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Histological and Cytological Changes Associated with Susceptible and Resistant Responses of Chili Pepper Root and Stem to *Phytophthora capsici* Infection

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Microscopic study of chili pepper (*Capsicum annuum* L.) infected with *Phytophthora capsici*, causing Phytophthora blight of chili pepper, was conducted to compare histological and cytological characteristics in the root and stem of susceptible (*C. annuum* cv. Bugang) and resistant (*C. annuum* cv. CM334) pepper cultivars. The susceptible pepper roots and stems were extensively penetrated and invaded by the pathogen initially into epidermal cells and later cortical and vascular cells. Host cell walls adjacent to and invaded by the infecting hyphae were partially dissolved and structurally loosened with fine fibrillar materials probably by cell wall-degrading enzymes of the pathogen. In the resistant pepper, the pathogen remained on root epidermal surface at one day after inoculation, embedded and captured in root exudation materials composed of proteins and polysaccharides. Also the pathogen appeared to be blocked in its progression at the early infection stages by thickened middle lamellae. At 3 days after inoculation, the oomycete hyphae were still confined to epidermal cells of the root and at most outer peripheral cortical cells of the stem, resulting from their invasion blocked by wound periderms formed underneath the infection sites and/or cell wall appositions bounding the hyphal protrusions. All of these aspects suggest that limitation of disease development in the resistant pepper may be due to the inhibition of the pathogen penetration, infection, invasion, and colonization by the defense structures such as root exudation materials, thickened middle lamellae, wound peridems and cell wall appositions.

Keywords **:** *Capsicum annuum* L., defense structures, *Phytophthora capsici*, resistant, root exudation materials

Phytophthora blight of peppers, caused by the oomycete pathogen *Phytophthora capsici*, is a devastating disease on chili pepper worldwide as well as in Korea (Hwang and Kim, 1995). It has been reported to be responsible for major production losses of chili pepper (*Capsicum annuum* L.). Management of the Phytophthora blight has included cultural practices, use of chemicals and crop rotation (Ristaino and Johnston, 1999). However, cultural control methods including crop rotation have not been much effective because the oospores of *P. capsici* resistant to desiccations, cold temperature and other unfavorable environmental conditions, are able to survive in soil in absence of host plant for several years (Hwang and Kim, 1995). Resistance of *P. capsici* to fungicides metalaxyl and mefenoxam has been reported both in the laboratory and field conditions (Bower and Coffey, 1985; Bruin and Edgington, 1982; Lamour and Hausbeck, 2001; Parra and Ristaino, 2001). It is therefore necessary to develop alternative strategies for the control of the Phytophthora blight in pepper production. Alternative strategies such as use of genetically resistant cultivars promise to be the most effective and environmental-friendly in controlling the Phytophthora blight. However, prolonged incubation periods or high inoculum concentrations of *P. capsici* can occasionally overcome resistance in pepper, resulting in the production of symptoms on some resistant plants (Barksdale et al., 1984; Hwang and Kim, 1995; Smith et al., 1967).

Although several ultrastructural and histological researches have been conducted on the interaction of *P. capsici* with pepper plants, most of their inoculation methods and plant tissues used have been somewhat artificial (mechanical wound inoculation with the pathogen mycelium or dipping in zoospore suspension), rather than showing the interactions in the natural infection processes (Hwang et al., 1989; Jones et al., 1974; Kim et al., 1989; Lee et al., 2000). In this study, inoculation followed the natural way of inoculation processions from their attachment to plant roots by pouring a zoospore suspension of *P. capsici* in media where the plants were growing. The objective of this study was to examine the differences in histological and cytological characteristics of susceptible and resistant chili pepper responses to *P. capsici* infection to give a basic information for the disease management.

Materials and Methods

Inoculum preparation. An isolate of *Phytophthora capsici* P-20156 was kindly provided by Dr. H. J. Jee (National

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Academy of Agricultural Science, Rural Development Administration, Korea). The pathogen was grown on 10% V8 juice agar (100 ml of V8 juice, 900 ml of distilled water, 1 g of $CaCO_3$, 15 g of agar in 1 L medium) at 25°C in the dark for 4 days. Pathogen mycelia were collected from the culture rubbed with a glass spreader, and incubated at 25°C for 2 days under a continuous fluorescent light to stimulate sporulation. The culture plates were immersed in sterile water at 4°C for 60 min, followed by incubating at room temperature for 60 min to release zoospores, which were collected by filtering through two layers of cheesecloth. The concentration of zoospore suspension was adjusted to 1×10^4 zoospores per milliliter using a hemacytometer.

Plant growth and pathogen inoculation for disease development on susceptible and resistant chili pepper. Chili pepper cv. Bugang susceptible and cv. Criollo de Morelos 334 (cv. CM334) resistant to *Phytophthora capsici* were used in this study, of which the seeds were kindly provided by Dr. B. D. Kim (Seoul National University, Korea). Plastic pots of 6-cm diameter were filled with 500 g sterilized sand and potting mixtures. Six-week-old chili pepper seedlings were transplanted to pots and inoculated with 5 ml of zoospore suspension of *P. capsici* into each pot two days later. The inoculated plants were placed at 25±2°C in a greenhouse. Disease severities on above-ground plant parts were evaluated until 13 days after inoculation using a scale modified from Ristaino (1990) as follows: 0=no visual disease symptoms, 1=leaves slightly wilted with brownish lesions beginning to appear on stem, 2=30-50% of entire plant diseased, 3=50-70% of entire plant diseased, 4=70-90% of entire plant diseased, 5=plant dead. Pepper seedlings treated with sterilie water instead of zoospore suspension served as control. Twelve plants were used for each treatment.

Plant growth and pathogen inoculation for microscopy. Chili pepper (cv. Bugang and cv. CM334) seeds were sown in a 2.5 cm (diameter)×6 cm (height) glass vial containing 8 ml of 1.5% water agar after the seeds were surface-sterilized consecutively by 1.0% sodium hypochlorite and 70% ethanol for 20 sec each followed by two times of washing in sterilized distilled water. Pepper seedlings germinated were cultured at 28°C under a fluorescent light for 16 h per day in a growth chamber. Ten-day-old pepper seedlings were inoculated with the pathogen by pouring one ml of zoospore suspension on their rhizosphere in each vial.

Statistical analysis. Analysis of variance was carried out using Statistical Analysis System 6.08 (SAS Institute, Cary, NC). Duncan's multiple range test was employed for significant difference in disease severity between the susceptible and resistant pepper cultivars.

Specimen preparation for light and transmission electron microscopy. Root and stem tissues of pepper plants inoculated with *P. capsici* were cut into small pieces (5 mm in length) with a sterile razor blade 1 and 3 days after inoculation. The specimens were fixed with modified Karnovsky's fixative consisting of 2% (vol/vol) glutaraldehyde and 2% (vol/vol) paraformaldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) and vacuum infiltrated (Karnovsky, 1965). Prefixed and infiltrated specimens were placed at 4°C overnight, and washed three times with same buffer for 10 min each. The specimens were postfixed with 1% (wt/vol) osmium tetroxide in the same buffer at 4°C for 2 h, and washed briefly twice with distilled water. The postfixed specimens were *en bloc* stained with 0.5% uranyl acetate at 4°C overnight. They were dehydrated in a graded ethanol series (30, 50, 70, 80, 90 and 100%), and three times in 100% ethanol for 10 min each. The specimens were further treated with propylene oxide as a transitional fluid two times each for 15 min, and embedded in Spurr's medium (Spurr, 1969), followed by polymerization at 70°C for 8 h. The embedded specimens were sectioned with a glass knife on an ultramicrotome (MT-X, RMC Inc., Tucson, AZ) for light microscopy. Sections (800 nm in thickness) of the pepper samples were mounted on a glass slide by heating at 40°C on a slide warmer. Sections were stained with 1% toluidine blue O for a few seconds at 40°C for general staining. Some sections (especially one day after inoculation) were stained with 0.25% Coomassie brilliant blue in 7% acetic acid for 10 min at 50°C, Schiff's reagent for 30 min after treating 1% periodic acid for 10 min, and 0.1% aniline blue in 0.15 M K_2HPO_4 (pH 8.2) for 5 min, to visualize proteins, polysacharrhides and callose, respectively (Lumini et al., 2007; Ruzin, 1999). Sections were observed under a compound light microscope (Axio, Zeiss, Oberkochen, Germany) equipped with a CCD camera (AxioCam HR, Zeiss, Oberkochen, Germany), and Axio vision V4.5 acquisition software. For visualization of callose, the sections stained with aniline blue were observed using a fluorescence microscope equipped with the filter set 38HE (excitation filter, BP 470/40 (HE); interference beam splitter, FT 495 (HE); and emission filter, BP 525/50 (HE)).

Ultrathin sections (approximately 80 nm in thickness) were mounted on copper grids, and double-stained with 2% (wt/vol) uranyl acetate and with Reynolds' lead citrate for 7 min, respectively. Sections were examined under a transmission electron microscope (JEM-1010, JEOL Ltd., Tokyo) operating at an accelerating voltage of 80 kV. Digitalized images were recorded by means of the CCD

camera (ES1000W, Gatan Inc., Pleasanton, CA).

Results

Disease development in the susceptible and resistant pepper cultivars. Typical symptoms of Phytophthora blight appeared at 4 days after inoculation on the susceptible pepper cv. Bugang, and progressed with time after inoculation to reach maximum severity (all plants dead) 11 days after inoculation (data not shown). However, no disease was developed on the resistant pepper cv. CM334 up to the end of disease observation date (13 days after inoculation), showing a very significant difference (P=0.01) by Duncan's multiple range test between the two pepper cultivars.

Light microscopy of susceptible and resistant chili pepper roots infected with *P. capsici*. Although no distinct Phytophthora blight symptoms were viewed at one day after inoculation, light microscopy of the susceptible and resistant pepper roots revealed that the pathogen was successfully inoculated on both susceptible (cv. Bugang) and resistant pepper (cv. CM334) cultivars (Fig. 1). In the susceptible cultivar, epidermal cells including root hairs were penetrated by the oomycete hyphae (Fig. 1A, B), while in the resistant cultivar, the pathogen remained on the epidermal surface, showing the failure of penetration into the epidermal cells (Fig. 1C, D). In contrast to the susceptible pepper, middle lamellae underneath the root epidermis were densly stained, probably indicating cell wall encrusting in the resistant pepper. In addition root exudation materials were largely accumulated on the epidermal surface, in which the inoculated pathogen was embedded and appeared to be immobilized. The root exudation materials together with the middle lamellae underneath epidermal cells were positively stained blue with Coomassie brilliant blue (Fig. 1E) and reddish purple with Shiff's reagents (Fig. 1F), but not with aniline blue (data not shown), indicating their chemical components of proteins and carbohydrates, but of no callose.

At 3 days after inoculaiton when susceptible pepper leaves slightly wilted with superficial brownish lesions beginning to appear on the stem, but no symptoms appeared on resistant pepper leaves and stems, light microscopy of the roots also showed differences in pathogen's infectivity between the susceptible and resistant pepper cultivars (Fig. 2). In the susceptible pepper, the oomycete hyphae invaded and spread throughout the cortical and even into xylem tissue of the stele (Fig. 2A, B), while the oomycete hyphae were still confined in epidermal cells, showing no invasions into the cortical cells of the resistant pepper (Fig. 2C, D). New cell walls probably derived from cell divisions were formed across cortical cells just underneath the epidermal

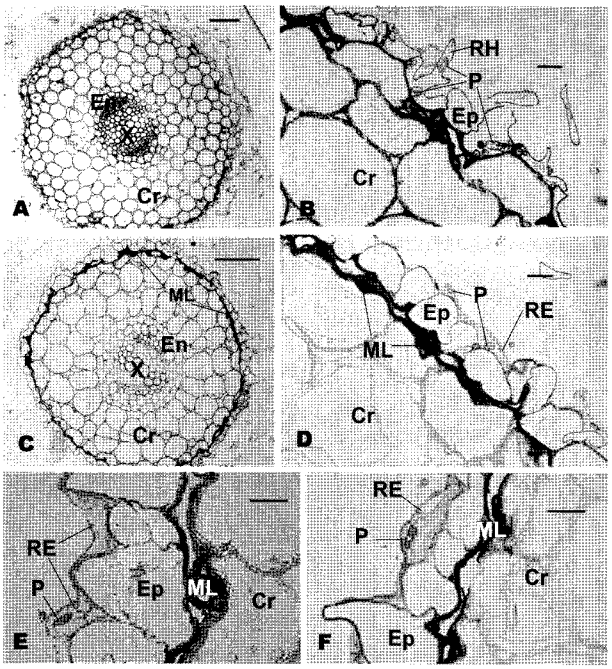

Fig. 1. Light micrographs of susceptible (A, B) and resistant (C-F) pepper roots inoculated with *Phytophthora capsici* at one day after inoculation, showing overviews of cross sections (A, C) and detailed views of the inoculation sites (B, D, E, F). The sections are stained with toluidine blue O (A-D), Coomassie brilliant blue (for staining proteins) (E) and Schiff's reagent (for staining polysaccharides) (F). Note in B that the pathogens (P) penetrated into epidermal cells (Ep) including root hairs (RH), but that no pathogen penetrations occurred in D-F, in which the pathogens (P) were embedded in root exudation materials (RE). Middle lamellae (ML) between epidermal (Ep) and cortical (Cr) cells were thickened and densely stained, showing same staining reactions as root exudation materials (RE). En: endodermis, X: xylem. Bars = 100 μm (A, C) and 10 μm (B, D-F).

cells adjacent to the pathogen, which were probably in the process of wound periderm formation.

Electron microscopy of susceptible and resistant chili pepper roots infected with *P. capsici*. In electron microscopy of the susceptible pepper roots at one day after inoculation, the oomycete hyphae penetrated into the epidermal cells through intercellular spaces, making a dissolution of middle lamella probably by cell wall lytic enzymes derived from the penetrating hyphae (Fig. 3A). Only a small amount of root exudation materials were deposited on the surface of the susceptible root epidermis, and also accumulated only on a part of the oomycete hyphae. On the other hand, the oomycete hyphae were completely embedded in root exudation materials which were thickly accumulated with an approximate maximum thickness of 3.0 μm on the resistant root surface (Fig. 3B). The middle lamella between epidermal and cortical cells was thickened with the accumulation of electron-dense

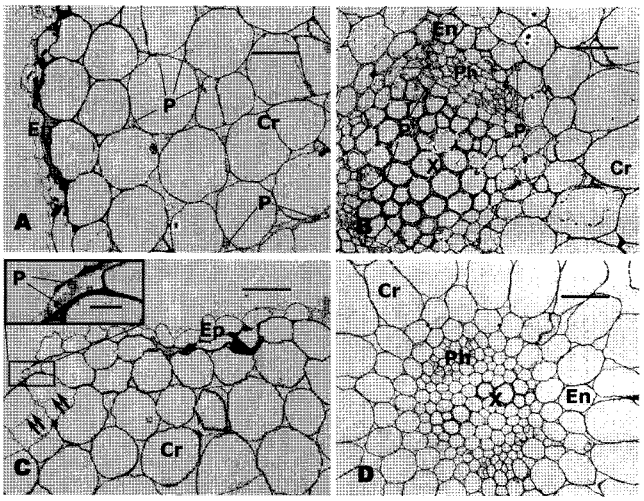

Fig. 2. Light micrographs of susceptible (A, B) and resistant (C, D) pepper roots inoculated with *Phytophthora capsici* at 3 days after inoculation, showing the pathogens (P) invading in cortical (Cr) and vascular tissues (xylem (X) and phloem (Ph)) in A and B, but remaining in epidermal cells (Ep) in C and inset of C (the rectangular area magnified), and no pathogen invading in the internal root tissues such as cortex (Cr), endodermis (En), phloem (Ph) and xylem (X) in D. Note new cell walls (double arrows) across cortical parenchyma cells underneath the infection site, formed by periclinal cell divisions (probably in the process of wound periderm formation). Bars=50 μm.

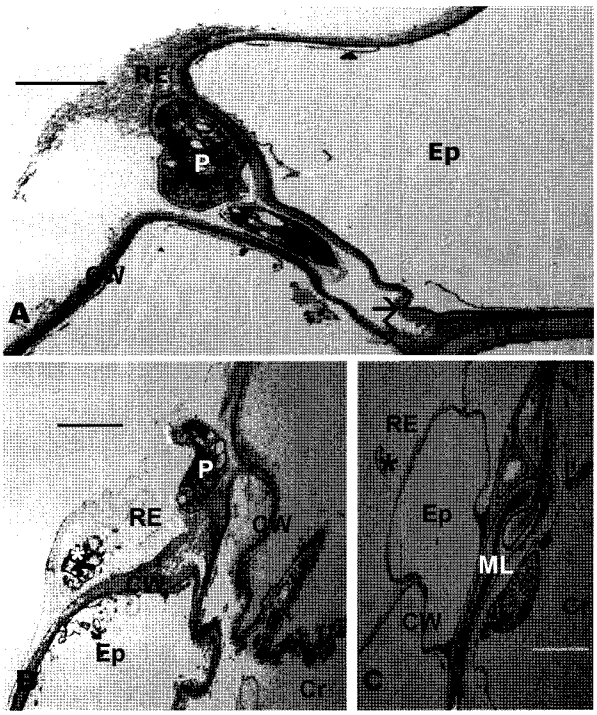

Fig. 3. Electron micrographs of susceptible (A) and resistant (B, C) pepper roots inoculated with *Phytophthora capsici* at one day after inoculation, showing the oomycete hyphae (P) penetrating epidermal cells (Ep) intercellularly by dissolving cell walls (CW) (arrows) schizogenously in A, but remaining on the surface of epidermal cells (Ep) embedded in root exudation materials (RE) in B and C. Note the dissolved oomycete hyphae (asterisks) and thickened middle lamella (ML) between epidermal (Ep) and cortical (Cr) cells in the resistant pepper. Bars=2 μm (A, B) and 5 μm (C).

materials and/or overlapping of bent cell walls (Fig. 3C), which showed structural features similar to the light microscopic view (Fig. 1D). No enzymatic dissolution of epidermal cell walls was indicated but a portion of the pathogen inoculated was almost completely dissolved within the root exudation materials.

Invasive hyphal growths were frequently observed in susceptible pepper root tissues at 3 days after inoculation (Fig. 4A, B). A haustorium-like hyphal cell was formed inside of the cortical parenchymal cell, of which the cytoplasm turned to be electron-dense, which was probably at the initial stage of the pathogen establishment (Fig. 4A). The root cortical cells were colonized by the oomycete hyphae that have grown inter- and intracellularly, showing somewhat cell wall loosening indicated by fibrilar-like cell wall matrix (Fig. 4B). In the resistant pepper roots at 3 days after inoculation, hyphal protrusions into cortical cells were also formed as an invasive growth of the pathogens from the infection sites (epidermal cells) (Fig. 4C, D). However, cell wall appositions were formed ahead of the hyphal protrusions, showing the restriction of the hyphal growth. The hyphal cells appeared to be damaged, indicated by disorganized appearances such as condensed cytoplasm, numerous small vacuoles formed from the central vacuole, and degenerated cell organelles such as nucleus containing granular-like nucleoplasm. Intrahyphal hyphae were frequently formed inside the degenerated and degenerating hyphal cells.

Light microscopy of susceptible and resistant chili pepper stems infected with *P. capsici.* No pepper stem tissues infected with the pathogen were observed by light microscopy at one day after inoculation, regardless of the susceptible and resistant pepper cultivars (data not shown). However, at 3 days after inoculation, the susceptible pepper stem tissues were invaded by the oomycete hyphae, colonizing all over cortical and even vascular tissues (Fig. 5A, B). In the resistant pepper, only epidermal cells and sometimes outer peripheral cortical cells were invaded by the oomycete hyphae at 3 days after inoculation, but not inner cortical or vascular cells (Fig. 5C, D). In case of the epidermal cell-limited pathogen invasion, cross cell walls were formed by periclinal cell divisions of cortial cells confronting the infection site, which was structurally similar to wound periderm formation processes.

Electron microscopy of susceptible and resistant chili

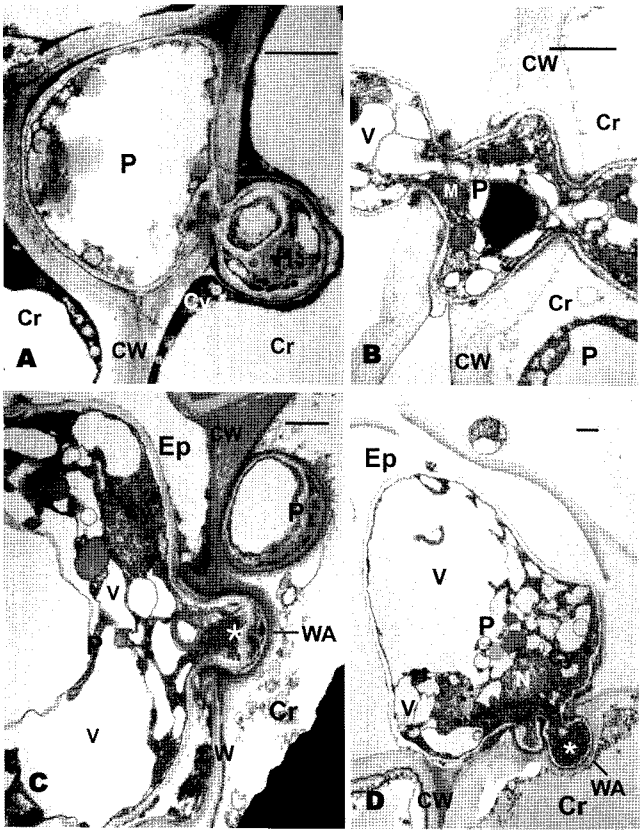

Fig. 4. Electron micrographs of susceptible (A, B) and resistant (C, D) pepper roots inoculated with *Phytophthora capsici* at 3 days after inoculation, showing the oomycete hyphae (P) invading cortical cells (Cr), forming haustorium-like hyphal cell (H) in A and invading through cortical cell walls (CW) in B, and forming hyphal protrusions (asterisks) bounded by cell wall apposition (WA) formed in cortical cells (Cr) in C and D. Hyphal cells bounded by cell wall appositions appear to be degenerated with disorganized cytoplasm (Cy), broken vacuoles (V) and degraded nucleus (N). Bars=1 μm.

pepper stems infected with *P. capsici.* No infected stem cells and tissues were found both in the susceptible and resistant pepper cultivars at one day after inoculation (data not shown). At 3 days after inoculation, however, pathogen invasions were found in cortical and vascular tissues of the susceptible pepper cv. Bugang (Fig. 6A, B), but only in epidermal cells of the resistant pepper cv. CM334 (Fig. 6C). No defense structures were formed in the susceptible pepper stem cells, but their cytoplasm with pathogen invasions in surrounding intercellular spaces were degenerated (Fig. 6A). In the resistant pepper, cell wall appositions were formed around the invading pathogen in the epidermal cells (Fig. 6D). The hyphal cells confronting with wall appositions appeared to be degenerated with condensed necrotic cytoplasm. Intrahyphal hyphae were frequently formed inside the degenerated and degenerating hyphae in the resistant pepper stem.

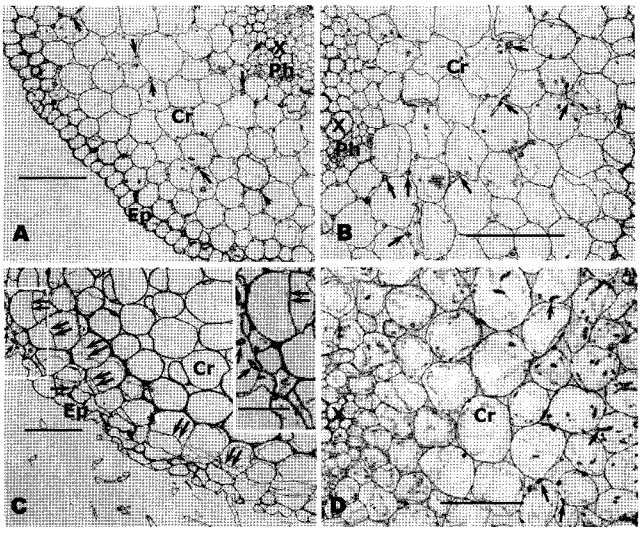

Fig. 5. Light micrographs of susceptible (A, B) and resistant (C, D) pepper stems inoculated with *Phytophthora capsici* at 3 days after inoculation, showing the pathogens (arrows) invading extensively in cortical (Cr) and vascular tissues such as xylem (X) and phloem (Ph) in A and B, but remaining in epidermal cells (Ep) in C and inset of C (the rectangular area magnified), and outer cortical cells (Cr) in D. No pathogen invasions occurred in inner cortical cells and vascular tissues such as xylem of the resistant pepper stem. Note new cell walls (double arrows) across cortical parenchyma cells underneath the epidermal cells, formed by periclinal cell divisions (probably in the process of wound periderm formation). Bars=100 μm.

Discussion

There was a very significant difference in the development of Phytophthora blight between the susceptible (cv. Bugang) and resistant (cv. CM334) pepper cultivars inoculated with zoospore suspension of *P. capsici*. All plants were dead by the pathogen infection in the susceptible cultivar, but at least superficially no indication of the disease development was observed in the resistant pepper cultivar up to the end of the symptom examination (13 days after inoculation). *C. annuum* cultivar 'Criollo de Morelos 334' (cv. CM334) is one of the efficient resistance sources against the most aggressive *P. capsici* isolates in pepper (Bonnet et al., 2007; Oelke et al., 2003; Palloix et al., 1988; Thabuis et al., 2004). This suggests that CM334 pepper should be a good model plant for the study of defense mechanisms against *P. capsici*.

When the pepper root and stem cells and tissues were viewed by light and electron microscopy, infections occurred in both cultivars; however, the degree of infection based on the structural features differed greatly between the two. The susceptible pepper roots and stems were extensively invaded by the pathogen into the vascular system, which may be related to the wilt symptom expression in this

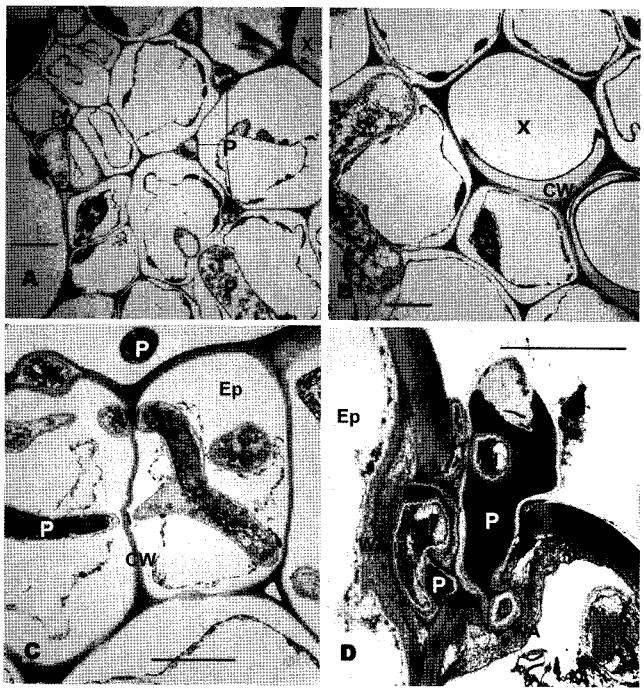

Fig. 6. Electron micrographs of susceptible (A, B) and resistant (C, D) pepper stems inoculated with *Phytophthora capsici* at 3 days after inoculation, showing the pathogen hyphae (P) invading vascular tissues such as phloem (Ph) and xylem (X) in A and B, but invading only epidermal cell (Ep) in C and D. Note cell wall appositions formed in epidermal cells confronting to the necrotized pathogen hyphae (P) containing intrahyphal hyphae (IH). Bars=5 μm.

pepper cultivar. Only outer root and stem tissues of the resistant pepper cultivar were colonized by the pathogen, indicating that infection was blocked by host defense mechanisms from the early infection stages. Host cell walls adjacent to and invaded by the infecting hyphae were partially dissolved and structurally loosened with fine fibrillar materials possibly by cell wall-degrading enzymes from the oomycete pathogen (Benhamou and Côté, 1992; Yoshikawa et al., 1977), which was more prominent in the susceptible pepper than in the resistant pepper. This suggests that the pathogen activity may be much higher in the susceptible than in the resistant pepper. Extrahaustorial matrix composed of electron-dense materials, which is associated with the penetration of host cell wall by oomycetes pathogens (Enkerli et al., 1997), was formed around the pathogen haustoria in both susceptible and resistant pepper roots infected with *P. capsici*, indicating its establishment as a parasite. However, the intrahyphal hyphae were formed much more frequently in the resistant than in the susceptible pepper (unpublished data). Intrahyphal hyphae are known to be induced in stressful conditions (Brown and Wyllie, 1970; Cromey and Cole, 1985; Lim et al., 1983; Shankar et al., 1998; Tommerup and Abbott, 1981), suggesting the pathogen may be situated at harsh microenvironmental conditions in the resistant pepper. The structural features of hyphal cells showed cellular degenerations with disorganized appearances of the condensed cytoplasm containing unintact organelles such as broken vacuoles and nucleus with granular-like nucleoplasm.

Zoospores of *P. capsici* are able to directly penetrate the intact cuticle of the plant epidermal surface within an hour (Hausbeck and Lamour, 2004). In our study, the pathogen penetrated readily into the root epidermal cells including root hairs of the susceptible pepper cultivar cv. Bugang. On the other hand, the pathogen mobilization for initial penetration might be inhibited in the resistant pepper as it was embedded in root exudation materials that were thickly accumulated on the root surface and stained by Coomassie brilliant blue and Schiff's reagent, indicating their components to be proteineous materials and polysacharrides, respectively. There are a wide variety of compounds including amino acids, sugars, proteins, glycosides, etc. in plant extracts and exudates released from seeds and seedlings that have antimicrobial properties and inhibit the growth of root pathogens (Casey et al., 1998; Kraft and Boge, 1996; Nóbrega et al., 2005; Okubara and Paulitz, 2005; Schroth and Hildebrand, 1964; Terras et al., 1995). With the exudation of a wide variety of compounds, roots may regulate the soil microbial community in their immediate vicinity (Walker et al., 2003). Similarly in our study, the Phytophthora blight pathogen might be inhibited in its penetration into epidermal cells of the resistant pepper cv. CM334 not only by physical immobilization captured in the root exudation materials but also by chemical influences as indicated by the dissolution of the oomycete hyphae in the resistant pepper (Fig. 2B, C).

Thickening of the middle lamellae between epidermal and cortical cells was previously unknown structural feature in relation to plant resistance. Like root exudation materials, the thickened middle lamella may serve as a defense boundary against the initial pathogen infection, inhibiting the penetration from the epidermis into the cortex. As the middle lamellae were more densely stained by Coomassie briliant blue and Schiff's reagent than the root exudation materials (Fig. 1E, F), they may be formed by the deposition of materials like root exudates. Conversely, this suggests that the root exudation materials may be derived from the secretion of the materials in the thickened middle lamellae. Besides the material deposition in the thickened middle lamellae, they were constituted with overlapped cell walls in some portions, which may provide the more strengthened boundary against the pathogen infection.

One of the most prominent structural features found in the resistant pepper roots and stems was the formation of

cell wall appositions bounding on the hyphal protrusions at 3 days after inoculation. The oomycete hyphae adjacent to wall appositions were degenerated, showing destructed cytoplasmic contents including numerous vacuoles and degraded nuclei, suggesting the failure of the pathogen infection enough to produce disease symptoms in the resistant pepper roots and stems. Wall apposition formation is proved as one of the prevalent host reactions against the infection of *Phytophthora* spp. on tomato and pepper (Hwang et al., 1989; Hwang et al., 1994; Lee et al., 2000; Lee et al., 2001). In our study, wall appositions were mostly formed at the beginning of intracellular hyphal growth of the pathogen into the outmost cortical cells, restricitng its invasion from the epidermal cells.

New cell walls in cortical cells underneath the infection sites (epidermal cells infected by the pathogen) were formed by cell divisions only in the resistant pepper but not in the susceptible pepper 3 days after inoculation, which is structurally identical to those formed in ginseng roots inoculated with an avirulent isolate of *Cylindrocarpon destructans* that are suggested to be in the process of wound periderm formation (Kim et al., 2009). Wound periderm was reported to be formed as a histological defense structure by wounding and/or pathogen invasion (Biggs and Britton, 1988; Jeon and Kim, 2008; Kim et al., 2004, 2008, 2009). Cell walls formed by cell divisions have a function as a mechanical barrier to the pathogen infection.

The pathogen invasion was restricted in the epidermal cells of the resistant pepper roots until 3 days after inoculation, while in the susceptible pepper, the hyphal colonization occurred in the cortical cells, reaching to xylem tissues of the stele. Pathogen invasion in the susceptible stem tissues was also as extensive as in the root tissues; however, the pathogen invaded into cortical cells of the resistant pepper stem tissues, even though its spread was limited to ourter peripheral regions of the cortex. The difference in the suppression of disease development in the resistant pepper stems from the roots may be due to the absence of root exudation materials in the stems. Also this suggests the resistant responses in the resistant pepper are more discriminative in roots than in above-ground plant parts. Therefore, the soil-drenching inoculation method that may limit infection sites to underground plant parts (roots) may be more reliable than the stem-inoculation method for evaluation of the disease resistance as suggested by Kim et al. (1989).

The structural features of the oomycete hyphae in the susceptible pepper cv. Bugang differed from those in the resistant pepper cv. CM334. Although intrahyphal hyphae were formed in both pepper cultivars, their frequencies were much high in the reistant pepper. As their formation is known to be connected with aged stages of endophyte and host cells (Chan and Stephen, 1967; Lim et al., 1983), the penetrating hyphae in the resistant pepper of our study might be exposed to unfavorable conditions derived from host defense responses such as wall apposition formation.

In conclusion, all of these aspects suggest that limitation of disease development in the resistant pepper may be due to root exudates and mechanical barriers such as wall apposition and wound periderm that inhibit pathogen penetration, infection, invasion and colonization.

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