Cey. J. Sci. (Bio.Sci.) Vol. 27(1), 1999, 27-32

ROLE OF VESICULAR ARBUSCULAR MYCORRHIZAE IN NITRATE REDUCTION SYSTEM AND ZINC UPTAKE IN *ZEA MAYS*

S.Senthilkumar, P.Saravanakumar[1,*] and M.Sathiyanathan
Department of Biotechnology, JJ College of Arts and Science, Pudukkottai 622 404. India. [1]Department of Botany, University of Peradeniya, Sri Lanka

ABSTRACT

Corn (*Zea mays*) seedlings were inoculated with *Gigaspora margarita* and heavy metal stress was induced with zinc sulphate at 50 and 100 mM concentrations after ensuring the establishment of the fungus. In general, nitrate reductase activity and protein content were higher in all the mycorrhizae colonised seedlings. Zinc content was estimated both in leaves and roots of infected and non-infected seedlings. 30.5% of heavy metal accumulation was found in the roots and about 69.49 % in the leaves in the absence of Vesicular Arbuscular Mycorrhizae (VAM), whereas in the VAM colonised seedlings about 40.1% was accumulated in the leaves and 59.85% in the roots which point out to the retention of the observed heavy metal in the roots. This may indicate the synthesis of newer proteins that could bind the heavy metal and retain in the root, a possible mechanism of heavy metal resistance.

INTRODUCTION

Vesicular Arbuscular Mycorrhizal (VAM) fungi are obligate symbiosis that benefit plants by extending the functional root system and transporting nutrients to plants through hyphae (Cox *et al.*, 1975; Rogers and Williams, 1986; Koide and Elliott, 1989; Raju *et al.*, 1990; Wright and Upadhyaya, 1996). Besides, VAM helps plant to overcome adverse environmental factors like drought, salinity and heavy metal toxicity (Harley and Smith, 1983). Heavy metal toxicity is an important problem that the agricultural sector is facing. Use of chemicals to chelate the heavy metal in soil itself thus making it non-available to plants is no more considered as a viable solution. One of the biological considerations is that VAM encounter the phytotoxic effects of heavy metal and also help the plant in nutrient uptake during the adverse conditions (Arines *et al.*, 1989; Senthilkumar and Arockiasamy, 1997). It has been established that VAM fungi help alleviate the toxicity up to certain concentrations (Chandra and Kaurkehsi, 1994; Senthilkumar and Arockiasamy, 1995). Nitrate reduction in plants is a system that is very sensitive to heavy metals. However, increased nitrate reductase (NR) activity by VAM fungi has been reported by few workers (Wright and Abha Upadhyaya, 1996; Mathur and Vyas, 1996). The present study is aimed at finding out the NR activity, protein estimation and the distribution of heavy metal in the seedlings grown under different concentrations of zinc inoculated with *Gigaspora margarita* .

*Corresponding author. Tel.: 08-389130; fax: 8 388018; e-mail: saravan@botany.pdn.ac.lk

MATERIALS AND METHODS

The pots used for the experiment were repeatedly washed with tap water and dried in sunlight. The pots were filled with garden soil and sand at the ratio 1:1. Later, the pots were covered with aluminium foil and autoclaved for 3 hours at 20 lb pressure (Raju *et al.,* 1990). Sterilised sand mixture was taken in pots (3 kg / pot). In one set VAM was inoculated (pure culture of *Gigaspora margarita* was obtained from Prof. D.J. Bagyaraj, University of Agricultural Sciences, Bangalore, India) with 200 g of soil containing 30 spores of *Gigaspora margarita*. Certified and surface sterilised seeds of *Zea mays* were sown (7 seeds / pot) and the medium was irrigated with Cakmak and Marschegner (1996) nutrient solution. Medium without VAM treatment was kept as a control. Seedlings were maintained for 50 days. VAM inoculated seedlings were removed from each pot and the colonisation was ensured (Phillips and Hayman, 1970). After ensuring the colonisation, zinc sulphate was given along with nutrient solution at 50 and 100 mM. A similar treatment was followed for control seedlings. Ten days after the heavy metal treatment random sampling was carried out from the leaves and roots of infected and non-infected seedlings for biochemical analysis. Five replications were used for each treatment. *In vivo* NR activity was estimated by the method of Srivastava and Mathur (1981). Protein was estimated using the Lowry *et al.,* (1951) method and zinc content was analysed using atomic absorption spectroscopy after proper wet digestion (Peach and Tracey, 1956).

Random sampling was done for all the samples in triplicates. Estimations were carried out in triplicates and their mean values were subjected to Student's two mean 't'test (Gupta, 1977).

RESULTS AND DISCUSSION

Apart from the harmful effects to man and animals, the heavy metal also reduce plant growth (Mukerji and Mukerji, 1990). The comparative results of NR activity as affected by different concentration of zinc sulphate on roots and leaves are expressed in Table 1a and Table 1b. Lower concentration of zinc was found to promote NR activity and higher concentration caused definite inhibition of NR. There are several enzymes, which are very sensitive to heavy metal toxicity (Greger *et al.,*1991). It has also been proved that zinc interferes with NR activity (Falchuk *et al.*, 1977). NR activity was in the range of 0.003 to 0.15 μ mol. of nitrate produced h^{-1} g^{-1} F.wt. in shoots. Activity of this enzyme was found to be higher in 50 mM concentration in both the organs. A similar enhancement of nitrate reductase activity was reported in leaves and roots of VAM infected clover and this was attributed to improved phosphorus nutrition provided by VAM symbiosis (Shivashankar and Iyer, 1988). The effect of zinc on the protein contents of root are shown in Table 2.

Table 1 a

Effect of VAM on nitrate reductase activity in roots of *Zea mays* seedlings grown at different concentrations of zinc

Concentration of Zn (mM)	Contol NRA (μ mol. NO_2 formed $h^{-1}g^{-1}$ Fresh weight)	VAM-inoculated NRA (μ mol. NO_2 formed h^{-1} g^{-1} Fresh weight)	't' value
0	0.045	0.06	4.02*
50	0.051	0.07	7.47*
100	0.031	0.052	5.36*

*Significance at 5% level.

Table 1 b
Effect of VAM on nitrate reductase activity in leaves of *Zea mays* grown at g different concentrations of zinc

Concentration of Zn (mM)	Control NRA (μ mol. NO_2 formed $h^{-1}g^{-1}$ Fresh weight)	VAM-inoculated NRA (μ mol. NO_2 formed $h^{-1}g^{-1}$ Fresh weight)	't' value
0	0.004	0.08	5.31*
50	0.005	0.15	8.71*
100	0.003	0.005	4.44*

*Significance at 5% level.

Table 2
The effect of VAM concentrations in the root protein content of *Zea mays* grown at different concentrations of zn

Concentration of Zn (mM)	Control (mg/g F.wt.)	VAM-inoculated (mg/g F.wt.)	't' value
0	0.25 ± 0.0033	0.175 ± 0.0024	12.98*
50	0.22 ± 0.003	0.298 ± 0.0033	7.80*
100	0.03 ± 0.0008	0.15 ± 0.0008	13.66*

*Significance at 5% level.

The root protein was found to decrease, with increasing heavy metal concentrations in the absence of VAM fungi. However, increase in root protein content observed in the presence of VAM plays an important role in the synthesis of root protein and this may be as a response to heavy metal (Wright *et al.*, 1996) demonstrated this production of glomalin on mycorrhizal hyphae (12 - 63 μg protein/mg dried hyphae) which strongly that active mycorrhizal fungi are the major contributors of protein. The possibility that plants also produce a protein similar to the mycorrhizal fungi protein is being explored. Wright and Upadhyaya (1996) further stated that uncolonised Sudan grass does not produce the protein.

The heavy metal distribution in the roots and leaves of both infected and non-infected seedlings at two different concentrations is given in the Table 3. In the non-infected seedlings, about 69.49% of the heavy metal was distributed in the leaves and only 30.5% in the root system. The transport of various minerals from the soil to the aerial parts is physiologically well explained (Wright and Upadhyaya 1996). However, this natural phenomenon is altered and only 40.1% of the heavy metal is present in the leaves and the bulk 59.85% is retained in the root system because of the presence of VAM. The increased production of the root protein in the presence of VAM suggested the possibility that newly synthesised protein may play an important role in the retention of heavy metals in root. In the non-infected seedlings, a steady decrease in the root protein was observed from 0.03 to 0.25 mg/g fresh weight (Table 2). But the root protein of colonised seedlings showed a remarkable increase from 0.15 to 0.298 mg/g F. wt. At very high concentration, there was no increase in protein content.

Table 3
Absorption and transport of zinc by *Gigaspora margarita*

Concentration of Zn (mM)		Control (Zn content mg/g Fwt)	VAM-inoculated (Zn content mg/g Fwt)	't' value
Roots	50	0.08	0.35	7.47*
	100	0.10	0.44	13.01*
Leaves	50	0.18	0.20	4.51*
	100	0.23	0.33	8.66*

*Significance at 5% level.

There are several views regarding the accumulation of heavy metals in the presence of VAM rich as selective absorption of essential minerals by VAM fungi or accumulation of heavy metals in the cell wall having large number of complex sites for metallic cation binding, possibly by the special protein fraction.

Therefore, the fungus may provide the plant with additional metal binding sites or chelating the heavy metals by synthesising newer proteins (Cumming and Weinstein 1990; Senthilkumar and Arockiasamy 1995). Bradley (1982) further proposed that the endophyte may provide adsorptive surfaces within the cortical cells of the host root, avoiding metal toxicity. Though the nature of the binding protein was not studied, the present study suggests that VAM fungi play an important role in the synthesis of newer proteins and thus help the host to tolerate heavy metal toxicity.

ACKNOWLEDGEMENTS

We thank Prof.D.J.Bagyaraj, Department of Microbiology, University of Agricultural Sciences, Bangalore, India for providing VAM cultures.

REFERENCES

Arines, J., Vilorino,A. and Saing, M. (1989). Effect of different inocula of VAM fungi on manganese content and concentration in red clover plants. *New Phytologist* **112**: 215 - 219.

Bradley, R., Burt, A.J. and Read, D.J. (1982). The role of mycorrhizal infection in heavy metal resistance. *New Phytologist* **91**: 197-209.

Cakmak, K.I. and Marschener, H. (1986). Mechanism of phosphorus induced Zn deficiency in cotton. I. Zinc deficiency enhanced uptake rate of phosphorus. *Physiology of Plant* **68**: 483-490.

Chandra, S. and Kaurkehsi, H. (1994). Role of VAM in plants raised in stress areas. *Mycorrhiza News* **6**: 9-10.

Cox, G., Sanders, F.E., Tinker, P.E. and Wild J.A. (1975). Ultrastructural evidence relating to host endophyte transfer in a vesicular arbuscular mycorrhiza . In: *Endomycorrhiza* (Sanders, F.E., Mosse, B. and Tinker, P.B. [ed]) Academic Press, London, 297 – 312.

Cumming, J.R.R. and Weinstein, L.H. (1990). Aluminium mycorrhizal interactions in the physiology of pitch pine seedlings. *Plant and Soil* **125**: 7-18.

Falchuk, K.H.., Ulpino, L., Magnus, B. and Valee,B.L. (1977). *E.grscilis* RNA polymerase. I. A zinc metalloenzyme. *Biochem. Commun.* **74**: 1206.

Greger, M., Brammer, E., Lindberg, S., Larson,G. and Idestem Almquist,J. (1991). Uptake and physiological effects of cadmium in sugar beet related to mineral provision. *Journal of Experimental Botany* **42**:729 - 739.

Gupta, S.P. (1977). Statiatical methods. S. Chand and Co. New Delhi pp 320-332

Harley, A.J. and Smith, S.E. (1983). *Mycorrhizal symbiosis.* Academic Press, New York, pp.484.

Koide, R. and Elliott, G. (1989). Cost, benefit and efficiency of the VAM symbiosis. *Functional Ecology* **3**: 252 - 255

Lowry, O.H., Rosebrough, A., Farr, L.and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* **193**: 265 - 275.

Mathur, N. and Vyas, A. (1996). Changes in nitrate reductase and glutamate synthesise activities in *Zizipus mauritiana* by different VAM fungi. *Current Sciences* **70**: 1144-1145.

Mukerji, C. and Mukerji, S. (1990). Metabolism of germinating rice (*Oryza sativa* L.) seeds as influenced by toxic concentration of cadmium *Journal of Plant Physiology* **33**: 190 - 196.

Peach, K. and Tracey, M.V. (1956). *Modern methods of plant analysis* **Vol. I,** 473.

Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vascular fungi for rapid assessment of infection. *Transactions of British Mycological Society* **55**: 158 -161.

Raju, P.S., Clark, R.B., Ellis, J.R. and Duncan, R.R. (1990). Benefit and cost analysis and phosphorus efficiency of VAM mycorrhizal fungi colonisation with Sorghum (*Sorghum bicolor*) genotypes grown at varied phosphorus levels. *Plant and Soil* **124**: 199 –204.

Rogers,R.D. and Williams, S.E. (1986). Vesicular Arbuscular Mycorrhiza. Influence on plant uptake of Cesium and Cobalt. *Soil Biology and Biochemistry* **18**: 371 – 376.

Senthilkumar, S. and Arockiasamy, D.I. (1995). Distribution of zinc in various parts of *Sorghum bicolor* (L) Moench seedlings in the presence of VAM fungi. I. *Journal of Microbiology* **35** : 185 - 188.

Senthilkumar, S. and Arockiasamy, D.I. (1997). Effect of VAM fungi in alleviating the Zinc phytotoxicity in *Sorghum bicolor* (L) Moench. *Ecology and Environmental Conservation* **3**: 179 - 182.

Shivashankar, S. and Rohini Iyer (1988). Influence of VAM mycorrhizae on growth and nitrate reductase activity of Black pepper. *Indian Phytopathology* **41**: 428-433.

Srivastava, R.C. and Mathur, S.N. (1981). Effect of GA_3 on NR activity of *Phaseolus mungo* L. *Annals of Botany* **47**: 147.

Wright Sara, F, and Abha Upadhyaya. (1996). Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science* **161**: 575.

Wright Sara, F., Franke-Snyder, M., Morton, J.B. and Upadhyaya, A. (1996). Time-course study and partial characterisation of a protein on hyphae of arbuscular mycorrhizal fungi during active colonisation of roots. *Plant and Soil* **18**: 193 - 203.