysporum* penetration is using resistant cultivars for

planting (Diener and Ausubel, 2005). Therefore, a more efficient breeding program for resistant watermelons is necessary. In addition, DNA markers associated with resistance or tolerance to *Fusarium* wilt, could be used to rapidly assay large numbers of individual melon plants to ascertain the introgression of resistance (Joobeur et al., 2004). In this study, a G05-SCAR primer set GsF5/GsR5

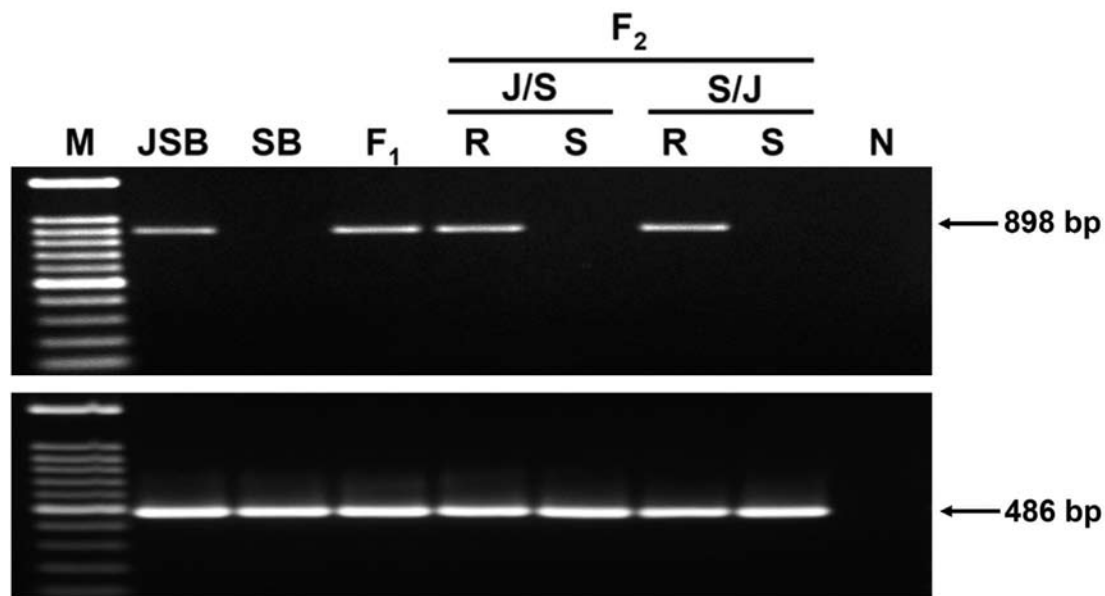


Figure 3. Ethidium bromide-stained patterns of SCAR PCR products using genomic DNA bulks of Fon-resistant (R) and -susceptible (S) hybrid populations of JSB and SB lines as templates. Equal amounts of gDNA from JSB, SB, 10 F_1 hybrids, and two crossing directions [JSB \times SB (J/S) and SB \times JSB (S/J)] of individual F_2 hybrids were pooled as corresponding DNA bulks according to Fon-resistant or -susceptible characteristics (as described in Materials and Methods.). The high-fidelity PCR was performed using the *PfuTurbo*[®] DNA polymerase with primer set GsF5/GsR5 (upper panel). In addition, the RPS10F/RPS10R primer set was used to amplify a 486-bp DNA fragment of the watermelon *Ribosomal protein S10* gene as a positive control on PCR assay with *Taq* DNA polymerase (lower panel). The locations of the 898-bp and 486-bp DNA bands associated with populations resistant to Fon and watermelons, respectively, are indicated on the right. N, negative control using sterile dH₂O as the PCR template. M, Gen-100 DNA ladder (GeneMark Technology Co., Ltd., Tainan, Taiwan).

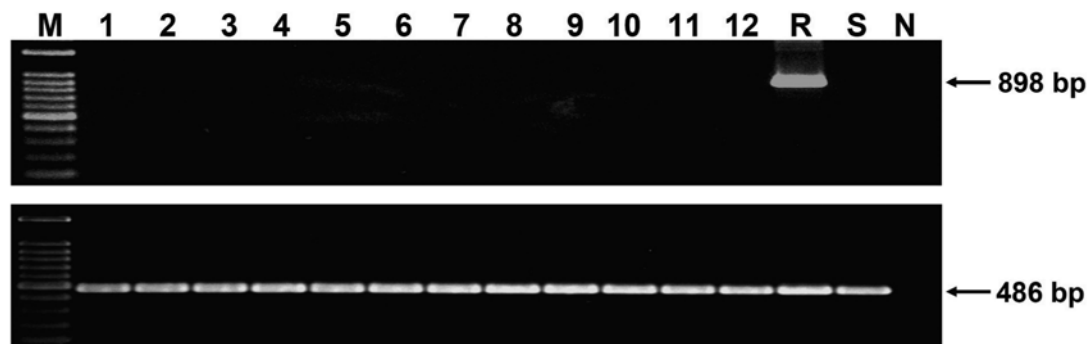


Figure 4. Ethidium bromide-stained patterns of SCAR PCR products using the genomic DNA of twelve commercial Fon-susceptible cultivars as templates. The tested gDNA samples were extracted from *Fusarium* wilt-susceptible Fine Light (lane 1), New Dragon (lane 2), Showing (lane 3), Empire No. 2 (lane 4), New Orchid (lane 5), Big Pink Orchid (lane 6), China Baby (lane 7), Dark Bell (lane 8), Red Delicious (lane 9), Cathay Belle (lane 10), New Crown (lane 11), and New Yellow Baby (lane 12) cultivars. The PCR results of JSB (R, Fon-resistant) and SB (S, Fon-susceptible) were also shown. The primers used for PCR with *Taq* DNA polymerase were the optimal primer set GsF5/GsR5 (upper panel) and the RPS10F/RPS10R primer set (lower panel) which generated a 486-bp DNA fragment of the watermelon *Ribosomal protein S10* gene as a positive control. The locations of the 898-bp and 486-bp DNA bands associated with JSB and watermelons, respectively, are indicated on the right. N, negative control using sterile dH₂O as the PCR template. M, Gen-100 DNA ladder (GeneMark Technology Co., Ltd., Tainan, Taiwan).

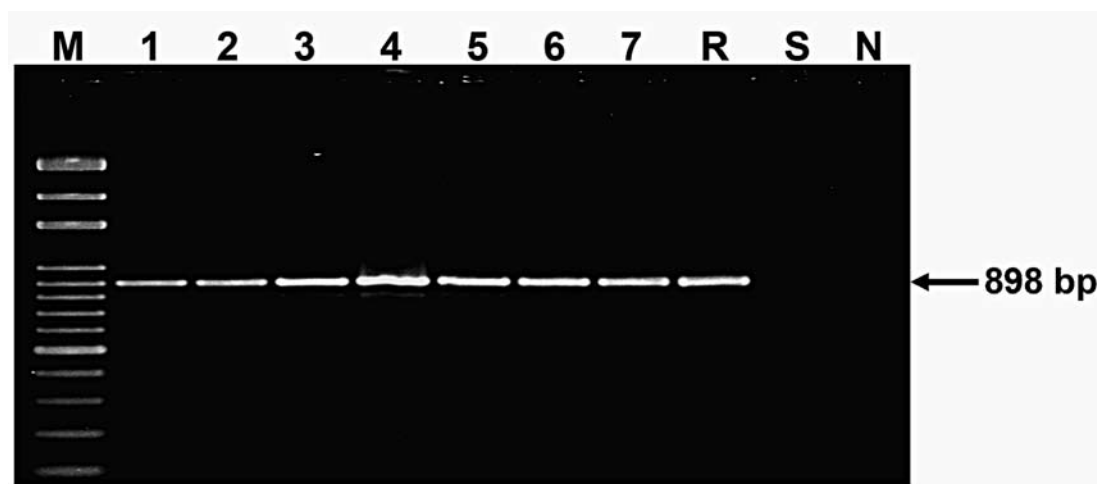


Figure 5. Ethidium bromide-stained patterns of SCAR PCR products using genomic DNA of seven Fon-resistant lines as templates. The tested gDNA samples were extracted from the Fusarium wilt-resistant Charlee (lane 1), Calhoun Gray (lane 2), Dixielee (lane 3), Charleston Gray (lane 4), Crimson Sweet (lane 5), Sugarlee (lane 6), and PI296341 (lane 7) lines. The PCR results of JSB (R, Fon-resistant) and SB (S, Fon-susceptible) were also shown. The primers used for PCR with *Taq* DNA polymerase were the optimal primer set GsF5/GsR5. The location of 898-bp DNA bands associated with watermelons resistant to Fon is indicated on the right. N, negative control using sterile dH₂O as the PCR template. M, Gen-100 DNA ladder (GeneMark Technology Co., Ltd., Tainan, Taiwan).

designed from the random primer OP-G05 amplified fragment (JSB-OPG05₈₉₈) sequence of JSB was developed to rapidly identify DNA bulks of Fon-resistant hybrids by PCR amplification (Figure 3). Furthermore, the corresponding OPG05₈₉₈ markers were not only present in the DNA of seven Fon-resistant lines (Figure 5) but also absent in that of twelve commercial Fon-susceptible cultivars (Figure 4). Therefore, the SCAR primer set GsF5/GsR5 seems to be a good tool to widely screen Fon-resistant lines by breeders.

PCR products similar to OPG05₈₉₈ could be generated by SCAR primer sets GsF6/GsR6 (16 nt each) and GsF7/GsR7 (17 nt each) in the PCR of both Fon-resistant JSB and Fon-susceptible SB DNA samples (Figure 2). These results suggested that the major difference of corresponding OPG05₈₉₈ fragments in these two lines was only in the first 10–15 nt or so. In addition, using other PCR primer sets designed from the sequence of JSB-OPG05₈₉₈ in between the random primer sites, we were unable to differentiate Fon-resistant JSB from Fon-susceptible SB lines. Actually, the sequences of the PCR products amplified from both Fon-resistant JSB and Fon-susceptible SB DNA samples by the GsF7/GsR7 primer set were almost identical except for an 18-nt insertion in the DNA fragment of the SB sample and a base substitution (data not shown). It is possible that these fragments are repeating sequences, and thus only small differences were found in the amplified fragments of JSB and SB samples when using GsF7/GsR7 primers. Therefore, in this study, we used the SCAR PCR technique to develop a molecular method to screen watermelons for Fusarium wilt resistance.

In this study, we used a BSA approach to accelerate the development of a SCAR PCR protocol. It provided

a rapid method to differentiate the Fon-resistant hybrid populations of JSB and SB lines (Figure 3). This approach is a highly efficient identification tool to screen molecular markers, especially when dealing with a single dominant gene (Liebenberg and Pretorius, 2004). In our study, we found it easy to amplify non-specific DNA bands in PCR using *Taq* DNA polymerase, especially for our bulked DNA samples. We therefore used *Pfu* DNA polymerase to perform PCR for our bulked DNA samples (Figure 3) because the proofreading activity of this enzyme could increase PCR specificity.

The genetic mechanisms of race-cultivar specificity in *F. oxysporum* are largely unknown, mainly due to the lack of a sexual stage in this fungus, which prevents genetic analysis (Huertas-González et al., 1999). However, according to the inoculation test, all F₁ plants were resistant to Fusarium wilt, and the F₂ population of SB × JSB, as well as the reciprocal crosses (JSB × SB), showed a 3:1 (resistant:susceptible) ratio. Therefore, the Fusarium wilt resistance of JSB can be controlled by a single dominant gene (Chen et al., 2003b), and BSA was thus used in this study.

The JSB line was originally derived from a sibling-crossed population of a mutant SB line, and in terms of horticulture traits, there are almost no microscopic differences in these two lines, except for the Fusarium wilt resistance (Chen et al., 2003a). That is why we obtained only eight markers associated with JSB (OPG05₈₉₈, OPY16₇₄₀, UBC38₃₅₀, UBC38₁₁₅₀, UBC52₉₈₀, UBC53₇₅₀, UBC53₁₀₀₀, and UBC53₁₄₀₀) and two markers associated with SB (UBC25₃₂₀, UBC38₅₀₀) (Table 1) from RAPD assays tested with a total of 300 different random primers. In addition, we used the AFLP (amplified fragment length polymorphism; originally designed by Vos et al., 1995)

technique to screen more DNA markers associated with Fon-resistant JSB. In our preliminary results, total 42 selective primers were used to perform AFLP analysis, but only two AFLP markers associated with Fon-susceptible SB were identified. These results demonstrated again that the genetic backgrounds of the SB and JSB lines are very similar.

In conclusion, in this study, we developed a SCAR-PCR system for rapid differentiation of Fon-resistant watermelons, and it may be further used for screening Fon-resistant lines to accelerate a molecular breeding program for watermelon. We will later screen more molecular markers associated with JSB, and those markers tightly linked to *Fusarium* wilt resistance will be used to isolate the *Fusarium* wilt resistance gene of JSB.

Acknowledgements. We are grateful to Drs. P. Peter Ueng and Yau-Wen Yang for critical reading and suggestions on this manuscript. This research was supported in part by a grant from the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taiwan, ROC (90AS-2.1.2-BQ-B7); by grants from the National Science Council, Taiwan, ROC (NSC 91-2313-B-005-094, NSC 92-2313-B-005-054, and NSC 93-2313-B-005-080); by the Ministry of Education, Taiwan, ROC under the ATU plan; and by National Chung Hsing University.

LITERATURE CITED

- Chen, K.S., P.-F.L. Chang, T.D. Liou, and J.W. Huang. 2003a. Identification of physiological races of *Fusarium oxysporum* f. sp. *niveum* and breeding for *Fusarium* wilt-resistant watermelon line. *Plant Pathol. Bull.* **12**: 247-254.
- Chen, K.S., T.D. Liou, P.-F.L. Chang, and J.W. Huang. 2003b. Selection for resistance of watermelon varieties (lines) to *Fusarium* wilt and their genetic analysis of inheritance. *Plant Pathol. Bull.* **12**: 173-180.
- Clercq, D.D., A. Ceustermans, M. Heyndrickx, J. Coosemans, and J. Ryckeboer. 2007. A rapid monitoring assay for the detection of *Salmonella* spp. and *Salmonella* Senftenberg strain W775 in composts. *J. Appl. Microbiol.* **103**: 2102-2112.
- COA. 2006. COA yearbook. Council of Agriculture, Executive Yuan, Annal Report of Agricultural Statistics, Taipei, Taiwan.
- Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA mini-preparation: version II. *Plant Mol. Biol. Rep.* **1**: 19-21.
- Dias, A.K.K., S.M. Aoki, J.F. Garcia, and C.M. Nunes. 2007. *Taenia solium* and *Taenia saginata*: Identification of sequence characterized amplified region (SCAR) markers. *Exp. Parasitol.* **117**: 9-12.
- Diener, A.C. and F.M. Ausubel. 2005. *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant Arabidopsis disease-resistance gene, is not race specific. *Genetics* **171**: 305-321.
- FAO. 2006. FAO yearbook (production). Food and Agriculture Organization of the United Nations, Rome.
- Forsyth, L.M., L.J. Smith, and E.A.B. Aitken. 2006. Identification and characterization of non-pathogenic *Fusarium oxysporum* capable of increasing and decreasing *Fusarium* wilt severity. *Mycol. Res.* **110**: 929-935.
- Hawkins, L.K. and F. Dane. 2001. Linkage mapping in a watermelon population segregating for *Fusarium* wilt resistance. *J. Amer. Soc. Hort. Sci.* **126**: 344-350.
- Huertas-González, M.D., M.C. Ruiz-Roldán, A. Di Pietro, and M.I.G. Roncero. 1999. Cross protection provides evidence for race specific avirulence factors in *Fusarium oxysporum*. *Physiol. Mol. Plant Pathol.* **54**: 63-72.
- Huaracha, E., M. Xu, and S.S. Korban. 2004. Narrowing down the region of the *Vf* locus for scab resistance in apple using AFLP-derived SCARs. *Theor. Appl. Genet.* **108**: 274-279.
- Joobeur, T., J.J. King, S.J. Nolin, C.E. Thomas, and R.A. Dean. 2004. The *Fusarium* wilt resistance locus *Fom-2* of melon contains a single resistance gene with complex features. *Plant J.* **39**: 283-297.
- Juchum, F.S., J.B. Leal, L.M. Santos, M.P. Almeida, D. Ahnert, and R.X. Corrêa. 2007. Evaluation of genetic diversity in a natural rosewood population (*Dalbergia nigra* Vell. Allemão ex Benth.) using RAPD markers. *Genet. Mol. Res.* **6**: 543-553.
- Liebenberg, M.M. and Z.A. Pretorius. 2004. Inheritance of resistance to *Uromyces appendiculatus* in the South African dry bean cultivar Kranskop. *S. Afr. J. Plant Soil.* **21**: 245-250.
- Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* **88**: 9828-9832.
- Nash, S.M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* **52**: 567-572.
- Ngezahayo, F., Y. Dong, and B. Liu. 2007. Somaclonal variation at the nucleotide sequence level in rice (*Oryza sativa* L.) as revealed by RAPD and ISSR markers, and by pairwise sequence analysis. *J. Appl. Genet.* **48**: 329-336.
- Notz, R., M. Maurhofer, H. Dubach, D. Haas, and G. Défago. 2002. Fusaric acid-producing strains of *Fusarium oxysporum* alter 2,4-Diacetylphloroglucinol biosynthetic gene expression in *Pseudomonas fluorescens* CHA0 in vitro and in the rhizosphere of wheat. *Appl. Environ. Microbiol.* **68**: 2229-2235.
- 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.
- Shi, J., W. Mueller, and C.H. Beckman. 1991. Ultrastructural responses of vessel contact cells in cotton plants resistant or susceptible to infection by *Fusarium oxysporum* f. sp. *vasinfectum*. *Physiol. Mol. Plant Pathol.* **38**: 211-222.
- Vos, P., R. Hogers, M. Bleeker, M. Reijmans, T. van der Lee,

- M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* **23**: 4407-4414.
- 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.
- Wechter, W.P., M.P. Whitehead, C.E. Thomas, and R.A. Dean. 1995. Identification of a randomly amplified polymorphic DNA marker linked to the *Fom 2* *Fusarium* wilt resistance gene in muskmelon MR-1. *Phytopathology* **85**: 1245-1249.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**: 6531-6535.
- Xu, Y., H.-Y. Zhang, G.-B. Kang, Y.-J. Wang, and H. Chen. 2000. Studies of molecular marker-assisted-selection for resistance to *Fusarium* wilt in watermelon (*Citrullus lanatus*) breeding. *Acta Genet. Sin.* **27**: 151-157 (In Chinese).
- Zhang, Z., J. Zhang, Y. Wang, and X. Zheng. 2005. Molecular detection of *Fusarium oxysporum* f. sp. *niveum* and *Mycosphaerella melonis* in infected plant tissues and soil. *FEMS Microbiol. Lett.* **249**: 39-47.

西瓜抗蔓割病品系之快速分子檢測技術之開發

林盈宏¹ 陳甘澍² 劉政道² 黃振文¹ 張碧芳¹

¹ 國立中興大學 植物病理學系

² 行政院農業委員會農業試驗所 鳳山熱帶園藝試驗分所

西瓜蔓割病係由 *Fusarium oxysporum* f. sp. *niveum* 所引起，為世界西瓜生產的重要限制因子之一。本病屬於維管束萎凋病，防治頗為困難，惟目前最好的防治方法即是選育及栽培抗病品種（系）。為提昇西瓜抗蔓割病選育之效率，本研究利用聚合酵素連鎖反應 (polymerase chain reaction) 技術搭配 GsF5/GsR5 引子對，用以快速判別抗病之西瓜品系。GsF5/GsR5 係由 OP-G05 隨機引子之增幅片段序列配合 SCAR (sequence characterized amplified region) 技術所設計之引子對，其增幅出之 898 bp 核酸片段對抗蔓割病之西瓜品系具專一性。本分子檢測法不僅容易快速判別西瓜雜交族群對蔓割病菌之不同感受性外，尚可用於判別西瓜商業品種的抗病與感病性。

關鍵詞： SCAR；分子標誌；分子檢測；西瓜；西瓜蔓割病菌；育種；聚合酵素連鎖反應；隨機增幅核酸多型性分析；鐮孢菌萎凋病。