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Effects of Arbuscular Mycorrhizal Fungus (*Glomus veruciforme*) on Changes of Some Physiological Parameters in Cadmium Treated Wheat Plants

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Abstract: Soil pollution by heavy metals has become a critical environmental concern due to its potential adverse ecological effects. In this study we have investigated the effect of arbuscular mycorrhizal fungus (*Glomus veruciforme*) on some physiological growth parameters, such as chlorophyll content, amount of total soluble sugar and total protein of wheat plants (*Triticum aestivum* L. cv. Azar2) and we compared these factors in mycorrhizal (AM) and non-mycorrhizal (non-AM) plants. The content of chlorophyll in the absence of Cd in AM-plants was higher than non-AM plants. In the presence of mycorrhiza the content of chlorophyll a and b was decreased by increase in Cd concentration in both AM and non-AM plants. Furthermore, the amount of total soluble sugars and total proteins of shoots and roots have been increased in parallel to exogenous Cd concentration. In roots of AM-plants, the increase of soluble sugars was more dramatic in the presence of 2500 μm, but in the roots of non-AM plants this increase was step-by-step. The increase of total soluble sugars was gradually and significant in shoots of both AM and non-AM plants. The content of total proteins in shoots was raised with the increased level of Cd treatment, gradually and non-significantly.

Key words: Physiological parameters, *Triticum aestivum*, *Glomus veruciforme*

INTRODUCTION

Studies of polluted soils have shown heavy metals contamination of the soils as well the uptake of these toxic elements by plants (Okoronkwo *et al*., 2005). The toxic elements accumulated in organic matter in soils are taken up by growing plants (Dara, 1993). Plants in certain mycorrhizal associations are less sensitive to cadmium stress than non-mycorrhizal (non-AM) plants (Suhutzendubel and Polle, 2002). Arbuscular Mycorrhizal (AM) associations are integral, functioning parts of plant roots and are widely recognized as enhancing plant growth on severely disturbed sites, including those contaminated with heavy metals. They are related to be present on the roots of plants growing on heavy metal contaminated soils and play an important role in metal tolerance and accumulation (Gaur and Adholeya, 2004). Accumulation of heavy metals in plants depends on many factors, including the transport of metals from the soil to the plant. Among these factors, bacteria and fungi have received special attention. Mycorrhizal fungi directly link soil and roots and can be of great importance in heavy metal availability and toxicity to plants (Krupa *et al*., 2003). Although the toxic effects of cadmium on biological systems have been reported by Obata and Umebayashi (1997), Das *et al.* (1997) and Sanita di Toppi and Gabbrielli (1999), the mechanisms of Cd toxicity are not completely understood yet. Cadmium can alter the uptake of minerals from the soil, or through a reduction in the population of soil microbes (Moreno *et al*., 1999). So, it can affect on mycorrhizal colonization and subsequent physiological events in both fungi and plants. Considering these facts, we studied the changes of some growth parameters as characters of physiological performance in AM and non-AM wheat plants under influence of some concentrations of cadmium. They include chlorophyll content, total soluble sugars and total proteins. The purpose of this study was the evaluation of *G. veruciforme* probable help to alleviate Cd toxicity in wheat plants.

MATERIALS AND METHODS

All of the experimental procedures were conducted in the Plant Physiology Lab of the Urmia University in autumn and winter of 2007. Seeds of wheat (*Triticum aestivum* L. cv. Azar2) were sterilized with 2.5% sodium hypochlorite solution for 15 min and then washed thoroughly with distilled water. Then the seeds planted in the sterilized soil/sand mixture. The mycorrhizal inoculum (*Glomus veruciforme*) used was stock culture prepared from Tabriz University (Iran). Mycorrhizal treatments were carried out by adding 50 g of inoculum per pot of these treatments which was placed bellow the seeds. Non-

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mycorrhizal treatments received the same quantity of autoclaved inoculum. The symbiotic fungal partner was produced in a soil/sand (1:1 v/v) mixture using maize as the host plant.

Inoculum (50 g) consisted of external mycelium, spores and colonized roots. Seeds of wheat (5 per pot) 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 $CdCl_2$. Plants were harvested and the roots and shoots were separated after 60 days.

Fresh weights of shoots and roots have been determined then these organs dried (at 70°C for 48 h) and weighed again. The contents of chlorophyll a and b in leaves were determined by the spectrophotometer according to Lichtenthaler and Wellburn (1985). Total soluble sugar content in shoots and roots was determined by anthrone method (Fales, 1951). Also, the total protein content in shoots and roots was determined by Lowry *et al.* (1951) method.

RESULTS

Both fresh and dry weights of AM plants were more than corresponding non-AM plants in all of the Cd treated and non-treated plants, significantly. These weights were decreased under Cd treatments in both AM and non-AM plants (Data are not shown here). The contents of chlorophyll a and b decreased significantly ($p<0.05$) by increase in Cd concentration. Table 1A and B show the effect of $CdCl_2$ on the content of chl a and chl b. The content of chlorophyll in the absence of Cd in AM-plants was higher than non-AM plants. In the presence of Cd, the amount of chlorophyll a and b decreased. This effect was more pronounced in the presence of 750 and 2500 μm treatments, but this decrease was not significant.

The amount of total soluble sugars in both roots and shoots increased in parallel of Cd concentration in AM and non-AM plants. In roots of AM-plants, this increase was more dramatic in the presence of 2500 μm, but in the roots of non-AM plants this increase was step-by-step. The increase of total soluble sugars was gradually and significant in shoots of both AM and non-AM plants (Table 2A and B).

The content of total proteins in shoots was raised with the increased level of Cd treatment, gradually and non-significantly. However, the content of total proteins in the roots of AM-plants increased in the presence of Cd (Table 3A and B).

Table 1A: The effect of Cd treatment on chlorophyll a content in the leaves of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Chlorophyll a content (mg g^{-1} FW)	Cd concentration-*G. veruciforme* treatment (μmol)	Chlorophyll a content (mg g^{-1} FW)	Cd concentration-Non-mycorrhizal treatment (μmol)
19.50±3.929	0	13.660±0.957	0
19.50±3.929	250	12.300±1.056	250
17.57±3.394	750	9.521±2.308	750
17.57±3.394	2500	9.521±2.308	2500

Table 1B: The effect of Cd treatment on chlorophyll b content in the leaves of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Chlorophyll b content (mg g^{-1} FW)	Cd concentration-*G. veruciforme* treatment (μmol)	Chlorophyll b content (mg g^{-1} FW)	Cd concentration-Non-mycorrhizal treatment (μmol)
8.23±1.976	0	4.30±0.363	0
8.23±1.976	250	4.30±0.363	250
7.72±1.452	750	3.40±0.609	750
7.21±0.823	2500	3.40±0.609	2500

Table 2A: The effect of Cd treatment on total soluble sugars content in the roots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Total soluble sugars content (mg g^{-1} DW)	Cd concentration-*G. veruciforme* treatment (μmol)	Total soluble sugars content (mg g^{-1} DW)	Cd concentration-Non-mycorrhizal treatment (μmol)
5.81±0.599	0	8.33±0.351	0
6.99±0.441	250	11.48±1.394	250
10.35±0.921	750	14.88±0.973	750
59.74±2.768	2500	16.61±0.814	2500

Table 2B: The effect of Cd treatment on total soluble sugars content in the shoots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Total soluble sugars content (mg g^{-1} DW)	Cd concentration-*G. veruciforme* treatment (μmol)	Total soluble sugars content (mg g^{-1} DW)	Cd concentration-Non-mycorrhizal treatment (μmol)
48.16±3.413	0	31.37±1.428	0
61.08±4.051	250	37.86±0.811	250
61.26±0.991	750	54.78±0.360	750
69.78±1.689	2500	80.35±4.800	2500

Table 3A: The effect of Cd treatment on total proteins content in the roots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Total proteins content (mg g^{-1} DW)	Cd concentration-*G. veruciforme* treatment (μmol)	Total proteins content (mg g^{-1} DW)	Cd concentration-Non-mycorrhizal treatment (μmol)
19.33±1.105	0	20.63±0.463	0
19.33±1.105	250	20.63±0.463	250
23.03±1.753	750	21.67±0.348	750
23.03±1.753	2500	21.67±0.348	2500

Table 3B: The effect of Cd treatment on total proteins content in the shoots of mycorrhizal and non-mycorrhizal wheat plants treated with $CdCl_2$

Total proteins content (mg g^{-1} DW)	Cd concentration-*G. veruciforme* treatment (μmol)	Total proteins content (mg g^{-1} DW)	Cd concentration-Non-mycorrhizal treatment (μmol)
23.00±0.300	0	23.00±0.300	0
27.17±1.241	250	26.47±0.233	250
29.20±1.504	750	28.10±1.021	750
31.67±3.605	2500	18.10±1.021	2500

DISCUSSION

Cadmium pollution of the environment has been rapidly increasing in recent decades as a result of rising consumption of Cd by industry. Sources of contamination by Cd are the mining and smelting of Pb and Zn, atmospheric pollution from metallurgical industries, the disposal of wastes containing Cd, sewage sludge application to land and the burning of fossil fuels (Ames *et al*., 1983).

We observed that the contents of chlorophyll a and b in both AM and non-AM plants decreased under cadmium toxicity and by increase in Cd concentration. Haghiri (1973) and Root *et al*. (1975) reported 50% of chlorophyll reduction in the leaves of plants exposed to 2.0 mg L^{-1} of Cd. Cadmium reduces chlorophyll formation by reducing the uptake of Fe^{2+} and Mg^{2+} (Haghiri, 1973; Khan and Frankland, 1983) and by reacting with essential thiol groups in both the protochlorophyllide reductase protein and other enzymes involved in the light dependent synthesis of δ-aminolaevulinic acid (Stobart *et al*., 1985). The decrease of photosynthetic pigments include chl a and b and carotenoids as a result of heavy metals application such as Cu, Zn and Pb has been reported in many species of plants (Van Assche and Clijsters, 1990). The higher amount of chlorophylls in mycorrhizal treatments can be related to the decreased transport of cadmium into leaves and to decreased destruction of chlorophylls, subsequently.

In this investigation, the amount of total soluble sugars increased in the roots and shoots of AM and non-AM plants. In roots of AM-plants specially in the presence of 2500 μm Cd, this increase was more obvious, that shows the need of fungal symbiont to sugar in the presence of 2500 μm Cd. Soltani *et al*. (2006) have reported that cadmium at 600 and 800 μmol had been resulted to an increased content of soluble sugars and malondealdehyde in leaves and roots of treated rape (*Brassica napus*) plants. Moreover, it is reported that, cadmium causes the increase of reductive sugars in the seedling of rice (Verma and Dubey, 2001).

In the present study, the amount of total proteins in shoots and roots of both AM and non-AM plants have been increased in response to cadmium stress. The increase of total proteins content can caused by raising in synthesis of some enzymes, such as antioxidant enzymes and also by the synthesis of proteins and polypeptides that contribute in defense system of cells. These proteinaceous molecules are binding to metals and produce metalothionins and phytochelatins and alleviate the poisonous effects of the metals (Chaoui *et al*., 1997).

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