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ORIGINAL PAPER

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Development of sequence characterized amplified region (SCAR) primers for the detection of *Phyto.5.2*, a major QTL for resistance to *Phytophthora capsici* Leon. in pepper

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Abstract *Phytophthora capsici* causes devastating disease on many crop species, including *Capsicum*. Resistance in *Capsicum annuum* is genetically and physiologically complex. A panel of *Capsicum* germplasm that included genotypes from both *C. annuum* and *C. chinense* showing highly resistant, highly susceptible and intermediate or tolerant responses to the pathogen, respectively, was screened with a series of randomly amplified polymorphic sequence primers to determine which genomic regions contribute to the highest level of resistance. One primer, OpD04, amplified a single band only in those *C. annuum* and *C. chinense* genotypes showing the highest level of resistance. The amplified product was cloned, sequenced and used to design longer primers in order to generate a sequence characterized amplified region marker which was then mapped in a reference mapping population and a screened population segregating for resistance to *P. capsici*. These primers were observed to define a locus on pepper chromosome 5 tightly linked to *Phyto.5.2*, one of six quantitative trait loci (QTL) previously reported to contribute to *P. capsici* resistance. These results indicate that the *Phyto.5.2* QTL may be widely distributed in highly resistant germplasm and provide improved resolution for this QTL. This work also defines the first breeding tools for this system, allowing for the rapid selection of genotypes likely to be highly resistant to *P. capsici*.

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Introduction

There are over 70 species of *Phytophthora* (Abad et al. 2002), many of which have devastating agronomic effects. *P. capsici* Leon. is a soil-borne oomycete pathogen that leads to *Phytophthora* stem, collar and root rots and crown blight in *Capsicum* species (Barksdale et al. 1984; Ristaino and Johnston 1999; Walker and Bosland 1999; Thabuis et al. 2003). *P. capsici* has a wide host range in the Solanaceae, including tomato and eggplant, and is also a major pathogen of squash, pumpkin, zucchini, cucumber and watermelon in the family Cucurbitaceae, of cocoa (Sterculiaceae) and of macadamia nut (Proteaceae) (Black et al. 1991; Biles et al. 1995; Ristaino and Johnston 1999; Holmes et al. 2001). Infection by this pathogen can result in severe, and even complete loss of pepper crops throughout the world. In recent years, the occurrence and severity of *P. capsici* infections in agricultural environments has increased for reasons that are not fully understood (Ristaino and Johnston 1999).

P. capsici can infect all parts of a pepper plant, including the roots, stems, leaves and fruit at any stage of growth, and can be seedborne, surviving in the soil and on host debris for months (Black et al. 1991; Biles et al. 1995; Oelke et al. 2003). Infection most commonly occurs during periods of heavy rainfall and high humidity in plantings that are over-crowded, over-fertilized with nitrogen or where poor drainage or excessive irrigation occurs (Black et al. 1991; Biles et al. 1995; Lefebvre and Palloix 1996).

To date, no effective management programs have been developed to control the spread of *P. capsici* in pepper crops. Chemical control, such as the use of pesticides, is limited and often ineffective against *Phytophthora* on pepper (Mozzetti et al. 1995; Oelke et al. 2003). Biological control has also been largely unsuccessful (Oelke et al. 2003). With the lack of dependable chemical and biological control measures, urgent

attention is now focused on the development of *Phytophthora*-resistant pepper varieties (Pflieger et al. 2001; Oelke et al. 2003). While breeding programs have been underway in many locations for at least two decades, none of the resistant varieties currently available show high levels of resistance due to the complex inheritance of resistance (e.g. Thabuis et al. 2004) and difficulty with reproducible phenotypic screens (Oelke et al. 2003).

Many studies have been published on the inheritance of resistance to *P. capsici* in pepper. Single-, two-, and multiple-gene systems have been reported (Pochard et al. 1983; Mozzetti et al. 1995; Lefebvre and Palloix 1996; Pflieger et al. 2001; Oelke et al. 2003; Thabuis et al. 2003, 2004). Additionally, Oelke et al. (2003) recently demonstrated the existence of different physiological races within *P. capsici*. This, along with the various and complex modes of inheritance, could explain the lack of varieties that show consistent high levels of resistance to *Phytophthora* infection across diverse geographical areas.

Capsicum annuum Criollo de Morelos 334 (CM334) is a small-fruited, pungent landrace from Morelos, Mexico. In numerous studies, CM334 has consistently shown a very high degree of resistance to *P. capsici* and is considered to be the most promising source of resistance currently known (Walker and Bosland 1999; Thabuis et al. 2003). At the present time, this source of resistance serves as the foundation for the major commercial breeding programs in both the public and private sector. The inheritance of resistance found in CM334 has been disputed for many years. Early work by Guerrero-Moreno and Laborde (1980) concluded that two recessive genes provided resistance in CM334. Later, Ortega et al. (1991) proposed a three-gene, multi-allelic system. In the earliest analysis of quantitative trait loci (QTL) in this system, three major QTL for resistance to *P. capsici* were reported (Lefebvre and Palloix 1996). Later studies confirmed a major QTL that was initially reported to span the entire length (107 cM) of pepper chromosome 5 when detected by interval mapping and ANOVA (Pflieger et al. 2001; Lefebvre et al. 2002). The remaining two QTL were located on pepper chromosome 11. More recently, six chromosomal regions, now designated *Phyto.4.1*, *Phyto5.1* and *Phyto.5.2*, *Phyto.6.1*, *Phyto.11.1* and *Phyto.12.1* located on chromosomes 4, 5, 6, 11 and 12, respectively, were identified to be involved in one or more components of resistance to *P. capsici* (Thabuis et al. 2003). This information has been used to evaluate the efficacy of various complex breeding schemes for resistance (Thabuis et al. 2004).

Marker-assisted selection (MAS) has been proposed for many years to offer the means to facilitate the management of complex traits such as resistance to *P. capsici* in pepper (e.g. Thabuis et al. 2004). An important limitation, however, is the ability to select for many chromosomal regions at once (Hospital and Charcosset 1997; Thabuis et al. 2004). One solution to this limitation is to combine phenotypic analyses with MAS for one or a few QTL that are the most critical for full recovery of the phenotype of interest. Phenotypic screens can reliably shift allele frequencies in favorable directions but have proven to be inefficient for capturing all of the favorable allelic configurations in one genotype with the desired agronomic or horticultural characteristics.

A common observation among plant breeders working to improve the response of *Capsicum* genotypes to *P. capsici* is while intermediate levels of resistance are readily recovered in segregants with desirable horticultural characteristics, it is not possible to recover levels of resistance equal to that of the resistant parent, CM334, without repeated backcrosses to the resistant parent—to the detriment of horticultural type (Thabuis et al. 2004). The study reported here was undertaken to test the hypothesis that it may be possible to efficiently identify one or a few QTL that are particularly critical for extreme resistance to *P. capsici* in *Capsicum*. Our strategy was to screen diverse germplasm that represent the phenotypic extremes of response to the disease and determine the relationship of chromosomal segments identified in this screen with known QTL. This may allow an efficient identification of the chromosomal regions that are most likely to be essential for maximum expression of the trait of interest. In this study, we employed PCR-based markers because many laboratories in the developed and developing world in both research and commercial settings are set up to use this technology. This paper describes a test of the strategy described above, which resulted in the development of a PCR-based sequence characterized amplified region (SCAR) marker for the detection of the *Phyto.5.2* QTL in *Capsicum*.

Materials and methods

Germplasm and populations

The germplasm used in this study was selected to represent sources of resistance to *Phytophthora capsici* in two *Capsicum* species, *C. annuum* and *C. chinense*. Accessions which were presented as homozygous and homogeneous were collected, planted, checked for type and self-pollinated to ensure pure lines for genetic studies. Resistant germplasm was checked in disease screens described below for uniformity and level of resistance. Highly resistant genotypes included in this study are *C. annuum* Criollos de Morelos 334 (CM334) obtained from The Asian Vegetable and Research Development Center (AVRDC, Shanhua, Taiwan), *C. annuum* CU44, a highly resistant breeding line derived from crosses between CM334 and *C. annuum Yolo B* and *C. chinense* PI 159234. Two identical sister lines from the original accession, CM334, designated CMA and CMB, served as checks. Additional lines used in the study included the susceptible *C. annuum* parents and check varieties *Early California Wonder 300* (ECW300),

Yolo B, *NuMex RNaky*, *NuMex Joe E. Parker* (JEP) and NY 99-210. The hybrid variety, *Paladin* (Syngenta, Greensboro, N.C.), known to have crown blight resistance to *P. capsici* but not extreme resistance to root rot, was provided by Steven Czaplewski, Syngenta, Naples, Florida.

To generate resistant and susceptible bulks for screens of molecular markers, CM334 was crossed with *Yolo B* and inbred to generate a large set of F_3 families. Segregants were subjected to phenotypic screens at the F_3 stage, and families that were highly resistant were self-pollinated. Remnant seed from families that were entirely susceptible was also planted and self-pollinated. Nine F_3 families were confirmed by phenotypic screening at the F_4 generation to represent the phenotypic extremes of this population—four resistant and five highly susceptible. DNA was extracted from at least six individual plants from each F_3 family as described below.

For the mapping studies, two previously constructed mapping populations, each involving a parent highly resistant to *P. capsici*, were used to evaluate candidate randomly amplified polymorphic DNA (RAPD) and SCAR markers. The first, the AC population, contains approximately 1,000 loci mapped in a *C. annuum NuMex RNaky* × *C. chinense* PI 159234 F_2 population (Livingstone et al. 1999; Paran et al. 2004). The second population is an intraspecific F_2 population consisting of 94 individuals derived from a single F_1 plant from a cross between *C. annuum* CM334 and *C. annuum NuMex Joe E. Parker* (CM334×JEP) screened with *P. capsici* in the greenhouse.

Phenotypic evaluation of resistance to *P. capsici*

The *P. capsici* isolate used in this study was cultured from infected pepper roots obtained from a commercial production field in California, maintained at Cornell University and California State University at Fresno and deposited in the American Type Culture Collection (ATCC, Manassas, Va.). Cultures were grown on V8 agar plates at 29°C in an incubator. To prepare inoculum for disease screens, cultures were flooded with ddH_2O, incubated for 1 h at 4°C and then held at room temperature for 30 min to promote sporulation. The spores were counted and the concentration adjusted to 1×10^4 spores/ml in distilled water. Inoculum (5 ml per cell) was injected via a syringe into each cell of a flat containing 2-week-old seedlings (PI 159234) or 5- to 6-week-old plants (all other material screened in this study) to be evaluated for disease response. The flats were then flooded for 48 h, drained and maintained in the greenhouse under supplemental light at 28°C. Symptoms apparent as wilt and death in fully susceptible genotypes typically developed 10 days post-inoculation (dpi), so initial screen scores were taken at 2 weeks dpi. Resistant plants were usually maintained until fruit set to determine the level of resistance. Plants were scored visually on a scale of 1–9 based on the timing and severity of symptom development. The resistant class was defined as individual plants that were scored 1 or 2 and survived through fruit set with minimal or no wilt symptoms

RAPD reactions

Fifteen lettered primer sets (OpA, -B, -C, -D, -E, -F, -I, -M, -N, -O, -P, -Q, -R, -S and -T) containing 20 decamers each (Operon Technology, Alameda, Calif.) were used to screen the germplasm panel for RAPD loci for a total of 300 primers. RAPD reactions were initially done on resistant and susceptible (S) bulks derived from crosses between S and CMA or CMB. Bulks were made using equalized amounts of DNA from each of five plants per F_4 family from four resistant families and five susceptible families, for a total of nine bulks.

Once candidate RAPDs were identified by comparing patterns obtained across the bulks with resistant and susceptible parents, the bulks were separated into individuals and tested again to confirm the presence of the band of interest in all resistant individuals and the absence of the band in all susceptible individuals. Reactions were performed in a 25-µl total volume per reaction. The components of each reaction were 2.5 µl 10× PCR buffer (Roche, Indianapolis, Ind.), 1.0 µl 2.5 m *M* dNTPs (Roche), 0.5 µl 10 m *M* primer (Operon), 0.25 µl *Taq* polymerase (Roche, 5 U/ml), 10 µl 20 ng/µl genomic DNA and 10.75 µl sterile water. The reactions were placed in a thermocycler (PTC-100, MJ Research, Waltham, Mass.) and cycled as follows: an initial denaturation of 4 min at 94°C; 44 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C; a final extension of 7 min at 72°C. The PCR products were electrophoresed on a 2% horizontal agarose gel and stained with ethidium bromide (EtBr) to visualize amplification products.

Cloning, DNA sequencing and SCAR primer design

Amplified bands from candidate primers were excised from the gel with a razor blade and the DNA extracted using the QIAquick Gel Extraction kit according to the manufacturer's instructions (Qiagen, Valencia, Calif.). Ligation of the PCR product was carried out overnight at 4°C with a TOPO-Easy Vector Cloning kit (Invitrogen, Carlsbad, Calif.) according to manufacturer's instructions. The purified ligation reaction was diluted with 50 µl water, and a 5-µl aliquot was subsequently mixed with 40 µl TOPO-10 competent cells and placed on ice for 1 min, then placed into a cuvette and electroporated (GenePulser; BioRad, Hercules, Calif.) for 5 min at 1.8 kV. One milliliter of LB medium was added immediately following electroporation and incubated for 1 h at 37°C with shaking, after which a 200-µl aliquot was plated onto LB agar plates containing ampicillin,

IPTG and X-gal and incubated overnight at 37°C. Up to six white colonies from each transformation reaction were streaked onto LB plates to produce single colonies.

Plasmid DNA was extracted from overnight cultures of transformed bacterial cells using the miniprep procedure (Sambrook and Russell 2001), and samples diluted 1:50 in sterile water. Ten-microliter aliquots of the diluted miniprep were mixed with 6 µl sterile water, 2 µl 10× buffer (Roche) and 2 µl *Eco*RI restriction enzyme (Roche) and the reactions incubated at 37°C for 1 h. The entire reaction, along with 5 µl of a 1-kb ladder (GibcoBRL, Gaithersburg, Md.), was electrophoresed on a 1% horizontal agarose gel and stained with EtBr. Two sequencing reactions were set up using 1 µg of plasmid DNA combined with 4 pmol of M13 forward and reverse primer, respectively. These reactions were brought up to a total volume of 18 µl with sterile water. The PCR reaction and sequencing were performed at the Cornell Bioresources Center. The contigs from the sequencing were aligned using EDITSEQ software (DNAStar, Madison, Wis.), and the forward and reverse primers were designed using PRIMERSELECT (DNAStar). Melting temperature, GC content and molecular weight were verified using OLIGO CALCULATOR software (http://micro.nwfsc.noaa.gov/protocols/.oligoTMcalc.html).

Optimization of SCAR primer PCR

PCR amplification reactions were run on a PTC-225 Peltier Thermal Cycler Tetrad DNA Engine (MJ Research, Watertown, Mass.) and optimized using the SCAR primers by varying $MgCl_2$ concentration and elongation temperature. The PCR reactions included 10 µl CMA DNA (20 ng/µl), 2.5 µl 10× buffer (no $MgCl_2$ added), 1 µl 2.5 m *M* dNTPs, 0.5 µl each of 10 m *M* forward and reverse SCAR primer and 0.25 µl *Taq* polymerase. A gradient of $MgCl_2$ concentrations (20, 40, 60, and 80 m *M*) was set up. The temperature gradient included the following temperatures 45°, 45.5°, 46.7°, 48.3°, 50.0°, 53.4°, 56.7°, 60.7°, 61.8°, 63.4°, 64.6°, and 65°C.

Linkage analysis and QTL detection

Pepper genomic DNA was extracted from immature leaf tissue of the mapping parents and individuals following the protocol used by Prince et al. (1997). Both the D04.717 RAPD and SCAR were mapped in the AC population to determine the relationship of the markers to previously described QTL for resistance to *P. capsici* using MAPMAKER V3.0 (Lincoln et al. 1993) as outlined by Livingstone et al. (1999).

The OpD04 RAPD marker and D04.717 SCAR marker were also mapped in the CM334×JEP F_2 population of 94 individuals with amplified fragment length polymorphic (AFLP) and RAPD markers. Linkage analysis was performed with using MAPMAKER V2.0 for Macintosh (Lander et al. 1987). Linkage group 5 (chromosome 5) was identified by using the GROUP command with a minimum LOD threshold of 3.5 and a maximum recombination fraction of 35%. The ordering of markers in this linkage group was done with the use of the COMPARE command at an LOD of 3.0. The Kosambi mapping function (Kosambi 1944) was used to convert recombination fractions to map distances in centiMorgans (cM).

Sixty-nine of the 94 F_2 individuals were advanced to the F_3 generation, and 24 plants of each of the F_3 families were tested with a *P. capsici* isolate. For each F_3 family, the resistance score was the number of resistant plants expressed as a percentage of the total number of plants tested. QTL detection was done by composite interval mapping (CIM) of the WINDOWS QTL CARTOGRAPHER v2.0 mapping program (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) (Basten et al. 1997; Wang et al., 2003).

Results

Identification of a RAPD marker consistently associated with extreme resistance to *P. capsici*

Three primers from the initial 300 of the primer set produced bands that were correlated with resistance to *Phytophthora* when evaluated in the bulked DNA samples from resistant and susceptible F_3 families. The most consistent of these was Operon primer D04 (OpD04). which produced a band of approximately 700 bp. Figure 1 shows this band amplified from DNAs of the initial bulked samples consisting of the resistant and susceptible F_3 families and parents. The band is present only in the resistant parents, resistant bulk, and all of the resistant individuals randomly selected from each resistant family that comprise the bulk. The band is absent in the susceptible parent, the susceptible bulk, and all of the susceptible individuals randomly selected from the susceptible families.

To test the apparent association between resistance to *P. capsici* and the locus detected by the OpD04 primer, the primer was tested with a number of other genotypes displaying a range of responses to the pathogen (Fig. 1, data not shown), including CMA, CMB, CU44, the susceptible parents, two additional susceptible varieties, *Yolo B* and ECW300, and *Paladin*. The band was present in CM and CU44, a highly resistant breeding line unrelated to the F_3 families derived from a cross between bell pepper genotypes and CM and absent in the susceptible varieties and in *Paladin*, a hybrid variety with intermediate resistance.

Sequencing and SCAR primer design

The band amplified by the OpD04 primer was cloned and sequenced, resulting in a sequence fragment

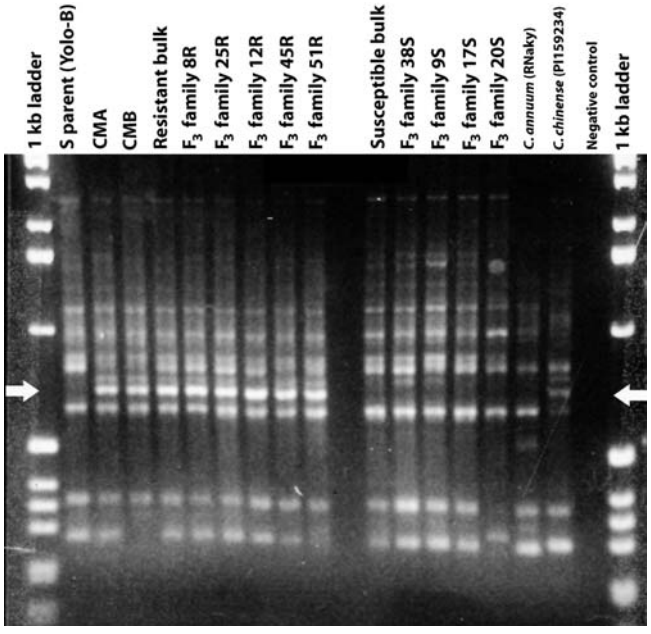

Fig. 1 Resistant and susceptible F_3 bulks screened with the D04 RAPD primer. Gel electrophoresis of the amplification products of Operon primer (Op) D04 on *Capsicum annuum* genotypes that are highly resistant and susceptible to *Phytophthora capsici*. *Lanes*: *S* Susceptible parent (*Yolo B*), *CMA*, *CMB* resistant parents, *resistant bulk* bulk of four highly resistant F_3 families, *9-8R*, *9-25R*, *0-12R*, *0-45R*, *0-51R* resistant individuals randomly selected from each of the F_3 families, *susceptible bulk*(bulk of DNAs from five susceptible F_3 families, *9-38S*, *0-9S*, *0-17S*, *0-20S* susceptible individuals randomly selected from each of the five F_3 families, *C. annuum* (*RNaky*) *C. annuum* mapping parent, *C. chinense* (*PI 159234*) *C. chinense* mapping parent, *negative control* water. *Arrows* indicate band of interest—approximately 700 bp

717 bp in length. Forward and reverse primers were developed using DNAStar software. The primer sequences were: OpD04.717-F: 5′-CCA TAA GGG TTG GTA AAT TTA CAA AG-3′ and OpD04.717-R: 5′-TCG AGA GAT AAT TCA GAT AGT ATA ATC-3′. These SCAR primers were used to amplify CMA, CMB, CU44, the susceptible parents, *Yolo B*, ECW300, and *Paladin* DNAs as was done with the RAPD marker previously described. The optimized protocol is described as follows. Each reaction consisted of 10 µl (40 ng/µl) genomic DNA, 10.75 µl sterile water, 2.5 µl 10× PCR buffer (12 m *M* $MgCl_2$ added), 0.5 µl each forward and reverse primer and 0.25 µl *Taq* polymerase. The optimized amplification program requires an initial denaturation of 2 min at 94°C; 32 cycles of 30 s at 94°C, 30 s at 45°C and 1 min at 72°C; a final extension of 5 min at 72°C. The results obtained with the SCAR primers were identical to those obtained with D04. The band amplified by these primers was present in the resistant parent and CU44 but was absent in genotypes showing intermediate (*Paladin*) or susceptible responses to the pathogen (*Yolo B*, *Cal Wonder 300*)(Fig. 2).

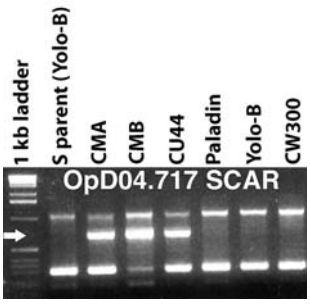

Fig. 2 The D04 RAPD and SCAR primers amplify a band only in highly resistant genotypes.Gel electrophoresis of the amplification products of the D04 SCAR primers on selected genotypes shown in Fig. 1. *Arrow* indicates band of interest

C. chinense PI 159234 is highly resistant to *P. capsici* and displays the dominant D04.717 RAPD and SCAR markers

C. chinense PI 159234 is an accession derived from a Peruvian landrace used widely in molecular mapping studies in *Capsicum*. Because *C. chinense* does not intercross easily with *C. annuum*, the PI 159234 genotype defines a case where there is a fair degree of reproductive isolation between these two *Capsicum* species. Anecdotal evidence suggested that PI 159234 could be highly resistant to *P. capsici* so we ran a disease screen on 22-week-old plants and based on the results, we rated the accession as uniformly highly resistant and equivalent to the resistant check, CM (data not shown). The D04.717 RAPD and SCAR markers were then evaluated on this genotype, and the dominant band associated with resistance was consistently present (Fig. 1). In order to determine if we could map this band in a well-developed reference mapping population derived from a cross between this accession and *C. annuum* var. *NuMex RNaky*, we first confirmed *NuMex RNaky* to be fully susceptible to *P. capsici* and that it uniformly lacked the D04.717 band (Fig. 1).

Molecular mapping of D04.717 RAPD and SCAR markers in a reference mapping population

In order to identify which, if any, of the previously identified QTL are linked to the loci identified by the D04 RAPD and SCAR primers and to confirm that these markers map to the same genomic location, we genotyped individuals in the AC population with both the D04.717 RAPD and SCAR primers (Livingstone et al. 1999) to determine the relation of the D04 loci to previously identified QTL for resistance or tolerance to *P. capsici*. When MAPMAKER V3.0 was applied, the OpD04.717 RAPD grouped to pepper chromosome 5 (LOD > 9.5) in a cluster of framework RFLP including CT155, TG363 and CD64a. The RAPD was most closely linked to CT155 with a revised LOD of 9.48 and a distance of 0.2 cM. It was also closely linked to CD64a

with a revised LOD of 9.19 and a distance of 0.4 cM (Fig. 3).

The D04.717 SCAR was assigned to pepper chromosome 5 with a LOD of 10.7 and grouped into the same framework RFLP cluster as its RAPD counterpart. It was most closely linked with CT155 with a revised LOD of 7.95 and a distance of 2.5 cM and with CD64a with a revised LOD of 7.82 and a distance of 2.5 cM (Fig. 3).

Because of the size of the QTL interval in the previously published work (up to 107 cM, Lefebvre et al. 1996) and the lack of common markers between maps, and to provide independent confirmation of our results, we mapped the D04.717 RAPD and SCAR markers in a second population that had been scored for response to *P. capsici* infection. The RAPD primer OpD04 produced a polymorphic band of 705 bp that mapped to the linkage group corresponding to chromosome 5, which is approximately 48 cM in this map. This linkage group contains the major *Phytophthora* resistance QTL (Fig. 4) in this population. The marker is flanked by a second RAPD, B04.451, at a distance of 4.8 cM and by an AFLP marker at a distance of 14.2 cM. The peak of the QTL falls around the B04.451 marker, 4.8 cM from D04.705. This marker defines this QTL peak as coincident with the QTL designated *Phyto.5.2* (Thabuis et al. 2003, 2004). These results demonstrate that the locus detected by D04-related molecular markers cosegregated with the chromosomal segment previously identified to contain *Phyto.5.2* in both interspecific and intraspecific mapping populations. The markers that we have identified in this study also fall near the peak of the major QTL for resistance to *P. capsici* in an independent mapping population.

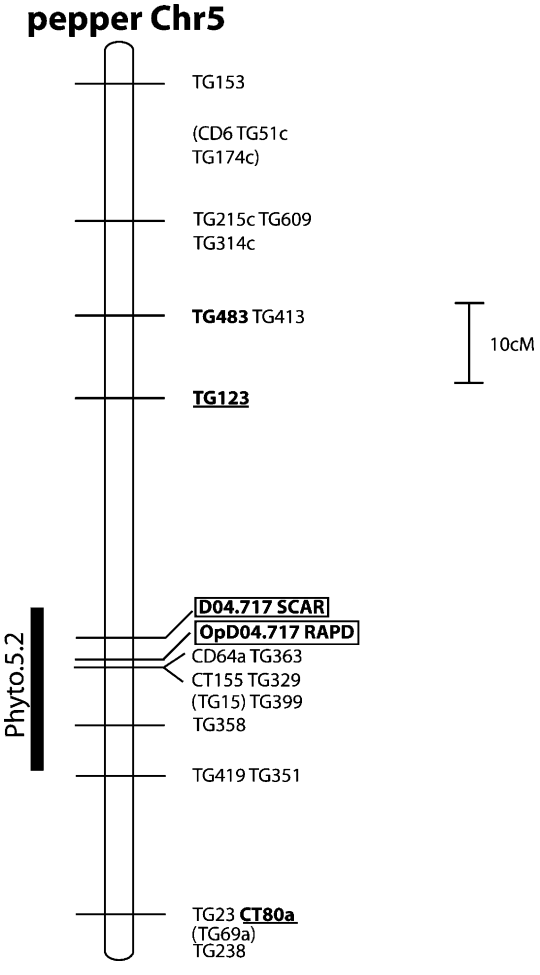

Fig. 3 D04 RAPD and SCAR markers map to pepper chromosome 5. RFLP map of pepper chromosome 5 from the *C. annuum* × *C. chinense* (AC) mapping population. The inferred position of the QTL previously reported by Thabuis et al.(Thabuis et al. 2003, 2004) are represented by the *darkly shaded bar*. Shared markers between the Thabuis et al. (2003) maps are RFLP markers highlighted in *bold*. The markers used to infer the placement of the QTL are *underlined*

Discussion

Resistance to *P. capsici* in pepper represents a very complex case where at least six regions of the pepper genome contribute to some or all of the components of the resistant response and expression of resistance is affected by plant development and environment (Thabuis et al. 2004 and references therein). Despite detailed information about QTL that contribute to this

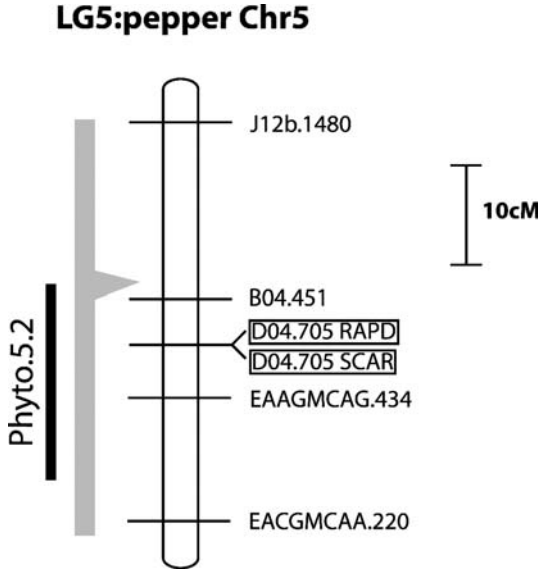

Fig. 4 D04 RAPD and SCAR loci on pepper chromosome 5 in a *C. annuum* mapping population segregating for resistance to *P. capsici*. The linkage group (LG5:Chr5) of the CM334 × JEP F_2 map is where the major QTL conferring resistance to *P. capsici* in pepper was located. The QTL, defined as significant above LOD = 3, spanned the linkage group (approx. 48 cM) indicated by the *lightly shaded bar*, and the peak LOD (9.02) is shown by the *attached arrowhead* to be within 6 cM of the D04 loci. The inferred position of the *Phyto.5.2* QTL from Thabuis et al. (2003, 2004) is represented by the *darkly shaded bar* (see also Fig. 3), which spans a portion of the QTL determined in the CM334 × JEP F_2 population, including the region containing the D04.705 RAPD and SCAR markers

trait and extensive investments in breeding varieties with resistance equal to that observed in the source of resistance, *C. annuum* CM334, resistance to *P. capsici* has not been achieved. In this study, we tested the hypothesis that a bulking approach may both contribute additional information on the extent to which a QTL is present in unrelated, highly resistant germplasm and assist in the identification of the specific QTL that should receive the highest priority in breeding programs.

We developed a large segregating population derived from the most promising source of resistance in *C. annuum*, CM334, and screened the F_3 generation to identify both the most resistant and the fully susceptible plant families. We used these families, together with other highly resistant and susceptible genotypes, to identify a RAPD locus that was present only in all highly resistant genotypes and absent in all of the test genotypes that showed intermediate (rated 3–7) or susceptible (rated 8 or 9) responses. Based on the D04 RAPD locus, a SCAR primer set was also developed. In subsequent studies with DNA samples provided by seed companies, this marker consistently appeared in the highly resistant proprietary breeding lines derived from CM334 and was absent in the susceptible proprietary inbred parents of commercial pepper hybrid varieties (data not shown). The results presented in Figs. 1 and 2 and those from the broader tests in proprietary germplasm obtained from several seed companies establish the potential utility of these markers to distinguish pepper genotypes with the highest levels of resistance to *P. capsici*. Furthermore, our results are consistent with the possibility that this QTL for resistance is present in other highly resistant genotypes, even those outside the species, *C. annuum*.

To determine whether these markers co-localized with a known QTL for resistance to *P. capsici*, we mapped the D04 RAPD and SCAR loci in two mapping populations—one, a well-developed reference population (Livingstone et al. 1999; Paran et al. 2004), which enabled us to infer the relationship of this locus to mapped QTL, and the other, a mapping population screened for resistance to *P. capsici* and analyzed for resistance QTL. In both populations, both the RAPD and SCAR loci mapped to the centromeric region of chromosome 5 within the interval defined by *Phyto.5.2* (Thabuis et al. 2003, 2004). This QTL has been implicated in resistance to several components of partial resistance, explaining from 5–42% of the phenotypic variance, depending on the population and resistance assay. In our study, these loci mapped within 5 cM of a RAPD marker, B04.451—which is similar to the marker B04_0.45 mapped in Thabuis et al. (2003), Pflieger et al. (2001) and Lefebvre and Palloix (1996)—at the QTL peak in our study and closely linked to the peak of the resistance QTL in previous works. Based on the comparative genetic maps published by Livingstone et al. (1999), this position in pepper places *Phyto.5.2* in an intergeneric disease resistance cluster as defined by Grube et al. (2000) with resistance genes, *R2*, Ny_{tbr} and *Gpa4* in potato and *Hero* in tomato, all of which occur in the segment of chromosome 4 that shows synteny with pepper chromosome 5.

These results confirm 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 six genomic positions identified in previous studies was consistently present in highly resistant germplasm. Theoretical studies suggest that the maximum number of genomic positions that can be selected simultaneously in a MAS breeding program is fewer than six (Hospital and Charcosset 1997). Therefore, from a practical point of view in this system, MAS should be integrated with a destructive phenotypic disease screen to achieve the best results. Because adjusting the intensity of a phenotypic screen can be difficult—resulting in survivors whose progenies show only intermediate levels of resistance—tools to distinguish among survivors of a destructive screen will be useful in this system. In practice, the marker published in this study will be most useful when applied in a population already subjected to a disease screen for resistance to determine which individuals among the survivors are most likely to be highly resistant and generate highly resistant progenies. Our results confirm previous work that identified *Phyto.5.2* as an important QTL for resistance to *P. capsici* in pepper and extend these results to suggest that this QTL may account for differences in the levels of resistance that are most economically significant. Furthermore, the results from this study define a selection tool for this QTL and suggest that future mechanistic studies on the components of resistance should focus on this position in the genome.

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References

Abad ZG, Abad JA, Cresswell T (2002) Advances in the integration of morphological and molecular characterization in *Phytophthora benus*: the case of *P. kelmania* and other putative new species. Phytopathology 92[Suppl 6]:S1

Barksdale TH, Poapvizas GS, Johnston SA (1984) Resistance to foliar blight and crown rot of pepper caused by *Phytophthora capsici*. Plant Dis 68:506–509

Basten CJ, Weir BS, Zeng ZB (1997) QTL CARTOGRAPHER: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, N.C.

Biles CL, Brunton BD, Wall MM, Rivas M (1995) *Phytophthora capsici* zoospore infection of pepper fruit in various physical environments. Proc Okla Acad Sci 75:1–5

Black LL, Green SK, Hartman GL, Poulos JM (1991) Pepper diseases: a field guide. Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan

Grube RC, Radwanski ER, Jahn M (2000) Comparative genetics of disease resistance within the Solanaceae. Genetics 155:873–887

Guerrero-Moreno A, Laborde JA (1980) Current status of pepper breeding for resistance to *Phytophthora capsici* in Mexico. In: Synopses 4th Meet Capsicum Working Group Eucarpia. I.V.T., Wageningen, pp 52–56

Holmes GJ, Lancaster ME, Rodriguez RJ, Redman RS (2001) Relative susceptibility of cucurbit and solanaceous crops to *Phytophthora* blight. Phytopathology 91[Suppl 6]:S39

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

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

Lefebvre V, Palloix A (1996) Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction pepper-*Phytophthora capsici* Leonian. Theor Appl Genet 93:503–511

Lefebvre V, Pflieger S, Thabuis A, Caranta C, Blattes A, Chauvert JC, Daubeze AM, Palloix A (2002) Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known-function genes. Genome 45:839–845

Lincoln SE, Daly MJ, Lander ED (1993) Constructing genetic linkage map with MAPMAKER/EXP V3.0: a tutorial and reference manual. Technical report. Whitehead Institute, Cambridge, Mass.

Livingstone KS, Lackney VK, Blauth JR, van Wijk R, Jahn MM (1999) Genome mapping in *Capsicum* and the evolution of the genome structure in the Solanaceae. Genetics 152:1183–1202

Mozzetti C, Ferraris L, Tamietti G, Matta A (1995) Variation in enzyme activities in leaves and cell suspensions as markers of incompatibility in different *Phytophthora*-pepper interactions. Physiol Mol Plant Pathol 46:95–107

Oelke LM, Bosland PW, Steiner R (2003) Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Capsicum annuum*. J Am Soc Hortic Sci 128:213–218

Ortega RG, Palazon-Espanol C, Cuartero-Zueco, J (1991) Genetics of resistance to *Phytophthora capsici* in the pepper line 'SCM-334'. Plant Breed 107:50–55

Paran I, Rouppe van der Voort J, Lefebvre V, Jahn M, Landry L, von Schriek M, Tanyolac B, Caranta C, Ben Chaim A, Livingstone K, Palloix A, Peleman J (2004) An integrated genetic linage map of pepper (*Capsicum* spp.). Mol Breed 13:251–261

Pflieger S, Palloix A, Caranta C, Blattes A, Lefebvre V (2001) Defense response genes co-localize with quantitative disease resistance loci in pepper. Theor Appl Genet 103:920–929

Pochard E, Molot PM, Dominguez G (1983) Etude de deux nouvelles sources de resistances a *Phytophthora capsici* Leon. chez le piment: confirmation de l'existence de trois composantes distinctes dans la resistance. Agronomie 3:333–342

Prince JP, Zhang Y, Radwanski ER, Kyle MM (1997) A versatile and high-yielding protocol for the preparation of genomic DNA from *Capsicum* spp. (pepper). HortScience 32:937–939

Ristaino JB, Johnston SA (1999) Ecologically based approaches to management of *Phytophthora* blight on bell pepper. Plant Dis 83:1080–1089

Sambrook J, Russel DW (2001) Molecular cloning: a laboratory manual, vol 1, 3rd edn. Cold Spring Harbor Laboratory Press, Plainview, pp 1.32–1.34

Thabuis A, Palloix A, Pflieger S, Daubeze AM, Caranta C, Lefebvre V (2003) Comparative mapping of *Phytophthora* resistance loci in pepper germplasm: evidence for conserved resistance loci across Solanceae and for a large genetic diversity. Theor Appl Genet 106:1473–1485

Thabuis A, Lefebvre V, Bernard G, Daubeze AM, 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. Theor Appl Genet 109:342–351

Walker SJ, Bosland PW (1999) Inheritance of *Phytophthora* root rot and foliar blight resistance in pepper. J Am Soc Hortic Sci 124:14–18

Wang S, Basten CJ, Zeng ZB (2003) WINDOWS QTL CARTOGRAPHER 2.0. Department of Statistics, North Carolina State University, Raleigh, N.C.