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Evaluation of Resistance in Hot Pepper Germplasm to Phytophthora Blight on Biological Assay

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Abstract. Phytophthora blight of pepper is the most economically important disease in the world cultivation regions. We investigated the phytophthora blight resistance of 300 accessions of Korean landrace of hot pepper germplasms collected from 83 local regions. The disease incidence rate was checked from 7 days to 28 days at an interval of 7 days after inoculation under greenhouse conditions. Among 300 accessions, the disease incidence rate of phytophthora blight of 67 accessions of pepper germplasm was more than 60.1%, while no disease was observed in 37 accessions at 7 days after inoculation. At 28 days after inoculation, five and eleven accessions of pepper germplasm were resistance and moderate resistance to *P. capsici*, respectively. Two hundred forty four susceptible accessions (81.3%) of pepper were scored as having more than 60.1% of disease incidence of phytophthora blight. This result suggests that five candidate pepper germplasm might be used as breeding resources for the phytophthora blight resistance breeding program. Also, further genetic studies should be carried out to verify this result, with the overall focus of providing information on important characteristics of pepper germplasm.

***Additional key words*:** *Capsicum*, Korean landrace, *Phytophthora capsici*

Introduction

The genus *Capsicum* belongs to the Solanaceae family which includes 20-30 species. This genus contains five economic species, i. e., *Capsicum annuum*, *Capsicum frutescens*, *Capsicum baccatum*, *Capsicum pubescens* and *Capsicum chinense*, *C. annuum* is known as pepper, sweet pepper or paprika, and is cultivated globally for use as vegetable species (Heiser, 1976; IBPGR, 1983; Tong and Bosland, 1999; Yoon et al., 2004).

Chili pepper is usually grown as an herbaceous annual in temperate areas and is a commonly grown dicotyledonous flowering plant (Bosland, 1996). Members of the genus *Capsicum*, 25 species, have been cultivated extensively, initially in the Americas and, subsequently throughout the world (Eshbaugh, 1993; Heiser, 1976). *Capsicum* is used as a food flavoring, as a coloring agent, as a pharmaceutical ingredient, and in other innovative ways (Bosland, 1994; Cronin, 2002; Krishna De, 2003; Wall and Bosland, 1993; Woodbury, 1980).

Among oomycetes, *Phytophthora* species cause the most serious pathogen in plants worldwide and cause the economically important soil-borne disease of pepper in Korea (Kamoun, 2001; Kim, 1993). Intensive studies have concentrated on the biology of *Phytophthora capsici*, evaluation of pepper germplasm for disease resistance, yield-loss assessment, and the testing of chemical, biological, and cultural measures of control (Chae et al., 2003; Hwang and Kim, 1995; Yoon et al., 2004)

Phytophthora blight, caused by *Phytophthora capsici* as a soil infection, is one of the most destructive and yield decreasing fact in the cultivating area of pepper crops (Barksdale et al., 1984; Kim et al., 1974; Leonian, 1992). It is estimated that the *Capsicum* agribusiness is worth about 100 million dollars per year. The main problems faced by growers are disease and a few insects (Kim and Park, 1988; SAGARPA, 2002; Um, 1998).

Although numerous attempts have been made to control the disease by chemicals (Hausbeck and Lamour, 2004; Jee et al., 1988; Oelke et al., 2003) and biological control (Ahn

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and Hwang, 1992; Oelke et al., 2003; Shen et al., 2002), these attempts often fail to control the disease under field conditions and may not persist long enough to affect the target pathogen. Because of the difficulty in controlling this pathogen, development of stable and sustainable red pepper cultivars resistant to the root rot is strongly required to achieve sustainable yields of red pepper (Gerhardson, 2002; Jee et al., 1988).

A number of studies have examined the mycological characteristics of *P. capsici*, and evaluated pepper germplasm for disease resistance. Some pepper accessions, such as AC2258 and CM334, have been reported to display high levels of resistance to *P. capsici* (Ogundiwin et al., 2005; Redondo, 1979; Smith et al., 1967; Yamakawa et al., 1979).

This study was carried out to evaluate hot pepper germplasm resistance against phytophthora blight in order to provide useful genetic resources for improving pepper breeding efficiency.

Materials and Methods

Plant materials

Capsicum annuum var. *annuum* comprised of 300 accessions of *Capsicum* originating from the Korean domestic landrace pepper were collected from 83 local regions and conserved

Table 1. Three hundred accessions of Korean landrace *Capsicum* germplasms were collected from 83 local regions in the Republic of Korea for the evaluation of Phytophthora blight resistance in this study.

Varieties or Accessions name	No. of accession	Varieties or Accessions name	No. of accession	Varieties or Accessions name	No. of accession
Andongjaerae	3	Gyeonggisoebulgochu	1	Pyeongchangjaerae	2
Anseongjaerae	4	Hadongjaerae	6	Samcheokjaerae	6
Asanjaerae	3	Hampyungjaerae	4	Sangjujaerae	3
Boryeongjaerae	1	Hancho	1	Seongnamjaerae	1
Boseongjaerae	5	Haneulcho	2	Seonsanjaerae	4
Bungeocho	1	Hapchunjaerae	1	Seoppulgochu	2
Byeolcho	1	Heungdeokjaerae	1	Seosanjaerae	1
Cheonanjaerae	3	Hongcheonjaerae	3	Seosoojaerae	1
Cheongnyangcho	1	Hwacheonjaerae	1	Seungjujaerae	1
Cheongsongjaerae	7	Hwaseongjaerae	1	Sinanjaerae	3
Chilgokjaerae	8	Iksanjaerae	5	Sinrimjaerae	1
Chilseongcho	6	Imsiljaerae	4	Subicho	2
Chungdojaerae	3	Injejaerae	9	Tongyeongjaerae	2
Daegwanryungjaerae	2	Jaewonjaerae	1	Uiseongjaerae	3
Daehwacho	4	Jangheungjaerae	2	Uljujaerae	2
Daepungjaerae	1	Jangseongjaerae	1	Wandojaerae	6
Dalseongjaerae	1	Jecheonjaerae	2	Wonseongjaerae	3
Damyangjaerae	4	Jejujaerae	5	Woolreungjaerae	1
Danyangjaerae	2	Jinanjaerae	1	Woomeongcho	1
Dongwonicho	1	Jindojaerae	4	Yangpyongjaerae	1
Ganghwajaerae	2	Jinjujaerae	1	Yangsanjaerae	1
Geumlungjaerae	2	Joongwonjaerae	2	Yeocheonjaerae	6
Geumsanjaerae	12	Jungseonjaerae	12	Yeojujaerae	1
Gimhaejaerae	2	Jungwonjaerae	2	Youngchunjaerae	4
Gokseongjaerae	2	Masanjaerae	1	Youngdeokjaerae	1
Gongjujaerae	4	Miryangjaerae	1	Youngiljaerae	11
Goryungjaerae	3	Mujujaerae	1	Youngjujaerae	1
Gosoojaerae	1	Namjijaerae	1	Youngjujaerae	3
Gunwijaerae	1	Namneungjaerae	1	Youngpungjaerae	5
Guryejaerae	5	Namwonjaerae	1	Youngwoljaerae	7
Gwangjujaerae	4	Namyangjaerae	1	Youngyangjaerae	6
Gwangyangjaerae	3	Pocheonjaerae	3	Unknown*	23

*Unknown: These accessions do not know the collection area.

in the National Agrobiodiversity Center of Rural Development Administration, Korea. The varieties name of Korean landrace was described as the collected 83 regions that included 12 varieties of Korean landrace, Bungeocho (1 accession), Byeolcho (1 accession), Cheongnyangcho (1 accession), Chilseongcho (6 accession), Daehwacho (4 accessions), Dongwonicho (1 accession), Hancho (1 accession), Haneulcho (2 accessions), Gyeonggiseoppulgochu (1 accession), Seoppulgoch -u (2 accessions), Subicho (2 accessions), and Woomeongcho (1 accession) (Table 1).

Five resistance varieties of commercial pepper, Yeogkang-hongjanggun, Buja, Umchungna, Powerspeed, and PR-Daechon and two susceptible varieties of commercial pepper, Hongboseg, and Gumdang, were used as control plants. Twenty seeds of each tested accession were replicated and disinfected with 2% NaOCl for 2 hrs. Disinfected seeds were washed with tap water three times before pre-germinating on moist filter paper in Petri-dishes at 30℃ for one-week in light condition. Eighteen seeds of pre-germinated pepper germplasms were sowed into 120 holes pots filled with peat moss as an artificial soil. The pepper seedlings were grown for 70 days in the green-house and watered every other day. None of the pepper germplasms were sprayed with any types of fungicides.

Preparation and inoculation of pathogen

The phytophthora blight pathogens, *Phytophthora capsici* (KACC 44716) were obtained from the Korea Agricultural Culture Collection (KACC) of Rural Development Administrations, Korea. We modified the production method of phytophthora sporangia reported by Jee et al. (1998). To induce zoosporangia production, the pathogen was grown on 10% V-8 agar for a week. The agar plate with fungal hyphae was then placed under florescent light. After one week, the sporangia were cold shocked (1 h at 4-8℃), followed by a one hr equilibration at room temperature. The sporangia were separated from the mycelia with a paint brush, and concentration was adjusted to the 10^5 zoosporangia/mL by a hemacytometer.

Disease evaluation on biological assay

Evaluation of phytopththora blight symptoms such as lesion appearance, lesion size and the severity of infection were carried out from 7 to 28 days after inoculation. We measured the lesion length occurring from the root crown (Fig. 1). The visible symptoms that appeared sequentially were crown rot, stem lesions, plant wilt, leaf defoliation, and damping-off. We determined the disease incidence of each tested accession of hot pepper germplasm using the following formula:

$$\text{Disease Incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of examined plants}} \times 100$$

Results

Phythophthora blight resistance level was graded with the disease incidence; resistance scored 0% of disease incidence, moderate resistance scored less than 20% of disease incidence, susceptible scored from 20.1% to 90% of disease incidence, and highly susceptible scored more than 90.1% of disease incidence (Fig. 2).

In susceptible and highly susceptible accessions, brownish lesions occurred on the pepper stems and extended rapidly into the upper parts of pepper plants; this was accompanied by a plant wilt, leaf defoliation, and damping-off (Figs. 2 and 3).

At 7 days after inoculation of *P. capsici*, the disease symptoms in stem and root of Phytophthora blight began to appear in the highly susceptible pepper germplasm, including the two susceptible varieties of commercial pepper, Hongboseg, and Gumdang (Fig. 1). Among the 300 accessions of pepper germplasm, 12 accessions were shown to be highly susceptible scored more than 90.1% of disease incidence in stem of phytophthora blight, while 37 accessions of pepper germplasm were scored as no disease symptoms (Table 2 and Fig. 1).

At 14 days after inoculation, 23 and 23 accessions of

Fig. 1. Photographs showing the preparation of sporangia from *Phytophthora capsici* (A). Incubation of 45 day-old pepper seedlings inoculated with 10^5 zoosporangia/mL (B). The occurrence of Phytophthora blight at 28 days after inoculation (C) .

pepper germplasm showed resistance and moderate resistance (scored less than 20% of disease incidence), respectively. 234 susceptible accessions of pepper germplasm were scored as having more than 20.1% of disease incidence. 20 accessions of pepper germplasm were shown to be highly susceptible symptoms as brownish lesions, plant wilt, and leaf defoliation and scored more than 90.1% of disease incidence (Table 2 and Fig. 3).

At 21 days after inoculation, 11 and 21 accessions of pepper germplasm showed resistance and moderate resistance, res-

Fig. 2. Grade of the lesion length of Phytophthora blight of pepper seedlings caused by *Phytophthora capsici* with a concentration of 10^5 zoosporangia/mL at 28 days after inoculation.

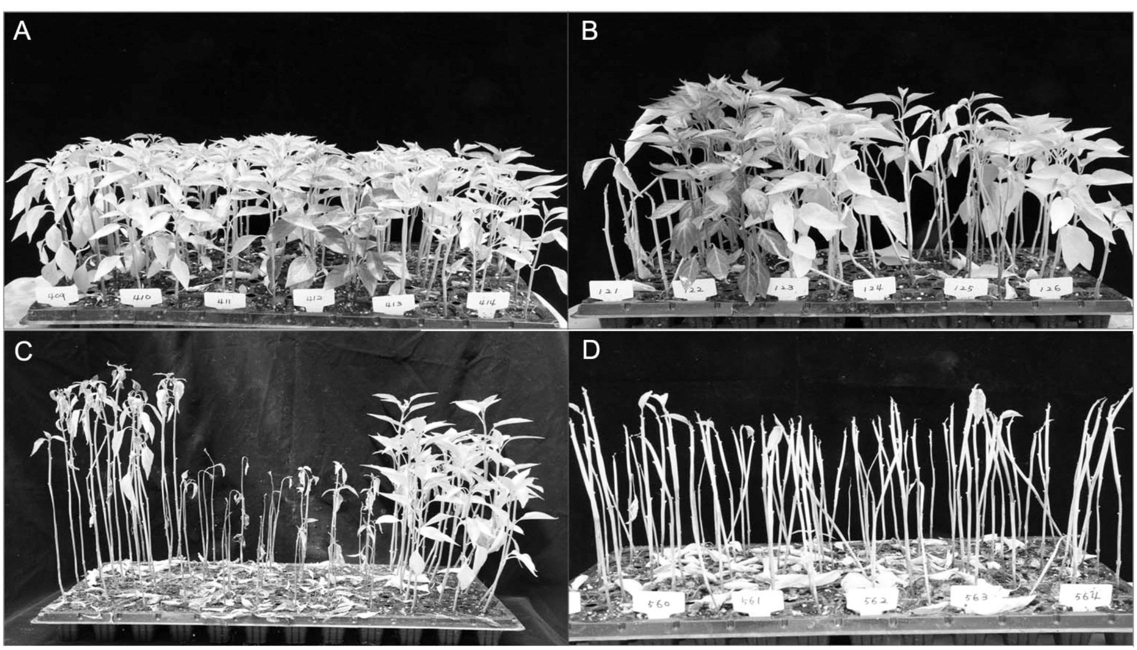

Fig. 3. Selection of the resistant accessions among the 300 accessions of the hot pepper germplasm, at 28days after inoculation with a concentration of 10^5 zoosporangia/mL of *Phytophthora capsici*. A, Resistance (0% disease incidence); B, Moderate resistance (1-20% disease incidence); C, Susceptible (20.1-90% disease incidence); D, Highly susceptible (more than 90.1%).

Table 2. Evaluation of 300 accessions of hot pepper germplasm for the disease incidence of Phytophthora blight caused by *Phytophthora capsici* lesion length of stem rot of pepper seedlings was measured from the root crown at 7-days intervals for 28 days.

Disease Incidence (%)	Accession number of hot pepper germplasm			
	7dyas	14dyas	21days	28days
Resistant (0)	37	23	11	5
Moderate Resistant (0.1-20)	53	23	21	11
Susceptible (20.1-90)	198	234	176	113
Highly Susceptible (90.1-100)	12	20	92	171

pectively. 176 susceptible accessions of pepper germplasm scored as having more than 20.1% of disease incidence. 92 accessions of pepper germplasm were shown to be highly susceptible, scored more than 90.1% of disease incidence (Table 2 and Fig. 3).

At 28 days after inoculation, only 5 accessions of pepper germplasm, Anseongjaerae (IT032469), Injejaerae (IT135643), Jungseonjaerae (IT032492), Seosoojaerae (IT113605), and Youngjujaerae (IT032490), were resistant to phytophthora blight. Also, 11 accessions of pepper germplasm, Cheongsongjaerae (IT113636), Goryungjaerae (IT032438), Guryejaerae (IT032442), Imsiljaerae (IT032477), Damyangjaerae (IT032448), Yangsanjaerae (IT032470), Youngiljaerae (IT032468, 032479), Youngjujaerae (IT032446), and Youngyangjaerae (IT032485), were moderate resistance (scored less than 20% of disease incidence) to *P. capsici*. 113 susceptible accessions (37.7%) of pepper scored from 20.1% to 90% of disease incidence. 171 accessions showed highly susceptible symptoms as brownish lesions, leaf defoliation, and damping-off and scored more than 90.1% of disease incidence (Table 2 and Fig. 3).

Discussion

Hot and sweet pepper is the most economically important crops in China, Indonesia, Korea, Mexico, and Southeast Asia (Kim and Park, 1988; Kim et al., 1974; Pae et al., 2003; SAGARPA, 2002). In Korea, the total annual production of pepper is approximately US 1.4 billion dollars (Ministry of Food, Agriculture, Forestry and Fisheries, 2008; Pae et al., 2003). In this study, 300 accessions of red pepper germplasm were inoculated with zoosporangia of *Phytophthora capsici* at seedling stages in a greenhouse to evaluate their resistance to phytophthora stem blight.

The five domesticated species are *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*, *Capsicum annuum*, *C. frutescens*, and *C. chinense* are grouped in a taxonomic complex that 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 (Baral and Bosland, 2004; Bosland and Votava, 2000; Eshbaugh, 1993; Idu and Ogbe, 1997; Jarret and Dang, 2004; Prince et al., 1995; Ryzlova and Kochieva, 2004). Horticultural, agricultural and biological diversity of the species, *C. annuum* have helped to make *Capsicum* a globally important crop as a fresh and cooked vegetable, as an ingredient for sauces and powders, and as a colorant, which is also used in cosmetics (Andrews, 1995, 1999; Bosland, 1994; Bosland and Votava, 2000).

Phytophthora blight of pepper caused by *P. capsici* is considered an important limiting factor that is causing decreasing yields in the cultivating areas (Barksdale et al., 1984; Hwang and Kim, 1995; Leonian, 1992; Ristaino and Johnston, 1999). The typical symptoms of *P. capsici* in pepper included foliar blight, fruit rot and a very characteristic stem rot at the root crown of the plant (Barksdale et al., 1984; Erwin and Ribeiro, 1996; Thabuis et al., 2003; Weber, 1932). Based on a survey of *Phytophthora* diseases in Korea from 1996 to 1999, 999 isolates were isolated from 66 host plants and classified into 17 species in order to study the genetic diversities of *Phtophthora* (Hong et al., 2000; Jee, 1998, 1999).

In this study, the symptoms of Phytopththora blight began to appear on pepper seedlings 6 days after inoculation of *P. capsici* in the highly susceptible accessions, including the two susceptible varieties of commercial pepper Hongboseg and Gumdang.

The pathogen can infect all parts of a pepper plant, including the roots, stems, leaves and fruit at any stage of growth and can be seedborne, surviving in the soil and on host debris for months (Biles et al., 1995; Black et al., 1991; Hwang and Kim, 1995; Oelke et al., 2003).

We found 5 accessions to be resistant, 11 accessions to have moderate resistance, 113 accessions that were susceptible and 171 accessions that were highly susceptible at 28 days after inoculation. The candidate 5 pepper germplasms resistant against *P. capsici* from the Korean domestic landrace pepper had a high population density of inoculums (10^5 zoosporangium/ml).

Numerous attempts have been made to control the disease by chemicals (Hausbeck and Lamour, 2004; Jee et al., 1988; Oelke et al., 2003) and biological control (Ahn and Hwang, 1992; Oelke et al., 2003; Shen et al., 2002; Strobel et al., 1999), but no effective management programs have been developed to control phytopthora blight caused by *P. capsici* in pepper crops.

Recently urgent attention has been focused on the development of phytophthora-resistant pepper varieties (Kim and Park, 1995; Oleke et al., 2003; Pflieger et al., 2001), and many studies have reported the inheritance of resistance to *P. capsici* in pepper. Single-, two-, and multiple-gene systems have been reported (Lefebvre and Palloix, 1996; Mozzeti et al., 1995; Oelke et al., 2003; Pflieger et al., 2001; Pochard et al., 1983; Thabuis et al., 2003, 2004).

Capsicum baccatum and *C. pubescens* are placed in other taxonomic complexes of the genus and are little used beyond Latin America, although *C. baccatum* var. *pendulum* (wild). Eshbaugh that has been extensively used as domesticated chili peppers. Therefore, when reviewing the literature, it is essential to determine whether the plants discussed are actually *C. annuum*, *C. frutescens* or another species (Heiser and Pickersgill, 1969).

In Korea, *Capsicum annuum* has been restricted to a very narrow genetic diversity due to consumer preferences. With the exemption of a few cultivars grown for fresh fruit, almost all cultivars are cultivated for their dried red fruits, with varying degrees of pungency (Chae et al., 2003; Pae et al., 2003).

The United States National Plant Germplasm System preserves an extensive *Capsicum* germplasm collection at the Southern Plant Introduction Station located in Experiment, Georgia. This collection contains approximately 3000 *Capsicum* accessions that include lines from all over the world (Bosland, 1996). In addition, Chae et al. (2003) characterized 225 accessions from 8 *Capsicum* species preserved at the Asian Vegetable Research and Development Center using 37 morphological traits, and these have been utilized as a useful genetic resources in the breeding of improved pepper cultivars adapted to subtropical and temperate regions. Since 1980, Embrapa's National Research Center for Vegetable Crops has conducted a pepper breeding program aimed 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 benefits from a large gene bank of genotypes native to Brazil and also introduced from abroad (Ribeiro et al., 2008).

The five candidate pepper germplasm might be used as breeding resources for the phytophthora blight resistance breeding program. Further genetic study should be carried out to verify our results with the overall focus of providing information on important characteristics of pepper germplasms.

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생물검정을 통한 고추 유전자원의 역병저항성 평가

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초　록. 역병은 세계적으로 고추에 발생하는 병 중 가장 문제시 되는 병해로 국내 83개 지역에서 채집한 재래종 고추 유전자원 중 300개를 선발하여 공시하였다. 파종 후 온실에서 70일간 육묘하였고 공시한 역병균의 유주자낭 농도를 10^5/mL으로 하여 주당 5mL 관주접종 후, 습실 처리하였고 1주 간격으로 4주간 이병주율을 조사하였다. 접종 7일 후 이병주율을 조사하였더니, 공시한 300개 고추 자원 중 67개 자원에서 이병주율이 60.1% 이상으로 높은 감수성을 보였으며, 37개 자원은 접종 후 7일까지 전혀 발병하지 않았다. 역병균 접종 28일 후, 공시한 300점의 고추자원 중 244개 자원은 60% 이상의 이병주율을 보여 역병에 대해 감수성을 나타내었으며, 11개 자원은 20% 미만의 이병주율을 보여 중도저항성을 보였고, 5개 자원은 전혀 발병하지 않아 저항성을 나타내었다. 5개 자원의 저항성 고추유전자원은 역병저항성 고추 품종 육종에 유용한 중간모본으로 사용될 것으로 생각된다. 본 실험의 정밀도를 높이기 위해 분자마커를 활용한 추가실험이 진행된다면 고추유전자원의 역병 저항성에 대한 중요한 정보를 제공할 수 있을 것으로 본다.

추가 주요어 : *Capsicum*, 한국 재래종, *Phytophthora capsici*