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Manganese Toxicity Effects on Chlorophyll Content and Antioxidant Enzymes in Pea Plant (*Pisum sativum* L. *c.v qazvin*)

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Abstract: Pea plants were grown in different concentrations of manganese (0, 25, 50, 100 and 200 ppm) during 15 days in culture solution. In this study, the effects of toxicity of manganese on chlorophyll content and antioxidant enzymes activity include catalase (CAT), ascorbate peroxidase (APX) and gauacol peroxidase (GPX) were investigated. The data showed that the low concentration of manganese (below 25 ppm) supplied in culture solution, induced decrease in chlorophyll content in pea plants and the high levels of manganese (above 50 ppm) inhibited chlorophyll synthesis in the leaves of this plants. Maximum and minimum rate of chlorophyll content were obtained in 25 and 200 ppm of manganese in culture solution, respectively. Our observations showed the close relationship between increase in manganese concentration and increase of antioxidant enzymes activity in pea plant grown in culture solution. The activity of APX in shoot and roots were higher than CAT and GPX. Data also suggest that the activity of antioxidant enzymes in shoot were higher than roots.

Key words: Toxicity, chlorophyll, antioxidant, pea plant, ascorbate peroxidase (APX)

INTRODUCTION

Manganese is one of the essential micronutrient for all plants, its Excess and deficiency induced unfavourable symptoms in plants (Miyake and Asada, 1992). Manganese has a vital role in redox reactions and cofactor for many enzymes include peroxidase, hydrogenase, oxidases and O_2 evolution (Foy *et al*., 1978). It is of particular importance in photosynthetic organisms where a cluster of Mn atoms in required as the catalytic center for light-induced water oxidation in photosystemII (Pittman, 2005). Manganese can be particularly toxic to plant growth and a variety of mechanisms exist to overcome such toxicity, including conversion of the metal to a metabolically inactive compound, such as a Mn^{2+}-chelate complex, or sequestration of the Mn^{2+} ion or Mn^{2+}-chelate complex in to an internal compartment such as vacuole, chloroplasts and endoplasmic reticulum (Csatorday *et al*., 1984; Hirschi *et al*., 2000; Schaaf *et al*., 2002; Fecht-Christoffers *et al*., 2003). However, the response of plants to excess Mn is affected by leaf age (Horst, 1988), temperature (Rufty *et al*., 1991), soil nutrient balance, soil PH, genotypes and light intensity (Alonso *et al*., 1998).

Csatorday *et al*. (1984) reported that inhibition of chlorophyll synthesis by Mn in a cyanobacterium, with accumulation of Mg protoporphyrin. Accumulation of Mg protoporphyrin and its monomethyl ester was also found in Fe and O_2 deficient whole plants (Granick, 1961; Spiller *et al*., 1982) and isolated plastids (Chereskin and Castelfranco, 1982). In generally accepted path of chlorophyll synthesis the probable site of Mn inhibition is an Fe requiring step following the insertion of Mg into the tetrapyrrole ring (Chereskin and Castelfranco, 1982).

Although, the physiological mechanisms of Mn toxicity and tolerance are unknown, several reports has suggests a role for excess Mn in the induction of oxidative stress.

The toxic effects of heavy metals, both essential and nonessential elements, have been linked to the production of free radicals (De Vos and Schat, 1991). Free radicals are usually formed as by-products of normal biological reactions, but their lifespan and diffusion into the cell space are closely controlled by the cell anti oxidant system. The involvement and role of antioxidants in protection against oxidative stress have been demonstrated using transgenic plants (Foyer and Mullineaux, 1994) and genetic variability in the content of the antioxidant elements has been reported in several species (light and excess). Because Mn might promote oxidative stress, it is important to characterize levels of antioxidants in leaves accumulating excess Mn.

In this study, the effects of different levels of Mn on antioxidants enzyme and chlorophyll levels were investigated.

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MATERIALS AND METHODS

To do the experiment seed of pea plants were surface strelized with 98% H_2SO_4 for 15 min, rinsed with tap water and ancubated at 251°C to germinate for 3 days. After germinating the seedlings were transferred to plastic pots and planted in the sand culture (4 seed in each pot and each treatment with 3 replicates) in the controlled condition (period of day/night, 12/12, at 251°C and relative humidity was between 50-60%) seedling were treatment with 5 levels of manganese (0, 25, 50, 100 and 200 ppm). after 15 days of treatment the pea plants with manganese, the plants were harvested and the shoot and root were separated.

Chl a and b in leaves were determined by the spectrophotometer according to Lichtenthaler and Wellburn (1985). Five gram fresh weight of leaves samples were ground in a mortar in 10 mL of 100% acetone. The extracts were filtered with filtering paper. Then determined the absorption of samples in wave length of 470, 645 and 662 nm via LKB spectrophotometer.

Enzyme assays: Oxidative enzymes were estimated in crude tissue extracts. For the preparation of crude tissue extracts, used the method of Change and Kao (1998). Five gram fresh leaf tissues of root and shoot weighted separately and ground in chilled pestle and mortar with 3 mL solution (inclued: Buffer trise-HCl, 0.5 molar with Ph = 7, $MgCl_2$ 3 milimolar and EDTA 1 milimolar). The extracts were cleared by centrifugating for a 10 min at 500 g and filtered.

CAT was estimated manometrically by an adaptation of the method described by Chance and Maehly (1959). The mine compartment of the flask contained 50 mM of extracted enzyme and 2.5 mL of 50 mM buffer phosphate (PH = 7.4) the side arm of the flask contained 0.1 mL of 0.1 M H_2O_2 decrease in absorption (wave length of 240 nm) by the deletion of H_2O_2 in 1 min were measured.

GPX was estimated by the method of Change and Kao (1998). The mine compartment of the flask contained 18.2 mL of 50 mM buffer phosphate, 1 mL of 0.1 M H_2O_2 and 0.3 mL of extracted enzyme and the end 1 mL of 1% gauacol were added. The enzyme activity by spectrophotometer in 1 min (wave length of 420 nm) were measured.

APX activity was estimated by method of Asada (2001). The reaction mixture contained: 2.5 mL of buffer phosphate (0.1 mM of ED TA, 0.5 Mm of ascorbate and 0.2 mL of 1% H_2O_2) and 0.1 mL of extracted enzyme. Enzyme activity were measured via oxidation of ascorbate by spectrophotometer (wave length of 240 nm).

RESULTS AND DISCUSSION

Chlorophyll: The data shown, that the pea plants were grown in 25 ppm of manganese concentration, contained higher content of chlorophyll to compared of other treatments. The content of chlorophyll below and above of 25 ppm manganese, supplied in medium were decreased gradually. In a low (below of 25 ppm) and excess (above of 50 ppm) of manganese the decreasing in chlorophyll were significant ($p<0.05$) (Fig. 1). The content of chla was higher than chlb in pea leaves.

The decrease in chlorophyll due to manganese deficiency would suggest that the manganese is precursor of chlorophyll synthesis (Page *et al*., 1966). Therefore, deficiency of manganese in leaves concluded the halt of chlorophyll synthesis process. In other hands, the decrease in chlorophyll content in excess of manganese may be due to the substitution of manganese in place of

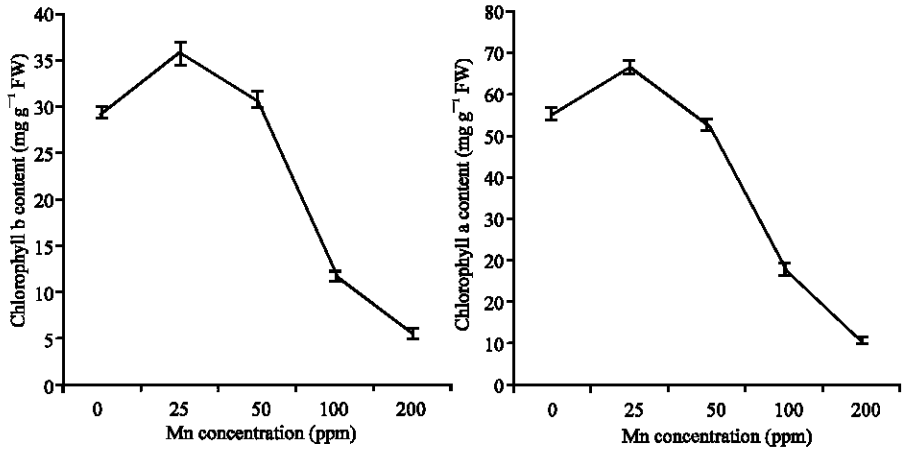

Fig. 1: The content of chlorophyll a and b in pea leaves has grown in medium containing 0, 25, 50, 100 and 200 ppm of Mn for the period of 15 days. The data indicates the mean±SE

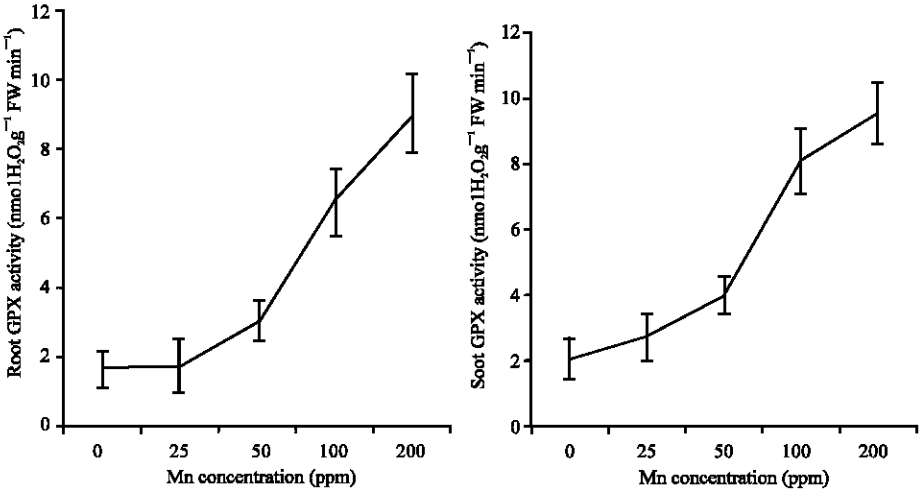

Fig. 2: The activity of GPX in shoot and root of pea plant has grown in medium containing 0, 25, 50, 100 and 200 ppm of Mn for the period of 15 days. The data indicates the mean±SE

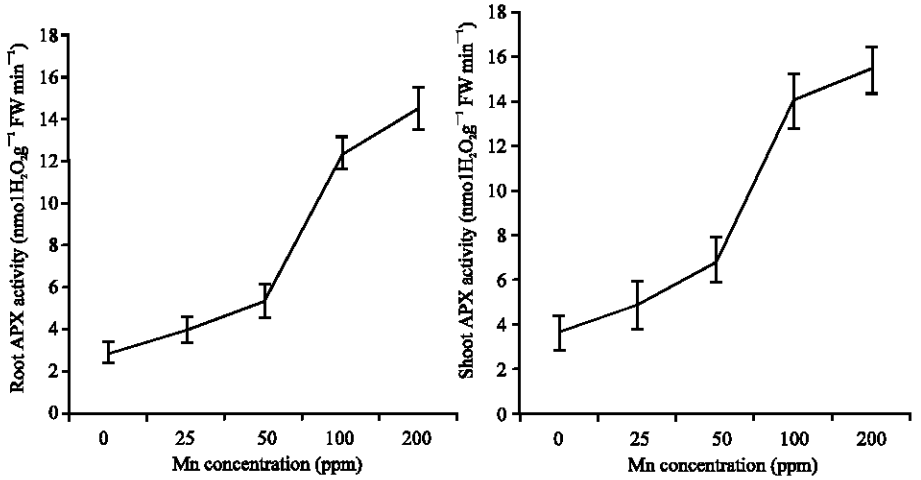

Fig. 3: The activity of APX in shoot and root of pea plant has grown in medium containing 0, 25, 50, 100 and 200 ppm of Mn for the period of 15 days. The data indicates the mean±SE

magnesium and iron in their respective porphyrin as suggested by Sideris and Young (1949). Also, decrease in chlorophyll content in excess of manganese may be due to in occurrence of changes in nitrogen metabolism involved in synthesise in compounds include proline for regulation of osmosis (Rosa-Ibara and Maiti, 1995).

Antioxidant enzymes: The data shown that the activity of all antioxidants enzymes (APX, GPX, CAT) were increased by excess of Mn in culture solution. But the rate of APX activity was higher to compared to GPX and CAT. In the all of treatments the activity of antioxidant enzymes in shoot were higher than the roots. Pea plants that grown in 200 ppm of Mn in medium, has shown highest rate in activity of antioxidant enzymes (Fig. 2-4).

Excess of Mn can induced oxidative stress in plants, oxidative stress can lead to formation of Reactive Oxygen Species (ROS) include superoxide radicals, hydroxile radicals and hydrogen peroxide (Demirevska-Kepova *et al*., 2004; Lei *et al*., 2007) that can damage of plant cells. Antioxidant enzymes may convert the H_2O_2 to the H_2O in the plant cells and neutralized the toxicity effects of H_2O_2 (Alonso *et al*., 1998). Therefore, in the condition of Mn toxicity, in order to protection the cells against oxidative stress, antioxidant enzymes, proportionately of Mn toxicity were increased.

The increase of activity of antioxidant enzymes in the shoot were higher than root in pea plants. Because manganese can translocated readily from roots to shoots (Mgema and Clarck, 1993; Charles, 1998) therefore,

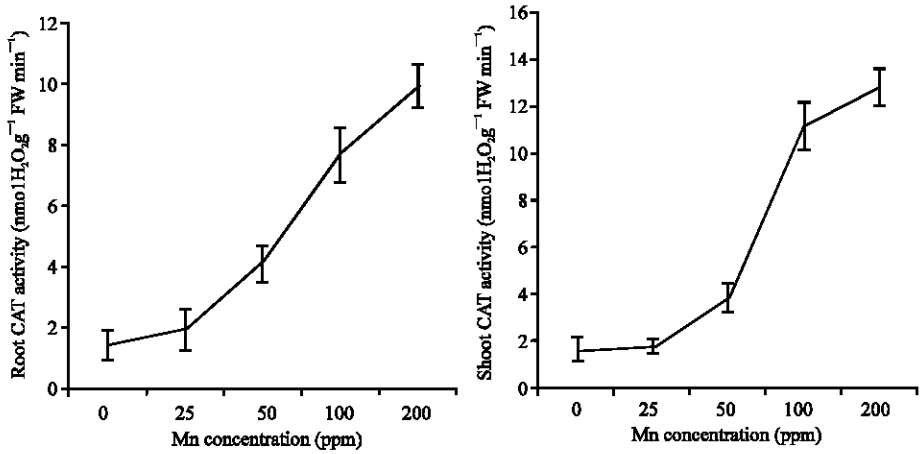

Fig. 4: The activity of CAT in shoot and root of pea plant has grown in medium containing 0, 25, 50, 100 and 200 ppm of Mn for the period of 15 days. The data indicates the mean±SE

accumulation of Mn and thus increasing in activity of antioxidant enzymes in shoot were higher than roots.

Our observations showed that in the level of 25 ppm of manganese in culture solution the pea plant were the best growth and higher content of chlorophyll and the activity of antioxidant enzymes were appropriated in this concentration. But in the below and above of 25 ppm of Mn concentration in the culture solution the chlorophyll content were decreased and the oxidative stress were increased and finally this condition lead to the decrease of growth in pea plant.

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