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AN INVERSE RELATION BETWEEN Pb^{2+} AND Ca^{2+} IONS ACCUMULATION IN *PHASEOLUS MUNGO* AND *LENS CULINARIS* UNDER Pb STRESS

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Abstract

This study focuses attention on accumulation of Pb^{2+} in root and shoot of *Phaseolus mungo* and *Lens culinaris* in relation with Ca^{2+} from nutrient solution in which $PbCl_2$ was added. The plants were cultivated in natural environment. It was observed that Pb^{2+} physically blocks water uptake which results in decline length of root and shoot with reduced leaf size of both species. This may be attributed with reduced fresh weight, and ratio of dry and fresh weight of two species. The accumulation trend of Pb^{2+} and Ca^{2+} showed a linear relation in between applied dose and accumulation of Pb^{2+} in *Phaseolus mungo* and *Lens culinaris* which inversely related with Ca^{2+} contents of both members of Fabaceae family. The reduced Ca^{2+} contents was due to chemical similarities in between the oxidation states of two ions but smaller ionic radius and high density of Pb^{2+} helps in accumulation of it in tissues of both species. These interactions may occur at the cellular and molecular level and are the abilities of Pb^{2+} to displace Ca^{2+} during specific physiological process. It is likely that Pb^{2+} blocks Ca^{2+} efflux from cells by substituting Ca in Ca^{2+} / Na during some enzymatic activity.

Introduction

Lead (Pb) is one of the most toxic metals in the environment (Azmat *et al.*, 2006) which may cause drastic morphological and physiological deformities in *Ipomoea lacunose* with a decrease in leaf net photosynthetic rate with increasing concentrations of Pb from 0 to 2000mg/L (Kambhampati *et al.*, 2005). Singh & Agrawal (2007) reported that an increase in heavy metal concentration in foliage of plants grown in sewage sludge-amended soil caused unfavorable changes in physiological and biochemical characteristics of plants leading to reductions in morphological characteristics, biomass accumulation and yield. The study concludes that sewage sludge amendment in soil for growing palak may not be a good option due to risk of contamination of Cd, Ni and Zn and also due to lowering of yield at higher mixing ratio (Singh & Agrawal., 2007). The excessive concentrations of Pb significantly decreased the dry matter mass of the above-ground plant parts and the root, leaf area, relative water content and transpiration intensity (Petrovic *et al.*, 1998; Kastori *et al.*, 1992). Lead nitrate (2.5 to 7.5 g/kg soil) treatment resulted in a decrease in plant height, root-shoot ratio dry weight and nodule number per plant. Toxicity tests were performed using seed germination, root elongation, shoot length and biomass as parameters for establishing growth inhibition of *S. nigrum* and *T. incarnatum* by arsenic, lead and zinc (Marques *et al.*, 2007). Haussling *et al.*, (1988) investigated the inhibition of root growth after exposure to Pb may be due to a decrease in Ca in the root tips, leading to a decrease in cell division or cell elongation. In root tips of Norway spruce 40% of the Ca taken up was used in root tips growth. In Norway spruce needles, the level of Ca and Mn decrease with Pb treatment, which could be a result of a decrease in number of root tips and sites for apoplastic solute flux through the endodermis. Pb physically blocks the access of many ions from absorption sites of the roots (Godbold & Kettner, 1991). Although Pb levels in root tips and the basal root may appear to be similar, Pb alters the levels of mineral elements in the roots. In root tips, the levels of Ca, Fe, Zn decrease after exposure to Pb. This study describes the adverse affects of Pb on growth factors of two important members of fabaceae family i.e., *Phaseolus mungo* and *Lens culinaris*, widely used as a meal in the world, as an important crop. Mechanism of accumulation of Pb in relation with important macronutrients Ca has been discussed in relation with growth, water contents and biomass production.

Materials and Methods

Phaseolus mungo and *Lens culinaris*, the two important pulses were chosen as experimental objects. All experiment were carried out in natural environment (temperature 25-35^{o}C and relative humidity 60-70%). Both species were grown in hydroponics in the Hoagland classical solution which is a well known nutrient solution. Pb was added as a $PbCl_2$ in nutrient solution @ 50, 100,150,200 and 250 ppm to examine the effects from germinated stage to Ca accumulation in seedlings of both species.

After 15 days of cultivation the plants were harvested and divided into two parts i.e., root and shoots, then dried at 60^{o}C till constant weight were achieved to determine water contents which were calculated by the difference in dry and fresh weight. The vegetative parts (root and shoot) were dry ash and digested with HNO_3 and HCl for quantitative determination of Ca and Pb in the plant tissues using Atomic absorption spectrophotometry. Pb accumulation, root absorption index (RAI) and the Pb concentration ratio (LCR) were determined by the method described by Akson & Visoottiviseth (2004). The relative growth rate of plants were determined by using the formula $\ln W_2 - \ln W_1 / T$ through fresh weight.

Results and Discussion

Growth of two species under Pb stress: Results reported in the Table 1 & 2 clearly indicate that both species were adversely affected by Pb accumulation. Uptake of Pb by shoots and roots of the plants was found to be proportional to the concentration of various $PbCl_2$ added and accumulation was more pronounced in roots than in shoots. Plant growth rate is a significant factor for reflecting the metal stress on growing species in contaminated soil and varied from specie to species (Liao *et al*., 2006; Weryszko-Chmielewska & Chwil, 2005; Tomar *et al.*, 2000). Results reported in Table 1 showed considerable reduction due to toxic metal Pb on relative growth rate, biomass production and water contents of *Phaseolus mungo* and *Lens culinaris* as reported earlier (Godbold & Kettner, 1991). Fresh weight of the plant were affected by the Pb (Table 1). The highest fresh weight production in leaves of *Phaseolus mungo* compared to *Lens culinaris* indicated that *Phaseolus mungo* was more tolerant as compared to *Lens culinaris* whereas fresh weight of both species showed acute toxicity of Pb on root and shoot. Relative growth rate of stress plants was considerably lower than that of none treated plant which may be attributed with the reduction in the root and shoot length. This results in growth inhibition, ratio of dry and fresh weight of total plant D_W/F_W (Table 1). It has been observed that root of both species were distinctly pretentious due to direct exposure of toxic metal with under ground parts which signify the role of root in plant growth and helped in preventing the excess of Pb in the shoot (Marques *et al*., 2007; Islam *et al*., 2007).

Table 1. Effect of Pb on ratio of Biomass of *Phaseolus mungo* and *Lens Culinaris*.

Pb (ppm)	Ratio of D_W/F_W root	Ratio of D_W/F_W shoot	Ratio of D_W/F_W leaf	Relative growth rate (fresh) $\ln W_2 - \ln W_1 / T$
Phaseolus mungo				
0	0.097 SD=$7.7X10^{-4}$ V=$7.7X10^{-4}$	0.0607 SD=$9.07X10^{-3}$ V=$7.4X10^{-3}$	0.134 SD= $3.5X10^{-3}$ V=$2.85X 10^{-3}$	-
50	0.079 SD=$2.31X10^{-2}$ V=$1.89X10^{-2}$	0.974 SD=$5.09X10^{-2}$ V=$4.16X10^{-2}$	0.102 SD=$1.53X10^{-2}$ V=$1.25X10^{-2}$	0.0281
100	0.074 SD=$1.38X10^{-2}$ V=$1.13X10^{-2}$	0.089 SD=$1.92X10^{-2}$ V=$1.57X10^{-2}$	0.085 SD=$3.8X10^{-2}$ V=$3.15X10^{-2}$	0.022
150	0.654 SD=$6.93X10^{-2}$ V=$5.66X10^{-3}$	0.079 SD=$1.44X10^{-2}$ V=$1.17X10^{-2}$	0.818 SD=$1.15X10^{-2}$ V=$9.39X10^{-3}$	0.161
200	0.366 SD=$8X10^{-3}$ V=$6.53X10^{-3}$	0.0847 SD=$2.89X10^{-2}$ V=$2.36X10^{-2}$	0.0926 SD =$2.09X10^{-2}$ V=$1.11X10^{-2}$	0.0219
250	0.059 SD=$5.131X10^{-3}$ V=$4.18X10^{-3}$	0.188 SD=$4.98X10^{-2}$ V=$4.07X10^{-2}$	0.331 SD=$1.361X10^{-3}$ V=$1.11X10^{-3}$	0.137
Lens culinaris				
0	0.508 SD=$4.725x10^{-4}$ V=$3.85x10^{-4}$	0.604 SD= $1.579x10^{-3}$ V= $1.579x10^{-3}$	0.148 SD= $2.08x10^{-4}$ V= $1.69x10^{-4}$	-
50	0.344 SD= $2.82x10^{-4}$ V= $3.46x10^{-4}$	0.8 SD= $4.82x10^{-3}$ V=$5.91 x10^{-3}$	0.637 SD= $7.81x10^{-4}$ V= $6.37x10^{-4}$	0.226
100	0.535 SD= $3.46x10^{-4}$ V= $2.82x10^{-4}$	0.595 SD=$1.91 x10^{-2}$ V= $1.56x10^{-2}$	0.634 SD=0 V=0	0.198
150	0.600 SD= $2.64x10^{-4}$ V=$2.16 x10^{-4}$	0.641 SD=0.0153 V=0.0124	0.710 SD= $1.15x10^{-4}$ V=$9.42 x10^{-5}$	0.0224
200	0.665 SD=$5.77 x10^{-5}$ V=$4.71 x10^{-5}$	0.629 SD= $4.57x10^{-3}$ V=$3.378.16 x10^{-3}$	0.723 SD=$1.0x10^{-4}$ V=$8.16 x10^{-5}$	0.0205
250	-	-	-	-

Water contents of two species under Pb stress: Water content of *Phaseolus mungo* and *Lens culinaris* in tissues were determined by difference in dry and fresh weight (Table 2). Results showed that Pb physically block the translocation of water from root to shoot which is related with the rate of photosynthesis (Haider *et al*., 2006), mainly associated with the water content and CO_2 absorption and also responsible for maintaining cell turgor and cell plasticity which also result in the reduction of water content uptake from root to shoot and decreased water potential in the cell. A decline in transpiration rate and water content in tissues occurs in plants growing under Pb exposure was reported by Kastori *et al*., (1992). Various mechanisms have been suggested for the Pb-induced decline in transpiration rate and water content. Pb treatment causes growth retardation,

Table 2. Translocation of water contents under the stress of Pb in *Phaseolus mungo* and *Lens Culinaris*.

Pb (ppm)	Water contents root (gm)	Water contents shoot (gm)	Water contents leaf (gm)
Phaseolus mungo			
0	0.0451 ± 0.0035	0.0185 ± 0.0042	0.0738 ± 0.0054
50	0.0463 ± 0.0036	0.1405 ± 0.0393	0.0659 ± 0.0098
100	0.0463 ± 0.004	0.1293 ± 0.0099	0.0656 ± 0.0011
150	0.0543 ± 0.0026	0.123 ± 0.0037	0.0615 ± 0.0068
200	0.0866 ± 0.0078	0.132 ± 0.02	0.0645 ± 0.0192
250	0.0350 ± 0.00158	0.0336 ± 0.0026	0.0151 ± 0.0019
Lens culinaris			
0	.0038 ± 0.0003	0.0636 ± 0.0034	0.003 ± 0.0002
50	0.00213 ± 0.0003	0.0193 ± 0.074	0.0019 ± 0.0002
100	0.0013 ± 0.0005	0.0231 ± 0.003	.00126 ± 0.0003
150	0.0015 ± 0.0003	0.0153 ± 0.006	0.0011 ± 0.0001
200	0.0017 ± 0.0016	0.0152 ± 0.016	.00096 ± 0.0001
250	-	-	-

which results in a reduced leaf area, the major transpiring organ were also observed in current study as reported earlier by Iqbal & Moshtaq (1987). Guard cells are generally smaller in size in plants treated with Pb. Pb lowers the level of compounds that are associated with maintaining cell turgor and cell wall plasticity and thus lowers the water potential within the cell.

Pb accumulation: The uptake and distribution of Pb in root and shoot in two species are presented in Table 3. Both plant species showed differences in their abilities to accumulate Pb. The total Pb uptake by *Lens culinaris* was greater than that of *Phaseolus mungo* (Table 3) showed the ability of accumulation of Pb diverse specie to specie. This is also supported by the fact that at 250 ppm total inhibition in seed germination was observed in case of *Lens culinaris*. This growth inhibition showed that Pb uptake by the plant was significantly influenced by the nature of species. These findings correspond to Walsh & Keeny (1975) and reduction in leaf size root, shoot length were the visual symptoms of Pb on both species which were more prominent in *Lens culinaris* as compared to *Phaseolus mungo* which results in the decline of ratio of fresh and dry weight of both species.

Root absorption index (RAI): The ratio of root Pb concentration (ppb) from nutrient solution Pb concentration (ppm) represents the translocation of Pb into root. A decrease in RAI value implies that Pb has accumulated more in roots as compared to shoot at a concentration range of 50 ppm to 250ppm in both species whereas 250ppm was lethal to *Lens culinaris* (Table 3). The value of accumulated Pb at 250ppm for *Phaseolus mungo* was 0.196 ppb but for the same concentration of Pb in case of *Lens culinaris* no germination showed that *Lens culinaris* was more sensitive as compared to *Phaseolus mungo*. The growth inhibition factor can easily be observed in case of *Lens culinaris* (Table 1).

Table 3. Accumulation of Pb in *Phaseolus mungo* and *Lens Culinaris*.

Pb (ppm)	% Accumulation of Pb in shoot	% Accumulation of Pb in Root	Total accumulation (TAR)	Root absorption Index of Pb (RAI)	Pb Conc. Rate In leaf
Phaseolus mungo					
0	-	-	-	-	-
50	0.91 ± 0.438	0.133 ± 0.053	10.828 ± 0.054	0.500	0.80
100	0.423 ± 0.13	0.074 ± 0.24	11.035 ± 0.366	0.300	1.660
150	0.28 ± 0.112	0.068 ± 0.0254	11.413 ± 0.18	0.246	1.594
200	0.255 ± 0.21	0.0482 ± 0.0028	14.341 ± 0.277	0.215	1.465
250	0143 ± 0.33	0.0295 ± 0.717	16.008 ± 0.606	0.196	1.408
Lens culinaris					
0	-	-	-	-	-
50	2.09 ± 0.022	0.0877 ± 0.0063	29.648 ± 0.583	0.940	0.978
100	1.479 ± 0.363	0.78 ± 0.004	33.205 ± 2.696	0.60	0.916
150	1.145 ± 0.029	0.0384 ± 0.0318	41.260 ± 0.708	0.433	1.000
200	0.902 ± 0.012	0.0234 ± 0.0236	51.429 ± 1.407	0.35	0.971
250	-	-	-	-	

Table 4. Accumulation of Ca in *Phaseolus mungo* and *Lens culinaris*.

Pb (ppm)	% Accumulation of Ca in root	% Accumulation of Ca in shoot	Total rate accumulation (TAR)	Root absorption Index of Ca (RAI)	Ca Conc. Rate In leaf
Phaseolus mungo					
0	-	-	30.462	-	1.115
50	0.640 ± 0.064	2.628 ±0.173	33.796 ± 0.153	2.40	1.083
100	0.268 ± 0.043	1.0218 ±0.0646	28.675 ± 0869	1.090	1.110
150	0.1619 ± 0.009	0.518 ± 0.0384	22.636 ± 0.22	0.640	1.135
200	0.0953 ± 0.0056	0.410 ± 0.007	23.843 ± 0425	0.425	1.880
250	0.0424 ± 0.0024	0.207 ± 0.0027	23.151 ± 0.88	0.28	1.428
Lens culinaris					
0	-	-	26.96	-	1.083
50	0.205 ± 0.015	6.007 ± 0.07	84.442 ± 1.562	2.20	1.200
100	0.116 ± 0.006	3.094 ± 0.759	68.391 ± 1.573	0.89	1.292
150	0.047 ± 0.003	1.797 ± 0.045	68.969 ± 1.129	0.533	1.275
200	0.024 ± 0.0024	1.195 ± 0.015	67.708 ± 1.893	0.36	1.25
250	-	-	-	-	

The Pb concentration ratio (LCR): The LCR (the ratio of shoot Pb concentration (ppb) to root) Pb concentration (ppm) indicates the mobility of Pb within plant species. LCR for *Phaseolus mungo* at 200 ppm was 1.408 whereas 0.971 for *Lens culinaris* which may be due to compact seedling size as compared to *Phaseolus mungo*. The difference in the values of Pb accumulated at same applied dose of toxic metal reflects that growth of *Lens culinaris* was negatively affected by metal and accumulation rate was vary in both species which results in reduced leaf size and undersized of root (Azmat *et al*., 2006). The Ca^{2+} contents of both species are inversely related with Pb concentration (Azmat *et al*., 2007) but in *Phaseolus mungo* the concentration of Ca was significantly higher as compared to *Lens culinaris* which may be attributed with the resistant nature of this specie due to high Ca^{2+} ion contents under Pb stress (Table 4) or it may be related with that the greater the concentration of Ca contents, more the survival of the plant in Pb contaminated environment.

Mechanism of accumulation and environmental perspective: The total uptake of Pb by *Phaseolus mungo* and *Lens culinaris* was higher at 250 ppm for *Phaseolus mungo* whereas *Lens culinaris* showed total inhibition in growth. Results showed that Pb toxicity was significant in both species. It was observed in all studied plants, that as the lead toxicity increased, calcium content of both species found to be decreased. The physiological deficiencies have been observed in both species which may be related with the reduced Ca^{2+} contents when soil supplies are interrupted by Pb soil conditions. Calcium is not readily translocated between plant tissues, and plants do not accumulate high calcium concentrations when it is in excess or by presence of some metal. Pb reduces accumulation of divalent cations (especially Ca and Mg) (Haider *et al*., 2006) by interfering with the membrane transport. Pb alters the pattern of Ca^{2+} fluxes across the plasma membrane, thus supposedly disturbing symplasmic Ca^{2+} homeostasis. Disturbance of the cell Ca^{2+} homeostasis appears to be an important feature of ion-related environmental stresses in general (salt, heavy metals, Pb) (Adam and Price, 1990). Transition metal transporters are of central importance in the plant metal homeostasis network which maintains internal metal concentrations within physiological limits (Krämer *et al*., 2007). Ca^{2+} is a divalent cation like Pb it is thought that Pb may replace the Ca due to their ionic similarities. The lowering of Ca^{2+}ion contents was due to chemical similarities in between the oxidation states of two ions but smaller ionic radius and high density of Pb helps in accumulation of it in tissues of both species. These interactions occur at the cellular and molecular level and are the abilities of Pb to displace Ca during specific physiological process. It is likely that Pb blocks Ca efflux from cells by substituting Ca in Ca^{2+} / Na during some enzymatic activity. These results are discussed in so far as they support the hypothesis that a rise in cytosolic calcium may play a role in the pathology of oxidative stress in plant cells through a mechanism analogous to the liver cell system (Hepler and Wayne, 1985).

Conclusion

The above investigations suggest that Pb toxicity were prominent in growth of both species and Pb accumulate more rapidly in root and translocate into shoot where it damage tissue structure of root and leaf. Pb blocks the Ca absorption in the plant and produced toxic effects on plant growth by reducing water uptake which ultimately effects the growth of seedling understudied. However these results were obtained from hydroponics experiment. Field experiments are now needed

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