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Notes

Evaluation on Red Pepper Germplasm lines (*Capsicum* spp.) for Resistance to Anthracnose Caused by *Colletotrichum acutaum*

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We evaluated 209 Capsicum accessions from the National Agrobiodiversity of RDA, for their reaction against of anthracnose disease caused by *Colletotrichum acutatum*. Two hundred nine accessions of *Capsicum* were almost composed with *Capsicum annuum* var. *annuum* originated from 37 countries. The percentage of infection due to *C. acutatum* ranged from 11.1% to 100% on immature and matured pepper fruits at 21 days after inoculation. On immature fruits of pepper, one hundred seventy three accessions of tested pepper were found infected with *C. acutatum*. Out of the 173 accessions, eighty five accessions were susceptible to anthracnose with dark brown to black lesions and slowly increased in size at 21 days after inoculation. Only thirty six accessions showed resistance against *C. acutatum*. On mature fruits of pepper, one hundred ninety eight accessions were infected with *C. acutatum* at 21 days after inoculation. Twenty one accessions showed a resistance reaction against *C. acutatum*. The results of this work indicated that all of resistance accessions to *C. acutatum* were have a genetic potential for the resources of resistance can be further used in pepper breeding programme against anthracnose and also demand more detailed investigation in this.

Keywords : anthracnose, capsicum, *Colletotrichum acutatum*, resistance

Chilli or red pepper is a commonly grown as an herbaceous annual dicotyledonous flowing plant in temperate areas (Bosland, 1996). Chilli belongs to members of the genus *Capsicum*, twenty five species, have been cultivated extensively, initially in the Americas and, later spread to European contact, throughout the worldwide (Eshbaugh, 1993; Heiser, 1976) is being used as a food flavoring, a coloring agent, a pharmaceutical ingredient, and in other innovative ways (Bosland, 1994; Cronin, 2002; Hoffman et al., 1983; Krishna De, 2003; Wall and Bosland, 1993; Woodbury, 1980). Numerous cultivars within the five domesticated species, *Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens*, have been grown exponentially (Heiser and Smith, 1953; Heiser, 1985; IBPGR, 1983; Smith and Heiser, 1957; Tong and Bosland, 1999; Yoon et al., 2004).

It is estimated that the *Capsicum* agribusiness is worth about 4% of gross agricultural production in Korea (Kwon et al., 2006). But, the main problems being faced by growers are the problems of managing diseases and few insect pests (Kim and Park, 1988; Um, 1998; SAGARPA, 2002).

Anthracnose, caused by various species of *Colletotrichum* spp. cause significant damage to a wide range of crops including pepper, strawberry, grapevine and apple (Berstein et al., 1995; Freeman and Katan, 1997; Hadden and Black, 1988; Kim and Nam, 1999; Park and Kim 1992). Especially, anthracnose is one of the major production constraints, due to loss more than 10% of the total annual pepper production, estimated to be more than 100 million US dollars, including Korea (Park and Kim 1992; Manadhar et al., 1995; Poonpolgul and Kumphai, 2007; Shin et al., 1999; Um, 1998). Typical anthracnose symptoms on pepper fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced marketability (Manandhar et al., 1995).

In Korea, four anamorphic species, *Colletotrichum acutatum*, *C. cocodes*, *C. dematium*, and *C. gloeosporioides*, and one teleomorphic species, *Glomerella cingulata* have been described as the causal agents of pepper anthracnose symptoms (Hong and Hwang, 1998; Kim et al., 1986; Kim et al., 2007; Park and Kim 1992). Among these, *C. acutatum* was currently reported as the dominant pathogens in Korea (Kim et al., 2008).

This study was carried out to evaluate resistance against anthracnose disease in pepper germplasm lines and to provide information on resistant sources for improving the efficiency of Chilli pepper breeding. For this purpose 209 accessions of three *Capsicum* species, *Capsicum annuum* var. *annuum*, *C. frutescens* and *C. pubescens*, from the National Agrobiodiversity Center of RDA, Korea, were

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screened against *C. acutatum* in Table 1. Ten pepper seeds of each accession were disinfected with 2% NaOCl for 4 hrs and washed with tap water at several times and pre-germinated on the moist filter paper in petri-dish at 30°C for one-week in light condition. Pre-germinated pepper seeds are then transplanted in peat moss artificial soil and pepper seedlings are grown for 21-25 days in the green house. Transplants are kept clean by managing weeds with mulching of black polyvinyl films. None of the pepper fields were sprayed with any types of fungicides. The pepper fruits were harvested at fruit breaker stage from immature-green to mature-red.

Based on conidia size, morphology, and pahogenicity, the isolates obtained from the pepper fruits were identified as either *C. gloeosporioides* or *C. acutatum* (Verma, 1973). The major population consists of the isolates of *C. gloeosporioides* in the late 1980th (Park and Kim, 1992), and recently *C. acutatum* was identified as a major pathogen in Korea pepper fields.

The dominant strain of anthracnose pepper disease, *Colletotrichum acutatum* KACC40042 (Kim et al., 2006; Wei et al., 2008), was obtained from Korean Agricultural Culture Collection (KACC, Rural Development Adminstration (RDA), Suwon, Korea. The anthracnose pathogen was grown on Potato Dextrose Agar (PDA) at 25°C under fluorescent lights. The conidia are harvested by placing 10 ml of sterilized distilled water onto 7 days old cultures, by scraping the surface of the media with an inoculating loop to release the conidia from the hyphae. Later added 10 ml of conidial suspension into a 500 ml of autoclaved new PDA medium, and poured approximately the medium into 30 sterile Petri plates. The plates were incubated for two days for mycelium grown on PDA medium under dark condition and later, induced the formation of perithecia for five days under light condition. An agar disk, 4 mm diameter was cut with a Cork borer from an actively growing of perithecial inoculums on the PDA medium.

We modified the inoculation methods for inoculums concentration, microdropping, high-pressure spraying, pinning, and microinjection as described by previous studies (Choi and Pae, 1987; Hong and Hwang, 1998; Pae et al., 1998; Yoon and Park, 2001).

The outbreak of the pepper anthracnose has severely decreased the pepper production. Thus, it is very important to identify the major pathogen of the pepper anthracnose for early diagnosis and the disease management in the fields, because each *Colletotrichum* species shows significant variation in the response to the host diversity of *Capsicum* varieties (Kim et al., 2008; Park and Kim, 1992).

The detached healthy fruits harvested from the pepper plants in a greenhouse were used for determining the resistance of each accession against the pathogens. Fruits were surface-sterilized in a 2,000 ppm NaDCC (Sodium Dichloroisocyanurate) solution for 30 min., rinsed in tap water and in sterile water for several times and placed on the sterile paper towel and then air dried. The surface sterilized fruits are wounded by a pin-pricking of the appliance (Accu-Chek Softclix) used for measuring the blood sugar and inoculated by placing 5 mm diameter of perithecia agar disk. Control fruits are inoculated with PDA disk (Choi and Pae, 1987). The inoculated fruits are incubated in a Lock & Lock container (21×28×9 cm) with high relative humidity (100%) at 22.5-27.5°C in normal light regimes and evaluated for disease reaction at weekly interval for three weeks after inoculation.

Evaluation of anthracnose symptoms such as lesion appearance, lesion size and the severity of infection was carried out from 7 to 21 days after inoculation. Lesion development of three fruits of immature (green) and matured (red) pepper of each accessions are assessed by the average percentage of diseased on each fruit by weekly checking for disease development.

In the present study, two hundred nine accessions of *Capsicum* were composed with 198 accession of *C. annuum* var. *annuum*, 10 accessions of *C. frutescens* and one accession of *C. pubescens*, originated from 37 countries including Korea and are preserved in the National Agro-biodiversity Center of RDA, Korea (Table 1).

The United States National Plant Germplasm System preserve an extensive *Capsicum* germplasm collection at the Southern Plant Introduction Experiment Station located in, Georgia. This collection consisted of approximately 3000

Table 1. Diversity of two hundred and nine accessions of Capsicum germplasms for the evaluation of resistance against anthracnose disease in this study

Species	Origins
Capsicum annuum var. *annuum* (198)	AFG(1), ARM(1), BGR(5), BOL(2), CAN(2), CHN(8), CSK(1), CZE(1), EGY(1), GEO(1), GTM(1), HUN(5), IDN(1), IND(1), ISR(2), KOR(95), LAO(4), MDA(1), MEX(2), MYS(3), NPL(6), PAK(1), PRI(1), ROM(1), RUS(9), THA(8), TJK(1), TUR(6), TWN(3), UKR(2), USA(1), UZB(21), VNM(1)
Capsicum frutescens (10)	LAO(8), MEX(1), TWN(1)
Capsicum pubescens (1)	GTM(1)

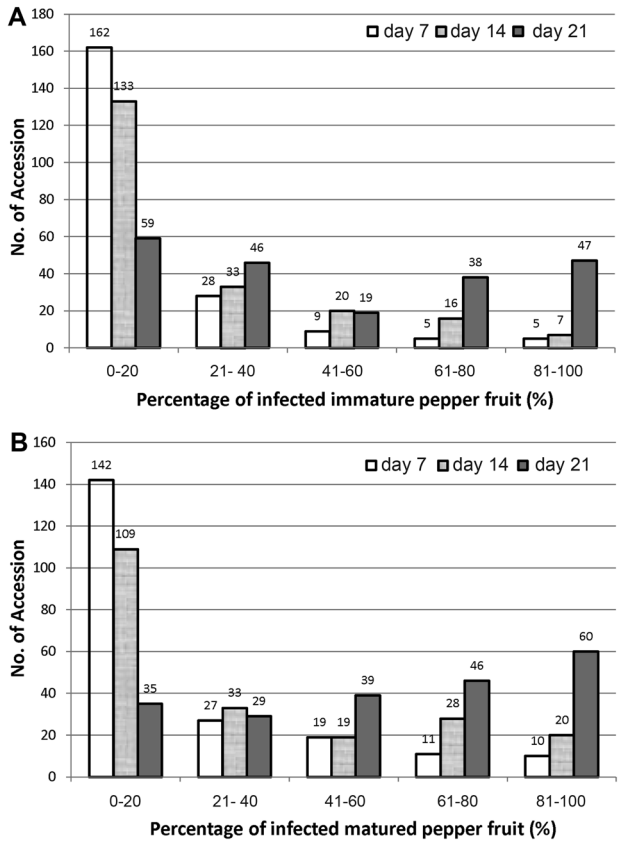

Fig. 1. Comparison of anthracnose resistance of pepper with immature (A) and matured (B) fruit of Capsicum germplasms at an interval of one week after inoculation with *Colletotrichum acutatum* at room temperature and under saturated humidity.

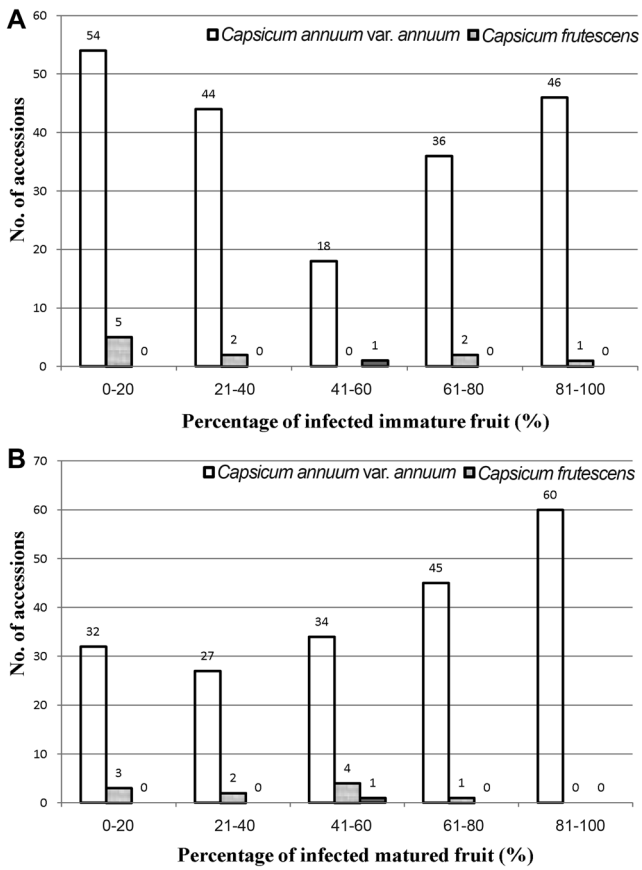

Fig. 2. Distribution of percentage ranges of infected immatured (A) and matured (B) fruit on Capsicum species for the evaluation of resistance against anthracnose disease caused by *Colletotrichum acutatum* at 21 days after inoculation.

Capsicum accessions that include lines from all over the world (Bosland, 1996). A total of 225 accessions from eight *Capsicum* species preserved at the AVRDC (Asian Vegetable Research and Development Center) were characterized using thirty seven morphological traits and were utilized a useful sources in the breeding of improved pepper cultivars adapted to subtropical and temperate regions (Chae et al., 2003).

The percentage of infection with *C. acutatum* ranged from 11.1% to 100% on both immature and matured pepper fruits at 21 days after inoculation (Fig. 1). The initial sunken symptoms were formed 7 days after inoculation on susceptible accessions and later these lesions increased in size, coalesced with other lesions leading to increased size on fruits (Fig. 3).

On immature fruits of pepper, one hundred seventy three accessions of tested pepper germplasm lines are infected with *C. acutatum*. Of the 173 accessions, eighty five accessions were susceptible to anthracnose disease and were scored from 60.1% to 100% and caused dark brown to black lesions and slowly increased in size at 21 days after inoculation (Fig. 1, A). However, thirty six accessions have shown resistance against *C. acutatum* and composed with thirty five accessions of *Capsicum annuum* var. *annuum* and one accessions of *C. frutescens* (Fig. 2, A).

On mature fruits of pepper, one hundred ninety eight accessions of tested pepper germplasm were found infected with *C. acutatum* at 21 days after inoculation. One hundred six accessions of infected pepper fruit produced more severe symptoms and scored from 60.1% to 100% (Fig. 1, B). In all of susceptible accessions the lesions increased in size, coalesced and damaged the entire fruit. Twenty one accessions have shown a resistance against *C. acutatum* and composed with ten accessions of *Capsicum annuum* var. *annuum* and one accessions of *C. frutescens* (Fig. 2, B).

Pepper anthracnose mainly damages immature green and matured red fruits and leaves as lesions. Thus, production in the tropics has serious disease and quality problems, which could be solved if the genetic diversity is rationally used in breeding programs. *C. gloeosporioides* attacks pepper fruits at both the green and red stage, while *C. capsici* mainly

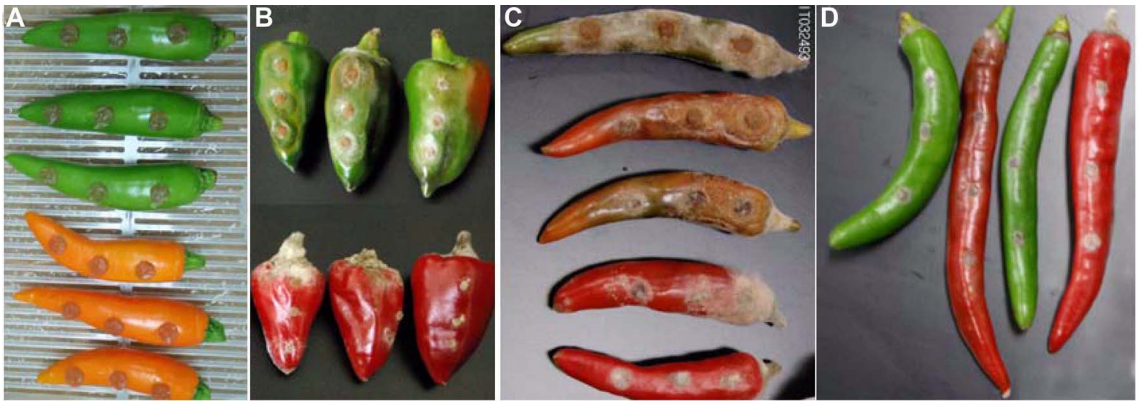

Fig. 3. Comparison of disease sympotoms developed on susceptible and resistant Capsicum accessions. Typical symptoms developed on accession of *C. annuum*, anthracnose disease caused by *Colletotrichum acutatum* at 21 days after inoculation. A, 7 days after inoculation; B, C, susceptible reaction; D, resistance reaction.

attacks pepper fruits at the red stage (Park et al., 1989; Park et al., 1990).

Thirty accessions of *Capsicum annuum* var. *annuum* and two accessions of *C. frutescens* showed complete resistance to *C. acutatum* in both immature and matured fruits (Fig. 2). The microscopic examination of susceptible cultivar revealed the surface and inner tissue of susceptible pepper fruits with pin-prick wounding, showed the typical symptoms, the mycelial growth and the propagation of acervuli and conidia (Fig. 4, B, D, F, and H). One the other hand, the resistant pepper fruits were devoid of such symptoms and mycological development on the pin-prick wounding tissue inoculated with the pathogen (Fig. 4, A, C, E, and G).

The five variously domesticated species are *Capsicum annuum*, *C. frufescens*, *C. chinense*, *C. baccatum* and *C. pubescus*. *Capsicum annuum*, *C. frufescens*, *C. chinense* are grouped in a taxonomic complex which has conventionally three, or perhaps two or one species (Pickersgill, 1988), with the three clusters of domesticated plants appearing to be more divergent than their wild progenitors (Bosland and Votava, 2000; Baral and Bosland, 2004; Eshbaugh, 1993; Heiser, 1985; Jarret and Dang, 2004; Idu and Ogbe, 1997; Park et al., 1999; Prince et al., 1995; Ry Zlova and Kochieva, 2004; Walsh and Hoot, 2001).

Capsicum pepper in Korea has a very narrow genetic base due to restricted consumer preferences with the exception of a few cultivars grown for their fresh fruits, almost all cultivars are cultivated for dried red fruits with varying degrees of pungency.

C. baccatum and *C. pubescu* are in other taxonomic complexes of the genus and little used beyond Latin America, although *C. baccatum* var. *pendalum* (wild). Eshbaugh, the variety that has been extensively domesticated chilli peppers. So in some literature caution is needed to ascertain whether the plants discussed are actually *C.*

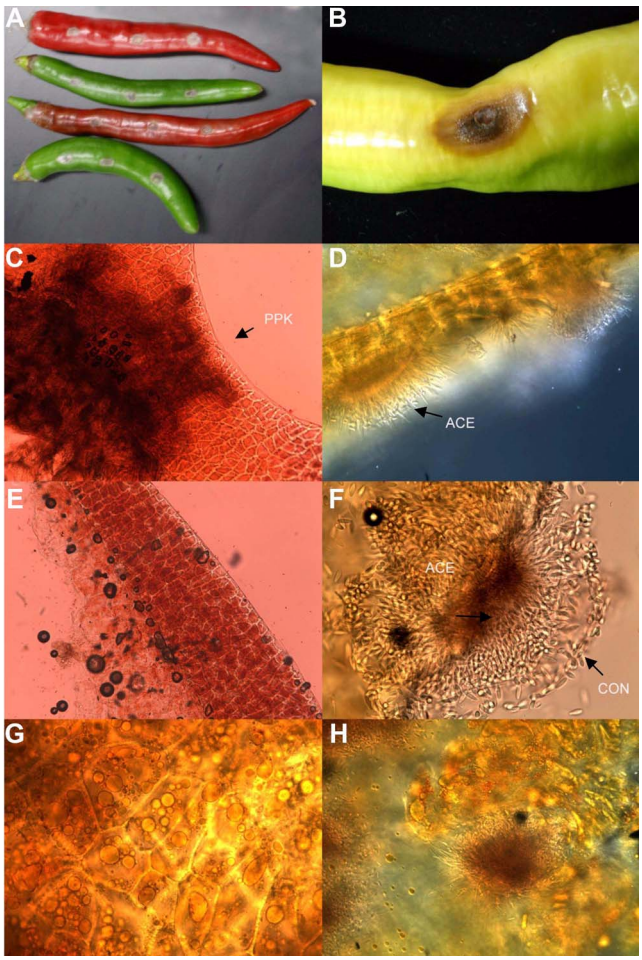

Fig. 4. The microscopic examination of resistant (left, A, C, E, and G) and susceptible (right, B, D, F, and H) pepper fruits with pin-prick wounding (PPK) in *Capsicum* germplasm. Typical symptoms developed on accession of *C. annuum*, anthracnose disease caused by *Colletotrichum acutatum* at 21 days after inoculation. ACE, Acervulus; CON, Conidia; PPK, pin-prick wounding site

annuum or *C. frutescens* itself or another of these species (Heiser and Pickersgill, 1969; Heiser, 1985).

In the species, *C. annuum* throughout the world, horticultural, agricultural and biological diversity has helped to make globally important crop as a fresh and cooked vegetable and a source of food ingredients for sauces and powders and as a colorant, which is used as well in cosmetics (Andrews, 1995, 1999; Bosland, 1994; Bosland and Votava, 2000).

Recently, it was found that a local Korean variety, *C. annuum* 'Daepong-cho', had resistance to *C. capsici*. Inheritance of resistance to *C. capsici* was analyzed in segregating population derived from 'Yeoju' × 'Daepoong-cho' and 'AR' × 'Daepoong-cho'. Out of the two lines, the resistance of 'Daepoong-cho' to *C. capsici* is controlled by a single recessive gene (Kim et al., 2008).

Several sources of resistance to C. capsici have been reported and, using these resources, researchers have studied the inheritance of anthracnose resistance (Cheema et al., 1984; Lin et al., 2002; Park et al., 1990; Pakdeevaraporn et al., 2005; Voorrips et al., 2004).

Anthracnose disease is one of the major economic constraints to pepper production worldwide, especially in tropical and subtropical regions. Accurate taxonomic information is necessary for effective disease control management. In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host (Park et al., 1989; Kim et al., 2004; Lin et al., 2004; Marin et al., 2004).

Management of the pepper anthracnose has been solely dependent upon the application of fungicides due to lack of resistant pepper cultivars (Hadden and Black, 1986; Manandhar et al., 1995).

Since 1980, Embrapa's National Research Center for vegetable crops has conducted a pepper breeding program aiming at demands from different market niches. The program traditionally emphasizes the development of hot and source specific sweet pepper populations, lines, cultivars, and hybrids with multiple disease resistance and high quality fruits, benefiting from a large gene bank of genotypes native to Brazil and also introduced from abroad (Ribeiro et al., 2008). In conclusion, 40 pepper germplasm accessions in immature fruits category and 25 accessions in mature fruit category showing resistance be further explored in contemporary durable resistance breeding programme for enhancing both disease resistance and quality of red pepper in Korea.

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