SNP Mapping to Locate Anthracnose Resistance in *Capsicum* spp.

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Abstract
Two SNP maps were constructed from two chili populations including *Capsicum annuum* x *C. chinense* 'PBC932', and *C. baccatum* 'PBC80' x 'CA1316', aiming to identify QTLs for anthracnose resistance. The 'PBC932'-derived map contained 12 linkage groups (LG) with 214 SNPs and 824 cM coverage. The 'PBC80'-derived map contained 12 LGs with 403 SNPs and 1,270 cM coverage. Based on the 'PBC932' map, two QTLs corresponding to the anthracnose resistances in mature green and ripe fruit stages were identified on the same location of LG2 between two SNPs within 14 cM. Based on the 'PBC80' map, three QTLs were identified in the ripe fruit stage, which corresponded to different resistance traits that were assayed by different inoculation methods (microinjection or MI and high pressure spray or HP) with two different pathotypes (PCa2 and PCa3). All the three major QTLs for the resistance traits assayed by PCa2/MI, PCa3/MI, and PCa3/HP were located on LG4 between two SNP markers within 17 cM.
Key words: *Capsicum baccatum*, *Capsicum chinense*, *Colletotrichum truncatum*, *Colletotrichum acutatum,* QTL

Introduction

Anthracnose, caused by a complex of *Colletotrichum* species, is a major fungal disease infecting chili fruit in the tropics and subtropics worldwide, especially Asia (Mongkolporn and Taylor 2011). Fruit yield losses, both pre- and post-harvested, due to anthracnose are severe in wet seasons, and the losses can be over 80% (Mahasuk *et al*., 2009a). Typical anthracnose symptoms appear as sunken necrotic tissues with concentric rings of acervuli, that are often moist (Than *et al*., 2008; Montri *et al*., 2009).

Breeding for the anthracnose resistance has been around in Asia for over two decades with not much success, due to the complexity of the causal pathogen and the host-pathogen interaction, and the lack of resistance in *Capsicum annuum* gene pool (Mongkolporn and Taylor 2011). Three chili varieties with immune resistance including *C. chinense* 'PBC932', and *C. baccatum* 'PBC80' and 'PBC81' were identified by the World Vegetable Center-AVRDC since 1998, and have been shared among Asian chili breeders.

The 'PBC932' and 'PBC80' have been the major sources of anthracnose resistance in Thailand, and were used as donor parents producing three chili populations to previously study the genetics of anthracnose resistance (Pakdeevaraporn *et al*., 2005, Mahasuk *et al*., 2009a, Mahasuk *et al*., 2009b, Mahasuk *et al*., 2013). A key genetic discovery in all three populations was that the resistances at different fruit maturity stages were controlled by different genes. Of the three populations, two were used to map QTLs conferring the resistance to anthracnose in this study, including an interspecific *C. annuum* 'Bangchang' x *C. chinense* 'PBC932', and an intraspecific *C. baccatum* 'PBC80' x 'CA1316'.
Single-nucleotide polymorphisms (SNPs) are the most abundant and stable form of genetic variation in most genomes, therefore high map resolution can be fast achieved (Ganal et al. 2009). Several high throughput SNP detection systems have been developed. Competitive allele-specific PCR (currently called Kompetitive Allele Specific PCR or KASP^{TM}) from KBioscience offers a user-friendly high-throughput assay. With the advantage of the high throughput KASP technology, two SNP maps were constructed aiming to identify QTLs for anthracnose resistances derived from two chili varieties ie. *C. chinense* 'PBC932' and *C. baccatum* 'PBC80'.

Materials and Methods

Mapping populations and phenotyping

Two F2 single crossed populations segregating for anthracnose resistance, *i.e.* interspecific *Capsicum annuum* cv. 'Bangchang' x *C. chinense* 'PBC932', and intraspecific *C. baccatum* 'PBC80' x 'CA1316', were used to map with the SNP markers. The anthracnose resistance was evaluated at mature green and ripe fruit maturity stages. The 'PBC932'-derived population was assayed with *Colletotrichum*

truncatum (former name *C. capsici*) isolate '158ci' by microinjection (MI) following Montri *et al*. (2009) . The 'PBC80'-derived population was assayed with two *Colletotrichum acutatum* pathotypes including PCa2 (Ca313) and PCa3 (CaMJ5) as identified by Mongkolporn *et al*. (2010) by MI and high pressure spray (HP) (Mahasuk *et al*., 2013).

Linkage map construction and QTL analysis

The SNP markers obtained from each chili population were mapped using JoinMap 3.0 (van Ooijen and Voorrips 2001). QTL analysis of the anthracnose resistance was performed using MapQTL 4.0 with interval mapping at LOD 3.0 (van Ooijen *et al*., 2002).

Results and Discussion

SNP maps and QTL locations

PBC932-derived map: Approximately 20% (214) of the total 1,024 SNPs developed from the *C. annuum* genome were mapped. The map contained 12 linkage groups (LG) covering 824 cM (Fig.1).

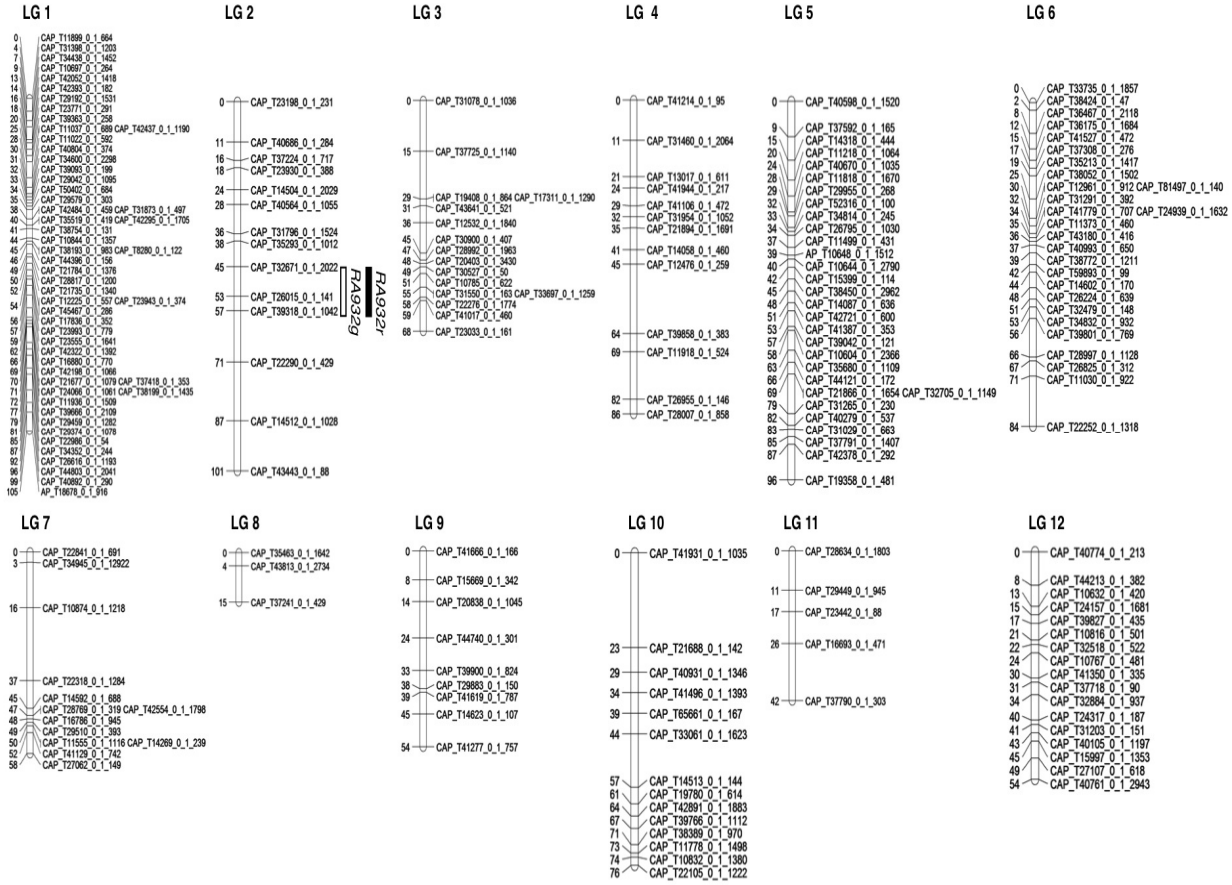

Figure 1. A SNP map of 'Bangchang' x 'PBC932' population, forming 12 linkage groups with 824 cM total coverage, constructed by JoinMap 3.0, LOD 6.0-10.0. The side bars indicate the locations of the QTLs for anthracnose resistance

Two major QTLs corresponding to the resistances to anthracnose in mature green and ripe fruit, were identified on the same location of the LG2 within two SNP markers at 14 cM. (Fig. 1).

The 12 LGs in both maps well corresponded to the assigned *Capsicum* chromosomes (P1-P12) (*Capsicum annuum* genome database; version 1.5, http://peppergenome.snu.ac.kr/), except for the LG3, LG8 and LG9 in the 'PBC80' map. The LG3 and LG8 were fused with the markers from P3, P5 and P9; and LG9 was fused with the markers from P3 and P5. Three major QTLs identified in the 'PBC80' were on the same location of LG4. Previous genetic analysis revealed that the resistance traits by different inoculation methods, MI and HP, appeared to be controlled by 2-linked genes, while the

resistance assayed with different *Colletotrichum* pathotypes, PCa2 and PCa3, appeared to be the same gene (Mahasuk *et al*., 2013).

PBC80-derived map: Approximately 35% (403) of the total 1,165 SNPs developed from the *C. baccatum* genome were mapped. The map contained 12 LGs covering 1,270 cM (Fig.2). Three major QTLs corresponding to the anthracnose resistance traits at ripe fruit stage, including the resistance to PCa2 by microinjection (PCa2/MI), resistance to PCa3 by microinjection (PCa3/MI) and resistance to PCa3 by high-pressure spray (PCa3/HP) were identified on the same location of LG4 flanked by two SNP markers 17 cM (Fig. 2).

Interestingly, P4 was also resided by other disease resistance genes, including tospoviruses and potyvirus (Djian-Caporalino *et al*., 2006). Previously, two QTLs for anthracnose resistance from different donor parent, *Capsicum baccatum* 'PBC81' were mapped on P9 and P12 (Lee *et al*., 2010; Lee *et al*., 2011). Our two minor QTLs were also on P9 and P12 (data is not shown). The SNPs that flanked all the identified QTLs in both maps will be greatly beneficial to select for the anthracnose resistance in chili breeding programs.

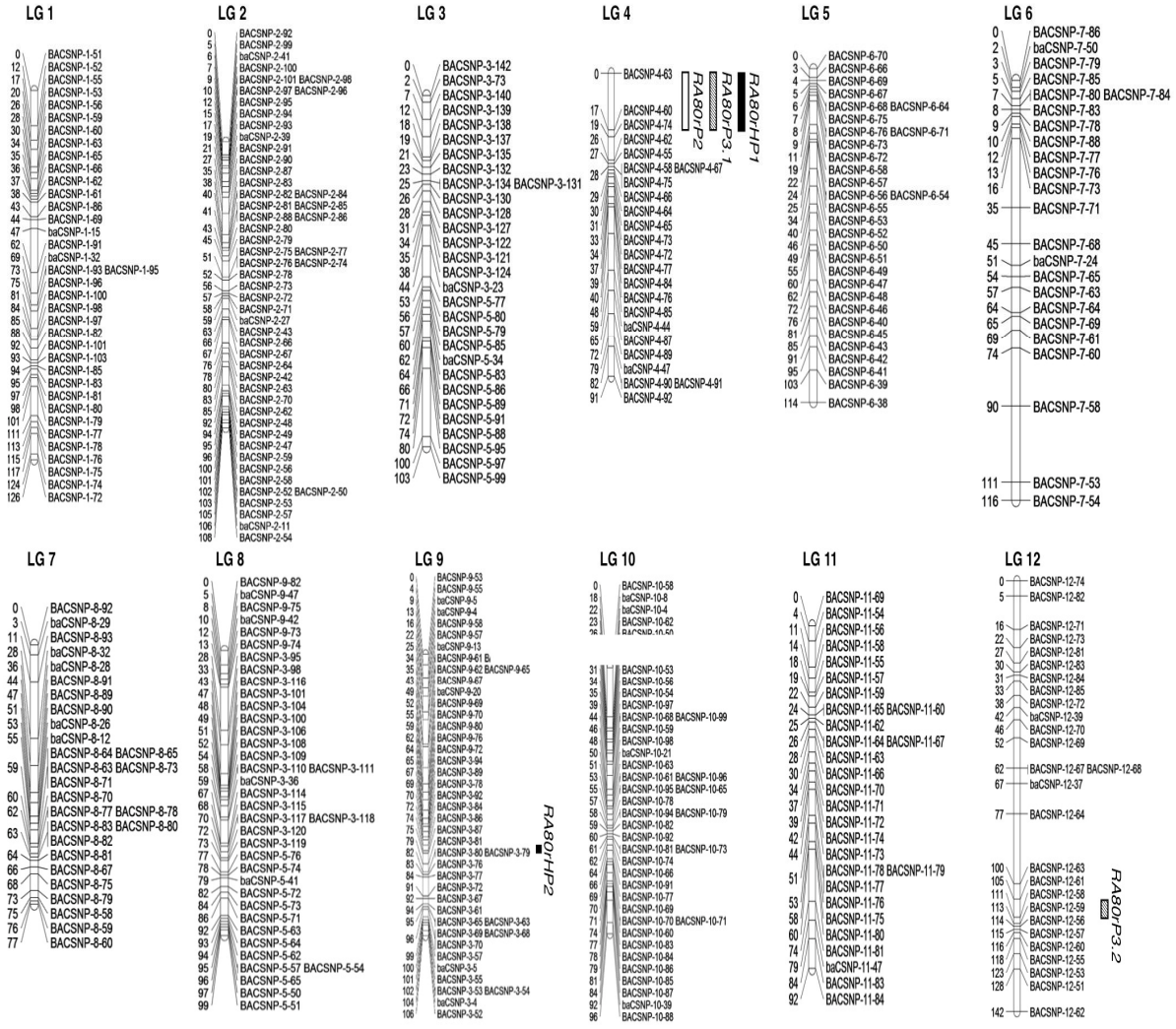

Figure 2. A SNP map of 'PBC80' x 'CA1316' population, forming 12 linkage groups with 1,270 cM total coverage, constructed by JoinMap 3.0, LOD 6.0-10.0. The side bars indicate the locations of the QTLs for anthracnose resistance

All the QTLs for anthracnose resistance identified in this study were derived from the same populations that had been genetically studied (Mahasuk *et al*., 2009a; 2013). The two major QTLs identified in the 'PBC932' responsible for the resistance at mature green and ripe fruit stages were on

the same location of LG2 or P2 (Fig 3, data is not shown). Genetically the genes responsible for the resistance traits on mature green and ripe fruit, *co1* and *co2,* were linked (Mahasuk *et al.*, 2009a). Therefore the *co1* and *co2* convincingly resided in the identified QTL area.

Conclusions

Two QTLs for anthracnose resistance in 'PBC932' were located on P2, while three QTLs identified in 'PBC80' were located on P4.

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