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Resistance to Anthracnose Caused by *Colletotrichum acutatum* in Chili Pepper (*Capsicum annuum* L.)

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Abstract

Pepper fruit anthracnose, caused by *Colletotrichum acutatum*, results in serious yield loss and affects crop quality in many Asian countries, making it a disease of economic consequence. A source resistant to *C. acutatum* was identified by the AVRDC within the line *Capsicum chinense* Jacq. PBC932. The resistant breeding line *C. annuum* AR is the BC_3F_6 generation derived from *C. chinense* Jacq. PBC932. The inheritance of resistance to *C. acutatum* was analyzed in segregating populations derived from the two crosses HN 11 × AR and Daepoong-cho × AR. Detached mature green fruits were inoculated using microinjection method. The disease response was evaluated as the disease incidence at 7 DAI. The segregation ratios of resistance and susceptibility to *C. acutatum* in the F_2 and BC_R populations derived from the two crosses fit significantly to a 1:3 Mendelian model. This indicates that the resistance of AR to *C. acutatum* is controlled by a single recessive gene.

Key words: *Capsicum annuum*, *C. chinense*, *Colletotrichum acutatum*, inheritance, anthracnose

Introduction

Pepper fruit anthracnose caused by several *Colletotrichum* spp. results in serious yield loss and affects crop quality in tropical and subtropical regions. The damage caused by this organism is very serious in many Asian counties, including South Korea and Taiwan, in which *C. acutatum* and *C. gloeosporioides* are the most destructive and widely distributed of several anthracnose pathogens. These pathogens primarily attack pepper fruits at both the green and red stages.

Several sources resistant to anthracnose have been used in studies of the inheritance of anthracnose resistance. The inheritance patterns vary depending on the resistance source and the *Colletotrichum* isolate. For instance, resistance to *C. capsici* was inherited through a single dominant gene as seen by Lin et al. (2002), and resistance to *C. dematium*, which is a synonym for *C. capsici*, was inherited partially dominantly as found by Park et al. (1990b). These authors also found that resistance to *C. gloeosporioides* was inherited as over-dominant or partially dominant in F_1 plants (Park et al. 1990a). In contrast, some reports have demonstrated that resistance to anthracnose is inherited recessively. For example, Cheema et al. (1984) found that resistance to *C. capsici* was inherited recessively with epistatic effects, and the resistance of *C. chinense* Jacq. PBC932 to *C. capsici* was observed to be inherited through a single recessive gene (Pakdeevaraporn et al. 2005). It has recently been reported that resistance to anthracnose can be inherited through multiple genes. To identify polygenic factors, Voorrips et al. (2004) conducted QTL analysis of the inheritance of resistance using an interspecific population derived from a cross between *C. annuum* and *C. chinense*.

The AVRDC has evaluated the resistance to anthracnose of many pepper accessions and has detected several resistance resources (AVRDC 1999). Of these, *C. chinense* Jacq. PBC932 has been used as one donor parent in the development of an anthracnose-resistant line (AVRDC 2000). A selected line from the BC_3F_4 generation, named AR, was introduced in Korea in

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2003. The present study was carried out to determine the inheritance of anthracnose resistance to *C. acutatum*, the causal pathogen of anthracnose in Korea and Taiwan, in the breeding line AR.

Materials and Methods

Plant materials

Three parents were used for this study of inheritance of resistance to *C. acutatum*. The resistant parent *C. annuum* AR, a BC_3F_6 generation line developed through self-pollination, was used. The two susceptible parents were *C. annuum* HN 11 and *C. annuum* Daepoong-cho, which are Korean elite and local lines, respectively. F_1 plants were obtained by crossing HN 11 and Daepoong-cho as female parents and AR as the male parent. F_2 populations were obtained by self-pollination of F_1 plants. The backcross populations BC_R and BC_S were produced by crossing F_1 plants to both parents only from the cross HN 11 × AR.

For the HN 11 × AR cross, populations consisting of resistant parents (5 plants), susceptible parents (6 plants), and the F_1 (6 plants), BC_R (46 plants), BC_S (17 plants), and F_2 (88 plants) generations were raised in a greenhouse at Seoul National University in Suwon, Korea, from September 2005 to July 2006. For the cross Daepoong-cho × AR, populations consisting of resistant parents (5 plants), susceptible parents (6 plants), and the F_1 (6 plants) and F_2 (131 plants) generations were raised in the same location from January 2006 to October 2006. Plants with viral disease symptoms were discarded.

Fungal isolate

Colletotrichum acutatum KSCa-1 was isolated from the experimental farm of Seoul National University as described previously (Yoon 2003). The isolate was purified using the single-spore isolation method with slight modifications (Ho and Ko 1997). The isolate was maintained on potato dextrose agar medium (Sigma Chemical Co., St. Louis, MO, USA) at 25°C under an alternating 16 h fluorescent light/8 h dark cycle in a incubation room for seven days, and the plates flooded with distilled water. Conidia were then collected by scraping the surfaces of the plates. The inoculum density was adjusted to 5×10^5 conidia/ml using a hemacytometer.

Inoculation

Inoculation was performed using the microinjection method developed at the AVRDC (1999). Three to five fruits per inoculation were harvested from individual plants at the mature green stage. The fruits were washed with distilled water to remove various germs on the fruit surface. Inoculation was conducted at a 0.7 mm depth using a microinjector (Hamilton PB600-1, Repeating Dispenser, Reno, NV, USA). Each fruit was injected with 2 μl of prepared conidial suspension, and two or three sites were inoculated depending on the fruit size. Inoculation was conducted with three to five replications. The inoculated fruits were placed on moistened paper towels with 100 ml distilled water in acrylic boxes. The boxes were sealed tightly with plastic wrap to maintain the relative humidity at greater than 95% and incubated for 48 h under the same conditions as those used for maintenance of the fungus. Finally, the plastic wrap was removed and the inoculated fruits were incubated for five more days under the same conditions.

Disease evaluation

Disease evaluation was conducted using the disease incidence method (percentage of infected sites per total inoculated sites) at 7 DAI as described previously but with slight modifications (Yoon et al. 2004). Chi-square goodness-of-fit tests were used for statistical analysis.

Results and Discussion

Anthracnose symptoms developed in inoculated fruits from the susceptible parents, HN 11 and Daepoong-cho, at 2 DAI, and there was no change in disease incidence after 7 DAI. Therefore, the time of disease evaluation for the inheritance study was set at 7 DAI.

Table 1 shows the disease incidence in the parents and F_1 plants. The resistant parent AR and the susceptible parents HN 11 and Daepoong-cho showed obvious differences in disease

Table 1. Disease incidence for the three parents and their F_1 progeny at seven days after inoculation with *C. acutatum* KSCa-1

Population	Disease incidence (%)[x]
Population 1	
AR	17.3 a
HN 11	56.6 b
F_1 (HN 11 × AR)	45.3 b
Population 2	
AR	18.5 a
Daepoong-cho	87.6 c
F_1 (Daepoong-cho × AR)	58.7 b

[x] Mean values within a column followed by the same letter are not significantly different based on Duncan's multiple range test ($P = 0.05$).

incidence. The F_1 plants were skewed clearly toward the same symptoms as the susceptible parent in the cross HN 11 × AR, but not in the cross Daepoong-cho × AR (Table 1).

Distributions of resistance and susceptibility scored by disease incidence were investigated in all of the segregating populations, including the F_2 and BC_R populations, and were skewed toward the susceptible parents HN 11 and Daepoong-cho (data not shown). These results suggest that the resistance of AR was inherited recessively.

Table 2. Segregation ratios of resistance and susceptibility to *C. acutatum* KSCa-1 in the crosses HN 11 × AR and Daepoong-cho × AR

Population	Expected ratio (R:S)	Observed frequency		x^2	Probability
		R*	S		
Population 1					
AR	-	5	0	-	-
HN 11	-	0	6	-	-
F_1 (HN 11 × AR)	-	0	6	-	-
F_2	1:3	24	64	0.242	0.622
BC_R	1:1	17	29	3.13	0.077
BC_S	0:1	0	17	-	-
Population 2					
AR	-	5	0	-	-
Daepoong-cho	-	0	6	-	-
F_1 (Daepoong-cho × AR)	-	0	6	-	-
F_2	1:3	25	106	2.45	0.118

*Less than 25.0% disease incidence evaluated as resistance.

On the criterion of 25.0% disease incidence, there were two peaks on both sides, but this result was not clear. Therefore, the criterion of resistance was determined to be 25.0% disease incidence.

In the cross HN 11 × AR, the segregation of resistance and susceptibility in the F_2 population was 24 to 64 (Table 2). The chi-squared and P values in the F_2 population were 0.242 and 0.622, respectively, which fits significantly to a 1:3 Mendelian model. The segregation ratios in the BC_R and BC_S populations were 17:29 and 0:17, respectively. In the cross Daepoong-cho × AR, the segregation of resistance and susceptibility in the F_2 population was 25:106, which fits significantly to a 1:3 Mendelian model. These results suggest that the resistance of AR to *C. acutatum* was inherited through a single recessive gene. However, a continuous distribution of disease incidence was displayed in all of the segregating populations (data not shown), which indicates that minor genes may have affected the resistance. Also, in the two crosses, F_1 plants showed somewhat different responses depending on the susceptible parent.

The resistance of *C. chinense* Jacq. PBC932 to *C. capsici* has been shown to be inherited through a single recessive gene (Pakdeevaraporn et al. 2005). As the resistance of AR was derived from *C. chinense* Jacq. PBC932, the present results correspond with those of a previous report, although the pathogens belong to different species. It will be necessary to confirm whether the genes that confer resistance to *C. capsici* and *C. acutatum* are identical. In several reports on the inheritance of resistance to *C. acutatum* or *C. gloeosporioides* in chili peppers (Park et al. 1990a; Voorrips et al. 2004; Yoon 2003), resistance was inherited dominantly. Therefore, this paper is the first to report that resistance to *C. acutatum* was inherited through a single recessive gene.

From a pepper breeding perspective, dominant resistance is more useful than recessive resistance because it will be manifested in F_1 hybrids even if only one parent has the allele additionally, the proportion of F_1 hybrids in worldwide markets is growing. Producing F_1 varieties using recessive resistance sources requires much time and effort. However, recessive resistance is more durable than dominant resistance. This information can benefit chili pepper breeding programs in the production of anthracnose-resistant F_1 varieties.

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