

## Possible Role of Arbuscular Mycorrhizal (AM) Fungi on Drought Tolerance in *Vigna unguiculata* subsp. *unguiculata* (L.) Walp and *Leucaena latisiliqua* L.

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**Keywords:** AMF, *Rhizobium* sp., drought tolerance, proline, *Vigna unguiculata*, *Leucaena latisiliqua*

### ABSTRAK

Ujian terhadap kultur pot telah dibuat dalam tanah yang disteril bagi menentukan kesan tiga kulat AM, *Acaulospora scrobiculata*, *Glomus aggregatum* dan *G. etunicatum*, kedua-duanya secara bersendirian serta dicantumkan, pada pertumbuhan tanaman serta rintangan kemarau *Vigna unguiculata* dan *Leucaena latisiliqua*. Semua tumbuhan diinokulat bersama *Rhizobium* sp. Semasa 45 hari pertama, semua pot diairkan kepada kapasiti lapangan dan tanaman kemudiannya bergantung kepada pusingan kemarau melalui air tertahan selama 7 hari. Tanaman tersebut seterusnya bergantung kepada 7 pusingan kemarau. Penginokulatan endofit tunggal *G. etunicatum* dalam *V. unguiculata* dan *A. scrobiculata* dalam *L. latisiliqua* menghasilkan pertumbuhan, biojisim, bilangan nodula, dan peratus pengkolonian akar yang lebih baik. Kandungan proline lebih tinggi dalam semua tanaman yang diinokulat bersama *G. etunicatum*. Sebaliknya menginokulatan *G. aggregatum* dalam *Vigna unguiculata* dan *G. etunicatum* dalam *L. latisiliqua* terhasil dalam kandungan tisu N, P dan K yang lebih tinggi. Dalam menginokulatan endofit berganda, *A. scrobiculata* dan *G. aggregatum* dalam *V. unguiculata*; *A. latisiliqua* dan *G. etunicatum* dalam cantuman *L. latisiliqua* didapati menjadi sangat berkesan.

### ABSTRACT

Pot culture experiments were carried out in sterilized soil to determine the effects of three AM fungi, *Acaulospora scrobiculata*, *Glomus aggregatum* and *G. etunicatum*, both individually as well as in combinations, on the plant growth and drought resistance of *Vigna unguiculata* and *Leucaena latisiliqua*. All plants were inoculated with *Rhizobium* sp. During the first 45 days all pots were watered to field capacity and the plants were then subjected to drought cycles by withholding water for 7 days. The plants were further subjected to 7 drought cycles. Inoculation of single endophyte *G. etunicatum* in *V. unguiculata* and *A. scrobiculata* in *L. latisiliqua* produced higher growth, biomass, nodule number, and percentage of root colonization. Proline content was higher in all the plants inoculated with *G. etunicatum*, whereas inoculation of *G. aggregatum* in *Vigna unguiculata* and *G. etunicatum* in *L. latisiliqua* resulted in higher tissue N, P and K contents. In double endophyte inoculations, *A. scrobiculata* and *G. aggregatum* in *V. unguiculata*; *A. scrobiculata* and *G. etunicatum* in *L. latisiliqua* combinations were found to be highly effective.

### INTRODUCTION

Arbuscular mycorrhizas (AM) confer several benefits on host plants. The AM fungi help plants not only in the better utilization of soil phosphorus (Hayman 1982; Koide 1991) through increased uptake but also of other elements such as N, K, Zn, Mg, Cu and S (Lambert *et al.* 1979; Abbott and Robson 1984; Stribley 1987; Barea 1991). Arbuscular mycorrhizas are also

known to increase resistance of plants to conditions such as drought (Safir *et al.* 1972) and extreme soil acidity (Mosse 1973). The improvement in the water relations of plants as a result of mycorrhizal infection has been reviewed by Cooper (1983) and Harley and Smith (1983). But it is difficult to distinguish direct mycorrhizal effects from those that could be mediated via improved mineral nutrition (Nye and Tinker

1977; Nelson and Safir 1982). In addition, other factors associated with AM colonization include changes in leaf elasticity, improved leaf water and turgor potential and maintenance of stomatal opening and transpiration (Auge *et al.* 1987). Increased root length and development of external hyphae may influence water relations of mycorrhizal plants (Kothari *et al.* 1990).

Although most studies on AM fungi have concentrated on their effect on plant nutrition, there is substantial evidence that the mycorrhizal associations may alter plant-water relations. Higher transpiration rates have been found in arbuscular mycorrhizal red clover, rangeland grass (*Bouteloua gracilis*), rose and apple, cow pea and cassava plants (Hardie and Leyton 1981; Allen 1982; Pai *et al.* 1994; Sundaresan and Sudhakaran 1996). In addition, more rapid recovery from water stress and higher soil moisture extraction at low soil water potential have also been observed in mycorrhizal plants (Safir *et al.* 1971; Hardie and Leyton 1981). These changes were broadly attributed to higher root hydraulic conductivity because of improved P nutrition of mycorrhizal plants, although the sizes of mycorrhizal and non-mycorrhizal plants were altogether different (Hardie and Leyton 1981). However, only a few studies have compared the role of different AM fungal species/isolates on imparting drought tolerance to host plants (Simpson and Daft 1990). The purpose of the present study was to compare the effects of AM fungal species, both individually as well as in combinations, in imparting drought resistance and also growth enhancement in *Vigna unguiculata* subsp. *unguiculata* and *Leucaena latisiliqua*.

## MATERIALS AND METHODS

### Plant Sources

Seeds of *Vigna unguiculata* subsp. *unguiculata* (L.) Walp and *Leucaena latisiliqua* (L.) Gills were procured from Tamil Nadu Agricultural University and Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India, respectively. Seeds were weighed and selected for uniformity, surface sterilized in 5% H<sub>2</sub>O<sub>2</sub> for 5 min, treated with boiling water for 30 sec (*V. unguiculata*) or scarified in concentrated H<sub>2</sub>O<sub>2</sub> for 30 min (*L. latisiliqua*) and then soaked in water for 24 h.

### Substrate

Seeds were directly sown into 10 x 15 cm polythene bags each filled with ca 1.5 kg sterilized

soil:sand mixture (3:1). The soil had pH 8.1 and electric conductivity of 0.2 mScm<sup>-1</sup>. The content of nitrogen, phosphorus and potassium was 104, 4 and 380 kg ha<sup>-1</sup> respectively.

### Inoculum

Soil with infected root bits, hyphae and spores was collected from the pot cultures of AM fungi, *Acaulospora scrobiculata* Trappe, *Glomus aggregatum* Schenck and Smith Emend. Koske and *Glomus etunicatum* Becker and Gerde., maintained in sterilized sand:soil (1:1 by volume) mixture, with cowpea as the host plant in the greenhouse of the Department of Botany, Bharathiar University, Coimbatore - 46, Tamil Nadu, India. Inocula of single and double and triple combinations were added respectively at the rate of 20, 10 + 10 and 6.7 + 6.7 + 6.7 gm soil per pot 5 cm below the soil surface. All the plants were additionally inoculated with a 20-ml suspension of nodulating bacteria which were grown in a yeast extract mannitol broth (Subbarao 1986). The bacteria were obtained from nodules of *Vigna unguiculata* and *Leucaena latisiliqua* growing at Bharathiar University campus, Coimbatore.

### Water Regime

During the first 45 days of the experiment, all plants were watered at field capacity. The plants were subjected to drought cycle by withholding water for 7 days. Similarly, plants were subjected to seven drought cycles.

### Treatments

The pot culture experiments comprised a 2 x 8 x 5 factorial of the following treatments: 2 sources of plant materials, 8 combinations of endophytes and 5 replicates. The treatments were arranged in randomized block design.

Plants were harvested along with their entire root system 101 days after sowing. Growth parameters such as root length, shoot length, leaf area, nodule and plant dry weights were measured.

Known weight of fresh soil sample (50 g) was dried in an oven at 105 °C until a constant weight was attained. It was cooled in a desiccator and weighed. The loss in weight denotes the moisture content

$$\text{Percentage moisture content} = \frac{\text{Wt. of fresh soil} - \text{Wt. of dry soil}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100$$

Relative Water Content (RWC)

$$\text{Relative water content (RWC)} = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100$$

Proline Estimation (Chinard 1952)

Fifty mg. of plant materials were homogenized in 10 ml of 3% aqueous sulphosalicylic acid, and filtered through Whatman No. 1 filter paper. To 2 ml of the filtrate, 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid were added. The mixture was heated in a water bath at 100°C for 1 h and cooled in running water. Then 4 ml of toluene was added and mixed vigorously with a test tube stirrer for 15 to 20 seconds and read with a spectrophotometer at 520 nm.

Plant Tissue N, P and K

Dry matter of 101-day-old plants was ground and digested in a triple acid mixture, and tissue P was determined by the molybdenum blue method as described by Jackson (1973). N was estimated following micro-kjeldahl digestion of the samples (Humphries 1956) and K was estimated by a flame photometric method (David 1962).

Root Colonization

The roots were cleared in 2.5% KOH at 90°C and stained with trypan blue (0.05% in

lactophenol). The percentage of root length infected was evaluated using the magnified intersection method as described by McGonigle *et al.* (1990).

RESULTS

Growth and Biomass

Growth, as measured by root and shoot lengths and dry weights, was invariably enhanced by endophyte inoculations, although the increase was not always statistically significant. Infection with either *Glomus etunicatum* or *Acaulospora scrobiculata* significantly increased the growth of *Vigna unguiculata* and *Leucaena latisiliqua*, respectively (Table 1, 2) while in double inoculations, *A. scrobiculata* and *G. etunicatum* on *V. unguiculata*; *G. aggregatum* and *G. etunicatum* on *L. latisiliqua* combinations gave maximum plant growth and biomass. The double and triple combinations of endophytes tend to eliminate the poor performance by a single species although some single species produced the largest plants. In general, the more consistent results were obtained from plants with mixed inoculations.

Root: Shoot Ratio

Though the double and triple endophyte inoculation for *V. unguiculata* (Table 1) and single endophyte *G. etunicatum* for *L. latisiliqua* (Table 2) increased root to shoot ratio, it generally decreased with endophyte inoculations but were

TABLE 1

Growth and biomass of *Vigna unguiculata* subsp. *unguiculata* treated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	Shoot Length (cm) (plant <sup>-1</sup> )	Root Length (cm) (plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> ) (plant <sup>-1</sup> )	Dry Weight (g plant <sup>-1</sup> )			R/S ratio
				Leaf	Shoot	Root	
Control	21.90d	24.00 d	4.65 c	0.024 d	0.298 c	0.049 b	0.178 a
V1	52.30 a	30.20 abcd	6.83 cd	0.040 c	0.638 b	0.088 b	0.142 a
V2	31.40 c	36.60 a	6.14 de	0.430 bc	0.588 b	0.092 b	0.157 a
V3	52.80 a	34.80 ab	9.26 ab	0.470 ab	0.767 a	0.109 b	0.141 a
V1 + V2	30.40 c	28.00 cd	8.39 abc	0.048 ab	0.526 b	0.198 a	0.200 a
V1 + V3	52.66 a	33.20 abc	7.57 bcd	0.044 bc	0.867 a	0.198 a	0.200 a
V2 + V3	22.54 d	35.30 ab	8.05 abc	0.045 abc	0.542 b	0.109 b	0.170 a
V1 + V2 + V3	37.60 b	29.60 cd	9.58 a	0.052 a	0.615 b	0.094 b	0.200 a

(V1: *Acaulospora scrobiculata*; V2: *Glomus aggregatum*; V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at P < 0.05.

TABLE 2  
Growth and biomass of *Leucaena latisiliqua* treated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	Shoot Length (cm) (plant <sup>-1</sup> )	Root Length (cm) (plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> ) (plant <sup>-1</sup> )	Dry Weight (g plant <sup>-1</sup> )			R/S ratio
				Leaf	Shoot	Root	
Control	12.58 d	32.80 c	9.00 ab	0.044 a	0.176 c	0.160 c	0.950 a
VI	16.30 a	40.86 ab	9.54 a	0.052 a	0.297 ab	0.264 b	0.910 a
V2	13.90 bcd	37.80 b	5.82 e	0.042 a	0.265 ab	0.236 c	0.940 a
V3	14.80 abc	42.80 a	6.22 de	0.048 a	0.285 ab	0.268 b	1.010 a
VI + V2	13.20 cd	41.60 ab	5.83 de	0.049 a	0.222 bc	0.213 d	0.960 a
VI + V3	13.80 bcd	42.60 a	8.30 abc	0.057 a	0.324 a	0.238 c	0.730 a
V2 + V3	15.30 ab	39.50 ab	8.00 bc	0.058 a	0.319 a	0.296 a	0.940 a
VI + V2 + V3	13.40 cd	37.60 cd	7.50 cd	0.056 a	0.310 a	0.266 b	0.890 a

(VI: *Acaulospora scrobiculata*; V2: *Glomus aggregatum*; V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at  $P < 0.05$ .

not statistically significant. Single inoculation of *G. etunicatum* on *V. unguiculata* and double inoculation of *A. scrobiculata* and *G. etunicatum* combinations on *L. latisiliqua* resulted in the lowest root to shoot ratio.

#### Moisture Content (Leaf, Shoot and Soil)

Leaf moisture content was lower in endophyte-inoculated *V. unguiculata* than the control plants (Fig. 1) whereas in *L. latisiliqua* (Fig. 2) double endophyte inoculations, with *A. scrobiculata* and *G. aggregatum* combinations, had higher leaf moisture content.

Single endophyte *A. scrobiculata* in *V. unguiculata* and double endophyte combinations, especially *A. scrobiculata* and *G. etunicatum* in *L. latisiliqua*, had higher shoot moisture content than other inoculations but was statistically significant (Fig. 1, 2).

Higher soil moisture content was observed in plants with single endophyte inoculation: *A. scrobiculata* on *V. unguiculata* and double endophytes, *G. aggregatum* and *G. etunicatum*, on *L. latisiliqua* (Fig. 1, 2).

#### Proline Accumulation

Proline accumulation was significantly higher in *G. etunicatum*-inoculated *V. unguiculata* and *L. latisiliqua*. While double endophytes *G. aggregatum* and *G. etunicatum* on *V. unguiculata* (Fig. 1) and triple endophytes *A. scrobiculata*, *G.*

*aggregatum* and *G. etunicatum* on *L. latisiliqua* (Fig. 2) resulted in higher accumulation of proline.

#### Nodulation

Nodule number was higher in *G. etunicatum* and *A. scrobiculata*-inoculated *V. unguiculata* (Table 3) and *L. latisiliqua* (Table 4) seedlings respectively. Double endophytes, *G. aggregatum* and *G. etunicatum* and triple endophytes *A. scrobiculata*, *G. aggregatum* and *G. etunicatum*, combinations inoculated seedlings had significantly higher nodule number in *V. unguiculata*, but not in *L. latisiliqua*.

#### Root Colonization

Mycorrhizal root colonization varied amongst different endophyte inoculations and host plants. Infection levels in *V. unguiculata* (Table 3) ranged from 20.0-97.7% of total root length. Higher root colonization was observed in single endophyte, *G. etunicatum* inoculation, whereas *L. latisiliqua* (Table 4) infection levels ranged from 10.4-48.7%. Combination of *A. scrobiculata*, *G. aggregatum* and *G. etunicatum* gave higher root colonization. No AM fungal contamination was evident in the control plants.

#### Mycorrhizal Dependency (MD)

The mycorrhizal dependency of the host species varied depending upon the endophytes and their combinations (Table 3 and 4). In a single endophyte, inoculation with *G. etunicatum*

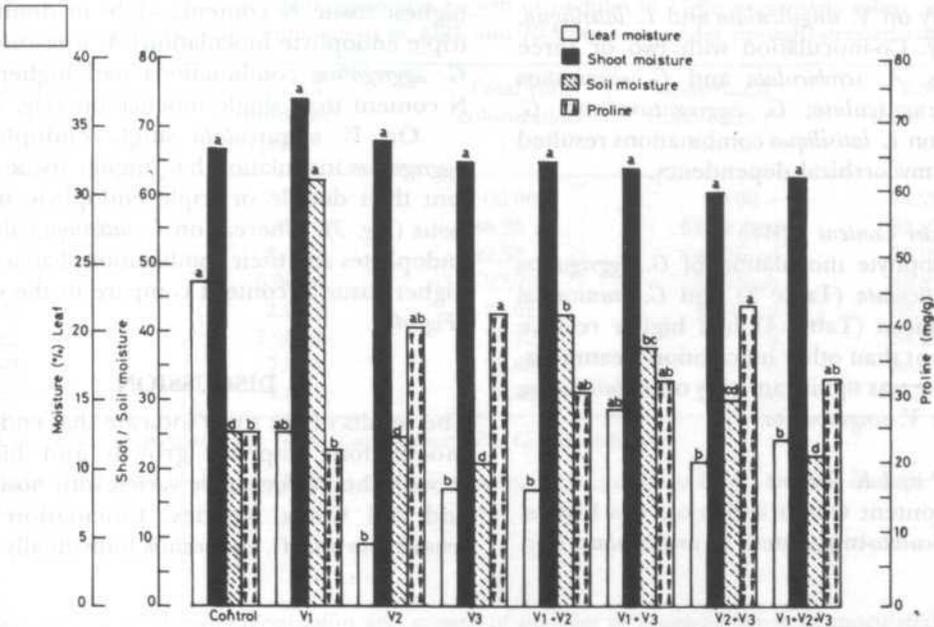


Fig. 1 Effect of VAM fungi (V<sub>1</sub>, Acaulospora scrobiculata; V<sub>2</sub> Glomus aggregatum; V<sub>3</sub> G. etunicatum) with Rhizobium sp. in different combinations on plant / soil moisture content and proline accumulation in *Vigna unguiculata*. subsp. *unguiculata*. Bars with the same letter(s) are not statistically significant according to DMRT at P ≤ 0.05

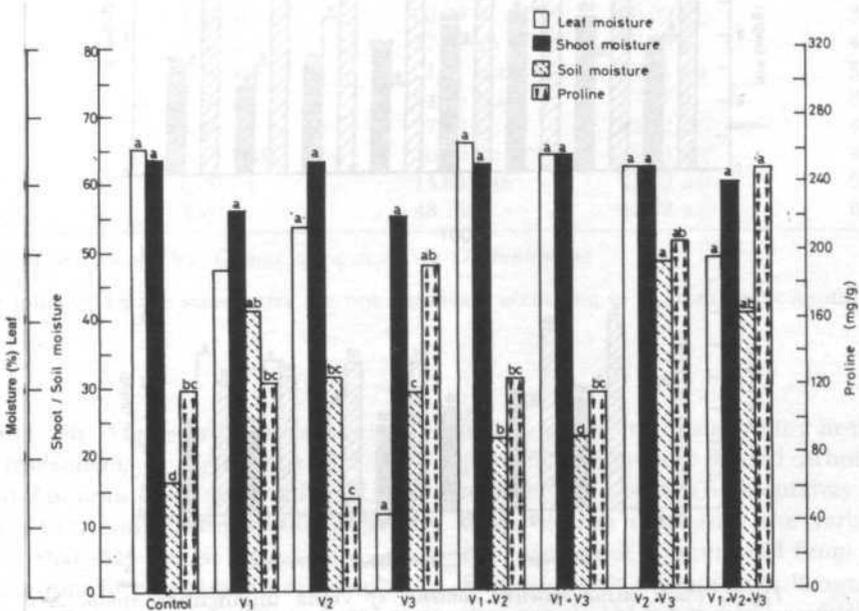


Fig. 2 Effect of VAM fungi (V<sub>1</sub>, Acaulospora scrobiculata; V<sub>2</sub> Glomus aggregatum; V<sub>3</sub> G. etunicatum) with Rhizobium sp. in different combinations on plant / soil moisture content and proline accumulation in *Leucaena latisiliqua*. Bars with the same letter(s) are not statistically significant according to DMRT at P ≤ 0.05

and *A. scrobiculata*, there was higher mycorrhizal dependency on *V. unguiculata* and *L. latisiliqua*, respectively. Co-inoculation with two or three endophytes, *A. scrobiculata* and *G. etunicatum* onto *V. unguiculata*; *G. aggregatum* and *G. etunicatum* on *L. latisiliqua* combinations resulted in highest mycorrhizal dependency.

*Relative Water Content (RWC)*

Single endophyte inoculation of *G. aggregatum* on *V. unguiculata* (Table 3) and *G. etunicatum* on *L. latisiliqua* (Table 4) had higher relative water content than other inoculation treatments. This increase was significant only on *L. latisiliqua*, but not on *V. unguiculata*.

*Tissue N, P and K Content*

Nitrogen content (shoot and root) was highest in *A. scrobiculata*-inoculated *V. unguiculata* (Fig.

3) plants. On *L. latisiliqua*, *G. etunicatum* had highest tissue N content, while in double and triple endophyte inoculations *A. scrobiculata* and *G. aggregatum* combinations had higher tissue N content than single inoculation (Fig. 4).

On *V. unguiculata* single endophyte *G. aggregatum* inoculation had higher tissue K content than double or triple endophyte inoculations (Fig. 3). Whereas on *L. latisiliqua* all single endophytes and their combinations had a slightly higher tissue K content compare to the control (Fig. 4).

**DISCUSSION**

The results of the study indicate that endophyte inoculations improve growth and biomass, though the performance varied with host plants and AM fungal species. Inoculation of *A. scrobiculata* and *G. etunicatum* individually and in

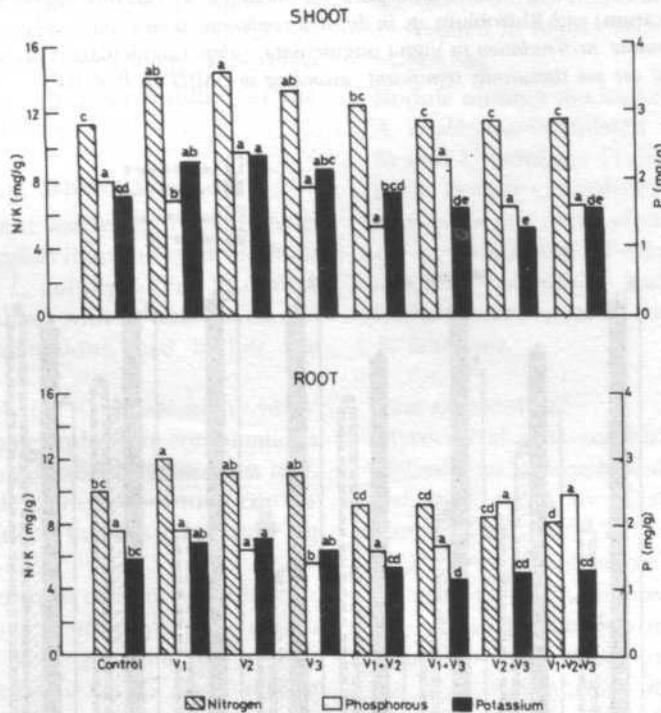


Fig. 3. Plant tissue nutrient contents of *Vigna unguiculata* subsp. *unguiculata* inoculated with ( $V_1$ , *Acaulospora scrobiculata*;  $V_2$  *Glomus aggregatum*;  $V_3$  *G. Etunicatum*) with *Rhizobium* sp. in different combination on plant / soil moisture content and proline accumulation in *Vigna unguiculata* subsp. *unguiculata*. Bars having the same letter(s) are not statistically significant according to DMRT at  $P \leq 0.05$

TABLE 3

Estimate of total VAM root colonization and growth of nodules in *Vigna unguiculata* subsp. *unguiculata* inoculated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	No. of nodules (plant <sup>-1</sup> )	Total root colonization (%)	Mycorrhizal dependency (%)	Relative water content (%)
Control	2.00 d	00.00 e	00.00 e	71.29 a
VI	5.80 c	88.35 ab	51.72 bcd	83.97 a
V2	3.00 d	82.52 ab	47.26 cd	93.02 a
V3	6.00 bc	97.70 a	59.75 ab	88.23 a
VI + V2	2.20 d	20.62 d	42.91 cd	88.72 a
VI + V3	7.00 ab	78.72 b	67.15 a	80.55 a
V2 + V3	7.80 a	78.68 b	41.26 d	86.47 a
VI + V2 + V3	7.80 a	48.62 c	52.90 bc	86.47 a

(VI: *Acaulospora scrobiculata*; V2: *Glomus aggregatum*; V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at  $P < 0.05$ .

TABLE 4

Estimate of total VAM root colonization and growth of nodules in *Leucaena latisiliqua* inoculated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	No. of nodules (plant <sup>-1</sup> )	Total root colonization (%)	Mycorrhizal dependency (%)	Relative water content (%)
Control	7.40 ab	00.00 e	00.00 c	38.93 b
VI	8.00 a	37.72 ab	39.39 a	42.83 b
V2	6.80 abc	21.67 bcd	32.71 ab	56.17 b
V3	7.80 a	24.56 bcd	37.97 a	89.74 a
VI + V2	5.40 de	27.98 bc	22.72 b	42.84 b
VI + V3	6.40 bcd	10.49 de	39.84 a	44.87 b
V2 + V3	6.00 cd	14.85 cde	45.89 a	51.47 b
VI + V2 + V3	4.20 e	48.73 a	40.82 a	61.16 b

(VI: *Acaulospora scrobiculata*; V2: *Glomus aggregatum*; V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at  $P < 0.05$ .

combinations on *Vigna unguiculata* and *Acaulospora scrobiculata*, *G. aggregatum* and *G. etunicatum* on *Leucaena latisiliqua* was found to be highly effective. Simpson and Daft (1990) also reported that AM fungi inoculation on maize and sorghum increased plant dry weight under water-stress conditions. It may in part be due to improvements in mineral nutrition and partly to the direct water-absorbing capacity of the mycelium, which acts to increase the conductivity of the plant root system and to maintain water flow to the plant even under conditions of water stress. Some inocula reduced leaf

area and dry weight suggesting in these cases, a parasitic effect due to limited carbohydrate availability in the plant (Bethlenfalvay *et al.* 1982). These results emphasize the variation in the effectiveness of different AM fungi (Abbott and Robson 1978; Carling and Brown 1980) and that not all AM fungi may be equally beneficial to all host species.

The root to shoot ratio of *V. unguiculata* reflects the high resistance of this species to drought stress. *V. unguiculata* is able to significantly increase the root to shoot ratio under drought stress, which is considered to be im-

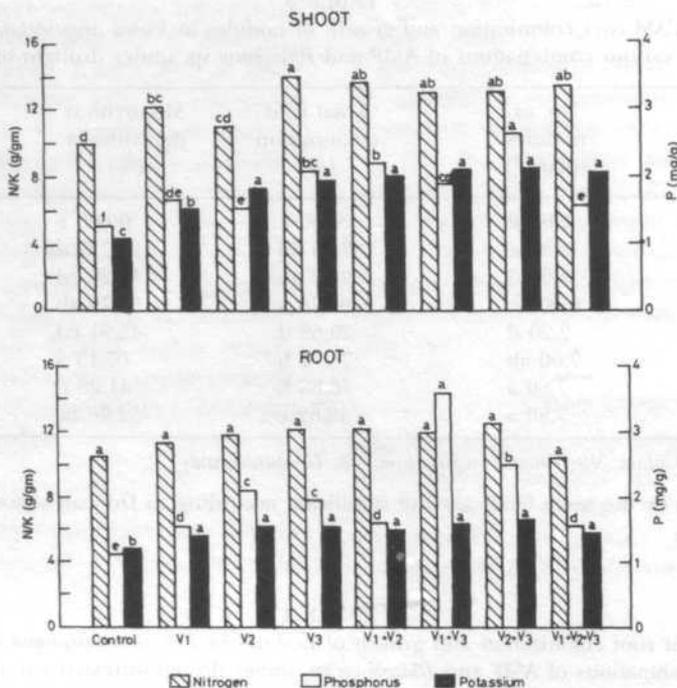


Fig. 4. Plant tissue nutrient contents of *Vigna unguiculata* subsp. *unguiculata* inoculated with ( $V_1$ , *Acaulospora scrobiculata*;  $V_2$  *Glomus aggregatum*;  $V_3$  *G. etunicatum*) with *Rhizobium* sp. in different combination on plant / soil moisture content and proline accumulation in *Leucaena latisiliqua*. Bars having the same letter (s) are not statistically significant according to DMRT at  $P \leq 0.05$

portant for drought prone areas. The root to shoot ratio of *L. latisiliqua* was high, but this species showed no capability to adapt to the environment. AM-inoculated seedlings of both plant species had a lower root to shoot ratio than uninfected plants, but the decreased root mass of the inoculated plants was probably functionally substituted by the external mycelium of the AM fungi. The length and biomass of the extraradical mycelium have been shown to increase under drought stress (Bethlenfalvay *et al.* 1988), and this could be a key factor in AM mediated drought stress.

The shoot and soil moisture contents were higher in mycorrhizal plants. Mycorrhizal plants, which frequently appear to be less prone to wilting and transplanting shock than uninfected plants (Barrows and Roncadori 1977; Levy and Krikun 1980; Janos 1980; Hardie and Leyton 1981; Cooper 1983). Safir *et al.* (1972) reported that soybean root resistance to water uptake was reduced by about 40% with mycorrhizal infection. This was made pos-

sible as a result of increase in surface area of the root system and absorption of the hygroscopic water by external hyphae. Nye and Tinker (1977) also suggested that hyphae ramifying into the soil are likely to increase the absorbing area for water uptake even further, and may also be able to bypass the dry zones that often surround the slow-growing roots during periods of drought. Allen (1982) calculated that the rate of fungus root water transport was  $2.5 \times 10^{-5}$  mg  $S^{-1}$  per hyphal entry point. Tisdall (1994) suggested that the hyphae present in the soil are produced by extracellular polysaccharides, to which microaggregates are attached and bound into stable macroaggregates, so that they do not collapse in water.

It is known that a number of physio-chemical factors are associated with drought tolerance in plants. Proline accumulation correlates with resistance to water stress in various plant species (Thakur 1980; Singh and Rai 1981; Fukutoku and Yoshio 1981). In the present

study, proline accumulation was higher in *G. etunicatum*-inoculated *V. unguiculata* and *L. latisiliqua* plants. Ramakrishnan *et al.* (1988) also reported that mycorrhizal maize plants yielded considerably greater amounts of proline accumulation under water stress. Different mechanisms have been suggested for proline accumulation, which occurred in desiccated leaves due to protein degradation. However, Singh *et al.* (1973) observed that the net protein synthesis continued slowly even as proline was accumulating under osmotic stress, suggesting that pre-existing protein is not the major source of proline. Therefore, some *de novo* synthesis seems to take place in water stressed leaves through inter conversion from other amino acids, especially glutamic acid (Srivastava and Kooner 1974). Some inocula yielded lower proline accumulation, suggesting that under moisture stress conditions, abscisic acid accumulation increases and transpiration rate is reduced, thus increasing the level of water in plants. Indirectly, proline has been shown to increase that water level by binding its active groups with water (Palfi *et al.* 1974).

Nodule water potential plays an important role in the nodular activity. Soil based stress, either directly affects the infection process and/or nodule functioning or indirectly plant growth and available photosynthates by acting upon the symbiosis (Singleton 1983). Plants inoculated with *G. etunicatum* and in combination with other endophytes on *V. unguiculata* and *A. scrobiculata* on *L. latisiliqua* had higher nodule number. However, the higher number of nodules formed in control plants were smaller. Similar results were reported in *Medicago sativa* and *Trifolium alexandricum* (Patterson *et al.* 1990). It may be due to the absorption of water by the external hyphae beyond the water depletion zone around roots and root hairs.

Percentage of AMF root colonization varied with different endophyte inoculations. In general, mycorrhizal inoculation had a higher root colonization than uninoculated control. Simpson and Daft (1990) reported similar increases in mycorrhizal root colonization in inoculated maize and sorghum plants. Higher root colonization makes more fungal-host contact and exchange of nutrients and water for better plant growth.

The mycorrhizal dependency varied among endophyte inoculations. Similar differences in

mycorrhizal dependency of other plant species such as citrus, wheat, forest plants, hardwood trees and crop plants have been reported by other workers (Menge *et al.* 1978; Janos 1980; Azcon and Ocampo 1981; Plenchette *et al.* 1983; Pope *et al.* 1983). It has been proposed that the length of the root hairs is indicative of the degree of mycorrhizal dependency (Baylis 1975). However, in the present study, it was observed that although mycorrhizal plants had higher root length, they were more dependent on mycorrhiza. This clearly indicates that root length alone can not be used as an index for assessing mycorrhizal dependency. Root production, root fibrosity and root geometry may be even more important factors in mycorrhizal dependency (Mosse *et al.* 1973; Plenchette *et al.* 1983).

Inoculation of *A. scrobiculata* in *V. unguiculata* and *G. etunicatum* in *L. latisiliqua* resulted in higher tissue P content. Michelsen and Rosendahl (1990) also reported similar results in *Acacia nilotica* and *L. leucocephala* under drought stress conditions. This can be attributed to the increase in surface area for absorption due to the extensive extramatrical network of mycelium produced by the mycorrhizal fungi in association with the host root system (Hayman 1978; Howeler *et al.* 1981).

Increased uptake of other nutrients, especially N and K, by mycorrhizal plants has also been reported by some workers. Dhillon and Ampornpan (1992) reported that inoculation of the AM fungi significantly increased the concentrations of N in rice than the control. The present study, in addition to confirming the above findings, adds that uptake of N and K by plants is greatly influenced by AM fungal species colonizing the roots. Stribley (1987) reported that P seems to be the most important nutrient involved. Other nutrients, such as N, K, S, Zn, Cu and Mn, are translocated along AM hyphae. Besides this increasing content of tissue N by direct absorption of extramatrical hyphae (Johanson *et al.* 1992, 1993; Frey and Schuepp 1993) which may also be increased indirectly through *Rhizobium*. The decrease in the nutrient content of *V. unguiculata* inoculated with double and triple endophytes could be due to the dilution effect of growth because dry matter usually accumulates faster than nutrient uptake (Jarrel and Beverly 1981).

The plants inoculated with multiple AM fungi in general had higher growth, proline and

nutrient content. Similar results were reported by Daft and Hogarth (1983) in maize and onion. In these cases, two situations may occur; two or more AM fungal species colonize the roots and add their P uptake abilities to give the plant a better phosphorus nutrition and water uptake. An alternative suggested by Daft and Hogarth (1983) claimed that each endophyte may be important at different times during the growing season. The results of the present study suggest that the inoculations of selected AM fungi offer resistance to drought. The introduced endophytes have to compete with the indigenous flora. Using a mixed inoculum, containing endophytes with differing strategies, might reduce variation and give more consistent benefits to the host plants. An ideal endophyte would need to possess several properties. For example, the ability to infect plants early in their growth period, efficiency in exploiting the soil, easy transfer of materials to the host quick, spread and multiplication, effective infection on a range of host plants under different environmental conditions. All these properties may not be found in one single endophyte and so a multiple inoculum would perhaps give more effective compromise.

### CONCLUSION

The present study clearly reveals that inoculation with *Glomus etunicatum* in *Vigna unguiculata* and *Acaulospora scrobiculata* in *Leucaena latifolia* would be the best for producing better growth, nodule number and percentage of root colonization. Proline content was higher in both plants inoculated with *Glomus etunicatum*. Multiple AM fungi inoculation had a positive role on drought tolerance in both plants in terms of growth, proline and nutrient content. Work is in progress to select the most effective AMF for drought tolerance under field conditions.

### ACKNOWLEDGEMENT

S. Grep thanks the University Grants Commission (UGC), New Delhi, for providing financial assistance.

### REFERENCES

- ABBOTT, L.K. and A.D. ROBSON. 1978. Growth of subterranean clover in relation to the formation of endomycorrhiza by introduced and indigenous fungi in field soil. *New Phytologist* **81**: 575-585.
- ABBOTT, L.K. and A.D. ROBSON. 1984. Colonization of the root system of sub-clover by three species of VA mycorrhizal fungi. *New Phytologist* **96**: 275-281.
- ALLEN, M.F. 1982. Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* (H.K.B.). *Lag Ex Steud. New Phytologist* **91**: 191-196.
- AUGE, R.R.M., K.A. SCHEKEL and R.L. WAMPLE. 1987. Leaf water and carbohydrate status of VA mycorrhizal rose exposed to drought stress. *Plant and Soil* **99**: 291-302.
- AZCON, R. and J.A. OCAMPO. 1981. Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. *New Phytologist* **87**: 677-685.
- BAREA, J.M. 1991. Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. *Advances in Soil Science* **15**: 1-40.
- BARROWS, J.B. and R.W. RONCADORI. 1977. Endomycorrhizal synthesis by *Gigaspora margarita* in poinsettia. *Mycologia* **69**: 1173.
- BAYLIS, G.T.S. 1975. The magnoloid mycorrhiza and mycotrophy in root systems derived from it. In *Endomycorrhizas. Proc. Symp. Univ. Leeds*, July 22-25, 1974, ed F.A. Sanders, B. Mosse and P.B. Tinker, p. 373-389. London: Academic Press.
- BETHLENFALVAY, G.J., M.S. BROWN and R.S. PACROVSKY. 1982. Parasitic and mutualistic associations between a mycorrhizal fungus and soyabean. *Phytopathology* **72**: 889-897.
- BETHLENFALVAY, G.J., M.S. BROWN, R.N. AMES and R.S. THOMAS. 1988. Effects of drought on host and endophyte development in mycorrhizal soyabean in relation to water use and phosphate uptake. *Plant Physiology* **72**: 565-571.
- CARLING, D.E. and M.F. BROWN. 1980. Relative effect of vesicular-arbuscular mycorrhizal fungi on the growth and yield of soyabeans. *Soil Science Society of American Journal* **44**: 528-532.
- CHINARD, F.P. 1952. Photometric estimation of proline and ornithine. *Journal of Biology and Chemistry* **199**: 91-95.
- COOPER, K.M. 1983. Mycorrhizal fungi can improve growth of horticultural crops. *Orchardist N.Z.* **56**: 40.
- DAFT, M.J. and B.G. HOGARTH. 1983. Competitive interactions amongst four species of *Glomus* on

- maize and onion. *Transactions of the British Mycology Society* **80**: 339-345.
- DAVID, D.J. 1962. Emission and absorption spectrochemical methods. In *Modern Methods of Plant Analysis*, ed. K. Peech and M.V. Tracy, Vol. 5, p. 1-25. Berlin: Springer-Verlag.
- DHILLON, S.S. and L.A. AMPORNAN. 1992. The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pre transplant rice *Oriza sativa* L. plants. *Biology and Fertility of Soils* **13**: 85-91.
- FREY, B. and H. SCHUEPP. 1993. A role of vesicular-arbuscular VA mycorrhizal fungi in facilitating interplant nitrogen transfer. *Soil Biology and Biochemistry* **25**: 651-658.
- FUKUTOKU, Y. and Y. YOSHIO. 1981. Source of proline-nitrogen in water-stressed soyabean *Glycine max* L. I. Protein metabolism and proline accumulation. *Plant and Cell Physiology* **22**: 1397-1404.
- HARDIE, K. and L. LEYTON. 1981. The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. I In phosphate deficient soil. *New Phytologist* **89**: 559-608.
- HARLEY, J.L. and S.E. SMITH. 1983. *Mycorrhizal Symbiosis*. London: Academic Press.
- HAYMAN, D.S. 1978. Mycorrhizal populations of sown pastures and native vegetation in Otago, New Zealand. *New Zealand Journal of Agricultural Research* **21**: 271-276.
- HAYMAN, D.S. 1982. Practical aspects of vesicular arbuscular mycorrhiza. In *Advances in Agricultural Microbiology*, ed. N.S. Subda Rao, p. 325-373. Oxford: IBH Publishing Co.
- HOWELER, R.H., D.G. EDWARDS and C.J. ASHER. 1981. Application of the flowering solution culture techniques to studies involving mycorrhizae. *Plant and Soil* **58**: 179-183.
- HUMPHRIES, E.C. 1956. Mineral components and ash analysis. In *Modern Methods of Plant Analysis*, ed. K. Peech and M.V. Tracey, Vol. I. p. 468-502. Berlin: Springer-Verlag.
- JACKSON, M.L. 1973. *Soil Chemical Analysis*. New Delhi: Prentice Hall.
- JANOS, D.P. 1980. Mycorrhizal influence tropical succession. *Biotropica* **12**: 56-64.
- JARREL, W.M. and R.B. BEVERLY. 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* **34**: 197-224.
- JOHANSON, A., I. JAKOBSEN and E.S. JENSEN. 1992. Hyphal transport of <sup>15</sup>N labelled nitrogen by vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. *New Phytologist* **122**: 281-288.
- JOHANSON, A., I. JAKOBSEN and E.S. JENSEN. 1993. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 3. Hyphal transports of <sup>32</sup>P and <sup>15</sup>N. *New Phytologist* **124**: 61-68.
- KOIDE, R. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* **117**: 365-386.
- KOTHARI, S.K., H. HARSCHNER and E. GEORGE. 1990. Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. *New Phytologist* **116**: 303-311.
- LAMBERT, D.H., D.E. BAKER and H. COLE. 1979. The role of mycorrhizal in the interactions of phosphorus with zinc, copper and other elements. *Soil Science Society of America Journal* **43**: 976-980.
- LEVY, J. and J. KRIKUN. 1980. Effect of vesicular-arbuscular mycorrhizal on *Citrus jambhiri* water relations. *New Phytologist* **85**: 25-31.
- MCGONIGLE, T.P., M.H. MILLER, D.G. EVANS, G.L. FAIR and J.A. SWAN. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495-501.
- MENGE, J.A., D.E. MUNNECKE, E.L.V. JOHNSON and D.W. CARNER. 1978. Dosage response of the vesicular arbuscular mycorrhizal fungi, *Glomus fasciculatum* and *G. constrictum* to methyl bromide. *Phytopathology* **68**: 1368-1372.
- MICHELSSEN, A. and S. ROSSENHAL. 1990. The effect of VA mycorrhizal fungi, phosphorus and drought stress on the growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. *Plant and Soil* **124**: 7-13.
- MOSSE, B. 1973. Advances in the study of the vesicular-arbuscular mycorrhiza. *Annual Review of Phytopathology* **11**: 171-196.
- MOSSE, B., D.S. HAYMAN and D.J. ARNOLD. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with P. <sup>32</sup>. *New Phytologist* **72**: 809-815.
- NELSON, C.E. and G.R. SAFIR. 1982. The water relations of well watered mycorrhizal and non-

- mycorrhizal onion plants. *Journal of American Society of Horticultural Science* 107: 271.
- NYE, P.H. and P.B. TINKER. 1977. *Solute Movement in the Soil-water System*. Oxford: Blackwell.
- PAI, G., D.J. BAGYARAJ, T. PADMAVATHI RAVINDRA and T.G. PRASAD. 1994. The water relations of mycorrhizal and non-mycorrhizal cow-pea (*Vigna unguiculata*) grown at three soil moisture regimes. *Indian Journal of Microbiology* 34: 317-322.
- OALFI, G., E. KOVES and R. NEHEZ. 1974. Main type of amino acid regulation in cultivars with deficient water supply and their practical application in agriculture. *Novenytermele's* 23: 219-228.
- PATTERSON, N.A., I. CHETT and Y. KAPULNIK. 1990. Effect of mycorrhizal inoculation on nodule initiation activity and contribution to legume productivity. *Symbiosis* 8: 9-20.
- PLENCHETTE, E., J.A. FORTIN and V. FURLAN. 1983. Growth responses of several plant species to mycorrhizal in a soil of moderate p-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil* 70: 199-209.
- POPE, P.E., W.R. CHANNEY, J.D. RHODES and S.H. WOODHEAD. 1983. The mycorrhizal dependency of four hardwood tree species. *Canadian Journal of Botany* 61: 412-417.
- RAMAKRISHNAN, R., B.N. JOHRI and R.K. GUPTA. 1988. Effect of vesicular-arbuscular mycorrhizal fungus on photosynthesis and photorespiration in water stressed maize. *Photosynthetica* 22: 443-447.
- SAFIR, G.R., J.S. BOYLER and J.W. GERDEMANN. 1971. Mycorrhizal enhancement of water transport in soyabean. *Science* 172: 581-583.
- SAFIR, G.R., J.S. BOYER and J.W. GERDEMANN. 1972. Nutrient status and mycorrhizal enhancement of water transport in soyabean. *Plant Physiology* 49: 700-703.
- SIMPSON, G. and M.J. DAFT. 1990. Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*. *Plant and Soil* 121: 171-178.
- SINGH, G. and V.K. RAI. 1981. Proline accumulation and drought resistance in *Cicer aurantium* L. *Biology Plant* 23: 86-90.
- SINGH, T.N., L.G. ASPINALL, L.D. PALEG and S.E. BOGGERS. 1973. Stress metabolism II. Changes in proline concentration in excised plant tissues. *Australian Journal of Biological Science* 26: 57-63.
- SINGLETON, P.W. 1983. A split-root growth system for evaluating the effect of salinity on the components of soyabean *Rhizobium japonicum* symbiosis. *Crop Science* 23: 259-262.
- SRIVASTAVA, A.K. and N.K. KOONER. 1974. Effect of osmotic potential on the mobilization of nitrogenous substances in the germinating seeds of *Phaseolus aureus* Roxb. *Indian Journal of Experimental Biology* 12: 278-281.
- STRIBLEY, D.P. 1987. Mineral nutrition. In *Ecophysiology of VA Mycorrhizal Plants*, ed. G.R. Safir, p. 193-211. Boca Raton, Florida: CRC Press.
- SUBBARAO, N.S. 1986. *Soil Microorganisms and Plant Growth*. Oxford: IBH Publishing
- SUNDARESAN, S. and P.R. SUDHAKARAN. 1996. Effect of water deficit stress on proline accumulation in two varieties of Cassava *Manihot esculenta* Crantz. Differing in their tolerance to drought. *Indian Journal of Experimental Biology* 34: 159-162.
- THAKUR, P.S. 1980. Comparative responses of two differentially sensitive *Zea mays* Linn. varieties to water stress. Ph.D. thesis, H.P. University, Shimla, India.
- TISDALL, J.M. 1994. Possible role of soil microorganisms in aggregation in soils. *Plant and Soil* 159: 115-121.

(Received 26 June 1997)

(Accepted 20 October 1997)