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Detoxification of Heavy Metals by The Interaction between Mycorrhizal Fungi and Jasmonic Acid : Modulation of The Antioxidant Defense System and Protein Profile in Maize

Samia M. El-Khallal and Hoda H. Abo-Ghalia
Botany Department, Faculty of Girls for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

THE EFFECT of exogenously applied arbuscular mycorrhizal (AM) fungi (*Glomus fasciculatum*) and jasmonic acid (JA) on the ability of maize plants to alleviate heavy metal stress, induced by 2 and 4mM Zn or Cr^{3+} ions was investigated. The results showed that both heavy metal ions induced oxidative stress response in maize plants treated or non-treated with AM fungi and / or JA. Therefore, a significant increase in the production of H_2O_2 and lipid peroxidation (as indicated by malondialdhyde, MDA) and a decrease in ascorbic acid (ASA) content in maize leaves was the physiological response of the metal-induced generation of O_2 radicals and subsequent cell or tissue damage. Results indicated that AM fungi and JA individually or in combination reduced Zn and Cr^{3+} toxicity, which is closely associated with the decrease in H_2O_2 formation and membrane lipid peroxidation. Moreover, Zn and Cr^{3+} stress induced alterations in the activities of the enzymatic antioxidant defense system such as guaiacol peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD). AM fungi and / or JA significantly increased activities of the antioxidant enzymes in leaves of stressed maize plants, which is considered to play an active role in the cellular defense strategy against oxidative stress and may have important ecological consequence for the adaptation of plants to heavy metal stress. On the other hand, the present results indicated that Zn and Cr^{3+} treatments caused a highly significant reduction in the contents of total soluble proteins and nucleic acids (DNA & RNA) compared with the corresponding control values. Despite of this, the plants treated with both AM fungi and JA had higher total protein and nucleic acid contents at all concentrations of Zn and Cr^{3+} ions. SDS – PAGE electrophoresis revealed that AM fungi and / or JA evokes changes in the pattern of protein profile of maize leaves in response to 4mM of both Zn and Cr^{3+} ions. Thus, disappearance of some bands and appearance of novel protein bands of low molecular weights in these plants could be attributed in heavy metal tolerance.

It was concluded that AM fungi and JA individually or in combination alleviated toxicity of both Zn and Cr^{3+} ions in maize plants mediated by the reduction in the level of H_2O_2 and MDA, induction in the activity of antioxidant defense system and novel proteins of low molecular weights. Finally, applying of mycorrhizal

fungi or JA reduced oxidative stress induced by Zn and Cr^{3+} ions, but the positive response was more pronounced when AM fungi and JA were applied together.

Keywords: Heavy metal stress, mycorrhizal fungi, jasmonic acid, *Zea maize*, Antioxidant system and Protein profile.

Heavy metal toxicity is one of the major environment health problems in modern society, with potentially dangerous bioaccumulation through the food chain. Rapid industrialization and urbanization have enhanced the levels of toxic heavy metals in the environment, posing a potential health hazard for all living organisms (Bonnet *et al.*, 2000; Yeh *et al.*, 2003). Exposure of plants to heavy metal ions causes growth inhibition, accompanied by the alteration of membrane permeability of cells leading to leakage of ions and pigment destruction (Luna *et al.*, 1994). Biochemical responses of higher plants to toxic metals are complex and several defense strategies have been suggested. These include complexation of metal ions, reduced influx of metals and enhanced production of antioxidants that detoxify free radicals produced in response to toxic metals (Hartley-Whitaker *et al.*, 2002). Mechanisms involved in oxidative stress are important, since heavy metals may cause formation of free radicals and activate different antioxidant system such as peroxidases, superoxide dismutases, catalase and glutathione (Wu *et al.*, 2003). In parallel to metal – induced tissue damage or cell death, alteration of both antioxidant enzyme activities and antioxidant levels as well as enhancement of both lipid peroxidation (Gallego *al.*, 2002) and phytochelatin synthesis (Gupta and Goldsbrough , 1991) have been observed. Therefore, the metal – induced phytotoxicity may be mediated by oxidative stress. At the molecular level, different types of the induced functional proteins were detected in plants as a consequence of their exposure to various ions of heavy metals. For example, plants contain chelators including small peptides, organic acid and amino acids that bind free metal ions. They contribute to metal detoxification by buffering cytosolic metal ions. The principle classes of known metal chelators are phytochelatins and metallothioneins (Suzuki *et al.*, 2002; Ederli *et al.*, 2004), which play a physiological role in heavy metal transport and detoxification. Moreover, Heat Shock Proteins (HSP_s) (Lewis *et al.*, 2001); pathogenesis –related proteins (Przymuinsiki and Gwozdz, 1999) and apoplastic proteins such as peroxidase and glucanases may be part of a general stress response and establish some degree of resistance against other stressors (Blinda *et al.*, 1997).

Zinc (Zn) is an essential micronutrient acting as a stimulator or an inhibitor for plant growth and metabolism depending on applied concentrations. Cakmak (2000) speculated that Zn stress may alter activities of a number of antioxidant enzymes, resulting in extensive oxidative damage to membrane lipid, proteins and nucleic acids. Chromium (Cr) occurs in several oxidation states ranging from Cr^{2+} to Cr^{6+}. Toxicity of Cr to plants depends on its valance state : Cr^{5+} is highly toxic and mobile wherase Cr^{3+} is less toxic . Both Cr^{3+} and Cr^{5+} are biologically

active oxidation states of Cr and are involved in redoxcycling with the production of reactive oxygen species (Scharma and Scharma, 1996). Chromium causes growth retardation (Han *et al.*, 2004), induce iron chlorosis and reduce activities of heme enzymes (Pandy and Sharma, 2003).

On the other hand, all organisms including microorganisms can achieve resistance to heavy metals by "avoidance" when the organism is able to restrict metal uptake or by "tolerance" when the organism survives in the presence of high internal metal concentrations. Among soil microorganisms, mycorrhizal fungi are the only ones providing a direct link between soil and roots, and can therefore be of great importance in phytoremediation, enhancing heavy metal availability and tolerance to plants (Tullio *et al.*, 2003; Gaur and Adholeya, 2004). Mycorrhiza have been reported to play an important role in phytoremediation of Cr-contaminated soils by enhancing Cr uptake and increasing translocation to shoot (Davies *et al.*, 2001) and had a positive effect on tissue mineral concentration, growth and gas exchange in Cr-treated plants (Davies *et al.*, 2002).

Jasmonic acid (JA) and its derivatives commonly termed jasmonates are hormonal regulators involved in plant responses to abiotic and biotic stresses as well as in plant development (Creelman and Mullet,1997). The role of jasmonates is well established as part of a complex signal transduction pathway activated upon local wounding of leaves (Ryan, 2000) . JA appears to promote the colonization and development of mycorrhizal structures in *Allium sativum* and mycorrhizal colonization has been reported to elevate JA biosynthesis in *Hordeum vulgare* (Hause *et al.*, 2002).The endogenous rise in jasmonates might be related to the fully established symbiosis rather than to the recognition of interacting partners or to the onset of interaction . Also it has been shown that applied exogenously jasmonates elicit several different physiological responses to stress. They can elicit components of the stress response and thereby increase plant resistance (Gao *et al.*, 2004). JA-Me strongly influences Cu and Cd toxicity in Arabidopsis plants, and this effect is partially metal – specific and depends on jasmonate concentrations and its internal or external origin (Maksymies and Krupa, 2002).

The present study was carried out to investigate the possible roles of a mycorrhizal fungus (*Glomus fasciculatum*) and jasmonic acid individually or in combination in alleviation of heavy metal stress induced by Zn and Cr^{3+} ions on maize plants. Changes in the activities of both enzymatic and non-enzymatic antioxidant defense system and protein profile were studied in stressed maize plants in an attempt to investigate the mechanism of heavy metal tolerance.

Material and Methods

Seeds of maize (*Zea maize* L.cv. Giza 2) were surface sterilized for 5 min. in 10%(w/w) Sodium hypochlorite, rinsed in distilled water and allowed to

germinate in sterilized water for 2 days. The uniform seedlings were selected and transplanted to clay pots (30 cm diameter) filled with 10 kg of sterilized sand loamy soil [1:2 (v/v)]. Each pot was planted with 6 seedlings which were thinned to 4 after one week post planting . The pots were divided into five groups; 20 pots for each. The pots of the 2^{nd} , 3^{rd} , 4^{th} and 5^{th} groups were subdivided into 4 sets and treated as follow :

- Plants of the first set were treated with the test solution of 2 and 4mM Zn or Cr^{+3} ions.
- Plants of the second set were inoculated with AM fungi and treated with the test solution of Zn or Cr^{+3} ions.
- Plants of the third set were sprayed twice with JA and treated with the test solution of Zn or Cr^{+3} ions .
- Plants of the fourth set were inoculated with AM fungi, sprayed with JA and treated with test the solution of Zn or Cr^{+3} ions.

On the other hand, the pots of the 1^{st} group were irrigated with tap water to serve as control. The plants were harvested ten weeks after sowing and were used for carrying out the different measurements.

Mycorrhizal inoculant

The AM fungus used was *Glomus fasciculatum*. It was produced by Biorize R and D, France as granular inoculum .The AM inocula (2500 spores / pot) were placed 2-3 cm below the planting holes in each pot at planting time.

Jasmonic acid application

Jasmonic acid (JA) at 25μM plus 0.05% tween 20 (100ml / pot) was applied twice to the foliage of maize plants at 2 and 4 weeks.

Heavy metal application

The heavy metal ions used in the present investigation were pure zinc (Zn) in the form of zinc sulfate ($Zn\ SO_4$) and chromium (Cr^{3+}) in the form of chromium sulfate ($Cr\ SO_4$). The ions of both heavy metals were applied as aqueous solutions of 2 and 4mM and were used for plant irrigation after two weeks planting for six weeks period.

Biochemical measurement techniques

Determination of hydrogen peroxide, lipid peroxidation and ascorbic acid

The H_2O_2 content was colorimetrically measured, as described by Jana and Choudhuri (1981). H_2O_2 was extracted by homogenizing 50 mg leaf tissue with 3ml of phosphate buffer (50mM, pH 6.5). The homogenate was centrifuged at 6.000 X for 25 min. to determine H_2O_2 contents; 3ml of extracted solution were mixed with 1 ml of 0.1 % titanium chloride in 20% (v/v) H_2SO_4. The mixture was then centrifuged at 6.000 X for 5 min. The intensity of the yellow colour of the supernatant was measured at 410 nm . H_2O_2 level was calculated using the extinction coefficient 0.28 $\mu mol^{-1}\ cm^{-1}$. The level of lipid peroxidation was measured as the amount of malondialdhyde (MDA), which was extracted with

10% trichloroacetic acid (TCA) and determined according to Heath and Packer (1968). The level of MDA was calculated using MDA's extinction coefficient of 155 mM cm^{-1}. Ascorbic acid (ASA) content was estimated according to Roe (1964), which is based on the reduction of 2,6- dicholorophenol indophenol (2,6-DCIP) by ascorbic acid. The quantity of ASA was calculated by comparing with the values obtained by the known quantities of standard ASA .

Enzyme extraction and assay procedures

Immediately after harvesting, leaves were frozen in liquid N_2. Frozen tissues were then ground to fine powder in a small mortar and pestle. The powder was then extracted with 50 mM K.phosphate buffer (pH 7.0), 0.1mM EDTA, 4% polyvinylpyrrolidone (PVP) and 0.2 mM ascorbic acid. The homogenates purified by centrifugation at 12.000 X at 4°C for 20min.

Catalase activity was determined by monitoring the decomposition of H_2O_2 at 240 nm following the method of Aebi (1984). Guaiacol peroxidase (GPX) activity was measured by monitoring the increase in absorbance at 470 nm as guaiacol reduction method (Zhang, 1992). Activity of ascorbate peroxidase (APX) was monitored as a decrease of ascorbate by measuring change in absorbance at 290 nm for 1min (Chen and Asada, 1989). Superoxide dismutase (SOD) activity was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Dhindsa *et al.*(1981). One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of reduction of NBT measured at 560nm. Activities of different forms of SOD were identified by using 3mM KCN or 5mM H_2O_2 as the final concentration in the reaction (Giannopolities and Ries, 1977). KCN inhibits Cu-Zn SOD but does not affect MnSOD or FeSOD , while H_2O_2 inactivates Cu / Zn SOD and FeSOD without affecting MnSOD.

Determination of nucleic acids

Air dried tissue was used for nucleic acids determination using the method suggested by Ogur and Rosen (1950) and Schenieder (1945). These methods depend on the insolubility of nucleic acids in cold perchloric acid, and specific reaction of RNA / DNA with orceinol and diphenylamine, respectively.

Extraction, estimation and characterization of proteins

Extraction of proteins was carried out by homogenising fresh maize leaves (1g) with 1ml of extraction buffer (100mM Tris–HCl (pH 7.5), 4mM 2-mercaptoethanol, and 0.1mM EDTA–Na. The homogenate was centrifuged at 10.000 X for 20min. The supernatant was used for quantitative estimation of total soluble proteins by using Bio – RAD reagent as described by Bradford (1976). Protein fractions were characterized and identified using one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS – PAGE). Poyacrylamide slab gel (12.5%) was prepared according to Laemmli (1970). Gel was stained by coomassie blue (0.5g/L) and destained with 5% MeOH/acetic acid

mixture . The destained gel was photographed, then scanned for quantitative determination using a laser densitometer.

Statistical analysis

The recorded data were treated statistically using the one way analysis of variance as described by Snedecor and Cochran (1969). The means were compared by L.S.D. using spps program version 8.

Results and Discussion

Changes in hydrogen peroxide, lipid peroxidation and ascorbic acid

It is clear from Table 1 that H_2O_2 was accumulated in leaves of maize plants in response to Zn and Cr^{3+} ions. Among the two metal treatments, the Cr^{+3} - treated plants registered higher values of H_2O_2 (265 and 490% for 2 and 4mM, respectively). On the other hand, accumulation of H_2O_2 during Zn and Cr^{3+} stresses decreased in plants treated with AM fungi and / or JA as compared to non-treated plants. These results indicate that rapid production of H_2O_2 is one of the most remarkable metabolic consequences during stress in plants. H_2O_2 is harmful and toxic to the cell, it leads to oxidative damage through a loss of structure and function of cells (Prasad *et al.*, 1995). Accumulation of H_2O_2 in leaves of maize plants in response to Zn and Cr^{3+} ions may be produced in a manner similar to H_2O_2 production in plants in response to other heavy metals (Cho and Park, 2000 ; Chien *et al.*, 2001). Results indicated that both AM fungi and JA individually or in combination alleviate toxicity of H_2O_2 by increasing activities of antioxidant enzymes which can decrease the level of H_2O_2 either by direct decomposition or oxidation.

Measurement of malondialdhyde (MDA) levels is routinely used as an index of lipid peroxidation under stress conditions. MDA level significantly increased in leaves of maize plants subjected to different concentrations of both heavy metal ions. The Cr^{3+} - treated plants recorded higher values of MDA than Zn – treated plants (Table 1), indicating that membrane lipid peroxidation was more active in response to Cr^{3+} ions. These results agree with the fact that Cr^{3+} can be reduced to Cr^{2+} by the biological reductants L. cystein and NADPH. The newly formed Cr^{2+} reacts with H_2O_2 which generally accumulated to produce hydroxyl radicals that are presumably responsible for tissue damaging effects (Pandy and Shyam , 2002). Zn stimulates lipoxygenase degradation of polyunsaturated fatty acids, as was evidenced by the accumulation of MDA in *Phaseolus vulgaris* seedlings (Wecks and Clijsters , 1997).

MDA content in leaves of AM fungus and / or JA – treated plants was observed to be lower than that in non—treated plants. The lowest MDA content was found in plants treated with both AM fungi and JA in response to Zn ions, compared with all stressed – plants (Table 1). Thus AM fungi and JA have

beneficial effects to prevent membrane damage during heavy metal stress may be through membrane stabilization and /or reduction of reactive oxygen species (H_2O_2, OH, O_2 .... etc) which induce lipid peroxidation. Unlike this, the striking increase in lipid peroxidation in both Zn and Cr^{3+} - treated plants was the physiological impact of the metal – induced generation of O_2 radicals.

TABLE 1. Changes in the hydrogen peroxide (H_2O_2), malondialdhyde (MD) and ascorbic acid (ASA) contents in leaves of maize plants treated with AM fungi and / or JA , and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions.

Treatments		H_2O_2 (μmol g^{-1} Fw)	MDA (μmol g^{-1} Fw)	ASA (μmol g^{-1} Fw)
Control		5.2	15.0	11.2
Zn	2mM	11.1**	22.0*	11.5
	2mM + AM fungi	8.5*	17.6*	14.7*
	2mM + JA	8.1*	18.2*	15.8*
	2mM + AM fungi + JA	7.4*	16.5	17.2**
	4mM	20.7***	35.0***	10.3*
	4mM + AM fungi	13.6**	28.3**	13.6*
	4mM + JA	14.8**	27.5**	13.7*
	4mM + AM fungi + JA	11.5*	23.6*	15.3**
Cr^{3+}	2mM	13.8**	25.0**	10.0*
	2mM + AM fungi	10.4*	21.3*	12.9*
	2mM + JA	9.8*	19.5*	13.6*
	2mM + AM fungi + JA	8.3*	18.6*	14.7*
	4mM	25.5***	41.0***	8.5**
	4mM + AM fungi	16.7**	32.0***	10.7
	4mM + JA	16.2**	30.0**	11.5
	4mM + AM fungi + JA	13.5*	26.8**	12.8*

* : Significant at $p < 0.05$, ** : highly significant at $p < 0.01$, *** : very highly significant at $p < 0.001$

Also, as shown in Table 1, ascorbic acid (ASA) content decreased in maize leaves with increasing the concentration of Zn and Cr^{3+} ions. However, the level of ASA significantly increased in leaves of AM fungi and / or JA, compared with non – treated plants. These results indicate that AM fungi and JA, especially when applied together may play an important role in increasing the level of antioxidant substance (ASA) in stressed maize plants, which is important to protect plants from oxidative stress. One of the important functions of ASA is the protection against oxidative damage of plant cells through the scavenging of H_2O_2 mediated by ascorbate peroxidase (El-Khallal., 2002). Moreover, heavy metal toxicity is considered to induce the generation of O_2 radicals which were assumed to be increased in the oxidation of ascorbic acid to dehydroascorbate, leading to reduction in the ASA content in plants (Rao and Sresty, 2000).

Changes in the activity of antioxidant enzymes

When plants encounter environmental stress, a number of defense responsive system begin to work to overcome them . One of these system is the activation of

antioxidant enzymes. Plant cells are equipped with several free radical detoxifying enzymes to protect them against oxidative damage induced by excess heavy metals . Therefore, activities of the enzymatic antioxidant defense system such as guaiacol peroxides (GPX), catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) were investigated in leaves of maize plants subjected to different concentrations of Zn and Cr^{3+} ions.

Peroxidase activity

Among various enzymes involved in the abolishment of O_2 free radicals, peroxidase can be considered as one of the key ones, since both of its extra and intracellular forms are participating in the breakdown of H_2O_2 (Foyer *et al.*, 1994).

Figure 1 shows that guaiacol peroxidase (GPX) increased in leaves of Zn – treated plants. Unlike Zn, Cr^{3+} ions strongly deceased activity of GPX in maize leaves and registered lower values when compared with the corresponding control. AM fungi and / or JA strongly increased GPX in leaves especially in response to Zn ions. It has been shown that increase in GPX activity is a defense response to most if not all metals which may cause damage or disturb normal function of the cells (Fang and Kao, 2000; Wu *et al.*, 2003; Metwally *et al.*, 2004). Results showed that induction in GPX activity in leaves of maize plants treated with both AM fungi and JA in response to heavy metal ions may be important for alleviating oxidative stress and increase heavy metal tolerance. Peroxidase activity usually increased in roots and nodules of mycorrhizal soybean plants (Porcel *et al.*, 2003) and pea seedlings (El Khallal, 2001) treated with JA under salt stress. On the other hand, plant exposure to toxic concentrations of Cr^{3+} produces diverse physiological response and reduces the activities of heme enzymes (Scharma and Scharma, 1996). Where Cr^{3+} - induced decrease in the availability of Fe for ferrochelatase catalyzed Fe incorporation in photoporphyrin IX could be a major factor limiting heme biosynthesis, manifested as decreased activities of CAT and POX in Cr^{3+} stressed plants. Also, the commonality of the ionic radii of Cr^{3+} and Fe^{3+} may lead to Cr^{3+} substitution for Fe^{3+} in the heme protein resulting in loss of their catalytic efficiency (Pandy and Sharma, 2003).

Catalase activity

Catalase (CAT) activity of leaves of maize plants decreased substantially with increasing concentrations of externally supplied of Zn and Cr^{3+} ions. AM fungi and/ or JA significantly increased CAT activity in leaves of heavy metal – stressed plants. High activity of CAT was found in Zn – stressed plants treated with both AM fungi and JA (Fig.1). These results indicated that inhibition in CAT activity in Zn and Cr^{3+} stressed plants might be related to the accumulation of H_2O_2 (Table 1) which increased during stress. It was suggested that heavy metals – induced decrease in CAT activity and other heme proteins resulting in

loss of their catalytic efficiency in tomato (Cho and Park, 2000) , cabbage (Pandy and Sharma, 2003) and *Phragmites australis* plants (Ederli *et al.*, 2004). However, induction in CAT activity in AM fungi and JA – treated plants is closely associated with the reduction in both H_2O_2 and MDA.

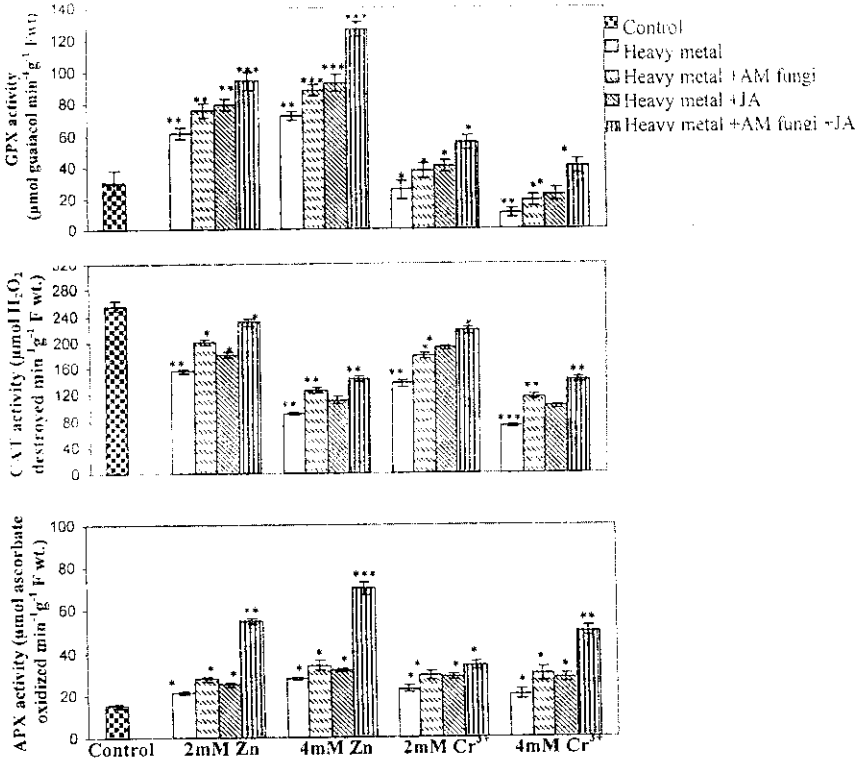

Fig. 1.Changes in the activities of guaiacol peroxidase (GPX), catalase (CAT) and ascorbate peroxidase (APX) in leaves of maize plants treated with AM fungi and or JA, and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions. * : Significant at $p < 0.05$, ** : highly significant at $p < 0.01$ and * : very highly significant at $p < 0.001$.**

Ascorbate peroxidese activity

Ascorbate peroxidase (APX) is one of the most important enzymes playing a crucial role in the eliminating poisonous H_2O_2 from plant cells, where APX has a higher affinity for H_2O_2 than CAT does (Foyer *et al.*, 1994). Unlike catalase, APX activity significantly increased in maize leaves in response to Zn and Cr^{3+} ions. The plants colonized with the AM fungi and sprayed with JA had a higher

APX activity at all concentrations of both heavy metals. Similar patterns of APX activity has been reported in shoots of *Salix vininalis* treated with Cd, Cu, and Zn ions (Landberg and Greger, 2002), in Cr^{3+} adapted sunflower callus (Gallego *et al.*, 2002), and in roots of green gram under Cr (VI) stress (Shanker *et al.*, 2004). On the other hand, induction of APX may have even more dramatic effect on the protection of plants against heavy metal stress as compared with catalases (Fig.2), because H_2O_2 generated at the intercellular space of the plant during environmental stress appears to diffuse first into the cytosol in which cytosolic APX is localized and only then into peroxisome in which catalase is typically found (Lee and Lee, 2000), thus cytosolic APX has a higher affinity for H_2O_2 than catalase does (Asada, 1992). Moreover, APX gene expression is rapidly induced by various stress conditions and may have an important role in stress tolerance (Mittler and Zilinskas, 1994). Thus, jasmonates induce an increase of the mRNA levels in tomato leaves (Orvar *et al.*, 1997) and stimulated APX activity in shoots and roots of canola under light and dark conditions (Comparot *et al.*, 2002). Also, inoculation with mycorrhizal fungi increased APX activity in the shoots of europaea plants and its activity coordinated with the higher content of Fe (Alguacil *et al.*, 2003).

Superoxide dismutase activity

Superoxide dismutase (SOD) activity in leaves of maize plants treated or non-treated with AM fungi and / or JA increased with increasing concentrations of externally supplied Zn and Cr^{3+} ions. Among the two metal ions, Zn-treated plants exhibited a higher value of SOD activity compared with Cr^{3+} treatments (Fig. 2). On the other hand, it is clear from Table 2 that 3 isoforms of SOD (Cu / Zn SOD, Mn SOD and Fe SOD) were present in maize leaves, and increase in the activity of one isoform might mask the decrease of another. Therefore, inhibition studies with KCN, which generally used to distinguish between Cu / Zn (CN – sensitive) and Fe or Mn SOD (CN – insensitive) showed that Cu / Zn SOD isoform being the most abundant and registered higher value when compared to the other isoform. While a significant inhibition in the activity was found in Fe SOD isoform especially in Cr^{3+} treatments (Table 2).

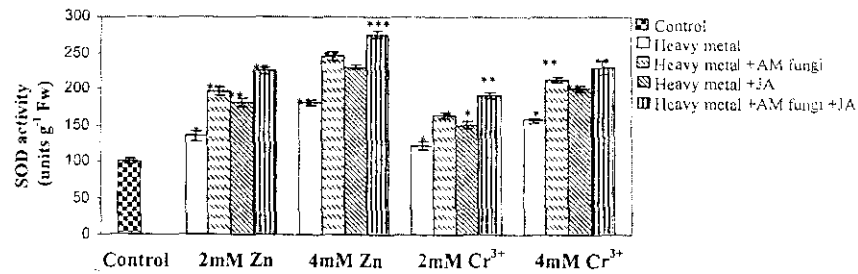

Fig. 2. Changes in the activity of superoxide dismutase (SOD) in leaves of maize plants treated with AM fungi and or JA, and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions. * : Significant at $p < 0.05$, ** : highly significant at $p < 0.01$, * : very highly significant at $p < 0.001$.**

Thus, results showed that the increment of total activity of SOD in Zn – treated plants appeared to be due to clear induction of Cu / Zn SOD isoform, where Cu / Zn SOD plays an important role in protecting plants against oxidative damage catalyzed by O_2 free radicals (Marschener and Cakmak, 1989). Also, inhibition in the activity of Fe SOD isoform in Cr-treated plants may be associated with the decrease in Fe uptake (Abo-Ghalia and El-Khallal, unpuplished). Stimulation of SOD activity was observed in roots and shoots of pigeon pea cultivars (Rao and Sresty, 2000) , barley plants (Wu *et al.*, 2003) and in roots of green gram (Shanker *et al.*, 2004) in response to different heavy metal ions. SOD activity increases in mycorrhizal roots of *Poxillus pinus* in response to Cd (Schutzendubel and Polle, 2002), and in roots of both light and dark grown canola seedlings treated with JA (Comparot *et al.*, 2002). Recently , Jung (2004) reported that total activities of catalase , peroxidase and superoxide dismutase increased greatly in *Arabidopsis thaliana* plants in response to methyl jasmonate.

TABLE 2. Changes in the activity of SOD forms (units g^{-1} Fw) in leaves of maize plants treated with AM fungi and /or JA, and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions.

Treatments		Cu/Zn SOD	Mn SOD	Fe SOD
Control		63.0	20.6	22.5
Zn	2mM	98.7**	22.7*	13.6**
	2mM + AM fungi	137.8***	34.0**	18.2*
	2mM + JA	125.6***	36.0**	17.4*
	2mM + AM fungi + JA	157.5***	44.0***	23.5
	4mM	134.0***	27.0*	18.0*
	4mM + AM fungi	177.5***	42.0***	25.0*
	4mM + JA	157.4***	51.7***	21.6
	4mM + AM fungi + JA	195.6***	48.6***	31.7**
Cr^{3+}	2mM	79.0**	31.0*	10.5**
	2mM + AM fungi	101.3**	45.0***	15.8*
	2mM + JA	93.0**	40.0***	16.5*
	2mM + AM fungi + JA	111.0***	61.0****	20.6*
	4mM	101.0***	47.0***	7.6***
	4mM + AM fungi	131.0***	63.7***	12.3**
	4mM + JA	134.0***	53.0***	11.5**
	4mM + AM fungi + JA	145.0***	74.5***	16.7*

* : Significant at $p < 0.05$, ** : highly significant at $p < 0.01$, *** : very highly significant at $p < 0.001$.

Finally, induction in the activity of antioxidant enzymes in maize plants especially inoculated with AM fungi and treated with JA may be important for removing excess of O_2 free radicals and induce heavy metal tolerance. On the other hand, accumulations of H_2O_2 and MDA contents in both Zn and Cr^{+3} treated plants indicated that antioxidant potential in the tissue of plants might not be enough to block the lipid peroxidation and then oxidative damage occur.

Nucleic acids and protein contents

Data in Table 3 show that nucleic acids (DNA & RNA) and total soluble protein contents in maize leaves significantly decreased with increasing concentrations of both heavy metal ions. The decrease in protein contents in the heavy metal- treated plants was probably due to adverse effects of reactive oxygen species ,which may be due to degradation of a number of proteins . Plants treated with AM fungi and/ or JA had a higher contents of nucleic acids and protein as compared with both heavy metal treated plants.

Reduction in nucleic acid and protein contents in plants in response to both heavy metal ions might result in an idleness of nucleic acid and protein synthesis machinery and blocking the formation of protein units having functional roles in the cells. Also, the transport of RNA from nucleus to cytoplasm and subsequent protein synthesis – machinery appeared to be disrupted (Jagetiya *et al.*, 1988). Francis *et al.*(1995) reported that metal ions caused a reduction in cell division and such reduction could be due to blocking the cell at G1 preventing DNA replication. In addition, inhibition in nucleic acids and protein contents were observed in plants in response to different heavy metal ions (Chien *et al.*, 2001; Saleh, 2001; Abd-Elhamid *et al.*, 2002; El-Ghamery *et al.*, 2002 and Khidr *et al.*, 2004).

TABLE 3. Changes in the nucleic acids (DNA & RNA) and soluble proteins (mg^{-1} Fw) in leaves of maize plants treated with AM fungi and / or JA , and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions.

Treatments		DNA	RNA	Total soluble protein
Control		0.51	0.94	21.0
Zn	2mM	0.28**	0.52*	15.0*
	2mM + AM fungi	0.33**	0.67*	20.8*
	2mM + JA	0.38*	0.63*	19.6*
	2mM + AM fungi + JA	0.42*	0.71*	23.7*
	4mM	0.13***	0.35**	9.3***
	4mM + AM fungi	0.24**	0.52*	16.8*
	4mM + JA	0.27*	0.48*	15.5*
	4mM + AM fungi + JA	0.33*	0.6*	18.5*
Cr^{3+}	2mM	0.21**	0.43**	13.2**
	2mM + AM fungi	0.28*	0.57*	18.3*
	2mM + JA	0.25**	0.53*	18.5*
	2mM + AM fungi + JA	0.32*	0.64*	21.5*
	4mM	0.09***	0.27***	7.6***
	4mM + AM fungi	0.13***	0.41**	12.5**
	4mM + JA	0.15**	0.38**	13.4**
	4mM + AM fungi + JA	0.21*	0.53*	16.2*

- : Significant at $p < 0.05$, ** : highly significant at $p < 0.01$, ***: very highly significant at $p < 0.001$.

Changes in protein profiles

The results of the SDS-PAGE electophoretic patterns of protein bands extracted from heavy metal stressed plants are shown in Fig 3. The relative molecular weights and percentages of polypeptide bands are recorded in Table 4. It can be seen that 4 polypeptide bands of molecular weights 61.3, 23, 13.6 and 12.5 KDa could be considered as main bands and were found in the differently treated plants. A set of 7 polypeptide bands (29.2, 21.0, 19.0, 17.6, 15.8, 14.4 and 11.5 KDa) were accumulated in plants treated or non-treated with AM fungi and/or JA in response to 4mM of both heavy metal ions. However, other polypeptide bands having molecular weights 81.5, 54.2, 49.6, 39 and 20.2 KDa were induced only by 4mM of Zn and Cr^{3+} ions. Also, especial band of molecular weight 69.2 was induced only in Cr-treated plants.

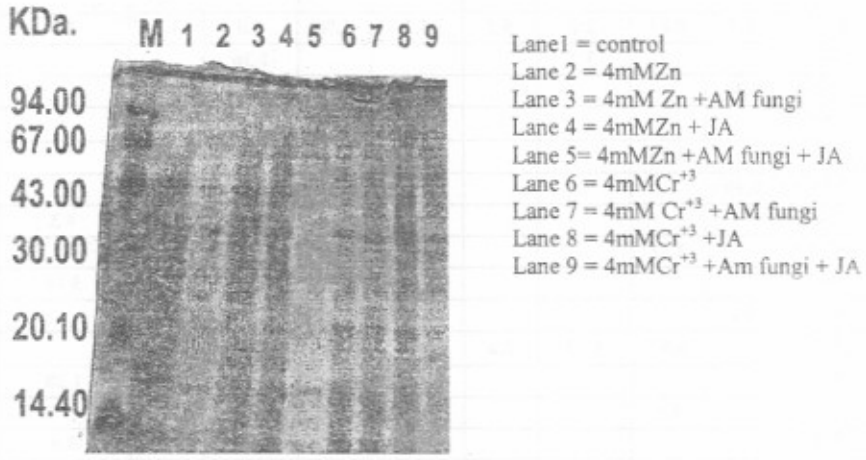

Fig. 3. SDS – PAGE of proteins extracted from leaves of maize plants treated with AM fungi and /or JA, and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions .

On the other hand, AM fungi and / or JA induced other polypeptide bands in maize leaves in response to Zn and Cr^{3+} ions. Thus, a set of 5 polypeptide bands of molecular weights 75.5, 30, 25.8, 24.5 and 11.0KDa were induced by both ions. However, novel proteins (80.6, 56.0, 36.6 and 33KDa) were induced in Zn-treated plants. In addition, polypeptide bands of molecular weights 63.0, 52.4, 46, 37.0, 10.8 and 10.2 KDa were found only in AM fungi and / or JA treated plants in response to Cr^{3+} ions.

TABLE 4. Comparative analyses of relative molecular weights and bands concentrations (%) of the different types of polypeptide bands in leaves of maize treated with AM fungi and / or JA and grown in soil polluted with different concentrations of Zn or Cr^{3+} ions for 8 weeks.

M. wt. (KDa)	H_2O	4 mM Zn				4 mM Cr^{3+}			
		Zn	Zn+AM fungi	Zn+ JA	Zn + AM fungi+ JA	Cr^{+3}	Cr^{+3}+ AM fungi	Cr^{+3} + JA	Cr^{+3} + AM fungi + JA
102.0			1.53						
81.5		0.47				1.03			
80.6				1.16					
75.5			0.98	1.5	1.84		1.6	1.75	2.03
69.2						1.44			
68.4	0.45								
63.0								2.03	
61.3	0.3	0.61	2.1	5.2	5.2	1.78	2.0	1.8	2.3
60.6			3.3				1.36		
58.0	0.34								
56.0					2.9				
45.2		0.93							
53.6	0.90								
54.2									4.3
49.6		2.11							
46.0							3.01	3.54	5.14
43.9	3.1								
41.5		0.95	1.19	0.6			0.88		
40.0								1.015	1.28
39.0		1.9				4.9			
37.0							2.46	3.01	3.0
36.6				1.2	1.3				
35.3		1.8	0.67						
33.0					0.88				
32.0	1.72								
30.0			1.53	1.16	1.84		0.97	1.31	1.06
29.0		0.47	1.41	1.59	1.26	0.61	1.32	1.16	2.04
27.5	4.5								
26.5	5.7								
25.8			4.2	2.7	3.54		0.32	1.72	0.81
24.5				0.61	1.26			1.31	1.84
23.0	1.68	0.13	1.25	1.25	3.22	2.46	3.18	3.61	4.01
21.0		2.51	2.76	3.01	3.91	1.71	1.61	3.81	2.94
20.2		0.71							
19.0		2.41	3.01	3.32	4.49	2.46	3.04	2.97	4.61
18.3	3.71								

TABLE 4. Contd.

17.6		0.95	1.13	0.86	1.43	1.31	1.12	1.06	1.21
16.8	8.2								
15.8		3.41	4.5	5.85	6.01	3.01	5.41	4.86	7.01
14.4		3.9	3.1	4.01	2.41	2.61	2.88	2.5	3.64
13.6	3.8	1.31	1.5	1.02	1.46	2.01	2.03	1.05	2.0
12.5	1.98	1.12	1.71	1.25	1.72	0.75	1.41	1.77	1.5
11.5		2.7	3.04	4.8	3.11	2.2	4.3	3.8	5.1
11.0					3.6			2.7	2.5
10.8							1.4		1.8
10.2							3.7		6.2

The present results revealed that heavy metal ions (Zn & Cr^{3+}), AM fungi and JA evokes changes in the pattern of protein profile of maize plants, therefore, induction of newly protein bands of low molecular weights (40-10.5) may play functional roles in heavy metal tolerance, which might be putative phytochelatins or heat shock proteins ($HSPs_s$) and have been documented by several investigators (Neumann *et al.*, 1994; Przymusinski and Gwozdz, 1994 & 1999; El-Ghamery *et al.*, 2002; Abd El-Hamid *et al.*, 2002; Ederli *et al.*,2004). Also, there have been several reports of an increase in HSP expression in plants in response to heavy metal stress. Tseng *et al.* (1993) showed that heavy metal stress increased the level of mRNA of low molecular weights (16-20KDa) in rice plants. Small HSP17 was expressed in roots of *Aremeria maritime* plants grown on Cu/rich soil (Neumann *et al.*, 1995) and increased in cell culture of *Silene vuglaris* and *Lycopersicon peruviamum* in response to a range of heavy metal - treatments (Wollgiehn and Neumann, 1999). Moreover, Suzuki *et al.* (2002) suggested that CdI19 were induced by different heavy metal ions and plays an important role in the maintenance of heavy metal homeostasis and / or in detoxification of heavy metal homestasis and / or detoxification by encoding plasma membranes with the capacity to serve as an initial barrier against inflow of free heavy metal ions into cells. In addition, JA induce expression of a variety of genes involved in activation of the defense mechanisms, especially genes for pathogenesis – related (PR) proteins (Harms *et al.*, 1998) and existence of a specific heavy metal responsive gene (Zhang *et al.*, 2001). However, some indirect data (Hensel *et al.*, 1999) indicating inducible action of heavy metals on the synthesis of (PR) protein and expression of markers genes of hypersensitive responsive (HR) (Pointer *et al.*, 1999; Siediecka *et al.*, 2001) support the hypothesis that there is a cross talk in action of heavy metals and JA. In barley , expression of a JA- induced gene , *JIP23* , is induced in arbuscule – containing root cortical cells , indicating that arbuscule formation is accompanied by JA production (Hause *et al.*, 2002). On the other hand , the increased phosphate supply due to arbuscular mycorrhization increases the N-status of plants (Abo-Ghallia and El-Khallal, 2005) , which may result in increased biosynthesis of storage protein in roots, modulated by jasmonates (Jia *et al.*, 2004) .

It was concluded that Zn and Cr^{3+} ions induced oxidative stress in maize plants, characterized by the increased H_2O_2 production and lipid peroxidation and alters activities of antioxidant defense system. Thus, the reduction in growth and the changes in metabolic activities of maize plants subjected to both heavy metal ions (Abo- Ghallia and El-Khallal, 2005) might result from accumulation of H_2O_2 and subsequent lipid peroxidation, which cause oxidative damage. Treatement with AM fungi and / or JA individually or in combination can alleviate toxicity of Zn and Cr^{3+} ions through hyperactivity of antioxidant enzymes and induction of proteins of low molecular weights, which could play an important role in the mechanism of heavy metal tolerance and protecting maize plants from Zn and Cr^{3+} toxicity . In addition , the results obtained in this study indicate that JA and AM fungi may be interact with each other by regulating antagonistically or synergistically the expression of genes involved in plant defense against heavy metal stress .

References

Abd El-Hamid, M.; El-Nahas, A.I. and Abd El-Hamid, A.M. (2002) Physiological and cytological studies on the effect of ions of some heavy metals on *Pisium sativum*. IV. The impact on nucleic acids content, mitotic activity and protein banding patterns of two cultivars of *Pisium sativum* (cv. B- master and cv. Sugary). *Proceedings , Plant and Industrial Pollution. The Egyptian Botanical Society, Cairo, March 6th* 2002, pp. 73-100.

Abo-Ghalia, H.H. and El-Khallal, S.M. (2001) Interaction between mycorrhizal fungi and jasmonic acid and their effects upon the growth, and metabolic activities of lupine plants grown under saline conditions. *Egypt. J. Botany*, **41** (2),201.

Abo-Ghalia, H.H. and El-Khallal, S.M. (2005) Alleviation of heavy metal stress by arbuscular mycorrhizal fungi and jasmonic acid in maize plants. *Egypt. J.Botany*, **45**,55.

Aebi, H. (1984) Catalase in vitro. *Meth Enzymol.* **105**, 121.

Asada .K. (1992) Acerbate peroxidase – a hydrogen peroxide – scavenging enzyme in plants . *Physiol Plant* . **85**, 235 .

Alguacil, M. M.; Hernàndez, J.A.; Caravaca, F.; Portillo, B. and Roldàn, A. (2003) Antioxidant enzymes activities in shoots from three mycorrhizal shrub species afforested in a degraded semi – aired soil. *Physiol Plant.* **118**, 562.

Blinda, A.; Koch, B.; Ramanjulu, S. and Dietz, K.J. (1997) De novo synthesis and accumulation of apoplastic proteins in leaves of heavy metal – exposed barley seedlings. *Plant Cell and Environ.* **20**, 969.

Bonnet, M.; Camares, O. and Vessire, P.H. (2000) Effects of zinc and influence of *Acremonium lolü* on growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne*). *J. Exp. Bot.* **51** (346),945.

Bradford, M. (1976) A rapid and sensitive method for the quantitative of microgram quantities of protein utilizing the principle of protein – dye binding. *Anal Biochem.* 72,248.

Cakmak, I. (2000) Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.* **146**, 158.

Chen, G.X. and Asada, K. (1989) Ascorbate peroxidase in tea leaves: Occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant Cell Physiol.* **30**,987.

Chien, H,F.; Wang, J.W.; Lin, C.C. and Kao, C.H. (2001) Cadmium toxicity of rice leaves is mediated through lipid peroxidation. *Plant Growth Regul,* **33**, 205.

Cho, U. and Park, O. (2000) Mercury-induced oxidative stress in tomato seedlings. *Plant Sci.*, **156**, 1.

Comparot, M.S.; Graham, C.M. and Reid, D.M. (2002) Methyl jasmonate elicits a differential antioxidant response in light – and dark – grown canola (*Brassica napus*) roots and shoots. *Plant Growth Reg.* **38**, 21.

Creelman, R.A. and Mullet, J.E. (1997) Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**,355.

Davies, F.T.; Puryear, J.D.; Newton, R.J.; Egilla, J.N.; and Saraiva Grossi, J.A. (2001) Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*). *J. Plant Physiol,* **158**,777.

Davies, F.T.; Puryear, J.D.; Newton, R.J.; Egilla, J.N.; and Saraiva Grossi, J.A. (2002) Mycorrhizal fungi increase chromium uptake by sunflower plants : influence on tissue mineral concentration , growth and gas exchange. *J.Plant Nutr.* **25**, 2389.

Dhindsa, R.S.; Plumb-Dhindsa, P. and Thorpe, T.A. (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superexide dimutase and catalase. *J.Exp. Bot.* , **23**, 93.

Ederli, L.; Reale, L.; Ferranti, F. and Rasqualimi, S. (2004) Responses induced by high concentration of cadmium in *Phragmites australis* roots. *Physiol Plant*, **121** (1),66.

El-Ghamery, A.A.; Mansour, M.M. and Abour-El Yousser, A. (2002) Effect of some heavy metals on mitotic activity, nucleic acids content and protein banding patterns in meristematic roots of *Nigella sativa* and *Tritacum sativum. Egypt J. Biotechnol,***11**, 266.

El-Khallal, S.M (2001) Some physiological roles of jasmonic acid in adaptation of pea seedlings to salt stress. *Egypt J.Biotechnol,* **10**,244.

El-Khallal, S.M. (2002) The influence of some phyto-growth regulators on the activity of antioxidant system in maize plant under water stressed conditions. *Bull. Fac. Sci. Assiut Univ.,* **31** (2-D),183.

Fang, W.C. and Kao, C.H. (2000) Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Sci.*, **158**, 71.

Foyer, C.H.; Lelandais, M.; and Kumert, K.J. (1994) Photooxidative stress in plants. *Physiol Plant,*. **92**,696.

Foyer, C.H.; lopez-Delgado H.; Dat, J.F. and Scott, L.M. (1997) Hydrogen peroxide and glutathionc – associated mechanism of a climatory stress toleranee and signaling. *Physiol Plant,* **100**, 241.

Francis, D.; Davies, M.S.; Braybrook, C.; James, N.C. and Herbert, R.J. (1995) An effect of Zinc on M-phase and G1 of the plant cell cycle in thc synchronous TBY-2 tobbacco and suspension.*J. Exp. Bot.* **46**,1887.

Gao, X.P.; Wang, X.F.; Lu, Y.F.; Zhang, L.Y.; Shen, Y.; Liang, Z. and Xhang, D. (2004) Jasmonic acid involved in the water stress induced betan accumulation in pear leaves. *Plant Cell Environ,* **27** (4), 497.

Galiego , S.; Benavides, M. and Tomaro, M. (2002) Involvement of an antioxidant defense system in the adaptive response to heavy metal ions in *Helianthus annuus* L. cells . *Plant Growth Regu.***36**, 267.

Gaur, A and Adholeya, A. (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Current Science.* **86** (4), 528.

Giannopolities , C.N. and Ries, S.K. (1977) Superoxide dismutase 1. Occurence in higher plants . *Plant Physiol.* **59**,309.

Gupta, S.C. and Goldsbrough, P.B. (1991) Phytochelatin accumulation and cadmium tolerance in selected tomato cell lines. *Plant Physiol.* **97**,306.

Han,F.X; Sridhar, M.; Monts, D. and Su, Y. (2004) Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea.New Phytologist,* **162** (2),484.

Harms, K.; Ramirez, I. and Rena-Cortès, H. (1998) Inhibition of wound – induced accumulation of allene oxide synthase transcripts in flax leaves by aspirin and salicylic acid.*Plant Physiol.* **108**. 1741.

Hartley-whitaker, J.; Ainsworth, G.; Vooijs, R.; Ten Bookum, W.; Schat, H. and Meharg, A.A. (2002) Phytochelatins involved in differential arsenate tolerance in *Holcus lanatus.Plant Physiol.* **126**, 299.

Hause , B.; Maier ,W.; Miersch, O.; Kramell,R. and Strack,D. (2002) Induction of jasmonate biosynthesis in arbuscular mycorrizal barley roots . *Plant Physiol,***130**,1215.

Heath,R.I. and Packer,L. (1968) Photoxidation in isolated chloroplasts : 1-Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistery and Biophysics.***125** ,1189.

Hensel, G.; Hunze, G. and Kunze, I. (1999) Expression of the tobacco gene CBP2O in response to developmental stage, wounding, salicylic acid and heavy metals. *Plant Sci.*, **148**, 165.

Jagetiya, B.L.; Aery, N.C.; Grover, I.S. and Thukral, A.K. (1988) Effect of different nickel salt on germination in moorg. *Environment and Develepment.* **189**,192.

Jana, S. and Choudhuri, M. A. (1981) Glycolate metabolism of the three submerged aquatic angiosperm during a ging. *Aquat Bot.*, **12**, 345.

Jia, Y.; Gray, V.M. and Straker .C.J. (2004) The influence of *Rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba* . *Ann.Bot.* (London), **94**, 251.

Jentschke, G. and Godbold, D.L. (2000) Metal toxicity and ectomycorrhizas. *Physiol Plant,* **109**,107.

Jung, S. (2004) Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant system . *Plant Physiol.* **42**,225.

Khidr, Z.A.; Ebad, F.A. and Mustafa, A. I. (2004) Alleviation of cadmium and lead toxicity in cotton plant using organic composit . *One Day Conference on Stress in Plants. Ain Shams Univ, Cairo,Egypt,* March 3,2004.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophagle T4. *Nature,* **277,** 680.

Landberg, T. and Greger, M. (2002) Differences in oxidative stress in heavy metal resistant and sensitive clones of *Salix viminalis* . *J. Plant Physiol,* **159**, 69.

Lee,D.H. and Lee,C.B. (2000) Chiling stress- induced changes of antioxidant enzymes in the leaves of cucumber . In gel enzyme activity assays . *Plant Sci.*, **159**,75.

Lewis, S.; Donkim, M.E. and Depledge, M.H. (2001) Hsp70 expression in *Entertomorpha intestinalis* (chlorophyta) exposed to environmental stressors. *Aquatic Toxicalogy,* **51**,277.

Luna, C.M.; Gonzalez, C.A. and Tripipi, V.S. (1994) Oxidative damage caused by an excess of copper in oat leaves. *Plant Cell Physiol.* **35**,11.

Maksymies, W. and Krupa, Z. (2002) Jasmonic acid and heavy metals in arabidopsis plant a similar physiological response to both stressors. *J. Plant Physiol,* **159**, 509.

Marchener , H. and Cakmak , I . (1989) High light intensity enhance chlorosis and necrosis in leaves of Zn , K and Mg deficient bean plants . *J. Plant Physiol* , **134**, 308.

Metwally, A.M.; El-Enany, A.F. and Dietz, K.J. (2004) Modulation of cadmium or copper in barley seedlings by salicylic acid. *One-Day Conference on Stress in Plants. Ain Shams Univ., Cairo, Egypt. March* **3**, 2004.

Mittier, K. and Zilinskas, B.A. (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought and following recovery from drought. *Plant, J.* **5**, 397.

Neumann, D.; Lichtenberger, O.; Günther, D.; Tschiersch, K. and Nover, L. (1994) Heat – Shock proteins induce heavy metal tolerance in higher plants.*Planta.* **194**, 360.

Neumann, D.; Nieden, U.Z.; Lichtenberger, O. and Leopled, F. (1995) How dose Armeria maritima tolerate high heavy metal concentrations?. *J. Plant Physiol.* **146**, 704.

Ogur, M. and Rosen, G. (1950) The nucleic acids of plant tissue. 1. The extraction and estimation of deoxy pentose nucleic acid and pentose nucleic acid. *Arch. Biochem,* **26**, 262.

Orlowska , E.; Ryszka. P.; Jurkiewicz, K. and Turnau , K. (2004) Effectiveness of arbuscular mycorrhizal fungi (AMF) strains in colonization of plants involved in phytostabilization of zinc wastes . *Geoderma . (in press) .*

Örvar, B.L.; Mc Pherson, J. and Ellis B.F. (1997) Pre-activating wounding response in tobacco prior to high-level ozone exposure prevents necrotic injury. *Plant J.* **11**, 203.

Pandy, V.D. and Shyam, R. (2002) Chromium ions inactivate electron transport and enhance superexide generation in vivo in pea (*Pisum sativum* L.cv. Azad) root mitochondria. *Plant Cell Enviviron.* **25** (5), 687.

Pandy, N. and Sharma, C.P. (2003) Chromium interference in ions nutrition and water relations of cabbage. *Environ Exp Bot.,* **49**, 195.

Pointer, D.; Gan, S.; Anasino, R.M.; Roby, D.; and Lam, E. (1999) Markers for hypersensitive response and senescence show distinct patterns of expression. *Plant Mol. Biol.* **39**,1243.

Prasad, T.K.; Anderson, M.D. and Stewart, C.R. (1995) Localization and characterization of peroxidases in the mitochondria of chilling – accumulated maize seedlings . *Plant Physiol* , **108** ,1597.

Porcel, R.; Barea, J.M. and Ruiz – Lozano, J.M. (2003) Antioxidant activities of soybean plants and their possible relation to the processes of nodule senescence. *New Phytol,* **157**, 135.

Przymusinski, R. and Gwozdz, E.A. (1994) Increased accumulation of the 16 × 103 Da polypeptide in lupin roots exposed to lead, copper and mtrite ions. *Environ. Exp. Bot.* **34** (1),63.

Przymusinski, R. and Gwozdz, E.A. (1999) Heavy metal – induced polypeptides in lupin roots are similar to pathogenesis- related proteins . *J.Plant.Physiol.*,**154**,703.

Rai, V.; Vajpayea, P.; Singh, S. and Mehortra, S. (2004) Effect of chromium accumulation on photosynthetic pigments , oxidative stress defence system, nitrate reduction , proline level and eugenol content of *Ocimum tenuiflorum* L .*Plant Science* **167**,1159.

Rao, K.V. and Sresty, T.V. (2000) Antioxidative parameters in the seedlings of pigeonpea (Cajanuscajan (L) Millspaugh) in response to Zn and Ni stresses. *Plant Sci.* **157**, 113.

Rout, G.R.; Samantaray , S. and Das, P. (1999) Chromium , nickel and zinc tolerance in *Leucaena leucocephala* (K8). *Silvae Genet,* **48**,151.

Roe, J.G. (1964) Chemical determination of ascorbic, dehydroascorbic and ketogluconic acid. In; D. Glick (ed). *Methods of Biochemical Analysis. 1. Interscience,* New York, 1464. pp. 115-139.

Ryan , C.A. (2000) The systemin signaling pathway : differential activation of plant defensive genes . *Biochem.Biophys.Acta.* **1477**, 112.

Saleh, A.H. (2001) Effect of Cd and Pb on growth, certain antioxidant enzymes activity, protein profile and accumulation of Cd, Pb and Fe in *Ràphanus sativus* and *Eruca sativa* seedlings. *Egypt. J. Biology,* **3**,126.

Scharma, D.C. and Scharma, C.P. (1996) Chromium uptake and toxicity effects on metabolic activites in wheat, *Triticum aestivum* L.cv. *Indian. J. Exp. Biol.* **34**, 689.

Schenider, W.C. (1945) Phosphorus compounds in animal tissues. 1- Extraction and estimation of deoxypentose nucleic acid and pentose nueleic acid. *J. Biol. Chem.* 293.

Schutzendubel , A. and Polle , A. (2002) Plant responses to abiotic stresses : heavy metal – induced oxidative stress and protection by mycorrhization . *J. Exp. Bot* . **55**(372) ; 1351.

Shanker, A.K.; Dijanaguiraman, M.; Sudhagar, R.; Chandrasekar, C.N. and Pathmanabhan, G. (2004) Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiate* (L.). R.Wilczek.cv (O4) roots. *Plants Sci.* **166** (4),1035.

Siedlecka, A.; Tukendorf, A.; Skorzynska – Polite, E.; Majksymiec, W.; Wojelk, M.; Baszynski. T. and Krupa, Z. (2001) Angiosperms (*Asteraceae,Convolvulaceae, Fabaceae* and *Poaceae.* other than *Brassicaceae*) In: *Prasad Marcel DeKKer, Inc,* New York, *pp.* 171-215.

Snedecor , G.M. and Cochran, W.C. (1969) Statistical Methods . 6 *th* Ed. Iowa Univ. Press, Ames, Iowa,U.S.A.

Suzuki, N.; Yamaguchi, Y. Koizumi, N. and Sdno, H. (2002) Functional characterization of a heavy metal binding protein Cd119 from Arabeidopsis. *The Plant J.* **32** (2) ,165.

Tseng, T. S.; Tzeng, S.S.; Yeh, C.H.; Chang, F.C.; Chen, Y.M. and Lin, C.Y. (1993) The heat – shock response in rice seedlings – isolation and expression of cDNAs that encode class. 1- Low moleculer weight heat – shock proteins. *Plant and Cell Physiol.* **34**,165.

Tullio, M.; Pierandrei, F.; Salermo, A. and Ren, F. (2003) Tolerance to cadmium of vesicular arbuscular mycorrhiza sporce isolated from a cadmium – polluted and unpolluted soil. *Biol Fertil. Soils.* **37**, 211.

Wecks, J.E. and Clijsters, H.M. (1997) Zn phytotoxicity induces oxidative stress in primary leaves of phaseolus vulgaris. *Plant Physiol Biochem.* **35** (5), 405.

Wollgiehn, R. and Neumann, D. (1999) Metal stress response and tolerance of cultured cells from *Silene vulgaris and Lycopersicon peruviamum* : Role of heat stress proteins. *J.Plant Physiol.* **154** , 547.

Wu, C.; Zang, G. and Dominy, P. (2003) Four barley genotypes respond differently to cadmium : Lipid peroxidation and activities of antioxidant capacity . *Environ.Exp.Bot.* **50** ,67.

Xiang , C. and Oliver , D.J. (1998) Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis* . *Plant Cell* .**10**, 1539.

Yeh, C.M.; Hung, W.C. and Huang, H.J. (2003) Copper treatment activities mitogen – activated protein Kinase signaling in rice. *Physiol Plant.* **119**,392.

Zhang , X.Z . (1992) "*Methodology of Crop Physiology*". Agricultural Press, Beijing, pp. 142-212.

Zhang, Y.; Chai, T.Y.; Dango, J.; Zhao, W.; An Chc ; Chen, Z. and Burkard, G. (2001) Cloning and expression analysis of the heavy – metal responsive gene PvsR.2 from Pea . *Plant Sci.* **161**, 783.

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إزالة سمية المعادن الثقيلة باستخدام كلا من الفطرة الجذرية وحمض الجاسمونيك . التغير فى نشاط مضادات الأكسدة والنمط البروتينى لنبات الذرة

سامية محب الخلال – هدى حسن ابو غالية
قسم النبات – كلية البنات – جامعة عين شمس– القاهرة – مصر .

يسبب التلوث بالمعادن الثقيلة ضررا بالغا لنمو النبات من خلال تنشيطها الفعال لشوارد الأكسجين الحرة المسببة لأكسدة الخلايا وموتها ولذلك أجريت هذه الدراسة فى محاولة لبيان تأثير المعاملة بكلا من الفطرة والمسبب الجذرية وحمض الجاسمونيك (٢٥ ميكرومول) على نشاط مضادات الأكسدة والتغيرات فى الأحماض النووية وأنماط البروتين لنباتات الذرة المعاملة بتركيزات ٢، ٤ مللى مول من أيونى الزنك والكروم وقد توصلت الدراسة إلى النتائج التالية :-

– أحدث معاملة نبات الذرة بتركيزات ٤،٢مللى مول من ايونى الزنك والكروم تراكما حادا فى مستويات فوق أكسيد الهيدروجين والمالون داى الدهيد وصاحب ذلك نقص فى محتوى حمض الاسكوربيك. و على العكس من ذلك فقد كان لتأثير المعاملة بالفطرة الجذرية وحمض الجاسمونيك دورا فعالا فى منع تراكمهم وصاحب ذلك إرتفاع محتوى حمض الأسكوربيك وقد أدى ذلك إلى تخفيف الضرر على حيوية الخلية من نشاط الشوارد الحرة.

– صاحب التلوث بأيونى الزنك والكروم تغيرا فى نشاط إنزيمات مضادات الأكسدة (جوياكول بيروكسيديز أوكسيد ديزميوتيز– كاتاليز – الاسكوربيت بيروكسيديز وسوبر أوكسيد ديزميوتيز) فى أوراق نبات الذرة المعاملة بأيونى الزنك والكروم فقد لوحظ نقص معنوي فى نشاط إنزيم الكاتاليز وإرتفاع نشاط إنزيمى الاسكوربيت بيروكسيديز والسوبر أوكسيد ديزميوتيز وبينما كانت هناك زيادة فى نشاط إنزيم الجواياكول بيروكسيديز فى أوراق النباتات المعاملة بالزنك صاحب ذلك نقص فى نشاطه فى أوراق النباتات المعاملة بايون الكروم.

– زادت نشاط الانزيمات المضادة للاكسدة زيادة ملحوظة فى النباتات المعاملة بالفطرة الجذرية وحمض الجاسمونيك (معا أو كلا على حدة) وذلك عند مقارنتها بالنباتات الغير معاملة. وكان لنشاط هذه الانزيمات دورا فعال فى إنحسار مسببات الأكسدة من نواتج الاكسجين الحرة مما زاد من حيوية الخلية.

– إنخفضت محتويات أوراق الذرة من البروتين والأحماض النووية (DNA, RNA) نتيجة المعاملة بأيونى الزنك والكروم وسجلت النباتات المعاملة بالفطرة أو بالهرمون معدلات أكبر من مثيلاتها الغير معاملة وقد أوضح الفصل الكهربى للبروتينات إستحثاث واضح للبروتينات ذات الأوزان الجزيئة المنخفضة (١٠–٣٠ كيلو دالتون) والتى ربما تلعب دورا فعالا فى زيادة تحمل النبات للمعادن الثقيلة.

ولذلك فقد انتهت هذه الدراسة إلى أن نباتات الذرة المعاملة بايونى الزنك والكروم وكلا من الفطرة الجذرية وحمض الجاسومنيك كان له الأثر الفعال فى نشاط إنزيمات مضادات الأكسدة وإستحثاث تكوين بروتينات جديدة (ذات أوزان جزيئية منخفضة) قد يكون لها الفضل فى إنحصار مسببات الاكسدة من الشوارد الحرة وزيادة تحمل النباتات للمعادن الثقيلة.