

Response of Siratro (*Macroptilium atropurpureum* Urb. Rabaceae) to Vesicular-arbuscular Mycorrhizal Fungi and *Rhizobium* sp. in Sterilized Soil

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ABSTRAK

Kajian telah dijalankan terhadap kesan penginokulan enam spesies kulat mikoriza arbuskulat vesikular iaitu, *Gigaspora margarita*, *Glomus mosseae*, *G. monosporum*, *G. versiformis*, *G. fasciculatum*, *G. deserticola* dan *Rhizobium* sp. ke atas pertumbuhan dan kandungan NPK dalam siratro (*Macroptilium atropurpureum*). Spesies mikoriza telah diasingkan daripada tanah hutan rizofera Western Ghats dan digunakan dalam kultur pot bersama *Sorghum bicolor* sebagai tanaman perumah. *Rhizobium* sp. yang diasingkan daripada nod segar Siratro telah dikultur dan dikekalkan dalam ekstrak yis bubur manitol. Pengaruh kulat VAM dan *Rhizobium* sp. sama ada bersendirian atau berkombinasi ke atas pertumbuhan dan kandungan nutrien Siratro menunjukkan peningkatan signifikan secara statistik selepas hanya enam hari tumbuhan ditanam. Penginokulan dual bersama kulat VAM dan *Rhizobium* sp. meningkatkan jumlah nod tanaman dan kandungan tisu NPK. Kandungan nutrien dan pertumbuhan tertinggi telah dibuktikan oleh tanaman yang diinokulat dengan *Rhizobium* bersama-sama dengan kulat VAM berganda.

ABSTRACT

The effect of inoculation of six species of vesicular-arbuscular mycorrhizal fungi viz., *Gigaspora margarita*, *Glomus mosseae*, *G. monosporum*, *G. versiformis*, *G. fasciculatum*, *G. deserticola* and *Rhizobium* sp. on growth and NPK content in siratro (*Macroptilium atropurpureum*) was studied. The mycorrhizal species were isolated from the rhizosphere forest soils of the Western Ghats and multiplied in pot culture with *Sorghum bicolor* as the host plant. *Rhizobium* sp. isolated from fresh nodules of siratro was cultured and maintained in yeast extract mannitol broth. The influence of VAM fungi and *Rhizobium* sp. either singly or in combination on growth and nutrient contents of siratro showed statistically significant increase after only 60 days of plant growth. Dual inoculation with VAM fungi and *Rhizobium* increased plant nodule number and tissue NPK content. Highest growth and nutrient content were exhibited by plants inoculated with *Rhizobium* together with multiple VAM fungi.

INTRODUCTION

The beneficial effect of vesicular-arbuscular mycorrhizal fungi (VAMF) and rhizobia on legume growth is well documented (Barea and Azcon-Aguilar 1983; Harley and Smith 1983; El-Hassanin and Lynd 1985; Kawai and Yamamoto, 1986; Ishac *et al.* 1987; Piccini *et al.* 1988). Phosphorus is often the growth-limiting nutrient since nodulating legumes require more P and nitrogen fixation (Mosse *et al.* 1976). Improved P nutrition often results in better nodulation and nitrogen fixation (Waidyanatha *et al.* 1979; Lynd *et al.* 1985). Earlier studies have shown that dual inoculation with VAMF and rhizobia

increased plant growth, nodule size, nodule dry weight and nitrogenase activity (Smith and Daft 1977; Smith *et al.* 1979; Asimi *et al.* 1980). The improved growth as a result of double symbiosis with VAMF and rhizobial inoculation is attributed to the improved phosphate uptake by VAMF (Barea and Azcon-Aguilar 1983). The effect of VAMF and rhizobia on temperate (Abbott and Robson 1978) and tropical forage legumes including siratro (Mosse 1977; Salinas *et al.* 1985; Ariens *et al.* 1991) has been shown to increase growth of these legumes. Since *Macroptilium atropurpureum* cv. Siratro is widely grown in Tamil Nadu in association with pasture grasses

and used as forage to supply protein to grazing animals (Kretschmer 1972; Hodges *et al.* 1982), the present study evaluates combinations of six VAMF and rhizobia for successful establishment of siratro in P-deficient soils, as a substitute for phosphorus fertilizer.

MATERIALS AND METHODS

Inocula of *Gigaspora margarita* Becker and Hall; *Glomus mosseae* (Nicol. and Gerd.), *G. monosporum* (Gerd. and Trappe); *G. versiformis* (Berch and Fortin); *G. fasciculatum* Thaxter *sensu* Gerd. and *G. deserticola* (Trappe, Bloss and Menge) were isolated from the rhizosphere of forest soil in the Western Ghats. The *Rhizobium* strain was isolated from fresh nodules of siratro and maintained in yeast mannitol broth for five days. Seeds of siratro were obtained from Tamil Nadu Agricultural University, Coimbatore.

Sandy loam soil collected from the experimental field of Botany Department, Bharathiar University, Coimbatore, was collected, air dried and freed of large organic debris using a 2-mm sieve. The soil was mixed with fine sand in a ratio of 4:1. The steam-sterilized soil, initially with pH of 7.5, N of 74 mg/g; P of 6.0 mg/g; K of 191 mg/g was packed in 26 x 14 cm polythene bags. The respective bags were inoculated either with a single VAM species alone or in combination with *Rhizobium* sp.; or with mixture of all the six VAMF with and without *Rhizobium* sp., or with *Rhizobium* sp. alone. One hundred grams of each VAMF inocula were placed as a thin layer 5 cm below the soil surface in the treatments. Steam-sterilized uninoculated soil served as control.

The polythene bags were arranged in a RCBD with four replications. Two surface-sterilized (5%, H₂O₂) seeds of uniform size were sown in each polythene bag. Twenty ml rhizobial suspension for yeast extract mannitol broth were given to 5-day old seedlings in the respective treatments. The plants in the greenhouse were watered daily to field capacity.

The plants were harvested at 20-day intervals for a period of 80 days. Roots were washed free of soil. Shoot length, root length, leaf area, nodule number, shoot and root dry weights (after drying at 65° C for 48 h) were recorded. VAM colonization was assessed after staining the root samples following the method of Phillips and Hayman (1970).

The 80-day-old plant materials were dried, ground and digested for the determination of tissue phosphorus (P) using the method applied by Jackson (1958), nitrogen (N) using the method of Humphries (1956) and potassium (K) using that of David (1962).

Data on plant growth, nodulation, VAM status and tissue nutrient content were subjected to analysis of variance (ANOVA), and the means separated using Duncan's new multiple range test at P = 0.05 level.

RESULTS

Root length

Siratro plants inoculated with *Glomus monosporum* had longer roots at 20 and 40 days after emergence (DAE) but at 60 and 80 DAE, plants inoculated with *Gigaspora margarita* had longer roots (Table 1).

Siratro inoculated with *Rhizobium* and *Gigaspora margarita* (at 20 and 40 DAE) and *Rhizobium* and *Glomus mosseae* (at 60 and 80 DAE) produced longer roots than the other endophytic inoculations. Plants inoculated with a mixture of all six VAMF species produced longer roots at 20 and 40 DAE than their combined effect with *Rhizobium*, whereas harvests on 60 and 80 DAE gave better growth only when associated with *Rhizobium* (Table 1).

Shoot Length

Shoot length at 20 and 40 DAE was greater in the presence of *Glomus deserticola*. Plants inoculated with *G. fasciculatum* produced better shoot length at 60 and 80 DAE. At day 20, the longest shoot was seen in plants with the single inoculation of *Rhizobium* sp. but at subsequent stages (40, 60 and 80 DAE) *G. deserticola* in association with *Rhizobium* sp. produced longer shoots than the other five endophytes studied. *Rhizobium* sp. co-inoculated with a mixture of all the six endophytes showed better shoot length on 40 and 80 DAE than with the endophytic mixture alone (Table 1).

Leaf Area

Siratro inoculated with *Gigaspora margarita* had greater leaf area than the other individual inoculation at all stages. *Rhizobium* sp. in association with *G. deserticola* produced great leaf area than the other five endophytes. *Rhizobium* sp. inoculated plants showed a more significant in-

TABLE 1
Effect of various inoculations on plant growth of *Macroptilium atropurpureum* at 20, 40, 60 and 80 days after emergence (DAE)

Treatment (a)	20D			40D			60D			80D		
	Shoot length (cm)	Root length (cm)	Leaf area (sq. cm)	Shoot length (cm)	Root length (cm)	Leaf area (sq. cm)	Shoot length (cm)	Root length (cm)	Leaf area (sq. cm)	Shoot length (cm)	Root length (cm)	Leaf area (sq. cm)
Control	2.40c	8.31cd	1.93b	4.90gh	13.77b	2.94g	12.17c	36.40d	4.76g	24.32bc	39.13cd	4.78c
VAM 1	4.10abc	14.00b	7.67a	15.75d	22.95ab	9.19abcd	19.42ab	59.95abc	9.38abcd	38.00abc	77.00ba	10.97a
VAM 2	5.42abc	12.00bc	4.03ab	10.12f	22.00ab	6.26def	37.95ab	49.90abc	7.32ef	42.40a	59.12ab	7.34b
VAM 3	4.47abc	14.95b	5.03ab	7.62g	29.87ab	6.83def	23.65ab	59.50abc	7.65def	34.25abc	60.97ab	7.81b
VAM 4	5.97abc	12.27bc	6.15ab	6.07gi	17.87ab	8.92abcd	29.90ab	51.00abc	9.25abcd	39.60ab	62.87ab	9.53ab
VAM 5	4.20abc	11.92bc	3.12ab	18.55bc	22.25ab	6.61def	40.25a	59.32abc	7.83cdef	43.75a	69.10a	9.41ab
VAM 6	7.82a	11.37bc	4.85ab	25.75a	20.00ab	6.37def	31.70ab	57.05abc	7.54def	39.75ab	66.15ab	9.60ab
VAM 1-6	5.20abc	16.75ab	5.08ab	11.60e	35.75a	7.41cdef	26.37ab	45.87abc	8.97 abcdef	30.20abc	55.02bc	8.99ab
R	6.92ab	17.00ab	6.35ab	13.50de	18.50ab	8.52abcd	20.25b	25.30c	9.54abc	40.12ab	34.85d	9.69ab
VAM 1+R	4.62abc	19.25a	3.27ab	16.56bcd	30.87ab	8.39abcde	31.85ab	51.75abc	9.11abcd	33.00abc	58.75ab	9.88ab
VAM 2+R	5.35abc	11.32bc	4.25ab	11.37ef	22.37ab	5.58ef	29.25ab	69.00a	7.50def	35.55abc	70.55ab	8.10ab
VAM 3+R	5.07abc	18.17ab	3.61ab	1.62h	20.83ab	5.21efg	31.12ab	62.05ab	8.41 abcdef	34.33abc	68.57ab	9.26ab
VAM 4+R	6.17abc	10.20cd	3.87ab	13.00de	19.84ab	4.22g	32.65ab	58.17abc	8.01bcdef	37.00abc	67.80ab	8.51ab
VAM 5+R	5.85abc	14.10b	3.41ab	13.50def	19.84ab	6.28def	31.37ab	49.75abc	6.81f	37.62abc	50.87bcd	7.61ab
VAM 6+R	4.30abc	12.82bc	7.85a	19.12bc	29.25ab	9.80a	32.62ab	59.10abc	9.93a	40.35ab	64.42ab	10.11ab
VAM 1-6+R	5.57abc	16.31ab	6.73ab	20.25b	30.47ab	9.46ab	29.12ab	57.20a	9.57ab	42.50a	67.00ab	16.37ab
NPS	5.04abc	13.77bc	4.31ab	10.25ef	21.62ab	6.25def	25.22ab	41.30bc	6.99f	27.50abc	51.90bc	7.84ab
FD	2.34c	7.49d	1.98b	3.53gh	12.19b	2.05h	11.53c	33.57d	3.94g	22.51c	36.25cd	4.61c

(a) VAM 1 - *Gigaspora* VAM 2 - *Glomus mosseae* VAM 3 - *G. monosporum*; VAM 4 - *G. versiformis* VAM 5 - *G. fasciculatum*
 VAM 6 - *G. deserticola* R - *Rhizobium*; FD - Formaldehyde; NPS Non-sterilized field soil.

Means followed by the same letter are not significantly ($p \leq 0.05$) different as determined by Duncan's new multiple range test.

TABLE 2
Effect of inoculation of various endophytes
on number of nodules per plant

Treatments (a)	Days after emergence			
	20D	40D	60D	80D
Control	0b	0b	0e	0g
VAM 1	0b	0b	0e	0g
VAM 2	0b	0b	0e	0g
VAM 3	0b	0b	0e	0g
VAM 4	0b	0b	0e	0g
VAM 5	0b	0b	0e	0g
VAM 1-6	0b	0b	0e	0g
R	2.75ab	3.25ab	3.00de	3.10f
VAM 1+R	8.00ab	8.00a	9.75ab	10.50b
VAM 2+R	3.25ab	6.00ab	6.50bcd	7.25e
VAM 3+R	4.25ab	4.50ab	6.50bcd	8.25cde
VAM 4+R	4.50ab	6.00ab	7.20bc	7.50de
VAM 5+R	2.75ab	7.00ab	7.25bc	7.25e
VAM 6+R	6.25ab	8.25a	9.75ab	10.25cd
VAM 1-6+R	8.00a	10.00a	11.25ab	14.75a
NPS	3.75ab	3.65ab	4.00cd	6.75e
FD	0.0b	0.0b	0.0b	0.0b

(a) see footnote to Table 1

crease in leaf area than that of the mixture of six VAMF. However, the endophyte mixture and *Rhizobium* sp. showed an additive effect on leaf area at all stages where the effect of endophytes is not significant when compared to *Rhizobium* sp. (Table 1).

Nodule Number

Siratro in association with *Rhizobium* sp. and with a mixture of all the six endophytes increased the nodule number to three times that of the rhizobial inoculation alone. *Gigaspora margarita* on 20 DAE and *G. deserticola* on 40 DAE produced more nodules in the presence of *Rhizobium* sp, whereas at 60 and 80 DAE both inoculations had the same nodule number. At all stages of growth the nodules of plants with a mixture of six endophytes together with *Rhizobium* sp. were four times the number of plants inoculated with only *Rhizobium* sp. (Table 2).

Dry Weight of Roots and Shoots

Inoculation with *Glomus mosseae* significantly increased the root dry weight compared with all other inoculations at all stages of growth. Shoot dry weight was higher in plants inoculated with *G. mosseae* at 20 and 40 DAE but was greater by *G. monosporum* at 60 and 80 DAE. Association

of *Rhizobium* sp. with *G. deserticola* produced greater shoot dry weight than all other endophytes with *Rhizobium*. The endophyte mixture increased dry weight of roots at all stages and shoot dry weight at 20 and 40 DAE. The root to shoot ratio was increased by both dual inoculation and also single VAMF inoculation compared to control, except at 60 DAE (Table 3).

Root Colonization

Glomus fasciculatum at 20 DAE, *G. mosseae* at 40 DAE, *G. versiformis* as well as *G. deserticola* on 60 DAE and *Gigaspora margarita* at 80 DAE showed higher root colonization than the others. *Rhizobium* sp. in association with endophyte mixture increased colonization in the early stages but decreased later (Fig. 1).

The endophytic inoculation increased the NPK concentration in plant tissue compared to uninoculated controls. Dual inoculation significantly increased the NPK concentration in plant tissues. A significant increase in NPK was observed in plants inoculated with *Rhizobium* sp. and multiple VAM fungi. Nutrient accumulation in plants grown in sterilized field soil containing indigenous VAM fungi paralleled dual inoculation (Fig. 2).

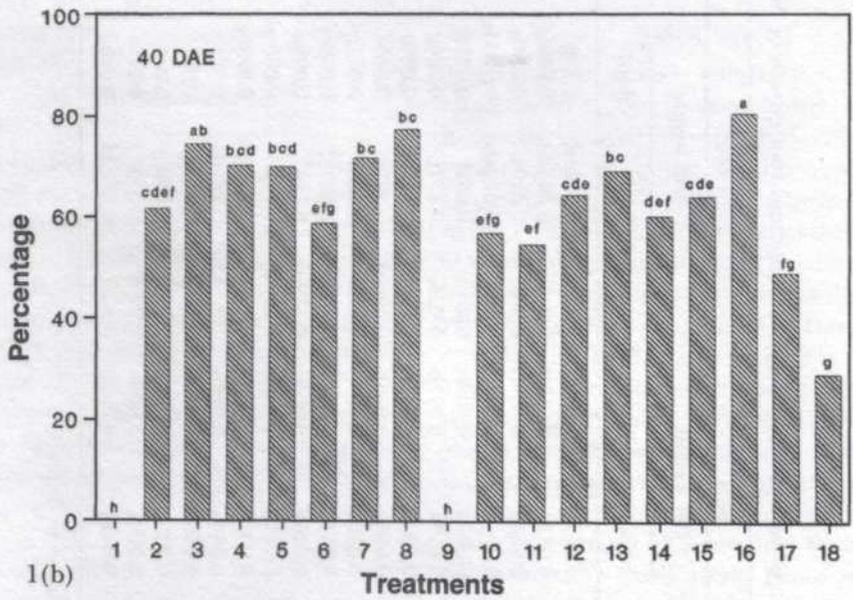
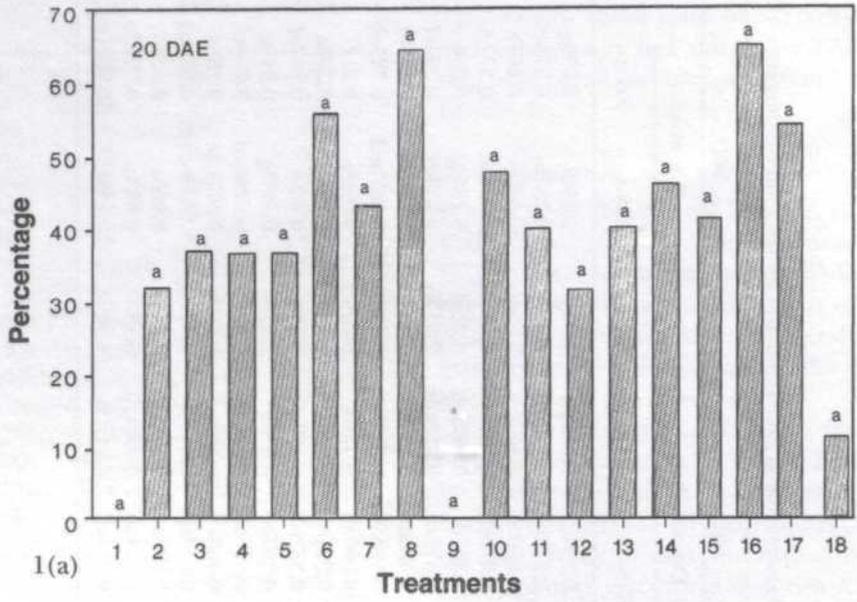
DISCUSSION

The increase in root length of siratro, inoculated with either *G. monosporum* or *Gigaspora margarita* compared with the remaining four endophytes, is similar to the observations of Lopes and Olivera (1980). *Gigaspora margarita* at 20 - 40 DAE and *Glomus mosseae* at 60 - 80 DAE increased root length when co-inoculated with *Rhizobium*. Similar results have been reported in *Phaseolus vulgaris* (Daniels-Hylton and Ahmed 1994) and *Vicia faba* (Ishac *et al.* 1994) by dual inoculation of *Rhizobium* sp. with VA mycorrhiza. This supports the observation that *Rhizobium* sp. and VA mycorrhiza increase plant growth to a greater extent than can be attributed to either of them when added singularly (Harley and Smith 1983; El Hassanin and Lynd 1985; Ishac *et al.* 1987; Kawai and Yamamoto 1986). The root to shoot ratio was increased by both dual inoculation and single inoculation of VAMF, which contradicts the findings that dual inoculation showed the lowest root-to-shoot ration (Piccini *et al.* 1988).

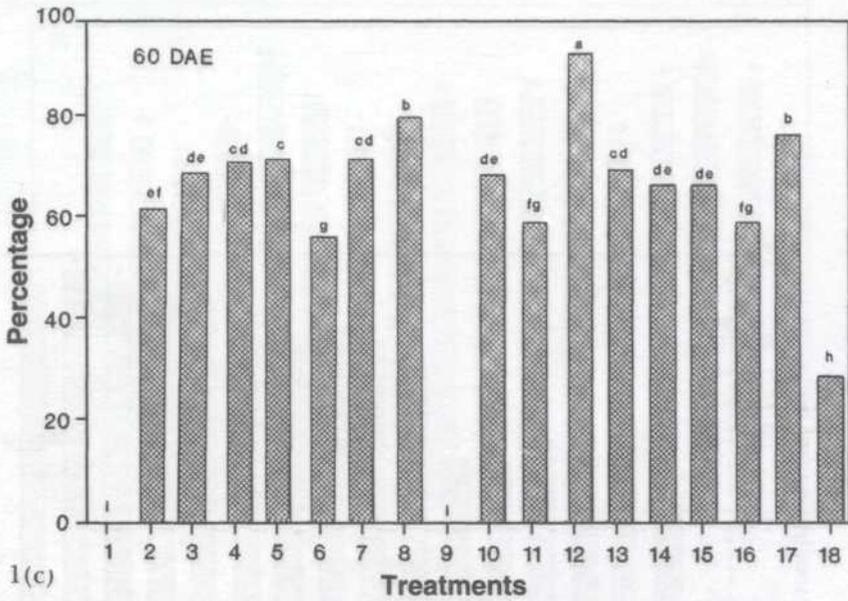
TABLE 3
Effect of various inoculations on shoot and root growth of *Macroptilium atropurpureum* at 20, 40, 60 and 80 days after emergence (DAE)

Treatment (a)	20D			40D			60D			80D		
	Dry weight		R/S	Dry weight		R/S	Dry weight		R/S	Dry weight		R/S
	Shoot	Root		Shoot	Root		Shoot	Root		Shoot	Root	
Control	0.044a	0.006b	0.144i	0.322cd	0.10g	0.036l	0.290c	0.057gh	1.921c	0.153i	0.070g	0.442b
VAM 1	0.041a	0.011b	0.245i	0.204def	0.030fg	0.145h	0.210bc	0.173def	0.839d	0.237hi	0.273ef	1.182b
VAM 2	0.064a	0.057b	0.867f	0.620a	0.124b	0.22g	0.668ab	0.467a	0.704e	0.685bc	0.467b	0.659b
VAM 3	0.034a	0.044a	3.33a	0.612ab	0.076de	0.138jh	0.675ab	0.294c	0.438hi	0.695bc	0.377cd	0.544b
VAM 4	0.057a	0.020b	0.336i	0.082f	0.031fg	0.379e	0.367d	0.092fgh	0.259j	0.435fg	0.256ef	0.585b
VAM 5	0.053a	0.011b	0.223i	0.137f	0.020g	0.140h	0.573ab	0.230cde	2.017b	0.553de	0.264ef	0.476b
VAM 6	0.100a	0.012b	0.115i	0.156ef	0.028a	0.164h	0.488ab	0.237cd	0.487h	0.49bef	0.239ef	0.484b
VAM 1-6	0.056a	0.033b	0.559h	0.123f	0.040f	0.321f	0.498ab	0.297bc	0.476h	0.667bcd	0.725ab	1.281a
R	0.036a	0.099b	0.275i	0.065f	0.011g	0.181h	0.666ab	0.023h	0.036m	0.699b	0.212	0.324b
VAM 1+R	0.051a	0.0044b	0.856g	0.157ef	0.059ef	0.380e	0.291bc	0.064fgh	0.256j	0.295gh	0.775a	2.583a
VAM 2+R	0.046a	0.064b	0.372e	0.124	0.098c	0.755d	0.387b	0.137efg	0.353i	0.409f	0.247ef	0.627b
VAM 3+R	0.041a	0.064b	1.586d	0.100f	0.080cd	0.800c	0.357b	0.039h	0.110l	0.407fg	0.040g	0.094b
VAM 4+R	0.050a	0.006b	0.116i	0.285cde	0.029fg	0.130jh	0.536ab	0.196de	0.366i	0.628bcd	0.254ef	0.444b
VAM 5+R	0.044a	0.081b	1.82c	0.284cde	0.028g	0.093k	0.468ab	0.252cd	0.540g	0.577cde	0.281ef	0.486b
VAM 6+R	0.053a	0.014b	0.255i	0.380c	0.027g	0.0751k	0.549ab	0.294c	0.561fg	0.606bcde	0.375cd	0.626b
VAM 1-6+R	0.058a	0.122b	2.35b	0.146	0.244a	1.660b	0.898a	0.395ab	0.442h	0.966a	0.326c	0.447b
NPS	0.020a	0.606b	0.290i	0.065f	0.210a	3.277a	0.534ab	0.099fgh	0.183k	0.883a	0.321de	0.378b
FD	0.041a	0.007b	0.165i	0.315cd	0.010g	0.034l	0.011e	0.049gh	4.350a	0.149i	0.0611g	0.413b

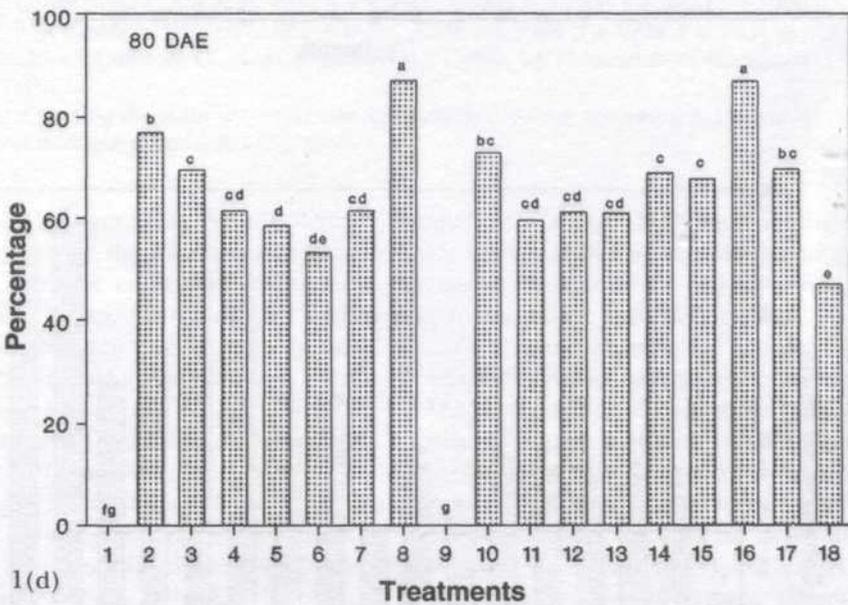
(a) See footnote to Table 1



RESPONSE OF SIRATRO TO VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI



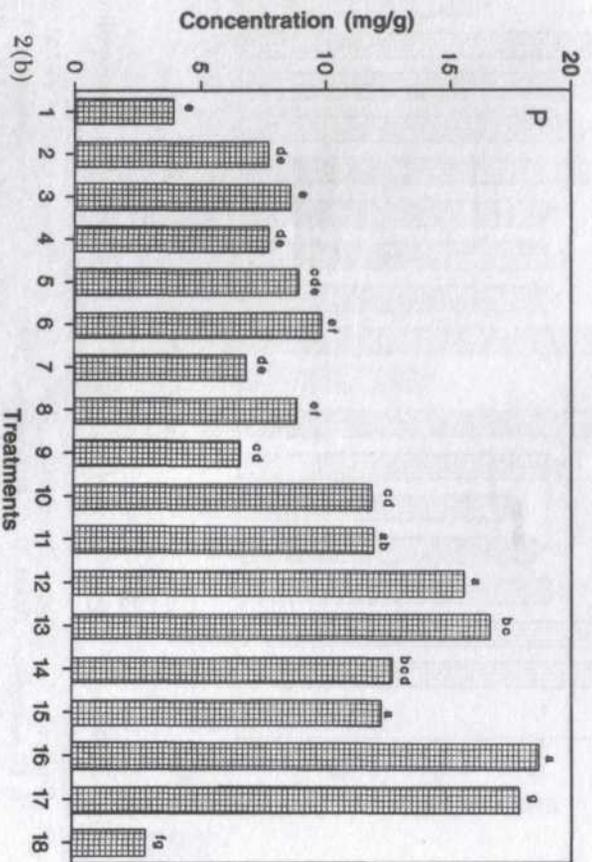
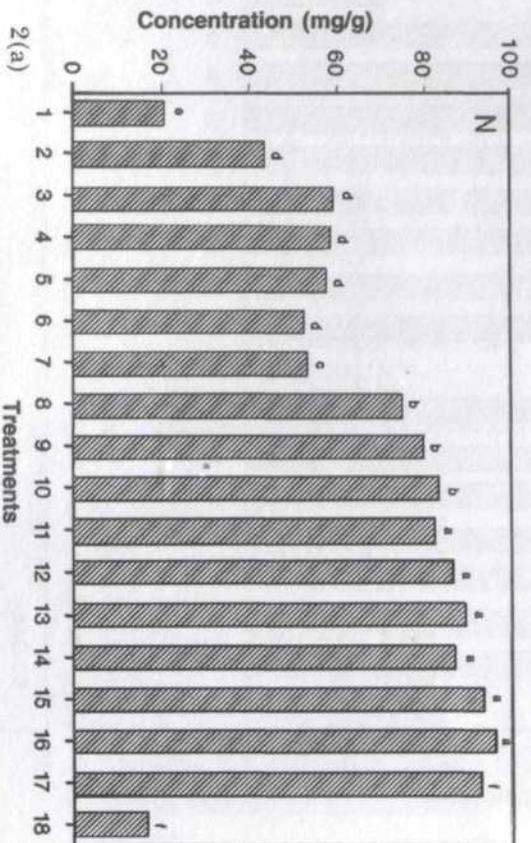
1(c)



1(d)

Fig 1. Influence of VAM fungi and rhizobia on root colonization in *Macroptilium atropurpureum*

1. Control, 2. *Gigaspora margarita*, (VAM 1), 3. *Glomus mosseae* (VAM 2), 4. *G. monosporum* (VAM 3), 5. *G. versiformis* (VAM 4), 6. *G. fasciculatum* (VAM 5), 7. *G. deserticola* (VAM 6) 8. VAM 1 + VAM 2 + VAM 3 + VAM 4 + VAM 5 + VAM 6, 9. *Rhizobium* (R), 10. VAM 1 + R, 16. VAM1 + VAM2 + VAM3 + VAM4 + VAM5 + VAM6 + R, 17. Non-pasteurised soil (NPS), 18. Formaldehyde-fumigated - (FD). Bars bearing the same letters are not significantly different according to Duncan's new multiple range test ($P \leq 0.05$)



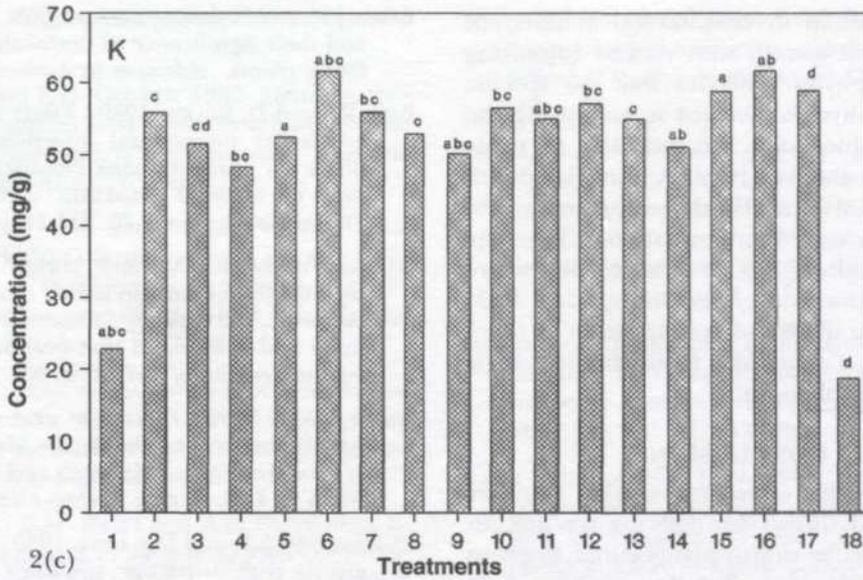


Fig 2. Influence of VAM fungi and rhizobia on nutrient (NPK) status of *Macropitium atropurpureum* in 80 days

1. Control, 2. *Gigaspora margarita*, (VAM 1), 3. *Glomus mosseae* (VAM 2), 4. *G. monosporum* (VAM 3), 5. *G. versiformis* (VAM 4), 6. *G. Fasciculatum* (VAM 5), 7. *G. deserticola* (VAM 6) 8. VAM 1 + VAM 2 + VAM 3 + VAM 4 + VAM 5 + VAM 6, 9. *Rhizobium* (R), 10. VAM 1 + R, 16. VAM 1 + VAM 2 + VAM 3 + VAM 4 + VAM5 + VAM6 + R, 17. Non-pasteurised soil (NPS), 18. Formaldehyde-fumigated - (FD).

Bars bearing the same letters are not significantly different according to Duncan's new multiple range test ($P \leq 0.05$)

The presence of longer roots in endophyte-inoculated plants rather than *Rhizobium* sp. at 20 and 40 DAE, and the combined influence of VAM and *Rhizobium* sp. at 60 and 80 DAE indicate that *Rhizobium* sp. might have supplemented nutrients for root growth only at later stages.

Unlike the roots, the shoot length increased in *G. deserticola* at early stages (20 - 40 DAE) and in *G. fasciculatum* at later stages (60 -80 DAE). Shoot length of siratro was higher in single inoculation with *Rhizobium* sp. than that of any endophyte studied up to 20 DAE. The *G. deserticola* and *Rhizobium* sp. combination was better than all other combinations. A plant inoculated with all the six VAMF together with *Rhizobium* sp. showed an additive effect on shoot growth.

Gigaspora margarita among single endophytic inoculation, and *G. deserticola* and *Rhizobium* sp. among dual inoculation, increased the leaf area whereas the endophytic mixture together with *Rhizobium* sp. increased the leaf area more than any of them individually. VA mycorrhizal

plants tend to have higher cytokinin activity in their shoots (Allen *et al.* 1980) and cytokinins promote leaf area by cell division and cell expansion (Bass and Kuoier 1989).

Gigaspora margarita at an early stage (20 DAE) and *Glomus deserticola* at later stages (40 DAE) and endophytic mixture (60 - 80 DAE) produced more nodules in the presence of *Rhizobium* sp. than other combinations. This supports the view that endophytes increase the nodule number when associated with *Rhizobium* sp. (Daniels-Hylton and Ahmed 1994). In the present study, *Glomus mosseae* produced greater root dry weight than all other endophytes at all stages. In the case of shoots, *Glomus mosseae* at early stages (20 and 40 DAE) and *G. monosporum* at later stages (60 and 80 DAE) produce greater dry weight. Medina *et al.* (1988) showed that *G. etunicatum* and *G. intraradices* produce higher shoot dry weight than other VAM inoculations. Though the endophytic mixture increased the root dry weight at all stages the shoot dry weight was reduced at later stages (60 and 80 DAE).

The variation in colonization at different stages of plant growth with various colonizing VAMF endophyte indicates that no specific VAMF endophyte is involved in colonizing the host root. Increased accumulation of tissue nutrients was observed in plants inoculated with a multiple VAMF and *Rhizobium* sp. compared to *Rhizobium* sp. or VAM inoculation. This supports the result of Piccini *et al.* (1988) where dual inoculation with *Rhizobium* sp. and VAM resulted in an increased accumulation of nutrients (N, P, K, Ca and Mg) in alfalfa than single endophyte inoculations.

CONCLUSION

The present study clearly reveals that *Gigaspora margarita* and *Glomus deserticola* are the best for producing quality siratro plants either singly or dually with *Rhizobium*. But the mixture of six VAMF and *Rhizobium* was found to enrich the NPK status of the legume. Work is in progress to establish quality siratro plants under field conditions with an effective VAMF and *Rhizobium* combination.

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REFERENCES

- ABBOTT, L.K. and A.D. ROBSON. 1978. Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. *New Phytologist* **81**: 575-587.
- ALLEN, M.F., T.S. MOORE and M. CHRISTENSEN. 1980. Phytohormone changes in *Boutelout gracilis* infected by vesicular-arbuscular mycorrhiza. I Cytokinin increase in the host plant. *Canadian Journal of Botany* **58**: 371.
- ARIENS, R.N., T.R. THIAGARAJAN, M.H. AHMED and W.A. MILAGHLIN. 1991. Co-selection of compatible rhizobia and vesicular-arbuscular mycorrhizal fungi for cowpea in sterilized and non-sterilized soils. *Biology and Fertility of Soils* **12**: 112-116.
- ASIMI, S., V. GIANINAZZI-PEARSON and S. GIANINAZZI. 1980. Influence of increasing soil phosphorus levels on interaction between vesicular arbuscular mycorrhiza and *Rhizobium* in soybean. *Canadian Journal of Botany* **58**: 2200-2205.
- BAREA, J.M. and C. AZCON-AGUILAR. 1983. Mycorrhizas and their significance in nodulating nitrogen fixing plants. *Advances in Agronomy* **36**: 1-54.
- BASS, R. and D. KULIER. 1989. Effect of vesicular-arbuscular mycorrhizal infection and phosphate on *Plantago major* Pleosperma in relation to internal cytokinin concentrations. *Physiologia Plantarum* **76**: 211-215.
- DANIELS-HYLTON, K.D.M. and M.H. AHMED. 1994. Inoculation response in kidney beans (*Phaseolus vulgaris* L.) to vesicular-arbuscular mycorrhizal fungi and rhizobia in non-sterilized soil. *Biology and Fertility of Soil* **18**: 95-98.
- DAVID, D.J. 1962. Emission and absorption spectrochemical methods. In *Modern Methods of Plant Analysis*, ed. K. Peech and M.V. Tracey, vol. 5 p. 1-25. Berlin: Springer-Verlag.
- EL-HASSANIM, A.S. and J.Q. LYND. 1985. Soil fertility effects with tripartite symbiosis for growth nodulation and nitrogenase activity of *Vicia faba* L. *Journal of Plant Nutrition* **8**: 491-499.
- HARLEY, J.C. and S.E. SMITH. 1983. *Mycorrhizal Symbiosis*. London: Academic Press.
- HODGES, E.M., A.E. KRETSCHMER, P. MISLERY JR., R.D. ROUSH, O.C. RUELKE and G.H. SNYDER. 1982. Production and utilization of the tropical legume *Aschynomene*. *Agricultural Experimental Station Circle S-2900*.
- HUMPHRIES, E.C. 1956. Mineral components and ash analysis. In *Modern Methods of Plant Analysis*, ed. K. Peech and M.V. Tracey, vol. 5 p. 468-502. Berlin: Springer-Verlag.
- ISHAC, Y.Z., M.J. DAFT, E.M. RAMADAN, M.E. EL-DAMORDASH and C.N. FARES. 1987. Effect of *Rhizobium* inoculation and/or endomycorrhizas on peanut growth. *2nd African Assoc. Biol. Nitrogen. Conf.* Cairo University, Cairo, Egypt. p. 589-596.
- ISHAC, Y.Z., J.S. ANGLE, M.E. EL-BOROLLOS, M.E. EL-DAMORDASH, M.I. MOSTAFA and C.N. FARES. 1994. Growth of *Vicia faba* as affected by inoculation with vesicular-arbuscular mycorrhizae and *Rhizobium leguminosarum* cv. *viciae* in two soils. *Biology and Fertility of Soils* **17**: 27-37.
- JACKSON, M.L. 1958. *Soil Chemical Analysis*. New Delhi: Prentice-Hall.
- KAWAI, Y. and Y. YAMAMOTO. 1986. Increase in the formation and nitrogen fixation by soybean nodules by vesicular-arbuscular mycorrhiza. *Plant and Cell Physiology* **27**: 399-405.
- KRETSCHMER, A.E. JR. 1972. Siratro (*Phaseolus atropurpureus* DC) A summer-growing perennial pasture legume for Central and South

Florida. *Florida Agriculture Experimental Station Circle*.

- LOPES, E.S. and E.D. OLIVERA. 1980. Efeito de species de mycorrhizas vesicular-arbuscular em Siratro (*Macroptilium atropurpureum*). *Bragantia* **39**: 241-245 (English abstract).
- LYND, J.Q., R.J. TYRL and A.A.C. PURCINO. 1985. Mycorrhizal-soil fertility effects on regrowth, nodulation and nitrogenase activity of siratro (*Macroptilium atropurpureum* (DC Urb.) *Journal of Plant Nutrition* **8**: 1047-1059.
- MEDINA, O.A., D.M. SYLVIA and A.E. KRETSCHMER JR. 1987. Growth response of tropical forage legumes to inoculation with *Glomus intraradices*. *Tropical Grasslands* **21**: 24-27.
- MEDINA, O.A., D.M. SYLVIA and A.E. KRETSCHMER JR. 1988. Response of siratro to vesicular-arbuscular mycorrhizal fungi. I. Selection of effective vesicular-arbuscular fungi in amended soil. *Soil Science Society of American Journal* **52**: 416-419.
- MOSSE, B. 1977. Plant growth responses to vesicular - arbuscular mycorrhiza. X. Responses of *Stylosanthes* and maize to inoculation in unsterile soils. *New Phytologist* **78**: 277-278.
- MOSSE, B., C.L. POWELL and D.S. HAYMEN. 1976. Plant growth response to vesicular-arbuscular mycorrhiza. IX Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. *New Phytologist* **76**: 331-342.
- PHILLIPS, J.M. and D.S. HAYMAN. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-161.
- PICCINI, D., J.A. OCAMPO and E.J. BEDMAR. 1988. Possible influence of *Rhizobium* on VA mycorrhiza metabolic activity in double symbiosis of alfalfa plants (*Medicago sativa* L.) grown in a pot experiment. *Biology and Fertility of Soils* **6**: 65-67.
- SALINAS, J.G., J.I. SANZ and E. SIEVERDING. 1985. Importance of VA mycorrhizae for phosphorus supply to pasture plants in tropical oxisols. *Plant and Soil* **84**: 347-360.
- SMITH, S.E. and M.J. DAFT. 1977. Interactions between growth, phosphate content and N₂ fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*. *Australian Journal of Plant Physiology* **4**: 403-413.
- SMITH, S.E., D.J.D. NICHOLAS and F.A. SMITH. 1979. Effect of early mycorrhizal infection on nodulation and nitrogen fixation of *Trifolium subterraneum* L. *Australian Journal of Plant Physiology* **6**: 305-306.
- WAIIDYANATHA, U.P., S.N. YOGARATNAN and W.A. ARIYARATHE. 1979. Mycorrhizal infection on growth and nitrogen fixation of *Pueraria* and *Stylosanthes* and uptake of phosphorus from two rock phosphates. *New Phytologist* **82**: 147-152.

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