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EXCESS COPPER AND MANGANESE ALTERS THE GROWTH AND VIGOUR OF MAIZE SEEDLINGS IN SOLUTION CULTURE

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SUMMARY

To elucidate the detrimental effect of excess Cu and excess Mn in maize (*Zea mays*, L) cv. Ganga2, seeds were germinated in petri-dishes under controlled conditions and supplied distilled water and nutrient solution at varying doses of Cu and Mn. Compared to the maize seedlings grown in complete nutrient solution, excess supply of Cu decreased the growth of shoot and root drastically whereas excess Mn was found to promote the growth of shoot and root significantly. The tissue concentration of Cu and Mn increased with increasing level of Cu and Mn. The concentration of iron decreased in shoots and increased in roots significantly with increasing supply of Cu and Mn. The activity of catalase- an iron containing enzyme, decreased at excess Cu and increased at excess Mn suggesting interference of excess nutrients in iron metabolism of plants. Decreased activity of amylase and proteinase in seeds was also observed at excess Cu and Mn.

Key words: **Amylase, catalase, copper, manganese, *Zea mays***

INTRODUCTION

Environmental pollution by metals has become extensive as mining and industrial activities have increased in the last two decades (Pinto *et al*. 2004). It has long been established that copper and manganese like other micronutrients are relatively toxic to plants when given in supra-normal doses (Marschner 2002). Inhibition of growth accompanied by chlorosis of young leaves is often apparent when copper and manganese are administered in toxic doses to the plants (Marschner 2002). Indirectly, these effects are principally caused by alterations in the concentration of essential mineral nutrients, decreased net photosynthesis as a consequence of stomatal closure, reduced intercellular spaces and alterations in the chloroplast structure (Demirevska-Kepova *et al*. 2004). Enzymes are one of the main targets of heavy metal ions and prolonged exposure of soils to heavy metals results in decline in soil enzyme activity. In addition, some transition metals like Fe^{2+}, Cu^{2+} and Mn^{2+} have the ability to stimulate the formation of free radicals and reactive oxygen species (Groppa *et al*. 2001, Hall *et al*. 2002). In order to cope with highly toxic metals or to maintain the level of essential metals within physiological ranges, plants have evolved complex mechanism that serve to control the uptake, accumulation and detoxification of metals (Stohs and Bagchi 1995). Previous studies have shown that toxic concentration of metals like iron (Fang and Kao 2000), copper (Chen *et al*. 2002) and manganese (Gonzalez *et al*. 1998) cause oxidative stress which results in enzymatic and non enzymatic antioxidative responses of plants and stimulation of lipid peroxidation.

Copper and manganese enrichment by seed pelleting or soaking the seeds in dilute solution of Mn, Cu or other nutrients have been reported as seed treatments for increasing seedling vigour (Khalid and Malik 1982,

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Tanyolac *et al*. 2007). In summer squash, seeds soaked for 24h in 500 ppm Cu or 2000 ppm Mn before planting had highest germination rate, plant growth and mean yield (Abed and Sharabash 1985). Ahmad and Trifu (1980) reported that in γ- irradiated winter wheat caryopsis application of 10 ppm $CuSO_4$ increased amylase and decreased catalase activity while $MnSO_4$ at 250 ppm decreased that of amylase and increased catalase.

The concentrations of copper and manganese were higher in the roots of plants than in the shoots. Manganese is usually transported in the xylem sap as a divalent cation (Mn^{2+}). The copper uptake is a metabolically mediated process and the element is taken up either as a divalent cation (Cu^{2+}) or as Cu chelate. The displacement of several ions from root exchange sites by copper and manganese has been reported because copper and manganese are very strongly bound in the root free spaces. These observations account for the frequently higher copper and manganese content in the roots than other plant tissues (Hill *et al*. 2000, Marschner 2002).

In the present study an attempt was made to examine the growth and some physiological changes in maize seedlings induced by excess Cu and/ Mn administered exogeneously in solution culture.

MATERIALS AND METHODS

Maize (*Zea mays* L.) cv. Ganga 2 seeds were germinated in petridishes lined with filter paper, moistened with distilled water in a BOD at 25 ± 1°C provided with light of 70 Wm^{-2} for 16h photoperiod. The seeds were surface sterilized by treating with 0.01% $HgCl_2$ for 5 min. and then washed thoroughly with tap water and rinsed with distilled water. After 3 days, seeds were given the following treatments: (i) Distilled water, (ii) Complete nutrient solution with normal Cu (0.5μM) and normal Mn (5μM) (Control), (iii) Complete nutrient solution with 0.5 X 10^2μM Cu and normal Mn (5μM), (iv) Complete nutrient solution with 0.5 X 10^4μM Cu and normal Mn (5μM), (v) Complete nutrient solution with normal Cu (0.5μM) and 5 X 10^2μM Mn, (vi) Complete nutrient solution with normal Cu (0.5μM) and 5 X 10^4μM Mn.

The composition of complete nutrient solution was: 2.0 mM KNO_3, 2.0 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 0.67 mM NaH_2PO_4, 0.05 mM NaCl, 0.05 mM Fe-EDTA, 5.0 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$, 16.5 μM H_3BO_3, 0.1 μM Na_2MoO_4, 0.05 μM $CoSO_4$, and 0.05 μM $NiSO_4$ (Hewitt 1966). The solution was changed daily without disturbing seeds/ seedlings and its volume was increased according to the requirement of seedlings. After 4 days (i.e.7 days growth), the seedlings were rinsed with 0.01 N HCl, washed under running tap water and finally rinsed with deionized water to avoid any surface contamination. The seedlings of all treatments were separated into shoot and root and dried in an electric oven at 70° C for 48 h. The concentration of copper, manganese and iron in shoot and root in maize were measured by atomic absorption spectrophotometer (Varian Techtron AA 120) after di-acid digestion (nitric acid: perchloric acid 10: 1). Homogenates of fresh shoot and seed tissue (20%) was prepared in glass-distilled water for enzyme extraction and protein determination by grinding the fresh leaf lamina in chilled pestle and mortar kept in ice bath. The homogenate was filtered with two-fold muslin cloth and the supernatant was stored at 2° C and used for enzyme assays within 4 h.

The activity of catalase in shoot was measured by the method of Euler and Josephson (1927) in 10 ml reaction mixture, standardized against 0.1 N $KMnO_4$, containing 0.5 mM H_2O_2 and 1 mM phosphate buffer pH 7.0 was stabilized at 25° C. The reaction was initiated by adding 1 ml of suitably diluted enzyme extract to the reaction mixture and was allowed to proceed for 5 min after which 5 ml of 2 N H_2SO_4 was added to stop the reaction. Corresponding blanks were run simultaneously in which sulfuric acid was added prior to the addition of enzyme extract. The amount of H_2O_2 reduced by the enzyme was determined by titrating the reaction mixture against 0.1 N $KMnO_4$. The activity of amylase (Katsumi and Fukuhara 1969) in seeds was assayed in reaction mixture containing 1.0 ml 0.1M acetate buffer pH 5.6, 0.1 ml 0.3% $CaCl_2$ and 1.0 ml suitably diluted enzyme extract. The reaction was initiated by addition of 1.0 ml 0.2% amylose and allowed to proceed for 30 minutes at 30° C. The reaction was stopped by 1.0 ml 10% TCA. A suitable aliquot of the supernatant was taken and the starch content was estimated using 1.0 ml I_2: KI (1:2) reagent colorimetrically. The activity of proteinase (Penner and Ashton 1967) in seeds was also determined in reaction mixture containing 1 ml 0.1 M phosphate buffer pH 7.0 and 1 ml suitably diluted enzyme extract. The reaction was started at 30^o C by addition of 1 ml 1% casein. After 30 minutes reaction was stopped by addition of 1 ml 20% TCA. For expressing enzyme activities on protein basis, soluble protein in enzyme extracts was determined in TCA precipitate according to Lowry *et al*. (1951) using bovine serum albumin (sigma) as a standard. All determinations were made in triplicate. The data have been analyzed statistically and mean values are given in the table and figure along with the standard error (SE±).

RESULTS AND DISCUSSION

The seedlings of maize, grown at different excess levels of copper and manganese were depressed in growth. The maize seedlings at adequate level of nutrient solution, grown previously with distilled water, stimulated the growth might be due to increase in concentration of essential nutrients in tissues and their participation without any hindrance in different metabolic functions such as reserve mobilization, solute translocation, respiration, growth and photosynthesis (Sandmann and Boger 1983, Marschner 2002). Excess supply of Cu at 0.5 X 10^2 and 0.5 X 10^4 µM restricted the growth of the seedlings and the leaves became chlorotic towards the apex (Tanyolac *et al.* 2007). In maize seedlings these symptoms of copper toxicity are somewhat superficially similar to that of Fe deficiency as has been suggested earlier by several workers (Daniels *et al*. 1972, Lanaras *et al*. 1993). The root growth was drastically reduced at 0.5 X 10^2 and 0.5 X 10^4 µM Cu (Table 1) as has been observed earlier in taro (Hill *et al*. 2000) and sunflower (Jiang *et al*. 2000). Excess supply of manganese induced the growth of both shoot and root almost equally at both the excess levels of manganese i.e. 5 X 10^2 and 5 X 10^4 µM, supports the view of Khalid and Malik (1982) on wheat and Abed and Sharabash (1985) on summer squash seeds soaked for 24h in 2000 ppm Mn before planting. The dry weight of both shoot and root of maize seedlings increased at normal supply of all nutrients including copper and manganese (control) from that of maize seedlings grown at distilled water (Table1). Compared to this, the dry weight was decreased at 0.5 X 10^2 and 0.5 X 10^4 µM Cu. The decline was higher in root as compared to shoot. The dry weight of both shoot and root increased with an increase in Mn supply at 5 X 10^2 and 5 X 10^4 µM Mn, supported the view of Abed and Sharabash (1985). The stimulatory effect of excess manganese on maize at seedling stage might be due to its higher requirement at adolescent stage for a number of metabolic processes leading to increased growth and development (Burnell 1988).

Increasing copper and manganese supply increased tissue copper/ and manganese in shoot and root (Table 2). A similar relationship between the supply and tissue concentration has been suggested earlier by several

Table 1. Effect of excess Cu and Mn on length and dry matter of maize seedlings in solution culture (±SE).

Plant part	**Treatments (µM)**					
	DW	**Cont.**	**Cont. + 0.5X10^2 µM Cu**	**Cont. + 0.5X10^4 µM Cu**	**Cont. + 0.5X10^2 µM Mn**	**Cont. + 0.5X10^4 µM Mn**
			Length (cm)			
Shoot	4.1±0.03	5.7±0.05	6.9±0.04	6.6±0.05	7.7±0.02	8.7±0.04
Root	3.9±0.02	4.3±0.02	6.4±0.07	2.0±0.00	5.0±0.04	7.4±0.06
			Dry weight (mg plant^{-1})			
Shoot	34.5±0.92	46.8±1.3	44.6±1.9	41.5±1.7	50.1±1.8	50.8±2.4
Root	17.7±0.04	18.2±0.07	20.8±0.81	15.5±1.2	20.4±0.08	25.0±1.1
Whole plant	52.2±1.9	65.0±2.1	65.4±2.01	57.0±1.6	70.5±2.3	75.8±2.1

Contol (cont.) = complete nutrient solution; DW = distilled water

Table 2. Effect of excess Cu and Mn on tissue concentrations of copper, manganese and iron in maize seedlings grown in solution culture (±SE).

Plant part	**Treatments (µM)**					
	DW	**Cont.**	**Cont. + 0.5X10^2 µM Cu**	**Cont. + 0.5X10^4 µM Cu**	**Cont. + 0.5X10^2 µM Mn**	**Cont. + 0.5X10^4 µM Mn**
				Copper (µg g^{-1} dm)		
Shoot	17±0.03	19±0.05	39±0.01	170±10.5	–	–
Root	33±0.12	65±0.72	151±17	511±23.1	–	–
				Manganese (µg g^{-1} dm)		
Shoot	23±0.92	28±0.03	–	–	87±11.8	192±12.4
Root	9±0.08	18±0.04	–	–	162±5.0	317±8.9
				Iron (µg g^{-1} dm)		
Shoot	75±2.5	126±12.5	84±4.7	76±5.3	87±3.2	76±2.1
Root	106±11	253±21.8	296±22.2	384±15.4	292±13.0	390±15.7

Contol (cont.) = complete nutrient solution; DW = distilled water

workers (Abed and Sharabash1985, Tanyolac *et al.* 2007). Despite its high concentration in root, relatively low total copper and manganese content may be attributed to smaller dry matter in root. However, marked decrease in total copper and manganese content of root is suggestive of damage to the uptake system of roots. From the observed results it appears that excess copper and manganese significantly restricted the translocation of Fe from root to shoot (Table 2) suggesting that these metals can displace Fe from physiologically active sites thus producing induced iron deficiency (Lanaras *et al.*1993, Alam *et al.* 2006).

The decrease activity of antioxidative enzyme catalase (Table 3) in shoot of maize seedlings in response to excess copper is suggestive of strong induction of oxidative stress as copper acts directly on the production of oxygen reactive species via Fenton and Haber Weiss reactions (Salin 1988) or might be due to complete or partial replacement of Fe from active sites or due to

Table 3. Effect of excess Cu and Mn on the specific activities of some enzymes in maize seedlings in solution culture (±SE).

Plant part	**Treatments (µM)**					
	DW	**Cont.**	**Cont. + 0.5X10^2 µM Cu**	**Cont. + 0.5X10^4 µM Cu**	**Cont. + 0.5X10^2 µM Mn**	**Cont. + 0.5X10^4 µM Mn**
				Catalase (µmol H_2O_2 decomposed)		
Shoot	240±10	246±14	232±12	185±7	280±17	349±21
				Amylase (mg starch hydrolysed)		
Seed	8.34±0.13	9.74±0.21	7.47±0.03	6.09±0.07	8.20±0.02	7.14±0.05
				Proteinase (mg protein hydrolysed)		
Seed	2.93±0.12	0.89±0.01	0.57±0.01	0.44±0.02	0.77±0.04	0.58±0.01

Contol (cont.) = complete nutrient solution; DW = distilled water hindrances in incorporation of Fe in the protein moiety of enzyme are in agreement with reports of Ahmad and Trifu (1980) in winter wheat and Luna *et al.* (1994) in oat leaves, whereas increase activity in excess Mn is in accord with the results on cotton (Sirkar and Amin 1974) and wheat (Ahmad and Trifu 1980).

The inhibited amylase activity (Table 3) in seeds at excess copper (Dasgupta and Mukherji 1977) and manganese (Ahmad and Trifu 1980) treated seedlings of maize might be due to enhanced peroxidant status by reducing the antioxidant glutathione pool, activating calcium dependant systems and affecting iron mediated processes (Pinto *et al.* 2003).

The lowered proteinase activity in seeds of excess copper and manganese (Table 3) in maize seedlings could result from various mechanisms including the oxidation and cross-linking of protein thiols, inhibition of key membrane proteins such as H^+ -ATPase or changes in the composition and fluidity of membrane proteins from disturbed membrane structure in such conditions (Quartacci *et al.* 2001).

REFERENCES

Abed, T.A. and Sharabash, M.T.M. (1985). Physiological response of summer squash to soaking seeds in Cu, Mn, or Zn solutions. *Ann. Agric. Sci. Moshtohor.* **22:** 473-486.

Ahmad, Z. and Trifu, M. (1980). Effect of complex treatment with γ- radiations microelements (Cu and Mn) and growth substances (indole-3-acetic acid) on the amylase and catalase activity in wheat seedlings. *Studies Univ. Babes- Batyai (Series) Biol.* **25:** 35-37.

Alam, S., Kodama, R., Akiha, F., Kamei, S. and Kawai, S. (2006). Alleviation of manganese phytotoxicity in barley with calcium. *J. Plant Nutr.* **29:** 59-74.

Chen, L.M., Lin, C.C. and Kao, C.H. (2002). Copper toxicity in rice seedlings: Change in antioxidative enzyme activities H_2O_2 and cell wall peroxidase activity in roots. *Bot. Bull. Acad. Soc.* **41:** 99-103.

Daniels, R.R., Stuckmeyer, B.E. and Peterson, L.A. (1972). Copper toxicity in *Phaseolus vulgaris* L. as influenced by iron nutrition. I: An anatomical study. *J. Americ. Soc. Hort. Sci.* **9:** 249-254.

Dasgupta, B. and Mukherji, S. (1977). Effects of toxic concentration of copper on growth and metabolism of rice seedlings. *Z. Pflanzenphysiol* **82:** 95-106.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Holzer, R. and Feller, U. (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. *Environ. Exp. Bot.* **52:** 253-266.

Euler, H.von and Josephson, K. (1927). Uber Katalase. *I. Leibigs Ann. Chem.* **452:** 158-187.

Fang, W.C. and Kao, C.H. (2000). Enhanced peroxidase activity in rice leaves in responses to excess iron, copper and zinc. *Plant Sci.* **158:** 71-76.

Gonzalez, A., Steffen, K.L. and Lynch, J.P. (1998). Light and excess manganese: Implications for oxidative stress in common bean. *Plant Physiol.* **118:** 493-504.

Groppa, M.D., Tomaro, M.L. and Benavides, M.P. (2001). Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. *Plant Sci.* **161:** 481-488.

Hall, J.L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53:** 1-11.

Hewitt, E.J. (1966). Sand and water culture methods used in the study of plant nutrition. *Common Wealth Agric. Bureaux*, London.

Hill, S.A., Susan, C.M. and Russell, S.Y. (2000). Taro responses to excess copper in solution culture. *Hort. Sci.* **35:** 863-867.

Jiang, W., Donghua, L. and Li, H. (2000). Effects of Cu^{2+} on root growth, cell division and nucleolus of *Helianthus annuus* L. *Sci. Total Environ.* **256:** 59-65.

Katsumi, M. and Fukuhara, M. (1969). The activity of α-amylase in the shoot and its relation to gibberellin induced elongation. *Physiol Plant.* **22:** 68-75.

Khalid, B.Y. and Malik, N.S.A. (1982). Pre-sowing soaking of wheat seeds in copper and manganese solutions. *Commun. Soil Sci. Plant Anal.* **13:** 981-986.

Lanaras, T., Moustakas, M., Symenoides, L., Diamantoglou, S. and Karataglis, S. (1993). Plant metal content, growth responses and some photosynthetic measurements on field-cultivated growing on ore bodies enriched in Cu. *Physiol. Plant.* **88:** 307-314.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin Phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Luna, C.M., Gonzzalez, C.A. and Trippi, V.S. (1994). Oxidative damage caused by excess of copper in oat leaves. *Plant Cell Physiol.* **35:** 11-15.

Marschner, H. (2002). Mineral Nutrition of Higher Plants. Academic Press, London.

Penner, D. and Ashton, F.M. (1967). Hormonal control of proteinase activity in squash cotyledons. *Plant Physiol.* **47:** 791-796

Pinto E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse, D. and Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *J. Phycol.* **39:** 1008-1018.

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

Quartacci, M.F., Cosi, E. and Navari-Izzo, F. (2001). Lipids and NADPH-dependent superoxide production in plasma membranes vesicles from roots of wheat grown under copper deficiency and excess. *J. Exp. Bot.* **52:** 77-84.

Salin, M.L. (1988). Toxic oxygen species and protective systems of the chloroplasts. *Physiol Plant.* **72:** 681-689.

Sandmann, G. and Boger, P. (1983). The enzymological function of heavy metals and their role in electron transfer processes of plants. In: A. Lauchli and R.L. Beileski (eds.), Inorganic Plant Nutrition, Encyclopedia of Plant Physiology, New Series, Vol. 15 B, pp. 563-596. Springer Verlag, Berlin.

Sirkar, S. and Amin, J.V. (1974). The manganese toxicity of cotton. *Physiol. Plant.* **54:** 531-543.

Stohs, S.J. and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med.* **18:** 321-336.

Tanyolac, D., Ekmekci, Y. and Unalan, S. (2007). Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. *Chemosphere.* **67:** 89-98.