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Effect of Mycorrhizal Fungi on Some Defense Enzymes Against *Gaeumannomyces gaminis* in Wheat

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Abstract: At this research, the effect of mycorrhizal fungi (*Glomus etunicatum*) On Pero Xidase (POX) and Phenylalanine Ammonia Lyase (PAL) activities and isozymatic pattern against *Gaeumannomyces graminis* were studied in wheat plants. Seeds were planted in inoculated soils in 4 treatment groups including Control (C), Mycorrhiza (M), Pathogen (P) and Pathogen- Mycorrhiza (PM). Plants were harvested 17 days after inoculation. POX activities in PM group were significantly greater than control group. Significant differences were not observed between P and C groups. POX activities significantly decreased in M group. PAL activities in M group were significantly greater than other groups. PAL activities in P and PM groups were significantly greater than C group. The appearance of new isozyme was induced in PM group. It is highly probable that induced POX isozymic activity and/or appearance of new isozymes may be responsible for elevated POX activity. Present results showed that the isozymatic patterns of POX were changed by inoculation of mycorrhiza and/or pathogen. The obtained results from this research is agreement with other researches about the enhancing effect of mycorrhizal fungi on PAL activity. The obtained results from the present research, confirm this opinion that defense related proteins is not induced in compatible interactions or is weak.

Key words: Mycorrhizae, peroxidase, phenylalanine ammonia lyase, defense responses, pathogen

INTRODUCTION

When a plant recognizes an invading pathogen, active defense mechanisms are induced which can include a hypersensitive response, accumulation of antimicrobial phytoalexins, synthesis of hydrolases and pathogenesis related proteins, reinforcement of cell walls through callose deposition and lignification and activation of defense related genes (Bruce *et al*., 1989; He *et al*., 2001).

Beneficial microorganisms that improve plant health trough the enhancement of plant resistance/tolerance against biotic stresses include bacteria, such as *Pseudomonas* spp. or *Bacillus* spp. and fungi such as *Trichoderma* sp., *Gliocladium* sp. or mycorrhizal fungi (Azcon-Aguilar and Barea, 1996, Pozo *et al*., 2002). Arbuscular mycorrhizal fungi which form symbiosis associations with root system of most agricultural, horticultural and hard wood crop species, have been suggested as widespread potential bioprotective agents (Pozo *et al*., 2002). Many authors have reported that Arbuscular mycorrhizal symbiosis can reduce root disease caused by several soil borne pathogens including *Phytophthora* species (Pozo *et al*., 2002), for example colonization of tomato plants by *Glomus mossae* has been demonstrated to reduce disease development in plants infected with *Phytophthora parasitica* (Pozo *et al*., 1996). How ever, the mechanisms underlying this protective effect are still not well understood (Pozo *et al*., 2002, 1996, 1999, Cordier *et al*., 1996, 1998, Trotta *et al*., 1996). At the present research, the effects of mycorrhizal fungi on defense system were studied against *Gaeumannomyces graminis* fungi in wheat plants.

MATERIALS AND METHODS

preparation of mycorrhizal inoculum: Inocula of the Arbuscular mycorrhizal fungi used were isolate of *Glomus etunicatum* from Jahad Research Center of Tehran university. All mycorrhizal inocula consisted of soil, spores, mycelium and infected root fragments from culture of zeamays.

Experimental design: *Gaeumannomyces graminis* var. Tritici. Was cultured on PDA medium. Inocula of this fungus was prepared as described by Simon (1987) and Wilkinson (1985).

Preparation of soil and inoculation of prepared soil: Wheat seeds (*Triticum aestivum* L.) were prepared from seed center of karaj and sterilized with nathrium hypocolorite 1% (v/v) for 15 min.

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Prepared soil contained sand, clay and pit (8:1:1, respectively) . This soil was inoculated with mycorrhiza and/or pathogen inocula (10% (v/v) and 0.5 % (w/w) respectively). Sterilized seeds were planted in inoculated soils in 4 treatment groups including control (C), mycorrhiza (M), pathogen (P) and pathogen-mycorrhiza (PM).Plants were cultured in natural conditions and harvested 17 days after inoculation.

Preparation of enzyme extracts and quantification of protein content: Enzymes were extracted at 4 centigrade degree in a mortar and pestle from 1 g (f.w.) root tissue using nathrium phosphate (0.1 M pH 7) as a extraction buffer containing 0.1 mM EDTA and pvpp 1% (w/v). The homogenate were centrifuged at 13000 rpm. for 30 min. at 4 centigrade degree and supernatants were used to measure enzyme activities. Protein content of the enzyme extracts were measured according to the procedure of Bradford (1976) using BSA as the standard.

Determination of peroxidase activity: POX activities were measured according to the procedure of Biles and Abeles (1991). The peroxidase activities were expressed as an increase in absorbance per min per microgram protein ($\Delta Amin^{-1}\ \mu g^{-1}$ pr.).

Determination of phenylalanine ammonia lyase (PAL) activity: The reaction mixture for PAL activity consisted of 6 µM phenylalanine (phe), Tris-HCl buffer (0.5 M pH 8) and 200 µL of enzyme extract. After 60 min at 37°C, the reaction was terminated by the addition of 50 µL of 5N HCl. PAL activity was assessed by measuring the amount of cinnamic acid produced at 290 nm and is expressed as microgram of cinnamate per microgram of protein (µgcin μg^{-1}pr.), according to the procedure described by Beaudoin-Eagan and Thrope (1985).

Electrophoresis of enzyme extracts: Non denaturing gel electrophoresis of enzyme extracts were carried out according to Laemmli (1970).The resolving and stacking gels were 10% and 4% (w/v), respectively. When the dye front had reached 0.5 Cm. from the bottom of the gel, electrophoresis was stopped and gel were immersed in POX reaction mixture containing 80 mL acetate buffer (0.2M, pH4.8), 8 mL of H_2O_2 3% (v/v) and 4 mL of benzidine (0.04 M in methanol 50% (v/v)) in dark conditions on shaker for 90 min relative mobility of each isozyme was calculated.

Statistical procedure: Analysis of variance was performed on all data sets. Duncan test with probability of 0.05 was used to show significant differences between treatments. All data are presented as mean±S.E.

RESULTS

POX activities in PM group were significantly greater than control group. Significant differences were not observed between P and C groups. POX activities significantly decreased in M group (Fig. 1).

PAL activities in M group were significantly greater than other groups. PAL activities in P and PM groups were significantly greater than C group. PAL activities in PM group were greater than P group but this difference was not significant (Fig. 2).

The appearance of new isozyme with RM 0.016 was induced in PM group. Isozyme with RM 0.06 was observed in all treatment groups. Isozyme with RM. 0.07 was strongly observed in C group and in other groups, was weak. Isozyme with RM 0.44 was slightly observed in P and PM groups. Isozyme with RM 0.8 was observed in all treatment groups including C, M, P and PM but this isozyme in PM and P groups was stronger than C and M groups (Fig. 3).

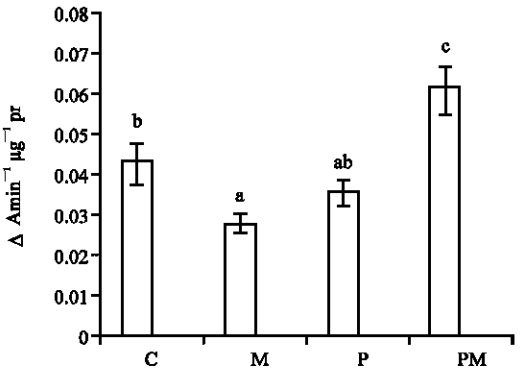

Fig. 1: Mean±S.E. of peroxidase activities in 4 treatment groups including control (C), mycorrhiza (M), pathogen (P) and pathogen- mycorrhiza (PM)

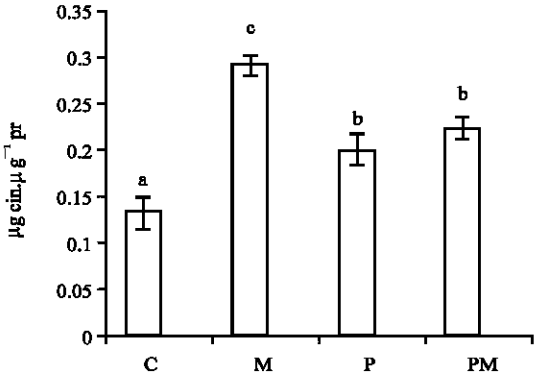

Fig. 2: Mean±S.E. of phenylalanine ammonia lyase activities in 4 treatment groups including control (C), mycorrhiza (M), pathogen (P) and pathogen-mycorrhiza (PM)

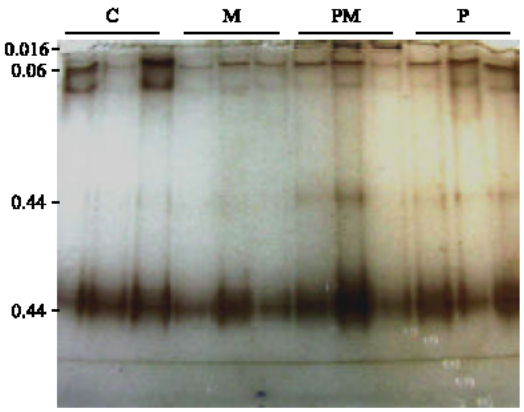

Fig. 3: Isozymatic pattern of peroxidase in 4 treatment groups including control (C), mycorrhiza (M), pathogen (P) and pathogen-mycorrhiza (PM)

DISCUSSION

Among the biochemical responses, the most rapid are increases in enzymatic activity including POX and PAL (Welbull and Niemeye, 1995). The activity of POX is a useful marker for localized and systemic acquired responses in plants challenged by a pathogen (Martinez *et al*., 2000). Local or systemic changes in POX and PAL activities upon *Fusarium* inoculation were associated with hypersensitive response induction in *Asparagus densiflorus* (He *et al*., 2001) increased activity of POX and PAL has been reported in plants treated with various abiotic and biotic inducers of resistance (Ruiz *et al*., 1999). Both the timing and the localization of increased POX activity may be important in limiting pathogen infections (Gay and Tuzan, 2000). POX may enhance defense by the production of toxic radicals (Asiegbu *et al*., 1994). POX activity was rather delayed or remained unchanged during the compatible interaction in susceptible plants (Mohammadi and Kazemi, 2002). The role of POX in plant defense mechanism has been attributed to its ability to oxidize key metabolites (i.e. phenolics) in plant or pathogen (Mohammadi *et al*., 2002).oxidative enzymes such as POX have been shown to be responsible for the oxidation of phenolics into antimicrobial quinones in plant cells attacked by phytopathogens and thus disease resistance during incompatibility (Reuveni, 1995). POX activity increased more rapidly in resistant than in susceptible plants after inoculation with the pathogen (Svalheim and Robertser, 1990, Mohammadi Kazemi., 2002). POX is also involved in lignin biosynthesis and degradation of cytotoxic levels of H_2O_2 generated in plant tissues as a result of pathogen attack (Van Loon, 1997, Svalheim and Robertsen, 1990). Pretreatment of susceptible wheat heads with heat killed mycelial wall preparation resulted in the elevation of POX activities, blockage in fungal spread and induced resistance in plant following inoculation with *Fusarium graminearum* (Mohammadi and Karzeni, 2002). It has been reported that muskmelon plants with higher POX activities were more resistant to pathogen than those with lower activities of the enzyme (Reuvani, 1995). POX is part of the pathogenesis related protein- 9 (PR-9) family (Chen *et al*., 2000). During the initial stages of colonization of *Allium porrum* by *Glomus versiform* before arbuscules form, chitinase and POX activities in roots were elevated compared to those from the uninoculated controls, At later stages, these activities in colonized roots dropped below levels in controls as colonization continued, (Spanu *et al*., 1989: Spana and Bonfante, 1988. Blee and Anderson, 1996). In mycorrhizal tobacco plants, transient increases of catalase and POX were observed to coincide with appressoria formation and fungal penetration into the root (Blilou *et al*., 2000). Interestingly, similar results have been shown in onion and been roots inoculated with arbuscular mycorrhizal fungi (Spanu and Bonfante 1988; Lambais, 2000).

In this research, the appearance of new isozyme with RM 0.016 was induced in PM group. It is highly probable that induced POX isozymic activity and or appearance of new isozymes may be responsible for elevated POX activity. Present results showed that the isozymatic patterns of POX were changed by inoculation of mycorrhiza and or pathogen. Remarkable increase in POX and appearance of 2 to 5 new isozymes were observed in cotton bolls inoculated with *Rhizoctonia solani* (Mellon and Lee, 1985). Elevated POX activity has been associated with the appearance of one or more POX isozymes in plant reacting hyper sensitively (Mohammadi and Kazemi, 2002). Alterations in the isoenzymatic patterns such as chitinases (Pozo *et al*., 1996), chitosanases (Pozo *et al*., 1998), and β 1,3 glucanases (Pozo *et al*., 1999) have previously been shown during mycorrhizal colonization of tomato roots with the induction of new isoforms.

PAL catalyses the first committed step in the biosynthesis of a wide array of phenolic compounds. Therefore, PAL has a major influence on phenylpropanoid biosynthesis. The phenylpropanoid pathway catalyzed by the PAL, leads to the formation of diverse derivatives such as phenolics, lignin, suberin, flavonoides, isoflavonoides, coumarins and soluble esters that many of these derivatives are involved in plant defense responses (Goldwasser *et al*., 1999). PAL was increased in both incompatible and compatible interactions between plants and pathogen (Chen *et al*., 2000). PAL could also

be activated by fungal elicitors or wounding (Chen *et al*., 2000, Redman *et al*., 1999). PAL activity is affected by a number of factors including light, temperature, growth regulators, inhibitor of RNA and protein synthesis, wounding, mineral nutrition and elicitor treatment (Mohr and Chaill, 2001).Activation of PAL in *Asparagus densiflorus* could directly affect accumulation of secondary toxic compounds, such as phytoalexins, which might be released in root exudates and on root segment surfaces from the inoculated plants to inhibit fungal spore germination and growth (He *et al*., 2001). Local or systemic changes in POX and PAL activities upon *Fusarium* inoculation were associated with hypersensitive response induction in *Asparagus densiflorus* (He *et al*., 2001). RNA blot analysis revealed change in the accumulation of PAL transcripts from the 28-day mycorrhizal roots compared to the uninoculated controls but, accumulation of PAL occurred only in arbusculated cells (Blee and Anderson, 1996). In mycorrhizal roots, the transient accumulation of salicylic acid was correlated to an increase of expression of genes encoding PAL and Lipid Transferring Proteins (LTP). Thus the induction of these activities in mycorrhizal symbiosis may be involved in the protector effect against fungal pathogens (Dumas-Gaudot *et al*., 1996). The obtained results from this research is agreement with other researches about the enhancing effect of mycorrhizal fungi on PAL activity. The obtained results from the present research, confirm this opinion that defense related proteins is not induced in compatible interactions or is weak.

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REFERENCES

Asiegbu, F.O., G. Daniel and M. Johnsson, 1994. Defence related reactions of seedling roots of Norway spruce to infection by *Heterobasidium annosum*. Physiol. Mol. Plant Pathol., 45: 1-19.

Azcon-Aguilar, C. and J.M. Barea, 1996. Arbuscular mycorrhizas and biological Control of Soil-borne plant pathogens-an over view of the mechanisms involved. Mycorrhiza, 6: 457-464.

Beaudoin-Eagan, L.D. and T.A. Thorpe, 1985.Tyrosine and phenylalanine ammonia-yase activities during shoot initiation in to bacco callus cultures. Plant Physiol., pp: 438-441.

Biles, C.L. andF.B. Abeles, 1991. Xylem sap proteins, Plant Physio., 96: 597-601.

Blee, K.A. and A.J. Anderson, 1996, Defense related transcript accumulation in *Phaseolus vulgar L.* colonized by the arbuscular mycorrhizal fungus *Glomus intraradices*. Plant Physiol., 10: 675-688.

Blilou, I., J.A. Ocampo and J.M. Garcia-Garrido, 2000. Induction of LTP (lipid transfer protein) and PAL (Phenylalanine ammonia-lyase) gene expression in Rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. J. Exp. Bot., 51: 1969-1977.

Bradford M.M., 1977, A rapid and sensitive method for the quantifiation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Bruce, J.R. and C.A. West, 1989. Elicritation of lignin biosynthesis and isoperoxidase activity by pectic Fragment in suspension cultures of castor Bean. Plant Physil., 889-897.

Chen, C., R.R. Belanger, N. Benchamou and T. Paulitz, 2000. Defense enzymes induced in cucumber roots by treatment with plant growth promoting Rhizobacteria (PGPR) and *Pythium aphanidermatum*, Physiol. Mol. Plant Pathol., 56: 13-23.

Cordier, C., S. Gianinazzi and V. Gianinnazzi-pearson, 1996. Colonization patterns of root tissues by *Phytophthora nicotiana* var. parasitica related to reduced disease in mycorrhizal tomato. Plant and Soil, 185: 223-232.

Cordier, C., M.J. Pozo, J.M. Barea, Gianinazzis and V. Gianinazzi -Pearson, 1998. Cell defense responses assocaiated with localized and systemic resistance to phytophthora induced in tomato by an arbuscular mycorrhizal fungus. Molecular Plant-Microbe Intera., 11: 1017-1028.

Dumas-Gaudot, E., S. Slezack, B. Dassi, M.J. Pozo, V. Gianinazzi-Pearson and S. Gianinazzi, 1996. Plant hydrolytic enzymes (Chitinases and B-1,3 glucanases) in root reactions to pathogenic and symbiotic microorganisms. Plant and Soil, 185: 211-221.

Gay, P.A. and S. Tuzun, 2000, Temporal and spatial assessment of defense responses in resistant and susceptible cabbage varieties during infection with *Xanthomonas campestris* pv. Campestris. Physiol. Mol. Plant Pathol., 57: 201-210.

Gold wasser, Y., J. Herschhorn, D. Plakhine, Y. Klefeld and B. Rubin, 1999. Biochemical factors involved in vetch resistance to *Orobanche aegyptiaca*. Physiol. Mol. Plant Pathol., 54: 87-96.

He, C.Y., T. Hsiangy and O.J. Wolyn, 2001, Activation of defense responses to fusarium infection in *Asparagalus densiflorus*, European J. Plant Path., 107: 473-483.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4, Nature, 227: 680-685.

Lambais, M.R., 2000. Regulation of Plant Defdense Related Genes in Arbuscular Mycorrhizae. In: Podila, G.K. and D.D. Douds (Eds). Current Advances in Mycorrhizae Research. St Paul, Minnesota: APS Press, 45-59.

Martinez, C., J.C. Baccou, E. Bresson, Y. Baissac, J.F. Daniel, A. Jalloui, J.L. Montillet, J.P. Geiger, K. Assigblse and M. Nicole, 2000. Salicylic acid mediated by the oxidative burst in a key molecule in local and systemic responses of cotton challenged by an aviralont race of *Xanthomonas campestris* pv. Malvacearu. Plant Phy., 122: 757-766.

Mellon, J.E. and L.S. Lee, 1985. Elicitation of cotton isoperoxidases by *Aspergillus flavus* and other fungi pathogenic September 19, 2006. to cotton, Plant Physiol. Pathol., 27: 281-288.

Mohammadi, M. and H. Kazemi., 2002. Changes in peroxidase and polyphenoloxidase activities in susceptible and resistant Wheat heads inoculated with fusarium graminearum and indced resistance. Plant Sci., 162: 491-498.

Mohr, P.G. and D.M. Cahill, 2001. Relative roles of glyceollin, lignin and the hypersensitive response and the influence of abscisic acid in compatible and incompatible interactions of soybeans with *Phytophthora sojae*. Physiol. Mol. Plant Pathol., 58: 31-41.

Pozo, M.J., E. Dumas-Gaudot, S. Slezack., C. Cordier, A. Asselin, S. Gianinazzi, V. Gianinazzi-Pearson, C. Azcon-Aguilar and J.M. Barea, 1996. Detection of new chitinase isoforms in arbuscular mycorrhizal tomato roots: Possible implications in protection against *Phytophthora nicotiana* var. parasitica. Agronomie, 16: 689-697.

Pozo, M.J., E. Dumas-Gaudot, C. Azcon-Aguilar and J.M. Barea , 1998. Chitosanase and chitinase activities in tomato roots during interactions with arbuscular mycorrhizal fungi or *Phytophthora parasitica*. J. Exp. Bot., 49: 1729-1739.

Redman, R.S., S. Freeman, D.R. Elifton, D.J. Morre, G. Brown and R.J. Rodringuez, 1999. Biochemical analysis of plant protection afforded by a non pathogenic endophytic mutant of *Colletotrichum magna*. Plant Physiol., 119: 795-804.

Reuveni, R., 1995. Biochemical Markers for Disease Resistance, In: Singh, R.P. and U.S. Singh (Eds), Molecular Methodes in Plant Pathology, Crc Press, Boca Raton, FL, pp: 99-144.

Ruiz, J.M., P.C. Garcia, R.M. Rivero and J. Romero, 1999. Response of phenolic metabolism to the application of carbendazim plus boron in tobacco. Physiol. Plant., 106: 151-157.

Simon, A., A.D. Rovira and R.C. Foster., 1987. Inocula of *Gaeumannomyces graminis* for field and glasshouse studies. Soil Biol. Biochem., 19: 363-373.

Spanu P. and F.P. Bonfante, 1988. Cell wall bound peroxidase activity in roots of mycorrhizal *Allium porrum*. New Phytol., 109: 119-124.

Spanu, P., T. Boller, A. Ludwing, A. Wiemken and A. Faccio, 1989. Chitinase in roots of mycorrhizal Allium porrum: Regulation and localization. Planta, 177: 447-455.

Svalheim, A. and B. Robertsen, 1990. Induction of peroxidases in cucumber hypocotyls by wounding and fungal infection. Physio. Plant., 78: 261-267.

Trotta, A., G.C. Rarese E. Gnavl, A. Fusconi , S. Samp and G. Berta, 1996. Interactions between the soil-borne root pathogen *Phytophthora nicotianae* var. parasitiea and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant and Soil, 185: 199-209.

Van-Loon, L.C., 1997. Induced resistance in plants and the role of pathogenesis related proteins, Eur. J. Plant Pathol., 103: 753-765.

Welbull, J. and H.M. Niemeyer, 1995. Changes in dihydroxy methoxybenzoxazinone glycoside content in wheat plants infected by three plant pathogenic fungi. Physiol. Mol. Plant Pathol., 47: 201-212.

Wilkinson, V.T., R.J. Cook and J.R. Alldredge, 1985. Relation of inoculum size and concentration to infection of wheat roots by *Gaeumannomyces graminis*. Phytopathology, 75: 98-103.