Nickel induced modulations in phosphorus metabolism and other growth parameters of wheat

Naveed Gulzar[1]*, Asif Hussain Hajam[1], Ishfaq Shafi Khan[2], Shabeer Ahmad Dar[2], Haneef Mohammad Bhat[2]

[1]Department of Botany, Jamia Hamdard, 110062 New Delhi India

[2]Centre of Research for development, University of Kashmir, 190006 Srinagar, India

Abstract

Nickel is well thought-out to be an indispensable micronutrient for plants, however at excess concentration, it proves toxic to the plants and toxicity of this metal has been attributed to its negative effect on various physiological and biochemical parameters as photosynthesis, mineral nutrition, sugar transport and plant water relationship. In this study, it was assessed that different concentrations of nickel appreciably effect the levels of soluble sugar, proline and glycinebetaine along phosphorus metabolism in two cultivars of wheat (UP2382 and VL616) but the cultivar VL616 was found to be more responsive to nickel stress. The present study encompassed two experimental lines simultaneously involving nickel treatment viz. 50 µM (T1), 100 µM (T2) and 150 µM (T3) in both cultivars (UP2382 and VL616). The results of the study indicated highly significant ($p < 0.001$) changes in the biological parameters evaluated in nickel treated wheat plants as compared to their controls. The study proposed that the Cultivar VL616 was more responsive to nickel stress as evidenced by the higher accumulation of sugar, glycinebetaine and proline contents and exhibited minimum decrease in the phosphate levels during the course of nickel treatments than UP2382 cultivar. There might be increase in Salicylic acid and Abcessic acid levels in cultivar UP2382 than in cultivar VL616 that makes the cultivar UP2382 less susceptible to nickel stress. Thus, Nickel stress leads to change in various biological parameters in wheat plants and by increasing the concentration of this micronutrient, there is variable changes in these parameters. However, plants combat abiotic stress factors by regulating the levels of these biological parameters.

Keywords: Nickel; Abiotic Stress; Wheat plants; Phosphorous; Micro nutrient

INTRODUCTION

Nickel, among one of the important metal pollutants on earth is of significant concern, because its concentration is rapidly escalating in soils of different parts of the world (Faryal et al., 2007; Atiq-ur-Rehman & Iqbal, 2008). Nickel forms an essential micronutrient for plant growth and it is also a component of the enzyme urease which is required for nitrogen metabolism in higher plants. For normal metabolic functioning in plants, micronutrients play an essential role but at higher concentration they are toxic to plants. The concentration of nickel in the environment is consequently elevated by emissions of nickel from the variety of natural and anthropogenic processes. Increase in concentration of Nickel through natural process includes weathering of minerals and rocks, whereas different compounds of nickel (such as nickel acetate, nickel carbonate, nickel hydroxide and nickel oxide) are used in a variety of industrial process makes up rise of this micronutrient and leads to toxicity (Cempel & Nikel, 2006). These compounds ultimately accumulate in the soil and environment, and can be easily taken up by plants. Thus, they can enter in the food chain and cause deleterious effects on animals and human lives (Cempel & Nikel, 2006). Plant growth and metabolism are greatly influenced by nickel concentrations. At lower concentration of nickel, it proves beneficial for growth and development of plants, but at higher concentrations, it showed deleterious effects on plants (Kochian, 1991; Welch, 1995; Hasinur et al., 2005). Ni is present in hydrated form in the soil solution $Ni(H_2O)_2^{6+}$ (Yusuf et al., 2011). Although, the toxic effects of excess nickel are evident through crop development, the germination stage is regarded as the most sensitive particularly to nickel. Increasing concentration of this micronutrient inhibits seed germination and seedling growth of different plant species (Farooqi et al., 2009). The Ni-induced growth inhibition has been attributed to down-regulation of protein synthesis and activities of some key enzymes responsible for mobilization of food

reserves during seed germination (Bishnoi et al., 1993). In addition to this, Ni is a well-known active competitor of a number of indispensable micro- and macro-elements and is supposed to reduce the uptake of elements in germinating seeds, resulting thereby in poor germination and seedling establishment (Korner et al., 1987; Kochian, 1991). In order to overcome the negative effects of high nickel concentrations in plants, addition of supplement Ca^{2+} could be used to overcome the negative effect.

Wheat is an important cultivable crop after Rice throughout the world. The current study was conducted to evaluate the effect of different concentrations of Nickel on different parameters in two cultivars of wheat. In this scenario, it was found that higher concentration of this micronutrient makes reasonable changes in phosphorus content and other parameters in both cultivars. However, Cultivar UP2382 showed more resistance to the Nickel stress than cultivar VL616.

MATERIALS AND METHOD

Authenticated seeds of two genotypes (UP2382 and VL616) of *Triticum aestivum* were taken from the Genetics Division, IARI, New Delhi, India. After thorough washing of seeds with water, seeds were surface sterilized with 0.01 % mercuric chloride. The seeds were washed again with distilled water and were sown in the pots containing the mixture of sand and vermiculite (1:1). After germination, three plants were maintained in each pot (Hummert International, Earth City, MO, USA) and plants were grown in Hoagland solution (Hoagland & Arnon 1950) of one-fourth strength for first 10 days. For next ten days in half-strength of Hoagland solution and in full strength for the last ten days. The plants were grown in the growth chamber under the controlled conditions of light (16h photoperiod), temperature (27^0C) and humidity (60%). Following treatments were given to 30 days old plants, T0 = 0 µM Ni, T1 = 50 µM Ni, T2 = 100 µM Ni, T3 = 150 µM Ni. The treated genotypes were taken in triplicates along with the control of each genotype during sampling. 48 plants of each variety were grouped into 4 groups, each group including 3 plants of each variety. Leaves of 30 days old plants were excised and used for experimental examination. The whole experimental procedure involves the utilization of three biological replicates.

Estimation of proline content

Proline content was determined by following the method of (Bates et al., 1973). 0.5 g of leaf sample was homogenized in 3% sulphosalicylic acid (10ml) followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant (2ml) was taken in test tube and 2 ml of acid ninhydrin along with 2 ml of glacial acetic acid were added. The mixture was incubated at 100 ˚C in a water bath for one hour and the tubes were placed in an ice bath to terminate the reaction. 4ml of toluene was added to each tube and mixed vigorously on a vortex for 10-30 seconds. The supernatant layer was taken from the mixture and absorbance measured at 520 nm using toluene as blank. The concentration of proline in samples was calculated against the standard curve of proline, expressed in µg g^{-1} fresh wt.

Soluble sugar content of leaves

Soluble sugar content was estimated by employing phenol-sulphuric acid method of (Dubois et al., 1956), Briefly, 0.5g of leaf samples were extracted by adding 80% (v/v) methanol keeping the solution at 70°C in a water bath. The resulting extract was centrifuged at 5,000 × g for 10 min at 4°C. The supernatant was collected and treated with 5% phenol and 98% sulphuric acid. The reaction mixture kept for 1h and then absorbance was measured at 485 nm. Soluble sugar content was calculated in $mMg^{-1}FW$.

Estimation of glycine betaine content

Glycine betaine content was determined following the procedure of (Grieve & Grattan, 1983). In brief, 0.5 g of leaf material was homogenized with 20 ml mili-Q water for 48 h and filtered. The filtrate was diluted with equal volume of 1M H_2SO_4 kept in ice-cold water for 1h for cooling and then cold potassium iodide-iodine reagent was added to it. The mixture was gently vortexed, stored at 4 °C overnight and centrifuged at 12 000 x g for 15 min at 4 °C. The precipitated per iodide crystals were collected and dissolved in 1,2-dichloroethane, and the absorbance was measured at 365 nm after 2 h using glycinebetaine (dissolved in 1 M H_2SO_4) as the standard. Glycinebetaine content was calculated in µg $g^{-1}FW$.

Estimation of phosphate content

Phosphate content was estimated by the method of (Ames, 1966). Briefly, 0.5g of leaf sample was taken in silica crucible. One ml of 10% $Mg(NO_3).6H_2O$ (in 95% ethanol) was added to it before powdering it to ashes in a mufle furnace at 600˚C for four hours. Reaction mixture (2.3ml) containing 10% ascorbic acid and 0.42% $(NH_4)6Mo_7O_{24}.4H_2O$ (made in 0.5M H_2SO_4) in 1:6 ratio and the solution was kept for 10 min at 45˚C to complete the reaction. The absorbance was measured at 820 nm and phosphorus content was calculated from the standard curve prepared using analytical-grade KH_2PO_4.Phosphate content was determined in µg $g^{-1}FW$.

Statistical analysis

We carried out the statistical analysis by using one-way ANOVA using Duncan's multiple range test (SPSS17.0); The results were compared for significance at $p < 0.05$ and the results were presented as mean ± SD (n = 3).

RESULTS AND DISCUSSION

Nickel stress-induced changes in the proline content

Proline content increased linearly with increase in the Ni concentration. Cultivar VL616 showed higher levels of this amino-acid than UP2382 at all treatments, signifying

the differential potential of the two cultivars in terms of regulating defense associated with proline during stress conditions (Table 1).

Change in sugar content of leaves during Ni stress

During our study there was a significant upsurge in the total sugar content with increase in nickel treatment with the maximum level exhibited by VL616 cultivar (Table 1).

Variation in Glycinebetaine content of leaves during Ni stress

There was considerable enhancement in glycinebetaine level with increase in the nickel concentrations. Cultivar VL616 was more responsive with the higher accumulation of this osmolytes (Table 1).

Changes in phosphorus metabolism under Ni stress

There was a considerable decline in phosphate level in plants under nickel treatments possibly due to decrease in uptake of phosphates from soil. Cultivar UP2382 was more responsive with maximum decline in Phosphorus content as compared to cultivar VL616 (Table 1).

Proline is one of the primary osmolytes which has a key role in osmotic adjustment besides functioning in antioxidant defense and stabilization of proteins and organelles (Sumitra et al., 2006; Anjum et al., 2014). Wheat plants exhibited considerable changes in the proline content in both cultivars after nickel treatments. The result of proline content showed the highly significant increase ($P < 0.001$) in T3 compared to their control in VL616 cultivar. However, both the cultivars (UP2382 and VL616) showed significant increase ($P < 0.5$) in proline content while T2 as depicted in (Table 1) The possible reason for increment in proline synthesis may possibly be the increased levels of this amino-acid in nickel-stressed plants. Our results regarding go in line with the earlier findings of enhanced proline content in Wheat cultivars by (Iqbal et al., 2014).

Sugars also play a crucial role in maintaining primary metabolic pathways and osmotic adjustment. Almost half of the total osmotic balance maintained by the plants under stress is due to soluble sugars only. Our results showed highly significant increase ($P < 0.001$) in soluble sugar content in T2 and T3 in cultivar (VL616), however cultivar (UP2382) in T3 showed significant up rise ($P < 0.05$) and non-significant ($P > 0.05$) in T2 as compared to their respective controls (Table 1). The increased content of sugars may be due to the altered carbohydrate metabolism in the plant to synthesize sugars for maintaining growth under stress. Our results were supported by the earlier studies of (Amini & Ehsanpourr, 2005).

Glycine betaine maintains osmotic balance and stability of both proteins and membranes (Chen and Murata, 2011). VL616 showed highly significant increase ($P < 0.001$) in Glycine betaine content in T3 as compared to their control. However, UP2382 showed significant increase ($P < 0.05$) as compared to their control in T3 and non-significant ($P > 0.05$) in T2 as compared to their control (Table 1). The increased level of glycine betaine may be attributed to the increased synthesis of this osmolyte under stress conditions similar increasing trend of glycine betaine has been earlier observed in sorghum, corn, tomato, cotton and wheat (Saneoka et al., 2001; Park et al., 2004; Desingh & Kanagaraj, 2007, and Wang et al., 2010).

Phosphorus is an essential element with both cell structural as well as functions utilities in the plant. It forms the integral part of nucleic acids and membranes and plays an indispensable role in photosynthesis and cell signaling. Nickel treatment induced substantial changes in the phosphorus metabolism of Wheat as revealed by the alteration in the phosphate levels and phosphate-metabolizing enzymes including acid phosphatase and alkaline phosphatase. Results showed that on monitoring the Phosphate levels in plants during nickel treatment, there was decrease in the phosphate during the nickel treatments. There is highly significant ($P < 0.001$) decrease in phosphate levels in cultivar (UP2382) as compared to their control as depicted in (Table 1). The response of wheat towards nickel treatment was both doze as well as cultivar-dependent. Ni, the micronutrient in lower concentrations has some role in growth and development in plants, but when the concentration of this micronutrient is elevated, it resulted in the stress levels in plants and at higher concentrations leads to inhibition of growth and development of chlorotic, necrotic and wilting processes in plants. Plants can efficiently develop symptoms of stress factors and resulted in the change in the concentrations of biochemical parameters. In this study, VL616 showed higher changes in the levels of various biochemical parameters than UP2382 cultivar, resulted that the cultivar VL616 was more prone to stress.

Table 1. Change in various Phosphate content and other biochemical parameters.

Parameters	To (Control)	T1 (50 µm Ni)	T2 (100 µm Ni)	T3 (150 µm Ni)
Proline	V1 6.3±0.32^{a} V2 6.82±0.10^{a}	V1 7.9±0.12^{a} V2 8.7±0.08^{b}	V1 8.7±0.14^{b} V2 9.4±0.16^{b}	V1 11.3±0.12^{b} V2 13.8±0.08^{bc}
Soluble sugar	V1 5.81±0.08^{a} V2 5.83±0.24^{a}	V1 6.5±0.20^{a} V2 7.10±0.08^{b}	V1 8.83±0.20^{b} V2 10.1±0.29^{bc}	V1 10.7±0.15^{b} V2 13.2±0.37^{bc}
Glycine betaine	V1 5.3±0.12^{a} V2 5.3±0.34^{a}	V1 6.8±0.08^{a} V2 9.5±0.20^{b}	V1 8.4±0.19^{a} V2 11.6±0.26^{b}	V1 10.4±0.06^{b} V2 14.0±0.28^{bc}
Phosphate	V1 13.3±0.33^{bc} V2 12.1±10.3^{bc}	V1 8.5±0.26^{b} V2 10.3±0.10^{bc}	V1 6.5±0.20^{a} V2 9.8±0.04^{b}	V1 5.4±0.16^{a} V2 6.2±0.24^{a}

Values are represented as mean ± SD (n = 3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); a $P > 0.05$ (non-significant), b $P < 0.05$ (significant in comparison to their control), bc $P < 0.001$ (highly significant in comparison to their control)

CONCLUSION

The results that were obtained in this study indicated that nickel toxicity in plants leads to an inhibition of growth, chlorotic, necrotic and wilting processes. Toxicity of this metal has been attributed to its negative effect on photosynthesis, mineral nutrition, sugar transport and water relations. Ni at different concentrations considerably influences the biochemical reactions of plants, including phosphorus metabolism and glycinebetaine and proline contents. In response to stress, plants show variable changes in these biochemical parameters. In this study, two strains of wheat showing different responses on nickel amendments of variable concentrations and the results determined that VL616 variety is greatly influenced by the stress levels as there is maximum change in these biochemical parameters as compared to UP2382 variety. These variable findings in results might be due to the presence of high content of flavonoids, phytoalexins, phenolic compounds and increase in defense signaling pathways in cultivar UP2382 than in VL616 to combat the stress as due to increasing stress levels of the heavy metals, UP2382 is found to be resistant than VL616.

The study indicated that nickel is considered to be an essential micronutrient for plants, however at excess concentrations this metal becomes toxic for majority of plant species. This study will help the researchers to use the micronutrients in a proper way and higher applications of this micronutrient will prove fatal for the plant growth and will lead to abiotic stress to plants.

ACKNOWLEDGEMENT

The author would like to thank gratefully the department of Botany, Jamia Hamdard University for providing all laboratory facilities in completing this work.

REFERENCES

Ames BN (1966) In Methods in Enzymology (Colowick, S.P., and Kaplan, N.O., Eds). Academic Press, New York Vol. 8: 115-118.

Amini F, Ehsanpour AA (2005). Soluble Proteins, Proline, Carbohydrates and Na+/K+ Changes in Two Tomato (Lycopersicon esculentum Mill.) Cultivars under in vitro Salt Stress. American J. of Biochem. and Biotech. 14: 212-216.

Anjum NA, Umar S, Iqbal M, Khan, NA (2011). Cadmium causes oxidative stress in mung bean by affecting the antioxidant enzyme system and Ascorbate–Glutathione cycle metabolism. Russ J Plant Physiology 58:92–99.

Atiq-ur-Rehman S, Iqbal MZ (2008). Level of heavy metals in the foliage of naturally growing plants collected from Korangi and Landhi areas of Karachi city, Pakistan. Pak. J. Bot. 40:785–789.

Bates LS, Waldren RP, Tease, ID (1973). Rapid determination of free prolinc for water stress studies; Plant Soil 39: 205-207.

Bishnoi N, Sheoran I, Singh R (1993). Effect of cadmium and nickel on mobilization of food reserves and activities of hydrolytic enzymes in germinating pigeonpea seeds. Biol. Plant 35, 583.

Cempel M, Nikel G (2006). Nickel: A Review of Its Sources and Environmental Toxicology. Polish Journal of Environmental Studies 15: 375-382.

Chen T, Murata N (2011). Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant Cell Environment 34: 1–20.

Desingh R, Kanagaraj G (2007). Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. Gen Appl Plant Physiology 33:221–234.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Farooqi ZR, Zafar Iqbal M, Kabir M, Shafiq, M (2009). Toxic effects of lead and cadmium on germination and seedling growth of Albizia lebbeck (L.) Benth. Pak. J. Bot. 41(1): 27-33.

Faryal R, Tahir F, Hameed A (2007). Effect of waste water irrigation on soil along with its micro and macro flora. Pak. J. Bot. 39:193–204

Grieve CM, Grattan SR (1983). Rapid assay for the determination of water-soluble quaternary ammonium compounds. Plant Soil 70: 303-307.

Hasinur R, Shamim S, Shah A, Shigenao KW (2005). Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. J. Plant Nutrition 28: 393-404.

Iqbal N, Umar S, Khan NA, Khan MIR (2014). A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism. Environ. Exp. Bot. 100:34–42.

Kochian LV (1991). Mechanisms of micronutrient uptake and translocation in plants, In: Micronutrients in Agriculture. (Ed.): J.J. Mortvedt. Soil Science Society of America, Madison, WI pp. 251-270.

Körner LE, Møller LM, Jensén P (1987). Effects of Ca2+ and other divalent cations on uptake of Ni2+ by excised barley roots. Physiol. Plant 71: 49-54.

Pandey N, Sharma CP (2002). Effect of heavy metals on growth and metabolism of cabbage. Plant Sci. 163: 753–758.

Park E, Jeknic J, Sakamoto Z, DeNoma A, Yuwansiri J, Murata R, Chen NTHH (2004). Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. Plant J.40:474–487.

Saneoka H, Ishiguro S, Moghaieb REA (2001). Effect of salinity and abscisic acid on accumulation of glycinebetaine and betaine aldehyde dehydrogenase mRNA in Sorghum leaves (Sorghum bicolor). J Plant Physiol. 158:853–859.

Sumitra K, Jutur P, Carmel BD, Reddy AR (2006). Salinity-induced changes in two cultivars of Vigna radiata: responses of antioxidative and proline metabolism. Plant Growth Regulation 50:11–22.

Welch RM (1995). Micronutrient nutrition of plants, Crit. Rev. Plant Sci. 14: 49-82.

Wang GP, Li F, Zhang J, Zhao MR, Hui Z., Wang W (2010a). Overaccumulation of glycine betaine enhances tolerance of the photosynthetic apparatus to drought and heat stress in wheat. Photosynthetica 48:30–41.

Yusuf M, Fariduddin Q, Hayat S, Ahmad A (2011). Nickel: An Overview of Uptake, Essentiality and Toxicity in Plants. Bull. Environ. Contam. Toxicol. 86:1-17.