J. Microbiol. Biotechnol. (2006), **16**(5), 724–730

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

SCAR Marker Linked with A1 Mating Type Locus in *Phytophthora infestans*

ZHANG, XUAN-ZHE, HYO-WON SEO[1], WON-GYEONG AHN[1], AND BYUNG-SUP KIM*

Department of Applied Plant Science, Kangnung National University, Gangneung, Gangwon-do 210-702, Korea
[1]Crop Division, National Institute of Highland Agriculture, Pyeongchang Gangwon-do 232-955, Korea

Received: October 11, 2005
Accepted: January 21, 2006

Abstract A sequence characterized amplified region (SCAR) marker, which was tightly linked with the A1 mating type locus in *Phytophthora infestans*, was developed. During the random amplified polymorphic DNA-based phylogenic studies of 33 isolates of *P. infestans* collected from year 2002 to 2004, we found an A1 mating type-specific DNA fragment. This 573-bp DNA fragment was generated only in the genomic DNA of the A1 mating types, when OPC-5 primer was used. Based on the specific DNA sequence, we designed the primer sets for generating the A1 mating type-specific 569-bp DNA fragment. When 33 genomic DNAs of *P. infestans* were subjected to PCR amplification using different primer combinations, the A1 mating type-specific DNA was amplified, when LB-1F and LB-2R primers were used. The specific 569-bp DNA fragment was generated only from all 18 A1 strains, but not from 15 A2 mating type strains. These results corresponded to the mating type discriminating bioassay of 33 isolates of *P. infestans*. Therefore, the primer combination of LB-1F/LB-2R was chosen as a SCAR marker. Overall, this study indicates that the SCAR marker could be developed into a useful tool for mating type determination of *P. infestans*.

Key words: *Phytophthora infestans*, mating type, SCAR marker

Late blight of potato and tomato, caused by *Phytophthora infestans*, is an extremely destructive disease, which can destroy potato fields in just a few days, when the condition is appropriate for disease incidence. The pathogen requires two mating types, A1 and A2, for sexual reproduction. Until the 1980s, the A2 mating type was restricted to central Mexico, and only the A1 mating type was found to be distributed worldwide [9]. Central Mexico is believed to be the center of origin of *P. infestans* [8]. Hohl and Iselin [17] reported the appearance of A2 isolates in Switzerland in 1981 for the first time. Since then, a number of reports have revealed that A2 mating types occur in many countries [2, 31]. In Korea, A2 mating type strains of *P. infestans* have been reported by Koh *et al.* [27] and So and Lee [36].

The two mating types of *P. infestans*, A1 and A2, are distinguished by the production of a specific pheromone and their response to the pheromone of opposite type [1, 26]. Sexual reproduction occurs in this heterothallic oomycete when individuals of opposite mating type come in contact, producing oospores that can survive in soil in the absence of a host [6, 34]. Sexual reproduction of *P. infestans*, associated with genetic recombination during meiosis in the antheridium or the oogonium, is a major mechanism of genetic variation in this diploid organism. The genetic mechanism for differentiating the A1 and A2 types in *Phytophthora* has been the subject of much speculation, particularly since oomycetes are diploid; therefore, mating-type alleles potentially operate in a heterozygous condition. However, other mechanisms of genetic variability may also have a significant role in creating new variants of this group of pathogens: Mutation, mitotic recombination, and parasexual recombination are the most common mechanisms of genetic variability in the absence of sexual reproduction [11].

Mating is important for fungal survival, because this increases the genetic fitness and diversity of *Phytophthora* species. Sexual spores produced from the mating process are often thick walled, and therefore more durable than vegetative structures. The genetic basis of mating type in *Phytophthota* has been most thoroughly addressed with *P. infestans*.

Judelson *et al.* [19, 20] initially identified B1, S1, and M15 markers via random amplified polymorphic DNA (RAPD) analysis, in which they were cloned and used to detect restriction fragment length polymorphism (RFLP) in hybridization assays or sequenced to develop allele-specific PCR polymorphisms that could be scored in a

*Corresponding author
Phone: 82-33-640-2353; Fax: 82-33-647-9535;
E-mail: bskim@kangnung.ac.kr

mapping population. Because the RFLP technique has excellent reproducibility, it has been used to analyze the genetic diversity of *P. infestans* by RG57 probe [6] and to identify many specific genes expression [3, 22–25, 29].

To overcome the reproducibility problem associated with the RAPD technique, RAPD markers have been converted to sequence characterized amplified region (SCAR) [32]. A number of SCAR markers have been developed for plant pathogenic fungi, crops and woody species such as *Erysiphe pisi* [18], lettuce [32], and apple [7].

Although amplified fragment length polymorphism (AFLP) and RFLP techniques have excellent reproducibility and consistency, these techniques are time-consuming and also require well-trained hands. Once the useful polymorphic DNAs in RAPD or AFLP were identified, they can easily be converted into co-dominant SCAR markers after DNA sequencing analysis of the polymorphic DNA fragment. The SCAR primer amplifies only a single locus at high annealing temperatures [21]. Because the SCAR marker involves a simple and easy processing procedure, it could be utilized as a friendly marker.

This study was carried out to identify the specific RAPD product linked with the A1 mating type locus, and to develop a SCAR marker for mating type determination in *P. infestans*.

MATERIALS AND METHODS

Source of Strains

The 16 A1 mating type and 13 A2 mating type isolates used in this study were isolated from potato and tomato leaflets that were collected from commercial fields and research stations in various locations of Korea from years 2002 to 2004. Standard isolates, DN3085 (A1) and TK301 (A2), were kindly provided by Dr. K. Y. Ryu (Rural Development Administration), and standard isolates KA-2 (A1) and BC-3 (A2) were isolated from potato and tomato plants in Korea. Fungi were isolated by placing potato tuber slices on diseased leaf fragments in Petri dishes, and then incubating them at 22°C. After 5–7 days, fungi grew on the surface of the tuber slices. Each isolate was derived by transferring hyphal tips on a selective medium of V-8 juice agar (200 ml of V-8 juice, 4.5 g of calcium carbonate, 20 g of agar, 800 ml of distilled water) containing 500 μg/ml of ampicillin, 200 μg/ml of vancomycin, 50 μg/ml of rifampicin, 100 μg/ml of pimaricin, 35 μg/ml of PCNB, and 10 μg/ml of benomyl. The purified isolates were maintained on V-8 juice rye agar medium at 22°C.

Mating-Type Determination

To determine the mating type of an isolate, a mycelial agar disk (7 mm in diameter) was cut from the colony edge of *P. infestans* that were grown on V-8 juice agar for 7–10 days. The agar disk of unknown mating type was placed at the center of the medium, and both standard A1 and A2 mating type isolates of *P. infestans* were then placed 3 cm apart from the center on opposite sides. The presence of oospores in the contact zone between standard and unknown isolates was examined to indicate sexual reproduction and to determine the mating type.

Genomic DNA Extraction and RAPD Analysis

All isolates of *P. infestans* collected from years 2002 to 2004 were grown in rye broth medium (500 ml of rye broth and 2.3 g of calcium carbonate), and the mycelia were harvested and freeze-dried. Total genomic DNA of each isolate was extracted by the method described by Goodwin *et al.* [12] with some modifications. The concentration of the RNase-treated DNA was determined by spectrophotometry. Based on the diversity of RAPD profiles and specific band markers linked with specific mating type, the OPC-5 primer was selected for determination of mating type among the 60 QIAGEN Operon primers, which were tested as described by Zhang *et al.* [37]. To identify the specific DNA product linked to A1 mating type loci generated by the OPC-5 primer (5'-GATGACCGCC-3'), RAPD DNA fingerprinting was performed for 33 isolates selected randomly among the total isolates described by Zhang *et al.* [37]. DNA amplification was performed in an GeneAmp PCR System (Applied Bioscience Inc., U.S.A.) with one cycle of initial denaturation at 94°C for 5 min, 36°C for 2 min, and 72°C for 2 min; 35 cycles of amplification at 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min; and a final cycle at 72°C for 10 min. Reactions were carried out in a 25 μl volume containing 1× *Taq* DNA polymerase buffer (10 mM Tris-HCl, 40 mM KCl, 1.5 mM $MgCl_2$, pH 9.0), 0.2 mM each of dNTPs, 0.4 μM primer, 1 U of *Taq* DNA polymerase, and 20 ng each of template DNA. Amplification products were separated on 1.2% agarose gels containing 0.5 μg/ml of ethidium bromide at a constant 60 V in 1× TBE buffer for 1 h at room temperature. DNA fragments were visualized on a UV transilluminator (Ultra-Violet Products Ltd., Cambridge, U.K.) at 302 nm, and the images were detected by autoradiography.

Subcloning and Sequencing of the A1 Mating Type-Specific Amplicon

After gel electrophoresis, an approx. 0.6 kb A1 mating type-specific DNA fragment of JBD 2-1 isolate was cut out from the agarose gel. The specific DNA fragment was extracted using an Agarose Gel DNA Extraction Kit (Roche, Germany), subcloned into the pCRII-TOPO cloning vector (Invitrogen, U.S.A.), and finally transformed into *E. coli* DH5α. The recombinant plasmids were extracted by using an Accuprep™ Plasmid Extraction Kit (Bioneer, Korea). After size confirmation, the insert DNA was sequenced by using an Accuprep™ DNA Sequencing Kit (Bioneer, Korea). The sequence of specific RAPD fragment was compared

by database information from NCBI (National Center for Biotechnology Information) using the BLAST search program.

Designing SCAR Primers and Amplification of Genomic Regions

Based on the sequences of a specific fragment (573 bp) of *P. infestans* A1 mating type isolates, 4 pairs of oligonucleotide primers (Table 2) were designed using Primer 3 ver 0.6 [35] and used for the selection of primers specific to the A1 mating type of *P. infestans*. PCR condition was one cycle of initial denaturation at 94°C for 5 min; 40 cycles of amplification at 94°C for 30 sec, 67°C for 30 sec, and 72°C for 30 sec; and full extension at 72°C for 7 min. Reactions were carried out in a 20 μl volume containing 1× *Taq* DNA polymerase buffer (10 mM Tris-HCl, 40 mM KCl, 1.5 mM $MgCl_2$, pH 9.0), 0.2 mM each of dNTP, 0.4 μM primer, 1 U of *Taq* DNA polymerase, and 20 ng each of template DNA. Amplification products were separated on 1.2% agarose gels containing 0.5 μg/ml of ethidium bromide at a constant 6 volt/cm in 1× TBE buffer for 40 min at room temperature. DNA fragments were visualized on a UV transilluminator (Ultra-Violet Products Ltd., U.K.) at 302 nm, and documented on Type 667 Polaroid film.

Results and Discussion

Mating-Type Determination

Among the 33 isolates of *P. infestans* used in this study, 18 isolates were A1 mating type, and 15 were A2 mating type. Isolates 1 and 19 were standard isolates in Japan for the determination of A1 and A2 mating types, respectively. Isolates 2 and 31 were typical standard isolates in Korea for A1 and A2 mating types, respectively (Table 1). A2 mating types were isolated in the potato fields of

Table 1. List of *Phytophthora infestans* isolates used in this study.

Isoolates	Pathogen no.	Isolate year	Mating type	Host	Location
DN 3085	1	-	A1	Potato	Japan
KA-2	2	-	A1	Potato	
KJ 20	3	2002	A1	Potato	Kim-je
NW 5	4	2003	A1	Potato	Nom-won
DH 2-6	5	2002	A1	Potato	Pyeong-Chang
SS 20	6	2002	A1	Potato	Je-Ju
AY 1	7	2003	A1	Potato	Nom-won
YY 1	8	2004	A1	Potato	Yang-yang
JP 12	9	2004	A1	Potato	Pyeong-Chang
JJ 5	10	2002	A1	Potato	Je-Ju
PN 1-4	11	2003	A1	Potato	Gang-neung
HJ 5	12	2003	A1	Potato	Gang-neung
HG 2-8	13	2004	A1	Potato	Pyeong-Chang
KD 4	14	2002	A1	Potato	Gang-neung
KJ 18	15	2003	A1	Potato	Kim-je
WS 6-5	16	2003	A1	Potato	Gang-neung
YY 1-5	17	2003	A1	Potato	Yang-yang
JBD 2-1	18	2004	A1	Potato	Gang-neung
TK 301	19	-	A2	Tomato	Japan
1-5-1	20	2002	A2	Tomato	Bu-yeo
MH 8	21	2003	A2	Potato	Mu-an
MH 2	22	2002	A2	Potato	Mu-an
BS 16	23	2003	A2	Potato	Bo-seong
BS 13	24	2003	A2	Potato	Bo-seong
BS 21	25	2003	A2	Potato	Bo-seong
BS 7	26	2002	A2	Potato	Bo-seong
DGL 1-1	27	2002	A2	Potato	Pyeong-Chang
DGL 2-2	28	2002	A2	Potato	Pyeong-Chang
DGL 2-4	29	2002	A2	Potato	Pyeong-Chang
DGL 3-12	30	2002	A2	Potato	Pyeong-Chang
BC-3	31	-	A2	Tomato	Bu-yeo
1-8-3	32	2002	A2	Tomato	Bu-yeo
T 3-5	33	2003	A2	Tomato	Gang-neung

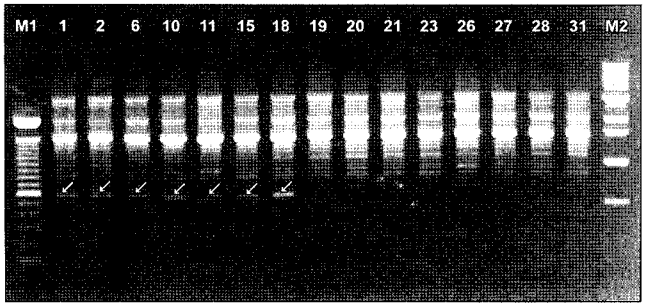

Fig. 1. RAPD profiles of *P. infestans* genomic DNAs amplified by the primer OPC-5.
About 0.6 kb of specific DNA fragments, indicated by arrows, was found only from A1 mating type isolates. Lanes M1 and M2 are 100 bp DNA ladder and 1 kb DNA ladder, respectively. The lane numbers indicate the pathogen numbers listed in Table 1.

```
001 GATGACCGCC TAGGTCCGCG TGCGGAAATA ATCGTTCTGG GAGATCGCGA
051 TTACTTCGCA TTCCTTCACT ATTGCACCGG ACGAACTGGG GCCGCTATTA
101 TCATGACGAG GATGGAAGGT ATTCAAAGCT CATGGGAGCC ATCACAAAAC
151 TCCAAAAGTG AGGTCCAAGC TTTGGTGTCG ATTCGACACT GTGAAGTATG
201 CGTTGATGAA TGGCGTTGAT GAACAGCAGG GAGATAAACT AATTTGTTGG
251 TTATACTTTA ATTCTTTGGT CGGATACAGA TTAGTTTCGT CTCACCCTCG
301 ACGCCTCTCA GATTGCGACT GCGATGCGAC GGTGTGCACT TATAGTGAGC
351 TGTATGTTGG TTGAATAGAG CGCGTTCATC GAGAGCAGCA GGTTTAGAGC
401 AGGAAGTACT CGCTGCTGCA CTGGCACTTA CAAGCATCAT GCCGATGCCG
451 TATGTTAACT CTCTTTTCGC CTCACGAGGA GTTTTAAATA AAGTCTCTGC
501 TTAGGAATCA CGCAATGCCA ATACTACTAC AAAATCGGTC GCTCTGCTTT
551 GTATCTCGTG TCT GATGACCGCC
```

Fig. 2. Nucleotide sequence (GeneIn Accession no. KS112618) of specific DNA fragment linked with the A1 mating type locus in *P. infestans*.
Underlined sequence is OPC-5 primer for the detection of a specific DNA fragment in *P. infestans* isolates of A1 mating type.

PyeongChang-gun of Gangwon-do in 2002, Boseong-gun and MuAn-gun of Jeollabuk-do in 2003, and the tomato fields of Buyeo-gun of Chungcheongnam-do in 2002 and Gangneung-shi of Gangwon-do in 2003. All isolates derived from tomato plants were A2 mating type.

P. infestans, the causal agent of late blight disease of both potato and tomato, has changed considerably in genotypic and phenotypic characteristics in the world [4, 14, 15]. Studies for genotypes of *P. infestans* were performed using isozyme [13], mitochondrial DNA haplotypes [15], RFLP [14], RAPD [30], and AFLP marker [24]. These genotype changes may be due to immigration, sexual crossing, or both. *P. infestans* is heterothallic, having different mating types, A1 and A2. A2 isolates of *P. infestans* collected from most European countries were intermediate or sensitive to metalaxyl [10, 16]. However, the predominance of A2 metalaxyl-resistant isolates was observed in many Canadian [33] and U.S.A. [5] populations. In Korea, A2 mating type isolates of *P. infestans* were found to be metalaxyl resistant [38].

Subcloning and Sequencing of the A1 Mating Type-Specific Amplicon

We have tried to find the genetic markers linked with mating type. During RAPD analysis using the OPC primers, we found that the OPC-5 primer repeatedly produced a 0.6 kb specific DNA fragment only in the A1 mating type isolates of *P. infestans* (Fig. 1). After subcloning the A1 mating type-specific DNA fragment of the JBD 2-1 isolate into the pCRII-TOPO vector, the DNA sequence of the RAPD fragment was determined to be 573 bp in length (Fig. 2). DNA sequence identity analysis using BLAST program indicated that there was no significant sequence homologue in the GenBank database.

Mating-Type Determination of *P. infestans* by SCAR Primer

Based on the sequence of the 573-bp RAPD fragment, we designed four pairs of primers and used these to distinguish two mating types of *P. infestans* by PCR amplification. The nucleotide sequence, length, annealing temperature, and G+C content of each primer set are listed in Table 2. The third forward primer was designed by extending the 10 bases of original RAPD primer (5'-GATGACCGCC-3') with the next 11 nucleotides of DNA sequences toward the 3' ends. The third reverse primer was designed by eliminating 4 bases of the 5' end of the original RAPD primer and adding the next 15 nucleotides toward the 3' end to the original 6 bases.

When genomic DNA of *P. infestans* and the first and second primer combination (Table 2) were used for PCR

Table 2. Four combinations of sequence characterized amplified region (SCAR) primers used in this study.

Primers	Sequence (5'-3')	Region of base	Annealing temperature (Tm; °C)	Length (base)	G+C content (%)
LB-1F	5'-GATGACCGCCTAGGTCCGCGT-3'	1–21	64.0	21	66.7
LB-1R	5'-GATGACCGCCAGACACGAGATA-3'	552–573	58.5	22	54.5
LB-2F	5'-ATGACCGCCTAGGTCCGCGT-3'	2–21	62.4	20	65
LB-2R	5'-ACCGCCAGACACGAGATACAA-3'	549–569	56.7	21	52.4
LB-1F	5'-GATGACCGCCTAGGTCCGCGT-3'	1–20	64.0	21	66.7
LB-2R	5'-ACCGCCAGACACGAGATACAA-3'	549–569	56.7	21	52.4
LB-3F	5'-TTAATTCTTTCGGTCGGATA-3'	258–276	55.01	20	35.0
LB-3R	5'-GCGAGTACTTCCTGCTCTAA-3'	394–413	55.03	20	50.0

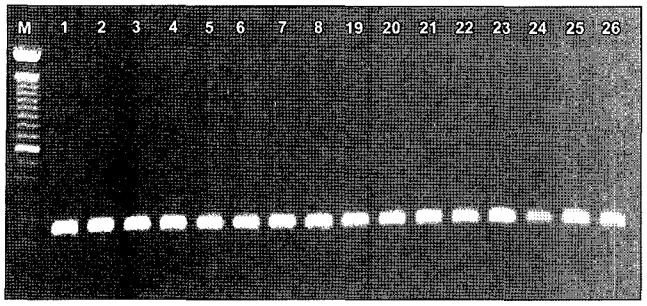

Fig. 3. Profiles of the genomic DNAs from different isolates of *P. infestans*, amplified by primers LB-3F and LB-3R.
The 156-bp fragments were generated on A1 and A2 isolates. Lane M is a 1-kb DNA ladder. The lane numbers indicate the pathogen numbers listed in Table 1.

amplification, the specific DNA fragment was not detected from most of A2 and some of A1 strains (data not shown). However, 156-bp DNA fragments appeared from the genomic DNA of *P. infestans* with A1 and A2 mating type strains, when amplified using the fourth primers LB-3F and LB-3R combination (Table 2 and Fig. 3). It would be of interest to investigate whether the specific 156-bp DNA fragment was unique to differentiate *P. infestans* from other *Phytophthora* species. A specific 569-bp DNA fragment was generated only from the A1 mating type when amplified using the third primers LB-1F and LB-2R combination (Table 2 and Fig. 4). This result indicates that only the third primer combination (LB-1F/LB-2R) can distinguish between A1 and A2 mating type isolates of *P. infestans*. Therefore, we finally decided on the third primer combination (LB-1F/LB-2R) as a SCAR marker. Thirty-three genomic DNAs of *P. infestans* were subjected to PCR amplification using the SCAR primer, and as expected, a specific 569 bp of PCR product was detected only from 18 A1 mating type isolates of *P. infestans*, but not from 15 A2 isolates (Fig. 5).

A genetic study on the mating type loci of *P. infestans* has previously been carried out by Judelson *et al.* [19], and 15 mating type loci linked with A1 and A2 were detected.

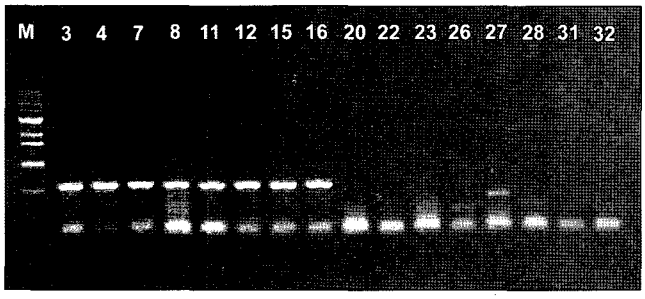

Fig. 4. Amplicon generated with the SCAR primer set LB-1F/LB-2R under optimal PCR condition.
The 569-bp fragments were generated only in the A1 mating type isolates, but not in the A2. Lane M is a 1-kb DNA ladder. The lane numbers indicate the pathogen numbers listed in Table 1.

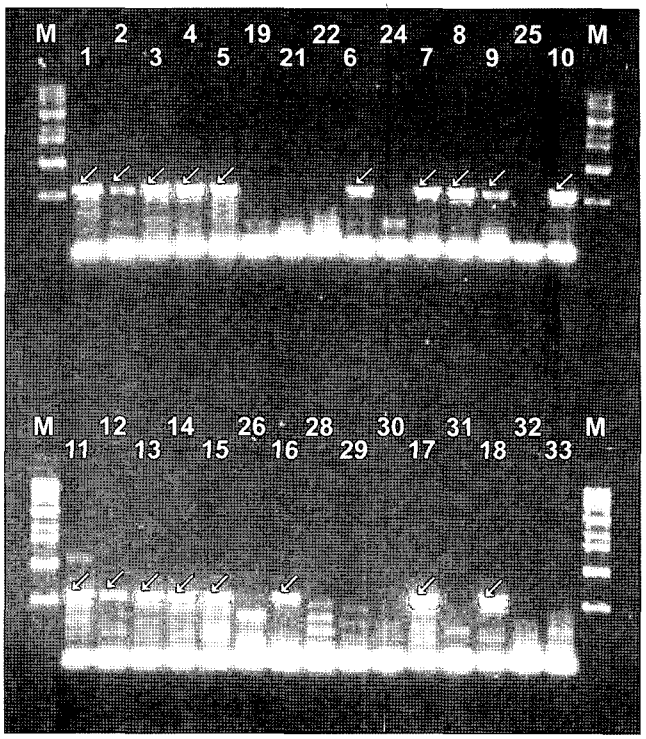

Fig. 5. Profiles of the DNA fragments amplified by the SCAR primer set LB-1F/LB-2R for *P. infestans* isolated from different locations in Korea.
DNA bands indicated by arrows are the A1 mating type-specific DNA fragments. Lane M is a 1-kb DNA ladder.

Therefore, they suggested that the mating type is regulated by a single locus, which displays a pattern of non-Mendelian inheritance.

To facilitate the analysis of many isolates, a PCR assay for determination of mating type of *P. infestans* was developed by using a SCAR primer based on the sequence of the 573-bp specific DNA fragment amplified by RAPD. The specific amplification of the 569-bp DNA fragment from the A1 mating type of *P. infestans* suggested that DNA sequences matching with the SCAR primer do not exist in the original genomic DNA of A2 strains. Furthermore, the specific DNA fragment linked with the A1 mating type was regulated only by a single locus, S1, as described by Judelson *et al.* [20].

In view of its distribution among natural populations of *P. infestans*, the SCAR marker may be a useful tool for studying and managing the late blight diseases in many parts of the world. Recently, the A2 mating type isolates has also been found to be distributed globally, although the two mating types of *P. infestans* were found together only in Central Mexico until the mid-1980s [9]. The potential for sexual recombination in commercial potato and tomato growing regions is significant, since the sexual spores (oospore) are long-lived sources of inoculum, and raises the possibility of developing new genotypes such as new

fungicide-resistant strains and more virulent strains of *P. infestans* [10, 28]. The PCR assay using the SCAR marker could be applied to DNA plant lesions to quickly uncover whether both mating types are present in the same field. This should help implement disease management strategies [10, 28].

REFERENCES

1. Brasier, C. M. 1992. Evolutionary biology of *Phytophthora*. Part: Genetic system, sexuality and the generation of variation. *Annu. Rev. Phytopathol.* **30:** 153–177.
2. Carlisle, D. J., L. R. Cooke, and A. E. Brown. 2001. Phenotypic and genotypic characterization of northern Ireland isolates of *Phytophthora infestans*. *Eur. J. Plant Pathol.* **107:** 291–303.
3. Cho, M. J., Y. K. Kim, and J. O. Ka. 2004. Molecular differentiation of *Bacillus* spp. antagonistic against phytopathogenic fungi causing damping-off disease. *J. Microbiol. Biotechnol.* **14:** 599–606.
4. Cooke, D. E. L., V. Young, P. R. J. Birch, R. Toth, F. Gourlay, J. P. Day, S. F. Carnegie, and J. M. Duncan. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995–1997). *Plant Pathol.* **52:** 181–192.
5. Deahl, K. L., S. P. DeMuth, S. L. Sinden, and A. Rivera-Pena. 1995. Identification of mating types and metalaxyl resistance in North American populations of *Phytophthora infestans*. *Am. Potato J.* **72:** 35–49.
6. Drenth, A., S. B. Goodwin, W. E. Fry, and L. C. Davidse. 1991. Genotypic diversity of *Phytophthora infestans* in The Netherlands revealed by DNA polymorphisms. *Phytopathology* **83:** 1087–1092.
7. Evans, K. M. and C. M. James. 2003. Identification of SCAR markers linked to Pl-w mildew resistance in apple. *Theor. Appl. Genet.* **106:** 1178–1183.
8. Fry, W. E., S. B. Goodwin, J. M. Matuszak, L. J. Spielman, M. G. Milgroom, and A. Drenth. 1992. Population genetics and intercontinental migration of *Phytophthora infestans*. *Annu. Rev. Phytopathol.* **30:** 107–129.
9. Fry, W. E., S. B. Goodwin, A. T. Dyer, J. M. Matuszak, A. Dremth, P. W. Tooley, L. S. Sujkowski, Y. J. Koh, B. A. Cohen, L. J. Spielman, M. G. Milgroom, K. L. Deahl, D. A. Inglis, and K. P. Sandlan. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways and implications. *Plant Dis.* **77:** 653–661.
10. Gisi, U. and Y. Cohen. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. Phytopathol.* **34:** 549–572.
11. Goodwin, S. B. 1997. The population genetics of *Phytophthora*. *Phytopathology* **87:** 462–473.
12. Goodwin, S. B., L. J. Spielman, J. M. Matuszak, S. N. Bergeron, and W. E. Fry. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology* **82:** 955–961.
13. Goodwin, S. B., R. E. Schneider, and W. E. Fry. 1995. Use of cellulose acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Dis.* **79:** 1181–1185.
14. Goodwin, S. B. and W. E. Fry. 1991. Global migration of *Phytophthora infestans*. *Phytopathology* **82:** 955–961.
15. Griffith, G. W. and D. S. Shaw. 1998. Polymorphism in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure culture or from host lesions. *Appl. Environ. Microbiol.* **64:** 4007–4014.
16. Hermansen, A., A. Hannukkala, R. H. Naerstad, and M. B. Brurberg. 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: Mating type, metalaxyl resistance and virulence phenotype. *Plant Pathol.* **49:** 11–22.
17. Hohl, H. R. and K. Iselin. 1984. Strains of *Phytophthora infestans* with the A2 mating type behaviour. *Trans. Br. Mycol. Soc.* **83:** 529–530.
18. Janila, P. and B. Sharma. 2004. RAPD and SCAR markers for powdery mildew resistance gene *er* in pea. *Plant Breeding* **123:** 271–274.
19. Judelson, H. S., L. J. Spielman, and R. C. Shattock. 1995. Genetic mapping and non-Mendelian segregation of mating-type loci in the oomycete, *Phytophthora infestans*. *Genetics* **141:** 503–512.
20. Judelson, H. S. 1996. Chromosomal heteromorphism linked to the mating type locus of the oomycete *Phytophthora infestans*. *Mol. Gen. Genet.* **252:** 155–161.
21. Kim, C. S., G. P. Lee, D. H. Han, K. H. Ryu, and C. H. Lee. 2000. SCARs markers derived from RAPD for cultivar identification in *Pyrus pyrifolia*. *J. Kor. Soc. Hort. Sci.* **41:** 125–128.
22. Kim, E. J., D. J. Kim, H. Y. Hwang, J. S. Yoon, Y. Yoon, and K. H. Baek. 2004. High-level expression of recombinant human interleukin-2 in Chinese hamster ovary cells using the expression system containing transcription terminator. *J. Microbiol. Biotechnol.* **14:** 810–815.
23. Kim, K. S. and Y. S. Lee. 2000. Rapid and accurate species-specific detection of *Phytophthora infestans* through analysis of ITS regions in its rDNA. *J. Microbiol. Biotechnol.* **10:** 651–655.
24. Kim, K. J., S. H. Eom, S. P. Lee, H. S. Jung, S. Kamoun, and Y. S. Lee. 2005. A genetic marker associated with the A1 mating type locus in *Phytophthora infestans*. *J. Microbiol. Biotechnol.* **15:** 502–509.
25. Kim, Y. T., B. K. Park, E. I. Hwang, N. H. Yim, S. H. Lee, and S. U. Kim. 2004. Detection of recombinant marker DNA in genetically modified glyphosate-tolerant soybean and use in environmental risk assessment. *J. Microbiol. Biotechnol.* **14:** 390–394.
26. Ko, W. H. 1988. Hormonal heterothallism and homothallism in *Phytophthora*. *Annu. Rev. Phytopathol.* **26:** 57–73.
27. Koh, Y. J., H. J. Chung, and W. E. Fry. 1994. Changes in frequencies and distribution of A2 mating types and metalaxyl-resistant isolates of *Phytophthora infestans* in Korea. *Korean J. Plant Pathol.* **10:** 92–98.

28. Lee, T. Y., E. Mizubuti, and W. E. Fry. 1999. Genetics of metalaxyl resistance in *Phytophthora infestans*. *Fungal Genet. Biol.* **26:** 118–130.
29. Lee. Y. S. and S. P. Lee. 2002. Development of rapid molecular detection marker for *Colletotrichum* spp. in leaf and fruit tissues of sweet persimmon. *J. Microbiol. Biotechnol.* **12:** 989–992.
30. Mahuku, G., R. D. Peters, H. W. Platt, and F. Daayf. 2000. Random amplified polymorphic DNA (RAPD) analysis of *Phytophthora infestans* isolates collected in Canada during 1994 to 1996. *Plant Pathol.* **49:** 252–260.
31. Mosa, A. A., M. Kato, N. Sato, N. K. Kobayashi, and A. Ogoshi. 1989. Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Japan. *Ann. Phytopath. Soc. Japan* **55:** 615–620.
32. 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.
33. Peters R. D., H. W. Platt, and R. Hall. 1998. Characterization of changes in populations of *Phytophthora infestans* in Canada using mating type and metalaxyl sensitivity markers. *Can. J. Plant Pathol.* **20:** 259–273.
34. Pittis. J. E. and R. C. Shattock. 1994. Viability, germination and infection potential of oospores of *Phytophthora infestans*. *Plant Pathol.* **40:** 387–396.
35. Steve. R. and H. J. Skaletsky. 1997. Primer3. Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.html.
36. So. M. S. and W. H. Lee. 1993. Occurrence of the A2 mating type of the potato late blight pathogen, *Phytophthora infestans*, in Korea. *Korean J. Med. Chem.* **5:** 72–75.
37. Zhang, X. Z., H. Y. Kim, and B. S. Kim. 2006. Analysis of genetic diversity of *Phytophthora infestans* in Korea by using molecular markers. *J. Microbiol. Biotechnol.* **16:** 423–430.
38. Zhang, X. Z., K. Y. Ryu, J. S. Kim, J. U. Cheon, and B. S. Kim. 2005. Changes in the sensitivity to metalaxyl, dimethomorph and ethaboxam of *Phytophthora infestans* in Korea. *Plant Pathol. J.* **21:** 33–38.