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RESEARCH PAPER

Indices for antioxidant enzymes activity and yield attributes of mash bean [*Vigna mungo* L. Hepper] genotypes under rhizospheric intensified lead (Pb) concentrations

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Key Message: During this study antioxidant enzymes production significantly reduced when mash bean was grown in soil having Pb^{2+} concentration more than 10 mg/kg soil. The Mash 88 proved to be the least sensitive and Mash ES-1 the most sensitive to higher Pb^{2+}concentrations.

Abstract: This study was designed to evaluate the toxicity extent of various Pb^{2+} concentrations with emphasis on genotypic variations. Four genotypes of mash bean i.e. Mash 80, Mash 88, Mash 97 and Mash ES-1 were grown in pots with four replicates. After twenty days of germination, various concentrations of lead (10, 20, 30, 40, 50 and 60 mg kg^{-1}) were added in soil as Pb $(NO_3)_2$. Increasing amount of Pb^{2+} from 10 mg kg^{-1} showed a gradual significant reduction in legume setting, grain numbers and total yield. The lowest overall reduction was observed under imposition of 10 mg kg^{-1} as 3.719% in legume number, 3.153% in grain number and 6.823% in total yield. The maximum reduction in yield attributes was recorded at 60 mg kg^{-1} in legume number (73.761%), in grain number (71.179%) and in total yield (92.326%). Among the genotypes, Mash 88 was the most productive for various yield attributes, while Mash ES-1 was least productive. The activity of antioxidant enzymes showed a linear correlation with metal concentration. Ascorbate peroxidase (APOX) increase was 11.611% at 40 mg kg^{-1}, 32.81% at 50 mg kg^{-1} and 49.503% at 60 mg kg^{-1}. Superoxide dismutase (SOD) increase was 14.911% at 40 mg kg^{-1}, 31.939% at 50 mg kg^{-1}) and 50.058% at 60 mg kg^{-1}. Catalase (CAT) increase was 7.092% at 30 mg kg^{-1}, 13.288% at 40 mg kg^{-1}, 18.239% at 50 mg kg^{-1} and 30.140% at 60 mg kg^{-1}. Superoxide dismutase and catalase activities were higher in genotypes Mash 88 as 7.451U mg/g and 34.544 U mg/g, respectively than that in other genotypes, while the least antioxidant activity was noted in Mash ES-1. These findings reflect the criteria for selection of the land regarding toxicity extent of Pb^{2+} to grow mash bean in future.

Keywords: Antioxidant enzymes, Genotypes, Lead, Mash bean, Toxicity, Yield

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Introduction

The metals having density more than 5 g/cm^3 are known as heavy metals (Morsy et al., 2012). In low pH soils, Heavy metals are considered to be readily soluble in water and easily flow through soil solution to plant (Delbari et al., 2019). Heavy metals are released into the environment and are a threat for sustainability of ecosystems. By contaminating the food chain it proves a source of toxicity not only for human health but also for the entire ecosystem functioning (Budijono, 2017; Ali & Khan, 2019). These metals contaminate soil, air and water which affect the plants, animals and humans health (Reis et al., 2010; Saha et al., 2016; Biswas et al., 2019; Aendo et al., 2020). Humans are exposed to Pb^{2+} by inhaling polluted air or by ingestion of contaminated food and water. Children are proved more sensitive to Pb^{2+} pollution because they can absorb five times more Pb^{2+} than adults (Christopher & Murray, 2015). Pb^{2+} accumulation in the body dysfunctions the organs including brain, kidney, liver and skeletal system (Canfield et al., 2005; Mohod & Dhote, 2013).

Main sources of Pb^{2+} emission are automobiles exhausts, fuel burning, industrial effluents, ores mining, fertilizer industries, municipal sewage sludge and pesticides (Tong et al., 2000; Sharma & Dubey, 2005). Lead (Pb) is rapidly increasing in agricultural soils (Hamid et al., 2010). Lead enters into water bodies by discharging of Pb^{2+} contaminated wastes into water (Renner, 2009; Mao et al., 2014; Zhang et al., 2018). Several methods are being used for the removal of heavy metals from water including chemical precipitation, membrane filtration, electrolysis, ion exchange and photocatalysis (Barakat, 2011; Eltayeb & Khan, 2019; Khan et al., 2019) filtration and adsorption, complexation of dry biomass (Calimli et al., 2019; Demirbas et al., 2019). Some of these methods have drawbacks of their high costs, less efficiency, and requirement of large amounts of chemicals. Therefore, there is a demand for developing low costs heavy metal treatment methods to remove heavy metals contamination (Rangreez et al., 2015).

Soil contamination by heavy metal is an important problem accounting for a reduction in plant yield. The

plants, when exposed to metal stress, have to adopt many internal changes as metal imposes oxidative stress on the plant (El-Soud, 2013). In plants, Pb interferes with many metabolic functions. It also affects germination, growth, dry biomass, nutrition uptake (Sharma and Dubey, 2005), photosynthesis and cell division (Ekmekci et al., 2009). Like other abiotic stresses, heavy metals cause the production of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide radical (Xu et al., 2008). ROS damages membrane lipids and can lead to the death of cells (Molassiotis et al., 2006). To protect from ROS, plants produce enzymatic antioxidants like catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), glutathione peroxidase (GSH-Px), peroxidase (POD), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR), as well as non-enzymatic antioxidants such as reduced glutathione (GSH) and ascorbate (AsA) (Asada, 1992). SOD is used for scavenging superoxide (O_2^-) radical (Reddy et al., 2005). H_2O_2 is scavenged by catalase. Peroxidases also scavenge H_2O_2 indirectly by combining it with antioxidant compounds such as ascorbic acid (Yingli et al., 2011).

The effects of Pb^{2+} stress has been studied on many plants including *Triticum aestivum* L. (Ekmekci et al., 2009), *Sesbania drummondii* (Venkatachalam et al., 2007), *Chenopodium album* L. and *Salsola passerine* (Hu et al., 2012), *Brassica juncea* and *Sesuvium portulacastrum* (Zaier et al., 2010). Although some plants have mechanisms for adaptation and tolerance of heavy metal, but heavy metals are toxic if their accumulation levels exceed the critical limit (Zhang et al., 2007). Mash bean [*Vigna mungo* (L.) Hepper] is an important pulse crop having potential of fixing atmospheric N_2 and thus enriches the soil with Nitrogen (Sen, 1996). It is used for food and fodder. It contains sufficient amounts of protein, fats, oil, carbohydrates and vitamins (James, 1981). For growing in polluted soil, the studies of biochemical responses of plants to environmental pollution. Keeping in view the value of mash bean [*Vigna mungo* (L.) Hepper] and considering the toxicity of ever increasing Pb $^{2+}$ concentration in environment, the present study was devised with the objective to find out the extent of lead (Pb) contamination in soil at which mash bean [*Vigna mungo* (L.) Hepper] plant can be grown for minimum loss of its production. Also among the tested genotypes to find out the susceptible and tolerant one to grow on Pb^{2+} contaminated soils.

Materials and Methods

A pot experiment was devised in quest of evaluation of yield attributes and antioxidant enzymes activity such as SOD, CAT and APOX in four [*Vigna mungo* (L.) Hepper] genotypes under various Pb^{2+} applied concentrations. Soil free from effluents hazards, after an initial survey, was selected. After drying in air it was ground, passed through 2mm sieve and mixed well. Seeds of four mash bean genotypes i.e. Mash 80, Mash 88, Mash 97 and Mash ES-1 were used in the experiment. The genotypes have their origin in Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan) and National Agricultural Research Centre (NARC) Islamabad (Pakistan). These were obtained from Pulse Section, Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan). For imposing metal pollution in soil, Nitrate of Lead (Pb) of Sigma Aldrich, Japan was used.

Experimental design and methodologies

Experiment was designed with complete randomization of treatments and genotypes to avoid unequal exposure of environmental factors. Each treatment was repeated four times. Soil was analyzed for physical and chemical properties according to methods described by Richards (1954) except otherwise mentioned. Only the reference number to each method is given here. Soil saturated paste (Method, 2a), saturation percentage (Method, 27a), soil saturation extract (Method, 27a), pH (Method, 27a), electrical conductivity (Method, 3a and 4b) and total metal concentration (Rowell, 1994). Pots of 30 cm diameter were filled with 10 kg sandy loam soils and lined with polyethylene bags ensuring seepage prevention. Seeds sterilized with 10% (V/V), Hydrogen peroxide, similar in size and weight of each genotype was germinated. After germination, thinning was performed to maintain one seedling in each pot in order to avoid the imbalanced uptake of nutrients by plants. After twenty days of germination, metals salts were applied in soil as a water solution of lead nitrate $(PbNO_3)_2$ (method similar to that used by Stoeva & Bineva (2003). Pots without the addition of metals salts acted as control. Lead (Pb) toxicity levels of 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 mg kg^{-1} soil were raised.

Yield attributes

For yield and its contributing factors, at the maturity of crop (90 days age) four plants per treatment in each replicate of all genotypes were randomly selected and number of legumes $plant^{-1}$, number of grains $fruit^{-1}$ and total yield $plant^{-1}$(g) were recorded

Enzymatic antioxidants

The data for antioxidant enzymes activity in fresh leaves was determined after twenty days of metal imposition.

Preparation of plant extracts

Each fresh plant sample (1.0 g) was crushed in a pestle and mortar using 3.0 mL of extractant buffer under ice cold conditions. The homogenate was centrifuged at 4 °C for 20 min at 13,000 rpm. The supernatant was used for analysis. The buffer for the estimation of APOX and CAT was 50 mM potassium phosphate buffer with pH 7.0 while for SOD activity sodium carbonate buffer of pH 10.2 was used.

Ascorbate peroxidase (APOX)

For estimation of APOX activity Nakano & Asada (1981) method was followed. Phosphate buffer (50 mM), 1 mM H_2O_2, 0.5 mM ascorbic acid and 70 µL of enzyme extract were mixed. The absorbance was recorded at 290 nm on

the spectrophotometer. One unit of ascorbate peroxidase was calculated as the amount of enzyme for oxidizing 1 µM of ascorbic acid min^{-1} g^{-1} fresh leaf. The APOX activity was expressed as UA mg g^{-1} protein

Catalase (CAT)

For estimation of catalase activity, Aebi (1984) method was followed. Reaction mixture (3 ml), 50 mM phosphate buffer, 15 mM H_2O_2, and 60 µL of extract were mixed. The absorbance was recorded for one minute on 240 nm at 25 °C. The enzyme required to release half peroxide oxygen was taken as a unit expressed as U mg g^{-1} protein.

Superoxide dismutase (SOD)

The superoxide dismutase activity was estimated by the method of Kono (1978). A mixture of 0.1 mM Ethylenediamine tetraacetic acid (EDTA), 50 mM sodium carbonate buffer, 24 µM Nitro Blue Tetrazolium (NBT), and 0.03% Triton X-100 were prepared. By addition of 1 mM hydroxylamine hydrochloride reaction was started. Added extract (70 µL), and the absorbance was noted at 560 nm for 2 min at 25 °C. The enzyme used to inhibit NBT reduction (up to 50%) was taken as one unit expressed as U mg g^{-1} protein.

Statistical analysis

The collected data were analyzed using the COSTAT computer package (CoHort Software, Berkeley, CA). At 5% level of probability, Duncan's New Multiple Range test was used to compare means (Duncan, 1955). Significant F values were tested by LSD tests at 0.05% significance level for testing their mean differences by using MSTAT-C Computer Statistical Programme (MSTAT Development Team, 1989).

Results

Number of legumes plant^{-1}

A definitive trend of reduction in number of legumes with increasing lead (Pb^{2+}) toxicity was observed (Table 1). Increasing amount of Pb^{2+} above 10 mg kg^{-1} appeared to be responsible for gradual and significant reduction in legume setting. The greatest promise, if the term may be used, in this fashion, in decreasing legume number by 73.761% was of 60mg kg^{-1} Pb^{2+} and minimum reduction (3.719%) was by 10 mg kg^{-1} Pb^{2+}. Individual genotypic responses also solidified this fact. However, the observations were excluded from the ongoing trends when 10 mg kg-1 Pb^{2+} increased the number of legumes in Mash 80 by 7.260% from untreated control plants. Among the genotypes, Mash 88 revealed maximum (8.416) and Mash ES-1 revealed minimum (6.500) value. Mash 97 differed from Mash 80 by 6.189 % over.

Number of grains fruit^{-1}

Plants grown in lead (Pb) contaminated soil produced a lesser number of grains (Table 2). Lead (Pb) established an inverse correlation between its concentration and grain development. Number of grains significantly decreased on exposure to concentrations of Pb^{2+}above than 10mg kg^{-1}. The maximum inhibitory effect of lead (Pb) (71.179%) was clear at 60mg kg^{-1} and minimum (3.153%) was by 10mg kg^{-1} concentrations. The augmentations of lead (Pb) effects could not be of suspected uniformity and an increase (7.127%) in grain frequency was observed by10 mg kg^{-1} in plants of MASH ES- 1. MASH 88 revealed maximum (5.408) and MASH ES-1 revealed minimum (5.018) value. MASH 88 differed significantly from MASH 80 by 4.643%.

Total yield plant^{-1}(g)

Lead (Pb) adversely affected the yield in a concentration dependent manner (Table 3). Yield was significantly affected in plants subjected to all levels of Pb^{2+}levels maximum (92.326%) being by 60 mg Pb kg-1 and minimum (6.823%) by 10 mg Pb kg^{-1} concentration. All the genotypes responded in a similar fashion to escalating levels of lead (Pb) toxicity. Of the genotypes, MASH 88 yielded maximum (2.613g) and MASH ES-1 revealed minimum (1.938g) production while the remaining genotypes lied between these two limits.

Ascorbate peroxidase (U mg/g protein)

Mean role of Pb^{2+}, after Duncan's Multiple Range test (Table 4), showed that APOX increased under the stimulus of Pb^{2+}. Lead (Pb) enhanced APOX in a concentration dependent manner statistically being effective from 40 to 60mg Pb^{2+}/kg soil concentrations. Mean and individual performance of genotypes to escalating levels of Pb^{2+} reflected 60 mg/kg concentration being optimum. Of all the concentrations, the three lower levels exerted decreasing influence on MASH 80 and MASH 88 by altering the APOX in a slightly descending way. So Pb^{2+} did not act only as a promoting agent but also as an inhibitory agent for its concentration. Statistically different sensitivity range to lead (Pb) was found in genotypes. Among the genotypes, MASH 97 was the most POD productive (46.366 U mg/g) and MASH-ES1 was the least productive (39.693 U mg/g). MASH 97 yielded 12.244% more APOX than that of MASH 80.

Catalase activity (U mg/g protein)

Table 5 reveals that Pb^{2+} produced catalase to an extent according to the levels of its concentration and induced statistically a substantial increase in CAT when applied in a concentration range of 30-60 mg/kg. The concentrations below this range did not reveal statistically any clear cut differences from lead untreated analogs. Lead concentration of 60 mg/kg was the optimum one causing a maximum increase (30.140%) while minimum (1.415%) one was caused by 10 mg Pb/kg concentration. So, 60 mg/kg concentration of lead, in this regard, was striking for the stimulation for catalase production. The response of all the genotypes showed similar fashion. Among the genotypes, Mash 88 revealed maximum (34.555 mg/g) and

Mash 80 revealed minimum (25.863 mg/g) and the rest of the two varieties differed equally from Mash 80.

Superoxide dismutase (U mg/g protein)

An exponential amplification of SOD by lead (Pb) treatment was recorded (Table 6). A definitive and statistically influential relationship of SOD and escalating lead (Pb) concentrations occurred from 40 to 60 mg/kg while the lower levels of lead (Pb) could not be justified statistically for their effect. Maximum (50.058%) increase in SOD was caused by 60 mg/kg and minimum (0.512%) was shown by 10 mg/kg concentration. 60mg/kg was optimum concentration which exerted the most important functioning in SOD induction in all the genotypes. SOD production got underway slightly by applying lower concentrations of lead (Pb) in Mash 80. Of the genotypes, Mash 88 revealed maximum (7.451) and Mash ES-1 revealed minimum (6.216 mg/g) Mash 97 had 6.820% less SOD than that of Mash 80.

Table 1 Number of legumes plant^{-1} of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Pb^{2+} (0, 10, 20, 30, 40 50 and 60mg Pb^{2+}/kg soil [values represent means ± SE]

Lead (mg kg^{-1} soil)	Mash 80	Mash 88	Mash 97	Mash ES-1	Treatment means
Control	23.00 ± 1.678	25.50 ± 1.568	23.50 ± 3.502	22.000±2.978	23.500^{a} ± 2.626
10	24.67 ± 1.626	24.334 ± 1.272	22.00 ± 1.218	19.500±1.134	22.626^{a} ± 2.452
	(-7.260)	(4.572)	(6.382)	(11.363)	(3.719)
20	21.00 ± 1.382	21.00 ± 1.382	18.67 ± 2.304	17.334±1.966	19.50^{b} ± 2.288
	(8.695)	(17.647)	(20.553)	(21.209)	(17.021)
30	16.67 ± 1.626	17.00 ± 1.274	14.164 ± 2.196	13.164±0.752	15.25^{c} ± 2.254
	(27.521)	(33.333)	(39.727)	(40.163)	(35.106)
40	10.50 ± 1.754	12.00 ± 0.762	10.50 ± 1.988	8.330±1.760	10.332^{d} ± 3.992
	(54.347)	(24.545)	(55.319)	(62.136)	(56.034)
50	7.994 ± 1.634	10.00 ± 1.632	8.50 ± 1.266	6.334±1.764	8.206^{e} ± 1.958
	(65.243)	(60.784)	(63.829)	(71.209)	(65.080)
60	6.33 ± 1.676	8.00 ± 1.718	6.00 ± 1.718	4.334±1.152	6.166^{f} ± 1.952
	(72.478)	(68.627)	(74.468)	(80.300)	(73.761)
Genotype means	15.736^{b} ± 7.21	16.832^{a} ± 6.746	14.762^{c} ± 6.698	13.00^{d} ± 6.674	15.082 ± 6.888
		(-6.964)	(6.189)	(17.386)	

Values represent means ± SE; Values in parentheses represent % increase (+)/ decrease (-) over control or over Mash 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes

Table 2 Number of grains fruit^{-1} of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Pb^{2+} (0, 10, 20, 30, 40 50 and 60mg Pb^{2+}/kg soil [Values represent means ± SE]

Lead (mg kg^{-1} soil)	Mash 80	Mash 88	Mash 97	Mash ES-1	Treatment means
Control	7.657 ± 0.717	7.345 ± 0.421	7.795 ± 1.098	7.015 ± 0.903	7.453^{a} ± 0.799
10	7.045 ± 0.445	7.290 ± 0.348	7.025 ± 0.347	7.515 ± 0.436	7.218^{a} ± 0.411
	(7.992)	(0.748)	(9.878)	(-7.127)	(3.153)
20	6.602 ± 0.621	6.765 ± 0.775	6.560 ± 0.318	5.772 ± 0.781	6.425^{b} ± 0.705
	(13.778)	(7.896)	(15.843)	(17.710)	(13.793)
30	5.077 ± 0.406	6.355 ± 0.450	5.227 ± 0.393	5.262 ± 0.174	5.480^{c} ± 0.622
	(33.694)	(13.478)	(32.944)	(24.989)	(26.472)
40	4.737 ± 0.376	5.015 ± 0.415	4.710 ± 0.559	4.535 ± 0.468	4.749^{d} ± 0.448
	(38.135)	(31.722)	(39.576)	(35.350)	(36.280)
50	3.045 ± 0.445	3.055 ± 0.270	3.365 ± 0.644	3.025 ± 0.173	3.122^{e} ± 0.405
	(60.232)	(58.407)	(56.831)	(56.878)	(58.110)
60	2.015 ± 0.438	2.540 ± 0.357	2.035 ± 0.452	2.005 ± 0.356	2.148^{f} ± 0.430
	(73.684)	(65.418)	(73.893)	(71.418)	(71.179)
Genotype means	5.168^{b} ± 2.028	5.408^{a} ± 1.925	5.245^{ab} ± 2.015	5.018^{b} ± 1.949	5.228 ± 1.960
		(-4.643)	(-1.489)	(2.902)	

Values represent means ± SE; Values in parentheses represent % increase (+)/ decrease (-) over control or over Mash 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes

Discussion

Yield and its contributing factors decreased with increasing metal concentration. Such findings regarding reduction in number of legumes and yield by metal treatment have been reported previously by many researchers (Salim et al., 1992; Khoshgoftarmanesh & Kalbasi, 2002; Singh et al., 2006). In the life of a plant, the shifting of floral primordia into flower comprises phases like flower induction, development, fruit setting, fruit development and seed development stages. The reduction in yield can be due to inactivation of photosynthetic enzymes, nutrition deficiency or water balance disturbance (Sharma & Dubey, 2005). Heavy metal imposition alters the ionic balance of soil and plant including micro and macronutrients of the plant. The change in nutrients status might be the cause of reduction in yield attributing factors by interfering with floral developmental stages (Hayati et al., 1995; Chaudhry et al., 2006).

Table 3 Total yield plant^{-1}(g) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Pb^{2+} (0, 10, 20, 30, 40 50 and 60mg Pb^{2+}/kg soil [Values represent means ± SE]

Lead (mg kg^{-1} soil)	Mash 80	Mash 88	Mash 97	Mash ES-1	Treatment means
Control	8.980 ± 1.122	9.530 ± 0.588	9.410 ± 2.292	7.844 ± 1.316	8.540^{a} ± 1.490
10	8.870 ± 0.938	9.030 ± 0.732	7.964 ± 0.512	7.454 ± 0.282	8.330^{b} ± 0.894
	(1.224)	(5.246)	(15.366)	(4.971)	(6.823)
20	6.930 ± 0.942	7.120 ± 0.670	6.140 ± 0.882	5.010 ± 0.1012	6.300^{c} ± 1.668
	(22.828)	(25.288)	(34.750)	(36.129)	(29.530)
30	4.260 ± 0.618	5.374 ± 0.598	3.714 ± 0.744	3.524 ± 0.294	4.218^{d} ± 0.910
	(52.561)	(43.609)	(60.531)	(55.073)	(52.818)
40	2.510 ± 0.182	2.970 ± 0.130	2.554 ± 0.740	1.824 ± 0.288	2.464^{e} ± 0.562
	(72.048)	(68.835)	(72.858)	(76.746)	(72.438)
50	1.244 ± 0.292	1.530 ± 0.204	1.444 ± 0.374	0.894 ± 0.288	1.292^{f} ± 0.350
	(86.146)	(83.945)	(84.654)	(88.602)	(85.548)
60	0.594 ± 0.126	1.034 ± 0.288	0.590 ± 0.164	0.524 ± 0.134	0.686^{g} ± 0.270
	(93.385)	(89.150)	(93.730)	(93.319)	(92.326)
Genotype means	5.770^{a} ± 3.378	5.226^{ab} ± 3.316	4.544^{b} ± 3.302	3.876^{c} ± 2.886	4.604 ± 3.220
		(-9.559)	(4.737)	(37.610)	

Values represent means ± SE; Values in parentheses represent % increase (+)/ decrease (-) over control or over Mash 80 for genotypes means. Values followed by dissimilar letters are different at P = 0.05 among means of treatments and genotypes

Ahanger et al. (2020) reported that variations in macronutrient levels may influence the energy balance and nutrient status of plants. Metal pollution may also decrease the yield and its components by creating nutritional deficiency at the stages of flower initiation, its development and fruit setting. Yield reduction might be the result of lowered photosynthesis and reduction in chlorophyll contents. Metal stress might have decreased the synthesis of chlorophyll and the photosynthetic efficiency of plants (Khan et al., 2015; Ahmad et al., 2018). Under stressful conditions, the chlorophyll breakdown is enhanced due to the up-regulation of the enzymes chlorophyllase (Dalal, 2012). Metal induces reduction in leaf area which in turn can lower the photosynthesis and ultimately reduces yield contributing factors. Leaf area reduction can be due to growth inhibition in metal treated plants (Pascual, 2009; Amalia et al., 2011). Reduced growth and biomass accumulation due to metal stress has earlier been reported by Ahmad et al. (2018). This Inhibition of growth may be due to reduced cell elongation and division after heavy metal imposition (Arduini et al., 1994).

Metal treatment lowers cytokinin contents which maintains stomatal opening (Bengtson et al., 1979). Decreased cytokinin level also creates nutrient deficiency like phosphorus in shoots (Thorsteinsson & Eliasson, 1990; Gniazdowska & Rychter, 2000). The decreased cytokinin levels might have decreased the nitrate reductase activity as well (Bueno et al., 1994). Yield reduction can be attributed to a reduced activity of nitrate reductase enzyme directly related to heavy metal stress and sink limitations. The decline in nitrate reductase activity due to stress is reported by Ahanger et al. (2020). The sink strength determines whether a flower will abscise or form a pod (Brun & Betts, 1984). The metal stress condition induces drought due to osmotic stress. The grain filling stage is regulated by four enzymes, i.e. starch synthase, sucrose synthase, starch branching enzyme, and adenosine diphosphate glucose pyrophosphorylase (Taiz & Zeiger, 2006). A reduction in the activity of these enzymes has been reported under the stress conditions having a negative impact on the yield (Ahmadi & Baker, 2001). The drought stress at the flowering stage may result in plant sterility (Yadav et al., 2004). Drought also decreases the rate of photosynthesis (Flexas et al., 2004) and leaf development (Rucker et al., 1995). The exposure of plant to drought conditions at flowering stage resulted in yield loss (Anjum et al., 2011). In cereals a significant reduction in the grain yield due to water stress may induce reduction in fertility of tillers and weight of grains (Samarah, 2005). Zeng et al. (2007) reported that lead accumulation in plants contaminated soil negatively affected the yield.

ROS are produced under metal stress conditions (Hussain et al., 2020). Ahmad et al. (2015); Ahanger et al. (2018) also reported increased ROS production in heavy metal stressed plants. Increased antioxidative enzymes such as SOD, CAT and POD were observed in leaf tissues of metal treated plants. Superoxide dismutase (SOD) is a prime defense ROS. Superoxide free radicals produced in cells act as precursors for many ROS (Alscher & Erturk, 2002). Enhancement of SOD may be ascribed to the production of more ROS or due to genes expression which encode SOD synthesis. The activities of CAT and POD were enhanced upon Pb exposure. Catalase is also involved in the main defense system against toxicity of ROS and CAT may play a key role in decreasing H_2O_2 levels in plant cells. CAT enzymes break down H_2O_2 to form water and oxygen. Low production of CAT at low concentrations of Pb probably is by inactivation of enzyme because ROS binds to the heme group of enzyme (Willekens et al., 1997).

Peroxidase (POD) eliminates ROS. Enhanced POD activity reveals its adaptation of plant to severe Pb metal stress. POD detoxifies H_2O_2 proxy radicals quenching (Radotic et al., 2000). POD decreases H_2O_2 by generating phenoxy compounds using lignin biosynthesis (Hu et al., 2012). These results are in agreement with other reports showing the positive effects of heavy metal treatment on antioxidative defense systems (Hou et al., 2007; Jin et al., 2008).

Table 4 Ascorbate Peroxidase Activity (APOX) (Umg/g^{-1}) of mash [*Vigna mungo* (L.) Hepper] grown in Pb^{2+} (0, 10, 20, 30, 40 50 and 60 mg Pb^{2+}/kg soil [Values represent means ± SE]

Lead (mg kg^{-1} soil)	Mash 80	Mash 88	Mash 97	Mash ES-1	Treatment means
Control	37.985 ± 3.120	40.312 ± 2.570	39.804 ± 4.308	33.180 ± 3.308	37.820^{d} ± 3.488
10	37.732 ± 2.370	39.043 ± 3.110	41.936 ± 3.299	33.941 ± 2.756	38.163^{cd} ± 3.098
	(-0.666)	(-3.148)	(5.356)	(2.293)	(0.922)
20	34.703 ± 2.206	35.278 ± 3.546	41.809 ± 2.878	34.491 ± 2.599	36.570^{d} ± 3.656
	(-8.640)	(-12.487)	(5.037)	(3.951)	(-3.305)
30	35.828 ± 3.726	39.525 ± 3.709	45.109 ± 3.334	41.750 ± 2.801	40.553^{cd} ± 3.567
	(-5.678)	(-1.952)	(13.327)	(25.829)	(7.226)
40	40.963 ± 3.870	48.662 ± 3.645	43.104 ± 2.901	36.225 ± 2.611	42.238^{c} ± 3.602
	(7.840)	(20.713)	(8.290)	(9.177)	(11.681)
50	44.813 ± 2.695	55.032 ± 3.639	54.245 ± 3.501	46.826 ± 3.309	50.229^{b} ± 3.609
	(17.975)	(36.515)	(36.280)	(41.127)	(32.810)
60	57.147 ± 4.137	59.025 ± 3.567	58.560 ± 2.701	51.437 ± 2.894	56.542^{a} ± 3.460
	(50.446)	(46.420)	(47.121)	(55.024)	(49.503)
Genotype means	41.310^{b} ± 4.110	45.268^{a} ± 4.169	46.366^{a} ± 3.989	39.693^{b} ± 3.789	
		(0.581)	(12.244)	(-3.914)	

Values represent means ± SE; Values in parentheses represent % increase (+)/ decrease (-) over control or over Mash 80 for genotypes means. Values followed by dissimilar letters are different at P = 0.05 among means of treatments and genotypes

Table 5 Catalase Activity (CAT) (U mg/g^{-1}) of mash [*Vigna mungo* (L.) Hepper] grown in Pb^{2+} (0, 10, 20, 30, 40 50 and 60 mg Pb^{2+}/kg soil [Values represent means ± SE]

Lead (mg kg^{-1} soil)	Mash 80	Mash 88	Mash 97	Mash ES-1	Treatment means
Control	28.290 ± 1.681	31.365 ± 1.561	28.905 ± 3.509	27.062 ± 2.871	28.905^{e} ± 2.621
10	28.291 ± 1.589	32.182 ± 1.747	29.522 ± 1.958	27.262 ± 1.477	29.314^{de} ± 2.181
	(0.003)	(2.605)	(2.134)	(0.739)	(1.415)
20	27.060 ± 1.445	33.412 ± 1.981	30.135 ± 1.738	28.492 ± 0.639	29.774^{de} ± 2.410
	(-4.348)	(6.526)	(4.255)	(5.284)	(3.006)
30	30.750 ± 3.377	33.621 ± 2.356	29.520 ± 1.628	29.931 ± 2.069	30.955^{cd} ± 2.568
	(8.695)	(7.193)	(2.128)	(10.601)	(7.092)
40	28.492 ± 2.387	34.846 ± 1.383	33.006 ± 1.378	34.642 ± 1.655	32.746^{bc} ± 2.663
	(0.714)	(11.098)	(14.188)	(28.010)	(13.288)
50	30.135 ± 1.487	36.900 ± 3.559	35.264 ± 1.629	34.412 ± 1.749	34.177^{b} ± 2.529
	(6.522)	(17.647)	(22.000)	(27.160)	(18.239)
60	37.515 ± 3.044	39.562 ± 2.129	37.926 ± 1.359	35.466 ± 1.746	37.617^{a} ± 2.288
	(32.609)	(26.134)	(31.209)	(31.055)	(30.140)
Genotype means	25.683^{c} ± 3.347	34.555^{a} ± 2.917	32.040^{b} ± 3.189	31.038^{bc} ± 3.181	31.891 ± 3.411
		(34.544)	(24.752)	(20.850)	

Values represent means ± SE; Values in parentheses represent % increase (+)/ decrease (-) over control or over Mash 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes

Table 6 Superoxide Dimutase (SOD) (U mg/g^{-1}) of mash [*Vigna mungo* (L.) Hepper] grown in Pb^{2+} (0, 10, 20, 30, 40 50 and 60 mg Pb^{2+}/kg soil [Values represent means ± SE]

Lead (mg kg^{-1} soil)	Mash 80	Mash 88	Mash 97	Mash ES-1	Treatment means
Control	6.105 ± 0.561	6.367 ± 1.126	6.413 ± 0.307	5.309 ± 0.622	6.049^{d} ± 0.721
10	6.038 ± 0.176	6.673 ± 0.639	6.154 ± 0.568	5.456 ± 0.398	6.080^{d} ± 0.564
	(-6.097)	(4.806)	(-4.038)	(2.769)	(0.512)
20	5.553 ± 0.084	6.586 ± 0.234	6.710 ± 0.171	5.522 ± 0.354	6.093^{d} ± 0.481
	(-0.042)	(3.440)	(4.631)	(4.012)	(0.727)
30	5.974 ± 0.851	7.195 ± 0.651	6.637 ± 1.052	5.017 ± 0.395	6.206^{cd} ± 0.777
	(-2.146)	(13.004)	(3.493)	(-5.500)	(2.595)
40	6.530 ± 0.879	6.932 ± 0.428	7.670 ± 0.767	6.673 ± 0.276	6.951^{c} ± 0.785
	(6.961)	(8.874)	(19.601)	(25.692)	(14.911)
50	7.115 ± 0.386	8.658 ± 0.729	8.727 ± 0.759	7.424 ± 0.643	7.981^{b} ± 0.804
	(16.544)	(35.982)	(36.083)	(39.838)	(31.939)
60	10.108 ± 0.613	9.749 ± 0.669	8.339 ± 0.544	8.113 ± 0.394	9.077^{a} ± 0.547
	(65.569)	(53.117)	(30.033)	(52.816)	(50.058)
Genotype means	6.774^{b} ± 1.217	7.451^{a} ± 1.079	7.236^{a} ± 1.154	6.216^{b} ± 1.145	
		(9.994)	(6.820)	(-8.237)	

Values represent means ± SE; Values in parentheses represent % increase (+)/ decrease (-) over control or over Mash 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes

Conclusion

Various concentrations of Pb^{2+}decreased yield components to a significant extent. The genotypes of the *Vigna* significantly differed in their responses to Pb^{2} stress. Mash 88 proved as the most productive while Mash ES-1 was the least productive. An adaptation mechanism to Pb stress was observed for *Vigna* plants, especially at higher doses

of heavy metal by antioxidant enzymes production. Plants adapted to higher doses of Pb treatment and the level of antioxidative enzymes was enhanced significantly. Increased SOD, CAT and POD activity is supposed to influence the antioxidant defense response of plants when exposed to Pb^{2+}toxicity. These findings show that enhanced antioxidant enzyme mechanisms heavy metal stress could help to overcome metal toxicity by ROS detoxification.

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