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Original article

Differential nickel tolerance of mung bean (*Vigna radiata* L.) genotypes in nutrient culture

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Abstract – Eight cultivars of mung bean (*Vigna radiata* L.) were tested for their tolerance to different levels of nickel (Ni) (0, 6, 12, 18 and 24 μM) in nutrient solution at pH 6.8. Seeds were germinated and grown in the presence of nickel under controlled environmental conditions. Standard growth parameters such as root length, shoot length, root/shoot dry biomass production and root/shoot tolerance index were used as markers of nickel toxicity. Measurements as early as 24 h after the beginning of treatment did not yield consistent results. However, root measurements 3, 6 and 9 days after the beginning of treatments yielded significant differences among cultivars which were similar to field performance in nickel-rich soils. The cultivars Dhauli and PDM-116 showed root growth while LGG-407, K-851, TARM-22 and TARM-1, TARM-21, TARM-26 exhibited a reverse trend in root growth in the presence of nickel (12 μM). The root tolerance index (RTI) and the shoot tolerance index (STI) with respect to Dhauli and PDM-116 were high indicating their tolerance to nickel; TARM-21 and TARM-26, however, showed a low RTI and STI. Based on the growth parameters eight cultivars of mung bean were ranked with respect to their tolerance to nickel: Dhauli > PDM-116 > LGG-407 > K-851 > TARM-22 > TARM-1 > TARM-21> TARM-26. Nickel induced greater G6PDH and GDH activity in Dhauli and PDM-116 as compared to LGG-407, K-851, TARM-22, TARM-1, TARM-21 and TARM-26. This method can be employed for quick screening of mung bean for nickel tolerance. (© Inra/Elsevier, Paris.)

nickel toxicity / glucose-6-phosphate dehydrogenase / glutamate dehydrogenase / mung bean / screening

Résumé – Tolérance au nickel de divers génotypes de haricot mungo (*Vigna radiata* L.) cultivés sur solution nutritive. On a testé la tolérance de huit cultivars de haricot mungo à divers niveaux de nickel (Ni) (0, 6, 12, 18 et 24 μM) présents dans une solution nutritive à pH 6,8. Les graines ont germé et se sont développées en présence de nickel en conditions de milieu contrôlées. Les paramètres standard de la croissance, tels que la longueur des racines, la longueur des pousses, la production de biomasse des racines et celle des pousses, l'indice de tolérance des racines et celui des pousses, ont été utilisés comme marqueurs de la toxicité du Ni. Les mesures faites 24 h après le début du traitement n'ont

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pas donné de résultats cohérents. La mesure des racines 3, 6 et 9 j après le début du traitement a donné des différences significatives entre cultivars qui avaient les mêmes performances au champ dans des sols riches en Ni. Les cultivars Dhauli et PDM-116 ont présenté une croissance racinaire en présence de Ni, tandis que la tendance était inverse pour LGG-407, K-851, TARM-22 et TARM-1, TARM-21 et TARM-26. L'indice de tolérance des racines (RTI) et celui des pousses (STI) indiquaient nettement une tolérance au Ni pour Dhauli et PDM-116 ; TARM-21 et TARM-26, en revanche, avaient un RTI et un STI faibles. La tolérance au Ni des huit cultivars, basée sur les paramètres de la croissance a donné le classement suivant : Dhauli > DDM-116 > LGG-407 > K-851 > TARM-22 > TARM-1 > TARM-21 > TARM-26. Le nickel a provoqué une plus grande activité enzymatique de la glucose-6-phosphate déshydrogénase et de la glutamate déshydrogénase chez Dhauli et PDM-116 comparé aux autres cultivars. Cette méthode peut être utilisée pour un criblage rapide du haricot mungo à la tolérance au nickel.(© Inra/Elsevier, Paris.)

haricot mungo / toxicité du nickel / glutamate déshydrogénase / glucose-6-phosphate déshydrogénase / criblage

1. INTRODUCTION

Nickel is known as an essential component of the enzyme urease and plays an important role in nitrogen and urea metabolism of higher plants [10, 12, 34]. It is widely distributed in plant tissues [16, 18]. Excess of nickel in soils causes reduced growth [19, 20]. Phytotoxicity, however, varies with the concentration of Ni in soil solution as well as with the plant species [20, 21]. Nickel is an antagonist to essential elements particularly in barley [13] and oat [11, 36]. There are many examples of toxic soil factors affecting plants through natural selection [14]. However, in soils which have varying levels of iron (Fe), calcium (Ca) and magnesium (Mg) plus high nickel as major soil components, the task of identifying and selecting desirable phenotypes is not a simple one due to mineral element interactions [15]. To overcome these confounding environmental effects the use of hydroponic culture has been employed to assess tolerance to toxic elements or the efficient utilization of nutrients in several crops [26]. Studies on hydroponics by non-destructive methods allow easy observations for screening of selected plants on the basis of relative growth rate. Therefore, identifying nickel-tolerant genotypes for better growth and productivity in Ni-toxic, -acidic and -infertile soils could be the best strategy to circumvent Ni toxicity. The present investigation was intended to identify the Ni-tolerant cultivars on the basis of root elongation, nickel accumulation, biomass production in hydroponic cultures and the enzyme activity. This study could also be a prerequisite for establishing a breeding programme for nickel tolerance in mung bean (*Vigna radiata* L.).

2. MATERIALS AND METHODS

2.1. Plant material and environmental condition

Seeds of eight mung bean (*Vigna radiata*) cultivars namely TARM-22, LGG-407, PDM-116, Dhauli, K-851, TARM-1, TARM-21 and TARM-26 were collected from the Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Orissa, India. Seeds were treated with detergent solution 'Teepol' (Glaxo, India) for 10 min and washed with running tap water for 15 min. Further, the seeds were sterilized with 0.1 % mercuric chloride solution for 20 min and were sown over plastic nets on glass trays (12 × 15 × 7 cm) (Borosil, India) containing the nutrient solution. The ratio of the seeds and the solution used were 1:30. The trays were kept in a growth room at 25 ± 2 °C under cool, white fluorescent lamp (55 μmol m^{-2} s^{-1}) with 16-h photoperiod. The nutrient solution consisted of 4.0 mM $CaNO_3$, 2.0 mM $MgSO_4$, 4.0 mM KNO_3, 0.4 mM $(NH_4)_2SO_4$, 2 μM $MnSO_4$, 0.3 μM $CuSO_4$, 0.8 μM $ZnSO_4$, 30 μM NaCl, 0.1 μM Na_2MoO_4, 1.43 μM KH_2PO_4, 10 μM H_3BO_3 and 20 μM Fe-Na-EDTA. The pH of the nutrient solution was adjusted to 6.8 using 0.1 N HCl or 0.1 N KOH and the solution was changed at 3-d-intervals in order to maintain the desired level of nutrients and pH. Nickel was supplied to eight cultivars in the form of nickel sulphate ($NiSO_4$) at 0, 6, 12, 18 and 24 μM. The experiment was laid out in a completely randomized block design (CRBD) with three replications. The experiments were repeated three times. The length

of the primary root and the shoot was measured at 3-d-intervals from the start of the experiments up to the 9th day. The rate of root elongation in each experiment was determined by subtracting the length of the root recorded on the 3rd and the 6th days from that of the 9th day. Tolerance index (TI) for the tested plants was calculated using the formula:

$$TI(\%) = \frac{\text{Mean root or shoot elaboration in solution with Ni}}{\text{Mean root or shoot elongation in solution without Ni}} \times 100$$

2.2. Enzyme assay

For these experiments, only shoots of plants treated with 12 μM Ni^{+2} were used. Plant tissues were homogenized in an ice-cooled mortar in 0.1 M Tris-HCl (pH = 7.8) containing 1 mmol/L EDTA and 1 mmol/L dithiothreitol. The homogenates were centrifuged at 10 000 rpm for 10 min and the supernatant was used for enzyme assays [29]. Activity of the following enzymes was measured according to Bergmeyer et al. [6]: glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and glutamate dehydrogenase (EC 1.4.1.2). The enzyme activity was referred to the protein content of each sample and expressed as percentage of control. Soluble proteins were determined in the supernatant according to Bradford [7] with bovine serum albumin as standard.

2.3. Statistics

Regression analyses were performed to assess the pattern of response of mung bean cultivars to different levels of nickel. Effects of nickel on growth variables at each level were noted with mean separation using the Waller-Duncan multiple range test of Harvard Graphic regression program.

3. RESULTS AND DISCUSSION

Eight cultivars of mung bean subjected to five levels of nickel responded differently in terms of seed germination, elongation of shoot and root and the total biomass production. Effect of different concentrations of nickel on the rate of germination are presented in *table I*. Among the concentrations tested, the rate of seed germination was found to be the best in 12 μM 3 days after the start of the experiment. At high concentration (18 μM) of nickel germination rate declined. The effects of the environment on germination are quite complex because of interactions and internal factors that modify germination patterns. Even though environmental conditions such as temperature, water and light may be adequate for germination, many other factors may delay or even prevent germination. High concentrations of heavy metals may, in some cases, prevent germination of seeds [1, 22, 25]. A good degree of variation in germination and growth parameters (length and biomass of root and shoot) was observed at 12.0 μM nickel; therefore, this concentration was chosen to compare the growth performance of eight cultivars. The results indicated that the root and shoot growth was affected by nickel in

Table I. Effect of different concentrations of nickel on the rate of seed germination of mung bean (*Vigna radiata* L.) after 3 days of culture (ten seeds per 300 mL nutrient solution; repeated thrice).

Cultivars	Percent of germination (%) Ni Concentration			
	0	6 μM	12 μM	18 μM
TARM-22	89.4 ± 0.74	87.4 ± 0.82	92.2 ± 0.41	28.2 ± 0.54
LGG-407	86.3 ± 0.56	89.6 ± 0.88	94.4 ± 0.45	18.4 ± 0.66
PDM-116	84.7 ± 0.42	89.2 ± 0.87	96.8 ± 0.37	32.5 ± 0.63
Dhauli	82.6 ± 0.54	84.2 ± 0.51	94.4 ± 0.42	34.4 ± 0.65
K-851	84.2 ± 0.14	85.7 ± 0.71	92.6 ± 0.32	22.2 ± 0.72
TARM-1	86.4 ± 0.26	88.7 ± 0.67	90.4 ± 0.86	20.8 ± 0.46
TARM-21	82.2 ± 0.67	84.2 ± 0.34	88.2 ± 0.83	24.6 ± 0.71
TARM-26	84.8 ± 0.72	86.4 ± 0.22	90.4 ± 0.45	26.4 ± 0.67

all the eight cultivars of mung bean studied (*table II*). Root length in 'Dhauli' and 'PDM-116', however, increased by 25.49 and 6.79 %, respectively, in the presence of nickel as compared to their respective controls, while in 'TARM-21' and 'TARM-26', the root length was reduced by 72.06 and 60.82 %, respectively; in the rest of the cultivars the effects on root length were intermediate. Shoot growth also varied from cultivar to cultivar in the presence of nickel as compared to the control. The root elongation method developed by Wilkins [37] to quantify the inhibitory effect of metal ions on root growth has been used widely in ecological studies for testing of tolerance of plants to metals. Lateral roots were higher in Dhauli and lower in TARM-26. The increase in lateral roots may be due to the impact of toxicity of heavy metals [4, 5]. The method is simple, rapid and easy to perform, thus far out-weighing its limitations [3]. Toylor and Foy [27] suggested that the root tolerance index (RTI) is one of the most important markers when screening genotypes and varieties for metal toxicity. Shoot tolerance indexes (STI) were also higher in 'Dhauli' and 'PDM-116' as compared to other cultivars. Tolerance indexes (TI) derived from ratios between data for treatment and control solutions have been useful to characterize individual populations for metal tolerance. Our observations, therefore, provide further evidence that 'Dhauli' and 'PDM-116' were the two cultivars which showed tolerance against 12 μM nickel having RTI values of 125.50 and 106.79, respectively (*figure 1*). The cultivars 'TARM-22', 'LGG-407' and 'K-851', became stunted and the leaves had a tendency to roll around the shoot. The nutrient solution containing 6 μM nickel did not affect shoot growth significantly. There were several reports on the growth of plants at lower concentrations of nickel either in solution or in soil [8, 9, 17, 24, 28].

Root and shoot biomass production were in accordance with root length. 'Dhauli' had a 46.06 % increase in root biomass as compared to control;

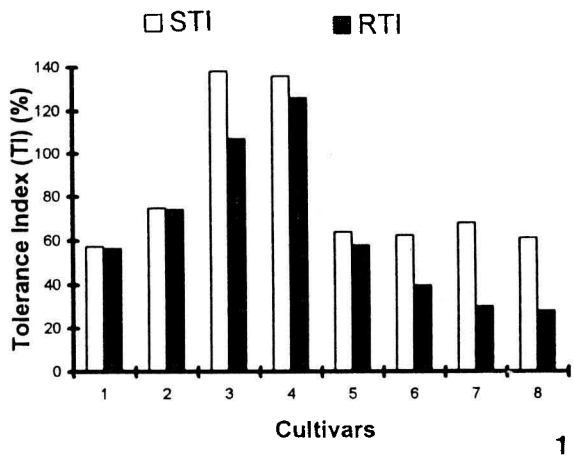

Figure 1. Tolerance index of shoot (S) and root (R) of eight mung bean cultivars (1. TARM-22, 2. LGG-407, 3. PDM-116, 4. Dhauli, 5. K-851, 6. TARM-1, 7. TARM-21, 8. TARM-26) in 12 μM of nickel.

Table II. Shoot length, root length and number of roots of eight mung bean cultivars in nutrient solution with 0 and 12 μM nickel after 9 days of growth. Values are means of 20 samples ± S.D.

Cultivars	Root growth (cm)*		Shoot length (cm)*		Number of lateral roots/plant*	
Nickel level	0	12 μM	0	12 μM	0	12 μM
TARM-22	5.21 ± 0.2b	2.93 ± 0.2b	14.01 ± 1.2d	8.03 ± 0.8b	4.23 ± 0.5b	2.36 ± 0.3a
LGG-407	4.37 ± 0.3a	3.23 ± 0. 5b	12.21 ± 1.1c	9.12 ± 0.3c	3.21 ± 0.3a	3.42 ± 0.5b
PDM-116	4.56 ± 0.3a	4.87 ± 0.4c	10.52 ± 1.2a	14.5 ± 0.9d	4.21 ± 0.2b	2.81 ± 0.4a
Dhauli	4.08 ± 0.3a	5.12 ± 0.7d	12.31 ± 0.9c	16.7 ± 0.8e	5.60 ± 0.6c	3.72 ± 0.4b
K-851	4.78 ± 0. 6a	2.77 ± 0.4b	11.33 ± 1.1b	7.23 ± 0.6b	3.23 ± 0.5a	2.51 ± 0.6a
TARM-1	4.95 ± 0.3a	1.96 ± 0.4a	10.32 ± 0.8a	6.43 ± 0.7a	4.12 ± 0.3b	2.36 ± 0.5a
TARM-21	6.23 ± 0.9b	1.88 ± 0.3a	12.82 ± 1.2c	8.71 ± 0.4b	3.72 ± 0.7a	2.31 ± 0.4a
TARM-26	6.48 ± 0.8b	1.81 ± 0.5a	11.21 ± 0.8b	6.84 ± 0.8a	3.14 ± 0.6a	2.13 ± 0.6a

Within a column means having a letter in common are not significantly different at 5 % level by Duncan's multiple range test.

Table III. Biomass yield of eight mung bean cultivars in absence and presence of 12 μM of nickel. Values are mean of 20 samples.

Cultivars	Biomass yield (mg per plant)					
	Root		Shoot		Shoots/Root	
	0	12	0	12	0	12
TARM-22	32.42e	18.46c	54.21d	36.41e	1.67	1.97
LGG-407	27.46b	22.42d	50.56d	38.23e	1.84	1.39
PDM-116	29.32c	33.21e	48.21b	57.23f	1.64	1.72
Dhauli	25.14a	36.72f	51.23d	63.32g	2.03	1.72
K-851	30.42c	16.32c	48.56b	33.46d	1.50	2.05
TARM-1	31.62d	12.36a	49.71c	27.57b	1.57	2.23
TARM-21	37.61e	13.48b	47.45a	28.81c	1.26	2.13
TARM-26	37.23e	12.52a	48.32b	26.27a	1.29	2.09

Within a column means having a letter in common are not significantly different at 5 % level by Duncan's multiple range test.

the cultivar 'PDM-116' showed 13.26 % increase in root biomass (*table III*). The results presented in *table II* indicate that in 'LGG-407', 'TARM-22', 'K-851', 'TARM-1', 'TARM-21' and 'TARM-26', sensitivity to nickel toxicity led to 18.35–66.37 % reductions in the root biomass as compared to the respective controls. 'Dhauli' showed an increase in the shoot/root biomass ratio in the presence of nickel compared to the control. The shoot biomass was more than the root biomass due to the fact that nickel was accumulated principally in the shoots and less in the roots even at higher concentrations [20, 23, 32].

The accumulation of nickel in root and shoot of 'Dhauli' and 'PDM-116' were found to be higher than other cultivars (*table IV*). Thus, Baker et al. [2] suggested two basic strategies of tolerance: metal exclusion, where metal uptake and transport is restricted, and metal accumulation where there is no such restriction and metals are accumulated in a detoxified form. Detoxification may result from cell wall binding, active pumping of ions into vacuoles, complexing by organic acids and possibly by specific metal-binding proteins, and alteration of membrane structure [33].

The relationships between nickel concentration and root length were different in different cultivars (*figure 2A–H*). The effect of nickel became accentuated over time, as can be observed by the increasing slope of the curves, and this trend was greater for 'TARM-26' and 'TARM-21' being susceptible to nickel. Cultivars like 'Dhauli' and 'PDM-116' were tolerant and other cultivars had intermediate response. Nickel induced a greater enzymatic activity of G6PDH and GDH in 'Dhauli' and 'PDM-116' than in the 'TARM-21' and 'TARM-26' (*table V*). The activity increased from 68.30 to 82.72 in the case of G6PDH and from 87.50 to 97.21 in the case of GDH as compared to control.

Table IV. Nickel content (μg/g) in eight mung bean cultivars in presence of 12 μM nickel after 9 days of culture. Values are means of ten replicates; repeated three times.

Cultivars	Ni content (μg/g) ± s.e.	
	Root	Shoot
TARM-22	4.5 ± 0.7	11.9 ± 2.3
LGG-407	6.9 ± 1.5	12.7 ± 1.9
Dhauli	9.9 ± 1.7	17.8 ± 3.5
PDM-116	8.6 ± 1.2	14.5 ± 2.7
K-851	7.2 ± 1.3	12.5 ± 1.2
TARM-1	6.2 ± 1.5	11.5 ± 4.7
TARM-21	7.1 ± 3.7	8.2 ± 1.5
TARM-26	4.2 ± 1.5	5.7 ± 2.1

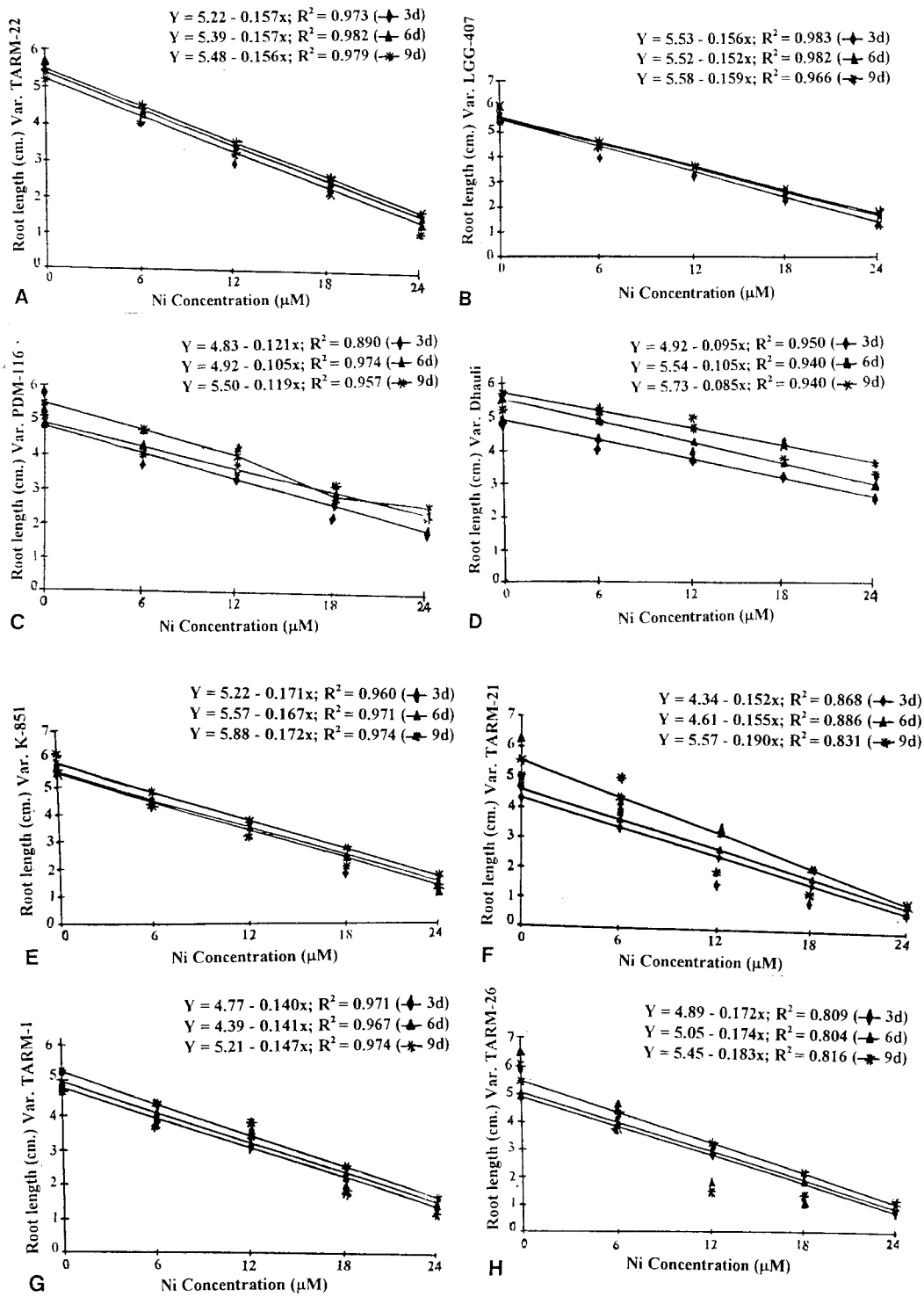

Figure 2. Relationship between different levels of nickel and root length at three time intervals for eight cultivars of mung bean culture on nutrient solution.

Table V. Effect of nickel (12 μM) on the enzyme activity of different cultivars of mung bean (*Vigna radiata* L.). Activities are expressed as *n* mole NAD (P)H oxidised per min per mg protein. Parenthesis indicates percent as compared to the control. Values given are the averages of three assays.

Cultivars	Enzyme activity	
	G6PDH	GDH
TARM-22	108.45 (10.27)	92.56 (27.93)
LGG-407	128.32 (16.42)	110.67 (29.39)
PDM-116	312.31 (68.30)	223.54 (87.50)
Dhauli	348.65 (82.72)	267.56 (97.21)
K-851	256.63 (35.4)	189.75 (18.33)
TARM-1	89.43 (18.84)	91.37 (22.57)
TARM-21	88.45 (20.98)	92.52 (22.78)
TARM-26	91.21 (13.52)	87.41 (19.99)

4. CONCLUSION

Some reports showed that once absorbed, toxic metals are not completely inert, but can stimulate the activity of certain enzymes like G6PDH and GDH [30, 31]. As nickel is termed as a heavy metal, it may enhance the enzymatic activity due to induced stress. Ni-induced reduction in growth of seedlings in nutrient solution was reported by Khalid and Tinsley [20], Wang [35] and Palacios et al. [23]. Nevertheless, the intrinsic mechanism by which nickel detoxification is effected by these tolerant cultivars, either before (extracellular level) or after absorption (cellular level), is still not fully understood. The linear regressions of root length versus concentration of nickel at 3, 6 and 9 days of exposure also confirmed varied response of nickel in eight cultivars of mung bean (*figure 2A–H*). Therefore, it was possible to categorise mung bean cultivars based on their relative tolerance to Ni-toxicity as Dhauli > PDM-116 > LGG-407 > K-851 > TARM-22 > TARM-1 > TARM-21 > TARM-26.

Among the tested cultivars, it appeared repeatedly that 'Dhauli' and 'PDM-116' were tolerant to nickel. Further studies are warranted to unravel the hidden facts on the mechanism of nickel tolerance in mung bean.

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