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SHORT COMMUNICATION

DIFFERENTIAL EFFECT OF LEAD ON NITRATE REDUCTASE ACTIVITY AND ORGANIC NITROGEN CONTENT IN MUNGBEAN(VAR. P-105) SEEDLINGS

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A concentration dependent decrease in nitrate reductase (E.C.1.6.6.1.) activity in root and leaf by the supply of 0.1 to 2.0 mM Pb(OAC) was noticed in *Vigna radiata* (L) Wilczek cv. P-105 seedlings raised for five days under continuous light of 5 K lux at 25± 2°C. Total organic nitrogen content in root and shood increased due to the metal supply whereas organic N content in the cotyledons decreased during the early growth phase. Data show that the enzyme activity was not directly related with the organic nitrogen content in the organs at this stage which was more dependent on the translocation of reserve food meterial from the germinating seeds.

Increasing Pb^{+2} level in the environment affects various plant processes adversely (Thapa *et al.*, 1988). Nitrogen metabolism is one of important metabolic processes which has special relevance to legumes. Nitrate reductase (E.C.1.6.6.1), is considered to be the rate limiting step in nitrate assimilation (Srivastava, 1980) and its activity is often correlated with the N-status of the plant (Mishra and Srivastava, 1983). However, in several other systems, usually in young seedlings, total organic nitrogen content could not be correlated with NR activity in the plant parts (Kumar *et al.*, 1993; Bharti and Singh, 1993). Nitrate reductase has been studied in certain species exposed to lead contamination. However, the enzyme shows considerable variation in response to the metal which is species and cultivar specific (Venketramana *et al.*, 1978; Burzynski and Grabowski, 1984; Sinha *et al.*, 1988 a, b; Kumar *et al.*, 1993; Bharti and Singh, 1993).

In the present investigation nitrogen assimilation was studied in roots and leaves of mungbean variety P-105 to determine its nitrate reduction efficiency and organic nitrogen content under lead contamination during early growth phase of the seedlings.

Seeds of *Vigna radiata* (L) Wilczek cv. P-105 obtained from CCSH Agriculture University, Hisar, were surface sterilized with 0.1% (w/v) $HgCl_2$ for 5 min. and then washed thoroughly with distilled water before planting. Seedlings were raised within small petriplates (4') containing wet Whatman No.-1 filter paper for 5 days at 25±2°C in a prefabricated growth chamber under continuous light of approximately 5 K lux. Seedlings were watered daily with distilled water containing different concentration of Pb^{+2} in the form of Pb (OAC) and 10 mm KNO_3.

In vivo NRA in the freshly harvested leaves and roots was determined by the method of Srivastava (1975).

In vitro NRA was also estimated in the crude homogenate by the method of Stevens and Oaks (1973).

Total organic nitrogen content in roots, shoots and cotyledons was estimated by microjeldahl method (Lang 1958) after digesting with concentrated H_2SO_4. Ammonium sulphate was used as a standard.

Lead acetate (0.1 to 2.0 mM) supplied to the intact seedlings of *Vigna radiata* cv. P-105 decreased *in vivo* nitrate reductase activity drastically in root and leaf tissues of the seedlings. Effect was, however, more pronounced in the roots than in the leaves (Table-I). At 0.1mM Pb^{+2} inhibition in the enzyme activity was 26% and 15% and the inhibition was increased further upto71 and 38% at 2.0 mM lead supply in roots and leaves

Table-I *In vivo* nitrate reductase activity in roots and leaves of 5 days old *Vigna radiata* (L) Wilczek cv. P-105 seedlings during lead supply.

Pb^{+2} (mM)	Nitrate reductase activity; n mole NO^-_2 hr^{-1} g^{-1} fr.wt. ± S.D.	
	Root	Leaf
0.0	1486.43±19.83 (100)	420.69±14.31 (100)
0.1	1100.80±20.20 (74)***	356.41±8.26 (85)**
1.0	624.02±26.23 (42)***	309.66±7.23 (74)***
2.0	427.70±9.91 (29)***	262.93±14.31 (62)***

Seedlings were watered daily with desired level of Pb (OAC) and KNO_3 (10.0mM) separately. Data ± SD are shown. Values relatives to control are given in brackets. Data are significant at p=0.01** and p=0.001***

respectiviely. *In vitro* NR activity could not be detected in either tissue possibly because of the presence of inhibitor(s) of the enzyme in the crude homogenate as observed by Puranik and Srivastava (1985) for bean leaves.

Total organic nitrogen content in roots and shoots of 5 days old seedlings. on the other hand increased significantly (at p = 0.001) with the supply of 0.1 to 2.0 mM Pb^{+2} (Table-II). A corresponding decrease in the organic N of the cotyledons during the metal supply was also noticed.

Inhibition in NRA by lead in seveal crop plants have been reported (Bharti and Singh, 1993; Kumar *et al.*, 1993) . The cause of inhibition of *in vivo* NRA due to lead supply may be multifacial. It may be due to (i) reduced supply of NADH; (Gengenbach et al., 1973), (ii) disorganization of chloroplasts (Rebechini and Hanzely, 1974) (iii) Lesser NO_3 supply to the site of the enzyme synthesis because lead treatment could create water stress in the plants (Durzynski and Grabowski, 1984.) and (iv) a direct effect of lead on the enzymatic protein synthesis/activity as it has a strong affinity for functional-SH group of the enzyme (Sinha *et al.*, 1988 b)

Data in (Table-II) show that exogenously supplied Pb^{+2} caused a significant ($p<0.1$) increase in the organic nitrogen of roots and shoots progressively with the higher dosage of the applied metal (0.1-2.0 mM). A slight increase in protein and nitrogen of *Zea* leaves due to Pb^{+2} supply (Sinha *et al.*, 1988a) and concentration dependent increase in soluble protein and organic nitrogen in Sesame roots and shoots during early growth phase (Kumar *et al.*, 1993; Bharti and Singh, 1993) have also been reported.

Total organic nitrogen content of young seedlings could not be correlated with nitrate reductase activity of the plant parts as reported for some plant species (Eilrich and Hageman, 1973; Mishra and Srivastava, 1983). These differences are possibly due to the different origin of nitrogen during germination and in the mature plants. It appears that the metal causes an increased translocation of the organic N from the cotyledons to the growing roots and shoots. Synthesis of new stress proteins in these organs during the metal stress, however, can not be eliminated. The results of the experiments demonstrated in this paper show that NRA of this cultivar is drastically inhibited by Pb^{+2} supply, however, the enzyme activity is not directly correlated with the organic nitrogen content of the organs which is more dependent on the translocation of reserve food material from the germinating seeds during early growth phase.

Table II. Total organic nitrogen content in different organs of *Vigna radiata* (L) Wilczek cv. P-105 seedlings during lead supply.

Pb^{+2} (mM)	mg nitrogen g^{-1} fr.wt. ± S.D.		
	Root	Shoot	Cotyledon
0.0	2.356 ± 0.23 (100)	3.730 ± 0.21 (100)	4.870 ± 0.34 (100)
0.1	3.040 ± 0.08 (129)**	4.812 ±0.09 (129) ***	3.867 ± 0.24 (79)*
1.0	3.849 ± 0.27 (163)**	5.028 ±0.16 (135)***	3.731 ± 0.18 (76)**
2.0	4.520 ± 0.18 (192)***	4.271 ± 0.12 (115)*	4.320 ± 0.15 (89) NS

Growth conditions and other details as in Table-I. NS= Non significant. Data are significant at p=0.01*, p=0.05** and p=0.001***

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