

Efficacy of AM fungi and its Interaction with *Rhizobium* in Winged bean (*Psophocarpus tetragonolobus* (L) D.C)



Botany

KEYWORDS : AM fungi, multiple inoculation, nodule number, P and N uptake.

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ABSTRACT

Pot experiments were conducted under greenhouse conditions to investigate the effect of three arbuscular mycorrhizal (AM) fungi (*Glomus fasciculatum*, *Glomus mossae* and *Acaulospora leavis*) individually and its interaction with rhizobium in a mixture, on the growth and mineral nutrition of Winged bean (*Psophocarpus tetragonolobus*). Inoculation of AM fungi along with rhizobium in different combinations of single and triple inoculation gave different results in growth parameters like plant height, dry weight of shoot and root, per cent root colonization, nodule number, spore number, P and N uptake. In conclusion, it was found that *Glomus fasciculatum* was found to be efficient AM fungi for winged bean and inoculation of all the three AM fungi and rhizobium resulted in highest growth parameters.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and also offer resistance to drought and salinity (Jeffries, 1987; Lakshman, 2009). Use of AM fungi in inoculated the plant growth is well documented. In the last three decades, there were lot of reports regarding the use of AM fungi to increase the plant growth and yield of agricultural crops, horticultural crops and forestry species. All AM fungi are obligate biotrophs that benefit the plant in several ways, for example by increasing nutrient capture, drought resistance, pathogen protection beneficial alterations of plant growth regulators and synergistic interactions with beneficial rhizosphere microorganisms (Bagyaraj, 1991).

Legumes are plants that bear seeds in pods. They markedly differ from non-legume crops because much of the nitrogen they require is produced through fixation of atmospheric nitrogen by bacteria inhabiting nodules borne on their roots. The AM fungi associated with legumes are responsible for adequate P nutrition. Increased P assimilation influence positively nitrogenase activity that in turn promotes root and mycorrhizal growth. The conducive effect of dual inoculation of roots with AM fungi and *Rhizobium* on growth, nutrient uptake and N₂ fixation in soybean (Bethlenfalvai et al., 1990), cowpea (Islam et al., 1990), and pea (Xavier and Germida, 2003) has been established. Olivera et al., (2004) reported that P application increased leaf area, plant dry biomass, nodule biomass, and shoot and root P content in common bean plants.

Winged bean (*Psophocarpus tetragonolobus*) is a tropical crop that is listed as one of the under exploited legumes. Winged beans have exceptionally high protein content and have been suggested as a potential food source for the tropics. It is unique among leguminous crops in that, several parts of the plant leaves, pods, seeds and tubers is edible and rich in protein. Mycorrhizal studies on this plant have not been reported so far. Therefore, the present study was undertaken.

MATERIALS AND METHODS

The rhizospheric soil samples were collected from legume growing areas of different localities of Dharwad. AM fungal spores isolated were mass multiplied by using Sorghum vulgare L. as a host plant in separate earthen pots. Pots measured 30 cm diameter and contained sterilized soil: sand mixture in the ratio of 3:1. Single or multiple AM fungal inoculum containing soil, hyphae, spores (215-250/ 50 g soil) and root bits was mixed with the soil in each experimental pot. *Rhizobium* inoculation was done by treating the seeds with a peat based culture before sowing.

AMF inoculum used for each treatment is as follows.

1. Control
2. AM fungi (*Glomus fasciculatum*)

3. AM fungi (*Acaulospora leavis*)
4. AM fungi (*Glomus mossae*)
5. *Rhizobium*
6. GF+AL+Rh
7. GF+GM+Rh
8. AL+GM+Rh
9. GF+AL+GM+Rh

Surface sterilized (seeds dipped in 1% mercuric chloride for three minute) seeds of Winged bean (*Psophocarpus tetragonolobus*) were sown in each pot above the soil inoculum and pots were arranged in random block design. Each treatment was maintained in triplicate. The spores were isolated from rhizospheric soil of Winged bean (*Psophocarpus tetragonolobus*) and spore count was recorded by wet sieving and decanting method (Gerdemann and Nicholson, 1963). The growth parameters of Winged bean (*Psophocarpus tetragonolobus*) such as plant height, dry weight of shoot and root, number of nodules spore number per cent root colonization, P and N uptake was determined. Phosphorus content in shoot was determined calorimetrically by the vanadomolybdate/phosphoric-yellow colour method outlined by Jackson (1973). Total nitrogen content was determined by the Microkjeldahl method (Bremner, 1960). Percentage mycorrhizal infection of the roots was determined by the root slide technique (Nicolson, 1960) after clearing the roots with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970).

$$\text{Root colonization} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

The data was statistically analyzed by using analysis of variance with the help of SPSS software. The mean values were compared by Duncan's multiple range tests at 0.05 level of significance

RESULTS AND DISCUSSION

In the present investigation, different AM fungi were inoculated to know the effect of single and multiple inoculation on winged bean. Table 1 revealed that inoculation of different AM fungi along with *Rhizobium* resulted increase in all growth parameters such as plant height, dry weight of shoot and root, root colonization, nodule number, spore number, P and N uptake than the control plants. But different AM fungi such as *Glomus fasciculatum*, *Glomus mossae* and *Acaulospora leavis* in combination with *Rhizobium* responded variedly in multiple combinations.

After 45 days highest growth parameters were observed in plants inoculated in combination of all the three AM fungi (*Glomus fasciculatum*, *Glomus mossae* and *Acaulospora leavis*). In case of triple inoculation, the combination of *Glomus fasciculatum* + *Glomus mossae* + *Rhizobium* showed highest growth in (48.0 cms) plant height when compared with the combination of *Glomus mossae* + *Acaulospora leavis* + *Rhizobium* (37.6cms)

and *Glomus fasciculatum*+ *Acaulospora leavis*+ *Rhizobium* (41.0cms), where as in single inoculation *Glomus fasciculatum* (53.6cms) was found to be efficient AM fungi when compared to *Glomus mossae*, *Acaulospora leavis*. Inoculation with *Rhizobium* alone did not show much increase in growth parameters. Other growth parameters such as dry weight of (2.20g)shoot and (1.76gm)root, (95.6)spore number, (75.6%)per cent mycorrhizal colonization, P (0.41) and N (0.31) uptake were also found to be highest in triple inoculated AM fungi and *Rhizobium* followed by single inoculation of *Glomus fasciculatum* (fig 2). There was a significant difference in all the growth parameters in all the treatments. The number of nodules was also found to be highest in multiple inoculation when compared to triple, single and uninoculated ones. Arbuscular mycorrhiza fungi give a major contribution to plant nutrition, promoting mostly the uptake of phosphorous (Bago et al, 2001). Many workers have reported enhancement of phosphate uptake and growth of legu-

minous plants by vesicular arbuscular mycorrhizal fungi (AMF) (Ezawa et al., 2000, Arihara and Karasawa, 2000).

Mycorrhizal fungi have been reported in roots of chickpea plants, improving the growth and yield of these plants, especially in phosphorus deficient soils (Zaidi et al., 2003). The important role of arbuscular mycorrhizas in the uptake by plants of nutrients of low solubility, especially phosphorus, is well recognized. For this reason, arbuscular mycorrhizas can often make very significant contributions to growth of N₂-fixing legumes. In some cases individual arbuscular mycorrhizal fungi had significantly different effects on the dry matter or concentration of P in the different bean varieties. However, this effect was small and would probably never be observed under field conditions, where all plants would have been infected by native soil AM fungi (Ibjijsen et al., 1996).

Table 1- Showing growth response of *Psophocarpus tetragonolobus* L. (Sweet) to single and multiple inoculation of AM fungi and *Rhizobium* at 45 days.

Treatments	Plant height	DWS	DWR	SN	No of nodules	% colonization	P uptake	N Uptake
Control	24.0±0.57a	0.36±0.33a	0.07±0.03a	54.6±0.33a	12.3±0.33a	34.6±0.33a	0.17±0.03a	0.08±0.03a
Gf	34.6±0.88e	1.36±0.33e	0.40±0.05c	75.0±0.57f	34.6±0.33e	58.0±0.00e	0.28±0.05e	0.17±0.03e
Gm	31.0±0.57d	1.13±0.33d	0.26±0.03b	70.3±0.33d	28.3±0.33c	52.3±0.33d	0.25±0.03d	0.15±0.03d
Al	28.6±0.33c	0.76±0.33c	0.15±0.05a	64.3±0.33e	22.3±0.33b	43.6±0.33c	0.22±0.33c	0.12±0.03c
Rh	27.3±0.33b	0.60±0.00b	0.13±0.03a	61.3±0.33b	33.3±0.33d	40.3±0.33b	0.20±0.03b	0.10±0.03b
Gf+Gm+Rh	48.0±0.57h	1.76±0.33g	1.46±0.03d	81.6±0.33g	41.3±0.33g	64.6±0.33g	0.36±0.03h	0.26±0.03h
Gf+Al+Rh	41.0±0.57g	1.70±0.00g	1.03±0.33c	75.3±0.33f	37.6±0.33f	58.6±0.33e	0.32±0.00g	0.23±0.03g
Gm+Al+Rh	37.6±0.33f	1.46±0.33f	0.08±0.03a	72.3±0.66e	35.3±0.33e	61.0±0.33f	0.30±0.06f	0.21±0.03f
Gf+Gm+Al+Rh	53.6±0.33i	2.20±0.57h	1.76±0.03e	95.6±0.33h	54.3±0.33h	75.6±0.33h	0.41±0.03i	0.31±0.03i

Gf- *Glomus fasciculatum*;Gm- *Glomus mossae* ;Al- *Acaulospora leavis*; Rh- *Rhizobium*; DWS- Dry weight of shoot; DWR- Dry weight of root;SN- Spore number. Means sharing a letter in columns are not significantly different according to Duncan's test P <0.05.

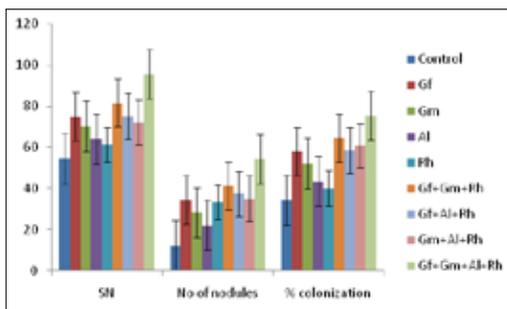


Fig 1. Showing the effect of different AM fungi (single, triple inoculation and multiple) on Spore number, no. of nodules and per cent root colonization in winged bean

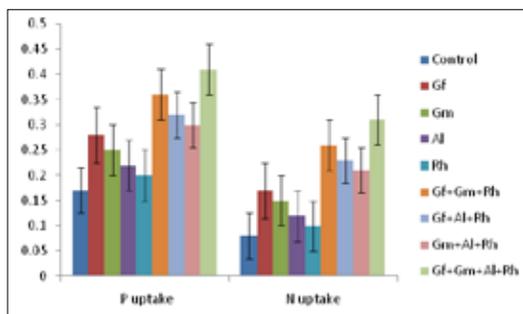


Fig 2. Showing the effect of different AM fungi (single, triple inoculation and multiple) on P and N uptake in winged bean

Arbuscular mycorrhiza is one of the most efficient ecological factors in improving growth and N content in legumes (Barea et al. 2002; Lakshman 1999). *Rhizobium*- associated plants are usually mycorrhizal. The mycorrhizal and *Rhizobium* symbiosis

usually acts synergistically on infection rate,mineral nutrition and growth of the plant. AM fungi improve P uptake in conditions where N and P are limited. The higher P concentration in the plant benefits the bacterial symbiont and nitrogenase functioning, leading to increased nitrogen fixation, which in turn promotes root and mycorrhizal development. (Lakshman, 1999).

From this investigation it can be concluded that *Glomus fasciculatum* was found to be most promising bio fertilizer for winged bean the next best fungus was *Glomus mossae*.

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