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Varietal response of Mung bean (*Vigna radiata* (L.) R. Wilczek) on chlorophyll pigments, Proline and Malondialdehyde content under lead stress condition

Saumya Shruti and Savita Jangde

Abstract

This study attempted to evaluate the response of lead on different varieties (HUM 1, HUM2, HUM6, HUM12 and HUM27) of Mung bean (*Vigna radiata*). Mung bean seeds were soaked in lead nitrate solution [Pb $(NO_3)_2$] of concentrations of 0 mM, 0.25 mM and 0.50 mM for 2 hours. Chlorophyll pigments, Proline content and Malondialdehyde content was estimated on the 14th day of early seedling stage. It was found that Chlorophyll pigments of Mung bean seedling significantly decreased with the increase in concentration of lead nitrate. However, a proportionately increased amount of proline and malondialdehyde content was observed with increasing concentration of lead nitrate. All the varieties of Mung bean responded differently to the lead nitrate concentrations. HUM 2 and HUM 6 were found to be more sensitive as compared to other varieties.

Keywords: Mung bean, Malondialdehyde, stress condition *Vigna radiata* L.

1. Introduction

The presence of a wide range of contaminants in sewage, used for irrigation, has a substantial impact on agricultural crop production (Yadav, 2010). Heavy metal contamination in agricultural fields (e.g., arsenic, lead, copper, cadmium, and nickel) has had major consequences affecting the production of crops (Singh *et al*., 2007). Heavy metals are inherent component of environment consists both of essential and non-essential types. It is the result of unplanned and unwanted accumulation of municipal waste disposal, mining activities, agricultural activities and excessive consumption of pesticides (Akinci and Akinci, 2010) [2]. Metal toxicity adversely affects soil biomass, soil fertility and crop prodcutivity (Sethi and Ghosh, 2013) [23].

Lead has been considered to be a cosmopolitan environmental pollutant amongst the heavy metal (Sharma and Dubey, 2005) [25]. Lead is a non- essential and obnoxious metal element to plants comes in soil through agrochemicals such as pesticides and fertilizers (Tao *et al*., 2015) [28]. Lead negatively affects plant growth and seed germination (Iqbal and Shazia, 2004). The negative impact on germination is among the most common toxic impact of heavy metals (Talebi *et al*., 2014) [27]. Lead causes structural changes in the photosynthesis apparatus and negatively impacts the photosynthetic pigments (Zulfiqar *et al*., 2019) [30]. Lead affects many biochemical processes, antioxidant production and lipid peroxidation in plants (Seneviratne *et al*., 2019) [22].

Plants exposed to lead toxicity affects chlorophyll pigments and overall it decreases the photosynthetic performance of plants (Albert *et al*., 2011) [3]. Lead stress in plants responsible for Reactive Oxygen Species (ROS) production in plants which causes oxidative stress, damages chloroplast membrane and disrupts the functioning of electron transport chain (Sharma *et al*., 2020) [24]. In response to different stress conditions, plants accumulate a significant amount of compatible osmolytes, such as proline (Hayat *et al*., 2011). Proline accumulated in cytosol and involves in osmotic adjustment against oxidative stress (Ku *et al*., 2012). Proline is used to offset cellular imbalances and protein denaturation caused due to lead stress (Liang *et al*., 2013) [18]. Proline tends to increase with increasing stress condition and serves as an important response against the environment stress condition (Choudhary *et al*., 2007) [9]. Malondialdehyde is a cytotoxic byproduct of lipid peroxidation that serves as an indication of free radical generation and subsequent tissue damage (Ohkawa *et al*., 1979) [19]. Therefore, increased proline and Malondialdehyde content considered as an indicator of environmental stresses.

The present study attempted to illuminate the different varietal responses to diffferent lead nitrate [Pb $(NO_3)_2$] concentrations on Chlorophyll pigments, Proline accumulation and lipid peroxidation (Malondialdehyde) activity in *Vigna radiata* L.

2. Material and Methods

Pure, healthy and disease-free seeds of Mung bean [*Vigna radiata* (L.) Wilczek] varieties HUM1(Malviya Jyoti), HUM2 (Malviya Jagriti), HUM6 (Malviya Janpriya), HUM12 (Malviya Janchetna), HUM16 (Malviya Jankalyani and HUM27 were procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Present study was carried out in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in the month of April 2021. Mung bean seeds were sterlized with 0.1% $HgCl_2$ for 3 minutes, subsequently rinsed five times with distilled water. Sterlized seeds were soaked for two hours in metal solutions [T_0 = distilled water (control), T_1 = 0.25 mM lead nitrate and T_2 = 0.50 mM Lead nitrate solution]. These lead nitrate concentration are lower and medium range of polluted soil condition. The control group was for comparison purposes. Twenty healthy and uniform seeds placed on each petri dishes seeds and allowed to germinate on well moistened filter paper. Each of petri dishes were continued to remain at room temperature (25±5° C). Seeds with more than 1mm radicle emergence was considered to be germinated. The following Biochemical observations were recorded on the 14th day after germination, *i.e.*, chlorophyll content, protein content, proline and malondialdehyde content.

2.1 Estimation of chlorophyll content

Fresh sample of 1 g weight was well mixed into a clean mortar. Tissues were grinded to fine pulp with the addition of 20 ml of 80% acetone. Sample was centrifuged at 5000 rpm for 5 minutes and then Supernatant was collected into the 100 ml volumetric flask. Same procedure was repeated until residue is colourless. Make up the volume to 100 ml with 80% acetone. the absorbance of the extracts were recorded at 645 and 663 nm, respectively according to Sadasivam *et al*., (1992) [21]. Chlorophyll content was calculated using given formula

$$\text{Chl a (mg of chl a/g of tissue)} = 12.7(\text{A at 663nm}) - 2.69(\text{A at 645nm}) \times \frac{V}{1000 \times W}$$

$$\text{Chl b (mg of chlb/g of tissue)} = 22.9(\text{A at 645}) - 4.68(\text{A at 663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/ g of tissue)} = 20.2(\text{A at 645}) + 8.02(\text{A at 663nm}) \times \frac{V}{1000 \times W}$$

2.2 Estimation of Proline Content

The proline content from the sample was extracted and estimated following the method of Bates *et al*. (1973) [6] and the absorbance was taken at 520nm.

2.3 Estimation of Malodialdehyde content

The amount of lipid peroxidation was measured by Malondialdehyde content estimation, according to the Heath and Packer, (1968) [14] method. Malondialdehyde is considered to be decomposition product of membrane lipid polyunsatuated components, uses reactive component as thiobarbituric acid (TBA) and absorbance was measured at 532 nm and extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4 Statistical Analysis

The ststistical analysis was performed using two factorials in completely randomized design and replicated thrice. The results expressed through graphs as the mean ± standard deviation (SD). Results analysed through two way ANOVA table and critical difference values were calculated at 5% level of Significance in order to compare the treatment means.

3. Results and Discussion

Effect of lead nitrate [$Pb(NO_3)_2$] on the different varieties of mung bean was at two increasing concentrations was recorded on after 14 days of lead nitrate exposure.

3.1 Chlorophyll a, b and total chlorophyll estimation

Lead stress [$Pb(NO_3)_2$] showed significant decrease in chlorophyll a, chlorophyll b and total chlorophyll pigment in every varieties. Among six genotypes, maximum Chlorophyll a was recorded in HUM 27 and least amount of Chlorophyll a in HUM 2. Maximum amount of Chlorophyll b and Total Chlorophyll was recorded from HUM 27 and least was recorded from HUM 2 (Fig 1-3). All six varieties showed maximum reduction in amount of chlorophyll a, Chlorophyll b and Total Chlorophyll at 0.5 mM treatment as compared to control. Among six varieties HUM 2 showed maximum reduction in Chlorophyll a, Chlorophyll b and Total Chlorophyll with 37.87%, 27.32% and 10.23% respectively, similar results were collaborated with Ahmed *et al*., (2008) [1] study in photosynthetic performance of two mung bean varieties exposed to lead and copper concentrations of 25 or 50 mg L^{-1} separately. However, presence of large amount of lead in the plant tissues have inhibitory effects on the pigment system of plant metabolism (Sengar and Pandey, 2020) [20]. Typically, lead accumulation in plants influence the level of chlorophyllase, an enzyme that negatively affects (degradation) chlorophyll content (Hu *et al*. 2012) [15]. Increasing concentration of $Pb(NO_3)_2$ (0.25 mM) to Pb $(NO_3)_2$ (0.5 mM) caused a progressive reduction in the observed photosynthetic pigments i.e., Chlorophyll a, Chlorophyll b and total Chlorophyll in all the six varieties of mung bean. Similar results were obtained by Ashraf *et al*., (2017) [4] on five aromatic rice cultivars and destruction of chlorophyll pigments in chickpea was also reported by Faizan *et al*., (2011) [11] after application of increased cadmium concentrations.

3.2 Proline content estimation

Among the six varieties of mung bean treated with increasing Pb $(NO_3)_2$ concentrated showed increased proline content as compared to control (Fig 4). Proline tends to be accumulate under conditions like heavy metal exposure and show a particular involvement in stress resistance (Chen *et al*., 2004; Gouia *et al*., 2003) [8, 12]. Proline accumulation from all six varieties seedling showed maximum in T2 as compared to control, which shows the protective nature of proline to protect enzymes and other cell metabolic events against harmful heavy metal damages (Shevyaakova *et al*., 2006). HUM 6 showed maximum accumulation of proline at 0.5 mM treatment with 81% difference observed from control. Similar results of proline accumulation with Pb $(NO_3)_2$ induction was also corroborated with the results of Ashraf *et al*., (2017) [4], who observed proline content in rice seedlinds were affected by the lead toxicity. Our results support the findings of Lamhandi *et al*., (2011) [17], who reported enhanced positive correlation of external lead concentrations and proline accumulation in 6 days old wheat seedlings.

3.3 Malondialdehyde content

Malondialdehyde content increased significantly in all the studied varieties and the highest amount accumulated in the T2. The increased Malondialdehyde indicated a concentration reliant free radical production (Fig. 5). The maximum amount of Malodialdehyde content was observed in HUM 6 at 0.5 mM treatment with 56% difference as compared to control. The concentration reliant free readical production increased with the exposure of Pb $(NO_3)_2$ has also been reported by Deshna and Bafna, (2013) [10] in *Vigna radiata* seelings. Malondialdehyde generation in this study is the result of increased oxidative stress and lipid peroxidation in plants and lead toxcity enhanced the oxygen radical production. Lipid peroxidation results in the breakdown of biological membrane functional and structural integrity, enhances plasma memnrane permeability, K^+ leakage and eventually cell death (Tewari *et al*., 2008). This increased lipid peroxidation could be considered as the one of the mechanism for manifestation of lead stress in plants (Verma and Dubey, 2003) [29].

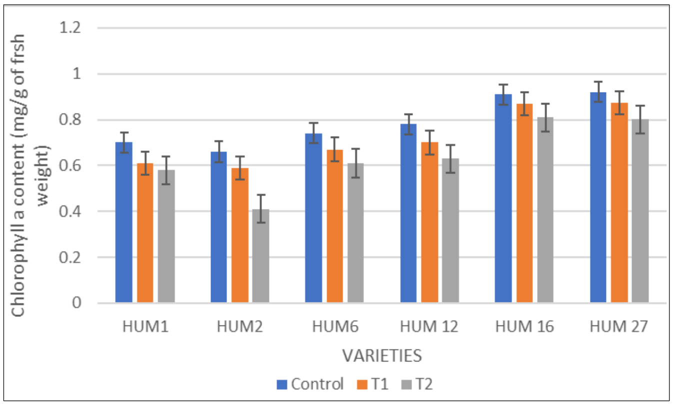

Fig 1: Effect of lead nitrate concentrations on chlorophyll a content in seedlings of Mung bean varieties. Vertical bars represent standard error

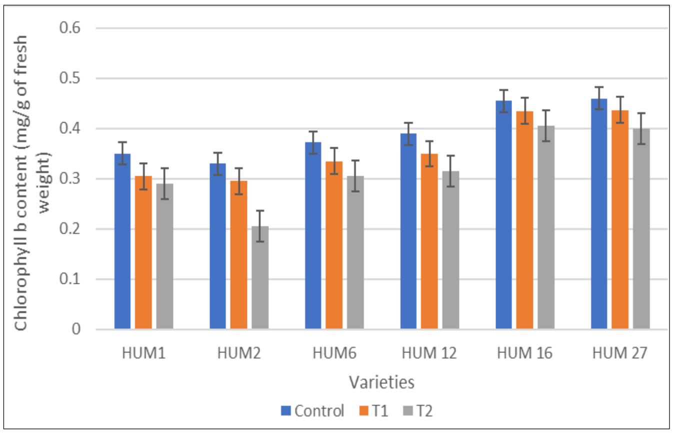

Fig 2: Effect of lead nitrate concentrations on chlorophyll b content in seedlings of Mung bean varieties. Vertical bars represent standard error.

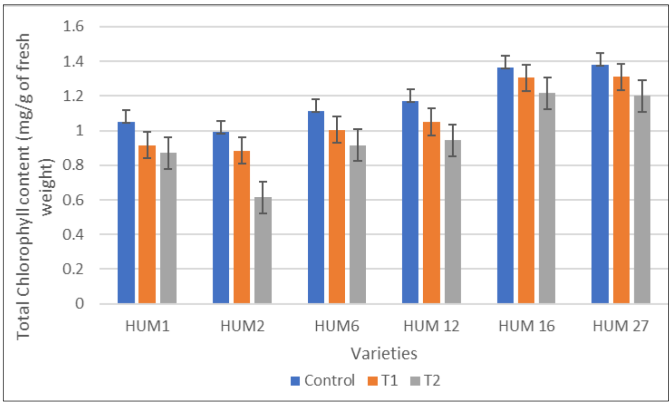

Fig 3: Effect of lead nitrate concentration on Total chlorophyll content in seedlings of Mung bean varieties. Vertical bars represent standard error

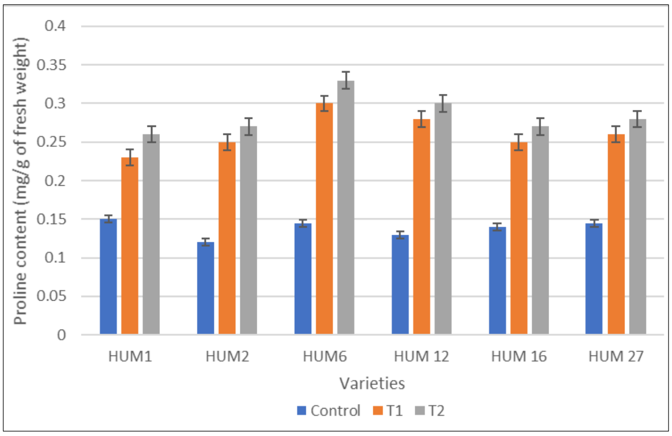

Fig 4: Effect of lead nitrate concentrations on the proline content in seedlings of Mung bean varieties. Vertical bars represent standard error

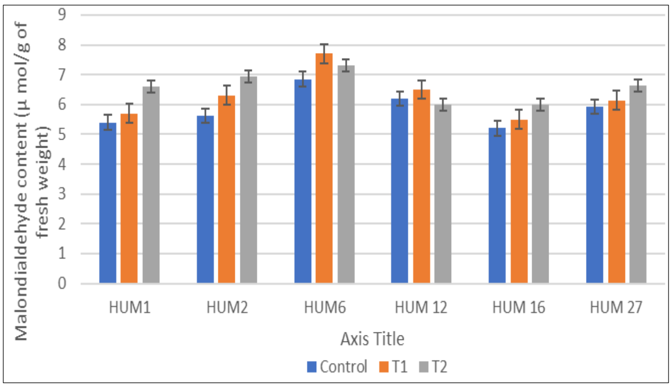

Fig 5: Effect of lead nitrate concentrations on the proline content in seedlings of Mung bean varieties. Vertical bars represent standard error

4. Conclusion

All six varieties of Mung bean in this study responded differently to lead toxicity during germination stage. This study revealed that activity of proline and Malondialdehyde increased with the increasing concentration of Pb $(NO_3)_2$ in all the selected varieties and maximum accumulation found in highest concentration (0.5 mM). Chlorophyll of lead stressed seedling decreased with increase in concentration, the reduced chlorophyll content reflects reduced growth of any plant. It was concluded from the study that HUM 2 and HUM 6 were most sensitive among the selected varieties as it showed maximum reduction in chlorophyll content and highest accumulation of proline and Malondialdehyde content respectively.

5. Acknowledgement

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