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Resistances to anthracnose (*Colletotrichum acutatum*) of Capsicum mature green and ripe fruit are controlled by a major dominant cluster of QTLs on chromosome P5

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ABSTRACT

Anthracnose (*Colletotrichum* spp.) is a serious disease worldwide in pepper (*Capsicum*) production. Inheritance of resistance to *Colletotrichum acutatum* from a *Capsicum chinense* accession (PBC932) was studied in a BC_1 population derived from a hybrid with *Capsicum annuum* line 77013 (susceptible) using a QTL analysis method. Resistance test was performed on detached mature green and mature red fruit under laboratory conditions by evaluated in disease incidence, true lesion diameter and overall lesion diameter. Based on a linkage map with 14 linkage groups, 385 markers (SSR, InDel and CAPS), 1310.2 cM in length, inclusive Composite Interval Mapping (ICIM) revealed main effect QTLs located in a close marker interval on P5 chromosome for all fruit stages and resistance criteria, and four minor-effect QTLs only at green mature stage. Identification of recombinant individuals suggested that resistance in green versus red fruit may be controlled by distinct genes within the QTL interval on P5.

1. Introduction

Pepper (*Capsicum* spp.) anthracnose (*Colletotrichum* spp.), causing pre- and post-harvest fruit rot, leads to severe economic losses in tropical and subtropical areas including China, Korea, India, Indonesia and Thailand, and has become one of the main barriers to pepper production (Kim et al., 2008b; Than et al., 2008a,b; Voorrips et al., 2004; Xia et al., 2011). Pepper anthracnose is caused by several *Colletotrichum* spp., including *Colletotrichum acutatum* (teleomorph *Glomerella acutata*), *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*), *Colletotrichum capsici* (a synonym of *Colletotrichum dematium*), and *Colletotrichum coccodes* (Park and Kim, 1992). *C. acutatum* and *C. gloeosporioides* are the most destructive and widely distributed (Sarath Babu et al., 2011; Voorrips et al., 2004). These pathogens attack pepper fruit at both the green and the red fruit stages, and can cause lesions on pepper leaves and stems. Typical anthracnose symptoms on pepper fruit are sunken necrotic tissues, with concentric rings of acervuli. These fruit blemishes lead to unmarketability (Than et al., 2008a,b).

In recent years, anthracnose in pepper has been more and more serious in China, especially caused by *C. acutatum* (Xia et al., 2011). Applications of fungicides and integrated pest management are used for disease control, which may have negative effects on farmer's income and their health. The most economic and environmentally friendly method is to develop resistant cultivars.

The main sources of resistance to anthracnose have been identified in *Capsicum baccatum* L. and *C. chinense* Jacq. by Asian Vegetables Research and Development Center (AVRDC) in 1999, and researchers have used these sources to study the inheritance of anthracnose resistance (Kim et al., 2010; Lee et al., 2010; Lin et al., 2002; Pakdeevaraporn et al., 2005; Voorrips et al., 2004). Genetic analyses of segregating populations showed that the resistance inheritance pattern varied depending on the *Colletotrichum* species and isolate, the resistance source, and also the fruit maturation stage.

Considering *C. acutatum*, the resistance derived from *C. chinense* 'PBC932′ into the line '0038–9155′ was shown to be controlled by two complementary dominant genes in green fruit, but two recessive genes in red fruit (Lin et al., 2007). Using another isolate ('KSCa-1′), the resistance from the same *C. chinense* accession in the pepper line 'AR', was reported as monogenic recessive in green fruit (Kim et al., 2008b). Using the same isolate and fruit stage, the

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resistance from *C. baccatum* (PI594137) was reported monogenic dominant (Kim et al., 2008a), whereas the resistance from *C. baccatum* ('PBC80′) was reported to be monogenic recessive in green fruit and monogenic dominant in red fruit (Mahasuk et al., 2009b).

Considering *C. capsici*, the resistance from the *C. chinense* 'PBC932′ was reported to be controlled by a single recessive gene in green as well as red fruit, as observed in the derived *C. annuum* L. lines 'AR' and 'Daepoong-cho' (Kim et al., 2008b; Mahasuk et al., 2009a; Pakdeevaraporn et al., 2005). Cheema et al. (1984) also reported a recessive resistance to *C. capsici* with significant epistatic effects. In contrast, dominant resistance to *C. capsici* isolates were reported in *C. annuum* breeding lines '83–168′ and 'Chungryong' (Park et al., 1990a,b; Lin et al., 2002).

Genetic mapping and Quantitative Trait Loci (QTL) analyses further specified the quantitative and polygenic or oligogenic nature of the resistances (Kim et al., 2010; Lee et al., 2010; Voorrips et al., 2004). One major QTL was detected for resistance to *C. capsici* and three minor QTLs additional to *C. gloeosporioidesin* an interspecific *C. annuum* Jatilaba × *C. chinense* PRI95030 population (Voorrips et al., 2004). Lee et al. (2010) identified two major linked QTL (CaR12.1 and CaR12.2) for *C. acutatum* resistance and two distinct major QTLs (CcRC and CcR9) for *C. capsici* resistance in an interspecific *C. baccatum* PBC81 × *C. annuum* SP26 population. Kim et al. (2010) identified two major QTLs and 16 minor QTLs that influenced *C. acutatum* resistance in an intraspecific *C. baccatum* × Golden-aji. These results confirm the specificity of resistance QTL as regard the *Colletotrichum* species, but are hardly comparable to the previous genetic analyses since parental relationships between resistance sources are not mentioned. Moreover the linkage groups carrying QTL were not assigned to pepper chromosomes. Endly, only red mature fruit were considered in these QTL analyses.

With the objective to breed *C. annuum* for resistance to *C. acutatum* which is prevalent in China, we focused on the *C. chinense* resistance source PBC932 because of its high resistance level and higher sexual compatibility with *C. annuum* compared to *C. baccatum*. In our study, we mapped QTL for resistance to *C. acutatum* from the *C. chinense* PBC932 accession in an interspecific cross with *C. annuum* and we focused on resistance alleles affecting fruit resistance at different maturity stage that would be of value in breeding programs.

2. Materials and methods

2.1.1. Mapping population

The female parent (P_1) was the inbred line 77013 (*C. annuum*), susceptible to *C. acutatum*, developed at the Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS). The paternal (P_2) was PBC932 (*C. chinense*), provided by Dr. Wang Tiancheng, AVRDC. The BC_1 population by backcrossing the interspecific F_1 to parent P_1 with186 individuals, 3 plants of each parental line and 9 F_1 plants were grown in a plastic greenhouse at IVF in 2012.

2.2. Pathogen

An isolate of *C. acutatum*, 'Ca', collected and isolated from pepper fruit in Hunan province using single-spore isolation method (Ho and Ko, 1997). It was assigned to the *C. acutatum* species by colony morphology and rDNA-ITS (ITS4/ITS5) sequence (Genebank accession No. KC936995).

2.3. Bioassay of anthracnose resistance

Artificial inoculation was performed on detached mature green fruit (green fruit that have reached their final size, 35–40 d after flowering) and mature red fruit (45–50 d after flowering, physiological maturity stage), using the microinjection method developed at AVRDC in 1999 with slight modification. The healthy fruit harvested from greenhouse-grown individual plants were washed in distilled water, 75% ethanol to remove various microorganisms on the fruit surface, and then air dried. Inoculation was conducted at a 1 mm depth using a microinjector (Micro Syringe^{TM} model 1705 TLL) and a dispenser (Hamilton PB600-1, Repeating Dispenser, Reno, NV, USA). Each fruit was injected with 1 μl of prepared conidial suspension with concentration of 5×10^5 spores ml^{-1} per site. One to four sites were inoculated per fruit depending on the fruit size so that the distance between two sited was at least 50 mm. The inoculated fruit were incubated with the inoculated sites facing upwards in plastic boxes (50 × 25 × 20 cm), on four layers of filter paper that was moistened with 150 ml distilled water. The boxes were sealed with plastic wrap to maintain the relative humidity above 95% and incubated under 28 °C in the dark for 48 h. The fruit was incubated for 5 more days under the same conditions after the plastic wrap was removed. Inoculation was conducted with three replications (3 plastic boxes), each with two pots fruit.

Three parameters were measured to evaluate the resistance phenotypes: the disease incidence (D) is the percentage of infected sites per total inoculated sites, the overall lesion diameter (O) is the average lesion diameter over all inoculated sites (in mm) including those that did not develop lesions, and the true lesion diameter (T) is the average lesion diameter over all lesions that developed (in mm) (Voorrips et al., 2004). Lesions showing bacterial rot were not measured. These three parameters were measured in green and in red mature fruit, resulting in six criteria GD, GO and GT for green fruit, RD, RO and RT for red mature fruit.

2.4. Data analysis

Distributions of disease incidence, overall lesion diameter and true lesion diameter at mature green and red stage were analyzed using R (2014). We first compared green and red fruit variables on BC_1 plants using the ANOVA model $y_{i,j,k} = m_{i,j} + \varepsilon_{i,j,k}$ with $y_{i,j,k}$ the phenotype value of measure type i (green or mature), of the kth repetition of genotype j, and $m_{i,j}$ the expected phenotypic value of measure type i on genotype j. Fisher tests were used to test for genotype effect and measure type effect. A Bonferroni procedure was then performed to test the assumption $m_{1,j} = m_{2,j}$ (equality of measures at green and red fruit stages) and to detect for which genotype green and red fruit variables were different.

Then we tested on each variable if a genotypic difference existed between BC_1 plants, and F_1 and P_1 plants with the model $y_{i,k} = m_i + \varepsilon_{i,k}$ were $y_{i,k}$ is the phenotypic value of the variable of the kth repetition of genotype i and m_i its mean. A Bonferroni procedure was then used to test which BC_1 decendant were significantly different from F_1 (respectively P_1) by testing the assumptions $m_i = m_{F1}$ (respectively $m_i = m_{P1}$).

Table 1
Values of resistance-related traits for parents and F_1 plants after inoculation with *C. acutatum*.

Method	P_1: 77013	F_1 (77013 × PBC932)	P_2: PBC932
GO (mm)	21.19 ± 0.82a	4.95 ± 3.2b	0.84 ± 1.06b
GT (mm)	21.22 ± 0.82a	7.60 ± 4.2b	2.10 ± 2.11b
GD (%)	99.87 ± 0.36a	47.92 ± 30.8b	12.19 ± 12.82b
RO (mm)	19.64 ± 2.27a	7.15 ± 4.3b	4.04 ± 2.50b
RT (mm)	19.87 ± 1.96a	8.17 ± 4.3b	5.75 ± 3.42b
RD (%)	97.82 ± 3.82a	57.06 ± 35.1ab	52.50 ± 30.92b

G, mature green fruit stage; R, mature red fruit stage; O, overall lesion diameter; T, true lesion diameter; D, disease incidence. *Note*: Different letters in each row mean significant difference based on ANOVA testing at 0.05 level.

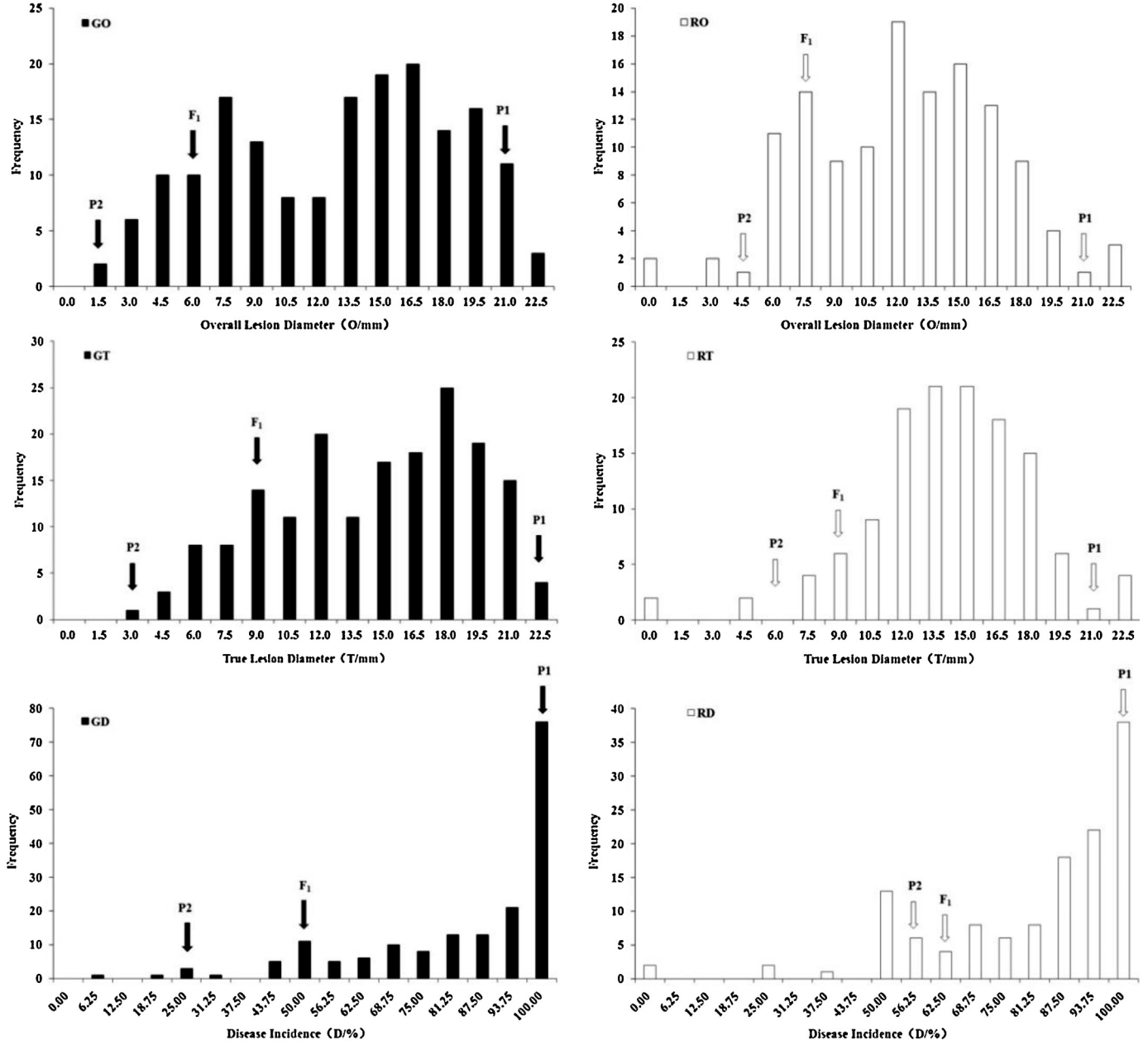

Fig. 1. Distribution of anthracnose (*C. acutatum*) resistance in the pepper BC_1 population for the different scoring methods. G, mature green fruit stage; R, mature red fruit stage; O, overall lesion diameter; T, true lesion diameter; D, disease incidence. P1: susceptible parental line 77013 (*C. annuum*), P_2: resistant male parental line PBC932 (*C. chinense*); F_1: 77013 × PBC932; BC_1 population: 186 individuals from (77013 × PBC932) × 77013.

2.5. Construction of linkage map

Total genomic DNA was extracted from F_1 and BC_1 mapping population seedlings by CTAB method with relatively minor modification (Fulton et al., 1995). Markers of 815 SSR (Huang et al., 2000, 2011; Lee et al., 2004; Minamiyama et al., 2006; Yi et al., 2006), 1 InDel (Wang, 2011) and 228 CAPS markers (Wu et al., 2009) were screened in parental lines and after genotyped and BC_1 population. The PCR amplifications of SSRs and InDel were performed as described by Huang et al. (2011) and Wang (2011). Primers of the InDel markers, F:GGTATCTTATTTCATAGGGACCAGGCA; R:TTTGCGGTAGTGACAACAACTTTACAGCCA (Wang, 2011). The PCR amplifications and restriction enzyme digestions of CAPS markers were performed as described by Wu et al. (2009).

Mapping was performed using JoinMap 4.0 software (Van Ooijen, 2006) with a minimum LOD score of 3.0, using Kosambi function (Kosambi, 1943). The linkage map constructed was compared to maps previously published (Lee et al., 2009; Mimura et al., 2012; Wu et al., 2009; Yi et al., 2006).

2.6. QTL analysis

The QTLs associated with anthracnose resistance were identified by the QTL ICIMapping software version 3.2 (Li et al., 2007) using ICIM-ADD and ICIM-EPI analysis methods. For detection of QTLs with additive effects (ICIM-ADD analysis), the *P* values for entering variables (PIN) and removing variables (POUT) were set at 0.0001 and 0.0002, and the scanning step was 0.1 cM; for the detection of QTLs with epistatic effects (ICIM-EPI analysis), the PIN

Table 2
T test and correlation analyses of phenotypic values of the BC_1 population ((77013 × PBC932) × 77013) between mature green and red fruit stages in the different scoring methods.

Method	*t* value	*Pr* > |*t*|	Correlation coefficient
GO/RO	2.51	0.014	0.371*
GT/RT	2.60	0.011	0.394*
GD/RD	1.45	0.149	0.277

G, mature green fruit stage; R, mature red fruit stage; O, overall lesion diameter; T, true lesion diameter; D, disease incidence.
* Significant difference at 0.05 level.

and POUT were set at 0.0001 and 0.0002, respectively, and the scanning step was 5.0 cM. A LOD threshold of 2.0 by permutation analyses was chosen to declare a putative additive QTL as significant, while 5.0 to a pair of putative epistatic QTL. The proportion of observed phenotypic variance explained (R^2) by each detected QTL and the corresponding additive effects were also estimated. The QTL nomenclature followed this: *An*thracnose *R*esistance at *G*reen (or *R*ed) fruit stage under *O*verall lesion diameter (or *T*rue lesion diameter, or *D*isease incidence) on chromosome P*x* (*x*: number of chromosome), for example $AnR_{GD}5$.

3. Results

3.1. Anthracnose resistance phenotyping and distribution of the BC_1 progeny

The mean disease scores of the resistant parent PBC932 were 12.19% for GD (Disease incidence in Green fruit stage), 0.84 mm for GO (Overall lesion diameter in Green fruit stage), 2.10 mm for GT (True lesion diameter in Green fruit stage), 52.50% for RD (Disease incidence in Red fruit stage), 4.04 mm for RO (Overall lesion diameter in Red fruit stage) and 5.75 mm for RT (True lesion diameter in Red fruit stage). These values were all significantly lower ($P = 0.05$) than those of the susceptible parent 77013 (GD: 99.87%, GO: 21.19 mm, GT: 21.22 mm, RD: 97.82%, RO: 19.64 mm and RT: 19.87 mm) ($P < 0.05$). The mean disease scores of the F_1 individuals fell in between PBC932 and 77013, but skewed toward PBC932 in all six phenotyping methods so that score values were not significantly different from those of the resistant parent (Table 1). These results suggest that the resistance from *C. chinense* PBC932, is completely dominant over the susceptibility, at both the mature green and red stages.

The distributions of the BC_1 individuals for anthracnose resistance in the different scoring methods are shown in Fig. 1. Most BC_1 individuals ranged between the parental lines for all the parameters. Distributions displayed a plurimodal (GO, RO, GT) or unimodal shape suggesting oligo or polygenic segregation. For disease incidence (GD and RD), more than 50% of individuals ranged in the most susceptible classes (90–100%), delivering a highly skewed and poorly informative segregation.

3.2. Genetic correlation analysis of resistance between mature green and red fruit stage

To determine whether resistance of green and red fruit depended on the same genetic control, *t*-test and correlation analysis of overall and true lesion diameters and disease incidence between the different fruit stages were conducted (Table 2). Correlations between green and red fruit for the three parameters were low (0.28–0.39) but significant, indicating that genes for green and red fruit resistance were no independently inherited. The relationships between green mature and red mature fruit stages are shown in Fig. 2. In these graphs, several BC_1 individuals located distantly from the linear regression curves, indicating that their resistance

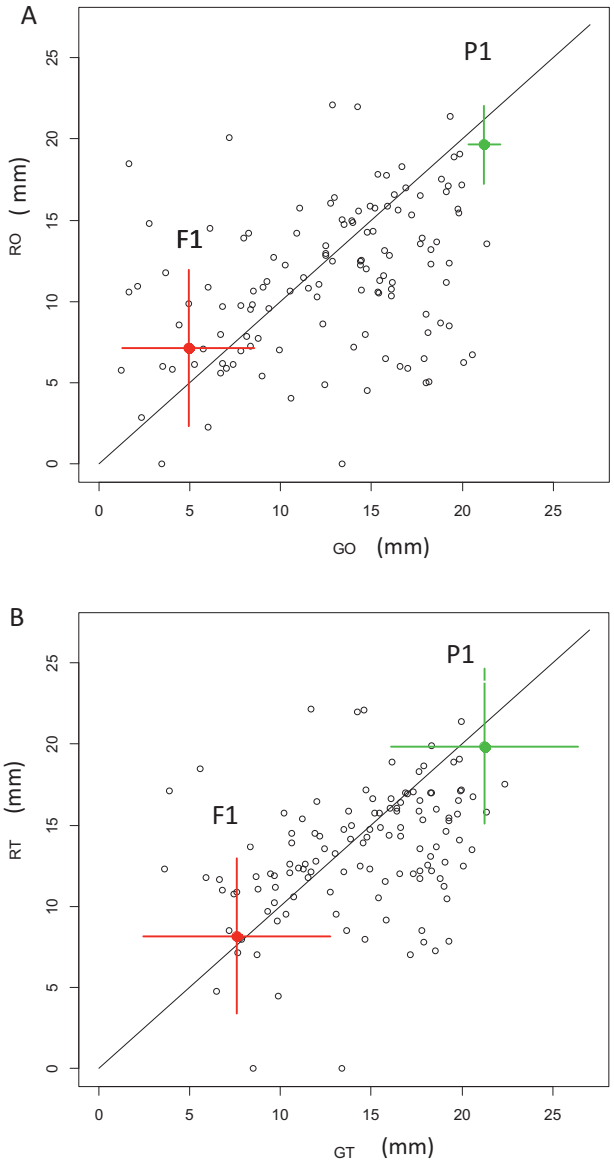

Fig. 2. Diagram representing the BC_1 individuals ((77013 × PBC932) × 77013) for their resistance phenotype measured in green mature fruit stage and red mature fruit stage. F_1: F_1 hybrid (77013 × PBC932); P_1: susceptible parent 77013 (*C. annuum*). Lines crossing at the F1 and P1 points indicate the standard errors of their means. A: relationship between GO (overall lesion diameter in green mature fruit stage) and RO (overall lesion diameter in red mature fruit stage) $RO = aGO + b$ ($a = 0.309$, $b = 7.526$) B: relationship between GT (true lesion diameter in green mature fruit stage) and RT (the true lesion diameter in red mature fruit stage). $RT = aGT + b$ ($a = 0.330$, $b = 8.623$).

phenotype diverge between green and red fruit stage. Bonferroni's multiple range testing at 0.05 level, confirmed that two of these individuals showed resistant phenotype in green (not significantly different from F_1 hybrid) but susceptible in red (not significantly different from or significant higher than the susceptible parent P_1) (Table 3). This suggests that resistance at the two maturation stages may recombine in a few individuals.

3.3. Linkage map

A total of 385 markers including 349 SSRs, 1 InDel and 35 CAPS, were assigned to 14 linkage groups spanning 1310.2 cM, with an average marker interval of 3.40 cM (supplementary data 1). The number of markers mapped per linkage group varied from 4 (P1b and P11b) to 68 (LG1), while the length of linkage

Table 3
Detection of BC_1 individuals with significantly distinct phenotypes for anthracnose resistance between green fruit stage and red fruit stage.

Plants	GO (mm)	RO (mm)	GT (mm)	RT (mm)
BC_1-58	2.76a *Nf*	14.80b *Np*	3.87a *Nf*	17.14b *Np*
BC_1-149	7.18a *Nf*	20.11b *Np*	11.68a *Nf*	22.17b *Np*

G, mature green fruit stage; R, mature red fruit stage; O, overall lesion diameter; T, true lesion diameter; D, disease incidence; P_1: inbred line 77013 (*C. annuum*), susceptible to *C. acutatum*; P_2: PBC932 (*C. chinense*), resistant to *C. acutatum*. *Note*: a, b: Different letters in each row mean significant difference between GO and RO, or GT and RT; *Nf*: not significant difference to the F_1 value, *Np*: no significant difference to the susceptible parent P_1 value (Bonferroni's multiple range testing at 0.05 level).

groups varied from 21.8 cM (P11b) to 152.1 cM (P4). Comparison of these maps using shared markers revealed that all the linkage groups matched to one unique pepper chromosome except LG1 linkage group, which markers belonged to both chromosomes 1 and 8 and could not be separated.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2014.10.033.

3.4. QTLs for anthracnose resistance

A total of nine to three additive QTLs on P3, P5, P7, P10 and P12 chromosomes were detected by ICIM-ADD analysis for all the indexes associated with *C. acutatum* resistance at mature green and red fruit stages, respectively (Table 4 and Fig. 3). No epistatic QTLs were detected by ICIM-EPI analysis.

Considering the green fruit, 5 QTLs were detected for GO ($AnR_{GO}3$, $AnR_{GO}5$, $AnR_{GO}7$, $AnR_{GO}10AnR_{GO}12$), one QTL for GT ($AnR_{GT}5$) and 3 QTLs for GD ($AnR_{GD}5$, $AnR_{GD}10$, $AnR_{GD}12$). The QTLs on chromosome P5 accounted for a high percentage the phenotypic variance for all traits (62.38%, 60.50% and 33.17% for GO, GT, and GD) and were located at the same marker interval between InDel and HpmsE116. The others QTLs showed minor effects with R2 values ranging from 2.52% to 5.4%. The total phenotypic variance explained by these QTLs was 73.88%, 60.50%, and 43.29% in GO, GT, and GD, respectively.

At the mature red stage, only one QTL on P5 chromosome was detected for each trait RO, RT, RD. Phenotypic variance explained by these QTLs was 15.24%, 15.90%, and 9.31% in RO, RT, and RD. These QTLs were co-located together, with a LOD max at the top of the chromosome, close to the major QTLs detected in green fruit. This indicates that the genome region at the top of chromosome P5, in the HpmsR116-InDel interval includes the major resistance gene(s) to anthracnose. The alleles increasing resistance at all the QTLs originated from the resistant parent PBC932, except for the minor QTLs on P7 (AnRgo7) and P10 (AnRGO10 and AnRGD10).

4. Discussions

4.1. Linkage map

The total length of our linkage map is similar to other interspecific maps (Kang et al., 2001; Lee et al., 2004, 2011; Livingstone et al., 1999; Prince et al., 1993; Yi et al., 2006). All the linkage groups were successfully assigned to the corresponding pepper chromosome using public SSR markers except LG1 which was a fusion of chromosome P1 and P8. Such pseudolinkage of chromosome 1 and chromosome 8 has been reported in interspecific (*C. annuum* × *C. chinense*) populations (Barchi et al., 2009; Lee et al., 2009; Yi et al., 2006). It was shown to result from the reciprocal translocation between P1 and P8 that occurred between *C. annuum* and *C. chinense* (Pickersgill, 1971; Wu et al., 2009).

4.2. Genetics of anthracnose resistance in pepper

The genetic analysis based on phenotype values of the F_1 and BC_1 individuals, which are either homozygous susceptible or heterozygous at the resistance loci, clearly showed that resistance of PBC932 to *C. acutatum* is mostly dominant. The QTL analyses further indicated that most of the genetic variation was explained by a major QTL in chromosome P5 at green as well as red fruit stages. This major QTL very probably corresponded to the major QTL for resistance to *C. capsici* and *C. gloeosporioides* detected by Voorrips et al. (2004) in the same species (*C. chinense*), but the lack of shared markers between the maps and of assignation of their linkage groups to pepper chromosomes made this comparison impossible. Minor QTLs in chromosomes P3, P7, P10 and P12 were also detected for resistance in green fruit but not red mature fruit, with resistant alleles originating from the susceptible parent at two of these loci. These results did not corroborate the previous studies which concluded to monogenic recessive (Kim et al., 2008b) or digenic dominant or recessive inheritance (Lin et al., 2007), but better fit the QTL analyses from Voorrips et al. (2004), Kim et al. (2010) and Lee et al. (2010) who detected one to two major QTLs, with additive to dominant effects, using distinct pepper accessions and *Colletotrichum* isolates.

The different phenotyping methods aimed at exploring distinct resistance components which may reduce the fruit damages caused by the fungus as expected by Voorrips et al. (2004). However, the major QTL on chromosome 5 was significant for all the components. Only the minor QTLs on chromosomes P10 and P12 did not affect the true lesion diameter, but significantly affected the disease index (frequency of successful infection) and the overall lesion diameter. Thus it could be expected that those QTLs primarily modulate the success of primary infection,

Table 4
QTLs identified for anthracnose resistance in BC_1 population (77013 × PBC932) × 77013.

QTLs	Chromosome	Position (cM)	Flanking markers	Interval (cM)	LOD	R^2 (%)	Add
$AnR_{GO}3$	P3	41.80	ES382-HpmsE126	0.5	2.30	2.93	1.878
$AnR_{GO}5$	P5	1.20	InDel-HpmsE116	9.6	32.26	62.38	8.61
$AnR_{GO}7$	P7	0.00	HpmsE057-Gpms161	6.9	2.21	2.52	−1.73
$AnR_{GO}10$	P10	90.30	Gp20068-C2_At4g03400	7.1	2.34	2.88	−1.85
$AnR_{GO}12$	P12	48.70	ES64-Epms745	0.8	2.70	3.17	1.97
$AnR_{GT}5$	P5	0.80	InDel-HpmsE116	9.6	31.91	60.50	7.28
$AnR_{GD}5$	P5	1.60	InDel-HpmsE116	9.6	12.26	33.17	0.25
$AnR_{GD}10$	P10	90.10	Gp20068-C2_At4g03400	7.1	2.26	4.72	−0.09
$AnR_{GD}12$	P12	49.90	ES118-ES181	1.5	2.84	5.40	0.10
$AnR_{RO}5$	P5	0.00	InDel-HpmsE116	9.6	4.49	15.24	3.59
$AnR_{RT}5$	P5	0.00	InDel-HpmsE116	9.6	4.70	15.90	3.12
$AnR_{RD}5$	P5	0.00	InDel-HpmsE116	9.6	2.65	9.31	0.13

Add, additive effect.

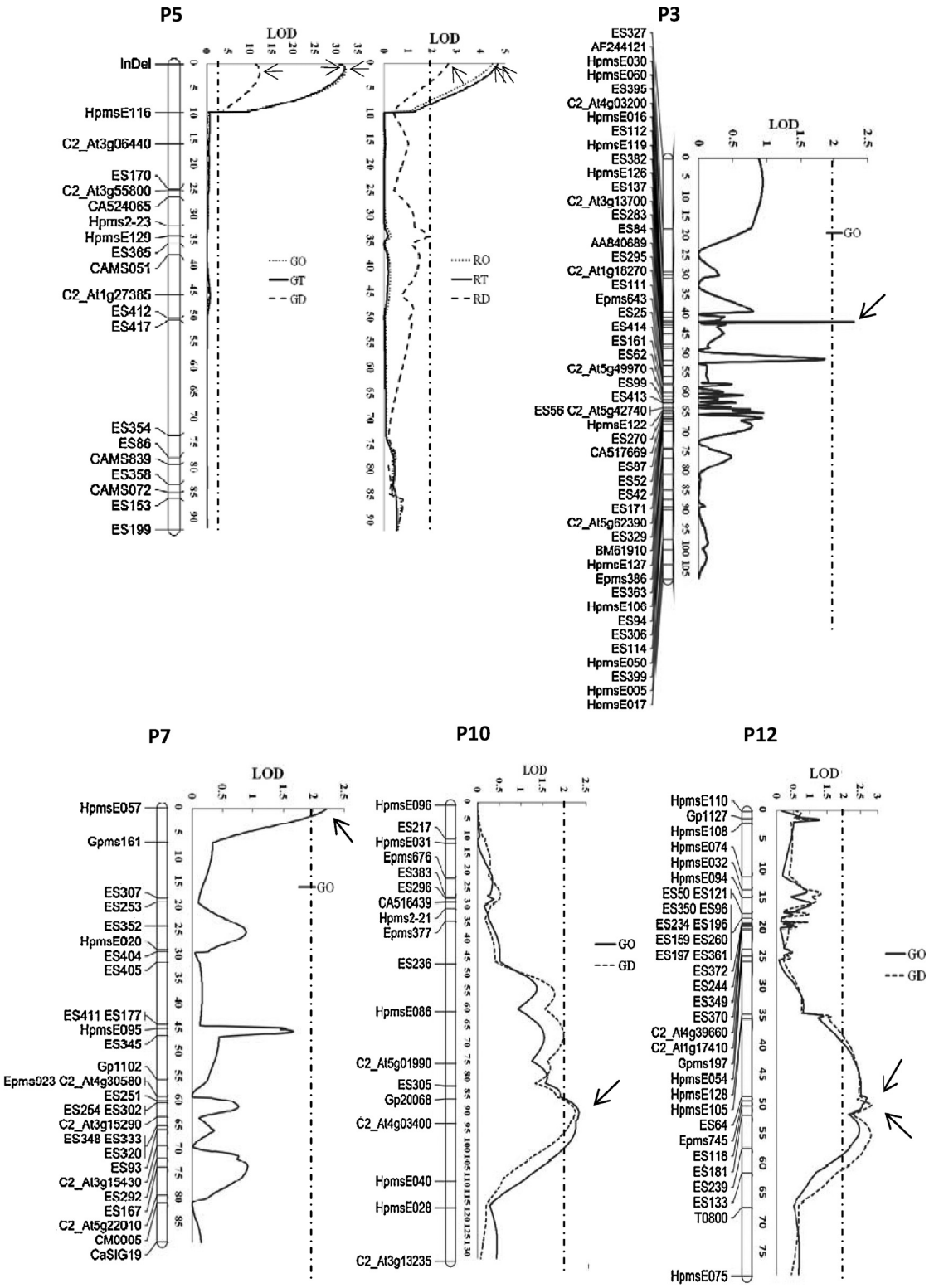

Fig. 3. LOD profiles of QTLs associated with anthracnose resistance. Arrows indicate the QTL loci with highest LOD value. G, mature green fruit stage; R, mature red fruit stage; O, overall lesion diameter; T, true lesion diameter; D, disease incidence. Only the chromosomes with significant QTLs are represented.

and consequently impact the overall lesion diameter. However, none of these minor QTLs displayed a significant effect on red mature fruit, indicating this resistance component is specific for green fruit stage, or too weak to be detected in red mature fruit.

4.3. Differences in genetics and expression of resistance between green and red fruit

Several authors gave evidence of differential reactions between green mature and red ripe pepper fruit toward anthracnose

resistance (Lin et al., 2007; Mahasuk et al., 2009b; Mongkolporn et al., 2010). Lin et al. (2007) concluded that the resistance of green fruit was dominant but recessive in red fruit. Mahasuk et al. (2009b) reported that genes in the green and red fruit derived from PBC80 (*C. baccatum*) were independent, but did not locate it in the pepper genome. Using a set of pepper accessions and *Colletotrichum* isolates, Mongkolporn et al. (2010) established a pathotype classification of the fungus and showed that isolates of *C. acutatum*, *C. capsici* and *C. gloeosporioides* are generally more virulent in red ripe fruit, while line PBC932 was more resistant to *C. capsici* at the mature green fruit stage than the ripe fruit stage. In our experiments, the lower phenotypic scores of the resistant parent PBC932 at green fruit stage (GO = 0.84, GT = 2.10, GD = 12.19) compare to red mature stage (RO = 4.04, RT = 5.75, RD = 52.50) already revealed that resistance expression is higher in green fruit. The weak (although significant) correlations between fruit stages for the phenotypic values of BC1 individuals also suggest differences. Considering QTL analyses, two obvious differences between fruit maturation stages raised: the minor QTLs were significant only at green fruit stage, and the R2 values of the major P5 QTLs were much higher at green fruit stage (33.17–62.38%) than red mature stage (9.31–15.24%). The same differences could be observed looking at the LOD values of these QTLs. On the one hand, the minor QTLs may express only at green fruit stage, the major QTL has a lower expression when the fruit becomes at maturity, so that most of the phenotypic variance may be explained by uncontrolled (environmental or error) variations. We also further looked to the P5 genomic region that controls most of the resistance whatever the parameter and fruit stage. The LOD max for all resistance components at green fruit stage (GO, GT, GD) was included within the InDel-HpmsE116 interval, but the LOD max for red fruit resistance components (RO, RT, RD) pointed on the InDel marker, that means at or above the upper limit of the linkage group. However, the confidence intervals of the QTLs overlap, so that QTL positions cannot be clearly differentiated. Looking further for putative recombinants in the BC_1 individuals based on phenotypes, only 2 individuals showed resistance at green fruit stage but susceptibility at red fruit stage. Those 2 individuals represented recombinant individuals and would indicate that resistance at green and red fruit stages are controlled by distinct genes within the same P5 genome interval. Mahasuk et al. (2009a,b) reported from phenotypic analyses that the genes in the green and red fruit derived from PBC932 were linked. In our research, we confirmed this result and precisely located these genes as major QTLs on chromosome P5. However to further confirm if resistance at the two fruit stages depend on a single or tightly linked QTLs, additional markers extending the linkage group have to be developed.

5. Conclusion

The resistance of pepper (*C. chinense* PBC932) to fruit anthracnose due to *C. acutatum* was shown to depend on a major QTL on chromosome P5 with dominant expression, and a few minor QTLs that partly originate from susceptible cultivars but express significantly only in green fruit. This is expected to make resistance breeding rather simple using backcross series to large fruited *C. annuum* cultivars, in which minor QTLs increasing green fruit resistance can be gathered. The markers InDel (Wang, 2011) and HpmsE116 should be efficient in markers assisted selection in order to introgress the quantitative resistance as a single major QTL cluster in new *C. annuum* genotypes. Phenotyping the first backcross generations will validate the efficiency of these markers. However, the results also indicate that resistance will be more efficient at the green-mature stage than in red mature fruit. Moreover recombinant individuals suggest that resistance at green versus red fruit stage may recombine at low frequencies. Fine mapping of the chromosome P5 telomeric region will deliver tools to further explore if distinct genes are included in the QTL region.

Conflict of interest

The authors declare that they have no conflict of interest.

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