A Comparative *In Vitro* Study on Biochemical Alterations in Two Cultivars of Black Gram Exposed to Nickel Stress

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Abstract: ***The present hydroponic study compares the tolerance ability of two cultivars (PU31C and Shekhar1C) of black gram [Vigna mungo (L.) Hepper] to Nickel(Ni) treatments. In this context different biochemical parameters were assessed and compared in 14 days treated seedlings under different Ni concentrations. After 14 days exposure to different concentrations of toxic Ni^{2+}, the two cultivars of V. mungo i.e. PU31C and Shekhar1C showed significant changes in their phytotoxicity and tolerance index values,, protein and sugar content. Sekhar1C variety seedlings showed more protein and reducing sugar level than PU31C variety, at toxic Ni (100 µM and 200µM) treatments. Whereas total sugar level in PU31C variety of black gram seedlings were significantly more as compared to Sekhar1C variety even at higher treatment dose of Ni. The present in vitro experiment showed hydroponically grown Sekhar1C variety of black gram seedlings under different treatment concentrations of Ni showed better stress tolerance than PU31C variety as evidenced from their tolerance index and phytotoxicity values.***

Keywords: Nickel, Protein, Phytotoxicity, Sugar, Tolerance index.

1. Introduction

Nickel (Ni) occurs abundantly in igneous rocks as a free metal or as a complex with iron. It stands at twenty-second position amongst most abundant elements in the earth crust [1]. Nickel (Ni) is considered to be an essential micronutrient for plants and in small amount is known to improve plant yield and quality [1]. But Ni at high concentrations in the soil environment becomes phytotoxic [2] [3]. Ni^{2+} form is stable over a wide range of pH and redox conditions prevailing in the soil. In general, naturally occurring concentration of Ni in soil and surface waters is lower than 100 and 0.005 ppm, respectively but anthropogenic activities further release Ni into the soil through various sources such as smelting, burning of fossil fuel, vehicle emissions, disposal of house hold, municipal and industrial wastes, metal mining, fertilizer application, and organic manures [1]. In plant tissues, the concentrations of Ni may vary from 0.1 to 5.0 ppm (dry wt. basis) with a threshold range of toxicity of 40-246 ppm, depending on plant species [4]. The most common symptoms of nickel toxicity in plants are inhibition of growth, chlorosis, necrosis and wilting [3][5] [6] [7]. Toxicity of this metal has been attributed to its negative effect on photosynthesis, mineral nutrition, sugar transport, water relations and induction of oxidative stress [4].

The impact of Ni toxicity on the biochemistry and physiology of different crop plants depends on the type of plant species, growth stage, cultivation conditions, Ni concentration and exposure time [8] [9] in the soil. It is now crucial to ascertain the role of different variety of crop for their tolerance to Ni stress, which is considered critical for raising a successful agricultural crop. The present *in vitro* study compares the tolerance ability of two cultivars (Sekhar1C and PU31C) of black gram (*Vigna mungo*) by assessing various biochemical and physiological changes in response to varying treatment doses of Ni..

2. Materials and Method

2.1 Plant Material

Dry seeds of black gram [*Vigna mungo* (L.) Hepper] cultivars (PU31C and Shekhar1) were collected from Orissa State Seed Corporation, Bhubaneswar.

2.2 Seedling growth

Germinated seeds were hydroponically grown in growth chambers in *in vitro* conditions. Well aerated hydroponic culture vessels containing half strength Hoagland's nutrient solution was taken as control and Hoagland's solution supplemented with different concentrations of Ni (Source: $NiCl_2$) viz. 1µM, 5µM, 10µM, 50µM, 100µM and 200µM for seedling growth. The seedlings were grown under white fluorescent tubes (36 W Philips TLD) with a photon flux density of 52 µ /m2s (PAR) with a 12h photo period inside the growth chamber for 14 days.

2.3 Analysis of Biochemical Parameters

2.3.1 Estimation of tolerance index and phytotoxity values

Tolerance indices (TI) and Phytotoxicity (%) in root and shoot were determined by following the formula as given by [10]. TI = (Mean root length of Ni treated seedlings/Mean root length of seedlings grown in control) × 100

2.3.2 Estimation of reducing sugar content

Alcoholic extract of seedlings were analyzed for reducing sugar content by Nelson-somogyi method [11]. Absorbancy was taken at 620nm. Reducing sugar content was as estimated using D-glucose as the standard.

2.3.3 Estimation of protein

Protein content of seedlings under different Ni treatments was done by using Folin-Ciocalteu reagent and taking absorbance of extracted leaf samples at 750nm [12].

2.3.4 Estimation of total sugar

Aliquotes from the 80% ethanol extract was taken for the estimation of total soluble sugar by anthrone reagent [13].

3. Results and Discussion

Treatments of different Ni^{2+} concentrations (1µM, 5µM, 10µM, 50µM, 100µM and 200µM) showed marked changes in the different biochemical parameters of 14 days grown *Vigna mungo* (L.) Hepper seedlings. A comparative analysis of two cultivars of black gram i.e. Sekhar1C & PU31C were made in respect to their tolerance index and different biochemical alterations induced by Ni.

3.1 Statistical Analysis

All of the treatments were conducted in triplicates and the data presented in the figures and tables are mean ± SEM (Standard Error of Mean) of three replicates.

3.2 Variations in *tolerance index and phytotoxity values*

Comparing the phytotoxicity values and Tolerance index (TI) values of two cultivars of black gram, it was noted that Sekhar 1C variety showed better tolerance to Ni than PU31C cultivar. TI value for shoot at high Ni treatment i.e. at 200µM was 74% in Sekhar 1C variety where as it was only 54% in PU31C cultivar. (Table 1). Enhanced root and shoot growth was observed at low treatment concentration of Ni as indicated by the negative values in Table 1 for concentration of 5µM and 10µM in both the cultivars of black gram. A sudden increase in shoot phytotoxicity was observed when treatment dose of Ni was increased from 100µM to 200µM. Lower metal concentrations favours plant growth as recorded in several plants by researchers [10] [14]

Table 1: Toxicological interpretations in two cultivars of 14 days grown *V. mungo* seedlings under Ni^{2+} stress.

SEKHAR 1C				
Ni^{2+} (µM)	Shoot Phytotoxicity (%)	Root Phytotoxicity (%)	Root Tolerance index (RTI)	Shoot Tolerance index (STI)
Control (0)	0	0	100	100
1	0.147275	16.89088	83.10912	99.85272
5	7.36377	-5.53064	105.5306	92.63623
10	4.712813	-7.17489	107.1749	95.28719
50	14.48208	9.865471	90.13453	85.51792
100	18.99853	65.47085	38.30846	94.71871
200	40.108	89.83558	29.43723	73.93939

PU31C				
Ni^{2+} (µM)	Shoot Phytotoxicity (%)	Root Phytotoxicity (%)	Root Tolerance index (RTI)	Shoot Tolerance index (STI)
Control (0)	0	0	100	100
1	4.59506	-12.8641	112.8641	95.40494
5	4.365307	-18.6893	118.6893	95.63469
10	6.375646	-9.46602	109.466	93.62435
50	-2.52728	17.96117	82.03883	102.5273
100	-5.22688	8.980583	91.01942	105.2269
200	43.5382	81.31068	16.55914	59.18122

NB: Negative values in the table indicate growth stimulation

3.1 Changes in Reducing Sugar Content

Reducing sugar content in 14 days old plants of *Vigna mungo* decreased with increasing the dose of Ni in the nutrient solution (Figure 2). It was found that maximum reducing sugar biosynthesis was observed in plants (both Sekhar1C & PU31C variety) treated with Ni^{2+} (200 µM). After 14 days treatment the decreasing trend of reducing sugar at different concentrations of nickel is as follows,
In Sekhar 1C variety of black gram :.
Ni^{2+} (200µM)> Ni^{2+} (100 µM) > Ni^{2+} (1 µM) > Control > Ni^{2+} (5 µM) > Ni^{2+} (50 µM) > Ni^{2+} (10 µM).

In PU 31C variety of black gram :.
Ni^{2+} (200µM)> Ni^{2+} (100 µM) > Ni^{2+} (10 µM) > Ni^{2+} (50 µM)> Ni^{2+} (5 µM) > Ni^{2+} (1 µM) > Control.

The reducing sugar content of PU31C variety is better than Sekhar1C variety.

In both the varieties (both Sekhar1C & PU31C variety) reducing sugar biosynthesis was enhanced in 14 days treated black gram seedlings when treated with Ni^{2+} (200 µM). There was no significant change in reducing sugar level when the seedlings were treated with Ni treatments up to 10 µM concentration. But beyond 100µM Ni treatment there found significant increase in the reducing sugar level as compared to control.

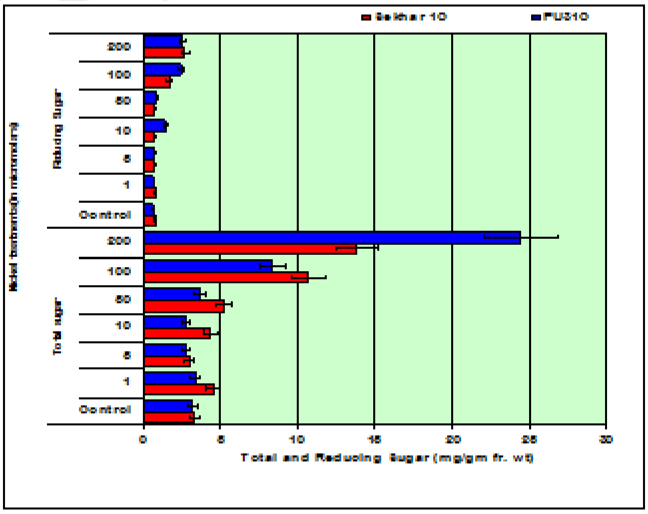

Figure 2: Clustered bar graph showing the comparative values of reducing and total sugar content in two cultivars of *Vigna mungo*

3.2 Changes in Protein Content

A gradual decrease in protein content was observed with increasing Ni treatments in 14 days treated varieties of black gram. After 14 days treatment of black gram seedlings the decreasing trend of protein at different concentrations of nickel is as follows (Table 2),

In shoot of Sekhar 1C variety of black gram :.
Ni^{2+} (1µM)> Ni^{2+} (5 µM) > Ni^{2+} (10 µM) > Control > Ni^{2+} (50 µM) > Ni^{2+} (200 µM) > Ni^{2+} (100 µM).
In shoot of PU31C variety of black gram :.

Control > Ni^{2+} (1 µM) > Ni^{2+} (200µM)> Ni^{2+} (5 µM) > Ni^{2+} (100 µM) > Ni^{2+} (10 µM) > Ni^{2+} (50 µM).

Table 2: Changes in protein content (mg g^{-1} fresh weight) of two black gram cultivrs under Ni stress. (Values are arithmetic mean ± standard error of mean of three replicates)

Ni treatments	Sekhar1C	PU 31C
Control	13.41±0.32	10.15±0.62
1µM	15.71±0.53	8.5±0.35
5µM	15.58±0.01	6.8±0.56
10µM	15.23±0.42	5.31±0.18
50µM	12.93±0.05	3.05±0.82
100µM	10.46±0.19	5.7±0.67
200µM	12.8±0.22	7.71±0.54

Protein synthesis and breakdown were affected by toxic heavy metals. Heavy metal induced increase in the reactive oxygen species can cause deleterious oxidation and degradation of proteins. Both decreases and increases in total protein content were reported in plants under heavy metal stress [15]. In this study, the decreasing amount of total soluble protein in the Ni-treated plants may be attributed to protein degradation due to oxidative damage.

3.3 Changes in Total Sugar Content

Total sugar content in 14 days old plants of *Vigna mungo* increased with increasing the dose of Ni in the nutrient solution (Fig. 2). It was found that Maximum total sugar biosynthesis was observed in *Vigna mungo* plants (both Sekhar1C & PU31C variety) treated with Ni^{2+} (200 µM). The order of increase in total sugar biosynthesis was as follows-
After 14 days treatment the decreasing trend of total sugar at different concentrations of nickel is as follows,
In Sekhar 1C variety of black gram :.
Ni^{2+} (200µM)> Ni^{2+} (100 µM) > Ni^{2+} (50 µM)> Ni^{2+} (1 µM) > Ni^{2+} (10 µM) > Control >Ni^{2+} (5 µM).
In PU 31C variety of black gram :.
Ni^{2+} (200µM)> Ni^{2+} (100 µM) > Ni^{2+} (50 µM)> Ni^{2+} (1 µM) > Control >Ni^{2+} (5 µM) > Ni^{2+} (10 µM).
The total sugar conent of PU31C variety is better than Sekhar1C variety

Total sugar content of these two varieties of black gram was highly increased with increased Ni content. PU31C variety showed more sugar content than Sekhar1C variety of black gram.

4. Conclusion

The above studies reveal some interrelationship between the different metabolic effects induced by nickel in a leguminous plant like *Vigna mungo*. Intensive future research on the effects of accumulation of heavy metal on plant metabolism is essential. Further the ability of different plants for increasing phytoaccumulation potential needs to be tried. Suitable post harvest bioremediation techniques should be adopted for disposal of plants and plant parts containing accumulated toxic nickel from the mining environment. These tolerant varieties of black gram seedlings should be grown in contaminated soil with *in situ* condition by the local farmers

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