http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (11): 1865-1869, 2007
ISSN 1028-8880

Effect of Arbuscular Mycorrhizal (*G. etunicatum*) Fungus on Antioxidant Enzymes Activity under Zinc Toxicity in Lettuce Plants

Shadi Farshian, Jalil Khara and Parviz Malekzadeh
Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

Abstract: Zinc is one of the eight trace elements which are essential for the normal healthy growth and reproduction of crop plants. Plants possess cellular mechanisms that may be involved in the detoxification of heavy metals and thus confer plants a better tolerance against them. Arbuscular mycorrhizal fungi colonization is one of these mechanisms. Here, the effect of mycorrhizal fungus *G. etunicatum* on Zn toxicity tolerance through enhanced activity of some of antioxidant enzymes has been studied. Treatments were applied in triplicates of two factorial analyses: a) mycorrhizal and non-mycorrhizal; b) 5 levels of Zinc (0, 1.5, 3.5, 5.5, 7.5 mM). Zinc was added to modified Hoagland's nutrient solution (with half P concentration). Plants were grown in growth chamber for 10 weeks. Toxicity symptoms such as necrosis and chlorosis appeared on the leaves. Activity of detoxifying enzymes Guaiacol peroxidase (GUPX) and Ascorbate peroxidase (APX) were measured. GPX activity in roots and shoots of mycorrhizal and non-mycorrhizal plants was increased. Also, APX activity increased in roots and shoots of mycorrhizal and non-mycorrhizal plants. Root length colonization (RLC) was measured by gridline intersect method. Mycorrhizal colonization decreased due to Zinc exposure. The results indicate the probable role of arbuscular mycorrhizal colonization in stress tolerance.

Key words: Lettuce, Zn toxicity, mycorrhizal arbuscular colonization, ascorbate peroxidase, guaiacol peroxidase

INTRODUCTION

Zinc is one of the eight trace elements which are essential for the normal healthy growth and reproduction of crop plants; the other elements are: boron, chlorine, copper, iron, manganese, molybdenum and nickel. The first symptom to present itself in most species exhibiting Zn toxicity is a general chlorosis of the younger leaves (Harmens *et al.*, 1993; Ren *et al.*, 1993; Fontes and Cox, 1995; Rout and Das, 2003). Depending on the degree of toxicity this chlorosis can progress to reddening due to anthocyanin production in younger leaves (Harmens *et al.*, 1993; Fontes and Cox, 1995; Lee *et al.*, 1996). Plants exhibiting Zn toxicity have smaller leaves than control plants (Ren *et al.*, 1993).

The volume of soil exploited by plant roots can be greatly increased by the external mycelium of arbuscular mycorrhizal (AM) fungi (Bolan, 1991; Marschner and Romheld, 1998) and enhanced absorption of relatively immobile micronutrients such as Zn, Cu and Fe from deficient soils by AM plants has been well documented (Li and Christie, 2001; Kothari *et al.*, 1991; Li *et al.*, 1997; Liu *et al.*, 2000). On the other hand, under conditions of high available soil Zn and Cu, the concentrations of these trace elements in shoots have been reported to be lower in mycorrhizal than in non-mycorrhizal plants (Dueck *et al.*, 1986; Leyval *et al.*, 1991; Weissenhorn *et al.*, 1995; Liu *et al.*, 2000). Reduced concentrations of micronutrients in mycorrhizal plants are sometimes attributed to a dilution effect linked to an increase in plant Dry Matter (DM) yield (Nielsen and Jensen, 1983). However, experimental results cannot always be explained by a dilution effect.

Elevated concentrations of trace metals such as Zn exist in many agricultural soils from past management practices and this may represent a risk to environmental quality and sustainable food production. Some studies have shown a positive impact of infection by arbuscular mycorrhizal fungi on the resistance of the host plants to Zn contamination of soils (Chen *et al.*, 2003; Heggo *et al.*, 1990; Hetrick *et al.*, 1994; Zhu *et al.*, 2001). Many heavy metals stimulate the formation of free radicals and reactive oxygen species.

(ROS), either by direct electron transfer involving metal cations or as a consequence of metal-mediated inhibition of metabolic reactions. If the scavenging system of a plant does not cope well with the formation of free radicals or ROS, it leads to uncontrolled oxidation and

Corresponding Author: Shadi Farshian, Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

radical chain reactions, which result in oxidative stress to the plant. The scavenging system controlling ROS comprises both nonenzymatic anti-oxidant (e.g., glutathione, ascorbate and carotenoids) and an enzymatic anti-oxidative system [e.g., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GTX) and glutathione reductase (GR)] (Elstner, 1982).

The present investigation was carried out to: (1) understand the responses of AM fungi to elevated Zn levels in soil; (2) evaluate mycorrhizal on Zn uptake and partitioning in the host plant with soil Zn contamination ranging from very low to heavy and (3) demonstrate Zn uptake via the mycorrhizal hyphae and estimate the hyphal contribution where the dilution effect due to enhanced plant growth has been eliminated.

MATERIALS AND METHODS

Surface sterilized seeds of lettuce (*Lactuca sativa* L.) were sown in the sterilized soil/sand mixture. The mycorrhizal inoculum used was stock culture *Glomus etunicatum* which was bulked in an open pot culture of *Zea mays* L. Mycorrhizal treatments were carried out by adding 20 g per pot of mycorrhizal inoculum from our stock culture collection which was placed below lettuce seeds. Non mycorrhizal treatments received the same quantity of autoclaved inoculum.

Plants (3 per pots) were grown for 60 days in a growth chamber in Urmia University with temperatures ranging from 19 to 25°C, a 16/8 h light/dark period and a relative humidity of 70-80%. One week after emergence of seed lings, plants received (30 mL per pot 3 times a week) modified Hoagland ´s nutrients solution (mg L^{-1}) with half P concentration, by adding 0 (control), 1.5, 3.5, 5.5, 7.5 mM $ZnSO_4$ to the nutrient solution. The plants were planted in February and harvested in March.

The symbiotic fungal partner, Glomus etunicatum, was produced in a soil:sand (1/1, v/v) mixture using maize as the host plant. Inoculum of Glomus etunicatum (30 g), consisting of spores, external mycelium and AMF colonized roots, was laid around the seed. The same amount of sterilized inoculum was laid into the control pots. The roots were cleared and stained by using the methods by Philips and Hayman (1970). The percentage of mycorrhizal colonization (F %) was estimated by the grid line intersect method (Giovannetti and Mosse, 1980). At the end of the experiment, plants were harvested 10 weeks after seed sowing. Plant shoots was separated, dried (70°C 48 h).

For extraction of antioxidative enzymes, roots were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12,000 g for 20 min and the resulting supernatant was used for determination of enzyme activity. The whole extraction procedure was carried out at 4°C. The decrease in the concentration of ascorbate was recorded at 290 nm. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of ascorbate (2.8 mM 1 cm 1 at 290 nm).

The whole extraction procedure was carried out at 4°C. APX and GUPX were assayed as described previously (Chang and Kao, 1998).

RESULTS

APX activity in mycorrhizal shoots increased significantly ($p<0.05$) by increase in Zn concentration Fig. 1. Figure 2 show that increase in APX activity by

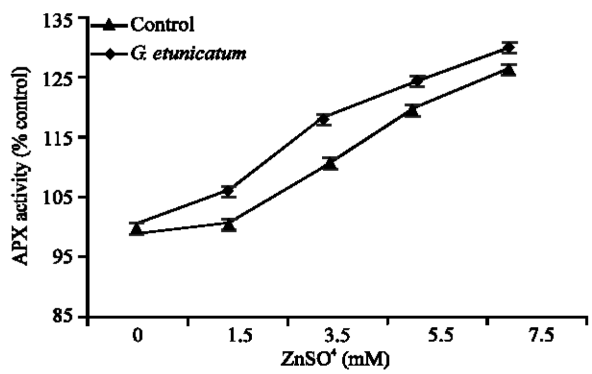

Fig. 1: The effects of Zn treatment on the activity of APX in the shoots of mycorrhizal and non-mycorrhizal Lettuce plants treated with $ZnSO_4$

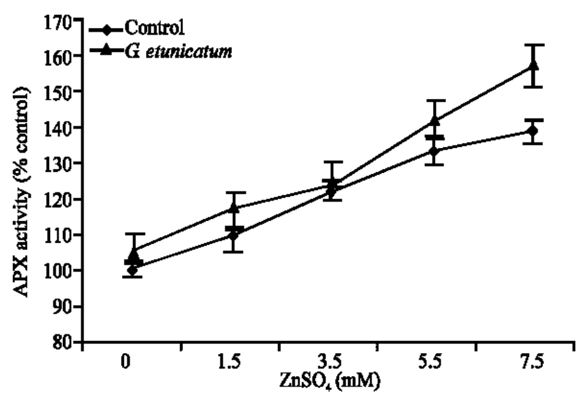

Fig. 2: The effects of Zn treatment on the activity of APX in the roots of mycorrhizal and non-mycorrhizal Lettuce plants treated with $ZnSO_4$

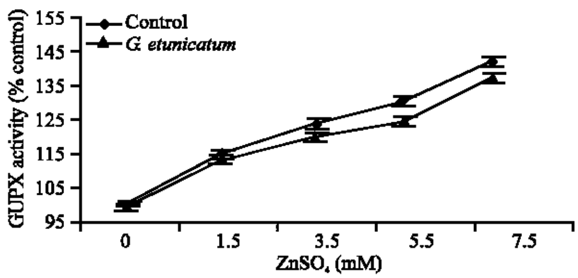

Fig. 3: The effects of Zn treatment on the activity of GUPX in the shoots of mycorrhizal and non-mycorrhizal Lettuce plants treated with $ZnSO_4$

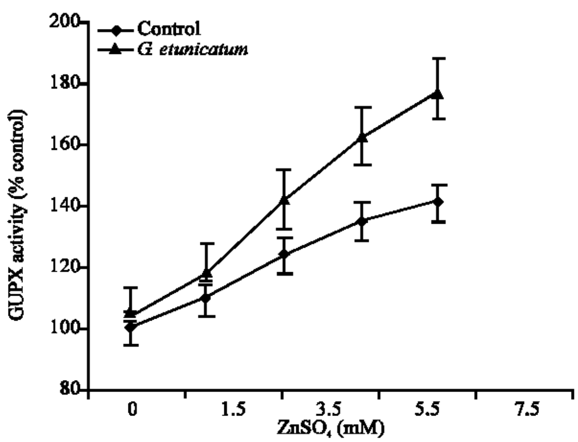

Fig. 4: The effects of Zn treatment on the activity of GUPX in the roots of mycorrhizal and non-mycorrhizal Lettuce plants treated with $ZnSO_4$

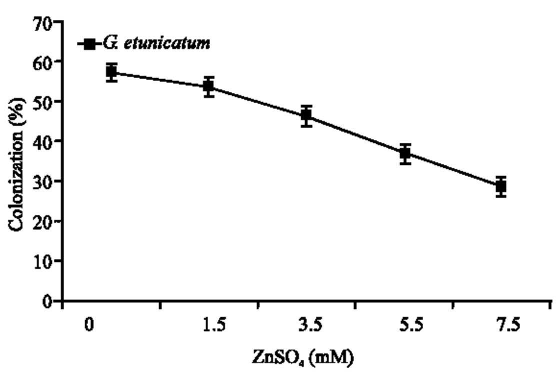

Fig. 5: The effects of Zn treatment on the percentage of root colonization with *Glomus etunicatum* in Lettuce plants

increased in Zn concentration in roots of these plants. Activity of this enzyme in non-mycorrhizal plant increased significantly ($p<0.05$), but Activity of this enzyme in roots and shoots of mycorrhizal plants higher than non-mycorrhizal plants.

Figure 3 and 4 show, GPX activity in mycorrhizal shoots and roots increased significantly ($p<0.05$) by increase in Zn.

Estimation of root length colonization by gridline intersect method, increase in Zn concentration, colonization percentage decreased significantly. The data show the possible role of mycorrhiza in plant protection against Zn toxicity.

Figure 5 shows that root colonization of Lettuce plants was significantly reduced and negatively correlated by the presence of heavy metals in nutrient solution, it was decreased from 57.3% in control to 28.6% in the 7.5 mM concentration.

The results indicate the probable role of arbuscular mycorrhizal colonization in stress tolerance.

DISCUSSION

The data of the present study showed that increased in Zn concentration in nutrient solution induced increased in antioxidant enzyme activity in shoots and roots of Lettuce plants. As shown in Fig. 1-4 APX activity in shoots and roots of lettuce plant increased in the presence of high Zn concentrations. It show that generation of reactive oxygen in the Zn toxicity. This finding supports results from some studies reporting the role of stress proteins such as phytochelatins and metallothioneines and antioxidant proteins (Rauser, 1999; Clemens, 2001) as possible mechanisms for protection against high toxic heavy metals (Ott *et al.*, 2002; Burleigh *et al.*, 2003; Tong *et al.*, 2004).

The protective mechanisms adapted by plants to scavenge free radicals and peroxides include several antioxidative enzymes such as SOD, APX, GUPX, CAT and POD. The antioxidative enzymes are important components in preventing the oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes is generally increased in plants when exposed to stressful conditions (Allen, 1995). Over expression of genes encoding these enzymes in several transgenic plant species conferring protection against free radicals has also been demonstrated (Allen, 1995).

Such a variation in response of these enzymes to Zn stress could be due to the variability of plant species in producing free radicals (Parviz Malekzadeh, In Press).

The present results indicate an enhancement in the activity of APX in response to zinc stress in L. *sativa*. Similar induction was reported in response to mild water stress (Baisak *et al.*, 1994).

The present results indicate an enhancement of GPX activity upon exposure to zinc, suggesting that this enzyme serves as an intrinsic defense tool to resist zinc induced oxidative damage in *L. sativa*. Induction of GPX activity in plants has also been reported under toxic levels of other metals like Al, Cu and Cd (Cakmak and Horst, 1991; Chaoui *et al*., 1997; Shah *et al*., 2001).

Antioxidant enzymes are considered to be an important defense system of plants against oxidative stress caused by metals (Weckx and Clijsters, 1996). The results of this study show differential responses of the anti-oxidative enzymes to zinc in the Lettuce plants. The production of anti-oxidant enzymes as a function of zinc concentration applied was evident in all tissues of the plants assayed during the present study.

Arbuscular Mycorrhizal Fungi (AMF) have multiple beneficial effects on the growth of plants. The fine hyphae of the fungi effectively mobilize water and nutrients such as phosphorus, nitrogen, potassium, calcium, zinc and copper from soil particles and these nutrients are then transferred to the host plants (Kothari *et al*., 1991; Jakobsen *et al*., 1992; Johansen *et al*., 1992; Smith and Read, 1997). AMF-colonized plants are generally more resistant to stresses caused by drought, salt, heavy metals or attack by pathogens. These positive effects of the fungi on the growth of plants often result from an improved nutrient supply and can partly be due to complex and not easily resolved interactions between the symbiotic partners.

ACKNOWLEDGMENTS

The authors are grateful to Shayan Farshian and Fatemeh Zabani for commenting on the English.

REFERENCES

Allen, R.D., 1995. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol., 107: 1049-1054.

Baisak, R., D.A. Rana, P.B.B. Acharya and M. Kar, 1994. Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Plant Cell Physiol., 35: 489-495.

Chen, B.D., X.L. Li, H.Q. Tao, P. Christie and M.H. Wong, 2003. The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere, 50: 839-846.

Bolan, N.S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil, 134: 189-207.

Burleigh, S.H., B.K. Kristensen and I.E. Bechmann, 2003. A plasma membrane zinc transporter from Medicago truncatula is upregulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol. Biol., 52: 1077-1088.

Cakmak, I. and W.J. Horst, 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (Glycine max). Physiol. Plant, 83: 463-468.

Chang, C.J. and C.H. Kao, 1998. H_2O_2 metabolism during senescence of rice leaves: Changes in enzyme activities in light and darkness. Plant Growth Regulation, 25: 11-15.

Chaoui, A., S. Mazhoudi, M.H. Ghorbal and E. El-Ferjani, 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Sci., 127: 139-147.

Clemens, S., 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta, 212: 475-486.

Dueck, T.A., P. Visser, W.H.O. Ernst and H. Schat, 1986. Vesicular-arbuscular mycorrhizas decrease zinc toxicity to grasses in zinc polluted soil. Soil Biol. Biochem., 18: 331-333.

Elstner, E.F., 1982. Oxygen activation and oxygen toxicity. Ann. Rev. Plant Physiol., 33: 73-96.

Fontes, R.L.F. and F.R. Cox, 1995. Effects of sulfur supply on soybean plants exposed to zinc toxicity. J. Plant Nutr., 18: 1893-1906.

Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. New Phytol., 84: 489-500.

Harmens, H., N.G.C.P.B. Gusmao, Den P.R. Hartog, J.A.C. Verkeij and W.H.O. Ernst, 1993. Uptake and transport of zinc in zinc-sensitive and zinc-tolerant Silene vulgaris. J. Plant Physiol., 141: 309-315.

Heggo, A., J.S. Angle and J.S. Chaney, 1990. Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol. Biochem., 22: 865-869.

Hetrick, B.A.D., G.W.T. Wilson and D.A.H. Figge, 1994. The in.uence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. Environ. Pollut., 86: 171-179.

Jakobsen, I., L.K. Abbott and A.D. Robson, 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1: Spread of hyphae and phosphorus inflow into roots. New Phytol., pp: 371-380.

Johansen, A., I. Jakobsen and E.S. Jensen, 1992. Hyphal transport of 15N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol., 122: 281-288.

Kothari, S.K., H. Marschner and V. Romheld, 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil, 131: 177-185.

Lee, C.W., M.B. Jackson, M.E. Duysen, T.P. Freeman and J.R. Self, 1996. Induced micronutrient toxicity in 'Towndown' Kentucky bluegrass. Crop Sci., 36: 705-712.

Leyval, C., K. Turnau and K. Haselwandter, 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: Physiological, ecological and applied aspects. Mycorrhiza, 7: 139-153.

Li, X., E. George and H. Marschner, 1991. Extension of the phosphorus depletion zone in VA mycorrhizal white clover in a calcareous soil. Plant Soil, 136: 41-48.

Li, X.L. and P. Christie, 2001. Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn contaminated soil. Chemosphere, 42: 201-207.

Liu, A., C. Hamel, R.I. Hamilton and B.L. Ma, 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza, 9: 331-336.

Malekzadeh, P., J. Khara and S. Farshian, 2007. Effect of Arbuscular Mycorrhiza (*Glomus etunicatum*) on Some Physiological Growth Parameters of Tomato Plant Under Copper Toxicity in nutrient Solution. Pak. J. Biol. Sci. (In Press).

Marschner, H. and D.J. Romheld, 1998. Role of root growth, arbuscular mycorrhiza and root exudates for the eciency in nutrient acquisition. Field Crops Res., 56: 203-207.

Nielsen, J.D. and A. Jensen, 1983. Infuence of vesicular-arbuscular mycorrhizal fungi on growth and uptake of various nutrients as well as uptake ratio of fertilizer P for lucerne (*Medicago sativa*). Plant Soil, 70: 165-172.

Ott, T., E. Fritz, A. Polle and A. Schutzendubel, 2002. Characterization of antioxidative systems in the ectomycorrhiza-building basidiomycete *Paxillus involutus* (Bartsch) Fr. and its reaction to cadmium. FEMS Microbiol. Ecol., 42: 359-366.

Phillips, J. and D. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-161.

Rauser, W.E., 1999. Structure and function of metal chelators produced by plants-the case for organic acids amino acids, phytin and metallothioneins. Cell Biochem. Biophys., 31: 19-48.

Ren, F., T. Liu, H. Liu and B. Hu, 1993. Influence of zinc on the growth, distribution of elements and metabolism of one-year old American ginseng plants. J. Plant Nutr., 16: 393-405.

Rout, G. and P. Das, 2003. Effect of metal toxicity on plant growth and metabolism: I. Zinc. Agronomie, 23: 3-11.

Shah, K., R.G. Kumar, S. Verma and R.S. Dubey, 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci., 161: 1135-1144.

Smith, S.E. and D.J. Read, 1997. Mycorrhizal Symbiosis. 2nd Edn., San Diego, London: Academic Press.

Tong, Y., R. Kneer and Y. Zhu, 2004. Vacuolar compartmentalization: A second-generation approach to engineering plants for phytoremediation. Trends Plant Sci., 9: 7-9.

Weckx, J.E.J. and HMM. Clijsters, 1996. Oxidative damage and defense mechanisms in primary leaves of Phaseolus vulgaris as a result of root assimilation of toxic amounts of copper. Physiol. Plant, 96: 506-512.

Weissenhorn, I., C. Leyval, G. Belgy and J. Berthelin, 1995. Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza, 5: 245-251.

Zhu, Y.G., S.E. Smith, F.A. Smith, 2001. Zinc (Zn)-phosphorus (P) interactions in two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. Ann. Bot., 88: 941-945.