Plant Pathol. J. 17(1) : 29-35 (2001)

The Plant Pathology Journal

Ultrastructure of Compatible and Incompatible Interactions of Pumpkin Stems Infected with *Phytophthora capsici*

Byung Kook Lee, Jeum Kyu Hong and Byung Kook Hwang*

Molecular Plant Pathology Laboratory, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea

(Received on December 23, 2000)

Early infection process of *Phytophthora capsici* in pumpkin stems was similar in the compatible and incompatible interactions 24 h after inoculation. Intercellularly growing hyphae penetrated host parenchyma cells by forming haustoria. An extrahaustorial matrix was found around the haustoria in both compatible and incompatible interactions. No wall appositions were observed at the infection sites in the parenchyma cells. In the compatible interaction, infecting hyphae grew well in the intercellular spaces between xylem vessels in stem tissues. Degraded host cell wall, plasmolysis of plasma membrane, and degenerated chloroplasts were pathological features of pumpkin stem tissues in both compatible and incompatible interactions. A characteristic host response in the resistant pumkin cultivar Danmatmaetdol was rapid cytoplasmic movement of host cells toward the oomycete haustoria.

Keywords **:** ultrastructure, pumpkin, *Phytophthora capsici*, compatible and incompatible interactions.

Phytophthora capsici Leonian is pathogenic on solanaceous and cucurbit plants, including pumpkin (*Cucurbita maxima* Duchesne) (Hwang and Kim, 1995; Lee et al., 2001). The soilborne oomycete pathogen has been known as a limiting factor for the production of pumpkin and pepper (Cho et al., 1997; Hwang and Kim, 1995; Lee et al., 2000). In our previous studies, isolates of *P. capsici* were found to cause stem rot, root rot, and foliar blight in tomato and pepper (Hwang and Hwang, 1993; Kim and Hwang, 1989; Kim and Hwang, 1992). In particular, differential interactions of *P. capsici* with the host plants were extensively examined at the genetical, cellular, biochemical and molecular aspects (Hwang et al., 1994; Hwang et al., 1996; Kim and Hwang, 1994; Kim and Hwang, 2000; Lee et al., 2000). Recently, we have examined the variation in virulence of *P. capsici* isolates from pumpkin and pepper plants and relative susceptiblity of pumpkin cultivars to *P. capsici* infection (Lee et al., 2001). Differential interactions of *P. capsici* isolates with pumpkin cultivars Hukpidan and Danmatmaetdol, which were susceptible and resistant to *P. capsici* infection, respectively, were precisely evaluated using different inoculation techniques. The differences in responses of pumpkin to *P. capsici* between the susceptible and resistant cultivars were quantitative rather than qualitative. The symptoms caused by various isolates of *P. capsici* developed more slowly on the resistant cultivar Danmatmaetdol than on the susceptible cultivar Hukpidan.

Several histological and ultrastructural studies have been done on the interaction of *P. capsici* with pepper plants (Hwang et al., 1989; Hwang et al., 1994; Jones et al., 1974; Jones et al., 1975; Kim and Hwang, 1989; Lee et al., 2000). Light microscopic examination of pepper stems infected with *P. capsici* revealed that hyphae colonized cortical parenchyma tissues and vascular bundles, followed by partial or complete disintegration of these tissues with intense, dark-brown color (Kim and Hwang, 1989). In our previous studies, reduced hyphal growth and sporangial formation were found in incompatible interactions after *P. capsici* infection on pepper stems (Lee et al., 2000). A main host response in the incompatible interaction also was the occlusion of cortical cells with amorphous materials. Plugging of the intercellular spaces in the cortex with electron-opaque materials was frequently observed in the incompatible interaction. Another common feature in the incompatible interaction was degeneration of mitochondria in the penetrating oomycete cytoplasm. However, there is little information about the fine structures of pumpkin-*P. capsici* interactions.

The research reported herein was conducted to examine the ultrastructural changes of pumpkin stem tissues infected with *P. capsici* in compatible and incompatible interactions.

Materials and Methods

Plant, pathogen, and inoculum. Two pumpkin (*Cucurbita maxima* Duchesne) cultivars, Danmatmaetdol and Hukpidan, used in this study were resistant and susceptible to the infection by isolate P98131 of *Phytophthora capsici*, respectively. Seeds of each cultivar were sown in plastic pots (5 × 15 × 10 cm) containing steam-

*Corresponding author.
Phone) +82-2-3290-3061, FAX) +82-2-925-1970
E-mail) bkhwang@korea.ac.kr

sterilized soil mix of compost soil, loam soil, and sand (1 : 2 : 1, v/v/v). Pumpkin plants were raised in the growth room at 25 ± 2°C under 16-h day illumination.

Isolate P98131 of *P. capsici*, which was isolated from a pumpkin plant, was grown on oatmeal agar at 28°C for 7 to 10 days and then incubated under fluorescent light at 28°C for 2 days to induce sporangial formation. Sterilized tap water was poured into the plates to harvest sporangia. The pathogens were kept at 4°C for 2 h, and then incubated at room temperature to release zoospores. Zoospore suspension was adjusted to 3×10^6 zoospores/ml with sterilized water.

The cultivars Danmatmaetdol and Hukpidan at the two-leaf stage were inoculated with *P. capsici* isolate P98131 by stem-wound method. The 1-cm longitudinal slits were made in the stems of pumpkin plants. Sterile cotton soaked with zoospore suspension (3×10^6 zoospores/ml) was placed on the stem slits. Inoculation sites were covered with plastic tape to maintain moist for 24 h.

Electron microscopy. Pumpkin stem tissues from the inoculated sites were cut into small pieces (5 mm in length) 24 h after inoculation of *P. capsici*. Tissue samples were fixed in a solution of 1% paraformaldehyde, 0.025% glutaraldehyde and 0.01 M phosphate

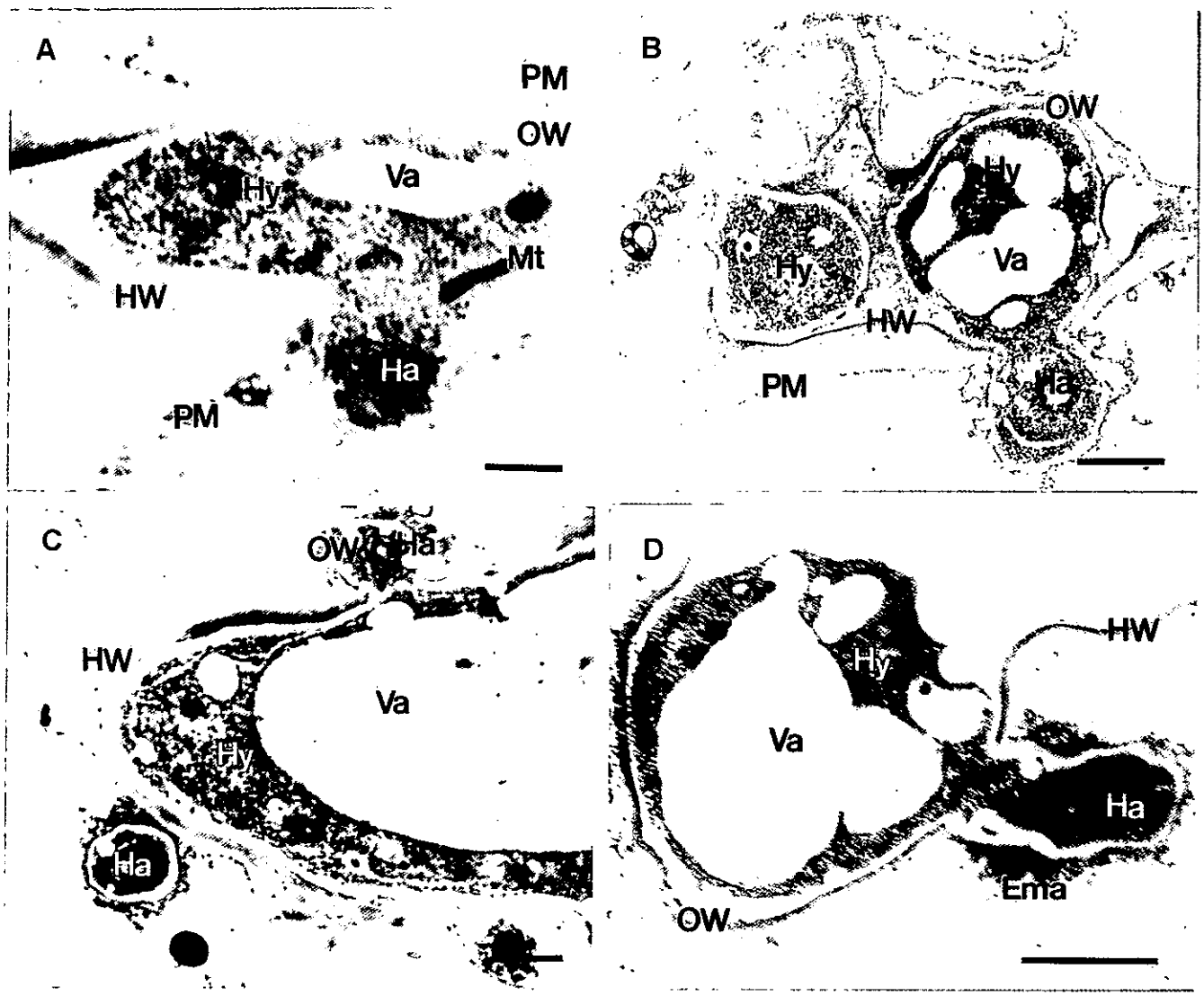

Fig. 1. Electron micrographs of oomycete hyphae penetrating parenchyma cells of pumpkin stem tissues of susceptible cultivar Hukpidan 24 h after inoculation with the isolate P98131 of *Phytophthora capsici*. (**A**) Host cell wall is perforated by haustorium and plasma membranes of infected and adjacent cells are separated from host cell walls. (**B**) A vacuolated hypha present in the intercellular space passes through the host cell wall. Plasma membrane of host cell is separated from host cell wall. (**C**) Penetration of host cell by haustoria from a highly vacuolated hypha. The haustorium is surrounded by an extrahaustorial matrix. (**D**) Interface of host cell with oomycete hypha. A well developed extrahaustorial matrix is present around the haustorium. Ema = extrahaustorial matrix; Ha = haustorium; HW = host cell wall; Hy = hypha; Mt = mitochondrium; OW = oomycete cell wall; PM = plasma membrane; Va = vacuole. Bars = 1 μm.

buffered saline (PBS, pH 7.2) for 10 h at 10°C, and then washed with the same buffer for 5 h at 10°C. The stem pieces were dehydrated in a graded series of ethanol, and embedded in a graded series of LR White resin. Polymerization was done under UV light (360 nm) for 48 h at −20°C and then for 48 h at room temperature.

Ultrathin sections from the trimmed pumpkin tissue blocks were made with a Diatome diamond knife on an ultramicrotome (LKB 2088 ultratome® V, Sweden). These sections were mounted on pioloform coated copper grids (100 meshes), and stained with uranyl acetate and Reynolds lead citrate for 10 min each. The stained sections were examined under a JEOL 1200 EX transmission electron microscope operated at 80 kV.

Results

Infection of pumpkin stems of susceptible (Hukpidan) and resistant (Danmatmaetdol) cultivars by *P. capsici* isolate P98131 resulted in the compatible and incompatible interactions, respectively. Infected stem tissues of both cultivars at the 2-leaf stage became severely compressed and collapsed 48 h after inoculation. However, rapid symptom development was found in the stems of the susceptible cultivar. To precisely examine early infection process of *P. capsici* within the stem tissues 24 h after inoculation, the stem tissues of the two cultivars were harvested from the

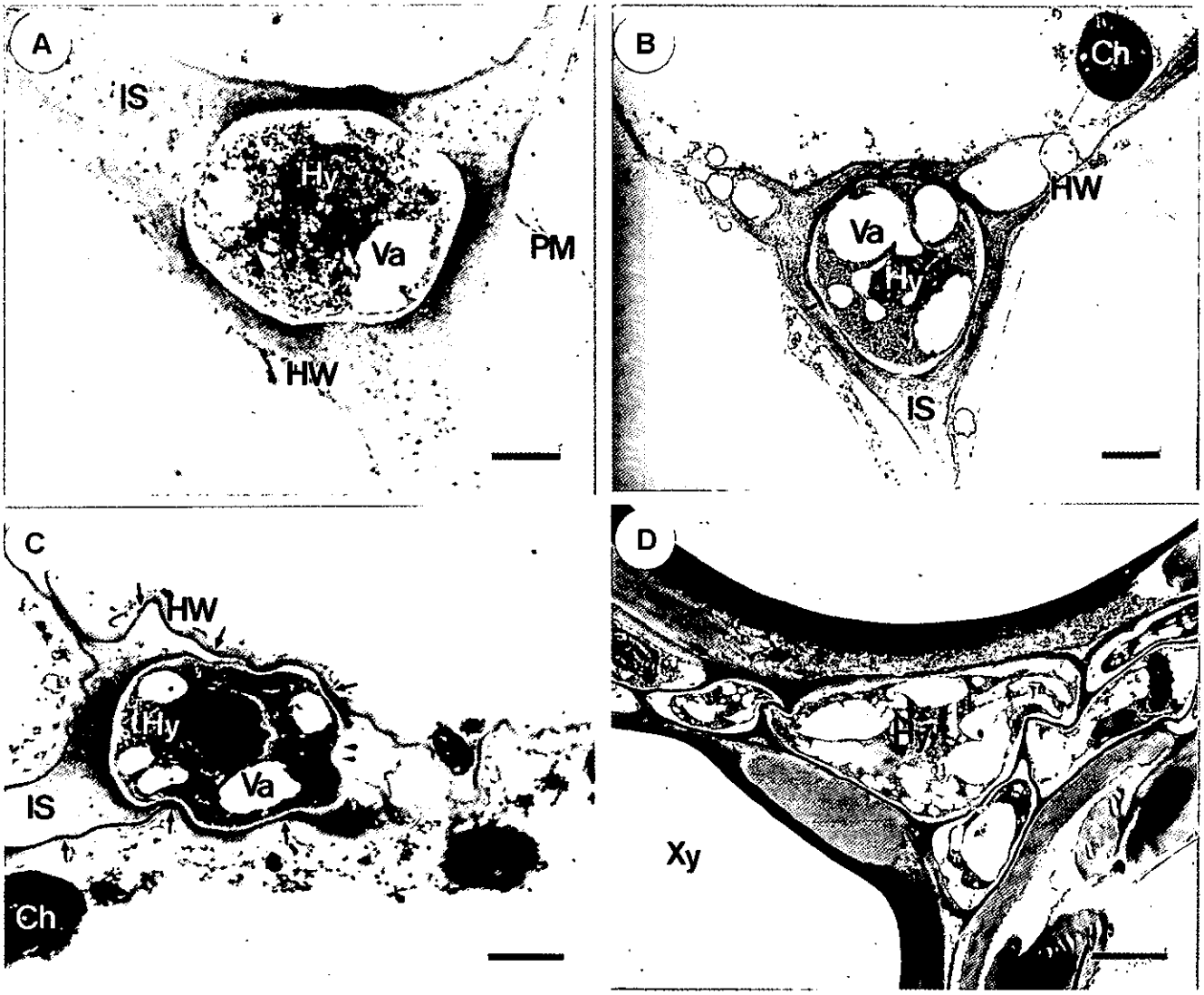

Fig. 2. Electron micrographs of oomycete cells in the intercellular spaces of pumpkin stem tissues of susceptible cultivar Hukpidan 24 h after inoculation with the isolate P98131 of *Phytophthora capsici*. (**A**) A hypha in the intercellular space of host cell. Intercellular space also is filled with electrondense materials. (**B**) A vacuolated hypha in the intercellular space with a thin host wall. Note a degenerated chloroplast in host cell. (**C**) Irregular and disintegrated host cell wall intimately contacted with the intercellular hypha (arrowheads). Some host wall are disrupted (arrows). Distorted chloroplasts are seen. (**D**) Several oomycete cells in the intercellular space between xylem vessels. Ch = chloroplast; HW = host cell wall; Hy = hypha; IS = intercellular space; Va = vacuole; Xy = xylem vessel. Bars = 1 μm.

inoculated sites, and then observed under a transmission electron microscope.

Ultrastructure of oomycete hyphae and infection sites in compatible interactions. At 24 h after inoculation, hyphae of *P. capsici* were frequently found in intercellular spaces of parenchyma cells of pumpkin stem tissues. Ultrastructural features of transverse sections of stem tissues of susceptible pumpkin (cv. Hukpidan) infected with the isolate P98131 of *P. capsici* are shown in Fig. 1. In the stem tissues of the susceptible cultivar, oomycete hyphae grew through the intercellular spaces, and developed haustoria to penetrate into host parenchyma cells. Plasma membranes of infected host cells were plasmolyzed and disorganized, which was characteristic of compatible interactions with *P. capsici* (Fig. 1A and 1B). Several haustoria were developed from highly vacuolated hyphae in contact with host cell walls (Fig. 1C). The penetrating hyphae were encased by thin layered extrahaustorial matrix, possibly containing cytoplasmic materials. However, host cell organelles were not observed in infected parenchyma cells (Fig. 1D).

Electron micrographs of oomycete cells in the intercellular spaces of stem tissues of susceptible pumpkin (cv. Hukpidan) are presented in Fig. 2. Intercellular spaces occupied by the hyphae were filled with amorphous materials (Fig.

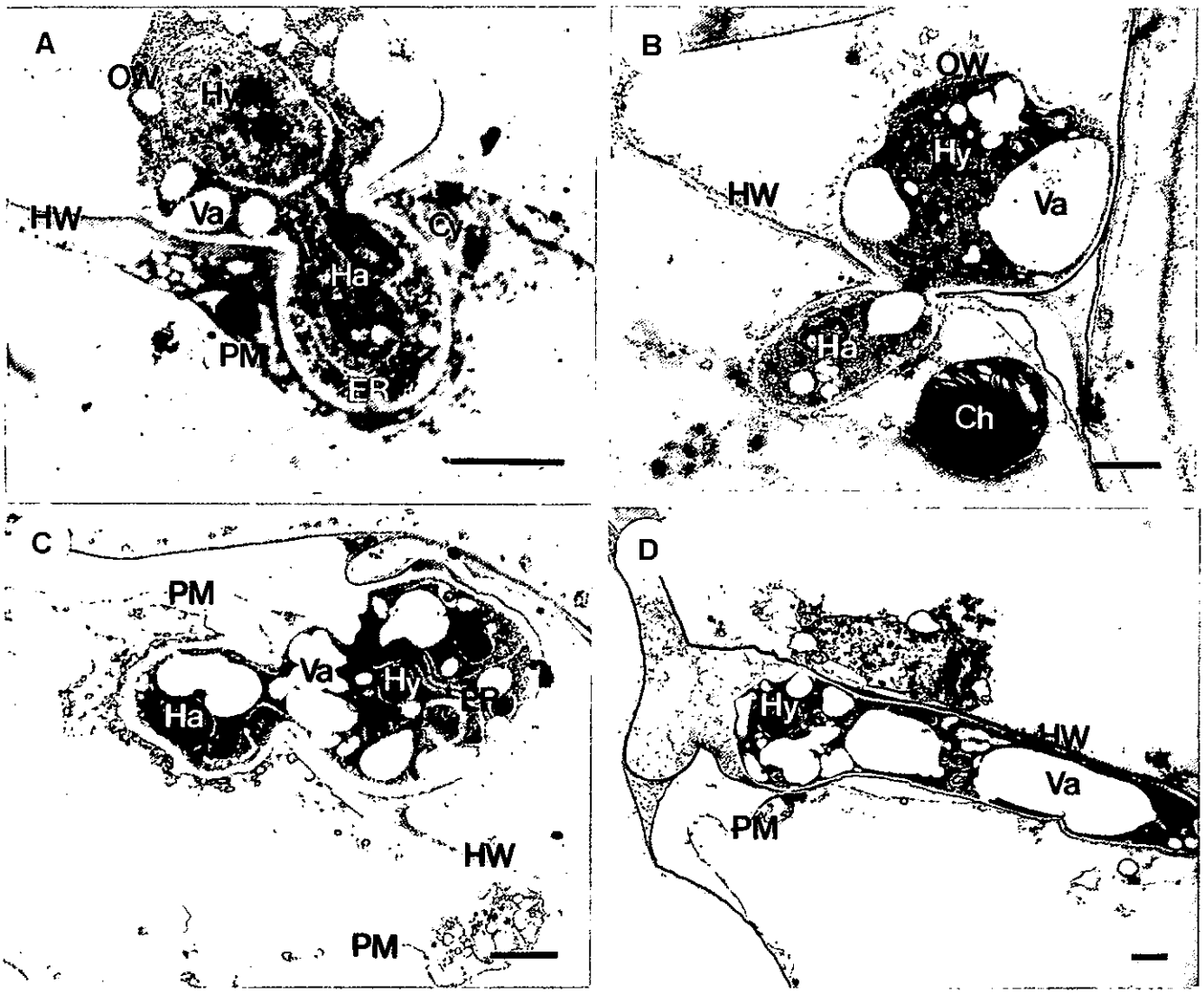

Fig. 3. Electron micrographs of oomycete hyphae penetrating parenchyma cells of pumpkin stem tissues of resistant cultivar Danmatmaetdol 24 h after inoculation with the isolate P98131 of *Phytophthora capsici*. (**A**) Host cytoplasmic materials aggregated around the penetrating haustorium. (**B**) Disintegration of host cell wall perforated by haustorium. (**C**) A haustorium surrounded by host plasma membrane with small vesicles. (**D**) Host cytoplasmic materials near the hypha in the intercellular space filled with electron-dense materials. Ch = chloroplast; Cy = cytoplasm; ER = endoplasmic reticulum; Ha = haustorium; HW = host cell wall; Hy = hypha; OW = oomycete cell wall; PM = plasma membrane; Va = vacuole. Bars = 1 μm.

2A). Host cell wall in contact with intercellular hyphae became irregular, partially dissolved and disintegrated (Fig. 2B). Chloroplasts within the host cells were distorted, and their membrane structures were disorganized (Fig. 2C). Infecting hyphae grew in the intercellular space between xylem vessels of the stem tissues. No distinct host responses such as wall appositions were found in the compatible interaction.

Ultrastructure of oomycete hyphae and infection sites in incompatible interactions. In the incompatible interactions, ultrastructural changes in oomycete hyphae and infection sites were found 24 h after inoculation with *P. capsici* (Fig. 3). Well developed oomycete hyphae were readily observed in parenchyma cells of stem tissues. Most hyphae penetrating host cells had numerous small or large vacuoles (Fig. 3A). A penetrating hypha formed a haustorium in contact with electron-dense materials. The host cell well invaded by the oomycete haustorium was partially degraded or thinned (Fig. 3B). A large, swollen chloroplast was present near the invading haustorium. Small vesicles aggregated around the host plasma membranes encircling an infecting hypha (Fig. 3C). Proliferous, vacuolated hypha grew in the intercellular space of host cell (Fig. 3D). Electron-dense and amorphous cytoplasmic materials were

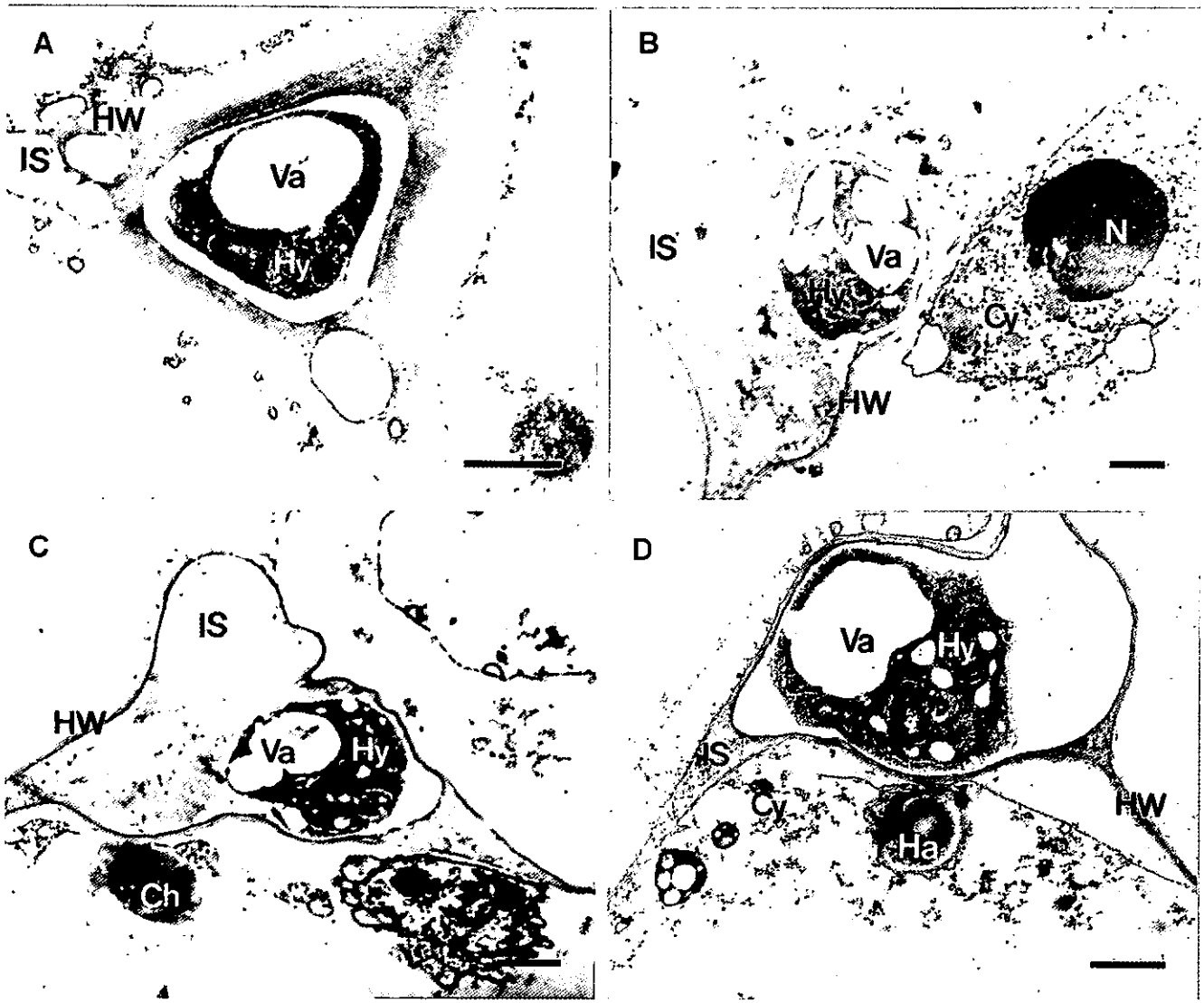

Fig. 4. Electron micrographs of oomycete cells in the intercellular spaces of pumpkin stem tissues of resistant cultivar Danmatmaetdol 24 h after inoculation with the isolate P98131 of *Phytophthora capsici*. (**A**) A hypha in the intercellular space. (**B**) Accumulation of host cytoplasmic material at the hyphal contact site. (**C**) An intercellular hypha in irregular and indistinct structures. Note the plasmolysis of plasma membrane in infected host cells. (**D**) Haustorium from the intercellular hypha is surrounded by the extrahaustorial matrix, and dense host cytoplasm accumulates around the haustorium. Ch = chloroplast; Cy = cytoplasm; Ha = haustorium; Hy = hypha; HW = host cell wall; IS = intercellular space; N = nucleus; Va = vacuole. Bars = 1 μm.

observed below host cell wall.

Electron micrographs of infected intercellular spaces of stem tissues in the incompatible interaction are illustrated in Figure 4. Some vacuolated and distorted hyphae were shown to grow compactly in the intercellular spaces surrounded by the host cell walls (Fig. 4A). The consistent feature of parenchyma cells of infected stems was plasmolysis of plasma membranes in infected and adjacent uninvaded cells (Fig. 4B, C, and D). Host cytoplasmic material with a nucleus aggregated at the contact site of oomycete hypha (Fig. 4B). An intercellular hypha in irregular and indistinct structures was observed in the plasmolyzed host cells (Fig. 4C). The invading haustorium, which was surrounded by the extrahaustorial matrix, was in intimate contact with host cytoplasmic materials (Fig. 4D). In general, the parenchyma cell walls were thinned.

Discussion

In our earlier study, the pumpkin cultivars Hukpidan and Danmatmaetdol have been demonstrated to respond differently to *P. capsici* infection (Lee et al., 2001). The symptoms caused by various isolates of *P. capsici* developed more slowly on the resistant cultivar Danmatmaetdol than on the susceptible cultivar Hukpidan. These findings led to the precise examination of whether or not ultrastructural changes differed between the compatible and incompatible interactions of pumpkin with *P. capsici*.

At 24 h after inoculation of pumpkin with the isolate P98131 of *P. capsici*, early infection process of the oomycete pathogen was similar in the compatible and incompatible interactions in this study. Intercellularly growing hyphae of *P. capsici* penetrated host parenchyma cells by forming haustoria. Formation of haustoria appeared to be essential and initial steps for hyphal growth of *P. capsici* in pumpkin cells, as previously observed in *P. capsici* -pepper interactions (Hwang et al., 1994; Jones et al., 1975; Kim et al., 1989; Lee et al., 2000). An extrahaustorial matrix, which consists of electron-dense materials associated with the host wall penetration of the oomycete pathogens (Enkerli et al., 1997; Coffey and Wilson, 1983), was found around the haustoria of *P. capsici* in both compatible and incompatible interactions. Formation of wall apposition has been demonstrated as one of the prevalent host reactions to *Phytophthora* spp. infection on host plants such as potato, acacia, soybean, tomato, and pepper (Hohl and Suter, 1976; Hwang et al., 1994; Lee et al., 2000; Miller and Maxwell, 1984; Tippet and Malajczuk, 1979). However, no wall apposition was shown at the infection sites in parenchyma cells of pumpkin stem tissues 24 h after inoculation. Although the timing of wall apposition formation may vary among host species, wall apposition toward the haustoria was not likely to be important in either compatible or incompatible interations of pumpkin to *P. capsici* infection. In contrast to our results, the incompatible interaction of tomato cells with *P. capsici* has been shown to be characterized by formation of wall apposition in the cortical parenchyma cells (Hwang et al., 1994). In the compatible interactions, infecting hyphae grew in the intercellular spaces between the xylem vessels of stem tissues, which was a unique feature not observed in the incompatible response to *P. capsici* infection.

In the incompatible interaction, ultrastructures in the penetrating oomycete hyphae were similar to those in compatible interactions. However, there were striking differences in the response of host cells 24 h after inoculation. Degraded host cell wall, plasmolysis of plasma membrane, and degenerated chloroplasts were pathological features of pumpkin stem tissues in both compatible and incompatible interactions. Interfaces of hyphae and host cell walls were palely stained due to the partial degradation, possibly by cell wall-degrading enzymes from the oomycete pathogen (Benhamou and Côté, 1992; Yoshikawa et al., 1977). However, these phenomena in the infected stem tissues were more pronounced in the compatible than in the incompatible interaction.

The infection process of oomycete hyphae in the compatible and incompatible interactions was similar to each other, but cellular responses of hosts and hyphae in resistant pumpkin tissues were quite different from those in susceptible ones. The invasion of *P. capsici* in the intercellular spaces and middle lamella was reduced in resistant stems. Colonization of vascular tissues by the infecting hyphae was rare in the resistant stems, as compared to the susceptible ones. One of the major characteristics in the resistant cultivar also was a rapid cytoplasmic movement of host cells toward the hausoria (Fig. 4). With the rapid cytoplasmic movement of infected cells in the incompatible interaction, swollen nuclei were found in the dense cytoplasm of the host cells adjacent to the oomycete pathogen. Cytoplasmic or nuclear movement was found in rust-infected cowpea leaf tissues (Heath, 1997; Heath et al., 1997; Skalamera et al., 1997; Skalamera and Heath, 1998). In our earlier study, different ultrastructural changes in mitochondria of *P. capsici* have been demonstrated in compatible and incompatible interactions with pepper stems (Lee et al., 2000). Taken together, the results in the present study suggested differential host responses and pathogen behavior in the compatible and incompatible interactions of *P. capsici* with pumpkin stem tissues.

Acknowledgement

We thank the National Instrumentation Center for Environ-

mental Management (NICEM), Suweon, for kindly providing facilities for the electron microscopy.

References

Benhamou, N. and Côté, F. 1992. Ultrastructure and cytochemistry of pectin and cellulose degradation in tobacco roots infected by *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 82:468-478.

Cho, Y. D., Kang, S. G. and Chung, J. H. 1997. Cultivars and sowing date for summer season production of sweet pumpkin in Cheju province. *RDA. J. Hort. Sci.* 39:33-38.

Coffey, M. D. and Wilson, U. E. 1983. An ultrastructural study of the late-blight fungus *Phytophthora infestans* and its interaction with the foliage of two potato cultivars possessing different levels of general (field) resistance. *Can. J. Bot.* 61:2669-2685.

Enkerli, K., Hahn, M. G. and Mims, C. W. 1997. Immunogold localization of callose and other plant cell wall components in soybean roots infected with oomycete *Phytophthora sojae*. *Can. J. Bot.* 75:1509-1517.

Heath, M. C. 1997. Signalling between pathogenic rust fungi and resistant or susceptible host plants. *Ann. Bot.* 80:713-720.

Heath, M. C., Nimchuk, L. N. and Xu, H. 1997. Plant nuclear migrations as indicators of critical interactions between resistant or susceptible cowpea epidermal cells and invasion hyphae of the cowpea rust fungus. *New Phytol.* 135:689-700.

Hohl, H. R. and Suter, E. 1976. Host-parasite interfaces in a resistant and a susceptible cultivar of *Solanum tuberosum* inoculated with *Phytophthora infestans*: leaf tissue. *Can. J. Bot.* 54: 1956-1970.

Hwang, B. K. and Kim, C. H. 1995. Phytophthora blight of pepper and its control in Korea. *Plant Dis.* 79:221-227.

Hwang, B. K., Kim, W. B. and Kim, W. K. 1989. Ultrastructure at the host-parasite interface of *Phytophthora capsici* in roots and stems of *Capsicum annuum*. *J. Phytopathol.* 127:305-315.

Hwang, B. K., Kim, Y. J. and Kim, C. H. 1996. Differential interactions of *Phytophthora capsici* isolates with pepper genotypes at various growth stages. *Eur. J. Plant Pathol.* 102:311-316.

Hwang, J. S. and Hwang, B. K. 1993. Quantitative evaluation of resistance of Korean tomato cultivars to isolates of *Phytophthora capsici* from different geographic areas. *Plant Dis.* 77:1256-1259.

Hwang, J. S., Hwang, B. K. and Kim, W. K. 1994. A light and electron microscopical study of compatible and incompatible interactions between *Phytophthora capsici* and tomato (*Lycopersicon esculentum*). *Korean J. Plant Pathol.* 10:83-91.

Jones, D. R., Graham, W. G. and Ward, E. W. B. 1974. Ultrastructural changes in pepper cells in a compatible interaction with *Phytophthora capsici*. *Phytopathology* 64:1084-1090.

Jones, D. R., Graham, W. G. and Ward, E. W. B. 1975. Ultrastructural changes in pepper cells in a compatible interaction with *Phytophthora infestans*. *Phytopathology* 65:1274-1285.

Kim, E. S. and Hwang, B. K. 1992. Virulence to Korean pepper cultivars of isolates of *Phytophthora capsici* from different geographic areas. *Plant Dis.* 76:486-489.

Kim, Y. J. and Hwang, B. K. 1994. Differential accumulation of β-1,3-glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 45:195-205.

Kim, Y. J. and Hwang, B. K. 2000. Pepper gene encoding a basic pathogenesis-related 1 protein is pathogen and ethylene inducible. *Physiol. Plantarum* 108:51-60.

Kim, Y. J., Hwang, B. K. and Park, K. W. 1989. Expression of age-related resistance in pepper plants infected with *Phytophthora capsici*. *Plant Dis.* 73:745-747.

Kim, W. B. and Hwang, B. K. 1989. Histological changes in the roots and stems of pepper plants infected with *Phytophthora capsici*. *Korean J. Plant Pathol.* 5:40-48.

Lee, B. K. Kim, B. S., Chang, S. W. and Hwang, B. K. 2001. Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. *Plant Dis.* 85:497-500.

Lee, Y. K., Hippe-Sanwald, S. and Hwang, B. K. 2000. Histological and ultrastructural comparisons of compatible, incompatible and DL-β-amino-n-butyric acid-induced resistance responses of pepper stems to *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 57:269-280.

Miller, S. A. and Maxwell, D. P. 1984. Ultrastructure of susceptible, host resistant, and nonhost interactions of alfalfa with *Phytophthora megasperma*. *Can. J. Bot.* 62:117-128.

Skalamera, D., Tibodh, S. and Heath, M. C. 1997. Callose deposition during the infection between cowpea (*Vigna unguiculata*) and the monokaryotic stage of the cowpea rust fungus (*Uromyces vignae*). *New Phytol.* 136:511-524.

Skalamera, D. and Heath, M. C. 1998. Changes in the cytoskeleton accompanying infection-induced nuclear movements and the hypersensitive response in plant cells invaded by rust fungi. *Plant J.* 16:191-200.

Tippett, J. and Malajczuk, N. 1979. Interaction of *Phytophthora cinnamomi* and a resistant host *Acacia pulchella*. *Phytopathology* 69:764-772.

Yoshikawa, M., Tsukadaira, T., Masago, H. and Minoura, S. 1977. A non-pectolytic protein from *Phytophthora capsici* that macerates plant tissues. *Physiol. Plant Pathol.* 11:61-70.