Ind J Plant Physiol. (October–December 2013) 18(4):372–376
DOI 10.1007/s40502-013-0053-8

SHORT COMMUNICATION

Effect of nickel on plant water relations and growth in green gram

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Received: 14 August 2012 / Accepted: 29 October 2013 / Published online: 10 December 2013

Abstract Effect of nickel on water potential (ψ_w), osmotic potential (ψ_s), turgor potential (ψ_p) and relative water content (RWC) was studied in the first trifoliate leaf of green gram [*Vigna radiata* (L.) Wilczek] in order to establish its possible influences on growth through altered water relations. Plants were grown on silica with nutrient solution containing 1, 10, 100 and 1,000 μM, Ni as $NiC1_2{\cdot}6H_2O$. The effect of Ni on water relations was highly concentration dependent. The growth promoting concentration of Ni (1 μM) resulted in the highest ψ_w and ψ_p, and lowest ψ_s. The growth inhibiting concentrations of Ni (10, 100 and 1,000 μM) significantly decreased ψ_w and ψ_p. At 1,000 μM Ni significantly increased ψ_s. At 1 μM Ni significantly increased RWC, while it was adversely affected at 10–1,000 μM Ni concentrations. One μM Ni treatment increased chlorophyll contents and growth, while higher concentrations (10–1,000 μM) of Ni significantly decreased the chlorophyll contents and growth.

Keywords Osmotic potential · Relative water content · Turgor potential · Water potential

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The discovery in 1975 that nickel is a component and integral part of the enzyme urease (Dixon et al. 1975; Fishbein et al. 1976), which is present in a wide range of plant species (Welch 1981), led to renewed scientific interest and research concerning the role of nickel in higher plants. Requirement of nickel in legumes has been shown regardless of the form of nitrogen nutrition (Eskew et al. 1984). The essentiality of nickel for non-legumes has also been established (Brown et al. 1987). Nickel is ubiquitous in plants and the level normally ranges from about 0.01 to 5 ppm with an average of about 1.5 ppm (Dunn 2007). Brown et al. (1987) have suggested that nickel may be considered as an essential element for plant growth. It has been accepted as a likely essential nutrient element for higher plants by many investigators (Bloom 2002; Marschner 2002) and later added to the US Department of Agriculture (USDA) list of essential plant nutrient elements (Hull 2003).

The effect of heavy metals on different physio-biochemical processes in plants is well studied (Manivasagaperumal et al. 2011; Najafi et al. 2011; Ezhilvannan et al. 2011). Several investigations have shown a nickel requirement for higher plants when the plants are grown solely on urea nitrogen sources. Gordan et al. (1978) grew *Lemna paucicostata* plants on urea as the sole sources of nitrogen. At level about 2 μM nickel sulphate significantly promoted growth. Cowpea grown in nutrient solution from which nickel was removed by ligand exchange technique, accumulated urea in most of the tissues (Walker et al. 1985). Eskew et al. (1984) observed increased leaf urease activity in soybean plants supplied with nickel. There are many reports that Ni depresses catalase activity (Dekock et al. 1979; Awasthi and Sinha 2013). Excess supply of nickel caused accumulation of antioxidants such as ascorbate and dehydroascorbate and decrease in the activity of catalase in the leaves, leading to accumulation of hydrogen peroxide.

Leaves of plants supplied with excess Ni showed decrease in water potential (ψ_w) and relative water content (RWC) and an increase in proline. Excess supply of nickel has been reported to interferes with plant water relations and induces oxidative stress Pandey and Pathak (2006). Inhibition of water uptake due to heavy metal toxicity in germinating seeds of different species has been reported by Parmar et al. (2012) and Doganlar et al. (2012). Bishnoi et al. (1993) observed that Ni and Cd slightly decreased the RWC and leaf water potential (ψ_w) at all application rates after 2 days of treatments. The present investigation was done to study the relationship between Ni concentrations, water relations, chlorophyll contents and growth of green gram plants.

The experiment was performed in culture room conditions in an improvised growth chamber, illuminated with cool white fluorescent lamps at Department of Botany, M. L. Sukhadia University, Udaipur (Rajasthan), India. The photoperiod was 12 h per day with a light intensity of 162 μmol m^{-2} s^{-1}. The average air temperature was 28.4 °C during light period and 21.8 °C during the dark period. The average relative humidity was 73.8 %. Seeds of green gram [*Vigna radiata* (L.) Wilczek var. K-851] were surface sterilized for 5 min in NaOCl (ca. 1 %, active chlorine), rinsed in distilled water, inoculated with *Rhizobium* culture and grown in plastic pots with 1.2 kg silica 10 seeds per pot. Pots were irrigated with deionized water for first two days and then supplied with half strength Hoagland's solution (−0.4 bar ψ_s) supplemented with 1, 10, 100 and 1,000 μM of nickel as $NiC1_2{\cdot}6H_2O$ at regular intervals. Control plants received only the basic nutrient solution (half strength Hoagland's solution). During the intervening period the amount of moisture lost on each day was replenished by irrigating the pots with deionized water. The seedlings were thinned 7 days after the sowing and 5 uniform plants were retained in each pot.

Chlorophyll ('a', 'b' and total) contents were estimated spectrophotometrically in acetone extract (Arnon 1949). Growth and Ni content of plants were analyzed just after fruiting. Dry weight of shoots and roots and the leaf area (leaf area meter, Systronics model 211) of first trifoliate leaves were estimated separately on three replicates of each treatment.

Ni contents were determined separately in roots, stems and first trifoliate leaves. Plant material was washed with distilled water, oven dried (80 °C), wet ashed with an acid mixture (HNO_3:$HClO_4$:H_2SO_4 = 10:4:1) and analyzed by atomic absorption spectrophotometer (Perkin Elmer, model 3300). Given values are the means of three replicated determinations per organ and treatment.

All determinations of water relations were carried out in first trifoliate leaves. 6 mm diameter disks were punched out of a leaf using a paper punch. One disk was put in a thermocouple psychrometer chamber (C-52 Sample chamber, Wescor Inc. Logan, Utah, USA) and equilibrated for 2 h. The ψ_w was read using a Microvoltmeter (HR-33T Dew Point Microvoltmeter, Wescor, Inc, Logan, Utah, USA). The tissue was frozen, thawed and equilibrated again in the thermocouple psychrometer chamber to determine ψ_s. Turgor potential was calculated as the difference between ψ_s and ψ_w. All determinations of water relations were carried out separately on leaves of 15, 18 and 21 days old plants.

Relative water content was determined by taking leaf disks (diameter 6.0 mm) from the leaves of same age and size as the ones used in the ψ_w measurement, following the modified method of Barrs and Weatherley (1962). The RWC was calculated as follows:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

The experiment was laid out in completely randomized design with three replications. Significance of differences in treatments for various parameters was determined by analysis of variance (Sokal et al. 1981). Standard error of mean (SEm±) and critical difference (CD) at 5 and 1 % levels were calculated to compare the differences among various treatments.

At 1 μM nickel treatment, increases in chl. 'a', chl. 'b' and total chl. were observed over the controls. Higher concentrations (10–1,000 μM) of nickel resulted in gradual decrease in the contents of chl. 'a', chl. 'b' and total chl. Chl. 'a'/chl. 'b' ratio decreased with increasing concentrations of nickel, except at 1 μM treatment (Table 1).

All growth parameters namely leaf area, dry weight of shoot and root significantly increased at 1 μM added nickel, which were 10.3, 18.9 and 22.5 % higher over the controls, respectively (Table 2).

Nickel concentrations of 10 μM and above proved to be toxic and retarded the growth significantly. Leaf area and dry weight of shoot and root showed a decreasing trend with increasing Ni levels. The reduction over the controls at 1,000 μM Ni in leaf area, shoot and root weight, dry weight were 76.2, 77.1 and 83.3 %, respectively. These plants exhibited severe chlorosis in trifoliate leaves and wilting with complete inhibition of podding (Table 2).

The Ni contents of plant organs increased linearly with Ni supply. Ni accumulated principally in roots, showing concentrations up to 40 times higher than that of upper plant parts. Except in control plants, where the trifoliate leaves had the highest Ni content, the Ni content in different organs generally followed the order: roots > stems > trifoliate leaves (Table 3).

In control plants, the water potential (ψ_w) was −10.7 bar, however, 1 μM Ni concentration resulted in a significant (0.01 level) increase (less negative) in ψ_w to −8.6 bar. Concentrations of Ni beyond 1 μM resulted in significant decrease in ψ_w over the controls (Fig. 1).

Table 1 Chlorophyll contents (chl. 'a', 'b' and total) of first trifoliate leaves (mg g^{-1} fr. wt.) of control and nickel treated plants

Treatment	Chl. 'a'	Chl. 'b'	Total Chl.	Chl. 'a'/Chl. 'b'
Control	1.340 ± 0.008	0.421 ± 0.015	1.763 ± 0.022	3.18
1 μM	1.565 ± 0.028 (+16.79)	0.512 ± 0.010 (+21.62)	2.079 ± 0.036 (+17.92)	3.06
10 μM	0.959 ± 0.028 (−28.43)	0.353 ± 0.012 (−16.15)	1.314 ± 0.041 (−25.47)	2.72
100 μM	0.650 ± 0.008 (−51.49)	0.20 ± 0.008 (−35.87)	0.922 ± 0.013 (−47.70)	2.41
1,000 μM	0.375 ± 0.019 (−72.01)	0.192 ± 0.006 (−54.39)	0.569 ± 0.014 (−67.73)	1.95
SEm±	0.021	0.011	0.028	
CD 5 %	0.067	0.036	0.090	
CD 1 %	0.097	0.052	0.131	

Values are the mean of three replicates ± standard deviation

Table 2 Leaf area (cm^2) of first trifoliate leaves and shoots and roots dry weight (mg pot^{-1}) of control and nickel treated plants

Treatment	Leaf area	Shoots	Roots
Control	12.6 ± 0.374	667 ± 16.997	120 ± 8.165
1 μM	13.9 ± 0.287	793 ± 20.548	147 ± 12.472
10 μM	10.0 ± 0.411	620 ± 21.602	92 ± 6.236
100 μM	7.0 ± 0.411	456 ± 17.327	50 ± 7.760
1,000 μM	3.0 ± 0.125	153 ± 12.472	20 ± 0.816
SEm±	0.339	19.300	8.020
CD 5 %	1.106	62.828	26.171
CD 1 %	1.610	91.419	38.080

Values are the mean of three replicates ± standard deviation

Table 3 Nickel content (μg g^{-1} dry weight) in different plant parts of control and nickel treated plants

Treatment	Stems	Roots	First trifoliate leaves
Control	1.2 ± 0.153	3.5 ± 0.500	4.3 ± 0.520
1 μM	9.5 ± 0.500	202.3 ± 2.517	8.2 ± 0.289
10 μM	13.8 ± 0.764	499.7 ± 3.512	12.0 ± 0.000
100 μM	30.0 ± .1.000	999.0 ± 3.606	25.3 ± 1.473
1,000 μM	35.3 ± 0.764	1,205.0 ± 4.583	30.1 ± 0.611
SEm±	0.403	1.880	0.439
CD 5 %	1.271	5.918	1.385
CD 1 %	1.808	8.418	1.970

Values are the mean of three replicates ± standard deviation

Osmotic potential (ψ_s) was significantly decreased (more negative) when plants were treated with low levels (1 and 10 μM) of Ni. Plants treated with 1,000 μM Ni level showed significant increase for ψ_s. Turgor potential was highest at 1 μM applied Ni. All other concentrations resulted in significant (0.01 level) decline in ψ_p, indicating turgor loss. The Influence of Ni on RWC was inhibitory except at 1 μM treatment.

In the present investigation RWC decreased significantly with increasing Ni concentrations except at 1 μM Ni treatment. Decreased water contents in plants grown in solutions containing toxic levels of Zn, Cd, Al, Pb or Cr have been reported (Barcelo et al. 1986a; Goransson and Eldhuset 1987).

All Ni concentrations affected water relations significantly, the effect being concentration dependent. The 1 μM Ni treatment resulted in the highest leaf ψ_w, ψ_p and RWC, and lowest ψ_s. Beyond 1 μM, Ni concentration, a linear decrease in ψ_p and RWC was observed with increase in metal concentrations. In the present study an increase in growth, chlorophyll contents, ψ_w, ψ_p and RWC was observed at 1 μM Ni concentration. Kirkham (1978) also found that non-growth reducing concentrations of cadmium increased ψ_p in *Chrysanthemum* leaves.

There is little information on the distribution of metals within leaves (Lei et al. 2008; Chen et al. 2009). Shewry and Peterson (1974) concluded from their studies with barley roots that more than two-third of internal Cr was in vacuoles, while the major proportion of the remaining metal was in cell walls. Accumulation of metals in vacuoles may be responsible for the decrease in ψ_s as observed in our experiment. In *Chrysanthemum* very low concentrations of Cd have been reported to affect root growth more significantly than shoot growth (Clark 1982). Under these conditions, decreased leaf ψ_s may be a consequence of change in assimilates partitioning, leading to increased sucrose levels in leaves (Samarakoon and Rauser 1979; Maheshwari and Dubey 2011). The increase in ψ_s in leaves of plants treated with 1,000 μM Ni concentration could be due to an interference of Ni in the transport of osmotically active substances, like organic acids and inorganic ions like K^+ and Na^+ (Barcelo et al. 1986b; Barcelo and Poschenrieder 1990).

In higher plants, roots are the first organ to contact the toxic metal concentrations and usually accumulate significantly higher metal concentrations than upper plant parts (Table 3). It is generally accepted that the impaired spatial distribution and the reduced root hair surface of metal stressed roots lead to bad root-soil contact, which lowers

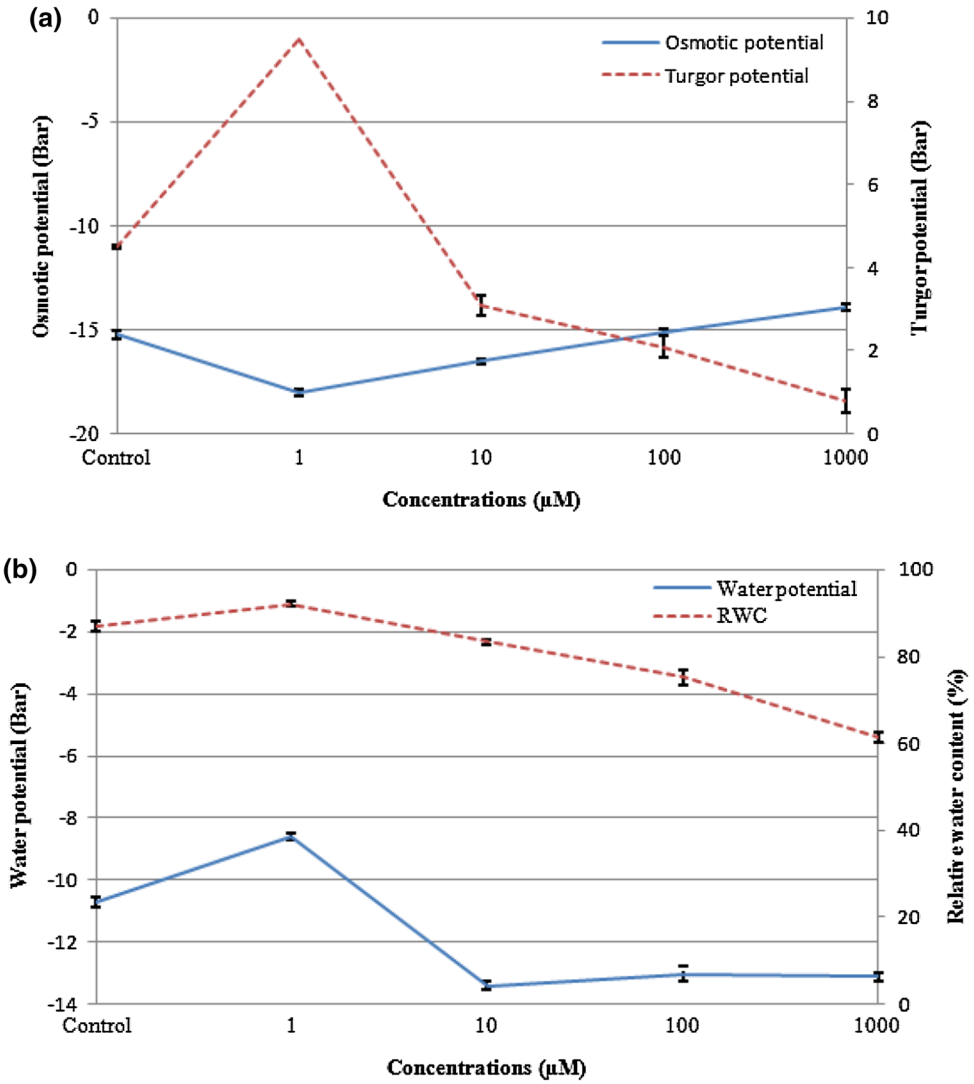

Fig. 1 **a**, **b** Water relations of first trifoliate leaf of control and Ni treated plants (individual points are means of three replicates)

the capacity of plants to explore the soil for water and affects water uptake of plants (Jagetiya 1998). It is suggested that reduced water uptake might be related to reduction in RWC at higher concentrations of Ni.

In the present study, Ni treated plants exhibited clear differences in their water relations. Significant enhancement in growth was observed at 1 µM Ni treatment. Higher concentrations (10–1,000 µM Ni) decreased growth significantly. Stimulation of growth due to trace quantities of Ni could be the result of increase in chlorophyll contents and/or by altered metabolism, which in turn positively influenced the ψ_p of plants.

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