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Varietal Difference in Mung Bean (*Vigna radiata* (L.) R. Wilczek) for Early Seedling Growth Under Lead Stress

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Abstract

Lead affects seedling growth and its related physio-biochemical functions negatively. The present study investigated the responses of six different varieties of Mung bean [*Vigna radiata* (L.) Wilczek] to three concentrations of 0.25 mM and 0.50 mM Pb $(NO_3)_2$ along with a control variant and there was varietal difference observed among these varieties, all responded differently to the lead toxicity at the early germination growth stages. Parameters were analyzed like germination percentage, RWC, plumule and radicle growth, plumule fresh and dry weight and radicle fresh and dry weight; MSI; biochemical parameters like and protein content, total soluble sugar and proline. Besides this antioxidant enzymes activity i.e., extent of lipid peroxidation through malondialdehyde content were also studied. The effect of metal toxicity was analyzed through MTI. They're existed significant differences among the varieties in the lead toxicity and had profound effect on all the morphological, biochemical and antioxidant enzyme parameters. Germination %, RWC, plumule length, radicle length, fresh and dry weight of seedling. Protein content, total soluble sugar decreased significantly, whereas, antioxidant enzymes proline and malondialdehyde content increased many folds with increasing toxicity of lead and recorded highest at 0.50 Mm Pb $(NO_3)_2$. The present study found that all the six genotypes of mung bean is sensitive for growth under lead toxicity even at lower concentration 0.25mM Pb $(NO_3)_2$ and at higher concentration 0.50mM Pb $(NO_3)_2$ was much affected by lead toxicity.

Key words: Antioxidant enzymes, Chlorophyll, Heavy metal, Lead, Malondialdehyde, Proline, Plumule, Radicle

Pulses present both environmental and nutritional benefits, they are often recommended in sustainable diet. Health organizations such as the Food and Agriculture Organization of the United Nations recommend pulses as staple foods to fulfil the basic protein and energy requirements of the human diet [1]. Mung bean [*Vigna radiata* (L.) R. Wilczek var. radiata] is cultivated over 6.9 million hectares area as a short duration leguminous crop, prominently in Asia [2]. It is a diploid, self-pollinating, fast-growing and short-duration crop and helps in the effective utilization of summer fellows to enhance the cropping intensity and crop production. Pulses are greatly affected by abiotic stresses like draught, salinity, waterlogging, heat, heavy metal affects the productivity of mung bean [3]. Arsenic, lead, copper, nickel, and other heavy metal contamination in agricultural fields has had a significant impact on crop output [4]. Both essential and non-essential forms of heavy metals are an intrinsic part of the environment [5]. According to Sethi and Ghosh [6], contamination with metals has a negative impact on soil biomass, soil fertility, and crop production. These morpho-physiological processes are highly affected by drought stress, which exhibit impact on yield.

Agricultural soils are contaminated by Pb compounds used as agricultural chemicals, such as Pb arsenate, which is used as a pesticide. Although it is a non-essential element for plants but they show a high tendency to uptake and accumulation in different plant organs [7]. Lead enters plants through their roots, causing morphological, physiological, biochemical, and molecular changes [8]. Presence of excess amount of lead resulted in some toxicity symptoms of inhibition of protein activity and disruption of structure due to formation of free radicles and Reactive Oxygen Species [9] Pb causes oxidative stress by creating excessive amounts of reactive oxygen species (ROS). Excessive ROS production degrades macromolecules, resulting in impaired physiological and biochemical processes [10], resulting in decreasing plant growth. Several mung bean types are innately tolerant to some degree of abiotic stress. The goal of the current study was to shed light on how different mung bean varietal reactions to varying lead nitrate [Pb $(NO_3)_2$] concentrations affected germination rates, relative water contents, metal tolerance indices, membrane stability indices, sugar and protein contents, proline accumulation, and malondialdehyde content (MDA).

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MATERIALS AND METHODS

The Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University provided healthy and clean genotypes of mung bean (HUM 1, HUM 2, HUM 6, HUM 12, HUM 16, HUM 27). To prevent any fungal infection, uniform-sized seeds from each genotype were chosen, and their surfaces were surface sterilized with a 0.1% solution of mercuric chloride for 3 min. This was followed by the prevalent four times of washing with distilled water in order to rinse out the mercuric chloride. To subject seeds to lead stress, mung bean seeds of all six genotypes were soaked in lead nitrate concentrations of 250 M and 500 M. The given concentration mimicked polluted soil representing the moderate and high concentration. The experiment was completely randomized with three treatments. Control treatments were provided with distilled water. After being soaked, all the seeds were moved onto petri dishes that contained germination paper. Before placing the seeds on the paper, a dropper was used to moisten it with distilled water. Following parameters were observed to access the effect of lead levels on early seedling growth.

Morphological parameters: Plumule length (cm), radicle length (cm), plumule fresh weight (mg), plumule dry weight (mg), radicle fresh weight (mg) and radicle dry weight (mg) were recorded at 7 days after germination.

Germination percentage: Numbers of germinated seeds were recorded after 24, 36 and 48 hours of seed germination. Germination percentage was calculated as per the Association of official seed analysis (1983).

Germination percentage = (No. of seed germinated / Total no. of seed sown) ×100

Relative water content: RWC is calculated for the approximation of seedling water status in terms of physiological consequences of cellular water deficit, as stated by Slatyer [11].

RWC = (Fresh weight− Dry weight) / (Turgid Weight−Dry weight) ×100

Metal tolerance index: Length of longest radicle in each treatment was measured and a tolerance Index was calculated according to the formula [12-13].

MTI = (Length of longest radicle in metal solution/ Length of longest radicle in control solution) ×100

Membrane stability index: Membrane Stability Index (MSI) was determined following the method of Premachandra *et al*. [14], as modified by Sairam [15]. Seedling sample (0.2g) of uniform size were placed in test tubes containing 10 ml of double distilled water in two sets. Test tubes in one set were kept at 40oC in a water bath for 30 min and electrical conductivity of the water containing the sample was measured (C_1) using a conductivity bridge. Test tubes in the other set were incubated at 100°C in boiling water in water bath for 15 min and electrical conductivity was measured as above (C_2). Membrane stability index (MSI) was calculated using the following formula:

$$MSI = [1 - C1/ C2] \times 100$$

Estimation of protein content (mg/g of fresh weight): Protein in a crude extract was determined according to the Coomassive Brilliant Blue G-250 dye binding method [16].

Estimation of total sugar content (mg/g of fresh weight): The amount of total soluble sugar was estimated by Hedge and Hofreiter [17].

Proline content estimation (mg/g of fresh weight): The proline content from the sample was extracted and estimated following the method of Bates *et al*. [18] and the absorbance was taken at 520nm.

Malondialdehyde (MDA) content (µ mol g^{-1} fresh weight): The level of lipid peroxidation was measured in terms of Malondialdehyde, and it was determined by the method of Hodges *et al*. [19].

The statistical analysis was done using standard statistical procedure using Two factorial completely randomized design (CRD). The observed tabular data were analyzed statistically by one way ANOVA table. Critical difference values were calculated at 5% level of Significance in order to compare the treatment means.

RESULTS AND DISCUSSION

Seed germination is highly affected by the lead stress through the treatment of 0.25mM $Pb(NO_3)_2$ (T1) and 0.50mM $Pb(NO_3)_2$ (T_2). All the six genotypes responded differently in respect to their treatments given. All these different genotypes showed highest germination percentage when no lead treatment was given but they showed a reduction in their germination percentage when applied 0.25mM $Pb(NO_3)_2$ (T_1) and this difference goes on wider when applied to 0.50mM $Pb(NO_3)_2$ (T_2). Higher percentage of decrease in germination indictor of inhibiting effect of lead on early growth and development of seedlings. In this experiment, during the first 24 and 36hours HUM 12 and HUM 27 showed least difference in their germination percentage as compared to control. During 72 hours, HUM 27 performed well with least difference. The decrease in seed germination percent of mung bean genotypes can be attributed to the accelerated breakdown of stored food materials in seed by the application of lead. Reduction in seed germination can also be attributed to alterations of selective permeability properties of cell membrane or due to impact on the activity of seed enzymes such as amylases and proteases causing decrease rate of food supply to the growing plumule and radicle. The maximum reduction in per cent germination was recorded in HUM 6 with 25%, 12.5% and 6.25% at 24, 36 and 72 hours respectively as highlighted from (Table 1). The decrease in seed germination due to heavy metal treatment is in conformity with the findings of other workers [20-22]. Plumule growth is the most distinguishable part of seedling growth when affected by any stress. Lead stress caused a significant decrease in plumule length of every genotype at 7 days after germination. Among treatments, plumule length was found longest in control, whereas the shortest plumule length was recorded in treatment T_2 $Pb(NO_3)_2$@ 0.50mM) in all the treatments. Among genotypes, significant differences in plumule length were observed and HUM6 and HUM16 showed higher plumule length than other genotypes in control treatment 7 days after germination. The maximum significant reduction in plumule length was observed in genotype HUM 12 with 7.46% in (Table 1). HUM 12, HUM27, HUM12 they performed equally but least difference was found in HUM6 when compared with control. There was reduction in the plumule length with increase in their ($Pb(NO_3)_2$ concentration [23]. Visual symptoms of lead toxicity were also estimated through the radicle length of seedlings. Radicles are considered to be the primary site of lead toxicity [20]. There was highest radicle length recorded when 0 mM Pb

$(NO_3)_2$ and least it was recorded from when treated with 0.50mM (Pb $(NO_3)_2$ (T2). Likewise, plumule length here, HUM 6 showed least difference in their treated ones when compared to control. Among genotypes, significant differences in radicle length were observed and HUM27 showed higher radicle length. The maximum significant reduction in plumule length was observed in genotype HUM 6 with 23%. As it was evident from (Table 2). The primary effect of lead toxicity in plants is a rapid decrease of radicle growth, which is thought to be attributed to cell division suppression in the radicle tip [24]. decrease in the length and in radicle dry mass of wheat under lead toxicity have been reported [25-26].

Table 1 The effect of lead stress on per cent germination in mung bean (*Vigna radiata* L.) varieties

		Duration of Germination (%)		
Varieties	Treatment	24 hours	36 hours	72 hours
HUM 1	0 mM	70	74	96
	0.25 mM	66	72	93
	0.50 mM	60	64	90
HUM 2	0 mM	60	73	93
	0.25 mM	50	60	90
	0.50 mM	40	53	86
HUM 6	0 mM	50	58	85
	0.25 mM	40	48	83
	0.50 mM	30	42	80
HUM 12	0 mM	66	80	96
	0.25 mM	63	76	93
	0.50 Mm	50	73	90
HUM 16	0 mM	90	93	96
	0.25 mM	80	90	96
	0.50 mM	70	80	80
HUM 27	0 mM	96	99	99
	0.25 mM	90	93	96
	0.50 mM	86	93	96
	Varieties (A) × Treatment (B) Interaction			
	C.D	1.63	1.632	1.64
	S.E(m)	0.57	0.56	0.58

C.D statistically measured significant and non-significant** at $p \leq 0.005$

Fresh weight of radicle and plumule showed significant decrease with the increase in the Pb $(NO_3)_2$concentration. Biomass of seedling is the most decisively factor of plant growth parameter. plumule fresh weight@ 0.50mM (Pb $(NO_3)_2$showed least increase in height as compared to control, and plumule fresh weight at 0.25mM Pb $(NO_3)_2$ showed little reduction in plumule fresh weight as compared to control. The maximum significant reduction in plumule fresh weight was observed in HUM 2 with 14.7% (Table 2). Least difference in plumule fresh weight was recorded from HUM16 and HUM 27. Highest reduction in plumule fresh weight recorded in HUM 1 with 12.5% Radicle fresh weight followed a similar trend as plumule fresh weight. Earlier studies also suggest reduction in fresh weight of plumule and radicle of plants [27] in mung bean [28]. The overall decrease in biomass of plants is directly related to the disturbed metabolic activities and due reduction in water absorption when grown in lead stress condition [29].

The significant decrease in dry weight of plumule and radicle in all these six genotypes of mung bean due to lead toxicity was observed. The treatment of lead 0.50mM Pb $(NO_3)_2$ (T_2) showed the highest reduction in the dry matter content in all the six genotypes of mung beans. HUM 16 and HUM 2 least difference in their dry weight of plumule when compared with control. Inn case of dry radicle weight there is not any specific variety performed well, but HUM 1, HUM 16 and HUM 27 performed better than the rest ones, they showed least difference when compared with control while maximum reduction observed in HUM 1 with 19.3% at 7 DAG. Among six genotypes, HUM 27 recorded highest plumule dry weight. There is reduction in the dry weight of plumule and radicle in lead stress condition is somehow because of increase in energy expenditure, lowered carbon intake and decrease in photosynthetic uptake [30]. HUM 6 least reduction in dry weight from control, Maximum reduction radicle dry weight from control observed in HUM 27 with 12.5% reduction at 7 DAG data depicted in (Table 2).

Most of the morphological parameters of any plants is controlled by the genetical setup but its expression is influenced by the growth× environment factor, here external factor constitutes of lead stress.

Relative water content is used to measure the relative turgidity of plant cells, and most suited measure to know the water status of plants in terms of cellular hydration. Data presented in (Table 2) stated the response of different genotypes to Metal Tolerance Index and all recorded differently with significant difference among them. When the concentration of lead is increasing in treatment, the RWC decreases. RWC @ 0.50mM Pb $(NO_3)_2$ (T2) showed reduction as compared to the control. Among genotypes, highest RWC recorded in HUM 27 and maximum reduction in RWC percentage found in HUM 6 with 14.3%. Pirzada *et al*. [31] reported similar results for relative water content in buckwheat.

As the ability to plants to survive in pollutant medium of growth is determined by their ability to tolerant the accumulated amount of metal present inside the plant body measured by Metal tolerance index. A close analysis of (Table 2) revealed that seedling growing at 0 mM Pb $(NO_3)_2$ (T_0) showed highest percentage but this showed reduction upon increasing the concentration from 0.25mM Pb $(NO_3)_2$ (T_1) to 0.50mM Pb $(NO_3)_2$ (T_2), it is because of increased amount of accumulated metal in them. There is reduction of tolerance index seen in from T_1 to T_2. The highest metal tolerance index was recorded in HUM 27 @ 0.25mM Pb $(NO_3)_2$ (T_1) and least was obtained in HUM 1. The reduction in tolerance index of metal upon increase in the concentration of applied treatment was also seen by Zacchini *et al*. [32]; de Souza *et al*. [33] in leguminous tree.

Table 2 The effect of lead stress on the seedling growth parameters in mung bean (*Vigna radiata* L.) varieties

		Seedling growth parameters					
Varieties	Treatment	Plumule length (cm)	Radicle length (cm)	Plumule fresh weight (mg of FW)	Plumule dry weight (mg of DW)	Radicle fresh weight (mg of FW)	Radicle dry weight (mg of DW)
HUM 1	0 mM	4.60	2.40	55.20	18.40	4.73	2.23
	0.25 mM	4.30	2.01	54.33	17.36	4.06	2.02
	0.50 mM	3.90	1.89	53.80	06.43	3.23	1.90
HUM 2	0 mM	4.70	2.60	55.53	18.80	4.71	2.20
	0.25 mM	4.47	2.32	53.53	18.30	4.14	1.90
	0.50 mM	4.00	1.60	52.90	17.90	3.58	1.22
HUM 6	0 mM	4.40	2.33	55.30	18.60	4.79	2.32
	0.25 mM	4.30	2.28	54.80	18.20	4.03	2.07
	0.50 mM	3.80	1.72	54.26	17.90	3.79	1.96
HUM 12	0 mM	6.37	4.40	82.40	21.30	12.46	5.50
	0.25 mM	5.99	4.04	80.56	20.70	12.09	5.05
	0.50 mM	5.54	3.87	79.70	19.50	11.57	4.79
HUM 16	0 mM	6.60	4.60	102.53	21.83	16.57	8.14
	0.25 mM	6.40	4.40	100.67	21.20	15.79	7.90
	0.50 mM	6.00	3.89	99.74	20.20	15.13	7.14
HUM 27	0 mM	6.70	4.73	95.80	22.20	15.50	7.26
	0.25 mM	6.40	4.43	94.43	21.70	14.56	6.23
	0.50 mM	5.90	4.13	93.03	20.93	13.93	5.79
		Varieties (A) × Treatment (B) Interaction					
C.D		**	0.46	0.25	0.197	0.189	0.115
S.E(m)		0.064	0.160	0.089	0.068	0.066	0.040

C.D statistically measured significant and non-significant** at p≤0.005

Table 3 The effect of lead stress on the physiological and biochemical parameters in mung bean (*Vigna radiata* L.) varieties

		Physiological and biochemical parameters						
Varieties	Treatment	Relative water content (RWC) (%)	Metal tolerance Index (MTI) (%)	Membrane stability index (MSI) (%)	Protein content (mg/g of FW)	Total soluble sugar (mg/g of FW)	Proline content (mg/g of FW)	Malondialdehyde content (μmol g^{-1} fresh weight)
HUM 1	0 mM	74.56		74.26	4.53	0.26	0.12	4.50
	0.25 mM	72.23	76.24	54.33	3.83	0.22	0.18	4.70
	0.50 mM	71.46	71.40	46.50	3.13	0.12	0.21	5.30
HUM 2	0 mM	74.92		72.50	4.44	0.25	0.06	4.30
	0.25 mM	72.47	82.56	54.43	3.83	0.22	0.18	4.70
	0.50 mM	69.68	80.14	41.60	3.34	0.13	0.20	5.10
HUM 6	0 mM	74.64		71.60	4.32	0.46	0.09	4.60
	0.25 mM	73.22	84.14	52.70	3.53	0.42	0.22	5.00
	0.50 mM	71.27	83.99	41.60	3.00	0.13	0.25	5.30
HUM 12	0 mM	78.60		76.30	4.65	0.24	0.07	4.30
	0.25 mM	77.45	81.46	65.36	3.55	0.21	0.18	4.60
	0.50 mM	76.46	77.82	57.60	3.82	0.14	0.20	5.30
HUM 16	0 mM	80.40		78.20	4.68	0.29	0.06	4.20
	0.25 mM	79.36	85.60	62.50	3.85	0.23	0.16	4.50
	0.50 mM	78.29	82.98	55.36	3.22	0.14	0.18	5.00
HUM 27	0 mM	81.05		77.50	4.77	0.28	0.07	4.10
	0.25 mM	79.10	86.36	59.70	3.35	0.22	0.19	4.50
	0.50 mM	77.51	82.10	48.30	2.82	0.13	0.21	5.06
		Varieties (A) × Treatment (B) Interaction						
C.D		0.436	2.873	0.227	0.034	0.002	**	**
S.E(m)		0.152	0.979	0.079	0.012	0.001	0.006	0.060

C.D statistically measured significant and non-significant** at p≤0.005

Mung bean seedlings recorded differently to the Membrane Stability Index (MSI) for the controlled ones and for the treated ones i.e., for @0.25mM Pb $(NO_3)_2$ (T1) and 0.50mM Pb $(NO_3)_2$ (T2). A glance at (Table 3) depicted MSI decreases on the increase in the Pb $(NO_3)_2$ concentration. The variety showing high MSI be the most stressed type variety as it indicates oxidative stress condition present inside cell, also an indicator of damage to cell wall. In our study highest MSI was recorded from HUM 2 then followed in HUM 6. least MSI is recorded from HUM 12, HUM 16 [34].

Protein and total soluble sugar present in the seedlings showed a gradual reduction with the increase in lead concentration form 0.25mM (Pb $(NO_3)_2$ (T_1) to 0.50mM (Pb $(NO_3)_2$ (T_2) as compared to control, but in total soluble sugar a slight significant increase in amount of glucose was explored in HUM 16 and HUM 27, there was significant amount of reduction seen in HUM 1 with 28.77%. The reduction in the photosynthetic pigments gradually reduced the glucose levels in the cells, thereby reduction in the glucose level of plant cell, least difference in total soluble sugar level was obtained from HUM27 and HUM 16, as compared to control (Table 3). There was lowest level of protein recorded from 0.50mM (Pb $(NO_3)_2$ (T2) in all these genotypes, similar result was obtained from [35]. There was maximum reduction in protein content in HUM 2 with 9.87%. Protein content reduction probably due to inhibition of protein synthesis or its degradation, this result was recorded from Lupinus albus under heavy metal stress [36]. Another reason for decrement in protein content may be due to oxidative stress. Least difference form control indicated by HUM 2 and HUM6.

Ahmad *et al.* [37] reported that plant's cell maintains osmotic balance of cell by the accumulation of glycine betaine, proline, other ions and soluble carbohydrates in order to tolerate to stress condition. Seedlings showed an increase in proline content with the increase in lead concentration, highest amount of proline accumulated in 0.50mM (Pb $(NO_3)_2$, resulted differently for each variety. Poline content was enhanced from 0.25mM Pb (NO3)2 to 0.50mM (Pb $(NO_3)_2$. Least accumulated proline recorded to be tolerant to stress condition, as to this, variety HUM 27 and HUM 1 showed least accumulation of proline. Higher proline concentration was observed in HUM 6 @0.25mM (Pb $(NO_3)_2$, HUM 6 and HUM 12@0.50mM (Pb $(NO_3)_2$. Results depicted in (Table 3) showed that proline concentration was significantly higher in the Lead-tolerant genotypes than in the non-tolerant genotypes. This result was in agreement to Al-Ghzawi *et al.* [38] when worked on lead toxicity in barley. Sofy *et al.* [39] worked on maize obtained the same results. Proline, assumed to be involved in the reconstruction of chlorophyll pigments, involve in the recycle of energy source and Kreb cycle.

Malondialdehyde (MDA) content in plants increased in case of stress affected condition, seedlings treated with lead @0.50mM (Pb $(NO_3)_2$ (T_2) showed the highest content of malondialdehyde accumulated as compared to @0.25mM (Pb $(NO_3)_2$ (T_1) and control, in which least amount of MDA was accumulated. Maximum amount of MDA content recorded from HUM 12 @.25mM (Pb $(NO_3)_2$ (T_1) and HUM 1, HUM2, HUM6 @0.50mM (Pb $(NO_3)_2$ (T_2) as presented in (Table 3). This increase in MDA accumulation in seedlings with the increase in (Pb $(NO_3)_2$ concentration, coincided with the result of Deshna *et al.* [40] in mung bean seedling and De Britto *et al.* [41] in *Capsicum annum*. The direct and indirect result of stress in seedlings lead to production of oxygen radicals and which ultimate results in increased lipid peroxidation and oxidative stress in plants. The increased level of MDA in seedlings indicated the presence of oxidative stress and this increased MDA, mechanism for the manifestation of stress due to (Pb $(NO_3)_2$.

CONCLUSION

Based on the aforementioned findings, when exposed to lead toxicity, all genotypes interaction resulted in distinct outcomes. Seedlings exposed to lead toxicity had reduced growth characteristics, lower nutritional content, and negative impacts on chlorophyll pigments. All six genotypes of *Vigna radiata* demonstrated a drop in germination percentage, seedling development, fresh and dry weight, and relative water content from 0.25mM (Pb $(NO_3)_2$ (T_1) to 0.50mM (Pb $(NO_3)_2$ (T_2). Reduction in chlorophyll a, chlorophyll b and total chlorophyll pigments observed with increase in concentration 0.25mM (Pb $(NO_3)_2$ (T_1) to 0.50mM (Pb $(NO_3)_2$ (T_2), which ultimately resulted in poor growth of seedlings. Overall reduction in protein content and total soluble sugar of seedlings over all genotypes, and maximum reduction was observed @ 0.50mM (Pb $(NO_3)_2$ (T_2). The activity of proline and malondialdehyde increased with the rise in lead concentration, and it was same among all genotypes. The increased MDA content indicates the production of free oxygen radical. As a result, plants defence mechanism against metal stress was seen, with increased lipid peroxidase synthesis and restricted growth due to a decrease in chlorophyll content.

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