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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (5): 687-690, 2005
ISSN 1028-8880

Phytotoxicity of Lead (Pb) to SDS-PAGE Protein Profile in Root Nodules of Faba Bean (*Vicia faba* L.) Plants

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Abstract: The present study was carried out to characterize and assess possible changes that might take place in the electrophoretic protein patterns involved in the N_2-fixing root nodules of *Vicia faba* L.(faba bean) in response to lead (Pb) toxicity. The banding patterns of the SDS-PAGE protein profile in root nodules revealed both qualitative and quantitative changes. Lead treatments (PbO), either applied foliar or in soil, inhibited the number of polypeptides synthesized in the root nodules. The band intensity of the large subunit (240 kDa) of nitrogenase enzyme was greatly inhibited by all PbO treatments. Moreover, the synthesis of the small subunits (52 to 73 kDa) of nitrogenase was completely inhibited by both soil applications (500 and 2000 ppm) of PbO. The foliar applications (500 and 2000 ppm) of PbO greatly stimulated a 23 kDa protein (from 4.79 to 13.1 and 19.4%, respectively). This protein was also stimulated (only 2-folds) in the root nodules by PbO soil treatments. In conclusion, the reduced productivity of *Vicia faba* L. crop under Pb stress could be attributed to the impairment of N_2-fixing enzymes.

Key words: Pb-stress, faba bean, root nodules, SDS-PAGE proteins, nitrogenase

INTRODUCTION

The term phytotoxicity has normally been associated with phenomenon whereby, a potentially harmful substance has accumulated in the plant tissue to a level affecting optimal growth and the development of the plant[1]. In recent decades, there has been increasing interest in heavy metals due to their toxicity to animals, plants and other living organisms, especially the long-term effect of these elements at high concentrations in the environment as they can persist in the soil for tens or thousands of years. The presence of a heavy metal like lead (Pb) in the soil always results in reduced plant growth[1-5] and impaired metabolism[3,6].

In plants, the reason for Pb toxicity is not clear. In animals, Pb toxicity interferes with iron (Fe) metabolism and the formation of heme. It inhibits two steps in the conversion of δ amino-laevulnic acid into heme[7]. Meanwhile, in the Fabaceae (Leguminosae) family, 90% of their members have root nodules in which nitrogen fixation occurs[8]. An important protein called leghemoglobin has a vital role in O_2 transport needed for bacteroid respiration. Yet, it is not known whether Pb has the same effect on the enzymes involved in heme synthesis in plant cells.

All biological N_2-fixing microorganisms depend upon the key enzyme nitrogenase. This enzyme is composed of two oxygen-sensitive nonheme iron proteins. The smaller is a Fe-protein (52-75 kDa); while, the larger is a MoFe-protein (240 kDa). Accordingly, the present study was an attempt to provide a partial characterization of possible changes that might take place in the electrophoretic protein patterns involved in N_2-fixing root nodules of *Vicia faba* L. plants in response to the phytotoxicity of Pb in air and soil.

MATERIALS AND METHODS

Plantation: Pure strain (Giza 5) of broad bean (*Vicia faba* L.) seeds were used as experimental plants and obtained from the Agricultural Research Center in Giza, Egypt. This study was conducted at Department of Botany, Faculty of Science, Sues Canal University, Ismailia, Egypt during the Winter and Spring of 2001. For plantation, 25 plastic pots (20 cm) were filled with homogenous pre-sieved garden soil (clay loam) obtained from the Botanical Garden of Botany Department in Ismaila, Egypt. Seeds were soaked in the pot soil about 6 cm deep and all pots were watered up to saturation, then kept in the open garden and

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irrigated regularly (twice/week) to field capacity until heavy (PbO) treatments.

Treatments: The five-week-old planted pots were randomly subdivided into five equal groups (5 pots each). One group was treated with pure water and sampled as control. Two groups were subjected separately to two foliar applications (T_1 and T_2) of aqueous solution (500 and 2000 ppm) of lead oxide (PbO). The other two groups were subjected to two separate soil treatments (T_3 and T_4) with the same PbO concentrations. Pots were kept in the open garden and irrigated regularly until sampling.

Protein electrophoresis

Preparation of total protein: From the fresh root nodules of both control and treated plants, total protein extracts were prepared by extracting appropriate portion of the fresh nodule paste with 0.125 M tris/borate (pH = 8.9). All extracts were kept for 24 h at 4°C and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for electrophoresis.

Gel electrophoresis: SDS Polyacrylamide Gel Electrophoresis (PAGE) was carried out with gel slabs according to the method of Laemmili[9]. Protein subunit bands were stained with Coomassie Blue R-250 by standard techniques. The gel was scanned using Gel-Pro-Analyzer ver. 3.1 (Media Cybermetics, 93-97).

RESULTS

Gel electrophotograph (Fig. 1) indicates obvious variations in the banding of protein patterns in the root nodules of *Vicia faba* plants in response to the investigated PbO treatments. The quantitative responses (Table 1) of the separated polypeptide molecular weights were expressed as variations in the number of bands and their relative concentrations (band % or intensity). The observed changes in protein profile were both quantitative (change in band intensity) and qualitative (disappearance of some bands or the appearance of new bands). Concerning the total number of protein bands (Table 1), PbO treatments either applied foliar or in the soil inhibited the number of polypeptides synthesized in the root nodules. The higher foliar PbO treatment (T_1) was the most inhibitory (15 out of 22 bands) to the synthesis of these polypeptides.

The band% (intensity) of the large subunit (240 kDa) of nitrogenase enzyme was greatly inhibited by PbO treatments. Moreover, the synthesis of the small subunit (52-73 kDa) of nitrogenase was completely inhibited by both soil applications (T_3 and T_4) of PbO. The foliar application of both 500 and 2000 ppm of PbO greatly stimulated a 23 kDa protein (from 4.79 to 13.1 and 19.4, respectively) (Fig. 2). The same protein was also stimulated by both soil applications (T_3 and T_4), by only two folds. The synthesis of other groups of polypeptides were relatively stimulated or inhibited in response to Pb stress. The only two new-synthesized proteins were a high molecular weight polypeptide of 178 kDa and a small one of about 5 kDa. Their synthesis was stimulated by PbO soil applications only.

Table 1: Comparative analysis of relative concentration (Band %) molecular weight (mol. wt.) and mobility rate (R_m) of root nodule proteins of *Vicia faba* L. plants in response to Pb toxicity

Band No.	Treatments and band (%) C	T_1	T_2	T_3	T_4	R_m	Mol. wt. (kDa)
1	8.02	14.3	10.7	10.8	8.44	0.02	249.19
2	10.7	7.90	5.46	5.28	5.02	0.04	240.67
3	-	-	-	-	2.88	0.06	178.43
4	1.11	0.50	1.33	1.17	1.22	0.10	135.16
5	1.18	0.66	0.93	-	-	0.15	99.33
6	1.65	0.87	-	-	-	0.18	89.46
7	1.19	1.47	-	1.12	0.95	0.20	77.28
8	1.97	0.78	0.58	-	-	0.24	57.71
9	2.77	1.42	-	-	-	0.26	50.60
10	2.12	1.95	2.42	3.97	3.68	0.28	43.45
11	2.14	0.56	1.08	2.12	1.84	0.34	35.11
12	6.29	4.04	4.17	6.29	6.07	0.38	34.58
13	2.45	2.39	1.31	2.72	2.76	0.40	33.71
14	4.79	4.02	1.85	3.26	4.81	0.44	32.35
15	1.28	2.11	-	1.83	1.20	0.48	31.21
16	1.42	-	1.57	2.13	1.58	0.52	29.80
17	4.97	-	-	-	-	0.57	25.21
18	4.79	13.1	19.4	8.75	8.96	0.59	23.31
19	4.89	4.75	9.08	6.70	6.19	0.63	20.45
20	0.61	1.20	3.31	1.17	1.11	0.67	16.56
21	0.70	-	-	2.15	2.53	0.89	5.97
22	-	-	-	1.82	2.37	0.90	4.71
23	1.64	-	-	1.98	-	0.92	3.83
24	8.74	13.10	11.1	10.3	12.7	0.96	3.52
Bands/Lane	22	18	15	18	18		

T_1 and T_2 = *Vicia faba* plants treated with two foliar applications (500 and 2000) of PbO;
T_3 and T_4 = *Vicia faba* plants treated with two soil applications (500 and 2000) of PbO;
C = untreated (control) plants

DISCUSSION

Heavy metals are generally found naturally at very low concentrations. Elevated concentrations are commonly associated with pollution from human activities. Contamination with heavy metals (Cd, Cu, Ni, Pb and Zn) can cause long-term suppression of carbon cycling, microbial biomass, nitrogen fixation, nitrification,

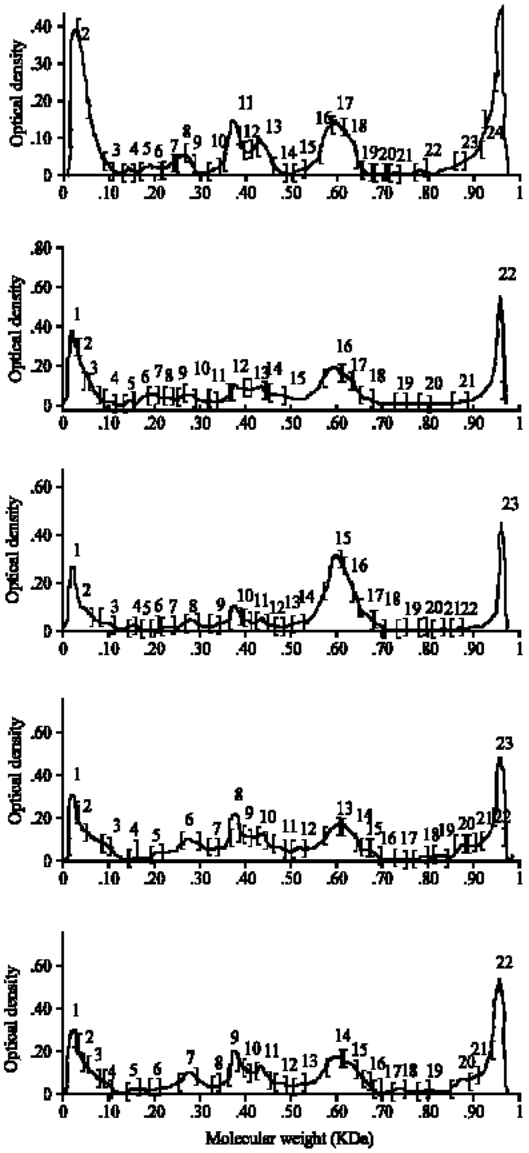

Fig. 1: Scan of the tracks in the electrophotograph (Fig. 2) of SDS-PAGE of total proteins in root nodules of *Vicia faba* L. plants. A, root nodules of untreated (control) plants; B and C, root nodules of plants treated with foliar application of 500 and 2000 ppm PbO; D and E, root nodules of plants treated with soil application of 500 and 2000 ppm PbO

dehydrogenase activity and mycorrhizal incidence[10]. Moreover, heavy metals can affect plant growth by interfering with enzyme activities or preventing the absorption of essential nutrients[11].

In the current investigation, both foliar and soil PbO applications (500 and 2000 ppm) inhibited the band intensity of the large subunit (240 kDa) of nitrogenase

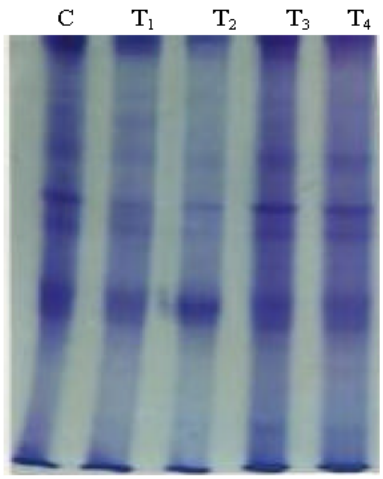

Fig. 2: Electrograph of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of total proteins in root nodules of *Vicia faba* plants. C, untreated (control) plants; T_1 and T_2, root nodules treated with foliar applications of 500 and 2000 ppm PbO; T_3 and T_4, root nodules treated with soil applications of 500 and 2000 ppm, respectively

enzyme; while, the band intensities of the small subunits (52-73 kDa) were inhibited by soil applications only. These quantitative changes in the electrophoretic protein profiles of *Vicia faba* plants were believed to be the result of subfractionation of protein bands[12] in response to the phytotoxicity of Pb stress[13]; where, new bands with a ltered relative mobility are formed instead of the final end product, the protein that should be formed[14]. Other Consistent results were reported in soybean (*Glycine max* L.); where, the nitrogenase activity was decreased in nodules treated with 200 μM Cd^{2+}[15]. Increased concentrations of Cu, Zn, Cd and Mn in wheat tissues decreased the activities of aspartate amino transferases, alanine amino transferases and peroxidases[16].

In white clover (*T. repens*), Nitrogen fixation by *R. leguminosarum* bv. Trifolii was found to be affected by the presence of heavy metals in sludge soils[17]. Decreased N_2 fixation and decreased clover yield occurred because the nodules were not effective in fixing N_2. In nodules where nitrogen fixation is occurring, the plant tissues contain the oxygen-scavenging molecule, leghaemoglobin. The function of this molecule in nodules is to reduce the amount of free oxygen and thereby to protect the nitrogen-fixing enzyme nitrogenase, which is irreversibly inactivated by oxygen.

Accordingly, the reduction in *Vicia faba* L. crop under Pb stress could be directly attributable to the

phytotoxicity of Pb to the synthesis (band intensity) of nitrogenase enzyme. Meanwhile, Pb toxicity may cause inactivation of nitrogenase enzyme by subfractionation of its protein band as well as interference with the function of leghaemoglobin protein in the root nodules.

REFERENCES

1. Beckett, P.H.T. and R.D. Davis, 1988. Upper critical level of toxic elements in plants. New Phytologist, 79: 95-106.
2. Agarwala, S.C., S.S. Bisht and C. Sharma, 1977. Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley. Can. J. Bot., 55:1299-1307.
3. Allinson, D.W. and C. Dzialo, 1981. The influence of Pb^{2+}, Cd^{2+} and Ni^{2+} on the growth of rye grass and oats. Plant Soil, 62:81-89.
4. De Filippis, L.E., R. Hampp and M. Zeigler, 1981. The effect of sub-lethal concentrations of zinc, cadmium and mercury on *Euglena*, II. Respiration, photosynthesis and photochemical activities. Arch. Microbiol., 128:407-411.
5. Woolhouse, H.W., 1983. Toxicity and Tolerance in the Responses of Plants to Metals. In: (Parson, A. and M.H. Zimmermann, Eds.). Encyclopedia of Plant Physiology, New Series, 12C, Springer-Verlag, Berlin, pp: 245-300
6. Chung, L.K., V.K. Gupta and S.K. Sawhney, 1992. Effect of cadmium on enzymes of nitrogen metabolism in pea seedlings. Photochemistry, 31: 395-400.
7. Mengel, K. and E. Kirkby, 1982. Elements with more toxic effects. In: Principles of Plant nutrition. Intl. Potash Inst., Worblaufen-Bern/Switzerland.
8. Allen, O.N. and E.K. Allen, 1981. The Leguminosae. A Source Book of Characterization, Use and Nodulation. Univ. of Wisconsin Press, Madison, USA.
9. Laemmili, U.K., 1970. Cleavage of structural proteins during the assembly of head bacteriophage T4. Nature, 227: 680-685.
10. Sims, G.K., 1990. Biological degradation of soil. Adv. Soil Sci., 11: 289-330.
11. Robb, D.A. and W.S. Pierpoint,1983. Metals and Micronutrients: Uptake and Utilization by Plants. In: Acad. Press, London.
12. Shehata, M.M., A.A. Habib, N.S. Khalifa and M.S. Salama, 2000. Cytological and biochemical effects of 5-Fluorouracil and cholchicine on Vicia faba plants. Egypt. J. Biotechnol., 7: 218-233.
13. Beltagi, M.S., 2001. Molecular responses of *Vicia faba* plants to heavy metal stress. Bull. Fac. Sci. Assiut Univ., 30: 219-227.
14. Abdel-Salam, A.Z.E., H.Z. Hassan, F.M.I. Badaway and W.M. Abdel-Naby, 1996. The mutagenic potentialities of three pesicides on three biological systems. Egypt. J. Genet. Cytol., 22: 109-128.
15. Tomaro, M.L., K.B. Balestrasse, M.B. Benavides and M. Gallego, 2003. Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. Func. Plant Biol., 30: 57-64.
16. Chakrabarti, C. and T. Chakrabarti, 1988. Effects of irrigation with raw and application of primary settled sewage sludge on wheat plant growth, crop yields, enzymatic changes and trace element uptake. Environ, Pollut., 51: 210-236.
17. Ghorbani, N.R., N. Salehrastin and A. Moeini, 2002. Heavy metals affect the microbial populations and their activities. 17th WCSS, August 2002, Thailand, pp: 14-21.