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Responses of *Pisum sativum* L. to Exogenous Indole Acetic Acid Application Under Manganese Toxicity

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Abstract Responses of pea (*Pisum sativum* L.) seedlings to manganese (50, 100 and 250 μM) and indole acetic acid (10 and 100 μM) treatments were investigated. Single and combined exposure of pea to manganese and 100 μM indole acetic acid decreased root and shoot fresh mass, chlorophyll, carotenoids, protein and nitrogen while ammonium content increased compared to the control. Combined treatment of pea with 250 μM manganese and 100 μM indole acetic acid decreased root and shoot fresh mass by 54% and 51%, chlorophyll and carotenoids by 31% and 26%, root and shoot protein by 47% and 44%, and root and shoot nitrogen by 44% and 40%, respectively. Activities of glutamine synthetase and glutamate synthase were decreased by the exposure of manganese and 100 μM indole acetic acid while glutamate dehydrogenase activity increased. Combined application of 250 μM manganese and 100 μM indole acetic acid decreased root and shoot glutamine synthetase activity by 44% and 39%, and glutamate synthase activity by 39% and 37% while root and shoot glutamate dehydrogenase activity increased by 47% and 42%, respectively compared to the control. In contrast, application of 10 μM indole acetic acid together with manganese decreased the negative impacts of manganese, and promoted seedling growth compared to the manganese treatments alone. This study has shown that 10 μM indole acetic acid protected pea seedlings appreciably from manganese toxicity by regulating ammonium content and the activities of enzymes of ammonium assimilation, while 100 μM of indole acetic acid exhibited opposite response under manganese toxicity.

Keywords Ammonium assimilation · Growth · Indole acetic acid · Manganese toxicity

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Manganese (Mn) is an essential micronutrient for plant growth and development, and is absorbed mainly as Mn^{2+}. However, excess Mn retards plant growth and development by interfering with metabolic processes (Foy 1984). The deleterious effects of Mn toxicity are often observed as stunted growth, chlorosis, crinkled leaves and brown lesions or "speckles" (Foy 1984). The mechanism of Mn toxicity in plants is still poorly known; however, its toxicity has been linked with generation of reactive oxygen species (ROS) that cause oxidation of biomolecules (Shi and Zhu 2008). In agriculture, nitrogen availability has a major influence on crop yield. Ammonium assimilation is an important metabolic process associated with the synthesis of several amino acids. However to date, little is known about the effect of Mn on NH_4^+ assimilation in plants.

Plant hormones are chemical messengers that regulate plant growth and development. Indole acetic acid (IAA) is a type of auxin that regulates plant growth and development (Chakrabarti and Mukherji 2003). However, several studies are available which show dual effects (positive or negative) of exogenous application of IAA on metabolism of plants as well as animals (Chakrabarti and Mukherji 2003; de Melo et al. 2004; Wang et al. 2007). Therefore, it would be noteworthy to know more about the effects of exogenous IAA application in plants.

We selected the pea (*Pisum sativum* L.) as a test species because it is an important leguminous crop, cultivated in most of the regions of the world for vegetable and pulse. In India, Mn-contamination of soil and water is a prevalent problem around various industries using Mn ores (Satyanarayana and Saraf 2007). This problem is further aggravated due to the use of Mn-containing water in irrigation. Deepali and Gangwar (2010) reported that textile and tannery effluents had 10 and 18 μM Mn/L while soil around these industries had 12 mM Mn/kg and 18 μM Mn/kg, respectively near Haridwar, India. The pea is also grown in Mn-contaminated soil which may have adverse effects on its productivity. Therefore, the aim of this study was to investigate the effects of exogenous IAA application on growth and NH_4^+ assimilation in pea seedlings under Mn toxicity.

Materials and Methods

Heathy and surface sterilized pea (*P. sativum* L. cv. Azad P-1) seeds were germinated and grown in sterilized Petri-plates (150 mm, Riviera) lined with Whatman No-1 filter paper, moistened with either 0.5 strength Hoagland's solution only (control) or with Mn (as $MnCl_2$; 50, 100 and 250 μM) and IAA (10 and 100 μM) alone or together in 0.5 strength Hoagland's solution (Arditti and Dunn 1969). During an 11-day seedling growth period, the growth chamber was set at 25 ± 2°C, 50%–60% of relative humidity and 150 μmol photons m^{-2} s^{-1} of light intensity with a 12 h photoperiod. In the present study, 12 treatments of Mn and IAA, alone or in combination, were prepared. Therefore, 12 Petri-plates were used, one for each combination. These selected treatments were given four times during 11-day seedling growth period at 3-day intervals; i.e., the first treatment was given during seed sowing (on the 1st day), second on the 3rd day, third on the 6th day and fourth on the 9th day. All the Petri-plates were kept wet by supplying Hoagland's solution daily or as needed. After 11 days, root and shoot samples from control and treated pea seedlings were harvested and different parameters were analyzed immediately.

For fresh mass measurement, ten seedlings were selected randomly from control and treated samples and then their root and shoot fresh mass was determined by using digital balance. Chlorophyll and carotenoids were estimated according to the method of Arnon (1949) and Ikan (1969), respectively.

Total protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Total nitrogen of each sample was estimated by the Kjeldahl method (Lang 1958). The NH_4^+ content was determined spectrophotometrically with the Nessler's reagent method (Molins-Legua et al. 2006).

For enzyme assays, control and treated samples were homogenized in respective chilled extraction buffer, the homogenate was centrifuged at 20,000*g* for 20 min at 4°C and the supernatant was used for enzyme analysis. Glutamine synthetase (GS, EC 6.3.1.2) activity was measured by following the method of Lillo (1984). One unit of enzyme activity is defined as 1 nmol γ-glutamylhydroxamate formed/mg protein/min. Glutamate synthase also called glutamine 2-oxoglutarate aminotransferase (NADH-GOGAT, EC 1.4.1.14) activity was determined by following the method of Singh and Srivastava (1986). One unit of enzyme activity is defined as 1 nmol NADH oxidized/mg protein/min. Glutamate dehydrogenase (GDH, EC 1.4.1.2) activity was assayed by the method of Singh and Srivastava (1983). One unit of enzyme activity is defined as 1 nmol NADH oxidized/mg protein/min.

The results presented are the means ± SE of three independent experiments. The significance of differences between the control and the treatments mean values was determined by the Duncan's multiple range test at $p < 0.05$ significance level.

Results and Discussion

Mn toxicity has become an important growth-limiting factor for plants in acidic soils (Foy 1984). Compared to the control, exposure of pea seedlings to 50–250 μM Mn alone and 100 μM IAA alone, as well as in combination, caused a decrease in growth and photosynthetic pigments in both roots and shoots (Fig. 1). Roots were more affected than shoots. Maximum decrease in root and shoot fresh mass was noticed under the treatment of 250 μM Mn + 100 μM IAA, where they were decreased by 54% and 51%, respectively compared to the control (Fig. 1). In the case of carotenoids, however, 50 μM Mn alone increased its content, while the combined application of 50 μM Mn and 100 μM IAA caused a decrease in carotenoids content compared to control (Fig. 1) This decrease in growth may be associated with reduced levels of protein and nitrogen, and decreased activities of the NH_4^+ -assimilating enzymes, GOGAT and GS (Figs. 2, 3).

Mn in excess has been reported to affect physiological and biochemical processes associated with plant growth and development (Shi and Zhu 2008). IAA in excess has been observed to decrease cell membrane integrity and to result in DNA fragmentation, chromatin condensation and loss of mitochondrial transmembrane potential, all leading to cell damage (de Melo et al. 2004). Furthermore, it has been shown that the occurrence of excess Mn and excess IAA toxicity was closely related with deceased contents of Mg and Fe, which may be related to reduced growth (Wang et al. 2007; Shi and Zhu 2008). Excess Mn and excess IAA

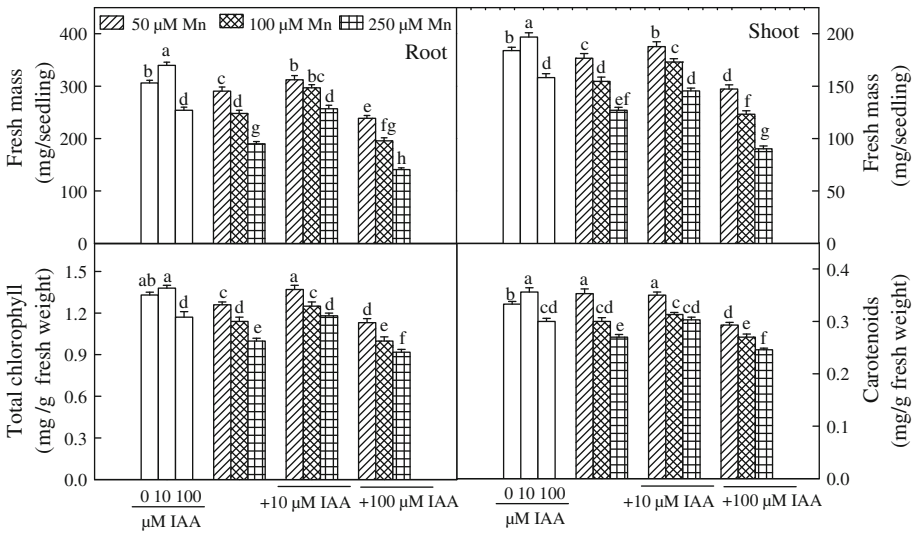

Fig. 1 Effects of exogenous IAA application on root and shoot fresh mass, total chlorophyll and carotenoids in pea seedlings under Mn toxicity. Values are presented as means ± SE of three independent experiments. Means with the *same letter(s)* are not significantly different at $p < 0.05$ according to the Duncan's multiple range test

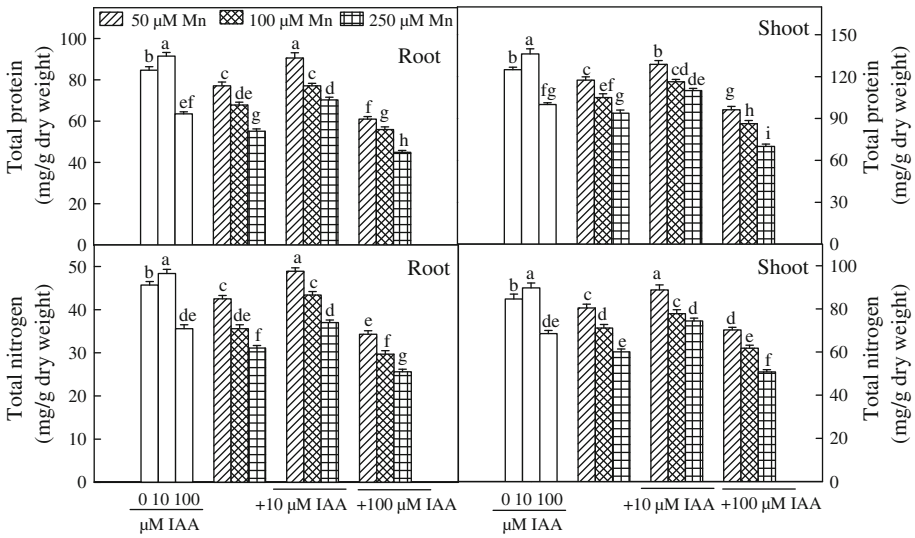

Fig. 2 Effects of exogenous IAA application on total protein and total nitrogen in root and shoot of pea seedlings under Mn toxicity. Values are presented as means ± SE of three independent experiments. Means with the *same letter(s)* are not significantly different at $p < 0.05$ according to the Duncan's multiple range test

toxicity may also be related to oxidative damage occurring in living systems through increasing cellular levels of ROS (Wang et al. 2007; Shi and Zhu 2008). This could partially explain the deleterious effects of Mn and 100 μM IAA on pea seedling growth.

In contrast, the application of 10 μM IAA together with Mn reversed the negative effects of Mn and promoted growth of pea seedlings compared to Mn treatments alone (Fig. 1). This 10 μM IAA-mediated alleviation of Mn toxicity may be related to increased activities of NH_4^+-assimilating enzymes, which perhaps maintained the nitrogen status of pea seedlings under Mn toxicity (Figs. 1, 2, 3). It has been reported that when salt stressed *Vigna radiata* plants were treated with IAA, showed better growth than IAA untreated plants (Chakrabarti and Mukherji 2003).

Protein and nitrogen contents may be regarded as reliable indicators of growth performance of plants under stress conditions. Total protein and total nitrogen both were lower in Mn and 100 μM IAA treated seedlings than their respective control values (Fig. 2). Exposure of pea seedlings to the highest degree of stress (250 μM Mn + 100 μM IAA), decreased root and shoot protein content by 47% and 44%, respectively, and nitrogen content by 44% and 40%, respectively compared to control. Decrease in protein content in pea seedlings under single and combined treatments of Mn and 100 μM IAA might be due to the increased degradation of proteins and accelerated protease activity (Balestrasse et al. 2006). Upon exposure of pea seedlings to Mn and 100 μM IAA, a decrease in nitrogen content suggested an interruption in nitrate uptake. A reduction in nitrogen content has been reported in *Vigna radiata* under stress conditions (Chakrabarti and Mukherji 2003). However, the treatment of 10 μM IAA together with Mn resulted in increased concentrations of total protein and total nitrogen in both

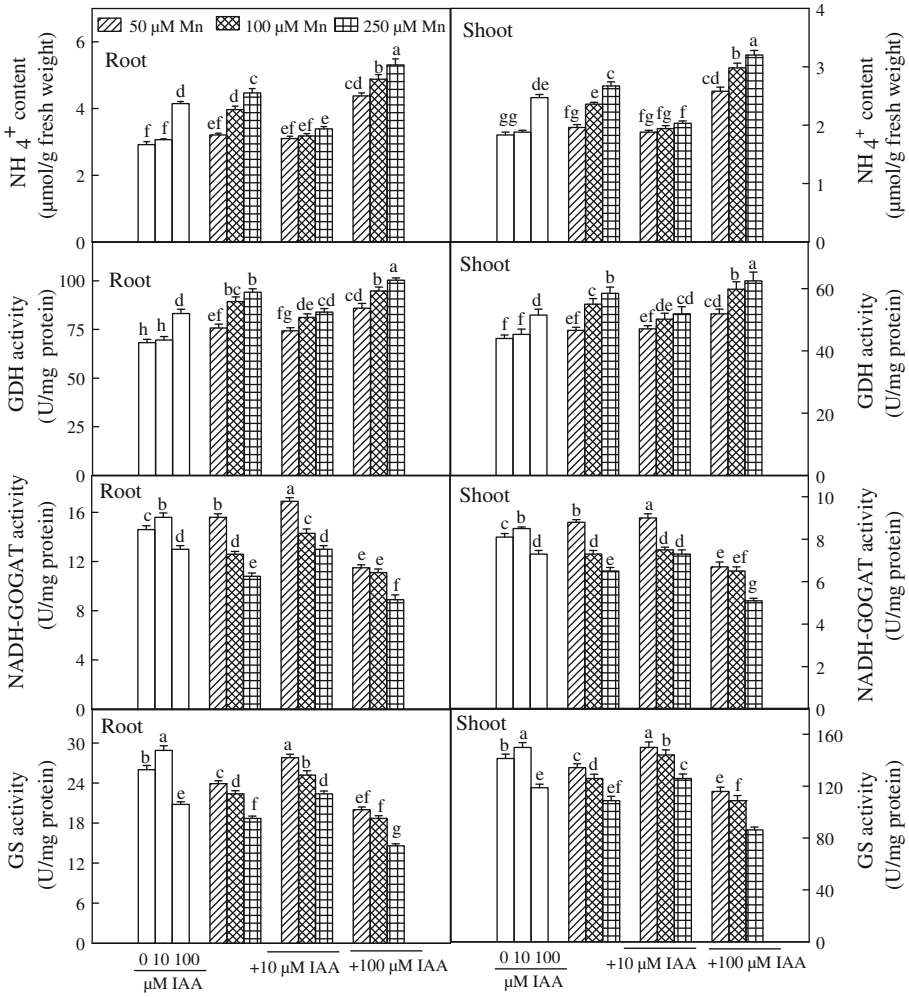

Fig. 3 Effects of exogenous IAA application on NH_4^+ content, and activities of GDH, NADH-GOGAT and GS in root and shoot of pea seedlings under Mn toxicity. Values are presented as means ± SE of three independent experiments. Means with the *same letter(s)* are not significantly different at $p < 0.05$ according to the Duncan's multiple range test

roots and shoots when compared to the respective Mn treatments alone (Fig. 2).

Nitrogen is considered to be a vital macronutrient that determines growth and productivity of plants. Its reduced form, ammonia, is toxic if it accumulates in cells, as it is known to affect ATP synthesis (Gerendás et al. 1997). In our study, NH_4^+ concentrations in both root and shoot were increased by exposure to Mn and 100 μM IAA alone, as well as in combination (Fig. 3). NH_4^+ content reached a maximum following exposure to 250 μM Mn + 100 μM IAA, increasing by 82% and 75%, respectively compared to the controls. This suggested a dysfunction of NH_4^+-assimilating enzymes. It has been reported that high concentrations of NH_4^+ lead to nutrient deficiency and chlorosis, inhibit secondary growth, alter intracellular pH and osmotic balance, and inhibit ATP synthesis (Gerendás et al. 1997). Therefore, the increased content of NH_4^+ observed in our study may be linked with declined growth of pea seedlings. On the contrary, 10 μM IAA alone did not significantly influence NH_4^+ content compared to the control (Fig. 3). Moreover, application of 10 μM IAA together with Mn, led to decreases in root and shoot NH_4^+ content compared to Mn treatments alone. This suggested the proper incorporation of NH_4^+ into an organic form (i.e., glutamate).

Glutamate dehydrogenase catalyses a reversible reaction involving the assimilation of NH_4^+ into glutamate and the deamination of glutamate into 2-oxoglutarate and NH_4^+ (Masclaux-Daubresse et al. 2006). Both Mn and IAA alone and in combination increased root and shoot GDH activity, with maximum activities observed in the combination treatment of 250 μM Mn and 100 μM IAA. Under Mn and 100 μM IAA treatments, increased GDH activity might have compensated for decreased GOGAT and GS activities (Fig. 3). It has been reported that under stress conditions that when the GS/GOGAT system is not fully operative, an increase in GDH activity may relieve pressure of accumulating toxic amounts of NH_4^+ and provide glutamate for the biosynthesis of several protective compounds (Skopelitis et al. 2006). However, it

seems that under Mn and 100 μM IAA treatments, increased GDH activity might not have been sufficient to remove excessive accumulation of NH_4^+ (Fig. 3). Glutamine synthetase and GOGAT both are considered prime enzymes for incorporation of NH_4^+ into glutamate (Masclaux-Daubresse et al. 2006). We observed that both GOGAT and GS activities (except GOGAT activity at 50 μM Mn alone) in root and shoot were decreased under single and combined treatments of Mn and 100 μM IAA (Fig. 3). GS activity was more affected than GOGAT activity. The maximum decrease in both GOGAT and GS activities was observed under the treatment of 250 μM Mn + 100 μM IAA (root and shoot GOGAT activity decreased by 39% and 37%, respectively and root and shoot GS activity decreased by 44% and 39%, respectively compared to control). Decreases in GOGAT and GS activities may lead to a disturbance in nitrogen status, which eventually affects the growth and development of pea seedlings. Under stress conditions, decreases in both GOGAT and GS activities may be attributed to oxidative modifications of these enzyme proteins. Balestrasse et al. (2006) reported that under Cd stress, GOGAT and GS proteins become oxidatively modified, leading to the loss of their functions. Both Mn and IAA in excess are known to stimulate excessive ROS generation and cause oxidative damage to proteins (Wang et al. 2007; Shi and Zhu 2008). Decreased activities of GOGAT and GS under stress conditions have been reported in earlier studies (Chakrabarti and Mukherji 2003; Balestrasse et al. 2006). In contrast, application of 10 μM IAA together with Mn increased GOGAT and GS activities in root and shoot compared to Mn treatments alone (Fig. 3). Under Mn + 10 μM IAA treatments, increased activities of GOGAT and GS suggested the proper incorporation of NH_4^+ into glutamate as indicated by low content of NH_4^+ when compared to Mn and Mn + 100 μM IAA treated seedlings (Fig. 3).

Our study has shown that IAA differentially modulated Mn toxicity in pea seedlings. Indole acetic acid (100 μM) itself produced negative effects on growth and NH_4^+ assimilation, and when it was given together with Mn, it increased Mn toxicity. However, application of 10 μM IAA together with Mn ameliorated Mn toxicity. Our results suggest that this was accomplished by the regulation of NH_4^+ content through increased activities of NH_4^+-assimilating enzymes. These data indicated that exogenous application of 10 μM IAA may be an alternative strategy to increase the tolerance of crops under metal stress.

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References

Arditti J, Dunn A (1969) Environmental plant physiology–experiments in cellular and plant physiology. Holt, Rinehart and Winston Inc, New York

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Balestrasse KB, Gallego SM, Tomaro ML (2006) Oxidation of the enzymes involved in ammonium assimilation plays an important role in the cadmium-induced toxicity in soybean plants. Plant Soil 284:187–194

Chakrabarti N, Mukherji S (2003) Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. Biol Plant 46:63–66

de Melo MP, de Lima TM, Pithon-Curi TC, Curi R (2004) The mechanism of indole acetic acid cytotoxicity. Toxicol Lett 148:103–111

Deepali, Gangwar KK (2010) Metals concentration in textile and tannery effluents, associated soils and ground water. New York Sci J 3:82–89

Foy CD (1984) Physiological effects of hydrogen, aluminium, and manganese toxicities in acid soil. In: Adams F (ed) Soil acidity and liming, 2nd edn. ASA-CSSA-SSSA, WI, USA, Agronomy Monograph, pp 57–97

Gerendás J, Zhu Z, Bendixen R, Ratcliffe RG, Sattelmacher B (1997) Physiological and biochemical processes related to ammonium toxicity in higher plants. Plant Nutr Soil Sci 160:239–251

Ikan R (1969) Natural products, a laboratory guide. Academic Press, New York

Lang CA (1958) Simple microdetermination of Kjeldahl nitrogen in biological materials. Anal Chem 30:1692–1694

Lillo C (1984) Diurnal variations of nitrite reductase, glutamine synthetase, glutamate synthase, alanine amino transferase and aspartate amino transferase in barley leaves. Physiol Plant 61:214–218

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandias M, Grandjean J, Valadier MH, Feraud M, Jouglet T, Suzuki A (2006) Glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink source nitrogen cycle in tobacco. Plant Physiol 140:444–456

Molins-Legua C, Meseguer-Lloret S, Moliner-Martinez Y, Campíns-Falcó P (2006) A guide for selecting the most appropriate method for ammonium determination in water analysis. Trends Anal Chem 25:282–290

Satyanarayana YV, Saraf R (2007) Iron and manganese contamination: sources, adverse effects and control methods. J Environ Sci Eng 49:333–336

Shi Q, Zhu Z (2008) Effect of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. Environ Exp Bot 63:317–326

Singh RP, Srivastava HS (1983) Regulation of glutamate dehydrogenase activity by amino acids in maize seedlings. Physiol Plant 57:549–554

Singh RP, Srivastava HS (1986) Increase in glutamate synthase (NADH) activity in maize seedlings in response to nitrate and ammonium nitrogen. Physiol Plant 66:413–416

Skopelitis DS, Paranychianakis NV, Paschalidis KA (2006) Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. Plant Cell 18:2767–2781

Wang H, Shan X, Wen B, Owens G, Fang J, Zhang S (2007) Effect of indole acetic acid on lead accumulation in maize (*Zea mays* L.) seedlings and relevant antioxidant response. Environ Exp Bot 61:246–253