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 (14): 2413-2417, 2007
ISSN 1028-8880

Effect of Cadmium Toxicity on the Level of Lipid Peroxidation and Antioxidative Enzymes Activity in Wheat Plants Colonized by Arbuscular Mycorrhizal Fungi

Azam Khalighi Jamal Abad and Jalil Khara
Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran

Abstract: High concentrations of heavy metals in the soil have an adverse effect on micro-organisms and microbial processes. Mycorrhizas are among the extracellular strategies to avoid metal toxicity. In the present study, the effect of cadmium on lipid peroxidation and antioxidative enzymes activity of AM (with *Glomus veruciforme*, *G. intraradices* and *G. etunicatum*) and non-AM Wheat plants was investigated. Wheat plants (*Triticum aestivum* L. cv. Azar2) which have been colonized by above species, were used in this study. They have been exposed to different concentrations of cadmium chloride for 60 days. The test solution contained: 0 (control), 250, 750 and 2500 μm cadmium. Toxicity symptoms such as chlorosis and necrosis appeared on the cadmium treated leaves. Activity of detoxifying enzymes Guaiacol peroxidase (GUPX) and Ascorbate peroxidase (APX) of mycorrhizal and non-mycorrhizal plants were increased. Also, the amount of malondialdehyde (MDA) increased in roots and shoots of mycorrhizal and non-mycorrhizal plants significantly as a result of Cd treatment, but it was more dramatic in mycorrhizal ones.

Key words: Wheat, cadmium, mycorrhiza, oxidative stress, MDA

INTRODUCTION

Among the heavy metals, cadmium (Cd) is of special concern due to its relatively high mobility in soils and potential toxicity at low concentrations (Das *et al*., 1997). Although Cd is not an essential mineral nutrient for plants, it is easily absorbed by the root system, causing a decrease in transpiration and photosynthesis (Bazzaz *et al*., 1974) and an increase in the respiratory rates. These effects seem to be related to Cd induction of premature senescence in plants (Van Assche *et al*., 1988). Plants in certain mycorrhizal associations are less sensitive to cadmium stress than non-mycorrhizal plants. Cadmium-induced changes in mycorrhizal roots were absent or smaller than those in non-mycorrhizal plants. A common consequence of most abiotic and biotic stresses that they result at some stages of stress exposure is an increased production of reactive oxygen species (Polle and Rennenberg, 1993). Reactive oxygen species may lead to the unspecific oxidation of proteins and membrane lipids or may cause DNA injury. As a consequence, tissues injured by oxidative stress generally contain increased concentrations of carbonylated proteins and malondialdehyde and show an increased production of ethylene (Dean *et al*., 1993; Ames *et al*., 1993). The concentrations and type of heavy metal and the species of plant are very important for these sorts of studies, because it is well known that different plants behave very selective to the elements and the threshold dose of any heavy metal may vary according to the plant species and the kind of heavy metal. The activation of the cellular antioxidant metabolism belongs to the general stress responses induced by heavy metals (Dietz *et al*., 1999). Although an active antioxidative metabolism does not represent a Cd tolerance mechanism in a strict sense, it is beneficial for plant performance under heavy metal stress. Inadequate activities of antioxidant defense systems cause oxidative damage, lipid peroxidation, membrane leakage in plants exposed to Cu, Fe and also to Cd. The present investigation was carried out to study the changes in lipid peroxidation and antioxidative enzymes (GUPX and APX) activity.

MATERIALS AND METHODS

Seeds of winter wheat (*Triticum aestivum* L. cv. Azar2) prepared from Agricultural Research Center of Urmia, were sterilized with 2.5% sodium hypochlorite solution for 15 min and then washed thoroughly with distilled water. The seeds were moistened in Petri dishes and incubated in 5°C for three weeks. The mycorrhizal inocula used were stock cultures prepared from Tabriz University (Iran). They include *Glomus veruciforme, Glomus intraradices* and *Glomus etunicatum*.

Corresponding Author: Azam Khalighi Jamal Abad, Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran
Tel: 09144545776 Fax: 98-441-2776707

Mycorrhizal treatments were carried out by adding 50 g of inocula per pot of these treatments which was placed bellow the seeds. Non-mycorrhizal treatments received the same quantity of autoclaved inocula. The symbiotic fungal partners, had been produced in a soil/sand (1:1 v/v) mixture using maize as the host plant, separately. Inocula (50 g) consisted of external mycelium, spores and AMF colonized roots. The germinated seeds of wheat (10 per pot) were sown in pots (with 12 cm diameter and 10 cm height) and seedlings thinned to 5 per pot after one week. The seedlings were grown for 60 days in a growth chamber with temperatures ranging from 23 to 27°C, a 14/10 h light/dark period and a relative humidity of 60-70%. One week after planting the seeds, plants received modified Hoagland's nutrient solution with half P concentration, 40 mL per pot 3 times a week. From the second week plants received test solution (40 mL per pot, 3 times a week) that contained 0 (control), 250, 750 and 2500 μm cadmium as $CdCl_2$. After 60 days, plants were harvested and the roots and shoots were separated.

In order to extraction of antioxidative enzymes, roots and shoots were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled mortar and pestle. Then the homogenate was centrifuged at 12000 g for 20 min and supernatant was used for determination of enzymes activity. The extraction procedure carried out at 4°C. APX and GUPX were assayed as described previously (Chang and Kao, 1998). The enzymes activity was calculated from initial rate of the reaction using the extinction coefficients of ascorbate and guaiacol (2.8 mM^{-1} cm^{-1} for ascorbate and 26.6 mM^{-1} cm^{-1} for guaiacol). MDA content was determined by spectrophotometer in 532 and 600 nm as Popham and Novacky (1991). All of the experimental procedures were conducted in the plant physiology lab of the Urmia University in spring and autumn 2006.

RESULTS

MDA content as an index of lipid peroxidation in roots and shoots of both AM and non-AM plants increased significantly ($p<0.05$) by increase in Cd concentration. Figure 1A and B show that increase in cadmium concentration leads to increase in lipid peroxidation in shoots and roots of AM and non-AM plants. MDA content of the roots was more than that of shoots in all of the experimental conditions. These amounts increased in response to Cd treatments in both shoots and roots in the absence of mycorrhizal fungi. The rate of this increase was more dramatic in the root samples. *G. etunicatum* had more potential to raise the level of MDA in response to Cd in the roots and shoots

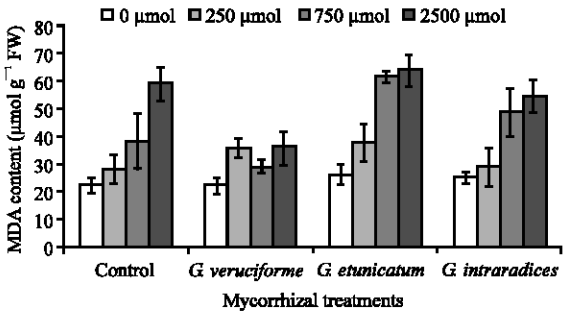

Fig. 1A: The effect of Cd treatment on MDA content in roots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

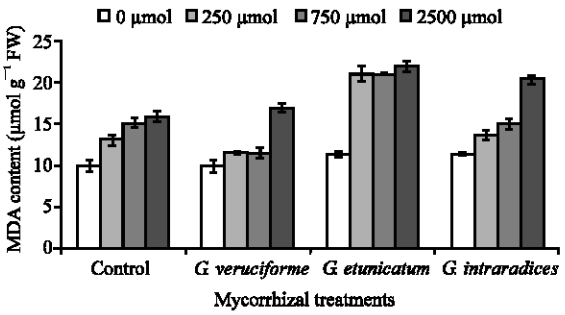

Fig. 1B: The effect of Cd treatment on MDA content in shoots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

of wheat plants, whereas in the absence of Cd, MDA content had no significant difference between fungal treatments. Similar trend was observed in the presence of *G. intraradices* and such enhancement effect is more prominent in the roots. On the other hand, MDA level of shoots have been increased in parallel of Cd concentration in the presence of *G. veruciforme*, but it can not been observed in roots. In the roots of symbiont plants with *G. veruciforme*, MDA content changes had no regular and distinct pattern in the response to Cd level. In a general view it seems that *G. etunicatum* fungi were more effective in the enhancement of lipid peroxidation. In contrast, *G. veruciforme* had the least effect. It can be suggested that all of above fungal treatments can increase the level of lipid peroxidation of wheat plants in response to Cd treatment.

Also, GUPX and APX activity in mycorrhizal and non-mycorrhizal plants increased significantly ($p<0.05$) in shoots and roots, but activity of these enzymes in AM plants were higher than non-AM plants. Figure 2A, B, 3A and B show that APX and GUPX activity in shoots and roots was increased significantly ($p<0.05$) by increasing

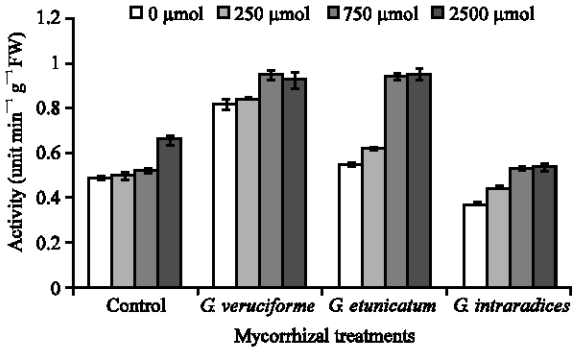

Fig. 2A: The effect of Cd treatment on the activity of APX in the roots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

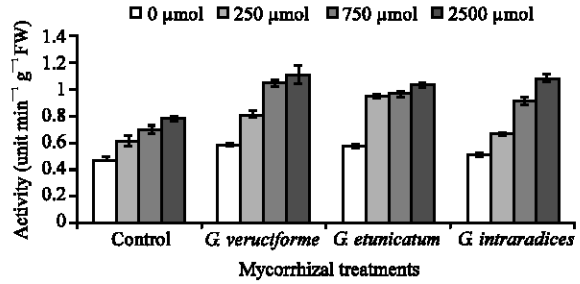

Fig. 2B: The effect of Cd treatment on the activity of APX in the shoots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Cd concentration. The comparison of APX activity in the roots and shoots shows no significant difference in the absence of fungal partner. However, the activity of this enzyme has been raised with the increased level of Cd treatment, gradually. This effect was step-by-step and was in accordance to Cd level in the shoots. However, a dramatic increase can be seen only in the presence of 750 µmol Cd in the roots, especially in the presence of *G. etunicatum*. Generally, it seems that fungal treatments cause an increase in the activity of APX and this enhancement effect is more significant in the presence of higher levels of Cd. There was no difference between roots and shoots, as it was seen in the control (non-AM) plants, but an ascending trend is evident in all of the conditions. *G. intraradices* had no noticeable alleviative effect on the activity of this enzyme, especially in the roots.

Higher activity of GUPX in the roots of control plants was observed in comparison with the shoots and its activity has been increased in both organs with an increase in Cd level. The positive effect of mycorrhizal treatments on the activity of GUPX was significant in all

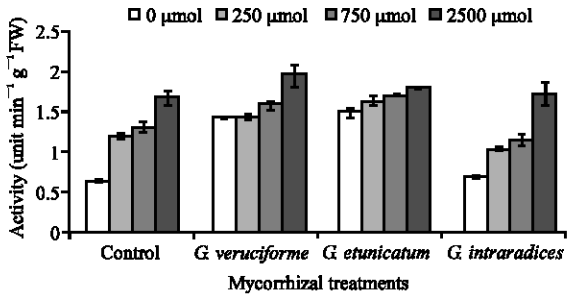

Fig. 3A: The effect of Cd treatment on the activity of GUPX in the roots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

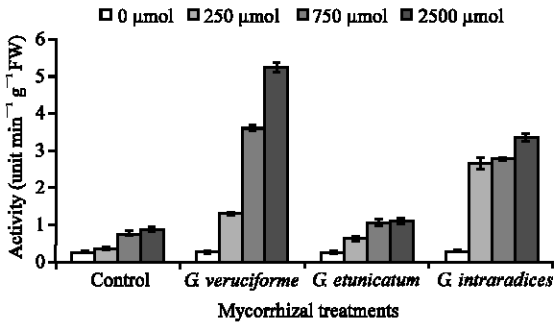

Fig. 3B: The effect of Cd treatment on the activity of GUPX in the shoots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

of the Cd concentrations. This effect was more prominent in the presence of *G. veruciforme*. For example, the activity of GUPX has reached to the mean of 5.5 unit min^{-1} g^{-1} FW in shoots as an effect of 2500 µmol Cd. It appears that *G. intraradices* could not increase the activity of GUPX in roots, whereas this effect was significant in the shoots under Cd stress. Cd level had no significant effect on the GUPX activity in the roots of wheat plants which had been inoculated by *G. etunicatum*. Shoots of these plants showed a similar trend. Overall, GUPX activity enhancement effect under the influence of Cd level is evident very well in the most of our mycorrhizal treatments and in the control plants.

The data of this study show the possible role of mycorrhization in plant protection against Cd toxicity.

DISCUSSION

Cadmium is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto *et al*., 2004). Krupa and Piotrowska-Seget (2003) reported that the concentration of cadmium in aboveground parts of *Pinus sylvestris* mycorrhized with

fungi was significantly lower than in non-mycorrhized seedlings. On the other hand, metals accumulated by mycorrhizal wheat plants were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them (Rabie, 2005).

The content of malondealdehyde (MDA) was increased in the presence of cadmium stress. Soltani *et al.* (2006) have reported that cadmium at 600 and 800 μmol increased content of soluble sugars and MDA in leaves and roots of treated rape (*Brassica napus*) plants. It has been reported that the activity of lipoxygenase increases as a consequent of an increase in lipid peroxidation due to Cd toxicity in treated plants (Sanita di Toppi and Gabbrielli, 1999). This enzyme catalyses the oxygenation of unsaturated and long chained fatty acids which containing one *cis* bond. Linoleic acid and Linolenic acid are the most common unsaturated fatty acids in the plant cells which are ideal substrates for lipoxygenase (Skorzynska-Pilot and Krupa, 2003). There had been characteristic differences among the wheat varieties examined in inducible enzymatics and malondialdehyde production reflecting different mechanisms in its stress induced reactions (Kiraly *et al.*, 2002). We observed that *G. etunicatum* had more capability to enhance the lipid peroxidation. MDA is a product of lipid peroxidation and an indicator of free radical production and tissue damage. Our experimental mycorrhizal plants had a better performance than non-AM ones as observed in fresh and dry weights (data have not shown here). It seems that tissue damage by free radicals is alleviated by some unknown direct and indirect mycorrhizal mechanisms, such as enzymatic pathways and detoxifying action of carotenoids (Sanita di Toppi and Gabbrielli, 1999) and so, presumably mycorrhizal colonization especially by *G. etunicatum* plays an important role in Cd tolerance in wheat plants.

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). In this study, Cd treatment resulted in an increase in the activities of APX and GUPX that can be considered as an indirect evidence for enhanced activities of free radicals under Cd stress.

Among our fungal species *G. veruciforme* and *G. etunicatum* had more enhancement effect on APX activity and so had more alleviative impact on Cd tolerance in wheat plants. APX activity in the roots of plants colonized by *G. intraradices* shows no increase in compare with the control plants and even it is lower than them. Probably this AM species has no enhancement effect on APX activity in roots. Also, *G. veruciforme* had the best positive effect on GUPX activity in both of shoots and roots.

Antioxidative enzymes are considered to be an important defense system of plants against oxidative stress caused by metals (Weckx and Clijsters, 1996). Since the peroxidase enzymes are related to free radical formation, it is evident that Cd induces the development of free radical reactions. The primary stress reaction and rapid changes appear in plants due to the applied element. The relationship between metal sensitivity and lipid peroxidation was clearly illustrated in response to cadmium, indicates that cadmium toxicity resulted in the increased peroxidation of ROS. Cadmium treatments had increased GUPX and APX activities in root of maize plants in presence of 0.25, 0.5 and 0.75 mM concentrations, but their activities had been constant in 1, 3 and 5 mM (Malekzadeh *et al.*, 2007)

Mycorrhizas are among the extracellular strategies to avoid metal toxicity (Marschner, 1995; Jentschke and Godbold, 2000). The use of mycorrhizal fungi as bioremediation agents has been reviewed by Donelly and Fletcher (1994). Arbuscular Mycorrhizal Fungi (AMF) have positive effects on the growth of plants. AMF-colonized plants are generally more resistant to stresses caused by drought, salt, heavy metals or attack by pathogens (Farshian *et al.*, 2007). The development of stress-tolerant plant-mycorrhizal associations may be a promising new strategy for phytoremediation and soil amelioration measures (Schutzendubel and Polle, 2002).

ACKNOWLEDGMENTS

We acknowledge of Miss Samaneh Rahmatzadeh and Ms. Latifeh Pourakbar for their help in experimental procedures of this study and Dr. Nasser Aliasgharzadeh from Tabriz University for providing mycorrhizal inocula.

REFERENCES

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

Ames, B., M. Shigenaga and T. Hagen, 1993. Oxidants, antioxidants and the degenerative diseases of ageing. Proceedings of the National Academy of Sciences, USA., 90: 7915-7922.

Bazzaz, F.A., G.L. Rolfe and R.W. Carloson, 1974. Effect of Cd on photosynthesis and transpiration of excised leaves of corn and sumflower. Physiol. Plant., 32: 373-376.

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

Das, P., S. Samantaray and G.R. Rout, 1997. Studies on cadmium toxicity in plants: A review. Environ. Poll., 98: 29-36.

Dean, R.T., S. Gieseg and M. Davies, 1993. Reactive species and their accumulation on radical-damaged proteins. Trends Biol. Sci., 18: 437-441.

Dietz, K.J., M. Baier and U. Kramer, 1999. Free radicals and Reactive Oxygen Species as Mediator of Heavy Metal Toxicity in Plants. Heavy Metal Stress in Plants. From Molecules to Ecosystem. Prasd, M.N.V. and J. Hagemeyer (Eds.) , Springer-Verlag, Berlin, pp: 73-79.

Donelly, P.K. and J.S. Fletcher, 1994. Potential use of mycorrhizal fungi as bioremediation agents. Bioremediation Through Rhizosphere Technology, 563: 93-99.

Farshian, S., J. Khara and P. Malekzadeh, 2007. Effect of arbuscular mycorrhizal (*G. etunicatum*) fungus on antioxidant enzymes activity under zinc toxicity in lettuce plants. Pak. J. Biol. Sci., 10: 1865-1869.

Jentschke, G. and D.L. Godbold, 2000. Metal toxicity and ectomycorrhizas. Physiol. Plant., 109: 107-116.

Kiraly, I. and P. Czovek, 2002. Changes of MDA level and O_2 scavenging enzyme activities in wheat varieties as a result of PEG treatment. Proc. 7th Hungarian Cong. Plant Physiol., 46: 105.

Krupa, P. and Z. Piotrowska-Seget, 2003. Positive aspects of interaction between plants and mycorrhizal fungi originating from soils polluted with cadmium. Polish J. Environ. Studies, 12: 723-726.

Malekzadeh, P., J. Khara, S. Farshian, A. Khalighi Jamal-Abad and S. Rahmatzadeh, 2007. Cadmium toxicity in maize seedlings: Changes in antioxidant enzyme activities and root growth. Pak. J. Biol. Sci., 10: 127-131.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Pinto, A.P., A.M. de Mota, A. Varennes and F.C. Pinto, 2004. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. Sci. Total Environ., 326: 239-247.

Polle, A. and H. Rennenberg, 1993. Significance of Antioxidants in Plant Adaptation to Environmental Stress. In: Plant Adaptation to Environmental Stress. Masfield, T., L. Fowden and F. Stoddard, (Eds.), London: Chapman and Hall, pp: 263-273.

Popham, P.L. and A. Novacky, 1991. Use of dimethyl sulfoxide to detect hydroxyl radical during bacteria-induced hypersensitive reaction. Plant Physiol., 96: 1157-1160.

Rabie, G.H., 2005. Contribution of arbuscular mycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy metal-polluted soil. Afr. J. Biotechnol., 4: 332-345.

Sanita di Toppi, L. and R. Gabbrielli, 1999. Response to cadmium in higher plants. Review. Environ. Exp. Bot., 41: 105-130.

Schutzendubel, A. and A. Polle, 2002. Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot., 53: 1351-1365.

Skorzynska-Pilot, E.W.A. and Z. Krupa, 2003. The activity of lipoxygenase in *Arabidopsis thaliana* L. Heynh. A preliminary study. Cell Mol. Biol., 8: 279-284.

Soltani, F., M. Ghorbanli and K. Manouchehri-Kalantari, 2006. Effect of cadmium on photosynthetic pigments, sugars and malondealdehyde content in *Brassica napus*. Iran. J. Biol., 19: 136-145.

Van Assche, F., C. Cardinales and J. Clijsters, 1988. Induction of enzyme capacity in plants as a result of heavy metal toxicity: Dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. Environ. Poll., 52: 103-115.

Weckx, J.E.J. and H.M.M. 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., 26: 506-512.