Indian J. Plant Physiol., Vol. 17, No. 1, (N.S.) pp. 71-74 (Jan.-Mar., 2012)

SHORT COMMUNICATION

EXCESSIVE COBALT INDUCED OXIDATIVE STRESS IN GROUNDNUT

PRATIMA SINHA* AND N. NAUTIYAL

Botany Department, Lucknow University, Lucknow –226007 (U.P.)

Received on 31st November, 2011, Revised and accepted on 7th February, 2012

In groundnut (*Arachis hypogea* L.), cv. Kaushal excess concentration of cobalt (Co) in the growing medium developed visual symptoms of toxicity that intensified with increasing level and duration of metal supply. The tissue concentration of Co increased and Zn and Fe decreased with increasing level of Co supply. There was a marked increase in the activities of antioxidant enzymes, *viz.* superoxide dismutase, ascorbate peroxidase, nonspecific peroxidase and H_2O_2 content which suggest induction of oxidative stress under excessive cobalt. The pattern of increase in proline content and decrease in dry matter, yield, lipid peroxidation, catalase activity with increase in Co concentration suggests that groundnut is sensitive to excessive Co supply.

***Key words: Arachis hypogea*, cobalt, SOD, catalase, proline, yield**

Heavy metal contamination of soils has markedly increased in the past few decades (Collins *et al.* 2010). Cobalt in lower concentration is considered an essential element for leguminous plants. It is a constituent of cobalmine (Vitamin B_{12}) and an essential cofactor in some metalo enzymes (Molas 2008). Excess Co induced toxicity in plants inhibits many metabolic and physiological processes. The chlorosis has been suggested to be due to the toxic effect of the metal on root development. It has been observed that expression of bean seedlings to heavy metal stress results in carbohydrate accumulation by fully expanded leaves, which is due to more photo assimilate exports *via* vein loading (Adriano 2001).

Excess concentration of Co is known to cause detrimental effect on plant growth and exerts deleterious effect on photosynthesis, respiration, transport of photo-assimilates, mineral nutrition of growing plants. It has been shown that excess Co induce generation of reactive oxygen species (ROS), which inhibit protein synthesis by oxidation of mRNA (Adriano 2001). Under stress conditions, a balance between generation and degeneration of ROS is controlled by antioxidative enzymes and non enzymatic low molecular mass antioxidants. Superoxide dismutase catalyses dissimulation of superoxide anion (O_2^-) to H_2O_2 and O_2, catalase and ascorbate peroxidase are responsible for scavenging H_2O_2 (Mathad and Pratima 2009). Induction of peroxidase activity has been observed due to different stress factors. An interaction of heavy metal ions with O-N-or S- ligands of the enzyme inhibited enzyme activity due to the inactivation of enzyme protein (Van Assche and Clijster 1990). In this study an effort has been made to understand the effect of excess Co on dry matter accumulation and antioxidant enzymes activity of groundnut plants grown at high levels of Co.

Groundnut (*Arachis hypogea* L.), was grown in refined sand in a glass house at ambient environment in polyethylene pots of 20 kg capacity. Each pot had a central drainage hole, whose rim was covered with glass

*Corresponding author: pratimasinha2006@rediffmail.com

wool over which a clean watch glass was inverted. The composition of the nutrient solution was described earlier by Sinha *et al.* (2012).

Initially, the seedlings were grown in the above nutrient solution for 30 days. Afterwards, pots containing the plants were separated into six lots with four replicates per lot and two plants in each pot. One lot was grown with full nutrient solution and served as control. The other five lots were supplied 0.05, 0.1, 0.2, 0.4, and 0.5 mM cobalt, respectively, in the form of cobalt sulfate based nutrient solution, and nutrient was supplied every day.

Visual symptoms on leaves were recorded periodically. Plants were sampled for dry matter at 10, 25 and 69 days after treatment (DAT) and dried in an hot air oven at 70°C for 48 h. The oven dried plant material was digested by wet digestion using nitric: perchloric acids (HNO_3: $HClO_3$) and the concentrations of cobalt, iron and zinc were estimated in clear digest using Atomic Absorption Spectrophotometer (Thermo Jarrell Ash, Franklin, MA, USA). Lipid peroxidation (MDA), proline content, catalase (CAT, E.C.1.11.1.6.), peroxidase (POD, E.C.1.11.1.7), superoxide dismutase (SOD, E.C.1.15.1.1), ascorbate peroxidase (APX, E.C. 1.11.1.11) were assayed by using the method of Sinha *et al.* (2012). The H_2O_2 content was determined by the method of Brennan and Frenkel (1977). The soluble protein content was determined in the enzyme preparations for expression of the specific activity. The experiment was arranged in a completely randomized design with three replications. Data were analyzed using ANOVA and tested for significance.

Cobalt-treated groundnut plants exhibited growth depression with chlorotic patches developed on the young leaves (0.5 mM Co at 18 DAT). Subsequently necrotic spot appeared on whole leaves, which enlarged, coalesced and ultimately lamina withered (Plate 1). Growth of plants was checked due to death of the growing point. At 0.4 mM Co treatment, only few flowers formed, which were smaller in size, lighter in colour and failed to produce pods. Flowering and fruiting were completely inhibited at 0.5 mM Co level. Chlorosis and necrosis might have occurred due to degradation of chlorophyll molecules or deficiency of Fe or Zn induced by Co treatment (Khan and Khan 2010).

The biomass decreased with an increase in Co supply from 0.05 to 0.5 mM and was more pronounced at 0.5 mM (Table 1). The reduction in biomass under excess

Plate 1. Young and emerging leaves showing chlorotic and bleached symptoms having reduced branching and size.

Table 1. Effect of excessive cobalt on dry matter and pod yield of groundnut

Co treatment (mM)	Shoot dry weight (g plant^{-1}) Days after treatment			Pod weight (g plant^{-1})
	10	25	59	
Control	2.45	6.88	22.24	3.44
0.05	2.28 (7)	4.76 (40)	15.86 (29)	3.02 (12)
0.1	2.26 (8)	3.78 (45)	10.95 (51)	1.84 (47)
0.2	2.17 (11)	3.45 (50)	9.06 (59)	1.06 (69)
0.4	2.06 (16)	3.15 (54)	6.5 (71)	0.83 (76)
0.5	1.93 (21)	2.77 (60)	3.2 (-86)	-
LSD (p=0.05)	0.09	0.11	0.54	0.21

Figures in parenthesis denote % decrease over the control

Co supply might be due to cumulative effect of inhibited physiological function and internal water deficit (Khan and Khan 2010). The accumulation of Co was maximum (Table 1) in roots and young leaves and lowest in stem.

With an increase in Co supply, iron and Zinc concentration decreased (Table 2) in plant parts, which, might be due to the competition between Co, Fe and Zn for the same physiological binding site as suggested by Mengel and Kirkby (2001). It has been reported that the high concentrations of Co in the roots and leaves can disrupt a range of metabolic processes due to competitive interactions (Liu *et al.* 2000).

Under stress environment, MDA levels are generally considered as an index of lipid peroxidation. MDA concentration increased with increasing Co levels (Table 3) indicating, the increased presence of free radicals in the tissues, and oxidative stress. Generation of free radicals (*OH, H_2O_2), or reactive oxygen species can convert fatty acid to lipid peroxides and destroy biological membranes (Panda *et al.* 2003).

There was more accumulation of proline in excess Co treated plants (Table 3), which may be due to reduction in water potential and play a role in detoxification of active oxygen species produced due to Co treatment (Mathad and Pratima 2009).

The activity of antioxidant enzyme ascorbate peroxidase (Table 3) increased with an increase in Co supply. Apel and Hirt (2004) reported that the APX found in organelles is involved in scavenging H_2O_2 produced under stress. In chloroplast, H_2O_2 can be detoxified by the ASA-GSH-NAPDH system that has been catalysed by APX (Tamas *et al.* 2005). Peroxidase (POX) activity increased with a simultaneous decline in CAT activity indicating an accumulation of H_2O_2 and

Table 2. Accumulation of Co, Fe and Zn in plant parts (25 DAT of Co) and in seeds of groundnut

Co treatment	Young leaves			Old leaves			Stem			Roots			Seeds		
(mM)	Co	Fe	Zn	Co	Fe	Zn	Co	Fe	Zn	Co	Fe	Zn	Co	Fe	Zn
Control	ND	351	73	NS	194	64	1.16	205	100	0.6	358	113	ND	80	61
0.05	273	191	53	28	192	52	28	210	70	201	417	97	ND	60	29
0.1	384	168	57	42	165	46	70	196	67	500	350	91	5	47	26
0.2	609	140	47	68	141	47	126	172	64	677	240	70	16	38	25
0.4	857	89	41	123	120	57	225	169	72	816	239	71	29	13	24
0.5	939	87	39	322	142	43	287	158	56	907	182	66	-	-	-
LSD (p=0.05)	23	12	21	18	20	10	21	15	11	32	27	9	4	9	6

Table 3. Effect of excess cobalt on activity of antioxidative enzymes, lipid peroxidation, proline and H_2O_2 concentration in groundnut leaves

Co treatment (mM)	Catalase (µmol H_2O_2 g^{-1} fw min^{-1})	Ascorbate peroxidase (µmol ascorbate min^{-1} mg^{-1} fw)	Lipid peroxidation (nmol MDA 100 mg^{-1} fw)	Proline (µmol proline 100 mg^{-1} fw)	H_2O_2 (µmol g^{-1} fw)
Control	278	0.27	19.98	66	7.8
0.05	269 (-36)	0.47 (+74)	18.91(-98)	88 (+33)	9.6 (+23)
0.1	250 (-10)	1.16 (+330)	17.05 (-15)	98 (+48)	13.2 (+62)
0.2	246 (-11)	2.06 (+663)	16.82 (-16)	119 (+80)	16.4 (+110)
0.4	142 (-49)	2.76 (+922)	14.73 (-26)	154 (+133)	17.6 (+126)
0.5	79 (-72)	3.19 (+1081)	10.39 (-48)	186 (+182)	20.0 (+156)
LSD (p=0.05)	19	0.18	1.21	22	1.9

Figures in parenthesis denote % increase (+) or decrease (-) over the control, fw = fresh weight

imposition of oxidative stress in Co treated groundnut plants.

Our study indicates that increase in concentration of Co led to decrease in biomass, yield, lipid peroxidation, and catalase activity thereby, suggesting that groundnut is sensitive to excessive Co concentration for oxidative stress.

ACKNOWLEDGEMENT

Authors are grateful to ICAR for providing financial support for this research work.

REFERENCES

Adriano, D.C. (2001). *Trace elements in terrestrial environment: Biochemistry,bioavaility, and risk of metals. 2nd edn.* Springer Verlag, New York.

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann. Rev. Plant Biol.* **55:** 373-379.

Brennan, T. and Frenkel, C. (1977). Involvement of hydrogen peroxide in regulation of senescence in pear. *Plant Physiol.* **59:** 411-416.

Collins, R.N., Estelle, B., Marie, C., Hicham, K., Olivier, P., Jean-Louis, M. and Barbara, G. (2010). Uptake, localization, and speciation of cobalt in *Triticum aestivum* L. (wheat) and *Lycopersicon esculentum* (tomato). *Environ. Sci. Technol.* **44:** 2904–2910.

Khan, M.R. and Khan, M.M. (2010). Effect of Varying Concentration of Nickel and Cobalt on the Plant Growth and Yield of Chickpea. *Australian J. Basic & Applied Sci*. **4:** 1036-1046.

Liu, J., Reid, R.J. and Smith, F.A. (2000). The mechanism of cobalt toxicity in mung beans. *Physiol. Plant.* **110:** 104-110.

Mathad, P. and Pratima, H. (2009). Copper toxicity causes oxidative stress in *Brassica juncea* L. seedlings. *Indian J. Plant Physiol.* **14:** 397-401.

Mengel, K. and Kirkby, E.A. (2001). Elements with more toxic effects. In Principles of plant nutrition, fifth ed. International Potash Institute Berne, Switzerland, pp. 657-670.

Molas, J. (2008). Comparison of the toxicity of co(ii)-gly and co(ii)-edta chelates to lucerne (*Medicago sativa* L.) plants. *Proceedings of ECO Pole.* **2:** 71-75.

Panda, S.K., Chaudhury, I. and Khan, M.B. (2003). Heavy metals induce lipid peroxidation and affects antioxidants in wheat leaves, *Biologia Plantar.* **46:** 289-294.

Sinha, P., Khutana, N. and Nutiyal, N. (2012) Induction of oxidative stress and antioxidant enzymes by excess cobalt in mustard. *J. Plant Nutr*. **35:** 952-960.

Tamas, L., Mistrik, I., Simonovicova M. and Siroka, B. (2005). Aluminum induced cell death of barley–root border cells is correlated with peroxidase and oxalate oxidase-mediated hydrogen peroxide production. *Plant Cell Repro.* **24:** 189-194.

Tewari, R.K, Kumar, P., Sharma P.N. and Bisht, S.S. (2001). Modulation of oxidative stress responsive enzymes by excess cobalt. *Plant Sci.* **162:** 381-388.

Van Assche, F. and Clijster, H. (1990). Effect of metals on enzyme activity in plants. *Plant Cell Environ*. **13:** 195-206.