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SHORT COMMUNICATION

ALTERED LEVELS OF METABOLITES UNDER THE INFLUENCE OF NICKEL IN GREEN GRAM

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Greengram [*Vigna radiata* (L.) Wilczek]cv K851, was grown with complete nutrient solution for 30 days. On 31st day, pots with plants were divided into four lots. One lot was allowed to grow and treated as control. In other three lots, nickel was supplied as $NiSO_4$ at 0.1, 0.2 and 0.5mM. At d 35(5 days after treatment), visible symptoms of excess Ni exhibited as chlorosis on young leaves at 0.5mM Ni and resembled with those of iron deficiency. The chlorosis later intensified and covered some of the middle leaves. Some brown necrotic irregular spots developed on both sides of the main veins on affected leaves, which were severely chlorotic. Compared to the control, reducing, non-reducing, total sugars, starch, phenols and non-protein nitrogen increased, whereas, concentration of protein nitrogen decreased with an increase in nickel supply. The accumulation of nickel was higher in roots than other plant parts.

***Key words*: Carbohydrate metabolism, green gram, nickel, seed weight.**

Nickel is an essential micronutrient for higher plant but is toxic to plant at excess level. The essentiality of nickel for the legumes has been shown (Eskew *et al.*1984, Gerendas *et al*.1999 and Marschner, 2002). Unlike most non-essential element nickel is fairly mobile in plants and readily accumulate in the seeds and fruits as observed in oats, beans and lupine (Govorina *et al.* 2003). Leaves have the highest nickel concentration (Robinson *et al*. 2003, Gerendas *et al*.1999). Nickel is readily transported in the xylem and pholem (Yang *et al*.1997) and in some plant species preferentially translocated into seed. It is distributed into the environment from natural source and industrial activities. Bisht *et al*. (1976) reported that extent of accumulation and depression in amino acids varied with genotype and concluded that excess cellular concentration either inhibits the utilization of amino acid in protein synthesis or promotes protein hydrolysis (Winkler *et al*. 1983). Normal balance of cellular nitrogen is also affected (Brown *et al*.1990).

The role of nickel in plant metabolism is not fully understood. Several enzyme activities and other metabolic processes are known to be affected in plants by the high nickel (Leblova and Zatovkalova 1988). High level of nickel also leads to accumulation of phenolics, starch and reducing sugars in maize seedling (Baccouch *et al.* 1998). In nickel treated roots the toxic effects were higher and increase in phenol content and extracellular peroxidase activity were associated with an early-induced cellular differentiation (Gabbrielli *et al.* 1999). However, it decreased the phenolic content in tea leaves, and enhanced the activity of phenylalanine ammonialyase (PAL). Polyphenol oxidase (PPO), however, was decreased (Basak *et al*. 2001). Polacco (1977) showed that nickel is required in urea utilization

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and urease synthesis, indicating its possible role in nitrogen metabolism in plants. Bick. *et al*. (1982) showed a direct relationship between total free amino acid nitrogen and nickel content in leaves. Excess supply of nickel resulted in accumulation of non-protein nitrogen and decrease in protein nitrogen content. In this paper the effects of Ni on yield and seed quality was examined in green gram.

Green gram [*Vigna radiata* (L.) Wilczek] cv. K 851 was grown in purified sand with pH about 6.5 at temperature (15-32ºC) for 30 days with complete nutrient solution 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 basal nutrient solution was 4 mM KNO_3, 4mM, $Ca(NO_3)_2$, 2mM $MgSO_4$, 1.5mM NaH_2PO_4, 100 mM Fe EDTA, 10 mM $MnSO_4$, 30mM H_3BO_3, 1mM $CuSO_4$, 1mM $ZnSO_4$, 0.2mM Na_2MoO_4, 0.1mm $CoSO_4$,0.1mM $NiSO_4$ and 0.1mM NaCl. On 31st day, pots with plants were divided into four lots. One lot was allowed to grow as such and was treated as control. In other three lots, Ni as nickel sulphate was supplied at 0.1, 0.2 and 0.5 mM. At d 50, concentration of reducing and non-reducing sugars (Nelson 1944), phenols (Swain and Hills 1959), starch (Montgomery 1957) and nitrogen fractions (protein and non-protein by Kjeldahl method) were measured colorimetrically in leaves and at d 67 in seeds after fixing in 80% boiling ethanol. All determinations were carried out in triplicate and data were analysed statistically (Panse and Sukhatme 1985). Standard error of the mean is presented along with the mean values in tables.

Greengram grown in sand culture at variable level of nickel developed visible symptoms as chlorosis on young leaves which later intensified and covered the middle lamina. At later stage necrotic spot developed on both side of the main vein on those affected chlorotic leaves. Excess supply of nickel affect metabolism of the plant and metabolite like phenol, starch and sugars.

Concentration of reducing and non-reducing and total sugars increased in leaves and seeds of plants with an increase in Ni supply (Table 1, 2). The increase in accumulation of reducing sugar was, however, more than that of non-reducing sugar. The accumulation of sugars under excess nickel was also reported for white bean (Rauser 1978), bush bean (Rauser and Samarkoon 1980) and maize (Baccouch *et al.* 1998). The concentration of starch in both leaves and seeds decreased with an increase in nickel supply from 0.1 to 0.5 mM (Table 1, 2). The changes in sugars and starch in leaves under excess nickel might be the consequence of either impaired carbohydrate metabolism at the source or at the sink site and thus responsible for inhibited growth. The decrease in starch content might be due to disturbed activity of starch synthase activity.

Table 1. Excess nickel and concentration of sugars, starch, phenol and nitrogen in seeds of green gram (37 days after treatment

	mM Ni			
Constituents	**0.0001**	**0.1**	**0.2**	**0.5**
Reducing sugars (% fw)	0.076±0.00231	0.083±0.00404	0.131±0.00867	0.103±0.00289
Non-reducing sugar (% fw)	0.122±0.0127	0.079±0.0226	0.021±0.00404	0.027±0.00981
Total sugar (% fw)	0.198±0.249	0.162±0.0104	0.152±0.00462	0.130±0.0127
Starch (% fw)	7.84±1.782	4.345±0.969	4.98±0.891	2.675±0.183
Phenols (% fw)	0.018±0.0010	0.026±0.0010	0.027±0.0005	0.039±0.0015
Non-protein N (% fw)	0.054±0.003	0.072±0.005	0.081±0.012	0.211±0.022
Protein N (% fw)	3.04±0.102	2.84±0.345	2.16±0.352	1.74±0.112

Table 2. Excess nickel and concentration of sugars, starch, phenol and nitrogen in leaves of green gram (20 days after treatment)

	mM Ni			
Constituents	**0.0001**	**0.1**	**0.2**	**0.5**
Reducing sugars (% fw)	0.076±0.00231	0.083±0.00404	0.131±0.00867	0.103±0.00289
Non-reducing sugar (% fw)	0.022±0.013	0.027±0.014	0.032±0.004	0.032±0.010
Total sugar (% fw)	0.098±0.010	0.110±0.001	0.135±0.001	0.163±0.013
Starch (% fw)	1.96±0.035	1.35±0.191	1.25±0.083	0.89±0.058
Phenols (% fw)	0.0018±0.000115	0.0026±0.00012	0.0027±0.0000577	0.0030±0.000115
Non-protein N (% fw)	0.033±0.00633	0.055±0.0318	0.055±0.0191	0.187±0.0318
Protein N (% fw)	2.707±0.904	1.628±0.348	0.999±0.0352	0.863±0.00866

The growth of plant ceased because of necrosis of the apical growing point of main shoot, which might be due to the accumulation of phenol and auxin in the affected plants. The phenol concentration in leaves and seeds of green gram increased with an increase in nickel supply (Table 1, 2). At 0.5 mM Ni, increase in phenol content was about 67% as compared to that of control plant. Excess nickel triggered the activity of extracellular peroxidases resulting in the production of phenolic componds. The accumulation of phenol is effective in producing superoxide radicals potentially capable of damaging membrane by lipid peroxidation or is responsible for creating risk of oxidative damage of the plasma membrane (Pandolfini *et al.* 1992, Kupper and Kroneck 2007).

As compared to the concentration of soluble protein in leaf extracts at control level its concentration decreased with an increase in Ni supply. The decrease in protein content in leaves might be the result of low RNA synthesis or higher RNAase activity. The perturbations in non-protein nitrogen and protein nitrogen in leaves and seeds is due to disturb nitrogen metabolism. The concentration of protein nitrogen in leaves of green gram decreased with an increase in nickel supply (Table 2). The concentration of non-protein nitrogen in green gram leaves at excess nickel levels increased compared to control. This increase was more marked at 0.5 mM Ni supply.

The dry weight of pods decreased significantly at excess Ni supply. The seed weight per plant at excess nickel also decreased and the reduction in pod and seed yield was most pronounced at 0.5 mM Ni supply (Table 3) and the quality of the seed has been deterioated. These observations are somewhat in consonance with the observation in beans (Piccini and Malavolta 1992) and in tomato (Palacios *et al.* 1999). The loss in seed

Table 3. Effect of excess nickel on some growth parameters and pod yield in green gram at maturity (37 days after treatment)

	mM Ni supply			
Yield components	**Control**	**0.1**	**0.2**	**0.5**
Number of pods plant^{-1}	16±0.577	13±1.155	10±1.732	5±0.00
Weight of pods (g plant^{-1})	3.17±0.112	2.50±0.393	1.72±0.216	31±0.003
Number of seeds plant^{-1}	49±0.577	50±1.155	35±2.309	16±0.00
Weight of seeds (g plant^{-1})	1.69±0.087	1.55±0.280	1.08±0.135	0.22±0.001
Weight of 100 seeds (g)	3.61±0.191	3.47±0.54	3.04±0.182	1.38±0.003

yield might be due to formation of a large number of immature flowers, which were shed prematurely, and most of them failed to open or produce bold seeds. The loss in seed weight might be due to formation of lesser number of endosperm cells per seed and their lowered capacity to store carbohydrate and protein.

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