

Inheritance of Anthracnose Resistance in a New Genetic Resource, *Capsicum baccatum* PI594137

Sang Hoon Kim¹, Jae Bok Yoon^{1*}, Hyo Guen Park¹

¹ Pepper and Breeding Institute, Business Incubator, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-853, Korea

Abstract

Pepper fruit anthracnose caused by *Colletotrichum species* is an economically important disease that causes serious yield loss and quality deterioration in many Asian countries including South Korea and Taiwan. Recently, *Capsicum baccatum* PI594137 was found to exhibit broad-spectrum resistance to *Colletotrichum acutatum*. The inheritance of anthracnose resistance to *C. acutatum* was analyzed in an intraspecific population derived from a cross between *C. baccatum* Golden-aji and PI594137. Detached mature green fruits were inoculated using the microinjection method. The disease response was evaluated as the disease incidence and the overall lesion diameter at 7 days after inoculation (DAI). The segregation ratios of resistance and susceptibility to *C. acutatum* in the F₂ and BC_s populations significantly fit a 3:1 Mendelian model. This result indicates that the resistance of PI594137 to *C. acutatum* is controlled by a single dominant gene.

Key words: *Capsicum baccatum*, *Colletotrichum acutatum*, inheritance, anthracnose, intraspecific population, broad-spectrum resistance

Introduction

Pepper anthracnose caused by several *Colletotrichum species* (mainly *C. acutatum*, *C. gloeosporioides*, and *C. capsici*) leads to serious problems in pepper production in tropical and subtropical regions. The damage caused by anthracnose is especially serious in many Asian countries including South Korea and Taiwan. In these countries, *Colletotrichum acutatum* is the most destructive of the several anthracnose pathogens present and is widely distributed. The pathogen primarily attacks pepper fruits at both the green and red stages.

Several anthracnose-resistant lines have been reported, and researchers have used these resources to examine the inheritance of anthracnose resistance. The inheritance patterns vary depending on the resistance sources and the *Colletotrichum* isolates. For example, resistance to *Colletotrichum capsici* is inherited through a single dominant gene (Lin et al. 2002), and resistance to *Colletotrichum dematium* (a synonym for *C. capsici*) is inherited as partially dominant (Park et al. 1990b). Resistance to *Colletotrichum gloeosporioides* is inherited as partially dominant or over-dominant in F₁ plants (Park et al. 1990a). In contrast, some studies have found recessive inheritance of resistance

to anthracnose. For example, Cheema et al. (1984) found that resistance to *C. capsici* was inherited recessively with epistatic effects, and Pakdeevaporn et al. (2005) found that the resistance of *Capsicum chinense* Jacq. PBC 932 to *C. capsici* was inherited through a single recessive gene. Kim et al. (2007) also found that the resistance of *C. annuum* AR, which was derived from the *C. chinense* Jacq. PBC 932, to *C. acutatum* was controlled by a single recessive gene. Another study found that resistance to anthracnose was inherited by polygenic genes (Voorrips et al. 2004). These authors identified polygenic factors using QTL analysis for the inheritance of resistance in an interspecific population derived from a cross between *C. annuum* and *C. chinense*.

Several anthracnose-resistant resources have been identified in *C. baccatum* and *C. chinense* germplasm by the AVRDC (Asian Vegetable Research and Development Center) of Taiwan (AVRDC 1999). Recently, a new resistant resource, *C. baccatum* PI594137, was identified at Seoul National University (Park 2005). This resource has broader-spectrum resistance to several *Colletotrichum* isolates than *C. baccatum* PBC 81 and *C. chinense* Jacq. PBC 932, which have been used as anthracnose-resistance resources. The present study was carried out to determine the inheritance mode of anthracnose resistance to *C. acutatum*, the causal pathogen of anthracnose in Korea and Taiwan, in an intraspecific population from a cross between the *C. baccatum* Golden-aji and PI594137.

*To whom correspondence should be addressed

Jae Bok Yoon Ph. D.

E-mail: yoonjb2@snu.ac.kr

Tel: +82-31-296-5797 / Fax: +82-31-296-5794

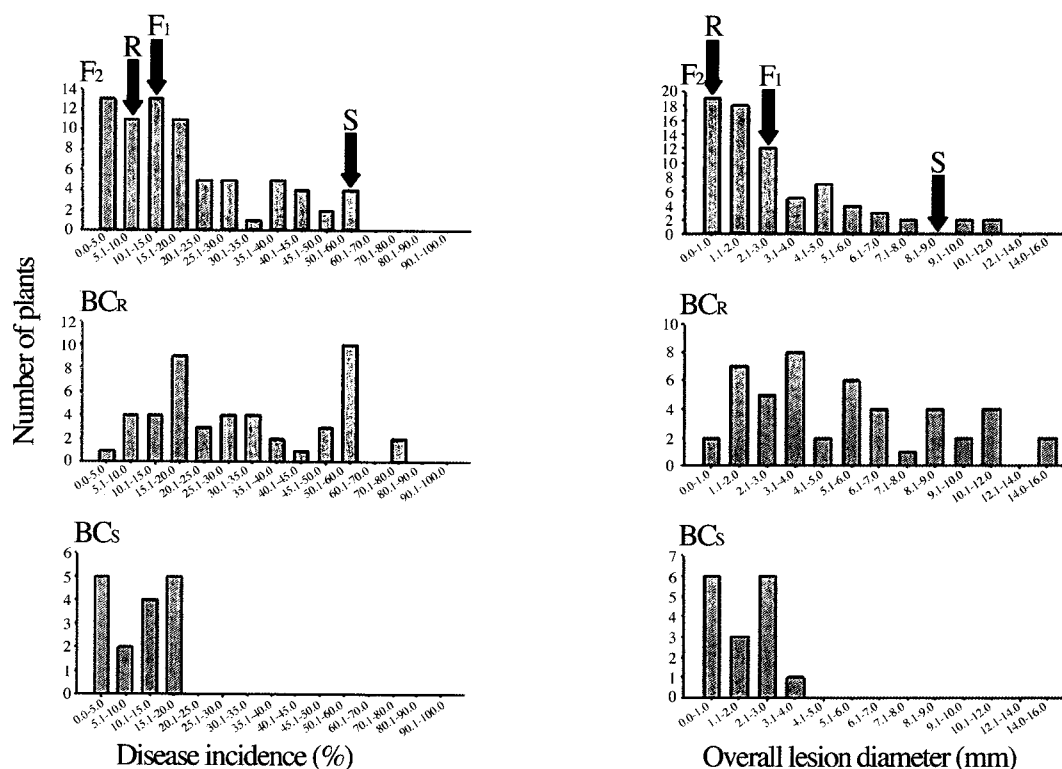


Fig. 1. Distributions of disease incidence and overall lesion diameter in F₂, BC_R, and BC_S populations derived from a cross of the *C. baccatum* Golden-aji and PI594137.

Materials and Methods

Plant materials

The parent lines *C. baccatum* Golden-aji and PI594137 were used to study the inheritance of resistance to *Colletotrichum acutatum*. F₁ plants were obtained by crossing Golden-aji as the female parent and PI594137 as the male parent. The F₂ population was obtained by self-pollinating F₁ plants. The backcross populations BC_R and BC_S were produced by crossing F₁ plants to both parents.

Populations consisting of a resistant parent (five plants), a susceptible parent (four plants), and F₁ (two plants), BC_R (15 plants), BC_S (47 plants), and F₂ (71 plants) generations were grown in a greenhouse at Seoul National University at Suwon, Korea from December 2005 to August 2006. Any plants with virus disease symptoms were discarded.

Fungal isolate

Colletotrichum acutatum KSCa-1 was isolated from a population at the experimental farm of Seoul National University, Suwon, Korea, 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) for seven days in an incubation room at 25 °C under an alternating 16 h fluorescent light/ 8 h dark regime. After the incubation, the plates were flooded with distilled water and conidia were collected by scraping the surface of the medium. The inoculum density was adjusted to 5×10^5 conidia/ml using a hemacytometer.

Inoculation

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

Disease evaluation

Disease evaluation was conducted at 7 DAI by assessing the disease incidence and overall lesion diameter as described previously (Voorrips et al. 2004) but with slight modifications. The chi-square goodness-of-fit test was used for statistical analysis.

Results and Discussion

Anthracnose symptoms on the susceptible parent, Golden-aji, began to develop at 3 DAI, followed by no further change in disease incidence, but disease symptoms overlapped after 7 DAI (data not shown). Therefore, the time of disease response evaluation for the inheritance study was set to 7 DAI. The mean values of disease incidence and the overall lesion diameter in parents and F₁ plants are shown in Table 1. The parents showed obvious differences, and the F₁ plants were clearly skewed toward the resistant parent in both disease incidence and overall lesion diameter (Table 1). These results suggest that the resistant parent, *C. baccatum* PI594137, is inherited dominantly. However, the susceptible parent, *C. baccatum* Golden-aji, did not show obvious susceptibility, as found in almost all *C. annuum* individuals (Table 1).

Table 1. Disease incidence and overall lesion diameter in *C. baccatum* Golden-aji, PI594137, and their F₁ progenies at seven days after inoculation with *Colletotrichum acutatum* KSCa-1

Population	Disease incidence (%) ^a	Overall lesion diameter (mm)	
		R	S
Golden-aji	50.3 b	8.9 b	
PI594137	8.1 a	1.0 a	
F ₁ (Golden-aji x PI594137)	12.0 a	2.6 a	

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

All segregating populations were investigated for distributions of resistance and susceptibility as scored by disease incidence and overall lesion diameter. The results showed that distributions in the F₂ and BC_R populations were skewed toward the resistant parent, PI594137 (Fig. 1). The segregation of resistance and susceptibility in the F₂ population as scored by a disease incidence of 30.0% was 58 to 16 (Table 2). The chi-squared and probability values in the F₂ population were 0.450 and 0.502, respectively, which significantly fit a 3:1 Mendelian model. The segregation ratios in the BC_S and BC_R populations were 25:22 and 16:0, respectively, which fit the expected segregation ratios, 1:1 and 1:0, respectively (Table 2). The segregation of resistance and susceptibility scored as an overall lesion diameter of 4.0 mm in the F₂ population was 54 to 20 (Table 2). The chi-squared and probability values in the F₂ population were 0.162 and 0.687, respectively. The segregation ratios in the BC_S and BC_R populations were 22:25 and 16:0, respectively, which also fit the expected segregation ratios, 1:1 and 1:0, respectively (Table 2).

These results indicate that the resistance of PI594137 to *Colletotrichum acutatum* was inherited as a single dominant gene.

The distributions of segregating populations and the results of the chi-square goodness-of-fit tests indicate that the resistance of PI594137 to *Colletotrichum acutatum* was controlled by a single dominant gene. However, the continuous distributions of the segregating populations suggest that minor genes could exist and affect the resistance. Furthermore, the susceptible parent, *C. baccatum* Golden-aji, did not show the severe susceptibility that is common in most *C. annuum* F₁ varieties. This suggests that *C. baccatum* germplasm could have basic resistance to anthracnose, unlike almost all other *C. annuum* germplasm.

A previous report stated that one anthracnose-resistance resource, *C. baccatum* PBC 81, was also inherited dominantly (Yoon 2003), but *C. baccatum* PI594137 has shown broader-spectrum resistance to several *Colletotrichum* isolates than PBC 81 (Park 2005). Therefore, the present results should be of benefit to pepper anthracnose-resistance breeding programs in many Asian countries.

Table 2. Segregation ratios of resistance and susceptibility as scored by disease incidence and overall lesion diameter in segregating populations derived from a cross of the *C. baccatum* Golden-aji and PI594137

Population	Expected ratio (R : S)	Observed frequency		χ^2	P value
		R	S		
Disease incidence ^a					
Golden-aji	-	0	4	-	-
PI594137	-	5	0	-	-
F ₁ (Golden-aji x PI594137) ^b	-	2	0	-	-
F ₂	3 : 1	58	16	0.450	0.502
BC _R	1 : 0	16	0	-	-
BC _S	1 : 1	25	22	0.191	0.662
Overall lesion diameter ^c					
Golden-aji	-	0	4	-	-
PI594137	-	5	0	-	-
F ₁ (Golden-aji x PI594137)	-	2	0	-	-
F ₂	3 : 1	54	20	0.162	0.687
BC _R	1 : 0	16	0	-	-
BC _S	1 : 1	22	25	0.191	0.662

^a Disease incidence lower than 30.0% was defined as resistance.

^b Overall lesion diameter of less than 4.0 mm was defined as resistance.

^c Individuals with virus symptoms were discarded.

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