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Biochemical changes in *Ziziphus xylopyrus* by VA mycorrhizae

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Abstract. The efficacy of six VAM species, *Acaulospora morrowae* Spain & Schenck, *Gigaspora margarita* Becker & Hall, *Glomus fasciculatum* (Thaxt. Sensu. Gerd.) Gerd. & Trappe, *G. macrocarpum* Tul. & Tul. *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders, and *Sclerocystis rubiformis* Gerd. & Trappe, collected from rhizosphere soils of *Ziziphus xylopyrus*, was evaluated for enhancement of NR, GS, GDH, PRO, PPO activities and protein, phenolic, and catechin contents in this fruit tree. Culturing was done under glass house conditions and analyses were performed 180 days after inoculation. All fungi showed beneficial effects, with *S. calospora* being the most promoting of all biochemical parameters.

Keywords: Glutamine dehydrogenase; Glutamine synthetase; Nitrate reductase; Peroxidase; Polyphenol oxidase; Mycorrhizae; VAM; *Ziziphus xylopyrus*.

Abbreviations: GDH, glutamine dyhydrogenase; **GS,** glutamine synthetase; **NR,** nitrate reductase; **PRO,** peroxidase; **PPO,** polyphenol oxidase; **VAM,** vesicular arbuscular mycorrhizae.

Introduction

Ziziphus xylopyrus is an important multipurpose fruit tree of the arid and semi arid regions of Asia. It is a source of fuel, fodder, and timber, besides producing the nutritious fruit known as ber. VAM fungi are well known to bring about physiological changes in plants by increasing various enzymatic activities (Mathur and Vyas, 1995). Nitrogen is the nutrient, of which availability is most likely limiting to plant growth (Mc Arthur and Knowles, 1993). Increased nitrogen uptake by VA mycorrhizae has been well recognized (Cliquet and Stewart, 1993). Nitrate reductase, glutamine synthetase, and glutamine dehydrogenase are important enzymes of nitrogen metabolism. Peroxidase and polyphenol oxidase are important components of the defence mechanism of plants against pathogens. Phenols and catechins are also important in plant disease resistance. (Protein levels in leaves determine the nutritive value of fodder). In view of these benefits, this investigation was undertaken to evaluate the efficiency of different VAM species towards biochemical enhancement in *Z. xylopyrus*.

Materials and Methods

Six VAM species—*Acaulospora morrowae* Spain & Schenck, *Gigaspora margarita* Becker & Hall, *Glomus fasciculatum* (Thaxt. Sensu. Gerd.) Gerd. & Trappe, *G. macrocarpum* Tul. & Tul. *Sclerocystis rubiformis* Gerd. & Trappe, and S*cutellospora calospora* (Nicol. & Gerd.) Walker & Sanders—were examined. Fungal samples were collected by wet sieving and decanting (Gerdemann and Nicolson, 1963) soil samples from the rhizosphere of *Ziziphus xylopyrus*. The fungi were identified following the synoptic key of Trappe (1982) and the manual of Schenck and Perez (1987) and maintained in pot cultures of *Cenchrus ciliaris*. Soil samples from the pot cultures, along with infected root segments of *C. ciliaris*, were used as inoculum. Twenty g of inoculum containing about 5,000 spores and infected root segments were inoculated in each 18-cm diameter pot containing sterilized soil. The soil used was sandy, with a pH of 8.2, available p of 2.30 ppm, total N of 2.15 ppm, and organic carbon of 0.12 percent. Five surface sterilized seeds (sterilized by 0.1% $Hgcl_2$ solution) of *Z. xylopyrus* were sown per pot. The pots were placed in a glass house at 60 percent humidity, 22–25°C and 12–14 h day length. The pots without VAM inoculum served as control.

All pots were supplied with half strength Hoaglands' nutrient solution every 15 days. After two weeks seedlings were thinned to one per pot. There were ten pots per treatment. After six months the plants were harvested. Phosphorus was estimated by the vanadomolybdate method (Jackson, 1973). Soluble proteins in leaves and roots were determined by the comassive blue method (Bradford, 1976) and total phenols were determined by using Folin ciocalteu's reagent (Bray and Thrope, 1954). For estimation of catechins, root samples were steamed and dried to constant weight. Catechins extracted in 50 per cent acetone were estimated by measuring absorbance by the yellow colour that developed with diazotized sulphanilamide at 430 nm (Gulati et al., 1993).

GS, GDH and NR Assay

Plant tissue, 0.5 g fresh weight per organ, was ground in liquid N_2 and extracted in 5 ml buffer. The GS, GDH extraction buffer (pH 8.0) contained 25 mm Tris, 1 mm

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EDTA, 1 mm DDT, 1 mm reduced GSH, 10 nM $MgSo_4$, 5 nM Glu, 1% PVP, and 0.5% Nonidet p-40. After centrifugation, the supernatants were used for enzyme and soluble protein assays as follows: GS was determined by the transferase assay (Shapiro and Stadtman, 1970). The reaction mixture contained 80 μmol of Mes, 60 μmol of L- Gln, 25 μmol of Na_2 ASO_4, 2.5 mol of hydroxylamine, 2 μmol of Mn Cl_2, and 15 μmol of ADP (Final pH 7.6). The reaction was initiated by adding 0.10 ml of enzyme extract and terminated after 10 min for shoots and 20 min for roots by adding 1 ml of ferric chloride reagent. The mixture was contrifuged and A_{500} was measured in the supernatant. NR activity was measured by the in vivo assay of Stewart and Orebamjo (1979) from small pieces of roots and shoots. GDH was assayed by determining the rate of 2-oxogluarate NADH oxidation. The reaction mixture contained 150 μmol of NH_4Cl, 1 μmol of $CaCl_2$, 0.3 μmol of NADPH, 20 μmol of 2 oxogluatrate and 100 μmol of Tris buffer (Final pH 8.2 and final volume 1 ml).

PRO and PPO Assay

Root pieces were homogenized in 0.1 M phosphate buffer, pH 7.0, with a pre-chilled mortar and pestle at 4°C. The homogenate was centrifuged at 5,000 *g* for 15 min and the supernatant was used for enzyme assay. Peroxidase activity was measured by incubating the enzyme with guaiacol and hydrogen peroxide (Racusen and Foote, 1965). The arbitrary unit of enzyme activity chosen was change in absorbance of 0.001 sec^{-1}. Polyphenol oxidase activity was measured at 420 nm, using the method of Mahadevan (1975). The activity is presented in terms of absorbance of 100 mg (fresh weight of tissues)$^{-1}$. At harvest time ten samples of each treatment were analyzed.

Results and Discussion

Observations regarding changes in NR, GS, and GDH activities are presented in Table 1. NR activity was in the range of 0.12–0.27 μmol nitrite produced h^{-1} g^{-1} fresh weight in roots and 0.17–0.35 μmol h^{-1} g^{-1} in shoots. Activity of this enzyme was higher in both organs in all VAM inoculated seedlings when compared with the control. However, the VAM species varied in their effects on this enzyme. Inoculated S. calospora plants showed a more than twofold increase in NR activity. A similar enhancement of NR activity in roots and leaves of VAM infected clover was attributed to an improved P-nutrition (Oliver et al., 1983). The highest GS activity in roots and shoots of *Z. xylopyrus* was observed with *S. calospora* inoculated plants, and the smallest increase was observed in *A. morrowae* inoculated plants. Smith et al. (1985) reported that *G. mosseae* contributed positively to GS activity in onion roots. In VAM infected roots, higher GS activity is probably accompanied by increased amino acid synthesis. Cliquet and Stewart (1993) reported increased ammonia assimilation, Gln production and xylem nitrogen translocation in VA mycorrhiza infected maize plants. VA mycorrhizal inoculation increased NADP–GDH activity more prominently in roots than shoots. *S. calospora* inoculated plants showed a more than threefold increased of NADP–GDH activity in roots of *Z. xylopyrus*. Cliquet and Stewart (1993) also observed an increased NADP–GDH activity in VA mycorrhizal roots. The presence of NADP–GDH, characteristic of fungi (Sims and Folkes, 1964) indicates that VAM colonized *Z. xylopyrus* roots have the potential for nitrogen metabolism through the fungal pathway.

Whatever the mechanism, the three key enzymes of inorganic nitrogen assimilation in *Z. xylopyrus* are enhanced most effectively by *S. calospora*. VAM inoculation also increased peroxidase and polyphenoloxidase activities in roots of *Z. xylopyrus* (Table 2). The increased peroxidase activity by VAM fungi may be due to an increased P-uptake resultant from the symbiosis. McArthur and Knowles (1992) reported lower peroxidase activity in low P roots than high P roots.

A positive correlation was observed between total phenol accumulation and PPO activity in VAM inoculated *Z. xylopyrus* roots (Table 2). *S. calospora* also was most efficient in enhancing phenolic accumulation. The increased phenolic accumulation might have resulted from an increased PPO activity. Accumulation of phenol in VA mycorrhizal plants has been reported (Krishna and Bagyaraj, 1984). as well as in tissues of a variety of plants during pathogenesis (Vidhyasekaran, 1989). PRO and PPO are important in the defence mechanism against pathogens. Both enzymes are involved in the oxidation of phenolic compounds to quinones, which are presumably toxic to

Table 1. Changes in GS, NR and GDH activities in *Z. xylopyrus* resultant from infection by different VAM fungi.

Treatment	Soluble protein (mg g^{-1} fw)		GS (μmol h^{-1} g^{-1} fw)		NR (μmol h^{-1} g^{-1} fw)		NADPH-GDH (μmol h^{-1} g^{-1} fw)	
	Root	Leaves	Root	Shoot	Root	Shoot	Root	Shoot
Acaulospora morrowae	1.14	9.14	71.06	76.18	0.14	0.21	0.29	1.15
Gigaspora margarita	1.76	12.22	103.20	116.07	0.23	0.28	0.72	1.20
Glomus fasciculatum	1.58	11.79	94.24	99.33	0.20	0.25	0.54	1.16
G. macrocarpum	1.51	10.46	90.18	93.26	0.17	0.22	0.42	1.17
Sclerocystis rubiformis	1.42	10.17	82.16	87.20	0.15	0.19	0.34	1.18
Scutellospora calospora	1.92	14.02	110.15	125.56	0.27	0.35	0.78	1.24
Control	0.72	7.13	55.18	56.23	0.12	0.17	0.20	1.12
L.S.D. at $P \leq 0.05$	0.25	2.23	0.12	0.14	0.02	0.02	–	–

Table 2. Changes in PRO and PPO activities in roots of *Z. xylopyrus* as a result of infection by different VAM fungi.

Treatment	PRO activity (Units mg^{-1} protein)	PPO activity (ΔA_{420}/100 mg fw)	Total-P (mg g^{-1} dw)	Total phenol (% dw)	Catechin (% dw)
Acaulospora morrowae	102.4	109.4	3.8	4.1	7.0
Gigaspora margarita	137.3	142.6	6.2	6.7	10.3
Glomus fasciculatum	120.5	130.2	5.4	6.0	9.3
G. macrocarpum	116.2	123.3	4.9	5.4	8.4
Sclerocystis rubiformis	112.4	118.5	4.3	4.9	7.9
Scutellospora calospora	178.0	183.6	7.6	7.9	11.2
Control	86.2	92.4	3.2	3.4	6.4
L.S.D. at $P \leq 0.05$	19.5	14.2	1.2	1.2	0. 5

pathogens (Mathur and Vyas, 1995). The considerable increase in PRO and PPO by *S. calospora* may be significant in protecting this fruit tree from attacking pathogens. Similarly, the increased catechin levels by the VAM inoculation (Table 2) may also enhance the pathogen defence mechanism of *Z. xylopyrus*. A nearly twofold increase in protein content was observed in leaves of *Z. xylopyrus* inoculated with *S. calospora* (Table 1). The higher protein accumulation in leaves of this plant may be advantageous when used as fodder for animals. Hence this VAM endophyte can also improve the nutritive value of *Z. xylopyrus* leaves.

It can be concluded that inoculation with *S. calospora* will cause elevation of assimilating enzymes most efficiently in *Z. xylopyrus*, which collectively will lead to increased biomass and more highly proteinaceous leafy fodder. It will also make the plant more resistant to pathogens as a result of increased PPO and PRO activities.

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