



Differential Responses of *Capsicum* Accessions to Foliar Blight and Root Rot by *Phytophthora capsici*

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Abstract

The oomycete *Phytophthora capsici* is one of the most destructive pepper pathogens, reducing product quality and resulting in major yield losses in *Capsicum* spp. To date, little is known about the defense responses of the host pepper against different *P. capsici* isolates. Although foliar blight and root rot are caused by the same pathogen, the host resistance responses may differ among disease symptoms and must be studied independently. In this study, 30 *Capsicum* accessions were inoculated with two *P. capsici* isolates by root drenching, and the severity of root rot was evaluated. In addition, one *P. capsici* strain was injected into the leaves of the same 30 accessions to evaluate the difference in host defense responses to foliar blight and root rot caused by the same strain. Among 30 accessions, 6 showed different resistance levels to the two *P. capsici* isolates. When comparing the resistance levels to root rot and foliar blight, 14 pepper accessions showed different resistance responses based on the inoculation site. These results suggest that the resistance to foliar blight in peppers differs according to the isolate of *P. capsici* and the inoculation site, even within the same pepper cultivar. The results of this study will provide useful information for the breeding of *Phytophthora* root rot and foliar blight-resistant *Capsicum* peppers.

Additional key words: defense response, leaf infiltration, *Phytophthora* blight, resistant screening, root drench

Introduction

Hot peppers (*Capsicum* spp.) of the nightshade family Solanaceae are economically important and widely cultivated horticultural vegetable crops. Hot peppers are characterized by their production of capsaicinoids, the compounds responsible for the hot pungent taste of peppers. Hot peppers are also rich in phenolic-derived compounds with unique physiological and pharmacological properties, some of which provide human health benefits (Howard and Wildman, 2007; Loizzo et al., 2015). According to the Food and Agriculture Organization of the United Nations, 40 million tons of fresh and dried hot pepper were produced on – 3.76 Mha worldwide in 2018, which consistently increased over the last decade (FAOSTAT, 2018).

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Phytophthora capsici (Leonian) is a soil-borne oomycete plant pathogen that is the most devastating pathogen affecting hot pepper production. *P. capsici* has a wide host range of more than 15 plant families, including Solanaceae, Fabaceae, and Cucurbitaceae, and can cause severe diseases such as foliar blight, crown blight, root rot, and fruit rot (Lamour et al., 2012; Yeom et al., 2012; Barchenger et al., 2018; Kim et al., 2019b). Significant yield losses due to *P. capsici* infection in open production and greenhouse cultivation have been reported in many countries, accounting for a worldwide economic loss of over one billion dollars per year (Hausbeck and Lamour, 2004). In the sequential production systems commonly used for pepper cultivation, soil-borne diseases such as *Phytophthora* blight often become a recurring issue, consistently impacting the yield and quality of the produce.

P. capsici can survive in the soil and host plant debris, spreading rapidly by wind-blown rain through the air and water movement in the soil under warm (25°C–28°C) and wet conditions. Thus, when *P. capsici* infection occurs in the field, it is often difficult to control (Lamour et al., 2012; Barchenger et al., 2018). Chemical control and cultural practices are used to manage *P. capsici*; however, these management practices are not completely effective. To date, the development of a disease-resistant cultivar is one of the best approaches to control *P. capsici* infection, but the availability of resistant breeding resources for disease management programs are limited in the pepper industry (Hausbeck and Lamour, 2004; Foster and Hausbeck, 2010; Granke et al., 2012).

Plants have developed a range of defense mechanisms to combat diverse environmental conditions, which provide tolerance and/or resistance to abiotic and biotic stresses (Lee and Yeom, 2015; Kang and Yeom, 2018; Kang et al., 2020; Lee et al., 2020). In peppers, the expression of resistance against *P. capsici* is determined by variable environmental factors including the variety, growth stage, temperature, inoculation method, and isolate. The development of functional gene-based markers and characterization of the genes that confer resistance against *P. capsici* are essential to facilitate efforts to breed disease-resistant pepper cultivars (Kim et al., 2017a, 2017b; Chunthawodtiporn et al., 2018; Kim et al., 2019b). Numerous molecular markers have been identified within a major quantitative trait locus (QTL) region; however, little progress has been made in the development of functional gene-based markers of *P. capsici* resistance, and no *P. capsici* resistance genes (R gene) have been characterized. In addition, the plant host defense responses to different strains of *P. capsici* are not well understood (Glosier et al., 2007). It appears that foliar blight and root rot are different *P. capsici* disease symptoms that should be studied independently (Foster and Hausbeck, 2010). Furthermore, resistance to *Phytophthora*-induced diseases such as root rot and foliar blight showed different patterns of complex inheritance (Reifschneider et al., 1992; Oelke et al., 2003; Thabuis et al., 2004; Ogundiwin et al., 2005; Yeom et al., 2011; Kim et al., 2019a, 2019b).

In this study, we conducted a thorough screening to identify sources of *P. capsici* disease resistance in *Capsicum* spp. that could be incorporated into new commercial pepper cultivars. The severity of root rot caused by different *P. capsici* isolates and correlations between root rot and foliar blight were evaluated in several *Capsicum* accessions. Further genetic studies to verify our results are warranted, with the overall aim to provide information on the important characteristics of pepper germplasm by revisiting classical genetic studies and demonstrating the contribution of genomics to the understanding of the molecular basis of resistance.

Materials and Methods

Plant Materials and Growth Environment

Thirty pepper accessions consisting of six landrace and 24 commercial hybrid cultivars were screened for resistance to *Phytophthora* diseases. *C. annuum* “CM334” with known resistance to *P. capsici* and *C. annuum* “ECW30R” with known susceptibility to *P. capsici* were used as negative and positive controls for disease development, respectively. Pepper seeds were sown in a Petri dish lined with a wet tissue layer for 2 weeks. After germination, the seedlings were transplanted into a 32-cell plug seedling tray in a plant growth chamber at 25°C with 16 h of light per day.

Pathogen Preparation

Two pathogen accessions (*P. capsici* Leon “KACC 40470” and “KACC 40476”) were obtained from the Korea Agricultural Culture Collection (KACC) of Rural Development Administrations (Table 1). *P. capsici* inoculum preparation was described previously (Yeom et al., 2011). *P. capsici* was grown in potato dextrose agar medium in the dark for 7 d at 28°C, and then 8-mm diameter mycelial plugs were cut and cultured on V8 juice agar media (20% V8 juice, 0.3% CaCO₃, and 1.8% agar) for 5 d. Next, the mycelia were scraped and incubated under light for 2 d to promote sporangium formation. Chilling stress was induced by flooding the plate with sterile water and incubating at 4°C for 90 min; then, plates were incubated at 28°C for 30 min to stimulate the release of zoospores. The zoospores were counted using a hemocytometer, and the concentration was adjusted to 1×10^5 zoospores per ml with distilled water. Seedlings at the four-true-leaf stage were inoculated by root drenching with *P. capsici* “KACC 40470” (hereafter, KACC 40470) and “KACC 40476” (hereafter, KACC 40476) at a density of 2.0×10^5 zoospores per plant. To induce *Phytophthora* foliar blight, *P. capsici* “KACC 40476” suspension concentrated at 1.0×10^5 zoospores per ml was infiltrated on the marked leaf region on the same four-true-leaf stage plant. For spray inoculation, 500 µl of a zoospore suspension of KACC 40476 (1.0×10^5 zoospores per ml) was sprayed on each pepper leaf (Oh et al., 2010). For dropping inoculation, a 0.5-cm-diameter paper disc of Whatman filter paper (Sigma-Aldrich, St Louis, MO) was placed on the surface of each leaf, and then 20 µl (approximately 2000 zoospores) of KACC 40476 inoculum was dropped on each paper disc (Monroy-Barbosa and Bosland, 2010). Next, we prepared additional inoculum for plug inoculation of *P. capsici* (Bouwmeester and Govers, 2009). A mycelial plug (1 cm diameter) of KACC 40476 was placed on the abaxial side of leaf. Growth conditions were maintained at 25°C under a 16:8-h light/dark cycle.

Disease Scoring

Evaluations of foliar blight and root rot were performed when 80% of the “ECW30R” positive control plants were

Table 1. Characteristics of the *Phytophthora capsici* strains used in this study

| KACC ^z No. | Location of isolation | Source | Pathogenicity | |
|-----------------------|------------------------|-----------------------------|-----------------------|---------------------------------|
| | | | Reported ^y | Tested in this study (root rot) |
| 40470 | Buyeo, Chungnam, Korea | <i>Solanum lycopersicum</i> | Unknown | Strong Pathogenicity |
| 40476 | Goesan, Korea | <i>Capsicum annuum</i> | Strong Pathogenicity | Strong Pathogenicity |

^zKorean Agricultural Culture Collection.

^yPathogenicity information provided by KACC.

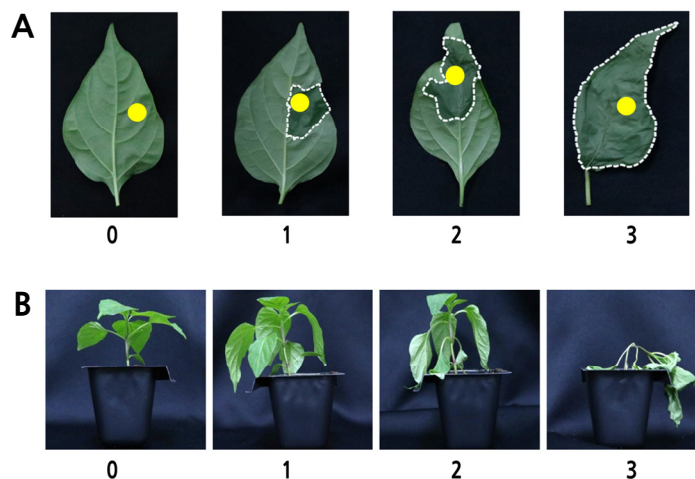


Fig. 1. The severity of *Phytophthora* blight on pepper plants rated using a scale of 0 to 3. (A) Foliar blight assessment of pepper plants. Evaluations were performed 6 days after inoculation (DAI): 0 = no symptoms or hypersensitive response, 1 = water-soaked lesion is less than 30% of leaf area, 2 = 30% to 60% of leaf area is wilted (scalded or necrotic), 3 = 60% or more of the leaf is wilted or the leaf has dropped off from the plant. (B) Root rot scale of pepper plants. Scoring was established 6 DAI using a scale of 0 to 3: 0 = no symptoms, 1 = stem lesion defoliated first and second leaves, or less than 30 % of entire plant diseased, 2 = stem lesion extending to second leaves, yellowing or defoliation of some upper leaves, or 30%-60% of entire plant diseased, and 3 = all leaves except uppermost leaf defoliated, more than 60% of entire plant diseased, or plant was dead.

diseased, which occurred 7 days after inoculation (DAI). A disease score for foliar blight was modified based on previous research (Monroy-Barbosa and Bosland, 2010; Jo et al., 2014). Foliar blight assessment was performed using a 0–3 scale in which 0 = no disease symptoms observed or small necrosis tissue; 1 = water-soaked lesion is less than 30% of leaf area; 2 = 30% to 60% of leaf area is wilted (scalded or necrotic); 3 = 60% or more of the leaf is wilted or the leaf has dropped off from the plant (Fig. 1A). Wilt induced by *P. capsici* was assessed using the following root rot severity scale. The plants were scored from 0–3 based on the proportion of defoliation symptoms observed on the entire plant following a previous study (Jo et al., 2014), in which 0 = no symptoms; 1 = stem lesion, defoliated first and second leaves, or less than 30% of entire plant diseased; 2 = stem lesion extending to second leaves, yellowing or defoliation of some upper leaves, or 30% to 60% of entire plant diseased; 3 = all leaves except uppermost leaf defoliated, more than 60% of entire plant diseased, or the plant was dead (Fig. 1B).

The lines were classified as resistant or susceptible based on the average severity of each line. The root drenching inoculation assay was measured by the average degree on 16–20 plants per cultivar; the leaf injection assay was measured by the average degree on 8–10 plants per cultivar. The trait classifications for peppers were evaluated as follows: 0 to less than 1 = resistance; 1 to less than 2 = moderate resistance; 2 to 3 = susceptible (Monroy-Barbosa and Bosland, 2010; Jo et al., 2014).

Data Analysis

The statistical analyses were performed using SAS software (version 9.4; SAS Inc., Cary, NC). The differences among average values of disease scores on the pepper cultivars were analyzed using Duncan's multiple range tests. There were no significant ($p < 0.05$) interactions between cultivar and biological replicate. Means across two biological replicates are reported.

Results and Discussion

Screening of Pepper Cultivars for Resistance to Two Isolates of *P. capsici*

In this study, 30 chili pepper accessions were tested for resistance and susceptibility to *Phytophthora* blight caused by *P. capsici*, including four *C. annuum* species, one *C. chinense* species, one *C. baccatum* species, and 24 Korean commercial pepper cultivars. Among the 30 accessions of pepper germplasm, the resistance level against *Phytophthora* blight of 23

Table 2. The resistance response of 30 *Capsicum* pepper varieties to root rot disease after inoculation with two isolates of *Phytophthora capsici*

| No. | Pepper accession | "KACC 40470" | | | "KACC 40476" | | | Known Trait ^z |
|-----|---|--------------|---------------------|----------------|--------------|--------|----|--------------------------|
| P1 | <i>C. annuum</i> "CM334" (NC) ^y | 0 | ± 0.00 ^x | R ^w | 0 | ± 0.00 | R | R |
| P2 | <i>C. annuum</i> "ECW30R" (PC) ^v | 2.8 | ± 0.11 | S | 3 | ± 0.00 | S | S |
| P3 | <i>C. annuum</i> "PR-Manidda" | 0.5 | ± 0.14 | R | 0 | ± 0.00 | R | MR/R |
| P4 | <i>C. annuum</i> "Dokyacheongcheong" | 0.2 | ± 0.09 | R | 0 | ± 0.00 | R | S/MR/R |
| P5 | <i>C. annuum</i> "Muhanjilju" | 0.1 | ± 0.07 | R | 0 | ± 0.00 | R | R |
| P6 | <i>C. annuum</i> "Ilsongjung" | 0.3 | ± 0.21 | R | 0 | ± 0.00 | R | MR/R |
| P7 | <i>C. annuum</i> "PR-Kstar" | 0 | ± 0.00 | R | 0 | ± 0.00 | R | R |
| P8 | <i>C. annuum</i> "PR-Shindaejang" | 0.2 | ± 0.27 | R | 0 | ± 0.00 | R | R |
| P9 | <i>C. annuum</i> "Asia-jumbo" | 0 | ± 0.00 | R | 0.1 | ± 0.08 | R | R |
| P10 | <i>C. annuum</i> "Dokjuyeokgang" | 2.3 | ± 0.61 | S | 0.6 | ± 0.60 | R | R |
| P11 | <i>C. annuum</i> "PR-Gugupalpal" | 0.8 | ± 1.06 | R | 0 | ± 0.00 | R | R |
| P12 | <i>C. annuum</i> "PR-Oganmanjok" | 0.8 | ± 0.07 | R | 0 | ± 0.00 | R | R |
| P13 | <i>C. annuum</i> "PR-Jeonguktongil" | 0.9 | ± 0.08 | R | 0 | ± 0.00 | R | R |
| P14 | <i>C. annuum</i> "PR-Daechon" | 0.2 | ± 0.14 | R | 0.1 | ± 0.07 | R | S/MR/R |
| P15 | <i>C. annuum</i> "Yeokganghonggianggun" | 0.4 | ± 0.05 | R | 0 | ± 0.00 | R | MR/R |
| P16 | <i>C. annuum</i> "PR-Mujeok" | 0.7 | ± 0.25 | R | 0.1 | ± 0.07 | R | MR |
| P17 | <i>C. annuum</i> "Yeokgangsumunjang" | 0 | ± 0.00 | R | 0 | ± 0.00 | R | MR |
| P18 | <i>C. baccatum</i> "PBC81" | 2.9 | ± 0.18 | S | 2.2 | ± 0.08 | S | S |
| P19 | <i>C. chinense</i> "PI159236" | 1.7 | ± 0.41 | MR | 1.7 | ± 0.52 | MR | S |
| P20 | <i>C. annuum</i> "Daedulbo" | 2.9 | ± 0.16 | S | 1 | ± 1.40 | MR | S |
| P21 | <i>C. annuum</i> "Bukang" | 3 | ± 0.06 | S | 2.4 | ± 0.28 | S | S |
| P22 | <i>C. annuum</i> "Supermanidda" | 2.5 | ± 0.30 | S | 0.8 | ± 0.14 | R | S |
| P23 | <i>C. annuum</i> "Nokgwang" | 2.5 | ± 0.48 | S | 2.7 | ± 0.21 | S | S |
| P24 | <i>C. annuum</i> "Tanegang" | 0.8 | ± 0.53 | R | 0 | ± 0.00 | R | ND ^u |
| P25 | <i>C. annuum</i> "AT-Shinhotan" | 1.7 | ± 0.08 | MR | 0.2 | ± 0.35 | R | ND |
| P26 | <i>C. annuum</i> "Jeoktoma-plus" | 1.4 | ± 0.11 | MR | 0.2 | ± 0.31 | R | ND |
| P27 | <i>C. annuum</i> "Mawuntan" | 2.7 | ± 0.21 | S | 1.5 | ± 0.64 | MR | ND |
| P28 | <i>C. annuum</i> "Meojjinsanaie" | 0.3 | ± 0.44 | R | 0 | ± 0.00 | R | ND |
| P29 | <i>C. annuum</i> "Subicho" | 2.9 | ± 0.14 | S | 2.6 | ± 0.64 | S | ND |
| P30 | <i>C. annuum</i> "MC4" | 0 | ± 0.00 | R | 0 | ± 0.06 | R | ND |

^zKnown resistance trait from previous studies or seed producers.

^yPC, positive control.

^xAverage disease severity score and standard deviation.

^wR, resistant; MR, moderate resistant; S, susceptible.

^vNC, negative control.

^uNot detected.

accessions was previously characterized (Jo et al., 2014), and the remaining 7 accessions had not been previously scored.

To determine disease resistance to *P. capsici* isolates by root inoculation, we investigated the severity of disease caused by root drenching with KACC 40470 and KACC 40476 in 30 *Capsicum* germplasms (Table 2). *C. annuum* “CM334” is reported to have the highest resistance level against multiple strains of *P. capsici* (Yeom et al., 2011; Kim et al., 2019b); thus, it was used as a negative control in this study. *C. annuum* “CM334” was confirmed to be resistant against both strains of *P. capsici*, with an average root rot severity score of 0. The positive control *C. annuum* “ECW30R” was confirmed to be susceptible to *P. capsici*, with average root rot severity scores of 2.8 and 3.0 when inoculated with KACC 40470 and KACC 40476, respectively.

The results of the inoculation with KACC 40470 revealed that 18 cultivars (60%), including “CM334”, had a mean root rot severity score of 0 to 1 and were considered resistant, while 9 cultivars (30%) including “ECW30R” had an average score between 2 and 3 and were considered susceptible. The results of the root inoculation with KACC 40476 revealed that 22 (73%) pepper cultivars showed resistance and 5 (16.7%) showed susceptibility. Although we did not measure the severity of pathogenicity for KACC 40470, the root inoculation results showed that comparatively, the pathogenicity of KACC 40470 was slightly higher than that of KACC 40476. Based on this result, we assumed that both KACC40470 and 40476 would have strong pathogenicity (Table 1). Comparing the root rot results between the two strains, 6 out of 30 pepper accessions showed different resistance levels to the two *P. capsici* isolates. Four pepper cultivars, “Dokjuyeokgang”, “Daedulbo”, “Supermanidda”, and “Mawuntan”, were scored as susceptible to KACC 40470 but showed resistance against KACC 40476. Two cultivars, “AT-Shinhotan” and “Jeoktoma-plus”, showed moderate resistance against KACC 40470 and resistance against KACC 40476 (Table 2). According to previous studies, there was a large difference in pathogenicity between *P. capsici* among isolates, and *P. capsici* isolates showed varying levels of virulence to pepper cultivars (Silvar et al., 2006; Candole et al., 2010; Jo et al., 2014). Consistent with previous studies, our results revealed that these two *P. capsici* isolates showed different pathogenicity against pepper cultivars. Eighteen cultivars including “CM334” were consistently resistant to both KACC 40470 and 40476. We also compared the root rot results in this study with those of previous studies (Table 2); differences in disease severity to *P. capsici* strains were detected in “PR-Manidda”, “Dokyacheongcheong”, “Ilsongjung”, “PR-Daechon”, and “Yeokganghongjanggun” cultivars. The majority of *P. capsici*-resistant pepper cultivars were derived from “CM334” by breeding programs in Korea. However, the *P. capsici* resistance in “CM334” is controlled by a QTL, and no pepper cultivars have shown complete resistance to all strains of *P. capsici*. These findings may provide relevant information about the differences in pepper resistance related to the pathogenicity of *P. capsici*, depending on the number of superior resistance genes introduced through the breeding of diverse pepper landraces (Foster and Hausbeck, 2010; Jo et al., 2014).

Evaluation of Pepper Cultivars for Resistance to *Phytophthora* Root Rot and Foliar Blight

To investigate the resistance response of pepper to root rot and foliar blight caused by *P. capsici*, we infiltrated KACC 40476 onto the 3rd and 4th true leaves of pepper plants. Disease resistance levels of the inoculated leaves of 30 pepper cultivars were observed 6 DAI (Fig. 2). Assessment of foliar blight severity was performed based on scores from 0 to 3, with 0 indicating no symptoms and 3 indicating the most severe symptoms. The pepper cultivars showed significant differences in resistance responses to foliar blight and root rot. Among the 30 pepper cultivars, “CM334” and “ECW30R” were consistently resistant and susceptible to both root inoculation and leaf inoculation, respectively. Only five cultivars

(16.7%) [P1 (CM334), P6 (Ilsonjung), P26 (Jeoktoma-plus), P14 (PR-Daechon), and P9 (Asia-jumbo)] were resistant to foliar blight, with scores between 0 and 1. Seventeen cultivars (56.7%) were deemed susceptible, with scores ranging from 2 to 3. Even though the leaf infiltration method by syringe is a commonly used method for plant pathogen inoculation (Kwon et al., 2021; Kang et al., 2022), this method could affect artificially effects during infection of zoospore compared to the root drench method. Therefore, to determine the effect of artificial inoculation by syringe on the phenotypic response of pepper against *P. capsici*, we used three different leaf inoculation methods, including leaf spray, dropping, and plug inoculation. These methods have been used to inoculate leaves of *P. capsici* in previous studies (Bouwmeester and Govers, 2009; Monroy-Barbosa and Bosland, 2010; Oh et al., 2010). We tested five cultivars; three cultivars [P11 (PR-Gugupalpal), P16 (PR-Mujeok), and P30 (MC4)] showed large differences in disease severity between root rot and foliar blight, and two cultivars were used as positive (P2) and negative control (P1). The phenotypic results of three leaf inoculation methods were consistent with leaf infiltration results, which showed that the responses of three tested cultivars (P11, P16, and P30) were susceptible to KACC 40476 with average disease scores of 3.0 displaying wilted leaves (Suppl. Fig. 1 and Suppl. Table 1). These results suggest that the leaf infiltration method could provide reliable results for screening of foliar blight resistance.

To examine the relationship between root rot resistance and foliar blight resistance, we compared the results of the root drenching inoculation and leaf infiltration of KACC 40476 (Fig. 3) and found that the resistance responses to *Phytophthora* root rot and *Phytophthora* foliar blight were inconsistent among *Capsicum* accessions. Twelve accessions (40%) showed resistant phenotypes (resistance or moderated resistance) to both root rot and foliar blight. Four accessions [P2 (ECW30R), P21 (Bukang), P23 (Nokgwang), and P29 (Subicho)] showed susceptibility to both inoculation methods (Figs. 2 and 3). The other 14 pepper accessions (46.7%) exhibited different disease resistance responses depending on the inoculation site (Fig. 3). Among these 14 cultivars, most (13 accessions) showed resistance or moderate resistance to root rot and susceptibility to foliar blight. Only one pepper accession (P18; *C. baccatum* “PBC81”) was susceptible to root rot and moderately resistant to foliar blight (Figs. 2 and 3). When comparing root rot response and foliar blight response, 66% of pepper accessions showed lower levels of resistance to leaf inoculation than root inoculation (i.e., R/MR, R/S, or MR/S). The major QTL that contributes to *P. capsici* resistance is identically expressed among plant organs, but resistance levels could differ depending on the presence or absence of minor QTL introduction (Liu et al., 2014; Rehrig

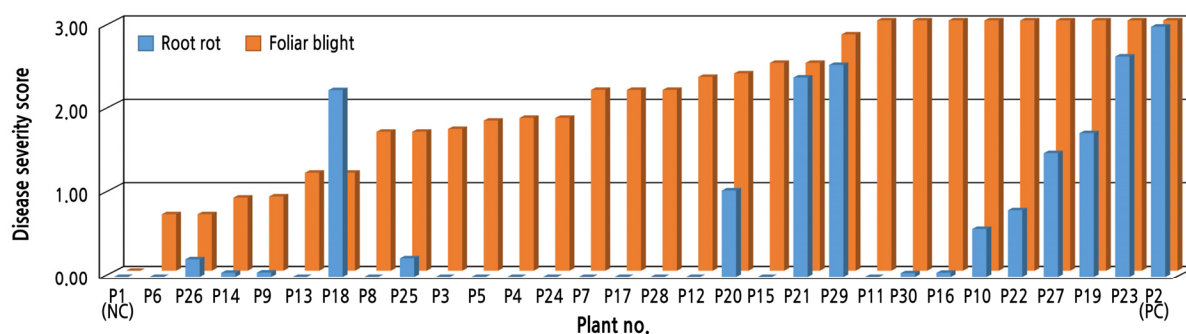


Fig. 2. *Phytophthora capsici* “KACC 40476”-induced root rot and foliar blight severity derived from the screening of 30 *Capsicum* spp. accessions. The values are means of two biological replicates with 16 plants each. Disease severity of the seedlings was rated on a scale of 0 to 3 at 6 days after inoculation: 0 to less than 1 = resistance; 1 to less than 2 = moderate resistance; 2 to 3 = susceptibility. On the x-axis, each pepper cultivar is numbered as shown in Table 2. The disease severity score is presented on the y-axis. PC, positive control; NC, negative control.

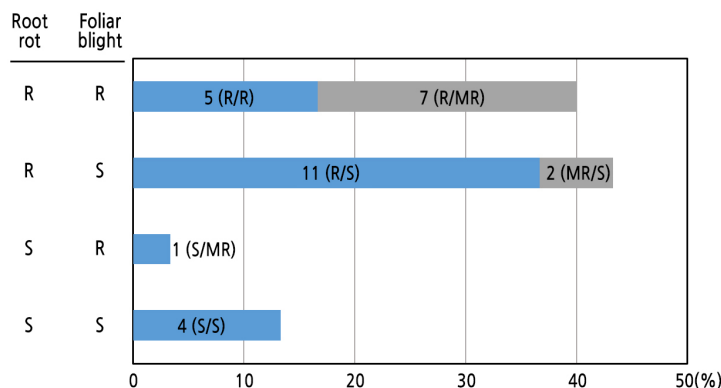


Fig. 3. Comparison of *Phytophthora*-induced root rot and foliar blight among pepper cultivars. Pepper seedlings were inoculated with *P. capsici* “KACC 40476” by root drenching and leaf injection. Disease severity was evaluated. R, resistance; MR, moderate resistance; S, Susceptibility.

et al., 2014; Kim et al., 2019b).

In this study, the severity of *Phytophthora*-induced diseases from *P. capsici* isolates KACC 40470 and KACC 40476 was determined in 30 *Capsicum* accessions. In addition, we compared the defense responses to root rot and foliar blight and found that some accessions showed different resistance responses depending on the inoculation method. Our findings suggest that the resistance to blight in peppers differs according to the isolate of *P. capsici* and the inoculation site, even within the same pepper cultivar. Therefore, pepper resistance is related to the pathogenicity of *P. capsici* isolates as well as the number of resistance genes introduced from resistant pepper germplasm during the breeding process. The major resistance genes could be identically expressed in all organs; however, resistance levels were different depending on the presence or absence of minor QTL in pepper accessions. The findings of this study contribute to our understanding of the molecular basis of resistance to *P. capsici* in peppers and can be applied to improve breeding methods.

Literature Cited

- Barchenger DW, Lamour KH, Bosland PW (2018) Challenges and strategies for breeding resistance in *Capsicum annuum* to the multifarious pathogen, *Phytophthora capsici*. *Front Plant Sci* 9:628. doi:10.3389/fpls.2018.00628
- Bouwmeester K, Govers F (2009) A novel method for efficient and abundant production of *Phytophthora brassicae* zoospores on Brussels sprout leaf discs. *BMC Plant Biol* 9:111. doi:10.1186/1471-2229-9-111
- Candole BL, Conner PJ, Ji PS (2010) Screening *Capsicum annuum* accessions for resistance to six isolates of *Phytophthora capsici*. *Hortscience* 45:254-259. doi:10.21273/HORTSCI.45.2.254
- Chunthawodtiporn J, Hill T, Stoffel K, Van Deynze A (2018) Quantitative trait loci controlling fruit size and other horticultural traits in bell pepper (*Capsicum annuum*). *Plant Genome* 11:160125. doi:10.3835/plantgenome2016.12.0125
- Food and Agriculture Organization of the United Nations (FAOSTAT) (2018) FAOSTAT. <http://www.fao.org/faostat/en/#data/QC>. Accessed 14 September 2020
- Foster J, Hausbeck M (2010) Resistance of pepper to *Phytophthora* crown, root, and fruit rot is affected by isolate virulence. *Plant Disease* 94:24-30. doi:10.1094/PDIS-94-1-0024
- Glosier BR, Ogundiwin EA, Sidhu GS, Sischo DR, Prince JP (2007) A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. *Euphytica* 162:23-30. doi:10.1007/s10681-007-9532-1
- Granke LL, Quesada-Ocampo L, Lamour K, Hausbeck MK (2012) Advances in research on *Phytophthora capsici* on vegetable crops in the United States. *Plant Dis* 96:1588-1600. doi:10.1094/PDIS-02-12-0211-FE
- Hausbeck MK, Lamour KH (2004) *Phytophthora capsici* on vegetable crops: research progress and management challenges. *Plant Dis* 88:1292-1303. doi:10.1094/PDIS.2004.88.12.1292
- Howard LR, Wildman RE (2007) Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum*

- annuum*). In RC Wildman, ed, Handbook of Nutraceuticals and Functional Foods, Ed 2, CRC Press, Boca Raton, FL, USA, pp 209-233. doi:10.1201/9781420006186.ch9
- Jo SJ, Shim SA, Jang KS, Choi YH, Kim J-C, Choi GJ (2014) Resistance of chili pepper cultivars to isolates of *Phytophthora capsici*. Korean J Hortic Sci Technol 32:66-76. doi:10.7235/hort.2014.13079
- Kang WH, Lee J, Koo N, Kwon JS, Park B, Kim YM, Yeom SI (2022) Universal gene co-expression network reveals receptor-like protein genes involved in broad-spectrum resistance in pepper (*Capsicum annuum* L.). Hortic Res uhab003. doi:10.1093/hr/uhab003
- Kang WH, Sim YM, Koo N, Nam JY, Lee J, Kim N, Jang H, Kim YM, Yeom SI (2020) Transcriptome profiling of abiotic responses to heat, cold, salt, and osmotic stress of *Capsicum annuum* L. Sci Data 7:17. doi:10.1038/s41597-020-0352-7
- Kang WH, Yeom SI (2018) Genome-wide identification, classification, and expression analysis of the receptor-like protein family in tomato. Plant Pathol J 34:435-444. doi:10.5423/PPJ.OA.02.2018.0032
- Kim DH, Kang WH, Yeom SI, Kim BD (2019a) Isolation of putative pepper defense-related genes against the pathogen *Phytophthora capsici* using suppression subtractive hybridization/microarray and RNA-sequencing analyses. Hortic Environ Biotechnol 60:685-699. doi:10.1007/s13580-019-00157-1
- Kim N, Kang WH, Lee J, Yeom SI (2019b) Development of clustered resistance gene analogs-based markers of resistance to *Phytophthora capsici* in chili pepper. Biomed Res Int 2019:1093186. doi:10.1155/2019/1093186
- Kim S, Park J, Yeom SI, Kim YM, Seo E, Kim KT, Kim MS, Lee JM, Cheong K, et al (2017b) New reference genome sequences of hot pepper reveal the massive evolution of plant disease-resistance genes by retroduplication. Genome Biol 18:210. doi:10.1186/s13059-017-1341-9
- Kim SB, Kang WH, Huy HN, Yeom SI, An JT, Kim S, Kang MY, Kim HJ, Jo YD, et al (2017a) Divergent evolution of multiple virus-resistance genes from a progenitor in *Capsicum* spp. New Phytol 213:886-899. doi:10.1111/nph.14177
- Kwon JS, Nam JY, Yeom SI, Kang WH (2021) Leaf-to-whole plant spread bioassay for pepper and *Ralstonia solanacearum* interaction determines inheritance of resistance to bacterial wilt for further breeding. Int J Mol Sci 22:2279. doi:10.3390/ijms22052279
- Lamour KH, Stam R, Jupe J, Huitema E (2012) The oomycete broad-host-range pathogen *Phytophthora capsici*. Mol Plant Pathol 13:329-337. doi:10.1111/j.1364-3703.2011.00754.x
- Lee HA, Yeom SI (2015) Plant NB-LRR proteins: tightly regulated sensors in a complex manner. Brief Funct Genomics 14:233-242. doi:10.1093/bfpg/elv012
- Lee J, Nam JY, Jang H, Kim N, Kim YM, Kang WH, Yeom SI (2020) Comprehensive transcriptome resource for response to phytohormone-induced signaling in *Capsicum annuum* L. BMC Res Notes 13:440. doi:10.1186/s13104-020-05281-1
- Liu WY, Kang JH, Jeong HS, Choi HJ, Yang HB, Kim KT, Choi D, Choi GJ, Jahn M, et al (2014) Combined use of bulked segregant analysis and microarrays reveals SNP markers pinpointing a major QTL for resistance to *Phytophthora capsici* in pepper. Theor Appl Genet 127:2503-2513. doi:10.1007/s00122-014-2394-8
- Loizzo MR, Pugliese A, Bonesi M, Menichini F, Tundis R (2015) Evaluation of chemical profile and antioxidant activity of twenty cultivars from *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chacoense* and *Capsicum chinense*: A comparison between fresh and processed peppers. LWT-Food Sci Technol 64:623-631. doi:10.1016/j.lwt.2015.06.042
- Monroy-Barbosa A, Bosland PW (2010) A rapid technique for multiple-race disease screening of *Phytophthora* foliar blight on single *Capsicum annuum* L. Plants. HortScience 45:1563-1566. doi:10.21273/HORTSCI.45.10.1563
- Oelke LM, Bosland PW, Steiner R (2003) Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Capsicum annuum*. J Am Soc Hortic Sci 128:213-218. doi:10.21273/JASHS.128.2.0213
- Ogundiwin EA, Berke TF, Massoudi M, Black LL, Huestis G, Choi D, Lee S, Prince JP (2005) Construction of 2 intraspecific linkage maps and identification of resistance QTLs for *Phytophthora capsici* root-rot and foliar-blight diseases of pepper (*Capsicum annuum* L.). Genome 48:698-711. doi:10.1139/g05-028
- Oh SK, Baek KH, Seong ES, Joung YH, Choi GJ, Park JM, Cho HS, Kim EA, Lee S, et al (2010) CaMsrB2, pepper methionine sulfoxide reductase B2, is a novel defense regulator against oxidative stress and pathogen attack. Plant Physiol 154:245-261. doi:10.1104/pp.110.1.62339
- Rehrig WZ, Ashrafi H, Hill T, Prince J, Van Deynze A (2014) CaDMR1 cosegregates with QTL Pc5.1 for resistance to *Phytophthora capsici* in pepper (*Capsicum annuum*). Plant Genome 7. doi:10.3835/plantgenome2014.03.0011
- Reifschneider F, Boiteux L, Della Vecchia P, Poulos J, Kuroda N (1992) Inheritance of adult-plant resistance to *Phytophthora capsici* in pepper. Euphytica 62:45-49. doi:10.1007/BF00036086
- Silvar C, Merino F, Diaz J (2006) Diversity of *Phytophthora capsici* in northwest Spain: analysis of virulence, metalaxyl response, and molecular characterization. Plant Dis 90:1135-1142. doi:10.1094/PD-90-1135
- Thabuis A, Palloix A, Servin B, Daubeze A, Signoret P, Lefebvre V (2004) Marker-assisted introgression of 4 *Phytophthora capsici* resistance QTL alleles into a bell pepper line: validation of additive and epistatic effects. Mol Breed 14:9-20. doi:10.1023/B:MOLB.0000037991.38278.82
- Yeom SI, Baek HK, Oh SK, Kang WH, Lee SJ, Lee JM, Seo E, Rose JK, Kim B-D, et al (2011) Use of a secretion trap screen in pepper following *Phytophthora capsici* infection reveals novel functions of secreted plant proteins in modulating cell death. Mol Plant Microbe Interact 24:671-684. doi:10.1094/MPMI-08-10-0183
- Yeom SI, Seo E, Oh SK, Kim KW, Choi D (2012) A common plant cell-wall protein HyPRP1 has dual roles as a positive regulator of cell death and a negative regulator of basal defense against pathogens. Plant J 69:755-768. doi:10.1111/j.1365-3113.2011.04828.x

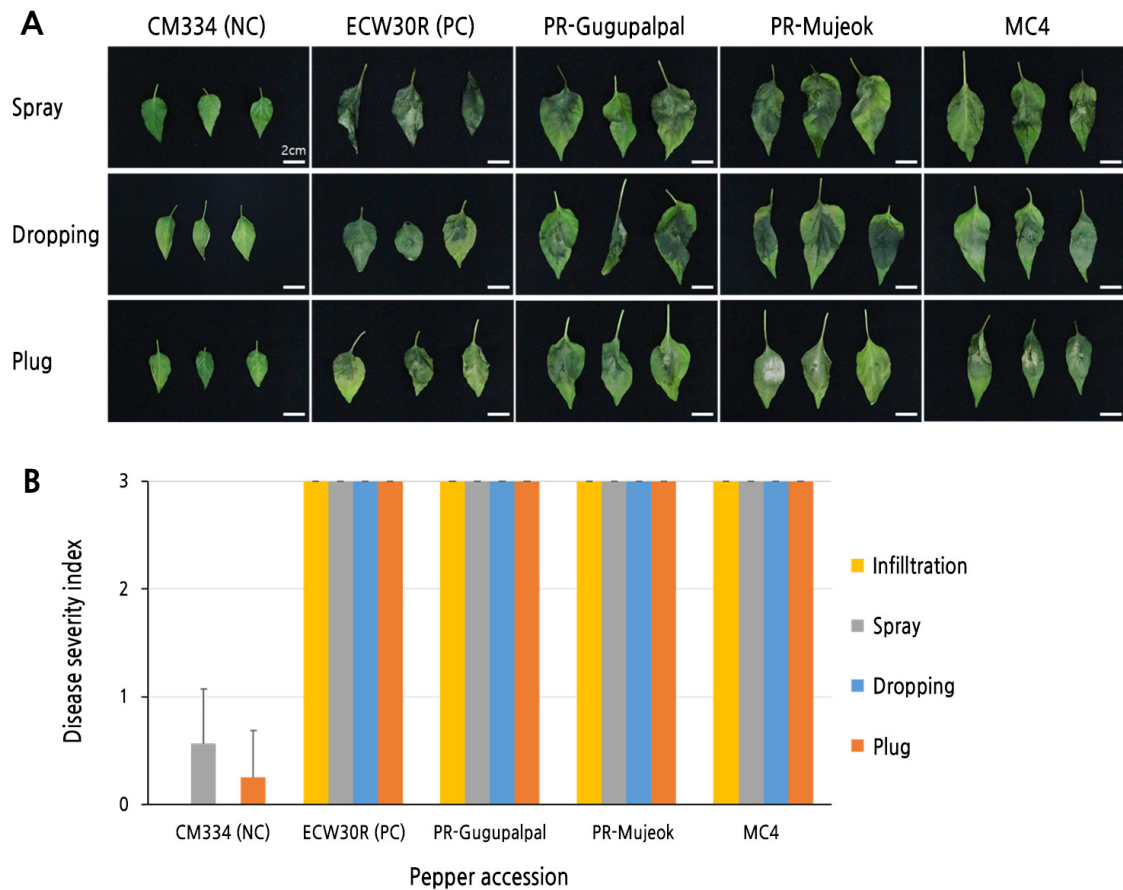
Supplemental Table 1. The responses of pepper varieties by inoculation methods of KACC 40476

| No. | Pepper accession | Root-rot | Foliar blight | | | | |
|-----|----------------------------------|--------------------------------------|-------------------|--------------------|-----------------------|-------------------|---|
| | | | Leaf-infiltration | Spray ^x | Dropping ^x | Plug ^x | |
| P1 | <i>C. annuum</i> "CM334" | 0 ± 0.00 ^z R ^y | 0 ± 0.00 R | 0.6 ± 0.51 R | 0 ± 0.00 R | 0.3 ± 0.44 | R |
| P2 | <i>C. annuum</i> "ECW30R" | 2.8 ± 0.11 S | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 | S |
| P11 | <i>C. annuum</i> "PR-Gugupalpal" | 0.5 ± 0.14 R | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 | S |
| P16 | <i>C. annuum</i> "PR-Mujeok" | 0.2 ± 0.09 R | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 | S |
| P30 | <i>C. annuum</i> "MC4" | 0.1 ± 0.07 R | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 S | 3 ± 0.00 | S |

^zAverage disease severity score and standard deviation.

^yR, resistant; MR, moderate resistant; S, susceptible.

^xAverage disease severity score represented as 32 leaves from 16 pepper plants for each method. Similar results were obtained from two independent experiments. One representative experiment is shown.



Supplemental Fig. 1. Confirmation of the phenotype through leaf-inoculation methods against KACC 40476. (A) Foliar blight symptoms of 5 pepper accessions through spray, dropping, and plug inoculation methods at 4 days after inoculation (DAI). The white scale bar in photo indicates 2 cm. (B) Foliar blight assessment of pepper plants at 4 DAI. NC: negative control; PC: positive control.