

Mini-Review

Cytology, Physiology and Molecular Genetics of Resistance to Phytophthora Blight in Pepper Plants

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Phytophthora blight of pepper, which is incited by *Phytophthora capsici* Leonian, is one of the most devastating soil-borne diseases of pepper in Korea (Hwang and Kim, 1995). Pepper plants can be infected by *P. capsici* in all stages of growth when environmental conditions are favorable. Phytophthora blight depends on soil as a source of initial inoculum. The repeated cultivation of pepper results in a build-up of inoculum in the soil. Disease is favored by prolonged periods of heavy rainfall accompanied by high winds from June to August in Korea. Stems of pepper plants are readily damaged by splitting, breaking, and lodging, thus accelerating development of Phytophthora blight.

The lack of effective measures to reduce soilborne inoculum of *P. capsici* helps explain why epidemics of Phytophthora blight occur frequently in pepper-growing areas of Korea. The severity of disease on peppers in the epidemic years should be due to the continuous planting of peppers in fields with a high inoculum of *P. capsici*. Consequently, the effective management of Phytophthora blight such as use of resistant pepper cultivars is considered of the utmost importance in sustaining acceptable production levels of pepper. To establish the most effective control strategies for Phytophthora blight of peppers using resistant cultivars, we need to know much more about disease resistance and defense-related genes of pepper to Phytophthora blight. In this paper, some informations about resistance of pepper to Phytophthora blight at the cytological, physiological and molecular aspects are presented.

Genetics of Resistance to Phytophthora Blight and Variation in *P. capsici* Isolates

Resistance of pepper to *P. capsici* was first found in 1960 in certain pepper genotypes by Kimble and Grogan (1960). This resistance was later confirmed to be governed by two distinct dominant genes that act independently without additive effect (Smith et al., 1967; Polach and Webster, 1972) or by a single dominant gene with modifiers (Barks-

dale et al., 1984). However, prolonged incubation period or very high inoculum concentration of *P. capsici* could occasionally overcome resistance and result in symptoms on resistant plants. Many studies have sought to identify a stable and durable source of resistance to *P. capsici* for use in breeding programs (Kimble and Grogan, 1960; Smith et al., 1967; Pochard and Daubeze, 1980; Barksdale et al., 1984). Resistance has failed to control Phytophthora blight effectively, especially during prolonged periods of rainy weather (Yang et al., 1989), because there may be virulent isolates in the *P. capsici* population able to attack the resistant pepper cultivars. In our earlier study, it has been suggested that age-related resistance, which is distinctly expressed as pepper plants mature, may be effective in reducing damage from this disease (Kim et al., 1989). Because the disease causes severe damage in pepper plants only at later growth stages in the field, the age-related resistance must be considered in breeding resistant pepper cultivars.

There are no pathogenic races of *P. capsici* on pepper plants, but several reports indicate varying degrees of virulence of isolates on different hosts. Polach and Webster (1972) defined 14 pathogenic isolates of *P. capsici* based on pathogenicity to different hosts, but not to different pepper cultivars. In our earlier studies, we examined the pathogenic variation on Korean pepper cultivars of *P. capsici* isolates from diverse geographic origins (Yang et al., 1989; Kim and Hwang, 1992). However, the Korean pepper genotypes did not differentiate any vertical pathotypes from the *P. capsici* isolates used. The great genetic variation observed in these same isolates from Korea, Europe, and New Mexico for restriction fragment length polymorphism of mitochondrial DNA (Hwang et al., 1991) suggests the possible existence of variation in virulence of naturally occurring *P. capsici* isolates. In recent study, however, we have demonstrated that there are differential interactions between *P. capsici* isolates and some pepper genotypes at late plant growth stages (Table 1) (Hwang et al., 1996)

Ultrastructure of Resistant Responses

Susceptible pepper plants have blighted foliage, leaf defoliation, crown rot, rapidly growing stem lesions, or damping-

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Table 1. Disease severity incited by six isolates of *Phytophthora capsici* on 17 pepper cultivars or accessions at the eight-leaf stage^a (Hwang et al. 1996)

Cultivar of accession	Disease severity ^b incited by isolates						Average
	S197	87E1	CBS178.26	87K14	87I33	87L19	
Hanbyul	5.0(S) ^c	5.0(S)	5.0(S)	5.0(S)	5.0(S)	5.0(S)	5.0
Cayenne Cajun 1	5.0(S)	4.7(S)	5.0(S)	5.0(S)	5.0(S)	5.0(S)	4.9
Cayenne Cajun 2A	4.3(S)	4.4(S)	4.5(S)	4.0(S)	4.8(S)	5.0(S)	4.5
Cayenne Cajun 1A	4.3(S)	4.5(S)	4.5(S)	4.2(S)	4.5(S)	5.0(S)	4.5
Cayenne Cajun 2	4.4(S)	4.4(S)	4.2(S)	4.4(S)	4.1(S)	5.0(S)	4.4
Peto Seed Cayenne	4.6(S)	4.4(S)	4.3(S)	5.0(S)	3.5(S)	5.0(S)	4.4
Tabasco	4.9(S)	4.0(S)	5.0(S)	5.0(S)	4.6(S)	3.4(S)	4.4
Bruce Foods	4.6(S)	4.4(S)	4.3(S)	4.8(S)	3.4(S)	5.0(S)	4.4
Durkee Cayenne	3.5(S)	4.3(S)	3.5(S)	5.0(S)	3.0(S)	5.0(S)	4.0
Kingkun	4.1(S)	5.0(S)	4.8(S)	4.6(S)	4.3(S)	1.3(R)	4.0
Capritto Cayenne	4.8(S)	4.3(S)	5.0(S)	3.6(S)	3.8(S)	1.3(R)	3.8
T.C. 3191	5.0(S)	5.0(S)	5.0(S)	0.3(R)	4.5(S)	0.0(R)	3.2
PI 189550	5.0(S)	4.5(S)	5.0(S)	3.0(S)	1.5(R)	0.5(R)	3.2
PI 201234	4.8(S)	4.6(S)	0.0(R)	0.0(R)	1.3(R)	0.0(R)	2.0
PI 188478	4.8(S)	4.5(S)	4.3(S)	0.5(R)	0.5(R)	0.3(R)	2.0
PM 217	5.0(S)	3.0(S)	0.0(R)	1.0(R)	4.0(S)	0.0(R)	2.0
PI 211238	2.0(R)	0.0(R)	5.0(S)	3.0(S)	0.0(R)	0.0(R)	2.0
Average	4.5	4.2	4.1	3.5	3.4	2.8	

^aPlants were inoculated with zoospore suspension (10^7 /ml) at the eight-leaf stage by the stem-wound technique.

^bDisease severity (D.S.) based on a 0-5 scale, where 0=immune or highly resistant, no visible symptoms and 5=highly susceptible, plant death.

^cS=susceptible (D.S.=2.1-5.0), R=resistant (D.S.=0.0-2.0).

off in juvenile plants (Kim et al., 1989). Many plants classed as resistant are symptomless or have foliage blight, crown rot and patches of superficial brownish-to-purple specking that develop slowly on the stems. The stem tissues infected by a virulent isolate of *P. capsici* showed typical symptoms of *Phytophthora* blight at 3 days after inoculation. The water-soaked lesions were rapidly developed on the stem tissues. The pepper plants infected by an avirulent isolate did not produce symptoms at 1 day after inoculation. Three days later, the pepper plants had superficial brown-purple specking that developed slowly on the stem surface.

Numerous ultrastructural studies have been made of the interaction of various host plants with *Phytophthora* spp. (Ehrlich and Ehrlich, 1966; Hanchey and Wheeler, 1971; Jones et al., 1974; Shimony and Friend, 1975; Hohl and Suter, 1976; Stössel et al., 1981; Allen and Friend, 1983; Feuerstein and Hohl, 1986; Hwang et al., 1994).

In the *P. capsici*-pepper combination, Jones et al. (1974) observed the ultrastructural changes in pepper cell organelles during infection by this oomycete on pepper fruit, e.g., highly lobed nuclei with prominent nuclei, parallel layers of rough endoplasmic reticulum, degenerated chloroplasts or ribosome-saturated disorganized cytoplasm. In our earlier study, light microscope examination of roots and stems of pepper plants infected with *P. capsici* revealed

that the oomycete hyphae colonized the cortical parenchyma cells, vascular bundles and pith, followed by the partial or complete disintegration of these tissues with intense, dark-brown staining (Kim and Hwang, 1989). Intercellularly growing hyphae of *P. capsici* penetrated the host cells by forming haustorium-like bodies (Hwang et al., 1989). At 24 h after inoculation of pepper stems with zoospores of *P. capsici*, the oomycete hyphae were found only in the intercellular spaces of cortical parenchyma cells (Hwang et al., 1990). Chloroplasts, numerous vacuoles, and cytoplasm in the cortical parenchyma cell were surrounded by plasma membrane in intimate contact with the host cell wall.

An extrahaustorial matrix and wall appositions around haustoria were visible in both compatible and incompatible interactions. The virulent isolate S197 of *P. capsici* formed normal haustoria and extrahaustorial matrix in the infected pepper stem tissue. In addition, the pathogen showed very rapid penetration progress and high colonization in compatible interaction, compared with incompatible interaction, as previously observed in other plant-pathogen interactions (Stössel et al., 1980; Cahill et al., 1989; Ward et al., 1989; Enkerli et al., 1997). Electron micrographs did not show notable difference in ultrastructure of *P. capsici*-infected tissues between compatible and incompatible interactions (Lee et al., 2000e). However, electron dense material depo-

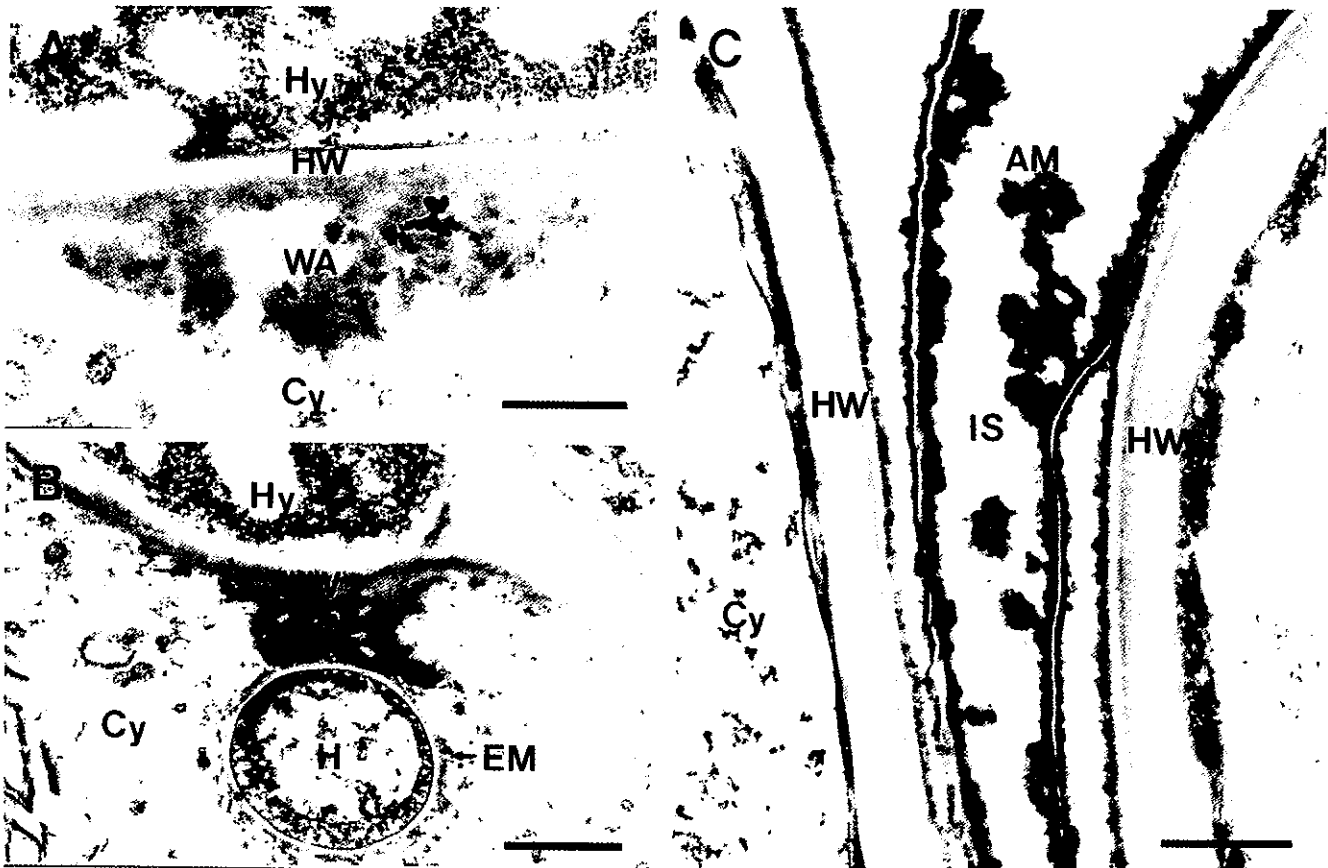


Fig. 1. Transmission electron micrographs of incompatible interactions of pepper (cv. Hanbyul) plants with *Phytophthora capsici* at 24 h after inoculation. (A) The initial stage of haustorium development. Wall appositions are present below the host cell wall intimately contacted with the fungal cell (Bar size=500 nm). (B) Hyphae with a mature haustorium. Note electron-dense wall appositions beneath the host wall. Some extra-haustorial matrix is seen around the haustorium (Bar size=500 nm). (C) Pepper stem tissues showing host reaction material in the intercellular space upon the pathogen infection. Bands of amorphous material lines the intercellular space (Bar size=500 nm) (Lee et al. 2000e).

sitions in intercellular space and abnormal structure of hyphal mitochondria were found in the incompatible interaction (Fig. 1). The electron-dense materials were usually found in cortical cells. This material, presumably rich in phenolic compounds, as judged by its texture and electron density, could act as a chemical barrier to *P. capsici* in the incompatible interaction.

Plant Nutrients Associated with Disease Resistance

The nutrition of a plant affects its resistance or susceptibility to disease, its histological or morphological structure, the function of tissues to hasten or retard pathogenesis, and the ability of pathogens to survive. Non-availability of nutrient elements needed to synthesize chemical and physical barriers, or the movement of nutrients into metabolic sinks around infection sites can result in susceptibility to disease. In contrast, resistance may be caused by the absence of nutrients essential for pathogenic activity.

Horsfall and Dimond (1957) proposed that the sugar content of plant tissue influences disease susceptibility. Since then, considerable research has been made concerning the availability of the nutrients for plant pathogens in plant tissues. The resistant pepper cultivar Kingkun contained less levels of fructose, glucose and sucrose in the stems at the different developmental stages than the susceptible pepper cultivar Hanbyul (Table 2) (Jeun and Hwang, 1991). These results suggest that low amounts of these carbohydrates in the resistant cultivar may partly affect the morphological constitution of the host-pathogen interface and the production of preformed fungistatic substances in relation to the inhibition of *Phytophthora* development in the pepper stems. Although the relationship between nutrition and pathogenesis is still unclear, these low levels of carbohydrates in the resistant cultivar may also provide an unfavorable energy source for nutrient utilization by *P. capsici*. These results are well supported by the results of Hwang et al. (1983), where the barley cultivar Rupee, highly resistant

Table 2. Amounts of fructose, glucose, sucrose and inositol in healthy stems of the pepper cultivars Hanbyul (susceptible) and Kingkun (resistant) at two plant growth stages (Jeun and Hwang, 1991)

Cultivar		Carbohydrate (mg g ⁻¹ dry weight) at the stage	
		Sixth leaf	Second branch
Hanbyul (susceptible)	Fructose	23.63 ± 1.60 ^a	32.00 ± 4.77
	Glucose	14.34 ± 0.94	17.49 ± 2.78
	Sucrose	21.23 ± 1.49	38.03 ± 1.53
	Inositol	1.40 ± 0.17	0.57 ± 0.17
Kingkun (resistant)	Fructose	13.59 ± 1.02	12.55 ± 1.07
	Glucose	6.24 ± 1.41	6.00 ± 0.29
	Sucrose	16.32 ± 1.45	20.17 ± 2.73
	Inositol	1.22 ± 0.47	0.62 ± 0.02

^aEach value represents the mean ± standard deviation of 3 replicate samples.

to powdery mildew, has lower level of soluble carbohydrates throughout the plant development than the susceptible cultivars.

In view of the slight change in the amount of total amino acids observed during plant development, it seems unlikely that there are relationships between the amino acid metabolism and the retardation of *Phytophthora* infection. In particular, the amount of total phenolic compounds in pepper stems was relatively low at the later growth stages of plants and also in the resistant cultivar Kingkun. Our results are not consistent with the general view of the significance of phenolic compounds in disease resistance (Schönbeck and Schlösser, 1976; Misaghi, 1982). Preformed antifungal substances, such as phenolics, may not contribute to the expression of resistance of pepper plants, but rather phytoalexins, which accumulate after exposure to *P. capsici*, may play an important role in this resistance (Hwang and Kim, 1990).

P. capsici cells require an adequate amount of inorganic nutrients in pepper tissues in order to multiply and to carry out their respective physiological functions. There are some reports that nitrogen and calcium may have direct effects of *Phytophthora* blight of pepper. Elenkov and Bakharieva (1975) reported that *Phytophthora* blight of pepper was increased by high nitrogen. The addition of small quantities of Ca, as CaCl₂, increased the percentage of pepper plants killed by *Phytophthora* blight (Muchovej et al., 1980). In our earlier study, the contents of macroelemental nutrients such as nitrogen, phosphorus, potassium, calcium and magnesium were drastically reduced in pepper stems at the later plant growth stage. These results suggest that the low concentration of these mineral nutrients in stems of mature pepper plants may provide a tissue environment less favor-

able for the development of *P. capsici*. However, no significant differences between the cultivars or the plant growth stages were found in the contents of silicon and microelemental nutrients such as sodium, iron, zinc and manganese.

Capsidiol Production Associated with Disease Resistance and Metalaxyl Treatment

Stössel et al. (1972) first reported that pepper fruits produced the antifungal sesquiterpenoid phytoalexin, capsidiol in response to infection by several pathogenic and non-pathogenic fungi. Other fungi and bacteria also elicit accumulation of capsidiol (Stössel et al., 1972; Ward et al., 1973). A series of extensive studies of the rate and magnitude of capsidiol accumulation coupled with ultrastructural studies to support a role for the compound in the restriction of fungal growth and development in infected pepper fruits and stems (Jones et al., 1974; Hwang et al., 1990). It also accumulated around sites of localized infection in leaves (Ward, 1976), but its accumulation or the sensitivity of fungi to capsidiol cannot explain the resistance or susceptibility of pepper to all fungi. The relation between capsidiol concentration and speed of oomycete invasion in stems of pepper cultivars susceptible or resistant to *P. capsici* has been assessed by Molot et al. (1981). In addition, phytoalexin production can be induced by fungicides. Reilly and Klarman (1972) demonstrated that several fungicides induced the phytoalexin glyceollin in soybean hypocotyl tissues. Cartwright et al. (1977) also reported that dichlorocyclopropanes may exert their systemic fungicidal activity against the rice blast disease by causing rice to synthesize the phytoalexins monilactones A and B.

In our earlier studies, it has been demonstrated that capsidiol concentrations in infected stems and roots of pepper plants were correlated with the degree of resistance expressed to *P. capsici* (Hwang and Kim, 1990; Hwang, 1995). In comparable infected organs, the resistant cultivar Kingkun always contained more capsidiol than the susceptible cultivar Hanbyul. The stem and root of the two cultivars accumulated more capsidiol and became increasingly resistant as plants matured. These results suggest that capsidiol production has a role in increasing the resistances of pepper plants with aging.

A combination of direct fungitoxic and indirect effects involving activation of natural defense mechanisms has been demonstrated in pepper plants treated with the systemic fungicide metalaxyl (Börner et al., 1983; Hwang and Sung, 1989). Metalaxyl treatment not only produced direct effects in the fine structure of the *P. capsici* cell but also the defense reaction of pepper plants (Sung and Hwang, 1988; Hwang et al., 1990). Due to the intimate nature of the interaction between *P. capsici* and pepper plant, fungicide inter-

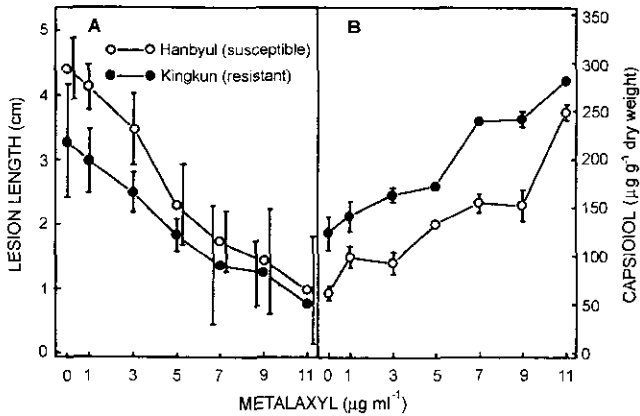


Fig. 2. Influence of concentration of a metalaxyl drench in soil on (A) disease development and (B) capsidiol production in the stems of pepper cultivars Hanbyul (susceptible) and Kingkun (resistant). Plants were inoculated at the eight-leaf stage with zoospore suspensions of *Phytophthora capsici* at the bottom of the stem. Data were obtained on day 4 after stem-inoculation. The vertical bars represent standard deviations (Hwang and Sung 1989).

ference with metabolism of the pathogen must inevitably lead to alteration of host physiology which results from disturbances at the pathogen-host interface. Enhanced capsidiol accumulation has been reported in response to the *P. capsici* infection in pepper plants treated with metalaxyl (Hwang and Sung, 1989; Hwang, 1995). Increasing concentrations of metalaxyl in soil treatments a day before stem inoculation with *P. capsici* gradually retarded the lesion development on the stems of pepper plants and stimulated capsidiol production in the infected stems (Fig. 2). In particular, the accumulation of capsidiol by metalaxyl treatment was more pronounced in the resistant cultivar Kingkun than in the susceptible cultivar Hanbyul.

In conclusion, disease resistance and metalaxyl treatment might make an important contribution to the effective prevention of *Phytophthora* blight in pepper plants by directly affecting the pathogen itself or indirectly enhancing capsidiol accumulation in infected tissue. However, there is a need for critical investigation of the natural sequence of events when resistance is expressed in pepper plants in relation to capsidiol production. Further detailed research on the relative rate of fungal expansion, capsidiol production and degradation at the metalaxyl-treated infection site is also needed.

Pathogenesis-Related Genes and Proteins

Plants contain many genes encoding defense-related proteins. These include resistance genes involved in gene-for-gene interactions leading to hypersensitive cell death, genes encoding signal transduction proteins, and downstream

defense genes, for example, those encoding pathogenesis-related (PR) proteins, enzymes involved in the generation of phytoalexins, the enzymes of oxidative stress protection, tissue repair, and lignification. Many of these genes are upregulated when the plant is attacked by pathogens. Just how many inducible defense genes exist in plant genomes is difficult to estimate, because it is likely that some, if not many, of these genes have dual or multiple functions.

Some plants defend themselves by a variety of constitutive defense barriers and active protective mechanisms accompanied by an array of biochemical and physical changes. One of the most-studied plant defense responses is the synthesis of a group of host-encoded proteins such as pathogenesis-related (PR) proteins (Jung and Hwang, 2000b). Recently, it has been suggested that several families of small, basic, and cysteine-rich antimicrobial proteins such as thionin (Bohlman et al., 1988), lipid transfer protein (Garcia-Olmedo et al., 1995), and plant defensin (Terras et al., 1992) may play a significant role in plant defense responses. Some PR proteins have indeed been found to have antifungal activity *in vitro* (Bol et al., 1990). The genetically engineered plants overexpressing PR proteins have been shown to be resistant to pathogen infection (Alexander et al., 1993). Moreover, PR proteins can be induced in plant tissues by treatment with abiotic elicitors. Some well-known chemical inducers of PR proteins include polyacrylic acid, ethylene, benzoic acid, salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA), benzo-(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH), and DL- β -amino-n-butyric acid (BABA) (Kessmann et al., 1994; Sunwoo et al., 1996).

By using two-dimensional SDS-polyacrylamide gel electrophoresis (PAGE), we have shown previously that some soluble proteins not detectable in healthy pepper stems were induced by *P. capsici* infection (Hwang et al., 1991). These findings have led us to suggest the possibility that some PR proteins such as β -1,3-glucanase and chitinase may be more distinctly induced and accumulated in resistant pepper tissues than susceptible ones by *P. capsici* infection.

β -1,3-Glucanases. β -1,3-Glucanase exists in multiple forms in a number of plant species. In tobacco, the vacuolar (basic isoforms) and wall-localized (acidic) β -1,3-glucanase activities are clearly distinct from one another in relation to their regulation during induced defense response (Van den Bulcke et al., 1989). β -1,3-Glucanase has been known to act as a synergist for the antifungal activity of chitinase (Mauch et al., 1988; Sela-Buurlage et al., 1993). In addition, the hydrolase solubilizes elicitor-active glucan molecules from the walls of invading fungal pathogens (Mauch and Staehelin, 1989), thus inducing its own pro-

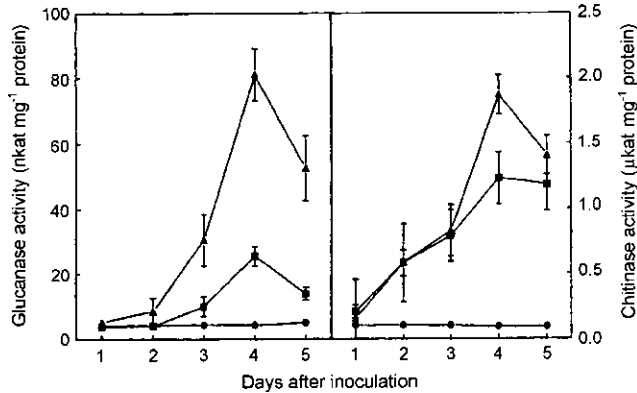


Fig. 3. Time-course activities of β -1,3-glucanase and chitinase in crude extracts of pepper (cv. Hanbyul) stems after inoculation with compatible (S197) (\blacksquare) or incompatible (CBS178.26) (\blacktriangle) isolate of *Phytophthora capsici*, and uninoculated pepper stems (\bullet) at the second-branch stage (Kim and Hwang 1994).

duction and that of other defense enzymes involved in the synthesis of antimicrobial phytoalexins and cell wall barriers. As β -1,3-glucanase is direct defense enzyme, in contrast to products of complex biosynthetic pathways such as phytoalexins, it may be valuable targets for engineering defense in transgenic plants.

P. capsici infection induced the synthesis and accumulation of β -1,3-glucanases in the stem tissues of pepper plants (Fig. 3) (Kim and Hwang, 1994). The hydrolase increase started soon after inoculation with *P. capsici*. After the appearance of symptoms on the pepper stems, the accumulation of β -1,3-glucanase became much pronounced in the incompatible interaction. We further isolated β -1,3-glucanase with antifungal activity from pepper stems (Kim and Hwang, 1997). The two acidic and four basic β -1,3-glucanases also were induced and accumulated in pepper leaves infected with *Xanthomonas campestris* pv. *vesicatoria* (Lee and Hwang, 1996). However, role of β -1,3-glucanase either in defense response or in physiological and developmental processes has not been well understood, since the enzyme activity exists in several isoforms that differ in amino acid sequences, cellular localization and inducibility (Domingo et al., 1994). More recently, a basic β -1,3-glucanase cDNA gene (*CABGLU*) was isolated from the cDNA library of pepper leaves (Jung and Hwang, 2000b). The deduced polypeptide of *CABGLU* which contains C-terminal extension N-glycosylated at a single site characterized as typical structure of class I β -1,3-glucanases has a high level of identity with tobacco basic β -1,3-glucanase (77.4%). Transcripts of *CABGLU* gene were somewhat more induced in incompatible interaction than in compatible interaction, when inoculated with *P. capsici* (Fig. 4). Accumulation of *CABGLU* mRNA was strongly induced in pepper leaves by ethephon and metnlyl jasmonate.

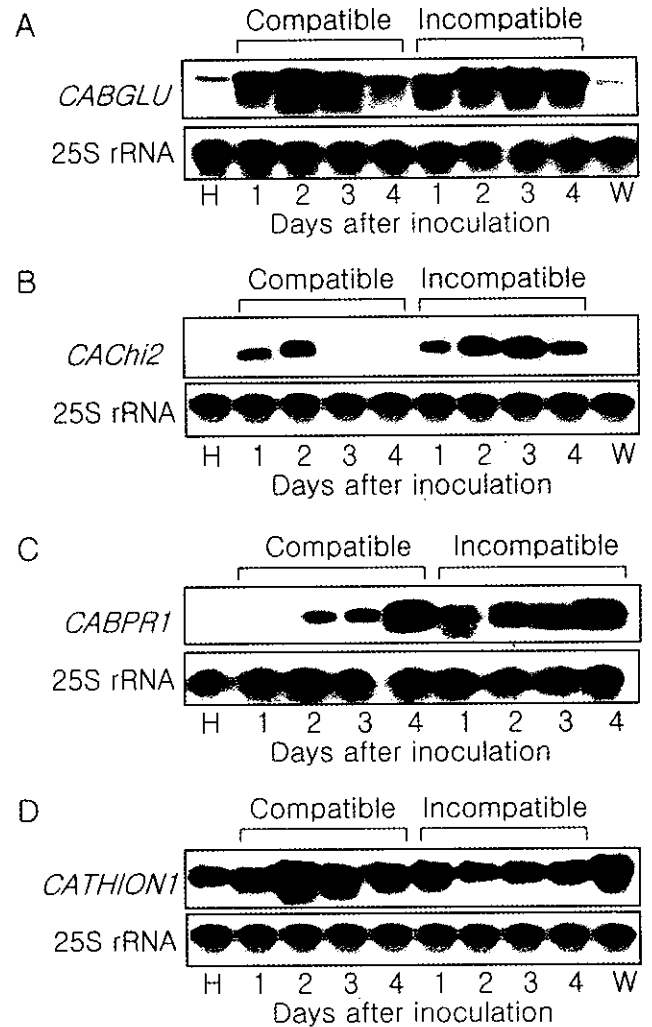


Fig. 4. Northern blot analyses of transcript levels of (A) β -1,3-glucanase (*CABGLU*), (B) chitinase (*CACHi2*), (C) PR-1 (*CABPR1*), and (D) thionin (*CATHION1*) cDNA genes in pepper stems inoculated with virulent isolate S197 and avirulent isolate CBS178.26 of *Phytophthora capsici*. Total RNA was isolated from pepper stems at various times after inoculation and 24 h after wounding. Thirty micrograms of total RNA from each sample were separated in each lane. A *Capsicum annuum* 25S rRNA probe was used as an internal standard (Jung and Hwang 2000a; Kim and Hwang 2000; Hong et al. 2000; Lee et al. 2000a).

Chitinases. Since the discovery of several isoforms of chitinase among PR-proteins (Kombrink et al., 1988), chitinases have been of particular interest in studies of plant resistance against fungal pathogens. The substrate for chitinase is chitin, the major structural component of the cell wall of many phytopathogenic fungi. Although oomycetes have been considered to be devoid of chitin in their cell walls, this has recently been challenged by the novel finding of Chérif et al. (1992) that cellulose and chitin occur in the hyphal walls of an oomycete pathogen *Pythium ultimum*. Chitinase has an antifungal activity and cause lysis of

fungal hyphal tips *in vitro*. In *in vitro* experiments, chitinase has shown the ability to partially hydrolyze fungal cell walls and to inhibit the growth of certain phytopathogenic fungi. The expression of chitinase genes in transgenic plants has provided further evidence for their role in plant defense (Punja and Raharjo, 1996).

In our earlier studies, the accumulation of chitinases in plant tissues by *P. capsici* infection has been demonstrated in pepper (Fig. 3) (Kim and Hwang, 1994). At the protein level, the chitinase activity induced by *P. capsici* infection was somewhat higher in the incompatible interaction than the compatible interaction at 4 days after inoculation, although the patterns of chitinase induction were similar in both compatible and incompatible interactions. Different isoforms of chitinases were purified from pepper stems (Kim and Hwang, 1996). The acidic isoform a1 (69 kDa, pI 5.0), basic isoform b1 (32 kDa, pI 9.0) and b2 (22 kDa, pI 9.1) were purified by chitin-affinity chromatography, with subsequent electroelution from nondenaturing polyacrylamide gel electrophoresis (PAGE) gels. The acidic isoform a1 has chitin-binding properties, but does not have antifungal activity. The basic isoforms b1 and b2 contain high ratios of cysteine and glycine at the N-terminal chitin-binding domain, exhibit chitinase activity, and show antifungal activities against some phytopathogenic fungi. None of the purified isoforms of chitinases inhibited hyphal growth of the oomycete *P. capsici* which lacks chitin. In contrast, zoospore germination and germ tube elongation of *P. capsici* were effectively inhibited by treatment with chitinases.

A chitinase cDNA clone (designated *CACHi2*) was isolated from the cDNA library of pepper leaves (Hong et al., 2000). The 1004-bp full-length *CACHi2* cDNA encodes a basic chitinase with an N-terminal 24 amino acid signal peptide followed by a catalytic region. The *CACHi2* is a class II chitinase, because it does not have chitin-binding domain and C-terminal extension sequences. Following *P. capsici* infection, the *CACHi2* chitinase mRNA was more highly expressed in the incompatible interaction than in the compatible interaction (Fig. 4). Treatment with ethylene-releasing ethephon resulted in a strong accumulation of the *CACHi2* transcripts in the pepper leaves. The *CACHi2* mRNA was usually localized in the vascular tissues and their expression was constricted in the phloem-related cells (Lee et al., 2000c). The spatial pattern of *CACHi2* mRNA expression was similar in both compatible and incompatible interactions but the temporal patterns were different from each other. The early induction of *CACHi2* mRNA was quite distinct in the incompatible interaction. Immunogold labeling data showed specific labeling of chitinase on the cell wall of the oomycete in both compatible and incompatible interactions at 24 h after inoculation. In particular, numerous gold particles were deposited on the cell

wall of *P. capsici* with a predominant accumulation over areas showing signs of degradation in the incompatible interaction. Chitinase labeling was also detected in the intercellular space and cytoplasm of host plants.

PR-1 protein. Proteins belonging to the PR-1 family were first identified in TMV-infected tobacco plants (Van Loon, 1976). These serologically related proteins have a molecular mass of 14-17 kDa and accumulate in many plant species subjected to pathogen infection and external stimuli. Many isoforms of PR-1 protein have been isolated, and many genes encoding them have been cloned and sequenced (Linthorst, 1991; Stintzi et al., 1993). Recently, increased resistance and significantly reduced disease symptoms were demonstrated in transgenic tobacco expressing PR-1a protein against *Peronospora tabacina* and *P. parasitica* var. *nicotianae* (Alexander et al., 1993). The suggested antifungal activity of PR-1 protein was confirmed by Niderman et al. (1995), who demonstrated antifungal activity of PR-1 against *Phytophthora infestans in vitro*. It cannot be excluded that proteins of the PR-1 family function directly and/or indirectly in plant defenses against fungal pathogens, although the mechanism of action is still not known.

There is considerable data available on PR proteins in dicotyledonous plants. However, there is more limited information about PR proteins in the pepper plants, especially the PR-1 protein. In previous studies, we showed that some PR proteins could be expressed in pepper plants upon pathogen attack and abiotic elicitor treatment (Kim and Hwang, 1996; Lee and Hwang, 1996; Hwang et al., 1997). More recently, Hwang et al. (1997) showed the accumulation of PR-1 protein in pepper stem tissues after treatment with DL- β -amino-n-butyric acid and challenge-inoculation with *P. capsici*. To examine further whether or not the PR-1 gene is induced by pathogen infection, Kim and Hwang (2000) cloned and characterized the PR-1 (*CABPR1*) cDNA gene from pepper leaves infected by *X. campestris* pv. *vesicatoria*.

P. capsici infection in pepper stems induced strong accumulation of the basic PR-1 mRNA in both the compatible and incompatible interactions, which suggested that the PR-1 genes may be required for the expression of symptoms and defense against fungal pathogens (Fig. 4) (Kim and Hwang, 2000). Furthermore, *P. capsici* infection also greatly stimulated ethylene biosynthesis, together with accumulation of PR-1 mRNA.

In situ hybridization and immunogold labeling were performed to examine the temporal and spatial expression pattern of PR-1 mRNA and PR-1 protein in pepper stem tissues infected by virulent and avirulent isolates of *P. capsici* (Lee et al., 2000d). The temporal expression of PR-1 mRNA varied greatly between compatible and incompati-

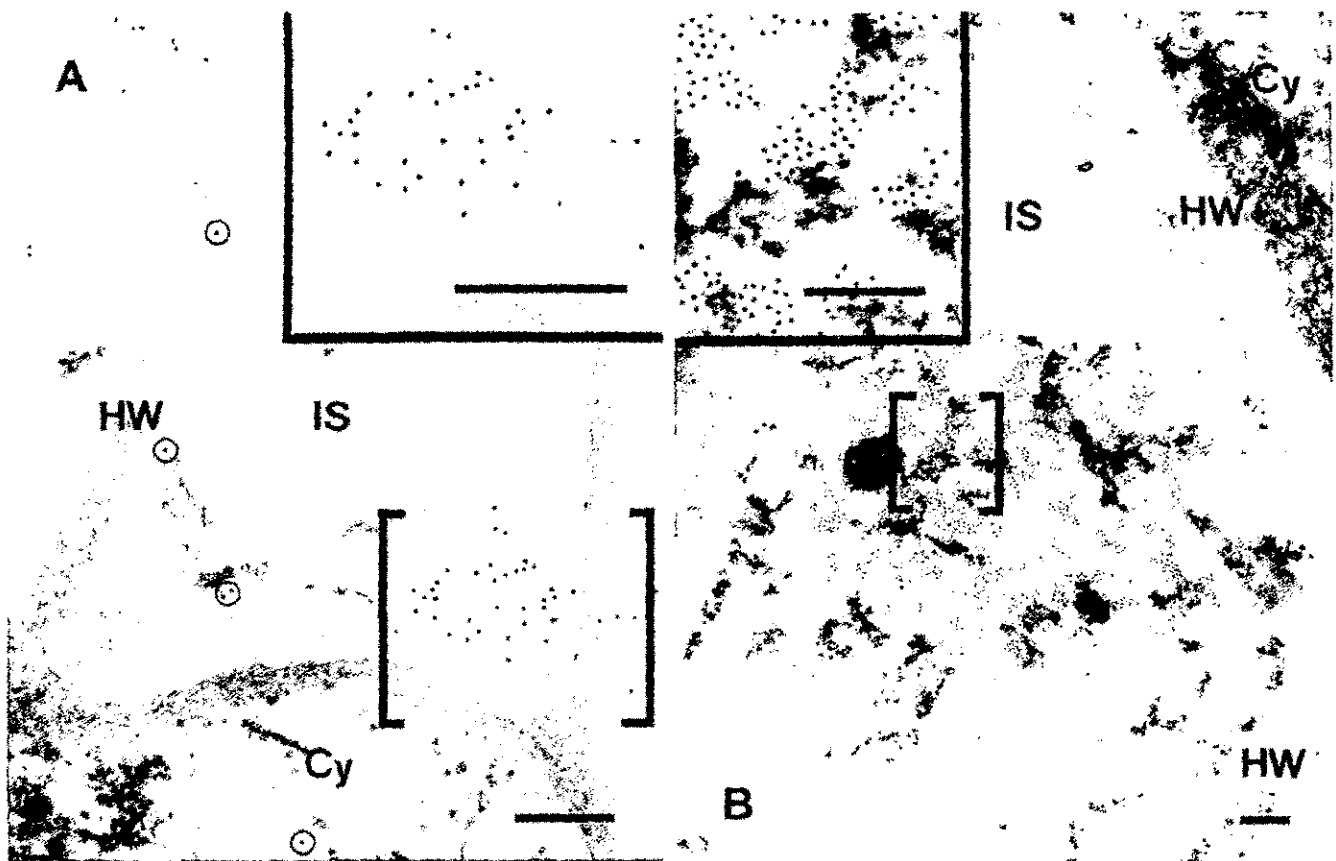


Fig. 5. Immunogold labeling with the polyclonal antibody against PR-1 protein of pepper stem tissue in the compatible (A) and incompatible (B) interactions 24 h after inoculation with the virulent isolate S197 and the avirulent isolate CBS178.26 of *Phytophthora capsici*, respectively. Bars: 500 nm. (A) A number of gold particles occur in the intercellular space (IS) of the infected pepper stem cells in the compatible interaction. Host cell wall (HW) and cytoplasm (Cy) remain unlabeled. The PR-1 protein is related to the fibrillar material in the intercellular space. **Inset** Higher magnification of the labeling. (B) Dense accumulation of gold particles in the intercellular space (IS) of infected stem tissue in the incompatible interaction. This PR-1 protein accumulation is related to the fibrillar and globular material filling the intercellular space. Host cell wall (HW) and cytoplasm (Cy) are almost free of labeling. **Inset** Higher magnification of the bracketed area (Lee et al. 2000d).

ble interactions (Fig. 5). An earlier expression of the PR-1 gene 6 h after inoculation was observed in the incompatible interaction. PR-1 mRNA was expressed in the phloem areas of vascular bundles in infected pepper stem tissues, but especially strongly in the incompatible interaction. PR-1 protein was predominantly found in the intercellular spaces of pepper stem cells in the compatible and incompatible interactions 24 h after inoculation. Strikingly, the immunogold labeling was associated with fibrillar and electron-dense material localized in the intercellular spaces. Dense labeling of PR-1 protein was also seen at the interface of the pathogen and the host cell wall, whereas few gold particles were detected over the host cytoplasm. However, PR-1 protein was not detected over the oomycete cell wall in either interaction.

Thionins. Thionins are a class of basic and cysteine-rich, low molecular weight proteins (about 5000 Da) found in a

variety of plants. They have been proposed to play an important role in plant defense (Bohlmann et al., 1988). The thionins were found in the vacuole and in the cell wall of plant tissues (Bohlmann et al., 1988). A main characteristic of most thionins is their toxic effect on different biological systems. Most of the thionins were toxic to both plant pathogenic fungi and bacteria. The only thionin known so far to have no toxic effects is the neutral seed protein crambin (Teeter et al., 1981). The common denominator for these toxic effects seems to be a destruction of the membrane (Bohlmann, 1994). In addition to the toxicity of thionins in whole organisms, Garcia-Olmedo et al. (1983) demonstrated inhibitory effects, for example, on protein synthesis. Structures of thionins contain 3-4 disulfide bonds important in maintaining their conformation and thus biological activity (Bohlmann and Apel, 1991).

The distribution and toxicity of thionins indicated that they may be defense proteins. Leaf thionins were supposed

Table 3. Effect of time of chemical treatment on protection of pepper plants (cv. Hanbyul)^a against *Phytophthora capsici* infection by DL- α -amino-n-butyric acid (AABA), DL- β -n-butyric acid (BABA), and γ -amino-n-butyric acid (GABA) (Sunwoo et al. 1996)

Days after chemical treatment	Disease severity ^b (mean \pm SD)			
	Water	AABA	BABA	GABA
1	4.7 \pm 0.18x	4.9 \pm 0.19x	2.5 \pm 0.87y	4.9 \pm 0.28x
4	4.7 \pm 0.19x	4.8 \pm 0.25x	0.7 \pm 0.80xy	4.7 \pm 0.34x
7	4.7 \pm 0.18x	4.8 \pm 0.25x	0.0 \pm 0.00y	4.7 \pm 0.29x
10	4.7 \pm 0.18x	4.6 \pm 0.29x	0.0 \pm 0.00y	4.9 \pm 0.09x
15	4.7 \pm 0.17x	4.7 \pm 0.29x	0.0 \pm 0.00y	4.9 \pm 0.10x

^aPepper plants at eight-leaf stage were uniformly sprayed with 1,000 g ml⁻¹ of aminobutyric acid at different time intervals before challenge inoculation with *P. capsici*.

^bDisease severities were recorded 12 days after challenge inoculation. Values for disease severity followed by the same letter in the same column are not significantly different at the 5% level according to Duncan's multiple range test.

to be post-infection defense proteins, as shown for the PR-proteins, whereas the seed-specific thionins are pre-infection defense proteins (Bohlmann, 1994). Bohlmann et al. (1988) reported that thionins of barley were detected in both compatible and incompatible interactions after infection by *Erysiphe graminis* f.sp. *hordei*. The distribution of thionins in the plant kingdom is very sporadic in distantly related plant families including tobacco, tomato, bell pepper, *Arabidopsis*, and barley.

In an earlier study, we cloned a novel thionin cDNA from pepper leaves infected by *X. campestris* pv. *vesicatoria* (Lee et al., 2000a). Infection by pathogens or treatment with abiotic elicitors led to a strong expression of thionin gene in pepper leaves. Transcripts of the thionin (*CATHION1*) were constitutively expressed in healthy pepper stems, but considerably accumulated upon infection by virulent and avirulent isolates of *P. capsici* (Fig. 4). In healthy, untreated pepper plants, transcripts of (*CATHION1*) gene were induced in an organ-specific manner, as revealed by RNA blot

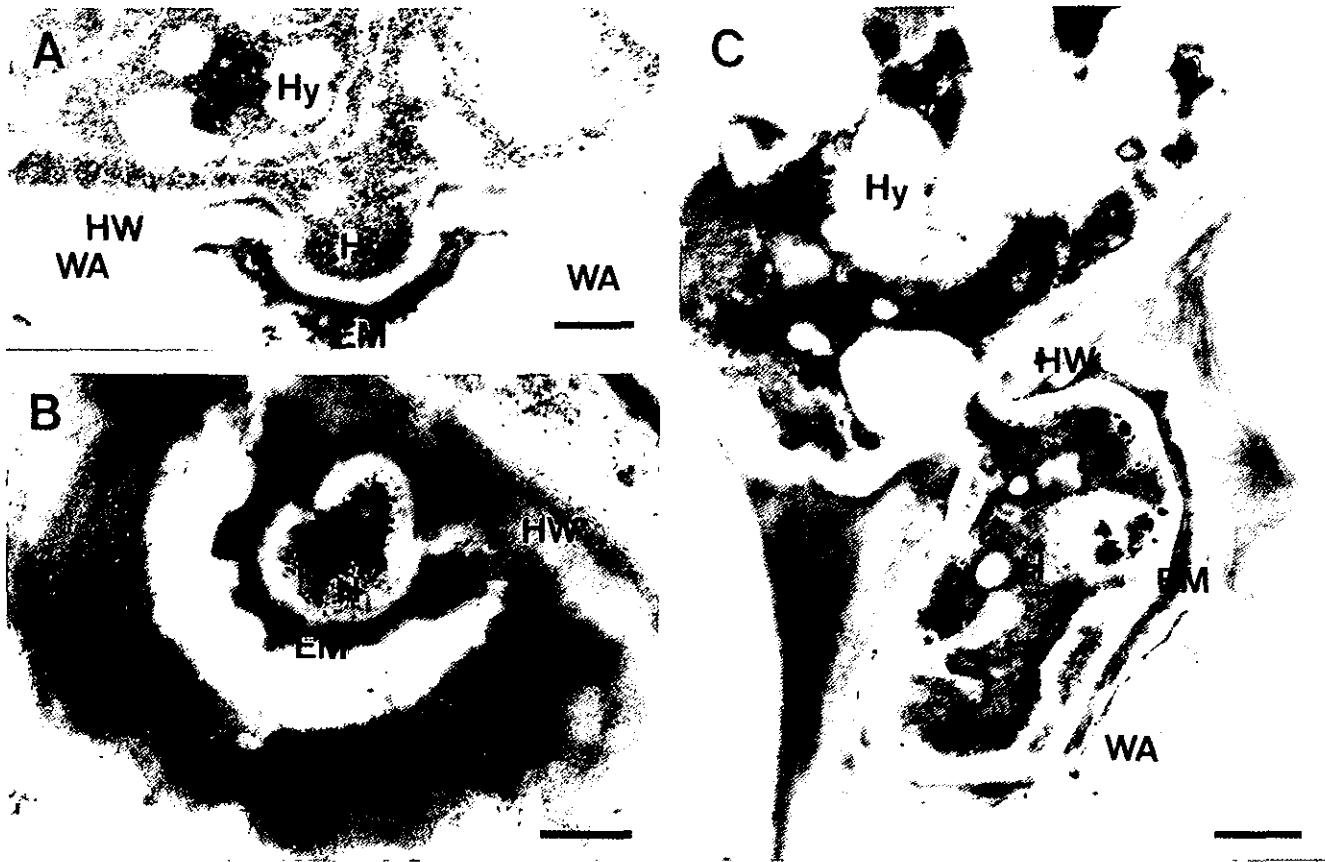


Fig. 6. Transmission electron micrographs of induced resistance reactions in stem tissues of pepper (cv. Hanbyul) at 24 h after challenge-inoculation. (A) The initial stage of haustorium development. Electron-dense extrahaustorial matrix strongly encompasses a growing haustorium. Wall apposition begin to form beneath host wall (Bar size=500 nm). (B) Extrahaustorial matrix and dense wall apposition surrounding the haustorium (Bar size=500 nm). (C) Hyphae with a mature haustorium. The haustorium is heavily encased by extrahaustorial matrix and host wall apposition. Degenerated fungal cytoplasmic materials are seen within the haustorium (Bar size=500 nm) (Lee et al. 2000e).

analysis and *in situ* hybridization (Lee et al., 2000b).

Induced Resistance against Phytophthora Blight in Pepper Plants

Localized infection of certain plants by pathogens can induce resistance to subsequent pathogen attack either locally in infected area or systemically in noninfected area of plants. This resistance is directed against not only the same pathogen but also other unrelated fungal, bacterial and viral pathogens. Induced resistance in a variety of host-pathogen systems is a well-documented phenomenon. In our previous study, we demonstrated that resistance to a virulent isolate of *P. capsici* was induced in pepper plants by inoculation earlier or simultaneously with an avirulent isolate (Hwang and Kim, 1992).

Induction of resistance can also be attained by abiotic inducers. DL- β -amino-n-butyric acid (BABA), a non-protein amino acids, has been demonstrated to act as an abiotic inducer of resistance in tomato against *P. infestans* and in tobacco against *P. tabacina*. Because BABA that has no fungicidal activity *in vitro* caused negligible or no growth inhibition of pathogens (Cohen, 1994), it is considered as an effective chemical capable of inducing resistance against plant pathogens. In recent studies, we reported that treatment of pepper plants with BABA induced resistance to subsequent infection by *P. capsici* (Table 3) (Sunwoo et al., 1996) or *Colletotrichum coccodes* (Hong et al., 1999). In contrast, the α -, and γ -isomers of aminobutyric acid were ineffective as inducers of resistance.

Reduced hyphal growth and sporangial formation were found in BABA-induced resistant and incompatible reactions after *P. capsici* infection. One of the most noticeable ultrastructural features of the BABA-induced resistant reaction was the formation of electron-dense wall appositions (Fig. 6) (Lee et al., 2000e). Another common feature in the BABA-induced resistant and incompatible reactions was degeneration of mitochondrial structure within penetrating hyphal cytoplasm. The mitochondrial structure in the BABA-induced resistant or incompatible reactions had no distinct double membrane layer and well-shaped cristae. Our recent studies have demonstrated that the treatment with BABA induced the synthesis and accumulation of β -1,3-glucanase and chitinase in the stem tissues of pepper plants (Hwang et al., 1997). In particular, accumulation of the two hydrolytic enzymes was remarkably pronounced in pepper stems challenge-inoculated with *P. capsici* after BABA treatment, suggesting a possible stimulation of PR proteins accumulation in the infected tissues by BABA treatment. BABA treatment did not stimulate capsidiol production in pepper stems, but prior to treatment led to high accumulation in *P. capsici*-infected ones. Unlike capsidiol

production, BABA treatment triggered a dramatic increase in the endogenous levels of salicylic acid (SA) in pepper stems. The increase in endogenous SA was much pronounced in *P. capsici* infected stems after BABA treatment. Taken together, the induction of resistance to *P. capsici* in pepper plants by BABA treatment positively correlated with the accumulation of certain β -1,3-glucanase, chitinase isoforms and SA. These results suggest strongly that SA may act as an endogenous signal for activating particular components of resistance to *P. capsici* and the induction of pathogenesis-related proteins such as β -1,3-glucanase and chitinase. In recent study, we have demonstrated that treatment with BABA was effective in suppressing the Phytophthora disease in field and plastic film house (Lee et al., 1999).

Conclusion

Phytophthora blight caused by *P. capsici* is currently one of the major factors limiting pepper production worldwide. Various control measures can be utilized to reduce Phytophthora blight damage in pepper production, but they do not offer complete control. To date, no single measure has been found to effectively control the Phytophthora disease. During the last decade, many pepper cultivars highly susceptible to Phytophthora blight have been cultivated intensively in Korea. Therefore, resistant pepper cultivars with high fruit quality should be developed by seed companies. The development of resistant pepper cultivars can be achieved by transgenic approaches to disease resistance in pepper plants. Some genes encoding defense- or pathogenesis-related proteins such as β -1,3-glucanase, chitinase, PR-1 protein, thionins, so on, have been cloned and characterized in pepper-*P. capsici* system. PR gene expression and disease resistance were induced in pepper by either *P. capsici* infection or an abiotic elicitor DL- β -amino-n-butyric acid. Integrating our knowledge of how these proteins function with the emerging understanding of other resistance mechanisms in pepper plants will lead to an integrated approach toward engineering resistance in pepper plants for control of Phytophthora blight.

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References

- Alexander, D., Goodman, R. M., Gut-Rella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl-Goy, P., Luntz, T., Ward, E. and Ryals, J. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. *Proc. Natl. Acad. Sci. USA* 90: 7327-7331.
- Allen, F. H. E. and Friend, J. 1983. Resistance of potato tubers to infection by *Phytophthora infestans*: a structural study of haustorial encasement. *Physiol. Plant Pathol.* 22:285-292.
- Barksdale, T. H., Papavizas, G. C. and Johnston, S. A. 1984. Resistance to foliar blight and crown rot of pepper caused by *Phytophthora capsici*. *Plant Dis.* 68:506-509.
- Bohlmann, H. 1994. The role of thionins in plant protection. *Crit. Rev. Plant Sci.* 13:1-16.
- Bohlmann, H. and Apel, K. 1991. Thionins. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:227-240.
- Bohlmann, H., Clausen, S., Behnke, S., Giese, H., Hiller, C., Reimann-Philipp, U., Schrader, G., Barkholt, V. and Apel, K. 1988. Leaf-specific thionins of barley a novel class of cell wall proteins toxic to plant-pathogenic fungi and possibly involved in the defense mechanism of plants. *EMBO J.* 7:1559-1565.
- Bol, J. F., Linhorst, H. J. M. and Cornelissen, B. J. C. 1990. Plant pathogenesis-related proteins induced by virus infection. *Annu. Rev. Phytopathol.* 28:113-138.
- Börner, H., Schatz, G. and Grisebach, H. 1983. Influence of the systemic fungicide metalaxyl on glyceollin accumulation in soybean infected with *Phytophthora megasperma* f.sp. *glycinea*. *Physiol. Plant Pathol.* 23:145-152.
- Cahill, D., Legge, N., Grant, B. and Weste, G. 1989. Cellular and histological changes induced by *Phytophthora cinnamomi* in a group of plant species ranging from fully susceptible to fully resistant. *Phytopathology* 79:417-424.
- Cartwright, D., Langcake, P., Pryce, J. and Leworthy, D. P. 1977. Chemical activation of host defence mechanisms as a basis for crop protection. *Nature* 267:511-513.
- Chérif, M., Benhamou, N. and Blanger, R. 1992. Occurrence of cellulose and chitin in the hyphal walls of *Pythium ultimum*: a comparative study with other plant pathogenic fungi. *Can. J. Microbiol.* 39:213-222.
- Cohen, Y. 1994. Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-amino-n-butanoic acids. *Phytopathology* 84:55-59.
- Domingo, C., Conejero, V. and Vera, P. 1994. Genes encoding acidic and basic class III β -1,3-glucanases are expressed in tomato plants upon viroid infection. *Plant Mol. Biol.* 24:725-732.
- Ehrlich, H. G. and Ehrlich, M. A. 1966. Ultrastructure of the hyphae and haustoria of *Phytophthora infestans* and the hyphae of *P. parasitica*. *Can. J. Bot.* 44:1495-1503.
- Elenkov, V. and Bakharieva, V. 1975. Mildew, *Phytophthora capsici*, a dangerous disease of pepper in Bulgaria. *Rastit. Zash.* 23:31-35.
- Enkerli, K., Hahn, M. G. and Mims, C. W. 1997. Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete *Phytophthora sojae*. *Can. J. Bot.* 75:1493-1508.
- Feuerstein, P. and Hohl, H. R. 1986. Histologie des Blattes und des Blattbefalls durch *Phytophthora palmivora* by *Piper nigrum* (Schwarzer Pfeffer). *Bot. Helv.* 96:95-108.
- García-Olmedo, F., Carbonero, P., Hernandez-Lucas, C., Paz-Ares, J., Ponz, F., Vicente, O. and Sierra, J. M. 1983. Inhibition of eucaryotic cell-free protein synthesis by thionins from wheat endosperm. *Biochim. Biophys. Acta Biomem.* 740:52-56.
- García-Olmedo, F., Molina, A., Segura, A. and Moreno, M. 1995. The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol.* 3:72-74.
- Hanchey, P. and Wheeler, H. 1971. Pathological changes in ultrastructure: Tobacco roots infected with *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 61:33-39.
- 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.
- Hong, J. K., Hwang, B. K. and Kim, C. H. 1999. Induction of local and systemic resistance to *Collectotrichum coccodes* in pepper plants by DL- β -amino-n-butyric acid. *J. Phytopathol.* 147:193-198.
- Hong, J. K., Jung, H. W., Kim, Y. J. and Hwang, B. K. 2000. Pepper gene encoding a basic class II chitinase is inducible by pathogen and ethephon. *Plant Sci.* 2000:39-49.
- Horsfall, J. G. and Dimond, A. E. 1957. Interaction of tissue sugar, growth substance, and disease susceptibility. *Z. Pflkrankh. Pflschutz.* 64:415-421.
- Hwang, B. K. 1995. Effects of age-related resistance and metalaxyl of capsidiol production in pepper plants infected with *Phytophthora capsici*. Pages 503-523 in: *Handbook of Phytoalexin Metabolism and Action*, ed. by M. Daniel and R. P. Putkayastha, pp. 503-523. Marcel Dekker, New York.
- Hwang, B. K., de Cook, A. W. A. M., Bahnweg, G., Prell, H. H. and Heitefuss, R. 1991. Restriction fragment length polymorphisms of mitochondrial DNA among *Phytophthora capsici* isolates from pepper (*Capsicum annuum*). *Syst. Appl. Microbiol.* 14:111-116.
- Hwang, B. K., Ebrahim-Nesbat, F., Ibenthal, W. D. and Heitefuss, R. 1990. An ultrastructural study of the effect of metalaxyl on *Phytophthora capsici* infected stems of *Capsicum annuum*. *Pestic. Sci.* 29:151-162.
- Hwang, B. K., Ibenthal, W. D. and Heitefuss, R. 1983. Age, rate of growth, carbohydrate and amino acid contents of spring barley plants in relation to their resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*). *Physiol. Plant Pathol.* 22:1-14.
- 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. and Kim, E. S. 1992. Protection of pepper plants against *Phytophthora* blight by an avirulent isolate of *Phytophthora capsici*. *Korean J. Plant Pathol.* 8:1-7.
- 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. and Kim, Y. J. 1990. Capsidiol production in pepper plants associated with age-related resistance to *Phytophthora capsici*. *Korean J. Plant Pathol.* 6:193-200.
- Hwang, B. K., Kim, Y. J. and Kim, C. H. 1996. Differential interactions of *Phytophthora capsici* isolates with pepper genotypes at various plant growth stages. *Eur. J. Plant Pathol.* 102:311-316.
- Hwang, B. K. and Sung, N. K. 1989. Effect of metalaxyl on capsidiol production in stems of pepper plants infected with *Phytophthora capsici*. *Plant Dis.* 73:748-751.
- Hwang, B. K., Sunwoo, J. Y., Kim, Y. J. and Kim, B. S. 1997. Accumulation of β -1,3-glucanase and chitinase isoform, and salicylic acid in the DL- β -amino-n-butylic acid-induced resistance response of pepper stems to *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 51:305-322.
- Hwang, B. K., Yoon, J. Y., Ibenthal, W. D. and Heitefuss, R. 1991. Soluble proteins, esterases and superoxide dismutase in stem tissue of pepper plants in relation to age-related resistance to *Phytophthora capsici*. *J. Phytopathol.* 132:1129-1138.
- Hwang, J. S., Hwang, B. K. and Kim, W. K. 1994. A light and electron microscopical study of compatible and incompatible interaction between *Phytophthora capsici* and tomato (*Lycopersicon esculentum*) *Korean J. Plant Pathol.* 10:83-91.
- Jeun, Y. C. and Hwang, B. K. 1991. Carbohydrate, amino acid, phenolic and mineral nutrient contents of pepper plants in relation to age-related resistance to *Phytophthora capsici*. *J. Phytopathol.* 131:40-52.
- 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.
- Jung, H. W. and Hwang, B. K. 2000a. Pepper gene encoding a basic β -1,3-glucanase is differentially expressed in pepper tissues upon pathogen infection and ethephon or methyl jasmonate treatment. *Plant Sci.* 2000:97-106.
- Jung, H. W. and Hwang, B. K. 2000b. Isolation, partial sequencing, and expression of pathogenesis-related cDNA genes from pepper leaves infected by *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Plant-Microbe Interact.* 13:136-142.
- Kessmann, M., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S. and Ryals, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annu. Rev. Phytopathol.* 32:439-459.
- Kim, E. S. and Hwang, B. K. 1992. Virulence to Korean pepper cultivars of isolates of *Phytophthora capsici* from different geographic area. *Plant Dis.* 76:486-489.
- 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.
- 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.* 45:195-209.
- Kim, Y. J. and Hwang, B. K. 1996. Purification, N-terminal amino acid sequencing and antifungal activity of chitinases from pepper stems treated with mercuric chloride. *Physiol. Mol. Plant Pathol.* 48:417-432.
- Kim, Y. J. and Hwang, B. K. 1997. Isolation of a basic 34-kilodalton β -1,3-glucanase with inhibitory activity against *Phytophthora capsici* from pepper stems. *Physiol. Mol. Plant Pathol.* 50:103-115.
- Kim, Y. J. and Hwang, B. K. 2000. Pepper gene encoding a basic pathogenesis-related 1 protein is pathogen and ethylene inducible. *Physiol. Plant.* 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.
- Kimble, K. A. and Grogan, R. G. 1960. Resistance to *Phytophthora* root rot in pepper. *Plant Dis. Rep.* 44:872-873.
- Kombrink, E., Schroder, M. and Hahlbrock, K. 1988. Several "pathogenesis-related" proteins in potato are β -1,3-glucanases and chitinases. *Proc. Natl. Acad. Sci. USA* 85:782-786.
- Lee, J. Y., Kim, B. S., Lim, S. W., Lee, B. K., Kim, C. H. and Hwang, B. K. 1999. Field control of *Phytophthora* blight of pepper plants with antagonistic rhizobacteria and DL- β -amino-n-butyric acid. *Plant Pathol. J.* 15:217-222.
- Lee, S. C., Hong, J. K., Kim, Y. J. and Hwang, B. K. 2000a. Pepper gene encoding thionin is differentially induced by pathogens, ethylene and methyl jasmonate. *Physiol. Mol. Plant Pathol.* 56:207-216.
- Lee, S. C., Lee, Y. K., Kim, K. D. and Hwang, B. K. 2000b. In situ hybridization study of organ- and pathogen-dependent expression of a novel thionin gene in pepper (*Capsicum annuum*). *Physiol. Plant.* 110:384-392.
- Lee, Y. K., Hippe-Sanwald, S., Jung, H. W., Hong, J. K., Hause, B. and Hwang, B. K. 2000c. In situ localization of chitinase mRNA and proteins in compatible and incompatible interactions of pepper stems with *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 57:111-121.
- Lee, Y. K., Hippe-Sanwald, S., Lee, S. C., Hohenberg, H. and Hwang, B. K. 2000d. In situ localization of PR-1 mRNA and PR-1 protein in compatible and incompatible interactions of pepper stems with *Phytophthora capsici*. *Protoplasma* 211:64-75.
- Lee, Y. K., Hong, J. K., Hippe-Sanwald, S. and Hwang, B. K. 2000e. 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.
- Lee, Y. K. and Hwang, B. K. 1996. Differential induction and accumulation of β -1,3-glucanase and chitinase isoforms in the intercellular spaces and leaf tissues of pepper by *Xanthomonas campestris* pv. *vesicatoria* infection. *J. Phytopathol.* 144:79-87.
- Linthorst, H. J. M. 1991. Pathogenesis-related proteins of plants. *Plant Sci.* 10:123-150.
- Mauch, F., Mauch-Mani, B. and Boller, T. 1988. Antifungal

- hydrolases in the pea tissue II: inhibition of fungal growth by combinations of chitinase and β -1,3-glucanase. *Plant Physiol.* 88:936-942.
- Mauch, F. and Staehelin, L. A. 1989. Functional implications of the subcellular localization of ethylene-induced chitinase and β -1,3-glucanase in bean leaves. *Plant Cell* 1:447-457.
- Misaghi, I. J. 1982. Alterations of phenol metabolism caused by disease. In: *Physiology and Biochemistry of Plant-Pathogen Interactions*, ed. by I. J. Misaghi, pp. 103-111. Plenum Press, New York and London.
- Molot, P. M., Mas, P., Conus, M. and Ferriere, H. 1981. Relations between capsidiol concentration, speed of fungal invasion and level of induced resistance in cultivars of pepper (*Capsicum annuum*) susceptible or resistant to *Phytophthora capsici*. *Physiol. Plant Pathol.* 18:379-389.
- Muchovej, J. J., Maffia, L. A. and Muchovej, R. M. C. 1980. Effect of exchangeable soil aluminum and alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green pepper. *Phytopathology* 70:1212-1214.
- Niderman, T., Genetet, I., Bruyere, T., Gees, R., Stintzi, A., Legrand, M., Fritig, B. and Msinger, E. 1995. Pathogenesis-related PR-1 proteins are antifungal: isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against *Phytophthora infestans*. *Plant Physiol.* 108:17-27.
- Pochard, E. and Daubeze, A. M. 1980. Recherche et évaluation des composantes d'une résistance polygénique: la résistance du Piment à *Phytophthora capsici*. *Ann. Amélior. Plant.* 30:377-398.
- Polach, F. J. and Webster, R. K. 1972. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. *Phytopathology* 62:20-26.
- Punja, Z. K. and Raharjo, S. H. T. 1996. Response of transgenic cucumber and carrot plants expressing different chitinase enzyme to inoculation with fungal pathogens. *Plant Dis.* 80: 999-1005
- Reilly, J. J. and Klarman, W. L. 1972. The soybean phytoalexin, hydroxyphaseollin, induced by fungicides. *Phytopathology* 62:1113-1115.
- Schönbeck, F. and Schlösser, E. 1976. Preformed substance as potential protectants. In: *Physiological Plant Pathology*, ed. by R. Hertefuss and P. H. Williams, pp. 653-678. Springer-Verlag, Berlin and New York.
- Sela-Buurlage, M. B., Ponstein, A. S., Bres-Vloemans, S. A., Melchers, L. S., Van den Elzen, P. J. M. and Cornelissen, B. J. C. 1993. Only specific tobacco (*Nicotiana tabacum*) chitinases and β -1,3-glucanases exhibit antifungal activity. *Plant Physiol.* 101:857-863.
- Shimony, C. and Freind, J. 1975. Ultrastructure of the interaction between *Phytophthora infestans* and leaves of two cultivars of potato (*Solanum tuberosum* L.) Orion and Majestic. *New Phytol.* 74:59-65.
- Smith, P. G., Kimble, K. A., Grogan, R. G. and Milett, A. H. 1967. Inheritance of resistance in pepper to *Phytophthora* root rot. *Phytopathology* 57:377-379.
- Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Geoffroy, P., Legrand, M. and Fritig, B. 1993. Plant pathogenesis-related proteins and their role in defence against pathogens. *Biochemistry* 75:687-706.
- Stössel, A., Unwin, C. H. and Ward, E. W. B. 1972. Postinfectious inhibitors from plants. I. Capsidiol, an antifungal compound from *Capsicum frutescens*. *Phytopathol. Z.* 74:141-152.
- Stössel, P., Lazarovits, G. and Ward, E. W. B. 1980. Penetration and growth of compatible and incompatible races of *Phytophthora megasperma* var. *sojae* in soybean hypocotyl tissues differing in age. *Can. J. Bot.* 58:2594-2601.
- Stössel, P., Lazarovits, G. and Ward, E. W. B. 1981. Electron microscope study of race-specific and age-related resistant and susceptible reactions of soybeans to *Phytophthora megasperma* var. *sojae*. *Phytopathology* 71:617-623.
- Sung, N. K. and Hwang, B. K. 1988. Comparative efficacy and in vitro activity of metalaxyl and metalaxyl-copper oxychloride mixture for control of *Phytophthora* blight of pepper plants. *Korean J. Plant Pathol.* 4:185-196.
- Sunwoo, J. Y., Lee, Y. K. and Hwang, B. K. 1996. Induced resistance against *Phytophthora capsici* in pepper plants in response to DL- β -amino-n-butyric acid. *Eur. J. Plant Pathol.* 102:663-670.
- Teeter, M. M., Marzer, J. A. and L'Itarlien, J. J. 1981. Primary structure of the hydrophobic plant protein crambin. *Biochemistry* 20:5437-5443.
- Terras, F. R. G., Schoofs, H. M. E., De Bolle, M. F. C., Van Leuven, F., Rees, S. B., Vanderleyden, J., Cammue, B. P. A. and Broekaert, W. F. 1992. Analysis of two novel classes of antifungal proteins from radish (*Raphanus sativus* L.) seeds. *J. Biol. Chem.* 267:15301-15309.
- Van den Bulcke, M., Bauw, G., Castresana, C., Van Montagu, M. and Vandekerckhove, J. 1989. Characterization of vacuolar and extracellular β -(1,3)-glucanases of tobacco: evidence for a strictly compartmentalized plant defense system. *Proc. Natl. Acad. Sci. USA* 86:2673-2677.
- Van Loon, L. C. 1976. Specific soluble leaf proteins in virus-infected tobacco plants are not normal constituents. *J. Gen. Virol.* 30:375-379.
- Ward, E. W. B. 1976. Capsidiol production in pepper leaves in incompatible interaction with fungi. *Phytopathology* 66:175-176.
- Ward, E. W. B., Cahill, D. M. and Bhattacharyya, M. K. 1989. Early cytological differences between compatible and incompatible interactions of soybean with *Phytophthora megasperma* f.sp. *glycinea*. *Physiol. Mol. Plant Pathol.* 34:267-283.
- Ward, E. W. B., Unwin, C. H. and Stössel, A. 1973. Postinfectious inhibitors from plant. . Tolerance of capsidiol by fungal pathogens of pepper fruit. *Can. J. Bot.* 51:2327-2332.
- Yang, S. S., Sung, N. K., Choi, D. I. and Kim, C. H. 1989. Pathogenic variation of *Phytophthora capsici* Leonian on red-pepper in Korea. *Korean J. Plant Pathol.* 5:370-376.