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Abstract *Phytopthora* root rot in pepper (*C. annuum*) is caused by *Phytophthora capsici* L., which exhibits a high level of pathogenic diversity. Resistance to this disease is conditioned by a number of quantitative trait loci. Pyramiding resistance alleles is desirable and could be simplified by the use of molecular markers tightly linked to the resistance genes. The purpose of this study was development of molecular markers linked to *Phytophthora* root rot resistance. An F_8 recombinant inbred line (RIL) population derived from a cross between YCM334 and a susceptible cultivar 'Tean' was used in combination with bulk segregant analysis utilizing RAPD and conversion of AFLP markers linked to *Phytophtora* root rot resistance into sequence-characterized amplified region (SCAR) markers. In conversion: one marker was successfully converted into a co-dominant SCAR marker SA133_4 linked to the trait. In bulked segregant analysis (BSA): three RAPD primers (UBC484, 504, and 553) produced polymorphisms between DNA pools among 400 primers screened. Genetic linkage analysis showed that the SCAR and RAPD markers were located on chromosome 5 of pepper. Quantitative trait locus (QTL) analysis showed that the SA133_4 and UBC553 were linked to *Phytophtora* root rot resistance. These markers were correctly identified as resistant or susceptible in nine promising commercial pepper varieties. These markers will be beneficial for marker-assisted selection in pepper breeding.

Keywords Marker-assisted selection · Phytophthora capsici · Marker · Pepper · Resistance

H. T. H. Truong · J. H. Kim · M. C. Cho · S. Y. Chae · H. E. Lee (✉)
Vegetable Crop Research Division, Horticultural Department, National Institute of Horticultural & Herbal Sciense (NIHHS), Rural Development Administration (RDA),
540–41 Tap-Dong, Suwon, South Korea 441-440
e-mail: helee72@korea.kr

H. T. H. Truong (✉)
Department of Horticulture Science, Agronomy Faculty, Hue University of Agriculture and Forestry (HUAF),
102 Phung Hung,
Hue, Vietnam
e-mail: truongthihonghai@huaf.edu.vn

Introduction

Phytophthora capsici L. causing root rot is an important soil-borne pathogen of pepper (*Capsicum annuum*). This soil-borne pathogen is difficult to eradicate because it can overwinter for lengthy periods in the soil (Hausbeck and Lamour 2004), limiting the usefulness of crop rotation as a management strategy (Lamour and Hausbeck 2003). Soil sterilization using methyl bromide was the primary method to control the disease. However, methyl bromide use was banned completely in 2005 (Siebring). The efficacy of fungicides applied as a drench for controlling *Phytophthora* root rot of pepper has been tested (Babadoost 2007; Hausbeck and

Cortright 2007; McGrath and Davey 2007; Matheron and Porchas 2008). Foster and Hausbeck (2010) have used fungicides and host resistance to manage *Phytophtora* crown and root rot in bell pepper. However, using chemicals increases production costs as well as environmental and health risks. The use of resistant cultivars is a simple and effective strategy. In Korea, *P. capsici* resistance breeding programs have been underway in many locations for decades, but no commercial varieties with high levels of resistance are currently available due to the complex inheritance of resistance and difficulty with reproducible phenotypic screening.

Inheritance of resistance to *P. capsici* in pepper has been studied; and specific and quantitative resistances have been reported (Sy et al. 2008; Monroy-Barbosa and Bosland 2008; Lefebvre and Palloix 1996; Thabuis et al. 2003; Truong et al. 2012). In addition, novel physiological *P. capsici* races have been identified (Monroy-Barbosa and Bosland 2011). This, along with the various and complex modes of inheritance, could explain the lack of varieties with high levels of resistance to *Phytophthora* infection across diverse geographical areas. Several resistant sources have been reported (Barksdale et al. 1984); however, *C. annuum* 'Criollo de Morelos-334' (CM334) is the best source of *P. capsici* resistance (Bosland and Lindsey 1991). CM334 shows a very high degree of resistance to different physiological *P. capsici* races (Oelke and Bosland 2003; Sy et al. 2007; Glosier et al. 2008) and even to the most virulent strains (Sy et al. 2005). The resistance from CM334 has been introduced into commercially grown cultivars via traditional breeding. However, the resistance has not been entirely successful due to the large number of physiological races (Monroy-Barbosa and Bosland 2011).

Marker-assisted selection (MAS) has been proposed for many years to facilitate breeding of complex traits such as resistance to *P. capsici* in pepper. However, the limitation is the ability to select for many chromosomal regions at once. One solution to this limitation is to combine phenotypic analysis with MAS for one or a few QTL that are most critical for full recovery of the phenotype of interest. The study reported here was to convert QTL that was particularly critical for resistance to *Phytophtora* root rot in *Capsicum* located in chromosome 5 (Truong et al. 2012). In a previous study, a PCR-based SCAR marker was developed for the detection of the *Phyto.5.2* QTL on chromosome 5 of pepper (Quirin et al. 2005). However, this marker was dominant, and not suitable for MAS, because heterozygous plants cannot be distinguished from homozygotes. Therefore, there was a need to identify a new PCR-based marker linked to *Phytophthora* root rot resistance. In this paper, we report the identification of RAPD markers tightly linked to the trait and conversion of AFLP markers to simple, co-dominant PCR-based marker.

Materials and methods

Plant materials and genomic DNA extraction

A mapping population of 126 F_8 RILs, resistant (YCM334) and susceptible (Tean) parents (Truong et al. 2012) and 21 pepper lines/cultivars were provided by National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Korea. Genomic DNA of the 126 individuals, parents and pepper lines was extracted from young leaves of greenhouse-grown plants following the protocol described by Raz and Ecker (1997).

Evaluation of *Phytophtora* root rot resistance

P. capsici isolate KACC 40158, which belongs to mating type A2 collected from Tamyang, Korea, was provided by Horticultural and Herbal Crop Environment Division, NIHHS, RDA, Korea) and used in current study. The isolates were cultured on V-8 juice agar from 7 to 10 days at 28°C. Mycelium plugs (0.5~1 cm in diameter) were taken from the edge of an actively growing colony and transferred to a new petri dish. Sterile distilled water was added to half of the petri dish, which contained cultured isolates, and then incubated at 28°C for 2 to 3 days under continuous light to promote sporulation. Sporangia in sterile distilled water were harvested and decanted through two to four layers of autoclaved cheesecloth to remove hyphal debris. The sporangial suspension was chilled at 4°C for 1 to 2 h and then transferred to 25°C for 1 h to induce zoospore release. Zoospore suspensions from each isolate were collected. Concentration of zoospore suspension was adjusted to 10^4~10^5 spores/ml using a haemacytometer. Three mililitres of zoospore suspension were irrigated into the soil surface of five-week-old pepper plants. Inoculated plants were scored for disease reactions 7, 14, and 21 days after inoculation, on a scale

of 0 to 5 (0=no symptoms, no necrosis, white/tan roots, healthy shoot; 1=leaf chlorosis but no necrosis; 2=leaf chlorosis, slight necrotic crown; 3=necrotic crown plus severe wilting; 4=severe necrosis, almost dead; 5=dead plant). Plants were scored individually. Plants with a score of 0 or 1 were considered to be resistant; plants with a score of 2 or higher were considered susceptible.

Bulk segregant analysis

An equal amounts of DNA from ten resistant RILs and ten susceptible RILs (Truong et al. 2012) were pooled into an R-pool and an S-pool, respectively, as described by Michelmore et al. (1991). These pools and parents were used to screen RAPD primers. Once DNA bands were found corresponding to the resistant parent and R-pool, or to the susceptible parent and S-pool, the bands were cloned and sequenced.

RAPD analysis

A total of 400 UBC (University of British Columbia) RAPD primers (synthesized by Bioneer, Korea) were screened on the resistant and susceptible parents, and R-and S-pools. The PCR reactions were performed in Eppendorf Mastercycler Gradient (USA). The 15 μl reaction volume included 2.5 mM $MgCl_2$ (Roche, Korea), 200 μM deoxyribonucleotide triphosphate mix (Roche, Korea), 10X PCR buffer, 25 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Genet Bio, Korea), and 0.5 μM random primer and 15–20 ng of genomic DNA. The amplification reactions were carried out using the following thermal profile: 94 °C for 3 min (1 cycle); 94 °C for 1 min, 37 °C for 1 min, 72 °C for 2 min (40 cycles); 72 °C for 7 min (1 cycle). Amplified products were incubated with a 1:10,000 dilution of the SYBR Green I nucleic acid gel stain (Invitrogen, USA) for 20 min and separated on a 1 % agarose gels using 0.5X TBE buffer for 4 h at 120 V and photographed under UV light. A 100 bp ladder was used as a molecular weight marker.

Conversion of AFLP and RAPD markers into SCAR markers

Cloning and sequencing AFLP and RAPD fragments

Three AFLP markers (a057_6, a133_4 and a119_7) linked to *Phytophthora* root rot resistance (Truong et al. 2012) were excised from the polyacrylamide gel with a razor blade. The DNA-containing gel was transferred into an Eppendorf tube, mixed with 50 μl sterile MiliQ water (Human Corporation, Korea), and kept at 4 °C overnight to release the DNA fragment from the gel. After the gel was spun down at 12,000 rpm for 1 min, the DNA-containing supernatant was transferred into a new Eppendorf tube and diluted with sterile MiliQ water (Human Corporation, Korea) in a 2 to 1 ratio and used as template for the subsequent amplification. The Eco*RI*+3 and Mse*I*+3 primers that revealed the polymorphic bands, were used to re-amplify the isolated DNA with the same reaction conditions as for the AFLPs (Truong et al. 2012). The re-amplified products were visualized on an agarose gel as described previously. The AFLP correct-size bands and RAPD fragment that linked to *Phytophtora* root rot resistance were excised from the gel under UV light and transferred into an Eppendorf tube and purified with a QIAquick gel extraction kit (Qiagen, Germany). The fragments were cloned using the TOPO TA Cloning kit following the manufacturer's instructions (Invitrogen, USA). Plasmid DNA was extracted using Core-one plasmid miniprep kit (Korea) and sent to the sequencing company CoreBio (Korea) for sequencing. The sequences were analyzed using the program BioEdit 7.0 (Hall 1999).

Inverse PCR

Inverse PCR (iPCR) primers were designed from resulting AFLP fragment sequences using Primo Inverse 3.4 (http://www.changbioscience.com/primo/primoinv.html). Primers were synthesized by Bioneer Corp. (Korea). The method utilized for performing the iPCR is diagrammed in Fig. 1. The primer sequences are given in Table 1.

Genomic DNA of the resistant parent YCM334 (5 μg) was digested with Eco*RI*, which does not cut the target sequences, overnight at 37 °C using 5 μl of the restriction enzyme buffer, 5 U enzyme and 30 μl of double-distilled water (ddH_2O). The digestion product was purified using QIAquick PCR purification kit (Qiagen, Germany). Ligation was carried out overnight at room temperature containing 10 μl digested DNA, 20 μlT4 DNA ligase buffer, 170 μl ddH_2O and 1 U of T4 DNA ligase. The ligated product was then purified using the QIAquick PCR purification kit (Qiagen, Germany). PCR was then performed. Each reaction contained 15–20 ng of ligation product,

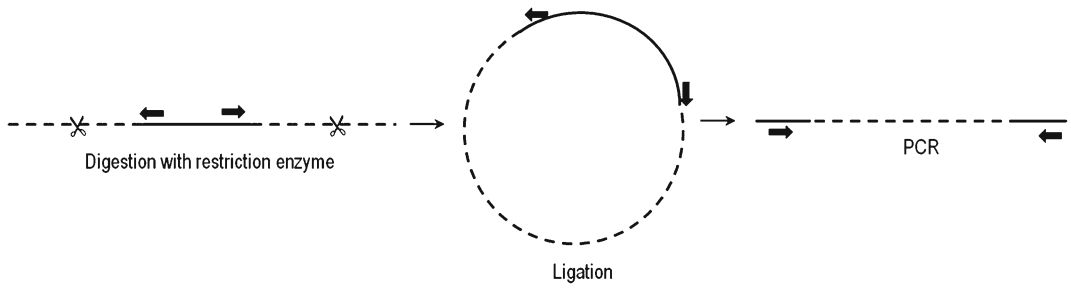

Fig. 1 Invert PCR method. Dash lines present unknown sequence. Continuous lines present known sequence. Black box arrows are invert PCR primer sites

200 μM deoxyribonucleotide triphosphate mix (Roche, Korea), 10 X PCR buffer, 1 U of *Taq* DNA polymerase (Roche, Korea), and 0.25 μM of each iPCR primer. PCR was performed on an Eppendorf Mastercycler Gradient (USA). The amplification profile consisted of an initial denaturation for 5 min at 94 °C followed by 35 cycles of PCR amplification under the following parameters: 20 s at 94 °C, 1 min at the annealing temperature of 50 °C, and 1 min of primer elongation at 72 °C. A final incubation at 72 °C for 10 min was programmed to allow completion of primer extension. Amplified products were visualized on an agarose gel as described previously. The sizes of the PCR products are listed in Table 1. The fragments were then excised from the gel under UV light and transferred into an Eppendorf tube and purified with a QIAquick gel extraction kit (Qiagen, Germany). The fragments were cloned using TOPO TA Cloning kit following the manufacturer's instructions (Invitrogen, USA). Plasmid DNA was extracted using Core-one plasmid miniprep kit (Korea) and sequenced (CoreBio, Korea). The sequences were analyzed using the program BioEdit 7.0 (Hall 1999).

SCAR analysis

New primers were designed from resulting iPCR circle and RAPD fragment sequences using the program Primer3 4.0 (Rozen and Skaletsky 2000). Each PCR reaction was carried out in a total reaction volume of 15 μl containing 15–20 ng of genomic DNA, 200 μM deoxyribonucleotide triphosphate mix (Roche, Korea), 10 X PCR buffer and 0.8 U of *Taq* DNA polymerase (Roche, Korea), 0.25 μM of each primer. Reactions were performed on an Eppendorf Mastercycler Gradient. The amplification profile consisted of an initial denaturation for 5 min at 94 °C followed by 35 cycles of PCR amplification under the following parameters: 1 min at 94 °C, 1 min at the annealing temperature 50–60 °C (depending on melting temperature of the primers), and 1 min of primer elongation at 72 °C. A final incubation at 72 °C for 5 min was programmed to allow completion of primer extension. Amplified products were visualized on an agarose gel as described previously.

High resolution melting (HRM) analysis

A standard PCR was performed in 10 μl reaction volumes with 50 ng of genomic DNA as template, 10 pmol of reverse and forward primers and 5 μl SsoFast EvaGreen supermix (Bio-Rad, USA). HRM was performed using CFX96 real-time PCR detection system (Bio-Rad, USA) and cycling condition for HRM was following the manual of SsoFast EvaGreen supermix (Bio-Rad, Hercules, CA) as follows: 98 °C for 2 min, then 40 cycles of denaturation at 98 °C for

Table 1 Inverse PCR primers for amplification and resulting PCR product size

Marker	Forward primer	Reverse primer	PCR product size	iPCR circle size
a133_4	GAATCACAAGGAAAAGAAAACAAG	TGAAAGGAGTCTCTGAATCCATAA	413	836
a57_6	CATGTCTTTTTTCACTGGGGGA	TTCATCGATGAAAATGAATCTGAAC	318	1,446
a119_7	GCTAGGTGCATCAAGAAGAATGT	AACACCTCACTAACATTCTTCTTG	204	na

na: not amplified

10 s, annealing/extension at 55 °C for 30 s, and one cycle of denaturation at 95 °C for 10 s, annealing/extention at 65 °C for 5 s, and denaturation at 95 °C for 20 s. The CFX Manager software 1.6 (Bio-Rad, USA) was used to discriminate genotypes.

Mapping position of SCAR and RAPD markers and QTL analysis

Linkage analysis was performed with MAPMAKER/EXP 3.0 (Lander et al. 1987) using the Kosambi mapping function to determine location of SCAR and RAPD markers. The logarithm of odds (LOD) score of 3.0 was used as a linkage threshold. Quantitative trait loci (QTL) detection was performed using composite interval mapping analysis (Zeng 1994) using QTL Cartographer (Basten et al. 2005) to determine association of the new markers mapped with *Phytophthora* root rot resistance. A 1,000-permutation test was performed to estimate the appropriate significance threshold for analysis. An LOD threshold of 3.0 corresponding to a genome-wide significance level of 0.05 was chosen. MAPCHART (Voorrips 2002) was then used to draw QTLs on the linkage group.

Results

Identification of RAPD marker linked to *Phytophtora* root rot resistance

DNA from parents was screened for informative polymorphism with a total of 400 UBC RAPD primers. Of these, 40 primers were detected to be polymorphic between the resistant and susceptible parents. These primers were screened on R- and S-pools and together with parents, but only three primers (UBC484, 504 and 553) showed polymorphism between R- and S-pools. These primers were then used to analyze the twenty individuals comprising the bulks to determine whether there was significant linkage to the resistance trait. However, only one marker UBC553 revealed a 100 % linkage in the individual plants comprising the contrasting bulks (Fig. 2).

Development of SCAR markers

Template DNA preparation is a limiting step in the iPCR procedure (Ochman et al. 1993), and preparation condition must be optimized. Restriction enzymes selected for the generation of the template must not cut within the characterized fragment. Furthermore, it is desirable that the overall iPCR product length be less than approximately 2 kb for convenience of cloning and sequencing. The sequences obtained from fragments a057_6, a119_7 and a133_4 (Table 1) associated with *Phytophthora* root rot resistance (Truong et al. 2012) were used to generate iPCR primers. A 6-bp-cutting (Eco*RI*) restriction enzyme for iPCR template generation was chosen. From 413- and 318-bp fragments generated from the AFLP markers a133_4 and a57_6, respectively, about 836 and 1,446 bp of additional DNA sequences, respectively, was obtained via iPCR (Fig. 3, Table 1). A DNA region appropriate for iPCR primers design was not identified for one of the three AFLP fragments (a119_7). In order to screen primers using a high resolution melting (HRM) system if primers are not polymorphic using regular PCR, four standard PCR primer pairs were designed covering the iPCR sequences: three primer pairs from 1,446-bp fragment and one primer pair from 836-bp fragment. None of the primer pairs obtained from a 1,400-bp fragment showed polymorphism between YCM334 and Tean. These primer pairs were used again to screen the resistant and susceptible parents using the HRM system, but no SNP was detected (data not shown). However, the primer pair obtained from an 836-bp fragment produced two polymorphic fragments in the parents indicating either a deletion or insertion mutation between the two alleles. The fragment amplified in the resistant parent was about 780 bp and in the susceptible parent was about

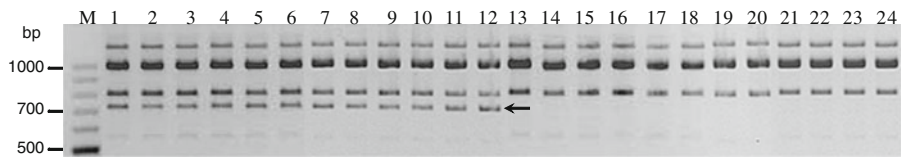

Fig. 2 Agarose gel electrophoresis of RAPD marker UBC553. M, 100 bp molecular weight marker; 1, YCM334; 2, R-pool; 3–12: resistant RILs; 13, Tean; 14, S-pool; 15–24, susceptible RILs. Arrow indicates polymorphic fragment

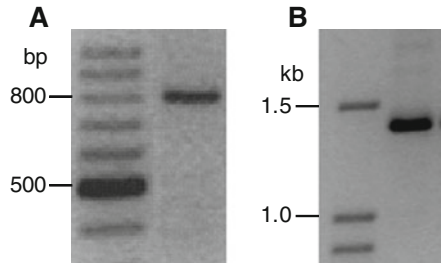

Fig. 3 Agarose gel electrophoresis of iPCR fragments obtained from the regions adjacent of AFLP markers a133_4 (**A**) and a57_6 (**B**) of resistant parent YCM334

800 bp, and a non-specific monomorphic fragment of about 1,200 bp was also amplified (Fig. 4). This SCAR marker was designated as SA133_4.

Based on the UBC553 fragment sequence, two primer pairs were designed. Of these, one primer pair was designed starting from 5′ and 3′ ends of the original RAPD primer. However, SCAR primer pairs for RAPD marker UBC553 were not polymorphic using different primer pair combinations and PCR conditions.

Mapping position of the SCAR and RAPD markers and QTL analysis

The SCAR and RAPD markers were used to characterize a mapping population consisting of 126 F_8 RILs (Fig. 5). These markers were co-located in the linkage group P5 (chromosome 5) of the 'YCM334 x Tean' core map (Truong et al. 2012). The SCAR marker SA133_4 was located a further 0.9 cM away from the original AFLP marker a133_4. The RAPD marker UBC553 was mapped at a distance of 1.7 cM from AFLP marker a119_7 which linked to *Phytophtora* root rot resistance, but markers UBC484 and UBC504 were not in the region of common QTLs for *Phytophthora* root rot resistance (Fig. 6). This is in agreement

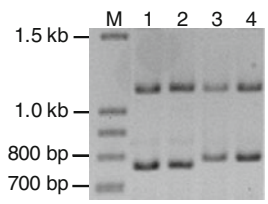

Fig. 4 Agarose gel electrophoresis of co-dominant SCAR marker SA133_4 linked to Phytophthora root rot resistance. M, 100 bp molecular weight marker; 1, YCM334; 2, R-pool; 3, Tean; 4, S-pool

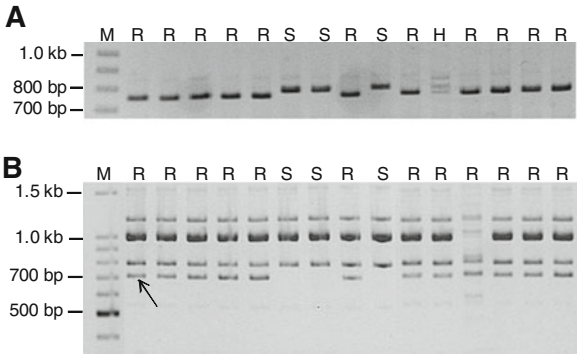

Fig. 5 Agarose gel electrophoresis of selected F_8 RILs screened using markers SA133_4 (**A**) and UBC553 (**B**). M, 100 bp molecular weight marker; R, resistance; S, susceptibility; H, heterozygosity. Arrow indicates polymorphic fragment

with the tight linkage to the resistance trait of the marker UBC553. The disease reactions of the F_8 RIL population evaluated in previous study were analysed with the marker genotypes and resulted markers SA133_4 and UBC553 were in the region of common QTL associated with the stable resistance reported by Truong et al. (2012) (Fig. 6).

Utilization of the markers for screening pepper hybrids

The SCAR and RAPD markers were used to identified presence of the resistance allele in nine commercial cultivars and were correctly identified as resistant or susceptible using markers SA133-4 and UBC553 (Table 2). Thus, these markers make it possible to monitor the *Phytopthora* root rot resistant alleles commonly used in breeding programs.

Discussion

Resistance to *Phytophthora* root rot in pepper is complex where at least five regions of the pepper genome contribute to the resistant response and expression of resistance is affected by environmental factors and plant development (Lefebvre and Palloix 1996; Thabuis et al. 2003; Truong et al. 2012). Based on detailed information about resistance QTL that contribute to this trait reported by Truong et al. (2012), an attempt has been made to convert AFLP markers linked to *Phytophthora* root rot into co-dominant, simple PCR forms. However, there are reasons why this approach will not be generally successful. Chiefly, the average size of the AFLP fragments of interest is short in length

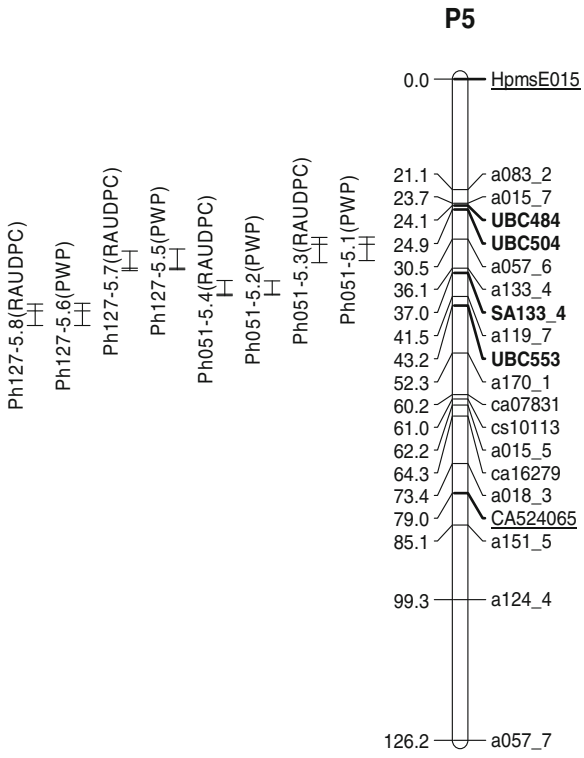

Fig. 6 Pepper chromosome 5 (P5) of the F_8 RIL mapping population reported by Truong et al. (2012). New SCAR and RAPD markers map to the chromosome 5 highlighted in bold. Underlined markers are consensus SSR markers in common with previous published pepper linkage maps. The position of the QTLs together with their confidence interval previously reported by Truong et al. (2012) are presented in the right of linkage groups and indicated by horizontal lines

and it is unlikely that appropriate PCR primers could be designed from such short DNA sequences or that the resulting PCR products could be distinguished by standard agarose gel electrophoresis. Consequently, it was necessary to consider a different approach, beginning with the generation of larger tracks of DNA sequence. Inverse PCR was the key to our success in converting the marker linked to *Phytophthora* root rot resistance. Of three AFLP markers selected, only one marker was successfully converted into co-dominant SCAR marker SA133_4 using the iPCR method. The marker amplified about 780 and 800-bp long bands in resistant and susceptible parents, respectively and was able to identify the heterozygote. In addition, a non-specific monomorphic fragment of about 1,200 bp was also amplified. Various PCR protocol changes attempted did not eliminate this non-specific amplification. These however did not interfere with the scoring for the polymorphic SCAR marker linked to *Phytophthora* root rot resistance and thus, the marker can still be used for MAS. Presence of non-specific amplification with SCAR markers has also been reported in other studies (Kelly and Miklas 1998; Horejsi et al. 1999; Gupta et al. 2006) which is attributed to the SCAR primers containing ubiquitous sequences in multiple genomic regions that may result in mismatch primer annealing during PCR.

Of the three RAPD markers that showed polymorphism between R- and S-pools among 400 RAPD primers screened, only one marker, UBC553, tightly linked to *Phytophthora* root rot resistance. Conversion of this marker into a SCAR marker failed, although an additional primer pair was design from the RAPD primer sequence site. The failure of the SCAR marker derived from RAPD marker UBC553 to produce polymorphism could be caused by mismatches in nucleotide in the priming sites as reported by Paran and Michelmore (1993), Horejsi et al. (1999) and Gupta et al. (2006).

To determine whether the SCAR and RAPD markers co-localized with a known QTL for *Phytophthora* root rot resistance, we mapped the three RAPD markers (UBC484, 504, and 553) and SCAR marker in a reference mapping population, which enabled us to infer the relationship of these loci and the mapped QTLs. All RAPD and SCAR markers were mapped to chromosome 5 of a reference map. Markers UBC553 and SA133_4 were located in the region of QTLs contributing to stable resistance on chromosome 5. This QTL has been implicated in resistance to several

Table 2 List of pepper lines/cultivars tested

Accession	Phenotype	Genotype/marker	
		SA133_4	UBC553
82PR 116	R	R	R
82PR 117	R	R	R
82PR 48	S	S	S
82PR 405	S	S	S
Tantan	S	S	S
Wonkang No. 3	R	R	R
Kyongbukte 1	S	S	S
Konesianha	R	R	R
Konchowang	S	S	S
Tean	S	S	S
YCM334	R	R	R

pathogen isolates, explaining from 20.0 % to 48.2 % of the phenotypic variance (Truong et al. 2012). In our study, SCAR marker SA133_4 was mapped a further 0.9 cM away from the original AFLP marker a133_4, and RAPD marker UBC553 was mapped closely to the AFLP marker a119_7. Both RAPD and SCAR markers were close to the peaks of the resistance QTL reported in previous works. This result confirms that by applying a bulk approach we were able to identify a marker for a QTL that is suitable for high-throughput analysis, even in a very complex system. Furthermore, using this approach we determined which of the five genomic positions identified in previous studies was present in our promising pepper lines/cultivars. The results confirm previous work that identification of QTLs located in the region of 24–44 cM on chromosome 5 (P5), which associated with stable resistance to *Phytophthora* root rot in pepper, were important QTLs (Truong et al. 2012). In previous studies, QTLs for *Phytophtora* root rot resistance against different pathogen isolates from different geographic regions have been identified on chromosome 5 (Thabuis et al. 2004; Bonnet et al. 2007; Kim et al. 2008). Thus, these results suggest that these QTLs may account for differences in the levels of resistance that are most economically significant. In addition, the SCAR marker SA133_4 has an advantage of identifying homozygous individuals from heterozygous plants in F_2 populations, which is impossible to achieve by evaluation of disease phenotype. Thus, combination of the use of the two markers SA133_4 and UBC553 will make it possible to select *Phytophthora* root rot resistant plants in a wide range of crosses in pepper breeding programs.

References

Babadoost, M. (2007). Fungicide efficacy for control of *Phytophthora* blight of bell pepper, 2008. Plant Disease Management Reports 2, V158. Online publication

Barksdale, T. H., Papavizas, G. S., & Johnston, S. A. (1984). Resistance to foliar blight and crown rot of pepper caused by *Phytophthora capsici*. *Plant Disease, 68*, 506–509.

Basten, C. J., Weir, B. S., & Zeng, Z. B. (2005). *QTL Cartographer: A reference manual and tutorial for QTL mapping Department of Statistic*. Raleigh, North Carolina: North Carolina State University.

Bonnet, J., Danan, S., Boudet, C., Barchi, L., Sage-Palloix, A. M., Caromel, B., Palloix, A., & Lefebvre, V. (2007). Are the polygenic architectures of resistance to *Phytophthora capsici* and *P-parasitica* independent in pepper? *Theoretical and Applied Genetics, 115*, 253–264.

Bosland, P. W., & Lindsey, D. L. (1991). A seedling screen for *Phytophthora* root rot of pepper, *Capsicum annuum*. *Plant Disease, 75*, 1048–1050.

Foster, J. M., & Hausbeck, M. K. (2010). Managing *Phytophthora* crown and root rot in bell pepper using fungicides and host hesistance. *Plant Disease, 94*, 697–702.

Glosier, B. R., Ogundiwin, E. A., Sidhu, G. S., Sischo, D. R., & Prince, J. P. (2008). A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. *Euphytica, 162*, 23–30.

Gupta, S. K., Charpe, A., Prabhu, K. V., & Haque, Q. M. (2006). Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. *Theoretical and Applied Genetics, 113*, 1027–1036.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Hausbeck, M. K., & Lamour, K. H. (2004). *Phytophthora capsici* on vegetable crops: Research progress and management challenges. *Plant Disease, 88*, 1292–1303.

Hausbeck, M. K. & Cortright, B.D. (2007). Evaluation of fungicides and applications for management of *Phytophthora* blight of pepper, 2006 Plant Disease Management Reports 1, V139. Online publication

Horejsi, T., Box, J. M., & Staub, J. E. (1999). Efficiency of randomly amplified polymorphic DNA to sequence characterized amplified region marker conversion and their comparative polymerase chain reaction sensitivity in cucumber. *American Society for Horticultural Science, 124*, 128–135.

Kelly, J. D., & Miklas, P. N. (1998). The role of RAPD markers in breeding for disease resistance in common bean. *Molecular Breeding, 4*, 1–11.

Kim, H. J., Nahm, S. H., Lee, H. R., Yoon, G. B., Kim, K. T., Kang, B. C., Choi, D., Kweon, O., & Cho, M. C. (2008). BAC-derived markers converted from RFLP linked to Phytophthora capsici resistance in pepper (Capsicum annuum L). *Theoretical and Applied Genetics, 118*, 15–27.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., & Newburg, L. (1987). MapMaker: an interactive computer package for constructing primarily genetic linkage maps of experimental and natural populations. *Genomics, 1*, 174–181.

Lamour, K. H., & Hausbeck, M. K. (2003). Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. *Plant Disease, 87*, 841–845.

Lefebvre, V., & Palloix, A. (1996). Both epistatic and additive effects of QTL are involved in polygenic induced resistance to disease: A case study, the interaction pepper-*Phytophthora capsici* L. *Theoretical and Applied Genetics, 93*, 503–511.

Matheron, M. E. & Porchas, M. (2008). Efficacy of fungicides for management of the soil-phase of *Phytophthora* blight on pepper plants, 2006. Plant Disease Management Reports 2, V083. Online publication.

McGrath, M. T. & Davey, J. F. (2007). Efficacy of fungicides for control of *Phytophthora* blight in pepper on crown rot tolerant and susceptible cultivars, 2006. Plant Disease Management Reports 1, V131. Online publication.

Michelmore, R. W., Paran, I., & Kesseli, R. V. (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. *Proceedings of the National Academy of Sciences, 88*, 9828–9832.

Monroy-Barbosa, A., & Bosland, P. W. (2008). Genetic analysis of *Phytophthora* root rot race-specific resistance in Chile pepper. *Journal of the American Society for Horticultural Science, 133*, 1–5.

Monroy-Barbosa, A., & Bosland, P. W. (2011). Identification of novel physiological races of *Phytophthora capsici* causing foliar blight using the New Mexico recombinant inbred pepper lines set as a host differential. *Journal of the American Society for Horticultural Science, 136*, 205–210.

Ochman, H., Ayala, F. J., & Hartl, D. L. (1993). Use of polymerase chain reaction to amplify segments outside boundaries of known sequences. *Methods in Enzymology, 218*, 309–321.

Oelke, L. M., & Bosland, P. W. (2003). Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Capsicum annuum*. *Journal of the American Society for Horticultural Science, 128*, 213–218.

Quirin, E. A., Ogundiwin, E. A., Prince, J. P., Mazourek, M., Briggs, M. O., Chlanda, T. S., Kim, K. T., Falise, M., Kang, B. C., & Jahn, M. M. (2005). Development of sequence characterized amplified region (SCAR) primers for the detection of *Phyto5.2*, a major QTL for resistance to *Phytophthora capsici* Leon in pepper. *Theoretical and Applied Genetics, 110*, 605–612.

Raz, V., & Ecker, J. (1997). DNA isolation from *Arabidopsis thaliana*. In B. Birren, E. Green, S. Klapholz, R. Myers, & J. Roskams (Eds.), *A laboratory manual, vol. 1* (pp. 24–25). New York: Cold Spring Harbor.

Rozen, S., & Skaletsky, H. J. (2000). Primer3 on the SSS for general users and for biologist programmers. *Methods in Molecular Biology, 132*, 365–386.

Paran, I., & Michelmore, R. W. (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics, 85*, 985–993.

Siebring. Soil sterilization. http://www.siebringmfg.com/soil_sterilization_equipment.htm

Sy, O., Steiner, R., & Bosland, P. W. (2008). Recombinant inbred line differential identifies race-specific resistance to *Phytophthora* root rot in *Capsicum annuum*. *Phytopathology, 98*, 867–870.

Sy, O., Steiner, R., & Bosland, P. W. (2005). Inheritance of *Phytophthora* stem blight resistance as compared to *Phytophthora* root rot and *Phytophthora* foliar blight resistance in *Capsicum annuum* L. *Journal of the American Society for Horticultural Science, 130*, 75–78.

Sy, O., Steiner, R., & Bosland, P. W. (2007). Recombinant inbred line differential identifies race-specific resistance to *Phytophthora* root rot in *Capsicum annuum*. *Phytopathology, 98*, 867–870.

Thabuis, A., Lefebvre, V., Bernard, G., Daubeze, A. M., Phaly, T., Pochard, E., & Palloix, A. (2004). Phenotypic and molecular evaluation of a recurrent selection program for a polygenic resistance to *Phytophthora capsici* in pepper. *Theoretical and Applied Genetics, 109*, 342–351.

Thabuis, A., Palloix, A., Pflieger, S., Daubeze, A. M., Caranta, C., & Lefebvre, V. (2003). Comparative mapping of *Phytophthora* resistance loci in pepper germplasm: Evidence for conserved resistance loci across *Solanaceae* and for a large genetic diversity. *Theoretical and Applied Genetics, 106*, 1473–1485.

Truong, H. T. H., Kim, K. T., Kim, D. W., Kim, S., Chae, Y., Park, J. H., Oh, D. G., & Cho, M. C. (2012). Identification of isolate specific resistance to *Phytophthora* root rot in pepper (*Capsicum annuum* L.). *Plant Pathology, 61*, 48–56.

Voorrips, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity, 93*, 77–78.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics, 136*, 1457–1468.