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Effect of lead on nitrate reductase activity and alleviation of lead toxicity by inorganic salts and 6-benzylaminopurine

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Abstract

A concentration dependent decrease in root nitrate reductase (E.C.1.6.6.1) activity (NRA) by 0.1 to 2.0 mM lead acetate was noticed in three cultivars of *Vigna radiata* (L.) Wilczek (K851, MH8320 and ML337). Leaf NRA, on the other hand, increased significantly with increasing lead concentration which was more pronounced in cvs. MH8320 and ML337 than in cv. K851. Total nitrogen content of root and shoot was generally increased due to supply of the lead acetate, whereas the total nitrogen content of the colyledons was hardly affected in cvs. MH8320 and ML337 and decreased in cv. K851 during the early growth phase. The inhibition of root NRA could be alleviated by addition of inorganic salts (K_2HPO_4 and KNO_3, but not $CaCl_2$) or 6-benzylaminopurine (BAP) in the incubation medium. Lead mediated inhibition of root NRA was similar in light and dark grown seedlings, but lead induced increase in leaf NRA was more pronounced in the light than in the dark.

Additional key words: $CaCl_2$, K_2HPO_4, KNO_3, nitrogen content, *Vigna radiata*.

Introduction

Nitrate is the predominant form of inorganic nitrogen available to most of the higher plants; it gets assimilated into nitrite by nitrate reductase, the rate limiting enzyme in the overall assimilation of nitrate. The response of nitrate reductase activity (NRA) to exogenous lead supply is different in different plant species, cultivars and organs (for review see, *e.g.*, Singh *et al.* 1997). A significant alleviation of lead induced inhibition of biomass accumulation (Singh *et al.* 1994a) and chlorophyll biosynthesis (Singh *et al.* 1996b) in mungbean seedlings by K_2HPO_4 and $CaCl_2$ has been reported in our previous studies. As an extention of the earlier reports, this paper is concentrated to the different effects of lead on root and leaf NRA in three mungbean cultivars commonly grown as major legume crops in north-west India and to the effect of nutritional amendments and growth regulators.

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Materials and methods

Three cultivars of *Vigna radiata* (L.) Wilczek K851, MH8320 and ML337 were procurred from CCS Haryana Agriculture University, Hisar. Seeds were surface sterilized with 1 % (m/v) $HgCl_2$ for 5 min and than washed thoroughly with distilled water before the planting. Seedlings were raised in Petri plates (diameter 10 cm) containing wet *Whatman No. 1* paper for 5 d in a growth chamber (temperature 25 ± 2 °C, continuous light of approximately 80 μmol m^{-2} s^{-1}). Seedlings were watered daily with distilled water containing different concentrations of Pb^{2+} in the form of lead acetate and 10 mM KNO_3. For certain experiments etiolated seedlings raised in the dark at the same temperature were excised for root and leaves which were 24 h floated in the light or dark on desired incubation solutions.

In vivo NRA in the freshly harvested leaves and roots was determined as described by Srivastava (1975) using exogenous nitrate for production of nitrite in the dark. NRA was also estimated *in vitro* by the method of Stevens and Oaks (1973) but no activity was detected in crude homogenate from mungbean tissues, probably because of the presence of inhibitor(s) of the enzyme in the crude homogenate as shown by Puranik and Srivastava (1985).

Total nitrogen content of roots and cotyledons was estimated by microkjeldahl method described by Lang (1958), after digesting tissues with concentrated sulphuric acid. Ammonium sulphate was used as standard.

Data presented in tables are means of at least tree replicate experiments with duplicate determination ± standard deviations. Paired *t*-test was applied to test the significance of differences induced by treatments.

Results and discussion

Supply of 0.1 - 0.2 mM lead acetate to intact seedlings caused a concentration dependent 36 - 78 % decrease in root NRA in cv. K851, whereas the same treatment caused 13 - 34 % increase in the leaf NRA (Table. 1). A similar decrease in the root NRA, *i.e.*, 27 - 63 % and 31 - 60 % was noticed in cvs. MH8320 and ML337, respectively. Leaf NRA, however, was increased by 1.5 to 2.0 fold in the cvs. ML337 and MH8320, respectively.

Total nitrogen content of roots of 5-d-old seedlings increased by 21 - 39, 11 - 25, and 25 - 56 % in cvs. K851, MH8320 and ML337, respectively, with supply of 0.1 - 2.0 mM of lead aceate (Table 2). An increase in the shoot nitrogen in cvs. K851 MH8320 and ML337, *i.e.*, 16 - 30, 6 - 16, and 6 - 31 %, respectively, was also noticed. However, in the cotyledons a lead-induced decrease in the nitrogen content was observed only in cv. K851, whereas that in MH8320 and ML337 was not statistically significant (Table 2). A slight increase in protein and nitrogen contents in maize leaves due to Pb^{2+} supply (Sinha *et al.* 1988b) and concentration dependent increase in soluble protein and organic nitrogen contents in sesame and mungbean roots and shoots in the presence of Pb^{2+} during early growth stages (Kumar *et al.* 1993, Bhatri and Singh 1993, Singh *et al.* 1994a) have been reported. Inhibition in

NRA by lead have been reported in many other plants (Venkataramana *et al.* 1978, Burzynski and Grabowski 1984, Brackup and Capone 1985, Sinha *et al.* 1988, 1994a, 1996a, Kumar *et al.* 1993, Bharti and Singh 1993, 1994, Bharti *et al.* 1996). The causes of the inhibition may be multifacial, *e.g.*, due to reduced supply of NAD(P)H, disorganisation of chloroplasts, less NO_3^- supply to the site of synthesis caused by water stress, and direct effect of lead on protein synthesis because it has a strong affinity for functional -SH group of the enzyme (see Singh *et al.* 1997).

Table 1. *In vivo* nitrate reductase activity [µmol(NO_2^-) g^{-1}(f.m.) h^{-1}] in roots and shoots of different mungbean cultivars as affected by lead acetate. Means ± SD; $n = 6$; differences from control significant at $P < 0.01$ - **, at $P < 0.001$ - ***.

	Pb^{2+} [mM]	K851	MH8320	ML337
Roots	0.0	2.95 ± 0.01	0.52 ± 0.02	1.21 ± 0.01
	0.1	1.90 ± 0.02***	0.37 ± 0.02**	0.83 ± 0.00***
	1.0	1.52 ± 0.02***	0.25 ± 0.00***	0.63 ± 0.19***
	2.0	0.63 ± 0.01***	0.19 ± 0.01***	0.48 ± 0.02***
Leaves	0.0	0.57 ± 0.00	0.26 ± 0.01	0.54 ± 0.03
	0.1	0.65 ± 0.00**	0.39 ± 0.03***	0.75 ± 0.02***
	1.0	0.68 ± 0.01***	0.54 ± 0.03***	0.92 ± 0.02***
	2.0	0.75 ± 0.02***	0.60 ± 0.02***	0.99 ± 0.01***

The responce of the leaf NRA to lead is different than that of mungbean cultivar P105 and sesame seedlings (Kumar *et al.* 1993, Bharti and Singh 1993, 1994, Singh *et al.* 1994a, Dabas and Singh 1995, Bharti *et al.* 1996). It may be due to a lower accumulation of Pb^{+2} in the leaves of these plants. Alternatively, there may be an

Table 2. Total nitrogen content [mg g^{-1}(f.m.)] in roots, shoots and cotyledons of different mungbean cultivars as affected by lead acetate. Means ± SD, $n = 6$; differences from control significant at $P < 0.05$ - *, at $P < 0.01$ - **, at $P < 0.001$ - ***.

	Pb^{2+} [mM]	K851	MH8320	ML337
Roots	0.0	5.243 ± 0.36	2.890 ± 0.09	2.373 ± 0.06
	0.1	6.363 ± 0.18**	3.206 ± 0.07**	2.977 ± 0.09***
	1.0	6.952 ± 0.08**	3.372 ± 0.12**	3.205 ± 0.07***
	2.0	7.279 ± 0.23**	3.622 ± 0.06***	3.715 ± 0.04***
Leaves	0.0	4.536 ± 0.10	3.684 ± 0.06	2.768 ± 0.10
	0.1	3.770 ± 0.27**	3.892 ± 0.03**	2.956 ± 0.03*
	1.0	5.243 ± 0.24*	4.080 ± 0.02***	3.164 ± 0.05**
	2.0	5.891 ± 0.07***	4.288 ± 0.07***	3.622 ± 0.12***
Cotyledons	0.0	7.659 ± 0.37	3.164 ± 0.07	4.540 ± 0.14
	0.1	6.952 ± 0.10*	2.966 ± 0.04	4.746 ± 0.08***
	1.0	6.422 ± 0.20**	2.727 ± 0.05***	4.450 ± 0.05
	2.0	6.068 ± 0.10**	3.102 ± 0.02	4.372 ± 0.02

induced synthesis of lead binding phytochelatins. An inactivation/degradation of some potential inhibitor(s) of the NRA in the leaf tissue by Pb^{2+} may also be a possible reason for the increased enzyme activity. Puranik and Srivastava (1985) have reported a light regulated inhibitor of NRA in the bean leaves.

Addition of 10.0 mM K_2HPO_4 alleviated Pb^{2+} caused inhibition of root NRA in mungbean cv. ML337 and significantly increased the leaf NRA, *i.e.*, 181 % over those with the lead supply alone (Table 3). Addition of 10 mM $CaCl_2$ had no effect on root NRA, but significantly increased leaf NRA in the absence as well as in the *presence of lead acetate. Exogenously supplied 10 mM* KNO_3 *enhanced NRA in both* roots and leaves (Tables 3 and 4). Effect of KNO_3 was more pronounced in light than in dark grown seedlings. It is interesting to note that lead caused inhibition of NRA is reversible and could be counteracted by addition of inorganic salts K_2HPO_4 and KNO_3 but not by $CaCl_2$ (Table 3). It is in accordance to our earlier studies related to lead effect on biomass and photosynthetic pigments in mungbean seedlings (Singh *et al.* 1994a, 1996). Kumar *et al.* (1993) have also been reported the alleviation of

Table 3. Effect of lead acetate (1 mM Pb^{2+}) on nitrate reductase activity [μmol(NO_2^-) g^{-1}(f.m.) h^{-1}] in roots and leaves of 5-d-old light and dark grown mungbean seedlings (cv. ML337) in the presence of 10 mM K_2HPO_4, 10 mM $CaCl_2$ or 10 mM KNO_3. Means ± SD; *n* = 6; differences from control significant at $P < 0.05$ - *, $P < 0.01$ - **, at $P < 0.001$ - ***.

		Roots $-\text{Pb}^{2+}$	$+\text{Pb}^{2+}$	Leaves $-\text{Pb}^{2+}$	$+\text{Pb}^{2+}$
Light	control	1.53 ± 0.15	0.72 ± 0.06*	0.63 ± 0.07	0.99 ± 0.05*
	K_2HPO_4	2.09 ± 0.25	1.24 ± 0.13	1.61 ± 0.08**	1.77 ± 0.11**
	$CaCl_2$	1.60 ± 0.17	0.80 ± 0.06	1.21 ± 0.09*	1.30 ± 0.12*
Light	control	1.40 ± 0.06	0.68 ± 0.04***	0.59 ± 0.02	0.99 ± 0.04*
	KNO_3	*1.67 ± 0.03***	*0.93 ± 0.06****	*4.34 ± 0.0.08****	*4.18 ± 0.08****
Dark	control	0.84 ± 0.01**	0.43 ± 0.01***	0.70 ± 0.02	0.94 ± 0.01**
	KNO_3	1.57 ± 0.05**	0.52 ± 0.03**	1.16 ± 0.03**	1.26 ± 0.08*

Table 4. Effect of lead acetate (1 mM Pb^{2+}) and 6-benzylaminopurine (BAP; 1 μM) on nitrate reductase activity [μmol(NO_2^-) g^{-1}(f.m.) h^{-1}] in roots and leaves of 5-d-old light and dark grown mungbean seedlings (cv. ML337) in the presence or absence of 10 mM KNO_3. Detached roots or leaves were floated on desired solution for 24 h in light. Means ± SD; *n* = 6; differences from control significant at $P < 0.05$ - *, $P < 0.01$ - **, at $P < 0.001$ - ***.

		Roots $-KNO_3$	$+ KNO_3$	Leaves $-KNO_3$	$+ KNO_3$
Dark	control	0.63 ± 0.02	1.57 ± 0.05	0.44 ± 0.11	1.16 ± 0.03
Light	control	0.95 ± 0.03***	10.40 ± 0.78***	1.57 ± 0.06**	9.90 ± 1.06**
	Pb^{2+}	0.27 ± 0.03*	0.55 ± 0.09	0.26 ± 0.01***	0.69 ± 0.07**
	BAP	0.62 ± 0.01***	9.40 ± 0.69***	0.92 ± 0.03***	7.79 ± 0.25***
	Pb^{2+} + BAP	0.36 ± 0.01***	3.24 ± 0.30**	0.76 ± 0.02***	0.85 ± 0.09**

lead caused inhibition of NRA in sesame roots by K_2HPO_4. A reduced endogenous lead accumulation in roots and leaf tissues of mungbean seedlings in the presence of K_2HPO_4 and $CaCl_2$ have also been reported earlier (Singh *et al.* 1994).

When root and leaves from dark grown mungbean seedlings were incubated in light for 24 h, a multifold increase in the activity was observed for root and leaf NRA in the presence of KNO_3 (Table 5). Lead acetate (1.0 mM) reduced NRA in both roots and leaves of the etiolated seedlings and addition of BAP alleviated this reduction to a good extent, in roots especially in the presence of KNO_3, and in leaves in the absence of KNO_3 (Table 5).

Table 5. Effect of inhibitors on nitrate reductase activity [μmol(NO_2^-) g^{-1}(f.m.) h^{-1}] in roots of dark grown mungbean seedlings (cv. ML337) in the presence of 1 mM Pb^{2+} and 1 μM BAP. Roots were floated on desired solution for 24 h in the light. For comparison also values for control dark and light grown seedlings are presented. Means ± SD; $n = 6$; differences from control significant at $P < 0.01$ - **, at $P < 0.001$ - ***.

Treatments	Pb^{2+}	BAP	Inhibitors	NRA
Dark	-	-	-	1.57 ± 0.56
Light	-	-	-	11.80 ± 0.78***
	+			0.62 ± 0.04**
	+	+	-	3.84 ± 0.17***
	+	+	chloramphenicol	1.64 ± 0.16
	+	+	cycloheximide	2.68 ± 0.11***
	Pb^{2+}	BAP	sodium tungstate	1.67 ± 0.36***

When roots from dark grown mungbean seedlings were incubated with inhibitors of protein synthesis, *e.g.*, chloramphenicol and cycloheximide, in the presence of lead and BAP, these inhibitors caused a complete restriction in the increase in NRA which was earlier observed due to the addition of BAP in the presence of Pb^{2+} (Table 5). Sodium tungstate, an inhibitor of nitrate reductase, also restricted this increase in the enzyme activity.

References

Bharti, N., Singh, R.P.: Growth and nitrate reduction by *Sesamum indicum* cv. PB-1 respond differentially to lead. - Phytochemistry **33**: 531-534, 1993.

Bharti, N., Singh, R.P.: Antagonistic effect of sodium chloride to differential heavy metal toxicity regarding biomass and nitrate assimilation in *Sesamum indicum* seedlings. - Phytochemistry **35**: 1157-1161, 1994.

Bharti, N., Singh, P., Sinha, S.K.: Effect of calcium chloride on heavy metal induced alteration in growth and nitrate assimilation of *Sesamum indicum* seedlings. - Phytochemistry **41**: 105-109, 1996.

Brackup, I., Capone, D.G.: Effect of several metals and organic pollutant on nitrogen fixation, acetylene reduction by root and rhizomes of *Zostera marina* L. - Environ. exp. Bot. **25**: 145-151, 1985.

Burzynski, M., Grabowski, A.: Influence of lead on nitrate uptake and reduction in cucumber seedlings. - Acta Soc. Bot. Pol. **53**: 77-86, 1984.

Dabas, S. Singh, R.P.: Differential effect of lead on nitrate reductase activity and organic nitrogen contens in mungbean (var. P105) seedlings. - Indian J. Plant Physiol. **38**: 155-157, 1995.

Kumar, G., Singh, R.P., Dabas, S.: Nitrate assimilation and biomass production in *Sesamum indicum* L. seedlings in lead enriched environment. - Water Air Soil Polut. **66**: 163-177, 1993.

Lang, C.A.: Simple microdetection of Kjeldahl nitrogen in biological material. - Anal. Chem. **30**: 1692- 1694, 1958.

Puranik, R.M., Srivastava, H.S.: Increase in nitrate reductase activity in bean leaves by light involves a regulator protein. - Agr. biol. Chem. **49**: 2099-2104, 1985.

Singh, R.P., Bharti, N., Kumar, G.: Differential toxicity of heavy metals to growth and nitrate reductase activity of *Sesamum indicum* seedlings. - Phytochemistry **35**: 1153-1156, 1994a.

Singh, R.P., Dabas, S., Choudhary, A.: Recovery of Pb^{+2} caused inhibition of chlorophyll biosynthesis in leaves of *Vigna radiata* by inorganic salts. - Indian J. exp. Biol. **34**: 1129-1132, 1996.

Singh, R.P., Maheshwari, R., Sinha, S.K.: Recovery of lead caused decrease in biomass accumulation of mungbean (*Vigna radiata* (L.) Wilczek) seedlings by K_2HPO_4 and $CaCl_2$. - Indian J. exp. Biol. **32**: 507-510, 1994b.

Singh, R.P., Sinha, S.K., Tripathi, R.D., Maheshwari, R., Srivastava, H.S.: Respone of higher plants to lead contaminated environment. - Chemosphere **34**: 2467-2493, 1997.

Sinha, S.K., Srivastava, H.S., Mishra, S.N.: Effect of lead on nitrate reductase activity and nitrate assimilation in pea leaves. - Acta Soc. Bot. Pol. **57**: 457-463, 1988.

Srivastava, H.S.: Distribution of nitrate reductase in aging bean seedlings. - Plant Cell Physiol. **16**: 995-999, 1975.

Srivastava, H.S.: Regulation of nitrate reductase activity in higher plants. - Phytochemistry **19**: 725-733, 1980.

Stevens, D.L., Oaks, A.: Influence of nitrate on the induction of nitrate reductase in maize roots. - Can. J. Bot. **51**: 1255-1258, 1973.

Venketramana, S., Veeranjaneyulu, K., Ramadas, V.S.: Heavy metal inhibition of nitrate reductase. - Indian J. exp. Biol. **16**: 615-616, 1978.