RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 10: 1152-1158 (October 2021)

Indole Acetic Acid (IAA) Mediated Amelioration of Lead (Pb) Stress- Physiological Indices of Mung Bean [*Vigna radiata* (L.) Wilczek]

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10.18805/LR-630

ABSTRACT

Background: Heavy metals have their adverse effects on growth and physiology of plant. Plant growth regulators help in improving the growth and physiological phenomenon in plants. A pot culture experiment was devised to explore the ameliorative potential of Indole Acetic Acid (IAA) for toxicity of rhizospheric lead (Pb) on two varieties of Mung bean [*Vigna radiate* (L.) Wickzek].

Methods: Seeds of two varieties *i.e.*, M- 8 and MN-92 were grown in earthen pots filled with sandy loam soil and were arranged under complete randomization. Fifteen days after germination, the lead (Pb) was added @ 10mg/kg and 20mg/kg soil as solution Pb NO_3. Indole Acetic Acid @100.0mM was foliarly sprayed twice at 15 and 30 days of plants emergence. Physiological parameters *i.e.*, Photosynthetic Rate, Transpiration Rate, Stomatal conductance, Sub Stomatal CO_2 Concentration and biomass production in the form of stem, root and leaf dry weights were determined at the age of physiological maturity for three replicates.

Result: By application of IAA, photostnthetic rate reduction was declined from 24.61% to 17.78% under 10mg Pb stress and from 55.54% to 27.35% under 20mg Pb stress. Stomatal conductance reduction was declined from 0.56% to 0.28% under 10mg Pb stress and from 3.37% to 1.68% under 20mg Pb stress. Alleviation of Pb stress by IAA for transpiration rate was non significant. Similarly, the role of IAA for alleviation of Pb stress in term of dry weights of stem,root and leaves were non significant statistically.

Key words: Biomass, Indole acetic acid, Lead, Mung, Photosynthetic rate, Stomatal conductance, Transpiration rate.

INTRODUCTION

Rapid increase in trends of urbanization and industrialization, has lead to more heavy metals in the environment during the past decades (Ashraf *et al*., 2019). The origins of these heavy metals are natural or anthropogenic activities (Pichtel, 2016). The uses of agricultural fertilizers and pesticides, sewage sludge, mining and smelting of metal ores and fossil burnings are the main sources of heavy metals. Plant growth hormones are the phytochemicals which can compensate the adverse effects of heavy metals on plants. Auxins are the phytohormones which have their effects on different phenomenon of plant life like cell elongation, cell division, cell differentiation and expansion of leaf. Auxins induce antioxidant enzymes activities such as catalase and peroxidase. The major hormone among the auxins which is present in plant is Indole acetic acid (Jutta, 2000). Plant hormones have been reported to increase plant yield (Savita *et al*., 2011) and stomatal movement (Petri *et al*., 2010). Plant growth regulators have become commercialized in many countries like EU, USA and Japan due to their positive influence on plant growth and yield (Jahan *et al*., 2019). Mung bean [*Vigna radiata* (L.) R. Wilczek] has its origin in 1,500 BC in subcontinent and was thereafter introduced to the continents of Asia, Africa, Australia and America. In Pakistan, Mung bean is cultivated on an area of 141,000 hectares with mean annual yield of 93,000 tons (Pakistan Economic Survey, 2012). Keeping in view the importance of mash bean, hazardous effects of heavy metals and role of IAA, the present experiment was designed with objective to find out the ameliorating potential of IAA for Pb toxicity on mash bean.

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How to cite this article: Saleem, S., Yasin, G., Haq, I.U., Altaf, A., Hussain, K. and Nawaz, K. (2021). Indole Acetic Acid (IAA) Mediated Amelioration of Lead (Pb) Stress- Physiological Indices of Mung Bean [*Vigna radiata* (L.) Wilczek]. Legume Research. 44(10): 1152-1158. DOI: 10.18805/LR-630.

Submitted: 13-05-2021 **Accepted:** 26-06-2021 **Online:** 28-06-2021

MATERIALS AND METHODS

The experiment was designed to find the ameliorating potential of indole acetic acid (IAA) for stress effects of rhizospheric lead (Pb) toxicity on two mung bean [*Vigna radiata* (L.) Wickzek] varieties. The pot culture experiment was conducted in Bahauddine Zakariya University, Multan Pakistan during 2016-17. Effluents hazards free sandy loam soil was selected for the experiment. Soil analysis revealed that the Ec of soil was 1.5 mM, pH 6.5 and saturation percentage was 56%. Earthern pots of 30 cm diameter were filled with 6 kg soil after mixing it well. Mung bean seeds of two varieties i-e M-8 and MN-92 varieties were obtained from Ayub Agriculture Research Institute, Faisalabad (Pakistan). Lead nitrate salt and IAA of Sigma Aldrich, Japan were purchased. Pots after filling were irrigated and left till getting of field capacity moisture contents. After germination normal agronomic practices like irrigation and two times pesticide spray of 5% Thiodon were performed. Arrangement of pots was complete randomization by design. To develop the metal stress, amounts of lead nitrate was added after calculation to develop lead @10 and 20 mg/kg soil. Control pots were left without addition of salt. Solutions of IAA (100.0 mM) were sprayed at the age of fifteen and thirty days of age.

Treatment plan consisted of following six treatments.

T_1 = Normal soil (Without addition of metal salts) +Distilled water foliar spray.
T_2 = Normal soil (Without addition of metal salts) + 100.0 mM IAA foliar spray.
T_3 = Lead (10.0 mg/kg soil) + Distilled water foliar spray.
T_4 = Lead (20.0mg/kg soil) + Distilled water foliar spray.
T_5 = Lead (10.0 mg/kg soil) + 100.0 mM IAA foliar spray.
T_6 = Lead (20.0mg/kg soil) + 100.0 mM IAA foliar spray.

There were five pots for each treatment. After thinning, three plants were maintained in each pot. Gas exchange parameters of leaf such as photosynthetic rate (A), internal CO_2 (Ci) concentration, stomatal conductance (gs), water use efficiency (A/E) and transpiration rate (E) were measured from youngest fully expanded leaf specified from the top of each plant which has a mean leaf area of 4.72 cm^2. For this, an open system LCA-4 ADC portable InfraRed gas analyzer (IRGA) of Analytical Development Company, Hoddesdon, England was used. Timing of measurements were from 11.00 a.m to 1.00 p.m. Followings specifications were adjusted: Molar flow of air was 403.3 mmol $m^{-2}s^{-1}$ per unit leaf area, 99.9 kg Pa atmospheric pressure, 6.0 to 8.9 mbar water vapor pressure of chamber, photon flux density at the surface of was 1711 µmol/m^2/s, leaf temperature range was 28.4-32.4°C and external CO_2 concentration was 370 mmol/ mol.; Biomass production as stem, root and leaf dry weight were evaluated after 10 days of PGRs spray completion (40 days old plants). Biomass was determined with electrical balance after drying samples for 48 hours at 80°C. The data of experiment were analyzed statistically using software of COSTAT computer package. Keeping significance level of 5%, Duncan's multiple range test was applied comparing mean values (Duncan, 1955). MSTAT-C Computer Statistical Programm was used. Wherever, F values were significant for testing of means using LSD tests.

RESULTS AND DISCUSSION

The analysis of variance (Table 1) related to photosynthetic rate showed that significant differences were found among different treatments and varieties. Responses of varieties to treatment showed non-significant differences separately. IAA treatment caused an increase in photosynthetic rate (23.18%). Low and high lead doses decreased the photosynthetic rate by a value of 24.61 and 55.54% respectively. IAA ameliorated the lead stress so growth was reduced by a value of 17.78 and 27.35% in 10 and 20 mg lead treated plants (Table 2).

The analysis of variance of transpiration rate (Table 1) showed that treatments differed non significantly and separate response of both variety to each treatment differed non significantly. While significant differences were observed between two varieties. Increase in transpiration rate by the application of IAA was (8.02%). Lead toxicity of low and high levels decreased the transpiration rate 2.87 and 14.12% respectively. IAA ameliorates the lead toxicity and metal effects on transpiration rate were reduced to values of 1.73 and 7.28% respectively (Table 3).

The analysis of variance (Table 1) showed that stomatal conductance significantly affected by different treatments. Results revealed that varieties and treatments were differed highly significantly. Separate responses of varieties to treatments revealed non-significant differences. Stomatal conductance was increased by exogenous IAA spray as 1.96%. Low and high lead treatments decreased the stomatal conductance 0.56 and 3.37% respectively. IAA application decreased the harmful effect of lead therefore effects of metal on stomatal conductance were lowered to values of 0.28 and 1.68% respectively (Table 4).

Table 1: Mean sum of squares for photosynthetic rate, transpiration rate, stomatal conductance, sub stomatal CO_2 concentration, Stem dry weight, root dry weight and leaf dry weight of mung bean [*Vigna radiata* (L.) Wickzek].

Source	df	MSS						
		Photosynthetic rate	Transpiration rate	Stomatal conductance	Internal CO_2 concentration	Stem dry weight	Root dry weight	Leaf dry weight
Treatment (T)	5	43.814***	0.234^{ns}	241.577 *	551.977^{ns}	0.924^{ns}	0.254^{ns}	0.204^{ns}
Variety (V)	1	34.251*	3.540*	224.044 ***	0747.111^{ns}	0.511^{ns}	0.138^{ns}	0.012^{ns}
T × V	5	2.387^{ns}	0.004^{ns}	32.177^{ns}	68.311^{ns}	0.007^{ns}	0.001^{ns}	7.427^{ns}
Error	24	4.908	0.720	66.941	260.916	0.405	0.777	0.662
Total	35							

***= Highly significant; *= Significant; ns= Not significant.

Table 2: Means for photosynthetic rate [A; µmol (CO_2) m^{-2} sec^{-1}] of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD=3.761).

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.955)
MN-92	9.280±0.860	10.266±2.039	6.833±1.150	4.000±0.953	7.951±3.121	6.500±1.350	7.435±2.339 [b]
% age difference		+10.62	-26.36	-56.89	-14.32	-29.95	
M-8	10.999±4.549	17.700±3.825	8.400±1.084	4.911±1.081	9.061±3.184	8.190±0.397	9.385±3.878 [a]
% age difference		+60.92	-23.63	-55.35	-17.61	-25.53	+26.22
Treatment mean (LSD=1.601)	10.135±3.074 [ab]	12.485±3.663 [a]	7.640±1.334 [b]	4.506±1.015 [c]	8.332±2.343 [b]	7.363±1.272 [b]	9.073±3.170
% age difference		+23.18	-24.61	-55.54	-17.78	-27.35	

Table 3: Means for transpiration rate [E; mmol (H_2O) m^{-2} sec^{-1}] of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD=3.761).

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.955)
MN-92	1.680±0.336	1.910±0.667	1.600±0.419	1.400±0.450	1.664±0.127	1.540±0.670	1.644±0.436 [b]
% age difference		+13.69	-4.76	-16.66	-1.19	-8.33	
M-8	2.350±1.095	2.446±1.385	2.250±0.802	2.000±1.101	2.300±1.374	2.210±1.014	2.271±0.930 [a]
%age difference		+4.08	-4.25	-14.89	-2.12	-5.95	+38.13
Treatment mean (LSD=1.601)	2.018±0.628	2.180±1.014	1.960±0.663	1.733±0.836	1.983±0.939	1.871±0.849	1.985±0.784
% age difference		+8.02	-2.87	-14.12	-1.73	-7.28	

The analysis of variance of sub stomatal CO_2 concentration (Table 1) showed that varieties, treatment response of each variety to treatments differed non significantly. Low and high lead treatments decreased the sub stomatal CO_2 concentration 1.70 and 5.98% respectively. IAA treatment increased the sub stomatal CO_2 concentration (1.70%). IAA alleviated the toxic effect of lead and CO_2 concentration reductions were 0.56 and 3.41% respectively under low and high levels of stresses (Table 5).

Reduction in photosynthetic rate, CO_2 assimilation and transpiration rate might be due to decline in water potential (Atteya, 2002). Water contents in metal treated plants are decreased (Poschenrieder *et al.* 1989) due to increasing resistance in water flow (Barcelo *et al.,* 1988) or alteration of cell wall properties by metal (Poschenrieder *et al.,* 1989). Conduction and transport of water is influenced in root by toxic metals (Barcelo and Poschenreider, 1990). Photosynthetic reduction might be due to chlorophyll decrease by metal. Hampp *et al.* (1974) have shown that enzymes of chlorophyll biosynthesis like 6-amino laevulinic acid dehydratase and porphobilinogenase are affected in by metal treatment. Lead is also found to depress the rate of photosynthesis (Carlson *et al.,* 1975). Decrease in activities of many enzymes of CO_2 fixation (Barcelo *et al.*, 1988); changes in the thylakoid structure (Fodor *et al.,* 1996) may contribute to reduction in photosyntheticactivity and growth. Reduction in plant growth perhaps is conducive to Reactive oxygen species (ROS) production as heavy metals toxicity as an efficient generator of toxic ROS inhibits photosynthetic ETC (Kappus, 1985).

Table 4: Means for stomatal conductance [gs; mmol (CO_2) m^{-2} sec^{-1}] of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD = 15.57).

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.989)
MN-92	349±6.082	360±8.736	345±8.717	337±3.124	374±3.786	340±24.006	346±7.437 [b]
% age difference		+0.27	-1.14	-3.43	-0.57	-2.57	
M-8	365±1.527	366±1.154	363±1.527	351±1.527	364±1.527	361±1.527	361±10.940 [a]
% age difference		+0.27	-0.54	-3.83	-0.27	-1.09	+4.33
Treatment mean (LSD=1.713)	356±9.786 [ab]	363±6.870 [a]	354±11.178 [abc]	344±8.342 [c]	355±10.014 [ab]	350±19.072 [bc]	354±12.207
% age difference		+1.96	-0.56	-3.37	-0.28	-1.68	

Table 5: Means for sub stomatal CO_2 concentration (Ci; µmol. mol^{-1}) of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD = 27.220).

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=11.112)
MN-92	349±5.567[ab]	355±10.440	343±16.502	319±22.810	346±1.732	333±20.223	341±17.399
% age difference		+1.71	-1.71	-8.59	-0.85	-4.58	
M-8	355±24.111	360±20.000	348±18.193	340±9.073	352±9.814	346±17.156	350±15.793
% age difference		+1.40	-1.97	-4.22	-0.84	-2.53	+2.63
Treatment mean (LSD=1.713)	351±15.811	357±14.529	345±15.715	330±19.322	349±17.280	339±18.293	345±16.996
% age difference		+1.70	-1.70	-5.98	-0.56	-3.41	

Table 6: Means for stem dry weight (g) of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD=1.072).

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg + Distilled water spray	Pb10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mMean (LSD=0.437)
MN-92	2.033±0.970	2.21±0.785	1.473±0.596	1.206±0.663	1.760±0.620	1.513±0.685	1.70±0.701
% age difference		+8.70	-27.54	-40.67	-13.42	-25.57	
M-8	2.313±0.455	2.516±0.484	1.700±0.300	1.366±0.550	2.02±0.670	1.75±0.607	1.946±0.564
% age difference		+8.77	-62.03	-40.94	-12.66	-24.34	+13.93
Treatment mean (LSD=0.758)	2.173±0.695	2.363±0.607	1.586±0.439	1.313±0.548	1.893±0.595	1.635±0.594	1.82±0.632
% age difference		+8.74	-27.01	-39.57	-12.88	-24.75	

Table 7: Means for root dry weight [g] of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD = 1.810).

	No metal + Distilled water spray	No metal + IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.989)
MN-92	1.822±0.871	1.930±1.000	1.544±0.691	1.392±0.911	1.676±0.794	1.555±0.794	1.644±0.732
% age difference		+5.92	-15.25	-23.60	-8.01	-14.65	
M-8	1.892±1.033	2.111±0.92	1.674±0.911	1.500±0.791	1.811±0.999	1.677±0.792	1.771±0.792
% age difference		+11.57	-11.52	-20.71	-4.28	-11.36	+7.72
Treatment mean (LSD=1.713)	1.862±0.851	2.021±0.862	1.600±0.722	1.441±0.764	1.741±0.800	1.612±0.711	1.711±0.750
% age difference		+8.53	-14.07	-22.61	-6.49	-13.42	

Table 8: Means for leaf dry weight (g) of mung bean [*Vigna radiata* (L.) Wickzek] plants grown under lead stresses and exposed to foliar spray of IAA (LSD=1.371).

	No metal + Distilled water spray	No metal + IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.955)
MN-92	1.796±0.800	1.900±0.900	1.546±0.690	1.396±0.850	1.676±0.820	1.546±0.691	1.605±0.720
% age difference		+5.79	-13.91	-22.27	-6.68	-13.91	
M-8	1.766±0.850	1.836±0.901	1.513±0.785	1.330±0.923	1.663±0.765	1.520±0.751	1.642±0.690
% age difference		+3.96	-14.32	-24.68	-5.83	-13.93	+2.30
Treatment mean (LSD=1.601)	1.781±0.738	1.868±0.805	1.530±0.661	1.365±0.794	1.666±0.709	1.533±0.646	1.624±0.695
% age difference		+4.88	-14.09	-23.35	-6.45	-15.92	

The data related to analysis of variance of stem dry weight (Table 1) showed that varieties, treatments and response of individual variety to every treatment differed non significantly. Foliar application of IAA non significantly increased the stem dry weight in two varieties (8.74%). Low and high lead treatments decreased the stem dry weight by values of 27.01 and 39.57% respectively. The lead toxicity was decreased due to foliar application of IAA and dry weight reductions were by values of 12.88 and 24.75% respectively (Table 6).

The analysis or variance of root dry weight (Table 1) showed that treatments, varieties and separate response of varieties to treatment differed non significantly. Application of IAA increased the root dry weight (8.53%). Low and high lead doses decreased the root dry weight 14.07and 22.61% respectively (Table 7). The analysis of variance of leaf dry weight (Table 1) revealed that varieties, treatments and individual response of all varieties to treatment were different to non significantly degree. Indole Acetic Acid application increased the leaf dry weight upto 4.88% (Table 8). Low and high lead treatments decreased the leaf dry weight 14.09 and 23.35% respectively. Application of IAA alleviated the lead stress. Therefore, leaf dry weight reductions were up to 6.45 and 15.92% respectively (Table 8). Biomass of plant organs decreased due to metal stress (Table 6-8). Such type of findings are reported also by Ouariti *et al.* (1997) and Fengxiang *et al*. (2003). Plant growth and plant water contents have relations with plant weights and biomass. Decline in biomass might be due to Inhibition of both cell elongation and division by heavy metals (Arduini *et al*., 1994) or due to reduction in nitrogen contents (Andrews *et al*., 1999). Decrease in dry mass production may also be due to decreased cytokinin as a result of limited nutrients supply (Van der Werf and Nagel, 1996). Reduced amount of cytokinin decreases leaf expansion in plants treated with metal (Gadallah and El-Enany, 1999). Decreased cytokinin contents also affect cell division and cell expansion (Downes and Crowell, 1998). IAA when applied on plants increases net photosynthesis rate and synthesizes more C: N ratio which results in growth enhancement (Sudadi, 2012).

CONCLUSION

From the results of experiment it can be infered that foliar application of 100 mM indole acetic acid (IAA) affected the photosynthetic rate and stomatal conductance to statistically significant level. The ameliorative effects of IAA on transpiraton rate and internal CO_2 concentration were statistically non significant. Similarly, IAA effects for mitigation of Pb stress in term of dry biomasses were to a non significant level.

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