sequence analysis of individual forms of genes belonging to a multigene family by designing alternative primers, and (iii) situations where the outcome of the genetic end product is dependent upon more than one gene and the expression of each gene involved needs to be monitored. However, the applicability of this technique is limited to only situations where the nucleotide sequence of the gene to be searched is known.

1. Frohman, M A., in *PCR Protocols* (eds Innis, M. A., Gelfand, D. H, Sninsky, J. J. and White, T. J), Academic Press, New York, 1992, pp 28–38.
2. Jefferson, R. A., Kavanagh, T. A., Bevan, M. W., *EMBO J*, 1987, **6**, 3901–3907.
3. Thompson, C. J., Movva, N. R., Tizard, R., Cramen, R., Davies, J. E., Lauwereys, M, Botterman, J., *EMBO J.*, 1987, **6**, 2519–2523.
4. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
5. De Vries, S., Hoge, H., Bisseling, T., in *Plant Molecular Biology Manual* (eds Gelvin, S. B., Schilperoort, R. A and Verma, D. S. P.), Kluwer Academic Publishers, The Netherlands, 1988, **B6**, pp 1–13.
6. De Block, M, Botterman, J., Vandewiele, M., Docky, J., Thoen, C., Gossete, V., Rao Movva, N., Thompson, C., Van Montagu, M. and Leemans, J., *EMBO J.*, 1987, **6**, 2513–2518.
7. Jefferson, R. A., *Plant Mol. Biol. Rep.*, 1987, **5**, 387–405.
8. Jefferson, R. A., *Nature*, 1989, **342**, 837–838.

ACKNOWLEDGEMENTS Grants from the Rockefeller Foundation (RF 89026#39), New York, from the Department of Biotechnology, Government of India, to SKS, and from the University Grants Commission, New Delhi, to PN in the form of an Associateship are acknowledged gratefully.

Received 23 August 1994; revised accepted 1 March 1995

Changes in nitrate reductase and glutamine synthetase activities in *Ziziphus mauritiana* by different VAM fungi

Nishi Mathur and Anil Vyas

Department of Botany, J. N. V. University, Jodhpur 342 001, India

Efficacy of different vesicular arbuscular mycorrhizae (VAM) species towards increasing nitrate reductase (NR) and glutamine synthetase (GS) activities in *Ziziphus mauritiana* was evaluated under glasshouse conditions. After 45 days of seedling growth, plant samples were analysed. In general GS activity was higher in all the treatments as compared to NR activity. Addition of VAM increased the activities of both these enzymes. However, different VAM species varied in their efficacy to increase these enzymatic activities. Among the five VAM species used during the present investigation, *Glomus fasciculatum* was found to be the most efficient VAM species for *Z. mauritiana* as it increased most effectively the activities of GS and NR in this multipurpose fruit tree of the Indian Thar desert. This VAM fungus also increased protein contents more than twofold in *Z. mauritiana*, which can be of great significance in producing highly proteinaceous leaf fodder of this desert plant.

NITROGEN is the most important mineral nutrient for plant growth[1]. Indirect increased nitrogen uptake by vesicular arbuscular mycorrhizae (VAM) has been well recognized[2,3]. Due to its beneficial effects, VAM are receiving considerable attention in agriculture and forestry[4]. *Ziziphus mauritiana* is an important multipurpose fruit tree of arid and semiarid regions. It is a source of fuel, fodder and timber. Cultivation of the plant is done mostly in P-deficient sandy soils of drought-prone areas. The primary stresses imposed on vegetation by environment are lack of water and nutrients[5]. The VAM fungi may be of particular significance in coping with P-deficiency stress in natural ecosystems[1]. There has been less work on nitrogen metabolism in plants colonized by VAM[2]. Nitrate reductase (NR) and glutamine synthetase (GS) are two major enzymes of nitrogen metabolism. Increased NR and GS activities by VAM fungi have been reported by few workers[1,2]. Efficiency of VAM symbiosis is affected by a variety of factors, including environmental conditions and the host plant; hence, response of different host–fungus combinations and effects of different environmental conditions must be analysed[3]. Keeping all these facts in mind, efficiency of different VAM species were studied towards increasing NR and GS activities in *Z. mauritiana* in order to select an efficient VAM strain for this neglected multipurpose fruit tree of the Indian Thar desert.

Five VAM species, namely *Glomus constrictum* Trappe, *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe, *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe, *Gigaspora margarita* Becker and Hall and *Scutellospora calospora* (Nicol. and Gerd.) Walker and Sanders collected from rhizosphere soils of *Z. mauritiana* were maintained on *Cenchrus ciliaris* as pot cultures. The soil from these pot cultures along with the roots of *C. ciliaris* was used as source of inoculum. Ten g of inoculum was used in each pot of 18 cm diameter, containing sterilized soil, by the layering method[6]. The surface-sterilized seeds of *Z. mauritiana* were sown in these pots and the pots were kept in a glasshouse having 60% humidity and 22–25°C temperature. After 15 days of germination, seedlings were thinned to one per pot. For all studies except that on nitrate reductase, 45-day-old plants were harvested, and the roots and shoots were separated, weighed, immersed in liquid nitrogen, stored at 80°C, and analysed within 48 h. There were 20 replications in each treatment.

Plant tissue, 0.5 g fresh weight of each organ, was

ground in liquid N_2 and extracted in 5 ml of buffer. The GS extraction buffer (pH 8.0) contained 25 mm Tris, 1 mm EDTA, 1 mM DTT, 1 mM reduced GSH, 10 mM $MgSo_4$, 5 mM Glu, 1% PVP and 0.5% Nonidet P-40. After centrifugation, the supernatants were used for enzyme and soluble protein assays as follows.

GS (ES 6.3.1:2) was determined by the transferase assay[7]. The reaction mixture contained 80 µmol of Mes, 60 µmol of L-Gln, 25 µmol of Na_2AsO_4. 2.5 µmol of hydroxylamine, 2 µmol of $MnCl_2$ and 15 µmol of ADP (final pH 7.6). The reaction was initiated by addition of 0.10 ml of enzyme extract and terminated after 10 min for shoots and 20 min for roots by addition of 1 ml of ferric chloride reagent. After the mixture was centrifuged, A500 was determined. NR activity was measured by *in vivo* assay[8] from small pieces of roots or leaf discs.

All enzyme assays were carried out at 30°C and were linear with respect to the length of incubation time and the quantity of enzyme assayed. Soluble protein was determined by Bradford assay[9].

The buffer-soluble protein concentration was low in roots (between 0.51 and 1.76 mg g^{-1} fresh weight) and high in shoots (between 6.80 and 13.50) of *Z. mauritiana* in all the treatments. Addition of VAM increases the soluble protein concentration in both the organs. Different VAM species varied in their efficacy to increase soluble-protein concentration in both the organs (i.e. root and shoot). Among the five VAM species used in the present investigation, *G. fasciculatum* (Thaxt. Sensu Gerd.) Gerd. and Trappe increased soluble-protein concentration most efficiently in both the organs, while *S. calospora* (Nicol. and Gerd.) Walker and Sanders responded least effectively (Table 1). This increase in soluble-protein concentration was similar to the earlier findings[2] of VAM-inoculated *Zea mays* L.

NR activity was in the range of 0.07–0.18 µmol of nitrite produced h^{-1} g^{-1} fresh weight in roots and 0.18–0.9 µmol nitrite produced h^{-1} g^{-1} fresh weight in shoots. Activity of this enzyme was also found to be higher in both organs in all the VAM plants compared to control. However, here again *G. fasciculatum* colonization resulted in maximum increase in the enzyme activity. A similar enhancement of nitrate reductase activity was reported in roots and leaves of VAM-infected clover and was attributed to improved P-nutrition provided by VAM symbiosis[10]. Mc Arthur and Knowles[1] observed 20% increased NR activity in VAM potato plants. Endomycorrhizal fungal species like *G. macrocarpum* and *G. mosseae* have also been known to reduce nitrate ions[11]. The results in the present investigation suggest that with a capacity for reducing nitrate it is likely that the symbiotic effectiveness of the VAM fungus *G. fasciculatum* is enhanced in terms of nitrogen assimilation and translocation to the host plant.

GS activity was higher in all the treatments in root and shoot as compared to NR activity. Similar trend was also noticed in behaviour of different VAM species towards increasing GS activity in roots and shoots of *Z. mauritiana.* Maximum GS activity in roots and shoots were observed in *G. fasciculatum*-colonized plants, while least in *S. calospora*-treated plants. Smith *et al.*[12] showed by separating enzymatically fungal and plant tissue that *G. mosseae* contribute directly to GS activity in onion roots. In VAM roots higher GS activity may be accompanied by increased amino acid synthesis and an increase in 15N–Gln translocation through the xylem. Cliquet and Stewart[2] reported increased ammonium assimilation, Gln production and xylem nitrogen translocation in VAM maize plants.

The present investigation reveals that different VAM fungi vary in their efficacy to increase GS and NR activities and also the soluble-protein concentration. However, GS and NR activities in *Z. mauritiana* roots and stem can be increased significantly by inoculating the plants with *G. fasciculatum*. Whatever the mechanism of increase, the two major enzymes of nitrogen metabolism were increased by VAM fungi in *Z. mauritiana* plants. The application of *G. fasciculatum* can be of great significance in producing highly proteinaceous leaf fodder of *Z. mauritiana* as this VAM fungi increased by more than twofold the protein contents in the leaves of this fruit plant. Further study on the symbiotic relationship of the VAM fungi and *Z. mauritiana* can

Table 1. Changes in glutamine synthetase and nitrate reductase activities and soluble-protein contents in *Z. mauritiana* by different VAM species

	Soluble protein (mg g^{-1} fresh weight)		Glutamine synthetase (µmol $h^{-1}g^{-1}$ fresh weight)		Nitrate reductase	
VAM species	Root	Shoot	Root	Shoot	Root	Shoot
G. constrictum	1 36	8 90	83 10	85.74	0 14	0 25
G. fasciculatum	1 76	13 50	108 00	124 52	0 18	0 29
G. mosseae	1 60	11 60	102 10	122 24	0 16	0 27
Gl. margarita	1 31	8 50	81 35	83 24	0 11	0 22
S calospora	0 98	8 00	71 94	77 94	0 08	0 19
Control	0 51	6 80	56 54	57 08	0 07	0 18
CD at 5% level	0.28	2 36	5.32	0 12	0 02	0 02

be of great significance in understanding the physiology of this multipurpose fruit plant of the Indian Thar desert.

1 Mc Arthur, D A J and Knowles, N. R., *Plant Physiol*, 1993, **101**, 147
2 Cliquet, B J and Stewart, G R., *Plant Physiol.*, 1993, **101**, 865.
3 Smith, S E. and Gianinazzi Pearson, V., *Annu. Rev Plant Physiol. Plant Mol Biol*, 1988, **39**, 221.
4 Peterson, R L., Piche, Y and Plenchette, C., *Biotech. Adv*, 1984, **2**, 101
5. Fisher, R A and Turner, N. C., *Annu. Rev Plant Physiol*, 1978, **29**, 277.
6 Mathur, N and Vyas, A, *J. Plant Physiol.*, 1995 (in press).
7. Shapiro, B M. and Stadtman, E. R., *Methods Enzymol.*, 1970, **A17**, 910
8. Stewart, G R. and Orebamjo, T, *New Phytol*, 1979, **83**, 311
9 Bradford, M. M, *Anal. Biochem* 1976, **72**, 248.
10 Oliver, A. J., Smith, S. E, Nicholas, D J. D, Wallace, W and Smith, F. A., *New Phytol.*, 1983, **94**, 63.
11 Ho, I. and Trappe, J. M, *Mycologia*, 1975, G. 4
12. Smith, S. E., St. John, B. J, Smith, F. A. and Nicholas, D. J D., *New Phytol.*, 1985, **99**, 211

ACKNOWLEDGEMENT NM thanks CSIR, New Delhi, for financial support.

Received 26 August 1994, revised accepted 22 March 1995