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By

Rajni Shukla

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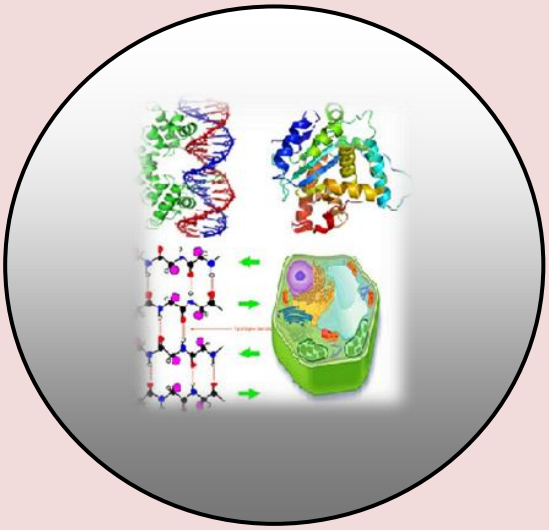

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RESEARCH PAPER

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Excess Nickel Altered the Translocation of Certain Nutrient and Metabolism of Antioxidative Enzyme in Cauliflower

Rajni Shukla

Department of Botany, C.B. Gupta Ag. P.G. College, B.K.T. Lucknow, U.P., India

ABSTRACT

A glass house experiment was conducted to with level of nickel control (0.0001mM Ni) while other two lots were supplied with excess nickel (Ni) at 0.1mM and 0.5mM using cauliflower (Brassica oleracea L. var. botrytis) cv. Snowball was grown in refined sand with complete nutrient solution for 79 days. At d 80 plants were separated into three lots. Excess nickel caused oxidative stress indicate by decrease in enzyme activities of catalase, peroxidase and acid phosphatase in leaves. The decreased activity of catalase may suggest interference of excess nickel in iron metabolism of plants. The appearance of metal specific toxicity is a likely result of damage predominantly due to enhanced generation of reactive oxygen species (ROS) at higher (0.5mM) nickel supply. Increase in Ni supply increased nickel concentration in all parts of cauliflower whereas the concentration of phosphorus (P), sulphur (S), iron (Fe) and manganese (Mn), Zinc (Zn) and copper (Cu) decreased significantly.

Keywords: Nickel, Manganese (Mn), Copper (Cu) and Iron (Fe).

INTRODUCTION

The accumulation of heavy metals in several plant species, of agriculture importance has been reported at several locations (Gragor et al. 1991) growing near industrial areas. Heavy metal stress may stimulate the formation of free radicals and reactive oxygen species (ROS) such as $\cdot O_2$ (super oxide radical), $\cdot OH$ (hyroxyl radical), H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen) in several plants (Alscher et al., 1997; Foyer et al.1997). Nickel salts are considered to be an occupational hazard and reported to produce undesirable effects and/or carcinogenicity in humans and animals (Obone et al., 1999). Nickel exposure causes formation of free radicals in various tissues in both human and animals which lead to various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulphhydryl homeostasis (Das et al., 2008).

During the period of metal treatment, plants develop different resistance mechanisms to avoid or tolerate metal stress, including the changes of lipid composition, the profiles of isozymes and enzyme activity, sugar or amino acid and the level of soluble proteins and gene expressions. These adaptations entail qualitative and/or quantitative metabolic changes that often provide a competitive advantage, and affect plant survival (Schützendübel and Polle 2002). Therefore, plant cells contain protective and repair systems that, under normal circumstances, minimize the occurrence of oxidative damage. Excess supply of nickel to plants also accelerates generation of reactive oxygen species (ROS) resulting into oxidative stress (Baccouch et al., 1998). The toxicity associated with nickel (II) is mainly due to generation of reactive oxygen species (ROS) with subsequent oxidative deterioration of biological macromolecules. Nickel can generate free radicals (FR) directly from molecular oxygen in a two-step process to produce superoxide anion and in continued process, produce highly toxic hydroxyl radical. The Ni-induced growth inhibition has been ascribed to down-regulation of protein synthesis and activities of some key enzymes responsible for mobilization of food reserves taking place during seed germination (Bishnoi et al., 1993). Several redox and non-redox metals like Fe (Fang and Kao, 2000), Cu (Teisseire and Guy, 2000), Zn (Rao and Sresty, 2000) and Cd (Romero- Puertas et al., 1999) are known to cause oxidative stress as indicated by lipid peroxidation and H2O2 accumulation in the cells (Schutzendube and Polle, 2002).

MATERIALS AND METHODS

Plant material and Growth Condition

Cauliflower (*Brassica oleracea* L. var. botrytis) cv. Snowball was grown in washed sand under controlled conditions in a glass house (Agarwala and Sharma 1976). Plants were grown in polyethylene containers of 10L capacity having a central drainage hole, covered with an inverted watch glass whose rim was lined with glass wool. The composition of the base nutrient solution was 4 mM KNO3, 4mM Ca(NO3)2, 2mM MgSO4, 1.5mM NaH2PO4, 100mM Fe EDTA, 10 mM MnSO4, 30mM H_3BO_3,1mM CuSO4, 1mM ZnSO4, 0.2mM Na_2MoO4, 0.1mM CoSO4 ,0.1mM NiSO4 and 0.1mM NaCl.

One lot was allowed to grow as such and was treated as control. In other two lots nickel was superimposed at 0.1 and 0.5 mM Ni as NiSO4. All the experiment was were carried out in triplicate.

Visual Observation and Zn, Fe, Cu, Mn Concentration

After 5 days, when plants showed depression in growth besides periodical record of visible symptoms,~~.~~ at d 90 and 110 (10 and 30 days after metal supply) plants were sampled for tissue estimation of phosphorous (Wallace 1951) and sulphur (Chesnin and Yein 1951) colorimetrically while of Fe, Mn, Zn and Cu were estimated by atomic absorption spectrophotometerAAS-4141. The concentration of iron, zinc, copper, manganese, phosphorous and sulphur was estimated in oven dried plant samples after di -acid digestion (HNO3:HClO4, 10:1) (Piper, 1942) by atomic absorption spectrophotometer.

Enzyme Extraction and Assay

Fourth fully expanded fresh leaf tissue (2.5 gm) was homogeniged in 10.0 ml chilled 50 m~~m~~M potassium phosphate buffer (pH 7.0) containing 0.5% (w/v) insoluble polyvinyl polypyrolidone and 1.0 mm phenyl methylsulfonyl floride in a chilled pestle and mortar kept in ice bath.

The homogenate was filtered through two muslin cloth and centrifuged at 20,000x g for 10 min. The supernatant was stored at 2ºC and used for enzyme assays wiin 4 hrs.

Assay of Antioxidative Enzymes

Catalase (CAT) and peroxidase (POD) were assayed in fresh leaf tissue extracts prepared by homogenizing samples in ice-cold glass distilled water (1:10) with a cold pestle and mortar at 4^0 C. The activity of CAT was assayed as described by Euler and Josephson (1927) in a reaction mixture (10 ml, standardized against 0.1 N $KMnO_4$) containing 500 µM of H_2O_2 and 1.0 mmol of potassium phosphate buffer (pH 7.0) was stabilized at 25^0C. The reaction was allowed to proceed for 5 min. and was stopped by adding 2.0 ml of 2N H_2SO_4. The final reaction mixture was titrated against 0.1 N $KMnO_4$. The activity of POD was assayed by the method of Luck (1963). The reaction mixture (10 ml) contained 5.0 ml of 0.1 M of potassium phosphate buffer (pH 7.0), 1.0 ml of 0.01% H_2O_2, 1.0 ml of 0.5% *p*-phenyldiamine. The reaction was started by adding 1.0 ml suitably diluted enzyme extract and allowed to proceed for 5.0 min. The reaction was stopped by adding 2.0 ml of 5N H_2SO_4 and the colour intensity was measured at 485 nm.

Acid phosphatase activity was assayed by the method of Schmidt (1955). The reaction mixture contained 0.5 ml 0.1M sodium acetate buffer pH 5.0 and 0.4 ml suitably diluted enzyme extract in a centrifuge tube. The reaction was initiated by the addition of 0.1ml. 0.1M sodium β-glycerophosphate at 30 ^oC and was stopped exactly after 20 min by adding 1 ml 10% (w/v) trichloroacetic acid (TCA). The corresponding blanks were run simultaneously with added TCA before the addition of the substrate. The contents were centrifuged at 400 x g for 10 min at room temperature. The amount of inorganic phosphate (Pi) liberated was estimated in a suitable aliquot of the supernatant by the method of Fiske and Subbarow (1925).

Statistical Analysis

All estimations made in triplicate. The data have been statistically analysed for standard error (± SE).

RESULT

Plant growth and Visible symptoms

The visible symptoms of nickel toxicity appeared after d 90-95 (10-15 days of metal supply) showed chlorosis of young leaves. In cauliflower, the growth depression and symptoms of excess nickel were less marked. Except for mild general yellowing of young leaves and no other specific effects appears that cauliflower is quite resistant to excess nickel. This may also indicate that plants are divergent in their sensitivity to nickel.

Activities of oxidative stress enzymes

Compared to the activity of catalase in control leaves its activity decreased at 0.1 and 0.5 mM Ni supply.

The activity of peroxidase decreased (Fig.3) in cauliflower leaves at 0.1 and 0.5mM Ni. Compared to the activity of acid phophatase (Fig 3) at control its activity decreased to 0.1 and 0.5 mM Ni.

Tissue Zn, Fe, Cu, P, S and Mn

At d 100, concentration of phosphorus decreased in all parts (except roots) from the values of phosphorus in respective to that of control level (Table3). The concentration of iron decreased in all parts except roots.

The concentration of sulphur in different part in cauliflower was decreased. The concentration of iron decreased in all parts except roots.

The concentration of zinc is decrease in all part of plant at 90 and 110 growth stage. The concentration of copper is decreased in all part of plant at different growth stage except root.

DISCUSSION

The visible symptoms of nickel toxicity appeared after d 90-95(10-15 days of metal supply) chlorosis of young leaves–(Gopal and Nautyal, 2012). The decrease in activity of catalase might be possible due to inhibitory role of excess heavy metals in inducing oxidative stress as-observed previously in sunflower (Gallego et al., 1996). The decrease in catalase activity in cauliflower (Fig 3) similar to the report on *Hyptis suaveolens* L. Poit. and *Helianthus annuus* L. (Pillay et al. 1996) and *Oryza sativa* L (Archana et al. 2006). The decrease in catalase activity in turn increase the H_2O_2 concentration creating oxidative stress enhancing the inactivating of catalase preventing synthesis of new enzyme (Dat et al. 1998).

Pandey and Pathak (2006) also noticed marked decrease at 10µM Ni supply. It might be possible due to higher accumulation of H_2O_2. The results are in contrast with the result of sunflower observed by Pillay et al. (1996). The decrease in acid phosphatase activity in nickel excess in cauliflower leaves might be due to disturbed phosphorous metabolism. This is in contrast with earlier report of (Pillay et al. 1996). The decrease in sulphur content in various parts of cauliflower is in consonance with the results on cabbage (Yang et al. ,1996) and cauliflower (Chatterjee and Chatterjee 2000), where excess Ni reduced the S content. The reduction in sulphur might be due to blockage of passage by the presence of Ni in excess amounts. This altered sulphur content in turn might be responsible for less available sulphur for different biomolecules to be utilized in various metabolic pathways as has been suggested for excess Ni (Gopal et al. 2001). The decrease in Mn in leaves might suggest a competition between them and the results are similar to those described by Taylor and Stadt, 1990; Lou et al.1991) These results are not in support with the observation of Piccini and Malavolta (1992) in bean, where no change was found in the concentration of Mn. Yang et al., (1996) have reported a decrease in Mn concentration in excess nickel application in maize. It appears that translocation of iron was disturbed in excess nickel supply. The disturbances in phosphorous and iron content due to excess Ni affect carbohydrate and nitrogen metabolism and this might be responsible for depressed growth and lowered biomass in excess Ni. Similar observation on lower Phosphorus content in excess Ni condition have been reported by Miller et al., (2000) in Virginia pine (*Pinus verginians Mill*). The accumulation in carbohydrate fraction (Fig 2) is in consonance with similar results on white bean (Rauser, 1978) bush bean (Rauser and Samarkoon, 1980).

The decrease in Mn (in cauliflower), Cu (in cauliflower) and Zn content (in all plants) in leaves might also suggest a competition between them and excess Ni. These results are not in support with the observations of Piccini and Malavolta (1992) in bean, where no change was found in the concentration of Mn and Zn but that of P was increased.

Yang et al., (1996) have reported decrease in Zn, Cu and Mn concentration in excess nickel condition in maize. In cabbage Fe and S decreased in excess Ni concentration by Yang et al., (1996).

CONCLUSION

The visible symptoms of nickel toxicity clearly indicate the inhibitory effect of excess nickel inhibit cauliflower growth and development Moreover, the present study showed a nickel mediated free radical reaction as manifested by an increase in the activities of POD and decrease the activity of CAT to help in detoxifying the H_2O_2 produced in response to Nickel treatment.

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Corresponding author: Dr. Rajni Shukla, Department of Botany, C.B. Gupta Ag. P.G. College, B.K.T. Lucknow, U.P., India
Email: rajnishukla.t@gmail.com