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BRIEF COMMUNICATION

Effect of 28-homobrassinolide treatment on nickel toxicity in *Brassica juncea*

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Abstract

Plants of *Brassica juncea* L. cv. T-59 were supplied with 50 or 100 μM nickel (Ni_{50}, Ni_{100}) at 10 d after sowing (DAS), and sprayed with 28-homobrassinolide (HBR) at 20 DAS. The plants treated with Ni alone exhibited reduced growth, net photosynthetic rate, content of chlorophyll, and the activities of nitrate reductase (E.C.1.6.6.1) and carbonic anhydrase (E.C. 4.2.1.1) at observed 40 DAS, whereas, the contents of peroxidase (PER), catalase (CAT), and proline were increased. However, the spray of HBR partially neutralized the toxic effect of Ni on most of the parameters. Moreover, the treatment of HBR in association with either of the Ni concentration boosted the contents of PER and CAT in leaves and that of proline both in leaves and roots.

Additional key words: carbonic anhydrase; catalase; chlorophyll; leaf; nitrate reductase; peroxidase; proline; protein; root; shoot.

Soil contamination with heavy metals has become a world-wide problem, leading to the loss in agricultural productivity and hazardous health effects as they become part of the food chain (Salt *et al*. 1995). However, their availability in the soil is determined by natural processes, especially the lithogenic and pedogenic ones, and by anthropogenic factors (Kevrešan *et al*. 1998). The heavy metals that affect (either positively or negatively) plants include Fe, Cu, Zn, Mn, Co, Ni, Pb, Cd, and Cr, but out of them, nickel has recently been defined as an essential micronutrient, because of its involvement in urease activity in legumes (Welch 1995). The requirement of Ni in the plants is generally low [1.7 μmol kg^{-1}(Ni) or even lesser] (Dalton *et al.* 1988). Symptoms of Ni toxicity can be observed between 0.19 to 0.85 mmol kg^{-1}(Ni) in plant dry biomass. These symptoms include the inhibition in root elongation, photosynthesis, and respiration, and interveinal chlorosis (Marschner 1995). Moreover, the toxic concentration of Ni also inhibits enzyme activities and protein metabolism (Kevrešan *et al.* 1998). However, Ni also accelerates the activities of anti-oxidative enzymes (Schickler and Caspi 1999, Prasad *et al*. 2005).

Brassinosteroids (BRs) are a new class of plant hormones possessing significant growth promoting activity (Mandava 1988). Due to their role in a wide range of physiological responses, they are essential regulators of plant growth and development (Clouse and Sasse 1998). Moreover, they ameliorate the abiotic and biotic stresses including those caused by salt, chilling, high temperature, drought, and pathogens (Sasse 2003). However, their role in plants subjected to heavy metal stress, particularly Ni, has remained elusive. Therefore, this study was undertaken to study the possible ameliorative role of 28-homobrassinolide (HBR) in plants grown under Ni stress.

Seeds of *Brassica juncea* Czern & Coss cv. T-59 were obtained from Indian Agricultural Research Institute, New Delhi, India. The healthy seeds were surface sterilized with 5 % hypochlorite solution followed by washings with double distilled water and sown in earthen pots (25 cm diameter), lined on its inner surface with polythene sleeves and filled with acid-washed sand (Hewitt 1966). Each pot was supplied with 200 cm^3 of full nutrient solution on alternate days, up to day 30. Thereafter, the quantity of nutrient solution was increased to 500 cm^3. De-ionized water (250 cm^3) was also given daily to each pot. The nickel (50 or 100 μM, Ni_{50} or Ni_{100}) in the form of nickel chloride was given through root at 10 DAS. However, 10^{-8} M HBR was applied through spray on leaf of 30-d-old plants (vegetative stage) with the precaution that HBR must not fall in the

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Abbreviations: BR – brassicines; CA – carbonic anhydrase; CAT – catalase; DAS – d after sowing; DM – dry mass; FM – fresh mass; HBR – 28-homobrassinolide; NR – nitrate reductase; P_N – net photosynthetic rate; PER – peroxidase.

pot and must stay on the plants. Control plants were sprayed with double distilled water. Each treatment was replicated five times. Length, fresh (FM) and dry (DM) masses of root and shoot, net photosynthetic rate (P_N), activities of carbonic anhydrase (CA), nitrate reductase (NR), catalase (CAT), and peroxidase (PER), and total chlorophyll (Chl), proline, and protein contents were assessed in 40-d-old plants. Chl content was estimated following Mackinney (1941). The procedures of Dwivedi and Randhawa (1974) and Jaworski (1971) were used for measuring the activities of CA and NR, respectively. The methods given by Chance and Maehly (1955) were employed to assess the activities of CAT and PER. P_N in intact leaves was measured by the portable photosynthetic system *LI-6200* (*LI-COR*, Lincoln, NE, USA). The proline and protein contents both in leaf and root were measured using the methods of Bates *et al.* (1973) and Lowry *et al*. (1951), respectively. Treatment means were compared by analysis of variance using the statistical package *SPSS*. The data were processed by one-factor analysis of variance. Standard error due to replicates was calculated.

The treatments significantly affected all the growth characteristics studied (Table 1). The application of HBR (10^{-8} M) alone had highly significant effects and increased all the growth parameters over the control. However, the treatment of plants with Ni_{50} or Ni_{100} alone inhibited growth of the plants. Ni_{100} was inhibitory and it decreased the root and shoot lengths, and FM and DM of roots and shoots by 33, 32, 49, 60, 52, and 52 %, respectively, below that of the control. However, the following treatment with HBR partly released the toxic effect generated by Ni, particularly that of Ni_{50}.

Table 1. Effect of HBR (10^{-8} M) on nickel (Ni_{50} or Ni_{100}) induced changes in shoot and root characteristics (FM = fresh mass, DM = dry mass), leaf chlorophyll (Chl) content, carbonic anhydrase (CA) activity, net photosynthetic rate (P_N), activities of nitrate reductase (NR), leaf catalase (CAT), and peroxidase (PER), and proline and protein contents in *Brassica juncea* cv. T-59 at 40 d after sowing (±SE).

	Control	HBR	Ni_{50}	Ni_{100}	Ni_{50} + HBR	Ni_{100} + HBR
Root length [cm]	16.30±0.14	19.60±0.24	13.50±0.57	10.80±0.14	15.50±0.05	14.20±0.08
Shoot length [cm]	21.30±0.49	24.8±0.23	15.60±0.18	14.30±0.31	17.90±0.37	16.20±0.15
Root FM [g]	0.83±0.01	1.22±0.02	0.68±0.01	0.42±0.01	0.74±0.01	0.69±0.01
Root DM [g]	0.29±0.01	0.49±0.01	0.16±0.01	0.11±0.01	0.25±0.00	0.20±0.01
Shoot FM [g]	1.40±0.01	1.88±0.02	0.88±0.05	0.66±0.01	1.09±0.03	0.96±0.01
Shoot DM [g]	0.42±0.01	0.67±0.01	0.27±0.01	0.20±0.01	0.35±0.02	0.23±0.01
Chl [g kg^{-1}]	1.30±0.01	1.60±0.01	0.90±0.02	0.80±0.01	1.2±0.01	1.10±0.01
CA [mol(CO_2) kg^{-1}(FM) s^{-1}]	2.70±0.05	3.50±0.03	2.10±0.03	1.80±0.03	2.40±0.03	2.20±0.03
P_N [mol(CO_2) kg^{-1}(FM) s^{-1}]	11.20±0.23	15.90±0.18	8.40±0.23	6.80±0.20	10.50±0.20	9.90±0.13
NR, leaf [nmol(NO_2) kg^{-1}(FM) s^{-1}]	123.3±2.2	161.4±1.8	88.6±1.2	66.9±1.4	110.8±1.8	86.1±1.4
NR, root [nmol(NO_2) kg^{-1}(FM) s^{-1}]	58.9±1.0	101.4±0.9	41.4±1.6	30.3±0.5	47.8±0.7	40.0±0.8
CAT [mmol(H_2O_2) kg^{-1}(FM)]	419.0±4.7	452.0±5.2	469.0±3.4	496.0±3.5	531.0±2.7	542.0±2.6
PER [unit g^{-1}(FM)]	12.20±0.16	18.50±0.39	22.00±0.32	26.60±0.25	25.50±0.17	30.40±0.21
Proline, leaf [mmol kg^{-1}(FM)]	8.50±0.50	11.10±0.62	12.20±0.99	15.00±0.15	14.10±0.21	16.70±0.23
Proline, root [mmol kg^{-1}(FM)]	12.20±0.26	15.50±0.27	20.40±0.36	26.30±0.42	25.10±0.13	28.30±0.60
Protein, leaf [%]	12.40±0.07	14.30±0.15	11.30±0.07	10.80±0.09	12.00±0.06	11.70±0.03
Protein, root [%]	9.30±0.04	11.10±0.05	8.20±0.02	7.10±0.03	9.20±0.03	8.80±0.07

The plants treated with HBR alone showed, as compared to the control, an increase in Chl content and P_N by 19 and 41 % (Table 1). However, the supply of Ni_{50} or Ni_{100} decreased the values of both these parameters. Ni_{100} was more injurious and it decreased the values by 37 and 38 % below that of the control, respectively. This effect of Ni was partially reversed by its combination with HBR, especially at Ni_{50}.

The activities of both CA and NR were significantly enhanced by the HBR treatment and the values were significantly higher than that of the control (Table 1). However, the treatment of plants with either Ni_{50} or Ni_{100} decreased their activities, Ni_{100} being more toxic. It decreased the values by 32, 45, and 48 % below that of the control for CA activity in leaf and NR activity in leaf and root, respectively. The toxic effect generated by Ni_{50} was partly neutralized by HBR spray.

Unlike the activities of CA and NR, the activities of CAT and PER were stimulated by both HBR and Ni_{50} or Ni_{100} (Table 1). The interaction of HBR on the activities of the enzymes was additive at Ni_{100}. This combination resulted in an increase in CAT and PER activities by 18 and 118 % over the control, respectively. The proline content followed a pattern similar to that of the activities of CAT and PER. The control plants possessed the lowest amount of proline (Table 1). The combination of HBR with Ni_{100} raised the values to a maximum of 96 and 130 % over the control, in the leaves and roots, respectively.

Protein content was decreased by the treatment with Ni_{50} or Ni_{100} both in the leaves and roots. Ni_{100} was most inhibitory which decreased the values by 12 and 23 % over the control, respectively. However, HBR not only significantly increased the values but also overcame the

inhibitory effect of Ni on the protein content to a limited extent, particularly at Ni_{50}.

We observed that the application of Ni^{2+} inhibited the growth, Chl content, and P_N (Table 1). Nickel adversely affects both the synthesis of 5-aminolevulinic acid and protochlorophyllide reductase complex (Stobart *et al*. 1985). This could have naturally resulted in the decrease in Chl content. Moreover, Ni also decreased P_N that may be the consequence of the inhibition of the key enzymes of photosynthetic carbon reduction (PCR) cycle viz. RuBP carboxylase, 3-PGA kinase, NADP- and NAD-glyceraldehyde-3-P-dehydrogenases, aldolase, and FBPase (Sheoran *et al*. 1990), in addition to the loss of Chl. However, HBR applied alone enhanced the Chl content and P_N and also partially overcame the ill effect of Ni, if given as a spray treatment with Ni. The increase in Chl content in the plants treated with HBR is in agreement with findings of Hayat *et al*. (2001) and Fariduddin *et al*. (2003). The increase in P_N by HBR may be the result of enhanced content of Chl (Table 1) and/or the activation of ribulose-1,5-bisphosphate carboxylase/ oxygenase, RuBPCO (Yu *et al*. 2004).

The activities of CA and NR decreased in plants treated with nickel. The toxic concentration of Ni inhibits mineral nutrition (Bercelo and Poschenrieder 1990), including the absorption of nitrate (Brown *et al*. 1990), therefore, a decrease in the activity of NR was observed. Brown *et al*. (1990) reported a similar response to Ni in barley. However, the observed enhancement in the activities of NR and CA (Table 1) in the plants supplied with HBR alone/in association with Ni^{2+} may have an impact on the uptake of NO_3 (Mai *et al*. 1989) and CO_2 assimilation mediated by RuBPCO (Yu *et al*. 2004), respectively. Moreover, Khripach *et al.* (1999) suggested that BRs affect the biosynthesis of enzymes by involving gene expression and/or the effect of BRs on cell membrane.

Unlike other parameters, the activities of peroxidase and catalase and proline content gave an increasing response to HBR and/or nickel treatments. The plants have natural endogenous defence systems to cope with the reactive oxygen species, the consequence of various stresses, including that generated by the heavy metals (Schützendübel and Polle 2002). The defence systems are (*a*) the metabolites including ascorbate, glutathione, tocopherol, and proline, and (*b*) enzymatic scavengers, include superoxide dismutases, CAT, PER, and glutathione reductases (Schützendübel and Polle 2002). The additive effect of HBR may be due to its action on transcription and/or translation (Kalinich *et al*. 1985, Bajguz 2000) by involving specific genes (Khripach *et al*. 1999). The application of BRs also caused the activation of anti-oxidative enzymes under water and salt stresses (Nunez *et al.* 2003) and increased superoxide dismutase and proline contents under NaCl stress (Clouse and Sasse 1998).

The decrease in protein content in Ni-treated plants (Table 1) was possibly due to a decrease in the metabolism of amino acids and that of nitrogen (El-Shintinawy and El-Ansari 2000). However, the treatment of plants with HBR alone or after Ni treatment increased plant protein content. The increase in protein content is possibly the result of the well documented effect of BRs on transcription and/or translation (Kalinich *et al*. 1985, Mandava 1988, Bajguz 2000, Fariduddin *et al*. 2004).

Our findings give a clear impression that the presence of Ni at a level that may cause stress in the plant adversely affects its metabolism and growth. Moreover, the metal also activates the system to improve the resistance capacity of the plants to the stress. However, HBR overcame the effect of Ni stress, though to a limited extent. The hormone may, therefore, be an ameliorative steroid to neutralize the toxicity of nickel in plants.

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