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Differential Induction of Phenylalanine Ammonia Lyase and Phenolics in Peppers (*Capsicum annuum*) in Response to Inoculation with *Phytophthora capsici*

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ABSTRACT

The biochemical response of pepper to *P. capsici*-22 infection, was investigated by comparing *P. capsici* -inoculated and non-inoculated roots, stems and leaves of resistant (CM-334) and susceptible (KM-Hot) cultivars. Two pepper cultivars with different resistance to *P. capsici*-22 were inoculated with 10^4 zoospore/mL to analyze the time course of phenylalanine ammonia-lyase (PAL) activity and total phenolic. Activity of PAL increased in inoculated roots, stems and leaves of two genotypes. PAL activity increased as early as 2 days after inoculation. The highest PAL content in control and infected leaves and stems was recorded in CM-334 cultivar. Induction of phenolic synthesis was found in the inoculated stems and leaves of two genotypes, with the highest rate of phenolic content observed in stems and leaves of the resistant genotypes, compared with controls. These results suggest that induction of PAL and accumulation of phenolics might have contributed to restrict the invasion of *P. capsici.* © 2011 Friends Science Publishers

Key Words: PAL; Pepper; Phenylalanine ammonia lyase; Phenolics; *Phytophthora capsici*

INTRODUCTION

Phytophthora root rot, caused by *Phytophthora capsici*, is one of the most economically destructive soil-borne diseases of pepper (*Capsicum annuum* L.) throughout the world. *Phytophthora* root rot, caused by *P. capsici,* is a devastating disease on peppers. *P. capsici* was first described on chili pepper in New Mexico (Leonian, 1922). The pathogen has reportedly caused severe epidemics in Central and South America, Europe, Asia, and throughout in the United States one a number of susceptible crops (Roberts *et al*., 2008). In Turkey it was first detected in the surroundings of Kahramanmaras city in 1970, from where it rapidly spread to other parts of the country (Cinar & Bicici, 1977).

The pathogen attacks different body parts (roots, stems, leaves & fruit) of plants. *P. capsici* is also pathogenic on, tomato, eggplant, cucumber, watermelon, pumpkin, squash, cocoa (Leonian, 1922; Kunimoto *et al*., 1976; Ristaino, 1990). *P. capsici* has unique attributes that make it an ideal model for detailed investigations of oomycete biology and host specificity. The pathogen moves from roots into stems - leaves and sections of the plant are killed. Roots, stems, foliage and fruit of mature pepper plants are susceptible. Infection can occur at any height on stems and roots. Stem lesions become dark brown to black and result in girdling and plant death. Infected roots are dark brown and mushy (Roberts *et al.*, 2008). Leaf spots are small at first, irregular to round, and water-soaked.

P. capsici may survive in seed and host plant debris in the soil by means of oospores (http://edis.ifas.ufl.edu/vh045). The pathogen produces spores of another type called zoospores that are contained within sac-like structures known as sporangia. Zoospores are motile and swim to reach and invade host tissue. Plentiful surface moisture is required for this activity. The sporangia are spread by wind-blown rain through the air (http://www.plantprotection.hu/modulok/angol/melon/phytophtora_mel.htm) and are carried with water movement in soil. The pathogen is also moved as mycelia (microscopic, fungal-like strands) in infected transplants and through contaminated soil and equipment. Since water is integral to the dispersal and infection of *P. capsici*, maximum disease occurs during wet weather and in low, water-logged parts of fields. Under ideal conditions, the disease can progress very rapidly and symptoms can occur 3-4 days after infection. Therefore, *P. capsici* can rapidly affect entire fields (Roberts *et al.*, 2008; Koç *et al*., 2011).

To date, no peppers cultivars with measurable resistance to *Phytophthora* root rot, the pathogen can survives in soils for several years, with only limited chemical control options for the disease is available. New strategies for the management of *Phytophthora* root rot are essential. Along with cultural management strategies,

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research is needed to assess the possibilities of using induced resistance in plants.

The use of resistant cultivars, crop rotation, modifications of cultural practices, as well as adequate doses of fungicides or biological control strategies, are the best approaches to control the *Phytophthora* disease (Black & Berke, 1998; Woo *et al.*, 2005). The exclusive use of fungicides has been unsuccessful to control the disease in certain pepper growing regions (Kim *et al.*, 1989; Parra & Ristaino, 1998). Despite extensive breeding efforts, no pepper cultivars with universal resistance to *P. capsici* have been commercially released (Oelke *et al*., 2003).

Numerous studies have been conducted since the first investigation by Kimble and Grogan (1960) to identify concerning the resistance of pepper to *P. capsici*. In the meantime, it was demostrated that resistance did not exist before inoculation and a reaction occurred in those plants with a resistant genotype after exposure to the pathogen. Although resistance of plants is highly dependent on environmental conditions, it has been detected that resistance is lower in early periods of development (Pochard *et al*., 1983).

In pepper resistance to *P. capsici* appears to be related to an active defense response against the pathogen. However, unlike other classical forms of resistance governed by single resistance genes, resistance in pepper to *P. capsici* weakens or dissipates with time, depending on the age, physiological conditions of the plant (Kim *et al.*, 1989).

In general, because of pathogen attack on host plants, the plant metabolism is getting altered obviously. However, the change of plant metabolism is varied significantly between susceptible and resistant plants. The studies concerned with obtaining pepper varities resistant to root rot have brought about necessity to examine and compare the metabolisms of pepper varities, where the resistibility to *P. capsici* are different. The roles of so many substances in plant, which play a role in the physiological and biochemical mechanisms and govern this disease are not fully understood yet.

The aim of this study was to investigate spatial and temporal changes in PAL activity and total phenolic content induced in susceptible and resistant pepper plants after infection with *P. capsici.* This information is critical to better understand resistance to *P. capsici* and how pepper respond in general to disease development. A better understanding of the plant's response to *P.capsici* infection will help develop strategies to improve plant defenses either through traditional breeding for plants with altered amounts and composition of phenylpropanoid compounds, or via genetic engineering.

MATERIALS AND METHODS

Plant material and growth conditions: Seeds of three *Capsicum annuum* cultivars, susceptible KM-Hot (Kahramanmaraş-Hot) and resistant PM 702 (Criollo de morelos = CM 334) were used in this study. The plants were maintained in a growth chamber under controlled environmental conditions (25±2°C & 16 h light/8 h dark period), for two months (approximately 7-8 true leaves).

Pathogenic *P. capsici* and seedling inoculation, and samples: *P. capsici*-22 (obtained from the Ankara Agricultural Faculty Collection, Ankara-Turkey) was grown on V_8 agar plates at 25°C in the dark. After zoospore production (Ward & Stoessl, 1974), the zoospores were collected and filtered through Whatman no: 54 to remove sporangial cases and mycelial fragments. Final concentration was adjusted to 10^4 zoospores per milliliter using a hemacytometer (Ward & Stoessl, 1974; Koç *et al*., 2011).

The roots of two month-old, uprooted seedlings (7 to 8 true leaves) were washed with tap water and disinfected by sodium hypochlorite (0.75%) for 1-2 min, before a final rinse. In 1 L of sterile distilled water which was added 1-2 drops of tween-20. Five washed seedlings were bunched together and wrapped with aluminum foil 3-4 cm above the root so the root necks plant crowns were at the same level.

Six groups of plants (30 seedlings in total) were put into a sterile glass bottles containing 400 mL of Hoagland solution. The plants were incubated for 3 days at 22±3ºC, 60% humidity and 14 h light period, so that they could acclimate. Six glass bottles were prepared for each pepper cultivar. Three days later, samples were taken out of seedling bunches from the plant breeding room, 100 mL zoospore suspension prepared at certain concentration (10^4) were put into 250 mL beakers and 100 mL sterile water was added. After seedling bunches were dipped into the solutions in the beakers, they were kept there for 1 h, and put into glass bottles again. Seedlings were taken from these glass bottles, and left to 22±2ºC, 60-65% humidity, and 14 h light periods on the 2nd, 4th and 6th days.

Despite the brown necrotic areas (the most severe damage) extending along the stems and roots of the pepper cultivars, the green parts of the plants exhibited less change. We then separated the stems and leaves into the upper parts. For this reason, while samples were taken, control and infected leaves (lower & upper parts), control and infected stem (lower, middle & upper) and roots were immediately separated, put into nylon bags, labeled and stored in a -70ºC deep freezer until analysis.

Phenylalanine ammonia-lyase (PAL; E.C. 4. 3. 1. 5) assay: PAL was extracted from the leaves, stems and roots (Ochoa & Salgado, 1992). PAL activity was quantified using the technique of (Ochoa & Gómez, 1993). The assay mixture contained 0.1 mL of extract, 1 mL 100 mM Tris-HCl buffer (pH 8.8), 0.5 mL of 10 mM L-phenylalanine, and 0.4 mL deionised water. The mixture was incubated for 1 h at 37°C and the reaction was terminated by adding 0.5 mL of 6 M HCl; then sample absorbance was measured at 290 nm. The calibration curve was constructed using cinnamic acid.

Total phenolic assay: Phenolics were extracted with 500 µL 80% methanol on an in a water bath (80°C) for 15 min and extracts was ultracentrifugated for 10 min at 500 g, then pellet was re-extracted with same method (Gayosa *et al.,* 2004). Phenol content was determined using the folin ciocalteu reagent (Singleton & Rossi, 1965). 100 µL of extracts 750 µL of Folin–Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 750 µL of (6%) Na_2CO_3 solution was added. After incubation for 90 min at room temperature, the absorbance against pre- pared reagent blank was determined at 765 nm with an UV-visible spectrophotometer (CECİL 5000). Total phenolic content of leaves and stems was expressed as mg. Total phenolic content of leaves, stems and roots was expressed as mg gallic acid was used as standard.

Statistical analyses: Trials were organized so to have three repetitions in randomized blocks experimental design. Data presented are mean values ± standard error measures for three replicates. Analysis of variance (ANOVA- factorial design) was employed to compare the means of two pepper cultivars and the significance of differences was determined by DUNCAN multiple comparison technique (Montgomery, 2005). Variance analysis was conducted by using Minitab 15.1 statistical software package, and DUNCAN tests were performed using the software package MSTAT-C statistics. The statistical significance is indicated by appropriate letters within the tables (α = 5%). Capital letters represent part differences in same cultivar, same group and same day (P< 0.05). Lower case letters represent differences in cultivars in same day, same group and same part (P< 0.05). Numbers represent differences in groups in same day, same cultivar and same part (P<0.05). Bold-lower-italic case letters represent days differences in same cultivar, same part and same group (P< 0.05).

RESULTS

An early induction of PAL was observed in the stem and root of peppers treated with *P. capsici* within two days. Increase in the PAL activity in the leaves of peppers was observed on the fourth day after inoculation. The highest PAL activity in non-inoculated (control) leaves and stems was recorded in CM-334 cultivar, which was significantly different than KM-Hot (P<0.05). Generally, pathogen inoculation increased PAL activity in both cultivars, compared with the non-inoculated controls (Fig. 1, 2 & 3).

In our study, PAL activity in the lower leaves of *P. capsici*-treated KM-Hot seedlings increased on days 4 and 6. It was observed that the PAL activity in middle and upper stems of inoculated susceptible cultivar (KM-Hot) increased with time (P<0.05). PAL activity of lower stems slightly decreased (P<0.05) with time (Fig. 1).

Increase in the PAL activity was observed in leaves of CM-334 inoculated with 10^4 zoospore/mL *P.capsici,* on the fourth day following inoculation, but there was a highly significant increase in induction of PAL reaching a peak on the sixth day. The highest PAL activity was recorded in lower leaves of CM-334 plants. When compared to the control, PAL activity on the sixth day after inoculation with *P. capsici* was significantly higher (P<0.05) in lower and upper leaves of CM-334 plants. The increases in their values were 206.387% and 113.213%, respectively (Fig. 1). The differences found on the fourth and sixth day was significant (P<0.05) in lower stems of CM-334 plants (Fig. 2). When compared to control lower stems, the greatest PAL activity in lower stems of CM-334 inoculated with *P.capsici* was detected on the sixth day (+102.189%) (Fig. 2).

P. capsici induced maximum production of the enzyme in lower leave and stems on the sixth day, that is in the challenge-inoculated set of plants, the enzyme induction was highest on six days after inoculation.

PAL activity in *P. capsici*-treated roots increased throughout the experimental period. In our study, PAL activity in roots of *P. capsici*-treated CM-334 and KM-Hot seedlings definitely increased on days 2, 4 and 6 (P<0.05). When compared to control roots, the maximum increase of PAL was observed in the roots of CM-334 seedlings infected with *P.capsici*, on the second (+ 98.7%), fourth (+133.80%) and sixth (+135.33%) days following the infection (P<0.05) (Fig. 3).

Large increases in the concentration of the phenolic were found only in the upper parts of *P. capsici* -inoculated leaves of two cultivars, where symptoms occurred. Higher concentrations were detected in the CM-334 upper stems samples than in KM-Hot six day after infection (Fig. 4, 5 & 6).

Decrease in the phenolic content was observed in lower leave of CM-334 and lower-upper leave of KM-Hot infected with *P. capsici,* on the second day following the infection (P<0.05), but there was a significant increase in induction of phenolic on the 6^{th} day (P<0.05) (Fig. 4). When compared to control, the maximum increase of phenolic content was observed in the upper stems of KM-Hot seedlings infected with *P. capsici*, on the sixth (+129.6%) days following the infection (P<0.05) (Fig. 5). Pathogen stress induced decrease in phenolic content in stems of CM-334. The phenolic content was lower compared with control, on the second day following the infection (P<0.05). The phenolic content reached maximum levels on the sixth day after challenge inoculation (Fig. 4, 5 & 6). When considering total phenolic contents showing a decrease only in roots. The roots in inoculated plants showed lower levels than control pepper roots, on the fourth and sixth days following the infection (P<0.05) (Fig. 6).

DISCUSSION

Many plant enzymes such as PAL are involved in defense reactions against plant pathogens. This enzyme has been correlated with defense against pathogens in several plants, including tomato (Borden & Higgins, 2002), pepper (Jung *et al*., 2004) and wheat (Mohammadi & Kazemi, 2002).

Fig. 1: PAL activity (Cinnamic acid (µmolg^{-1} FW) in leaves of peppers subjected to *P. capsici* (P <0.05) (I: Control Upper-leave, II: Infected upper leave, III: Control lower leave, IV: Infected lower leave)

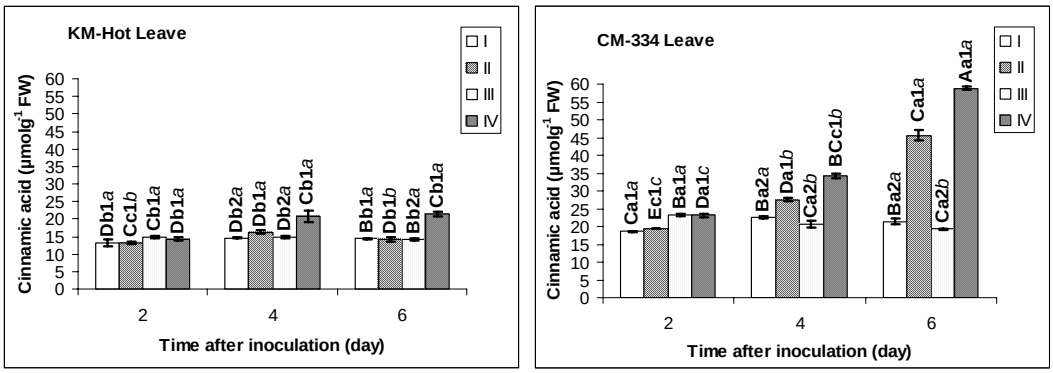


Fig. 2: PAL activity (Cinnamic acid (µmolg^{-1} FW) in stems of peppers subjected to *P. capsici* (P <0.05) (I: Control Upper-stem, II: Infected upper stem, III: Control middle stem, IV: Infected middle stem III: Control lower stem, IV: Infected lower stem)

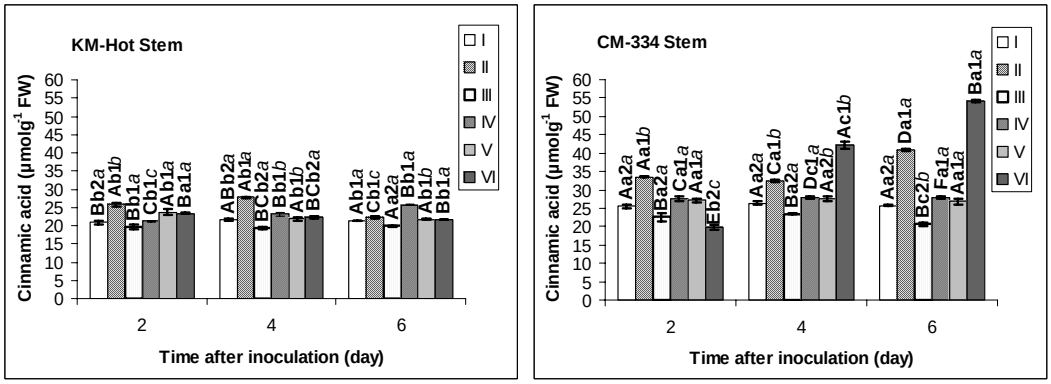
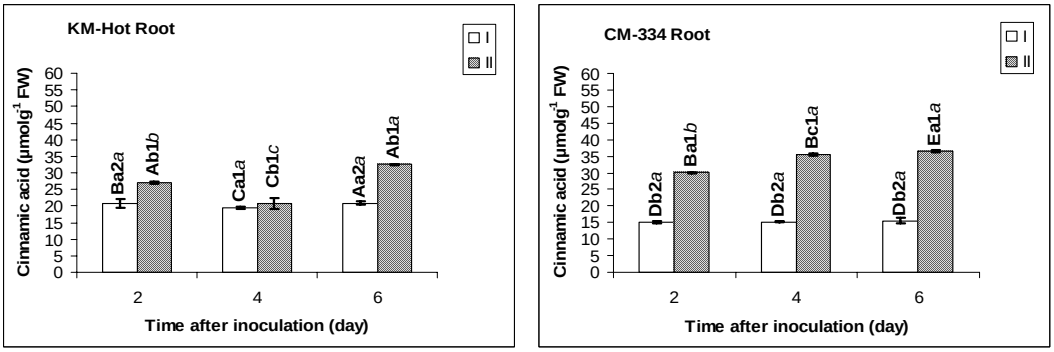


Fig. 3: PAL activity (Cinnamic acid (µmolg^{-1} FW) in roots of peppers subjected to *P. capsici* (P <0.05)(I: Control root, II: Infected root)



In our study, an increase in the PAL activity was observed in the stems and roots of pepper inoculated with *P. capsici* within two days of inoculation, making it one of the earlier responses to infection detected.

Increase in the PAL activity in the leaves of peppers was observed on the 4th day following the infection. An early induction of PAL is very important as the biosynthesis of lignin originate from L-phenylalanine (Paul & Sarma, 2005). Phenylpropanoid metabolism is defined as the sequence of reactions involved in the conversion of L-phenyl alanine to activated cinnamic acids (Hahlbrock & Grisebach, 1975). PAL accumulation in induced plants, may reduce phenylalanine, which is necessary for pathogen growth and development (Liu & Rahe, 1997). This is might be due to the inhibitory effect of PAL on pathogen growth. Transcinnamic acid which is an immediate precursor for the biosynthesis of salicylic acid, a signal molecule in systemic acquired resistance (SAR) (Klessig & Malamy, 1994).

Fig. 4: Changes in the content of phenolic compounds (Gallic acid (mgg^{-1} FW) in leaves of peppers subjected to *P. capsici* (P <0.05). (I: Control Upper-leave, II: Infected upper leave, III: Control lower leave, IV: Infected lower leave)

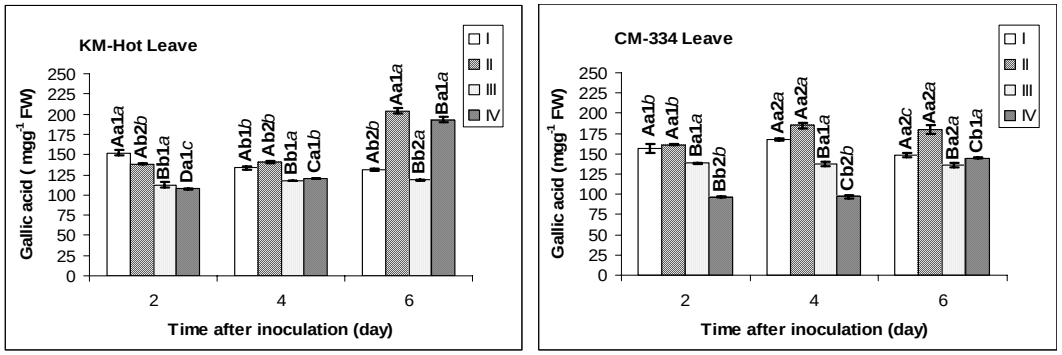


Fig. 5: Changes in the content of phenolic compounds (Gallic acid (mgg^{-1} FW) in stems of peppers subjected to *P. capsici* (P <0.05) (I: Control Upper-stem, II: Infected upper stem, III: Control middle stem, IV: Infected middle stem III: Control lower stem, IV: Infected lower stem)

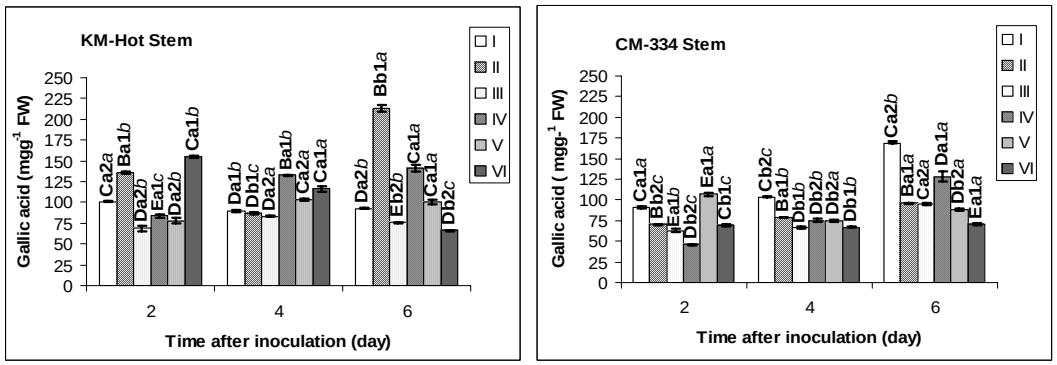
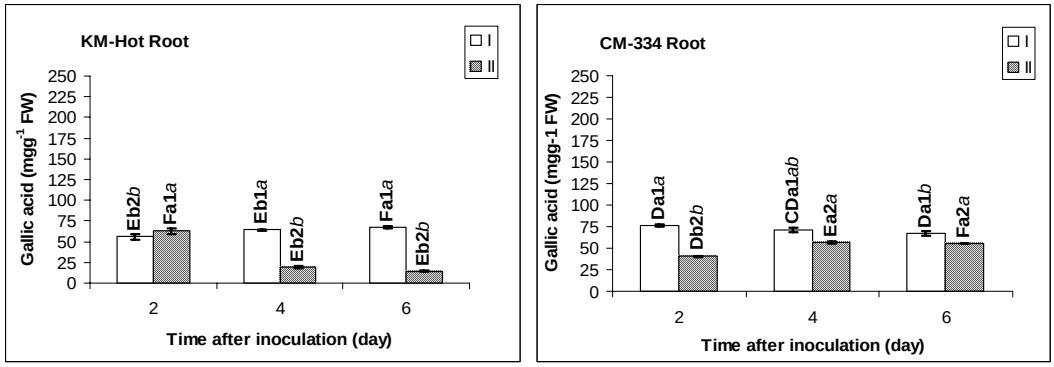


Fig. 6: Changes in the content of phenolic compounds (Gallic acid (mgg^{-1} FW) in roots of peppers subjected to *P. capsici* (P <0.05) (I: Control root, II: Infected root)



Initial pathogen infection may increase resistance to future pathogen attack. Thus, eventhough plants lack immune systems, they may develop eleborate mechanisms to protect themselves from disease-causing pathogens.

In this study, the resistant pepper cultivar responded was faster and greater than that of the susceptible cultivar, as indicated by increases in PAL. Since defence mechanisms induced in pepper depend on the level of PAL activity, their post-infectional response was related to an increase in enzyme activity (El Modafar *et al*., 2000; El Modafar *et al*., 2001; El Modafar & El Boustani, 2001). In our study, this differential response in PAL activity induction in resistant and susceptible cultivars could explain the difference in the speed and intensity of defence reactions induction observed in both cultivars.

Phenolic is used as indicator for any stress conditions that occur to plants. The first step of defense mechanism in plant involves a rapid accumulation of phenols at infection sites, which slows or restrict pathogen growth and development (Gogoi *et al.*, 2001). Altering the level of phenolic compounds in peppers may be disease susceptibility. The induced lignification has been proposed as a mechanism of disease in resistant plant against invasion of pathogen (Goodman *et al*., 1986; Lozovaya *et al.*, 2004) by making host walls more resistant to mechanical penetration. In our study, the changes in phenolic metabolism were localized in stems and leaves rather than in the lesion-containing areas of roots under the *P. capsici* inoculum. The infected roots, where symptoms were more visible did not show marked phenolic accumulation compared with the corresponding controls. This variability might be caused by mechanical damage, which is difficult to avoid during the sampling or pathogen challenge might cause damage and necrosis, which in turn decreases the accumulation of phenolic in pepper roots. No clear correlation was found between the PAL activity and phenolic levels in roots tissues. The present study showed that phenolic content accumulated significantly in leaves (specially upper) and stem (specially middle- upper) of pepper seedlings, compared with controls. Initial pathogen infection at lower sites may be increase resistance to invasion of pathogen through development of systemic acquired resistance. The phenolic content may contribute to enhance the mechanical strength of host cell wall, may also inhibit the fungal growth. The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Earlier studies also demonstrated that rapid esterification of phenolic compounds into the plant cell wall is a common and early response in the expression of resistance (Saikia *et al.,* 2006). All these results suggested that induction of PAL and phenolic by *P. capsici*-22 might result in the activation of defence.

In conclusion, *P. capsici*-22 was effective inducers of pathogen defence responses in pepper. This induced resistance by *P. capsici* was associated with the activation of defence-related enzymes such as PAL, accumulation of phenolics, and enhancement of antifungal activity of pepper, all of which may affect the growth and development of *P. capsici.* There is no doubt that PAL activity and phenolic content are a good indicator reflecting health and stress conditions of plants.

REFERENCES

Black, L.L. and T. Berke, 1998. *Breeding for Phytophthora Resistance in Pepper*, pp: 115–119. Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant, Avignon, France

Borden, S. and V.J. Higgins, 2002. Hydrogen peroxide plays a critical role in the defence response of tomato to *Cladosporium fulvum*. *Physiol. Mol. Plant Pathol.* 61: 227–236

Cinar, A. and M. Bicici, 1977. Control of *Phytophthora capsici* Leonian on red peppers. *J. Turkish Phytopathol.,* 6: 119–124

El Modafar, C., A. Tantaoui and E. El Boustani, 2000. Changes in cell wallbound phenolic compounds and lignin in roots of date palm cultivars differing in susceptibility to *Fusarium oxysporum* f. sp. *albedinis*. *J. Phytopathol*., 148: 405–411

El Modafar, C., A. Tantaoui and E. El Boustani, 2001. Differential Induction of Phenylalanine Ammonia Lyase in date palm roots in response to inoculation with Fusarium oxysporum f. sp. albenidis and to elicitation with fungal elicitor. *J. Plant Physiol.*, 158: 715–722

El Modafar, C. and E. El Boustani, 2001. Cell wall-bound phenolic acid and lignin contents in date palm as related to its resistance to *Fusarium oxysporum. Biol. Plant,* 44: 125–130

Gayosa, C., F. Pomar, F. Merino and M.A. Bernal, 2004. Oxidative metabolism and phenolic compounds in *Capsicum annuum* L. *var. annuum* infected by *Phytophthora capsici* Leon*., Sci. Hortic*., 102: 1–13

Gogoi, R., D.V. Singh and K.D. Srivastava, 2001. Phenols as abiochemical basis of resistance in wheat against karnal bunt. *Plant Pathol*., 50: 470–476

Goodman, R.N., Z. Kiraly and K.R. Wood, 1986. *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press, Columbia

Hahlbrock, K. and H. Grisebach, 1975. *In*: Harbourne, J.B., T.J. Mabry and H. Mabry (eds.), The flavonoids, pp: 866–915. Chapman and Hall, New York

Jung, W.J., Y.L. Jin, Y.C. Kim, K.Y. Kim, R.D. Park and T.H. Kim, 2004. Inoculation of *Paenibacillus illinoisensis* alleviates root mortality, activates of lignification-related enzymes, and induction of the isozymes in pepper plants infected by *Phytophthora capsici. Biol. Cont.,* 30: 645–652

Kım, Y.J., B.K. Hwang and K.W. Park, 1989. Experssion of age- ralated resistance in pepper plants infected with *P. capsici. Plant Dis*., 73: 745–747

Kimble, K.A. and R.G. Grogan, 1960. Resistance to *Phytophthora* root rot in pepper. *Plant Dis*. *Rep*., 44: 872–873

Klessig, D.F. and A.J. Malamy, 1994. The salicylic acid signaling in plants. *Plant Mol. Biol*., 26: 1439–1458

Koç, E., A.S., Üstün, C. İşlek and Y. Kaşko Arıcı, 2011. Defence responses in leaves of resistant and susceptible pepper (*Capsicum annuum* L.) cultivars infected with different inoculum concentrations of *Phytophthora capsici* Leon*. Sci. Hortic.,* 128: 434–442

Kunimoto, R.K., M. Aragaki, J.E. Hunter and W.H. Ko, 1976. *Phytophthora capsici*, corrected name of the cause of *Phytophthora* blight of macadamia racemes. *Phytopathology,* 66: 546–548

Leonian, L.H., 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici sp. nov. Phytopathology,* 12: 401–408

Liu, L. and J.E. Rahe, 1997. Altered root exudation and suppression of induced lignification as mechanisms of predisposition by glyphosate of bean roots (*Phaseolus vulgaris* L.) to colonization by *Pythium* spp. *Physiol. Mol. Plant Pathol.,* 51: 111–127

Lozovaya, V.V., A.V. Lygin, S. Li, G.L. Hartman and J.M. widholm, 2004. Biochemical response of soybean roots to *Fusarium solani* f. sp. *glycines* infection. *Crop Sci*., 44: 819–826

Mohammadi, M. and H. Kazemi, 2002. Changes in peroxidase and polyphenol activity in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Sci.,* 162: 491–498

Montgomery, D.C., 2005. *Design and Analysis of Experiments*, 6th edition. John Wiley and Sons, New York

Ochoa, A.N. and G.R. Salgado, 1992. Phenylalanine ammonia-lyase activity and capsaicin-precursor compounds in ñ-fluorophenylalanine-resistant and -sensitive variant cells of chili pepper (*Capsicum annuum*). *Physiol. Plant,* 85: 173–179

Ochoa, A.N. and P.J.E. Gómez, 1993. Activity of enzymes involved in capsaicin biosynthesis in callus tissue and fruits of chili pepper (*Capsicum annuum* L.). *J. Plant Physiol.,* 141: 147–152

Oelke, L.M., P.W. Bosland and R. Steiner, 2003. Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Capsicum annuum*. *J. American Soc. Hortic. Sci.,* 128: 213–218

Paul, D. and Y.R. Sarma 2005. Pseudomonas fluorescens mediated systemic resistance in black pepper (*Piper nigrum* L.) is driven through an elevated synthesis of defence enzymes. *Arch. Phytopathol. Plant Prot.,*38: 139–149

Para, G. and J.B. Ristaino, 1998. Insensitivity to Ridomil Gold (mefenoxam) found among field isolates of *Phytophthora capsici* causing *Phytophthora* blight on bell pepper in North Carolina and New Jersey. *Plant Dis.*, 82: 711

Pochard, E., A. Palloix and A.M. Daubeze, 1986. *The Use of Androgenetic Autodiploid Lines for the Analysis of Complex Resistance Systems in Pepper*, pp: 105–109. In dupication general de aragon, ed. VI Meeting on genetics and breeding on capsicum and eggplant. Zaragoza, España

Ristaino, J.B., 1990. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology,* 80: 1253–1259

Roberts, P.D., R.J. Mc Govern, T.A. Kucharek and D. Mitchell, 2008. *Vegetable Diseases Caused by Phytophthora Capsici in Florida*. Plant pathology department document SP159. Florida cooperative extension service, Institute of Food and Agricultural Sciences, University of Florida

Saikia, R., M. Yadavl, P.B. Singl, K.G. Dip, T. Singh and D.K. Aroral, 2006. Induction of resistance in chickpea by cell wall protein of *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina*. *Curr. Sci.,* 91: 1543–1546

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American J. Enol. Vitic.,* 16: 144–158

Ward, E.W.B. and A. Stoessl, 1974. Isolataion of the phyoalexin capsidiol from pepper leaves and stems. *66th Annual Meet. of the American Phytopathol. Soc*., pp: 11–15. Vancouver, Canada

Woo, J., J. Yu, K. Kil, P. Ro and K. Tae, 2005. Changes in pathogenesis-related proteins in pepper plants with regard to biological control of *Phytophthora* blight with *Paenibacillus illinoisensis*. *Biocontrology,* 50: 165–178

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