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Full Length Article

Alleviation of Nickel-Induced Stress in Mungbean through Application of Gibberellic Acid

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Abstract

Nickel (Ni) is the most eco-toxic and harmful metal for soil biological activity, plant metabolism and health of animals and human beings. Elevated level of Ni in plants causes many nutritional and physiological disorders as well as perturbs in normal balance of phytohormones. Exogenous application of phytohormones can regulate plant growth and reduce inhibitory effect of toxic metals on plants. Therefore present study was conducted to assess the effect of Ni contamination on growth and yield of mungbean and role of gibberellic acid (GA_3) to counteract negative effects of Ni contamination. Mungbean seeds were sown in potted soil contaminated with different levels of Ni (0, 20, 40, and 60 mg kg^{-1}). Two sets of Ni contamination levels were maintained, one set was kept without application of GA_3 while in second set 10^{-4} M GA_3 was applied as foliar spray at 15, 30 and 45 days after germination. Significant reduction in all growth and yield attributes of mungbean was recorded in Ni contaminated pots. However, application of GA_3 enhanced the length, fresh and dry weight of shoots and roots as well as grain yield of mungbean in Ni contamination. Moreover, Ni concentration was increased in roots and shoots of mungbean with increasing levels of Ni contamination from 0 to 60 mg kg^{-1}. But, application of GA_3 caused significant decrease in Ni concentration in roots and shoots of mungbean in Ni contaminated soil. It is concluded that use of GA_3 could be very effective to improve plant growth through reduced Ni uptake by plants in the Ni contaminated soils.

Keywords: Heavy metals; Reduction; Contamination; Phytohormones; Toxic; Mungbean

Introduction

Toxic metals' pollution is one of the major environmental issues. It leads to undesirable changes in physical, chemical and biological characteristics of air, water and soil, which adversely affect plants, animals and human beings (Samuel *et al*., 2014; Zhang *et al*., 2014). Nickel (Ni) is amongst the toxic metals and is considered as an essential element in traces for some species of animals, microorganisms and plants. However, its higher concentration in soil causes disturbance in normal metabolism of plants and toxicity symptoms (Ahmad *et al*., 2009; Ali *et al*., 2009). On an average, its concentration is 75 and 40 mg kg^{-1} in earth crust and soils, respectively. Major sources of Ni contamination in soil are agricultural waste, city effluents, impurities in fertilizers, bio-solids and burning of diesel oil (Pollard *et al*., 2014). The common symptoms of Ni phytotoxicity include inhibition of growth, seed germination and induction of chlorosis, necrosis and wilting (Leon *et al*., 2005; Ali *et al*., 2009; Ahmad *et al*., 2009). It causes oxidative stress in plants and interferes in photosynthetic activity and sugar transport mechanisms (Pandey and Sharma, 2002; Madhava Rao and Sresty, 2000). Metals toxicity causes perturbation in normal balance of phytohormones *i.e.* auxins, gibberellins, cytokinins, abscisic acid and ethylene (Rademacher, 1990; Gangwar *et al*., 2011). Phytohormones also known as plant growth regulators (PGRs) are the main players for regulation of plant growth and development (Khalid *et al*., 2001; Ghaafar and Rawi, 2011; Sabir *et al*., 2013). They are key for plants to respond an ever changing environment and external stimuli (Halter *et al*., 2005) and mediate plant growth in their specific synergistic and/or antagonistic cross-talk (Ali *et al*., 2013; Gangwar *et al*., 2011). In response to abiotic stresses biosynthesis of abscisic acid (ABA) increases while the level of cytokinin (CK) and gibberellins (GA) decreases (Veselov *et al*., 2003). Gibberellins (GA) are the versatile phytohormones, which play important role in seed germination, stem elongations, leaf expansion and development of reproductive parts of plants (Ghaafar and Rawi, 2011). Gibberellic acid (GA_3) helps the plants to adapt abiotic stresses and has shielding effects against metals toxicity (Tuna *et al*., 2008; Maggio *et al.,* 2010). Exogenous application of GA_3 increases the biosynthesis of salicylic

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acid (SA) and improves abiotic stress tolerance of plant which could be correlated with increased endogenous levels of SA (Gangwar *et al.*, 2011; Halter *et al.*, 2005). Exogenously applied GA_3 improves plant growth and development by modulating the endogenous levels of stress responsive phytohormones *i.e.* ABA and ethylene in abiotic stresses (Saeidi *et al.*, 2005; Gangwar *et al.*, 2011). Gibberellic acid improves plant growth, photosynthetic rate, sugar contents and antioxidant enzymatic activities of plants under heavy metal stress (Saeidi *et al.*, 2005). Savita *et al.* (2010) examined that GA_3 decreased the negative effects of Ni on peas and increased the seed germination, root and shoot growth in Ni contaminated soil. Exogenous application of GA_3 strongly inhibits Ni and Cd assimilation into plant tissues and increases sugar contents in plants as well as alters the carbohydrate accumulation pattern in plant under Cd and/or Ni stress (Picazo and Moya, 2007). Keeping in view the role of gibberellic acid to alleviate stress induced negative changes in plants, the present study was conducted to assess the role of gibberellic acid (GA_3) to counteract the negative effects of Ni contamination on growth and yield of mungbeen.

Materials and Methods

A pot experiment was conducted in the wire house under ambient conditions to assess the effect of gibberellic acid (GA_3) on growth and yield of mungbean (*Vigna radiate* L. var. AZRI-2006) in Ni contaminated soil. Sandy clay loam soil having EC 1.41 dS m^{-1}, pH 7.5, saturation percentage 37.5%, organic matter 0.60%, available phosphorous 7.30 mg kg^{-1}, extractable potassium 129 mg kg^{-1} and Ni below detectable range was used to fill the pots. Prior to pot filling soil was contaminated by using $NiSO_4$ salt as a source of Ni and finally four levels of Ni (0-no added Ni, 20, 40, and 60 mg kg^{-1}) contamination were maintained. Soil was allowed to equilibrate for two weeks after contamination with Ni and 10 kg soil was used to fill each pot lined with polyethylene sheet. Pots were irrigated with water before sowing and five seeds of mungbean were sown in each pot. After germination 3 plants were maintained in each pot by manual thinning. Two sets of Ni contamination levels were maintained, one set was kept without application of GA_3 while in second set, 10^{-4} M GA_3 was applied as foliar spray at 15, 30 and 45 days after germination. Experiment was conducted by following completely randomized design with three replications. Recommended dose of NPK fertilizers (22, 65 and 23 kg ha^{-1}) in the form of urea, di-ammonium phosphate (DAP) and sulfate of potash (SOP), respectively was applied and full dose of P and K was applied before sowing while N was applied in two splits. Pots were irrigated with tap water as when needed. Crop was harvested at maturity (74 days after sowing) and data regarding growth parameters *i.e.* plant height, fresh and dry shoot weight, root length, fresh and dry root weight as well as yield parameters *i.e.* number of grains per plant and grain yield per plant were recorded. For Ni determination, mungbean plants were separated into roots and shoots. Plant samples were wet digested using HNO_3 and $HClO_4$ (Rashid, 1986). After the wet digestion, the amount of Ni was determined using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 100, USA) as described by Wickliffe *et al.* (1994). Data was analyzed statistically by using computer based statistical software Statistix-8.1 (Analytical Software, Tallahassee, USA). Means were compared by applying new Duncan's Multiple Range Test (DMRT) (Steel *et al.*, 1997).

Results

Growth and yield attributes of mungbean were negatively affected at different levels of Ni contamination. However, the effect of various levels of Ni contamination was variable and reduction in growth and yield was increased by increasing levels of Ni contamination. Moreover, application of GA_3 reversed the toxic effects of Ni contamination and improved all the growth and yield parameters under different levels of Ni contamination. Data (Table 1) revealed that shoot length was significantly reduced up to 27, 36 and 66% at 20, 40 and 60 mg Ni kg^{-1} of soil, respectively as compared to control plants where neither GA_3 nor contamination were applied. But application of GA_3 significantly increased the shoot length by 32, 35 and 23% in Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown at same levels of contamination where no GA_3 was applied. Results presented in Table 1 showed that Ni contamination of 20, 40 and 60 mg kg^{-1} significantly decreased the root length up to 21, 22 and 64%, respectively as compared to control treatment. The root length was significantly increased by the application of GA_3 up to 23, 13 and 14% in Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown at same levels of contamination without GA_3 application.

Data (Table 1) showed that reduction in shoot fresh weight up to 36, 47 and 74% was observed by of 20, 40 and 60 mg kg^{-1} of Ni contamination, respectively as compared to treatment without contamination (control). But GA_3 improved the shoot fresh weight up to 17, 24 and 32% in Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown under contamination, where no GA_3 was applied. The shoot dry weight (Table 1) was significantly reduced by 36, 46 and 75% under Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown in control (neither GA_3 nor contamination). However, shoot dry weight was significantly increased up to 26, 22 and 29% by application of GA_3 in Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown where no GA_3 was applied.

Data regarding root fresh weight (Table 2) revealed

Table 1: Effect of gibberellic acid on shoot parameters of mungbean in Ni contamination

Treatments	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
	Without GA_3	With GA_3	Without GA_3	With GA_3	Without GA_3	With GA_3
Control	38.7 b	49.7 a	21.7 b	24.7 a	9.1 b	11.1 a
Ni (20 mg kg^{-1})	28.3 d	37.3 b	13.7 d	16 c	5.7 d	7.2 c
Ni (40 mg kg^{-1})	24.7 e	33.3 c	11.5 e	14.3 d	4.6 e	5.9 d
Ni (60 mg kg^{-1})	13.3 g	16.3 f	5.7 f	7.5 f	2.3 g	2.9 f

Data is mean of three repeats
Means sharing the same letter(s) do not differ significantly at $p\leq 0.5$

Table 2: Effect of gibberellic acid on root parameters of mungbean in Ni contamination

Treatments	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
	Without GA_3	With GA_3	Without GA_3	With GA_3	Without GA_3	With GA_3
Control	17 b	22.4 a	4.5 b	5.5 a	1.7 b	2.3 a
Ni (20 mg kg^{-1})	13.4 d	14.9 bc	3.3 d	4.4 bc	1.2 d	1.6 b
Ni (40 mg kg^{-1})	6.3 d	7.2 cd	3 d	4.1 c	1.0 e	1.5 c
Ni (60 mg kg^{-1})	16.5 e	4.2 e	1.3 e	1.5 e	0.4 f	0.6 f

Data is mean of three replicates
Means sharing the same letter(s) do not differ significantly at $p\leq 0.5$

that Ni contamination decreased the root fresh weight by 27, 33 and 71%, respectively as compared to plants grown in control (neither GA_3 nor contamination). However, increase in root fresh weight upto 33, 37 and 15% was observed by application of GA_3 at Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown where no GA_3 was applied. Decrease in root dry weight up to 29, 41 and 74% was recorded at 20, 40 and 60 mg kg^{-1} of Ni concentration, respectively as compared control where neither GA_3 nor Ni was applied. Nevertheless, application of GA_3 significantly increased the root dry weight up to 38, 45 and 22% in Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared to plants grown at same levels of Ni contamination without application of GA_3.

Number of grains per plant were significantly decreased at all levels of Ni contamination as compared to plants grown without Ni stress (Table 3). However, GA_3 improved the number of grains per plant in Ni contaminated soil as compared to plants growth in Ni contaminated soil without application of GA_3. It was observed that the grains weight per plant of mungbean was significantly decreased by 22, 31 and 51%, under Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively when compared with plants grown in control (where neither GA_3 nor Ni contamination) (Table 3). The grains weight of mungbean significantly improved up to 13, 29 and 20%, at Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively by application of GA_3 as compared to plants grown in Ni contamination where no GA_3 was applied.

Data presented in Table 4 show that GA_3 application was effective for decreasing Ni concentration in roots of mungbean. The application of GA_3 decreased the toxic effects of Ni on mungbean crop. Ni contents in root were significantly decreased by 34, 38 and 25%, with the application of GA_3 at 20, 40 and 60 mg kg^{-1} of Ni contamination as compared roots of plants grown at same levels of Ni contamination but without application of GA_3. Data regarding plant shoot analysis revealed that Ni concentration was significantly decreased with GA_3 application up to 31, 24 and 25% at Ni contamination of 20, 40 and 60 mg kg^{-1}, respectively as compared plants grown in Ni contamination where no GA_3 was applied.

Discussion

Nickel is a widely distributed metal in the environment and is a potentially toxic metal that negatively affects agricultural soils, plants, animals as well as in humans. The higher concentration of Ni in the plants can cause many nutritional and physiological disorders (Vinterhalter and Vinterhalter, 2005). Ni is considered to be an essential micronutrient for various plants (Vogel-Mikus *et al.,* 2005). However, higher concentration of this metal becomes toxic for the plant species. Effect of Ni contamination on plant growth inhibition has been reported by many researchers (Parida *et al.*, 2003; Vinterhalter and Vinterhalter, 2005).

Results of our study showed that Ni contamination significantly decreased the mungbean growth and yield. Nickel stress caused reduction in shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, number of grains per plant and grain weight per plant as compared to plants grown in pots without stress (control). This decrease in growth and yield attributes of mungbean might be attributed to inhibition of chlorophyll biosynthesis due to Ni toxicity that creates nutrient imbalance by replacing Mg^{+2} ions (Pandey and Sharma, 2002). Nickel phytotoxicity also decreases photosynthetic pigments (Ahmad *et al.*, 2007), chlorophyll content (Wani *et al.*, 2007) and induces oxidative stress (Kumar *et al.*, 2012), which might be the reasons of decreased growth and yield of mungbean. It is clearly evident from the work of other scientists that Ni toxicity decreases the tissue water contents, nutrient uptake, iron transport and causes mitotic

Table 3: Effect of gibberellic acid on yield parameters of mungbean in Ni contamination

Treatments	Number of grains plant^{-1}		Grains weight plant^{-1} (g)	
	Without GA_3	With GA_3	Without GA_3	With GA_3
Control	64 b	76.3 a	1.02 b	1.32 a
Ni (20 mg kg^{-1})	41.7 e	59 c	0.8 d	0.9 bc
Ni (40 mg kg^{-1})	33.3 f	50.7 d	0.7 d	0.9 c
Ni (60 mg kg^{-1})	18.3 h	28.7 g	0.5 f	0.6 e

Data is mean of three replicates
Means sharing the same letter(s) do not differ significantly at $p \leq 0.5$

Table 4: Effect of gibberellic acid on Ni concentration in root and shoot of mungbean in Ni contamination

Treatments	Ni Concentration (μg g^{-1})			
	Roots		Shoots	
	Without GA_3	With GA_3	Without GA_3	With GA_3
Control	6.8 g	3.9 h	3.3 g	2.1 h
Ni (20 mg kg^{-1})	15.9 d	10.4 f	7.8 d	5.4 f
Ni (40 mg kg^{-1})	19.8 c	12.4 f	10.1c	7.7 e
Ni (60 mg kg^{-1})	29.4 a	22 b	14.5 a	10.9 b

Means sharing the same letter(s) do not differ significantly at $p \leq 0.5$

inhibition (Gajewaska *et al.*, 2006; Saito *et al.*, 2010), which might result in decreased growth and yield of mungbean. Furthermore, reduced photosynthetic and transpiration activities (Ouzounidou *et al.*, 2006) and decreased enzymatic and antioxidant activities (Dubey and Pandey, 2011) due to Ni stress could also be the reason of decreased growth and yield of mungbean in Ni contamination. The plants grown on contaminated soil accumulates metal contents, which result in restricted growth of plants due to alterations in normal biochemical and physiological process like protein penetration, inhibition of enzyme activity, and impaired nutrition etc. (Arun *et al.*, 2005).

The improvement in plant growth and yield attributes in Ni contamination by GA_3 application might be due to involvement of GA_3 in regulation of various physiological and biochemical process of plants. It is reported that GA_3 increases total protein contents, total nitrogen contents, nitrate reductase activity, ammonium assimilating enzyme activity and increases the activities of glutathione reductase and dehydro reductase activities that might involve in enhancement of glutathione and ascorbate (Noctor and Foyer, 1998). Glutathione and ascorbate are antioxidants that play important role in scavenging of reactive oxygen species and prevent oxidative stress in plants (Noctor and Foyer, 1998; Saeidi *et al.*, 2005). Moreover GA_3 increases root biomass, shoot biomass, photosynthesis, water uptake, chlorophyll content, nutrient uptake and root length (Saeidi *et al.*, 2005; Gangwar *et al.*, 2011). The abiotic stresses cause disturbance in the normal balance of hormones of plants (Iqbal and Ashraf, 2013) and most likely GA_3 application regulated the hormonal balance of mungbean plants in Ni contaminated soil, which might be resulted in improved mungbean growth and yield. Moreover, Siddiqui *et al.* (2011) stated that the interactive effect of GA_3 with Ca^{2+} alleviates Ni toxicity in plants by the induction of antioxidative enzymes and proline accumulation, which might result in improved growth and yield of plants in Ni stressed soils.

Decrease in Ni concentration in root and shoot of mungbean plant by the application of GA_3 in Ni contaminated soil might be due to involvement of GA_3 in alteration of membrane permeability which affects the membrane transport processes like metals uptake (Rubio *et al.*, 1994; Picazo and Moya, 2007). Data regarding decrease in concentration of Ni in plants can be correlated with findings of Picazo and Moya (2007). They concluded that the application of GA_3 strongly inhibits Ni and Cd assimilation into plant and increases sugar contents in leaves, roots, and also changes carbohydrate accumulation pattern in plant.

Conclusion

Gibberellic acid (GA_3) increased the growth and yield of mungbean plant grown in normal soil as well as on contaminated soils. The application of GA_3 decreased the toxic effect of Ni on plant and improved the biomass of plant and reduced the concentration of Ni in plants. This approach could be very effective to reduce Ni uptake in plants in the Ni polluted soil but further work is needed to know the exact mechanism involved.

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