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Effects of Arbuscular Mycorrhizal Fungal Inoculation on Growth and Yield of *Flemingia vestita* Benth. ex Baker

Songachan, L. S.[1*] and Kayang, H.[2]

[1]Department of Botany, Banaras Hindu University, Varanasi- 221005, Uttar Pradesh, India
[2]Microbial Ecology Laboratory, Department of Botany, North Eastern Hill University, Shillong-793 022, Meghalaya, India.

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Abstract Pot experiment was conducted to investigate the effects of arbuscular mycorrhizal fungal inoculation on growth and tuber yield of *Flemingia vestita* under greenhouse condition.Three native AMF species (*Acaulospora scrobiculata*, *Glomus aggregatum* and *Glomus luteum*) and three commercial species (*Acaulospora laevis*, *Glomus fasciculatum* and *Glomus macrocarpum*) were used for inoculation. The results indicated that AMF inoculation increases plant growth and tuber yield compared to uninoculated ones. Plant growth in the form of plant height, leaf number and leaf area was greatest in *A. scrobiculata* inoculated plants, while root dry weight, tuber yield and P acquisition in roots and shoots was greatest in *G. macrocarpum* inoculated plants. Shoot dry weight was highest in *G. aggregatum* inoculated plants. From the present investigation, it was observed that *F. vestita* responds positively to AMF inoculation, the level of response however, depends on AMF species.

Keywords: Arbuscular mycorrhizal fungi (AMF), effect, inoculated, uninoulated

Introduction

Association of arbuscular mycorrhizal fungi (AMF) is of great economic significance on growth and nutrition of plants. Mycorrhizal inoculation enhanced nutrient uptake, especially phosphorus, as this fungal symbiosis increased the abilities of the host plants to explore a larger volume of soil than roots alone and to take up phosphate from a greater surface area (Joner *et al*., 2000). Studies have shown that different individuals of a plant species have distinct growth responses by inoculating different AMF species (Klironomos, 2003).

One critical step for applying arbuscular mycorrhizal technology is the appropriate selection of effective fungal isolates to be used as plant inoculants. The identification of efficient AMF is considered a prerequisite to inoculation

[*] **Coressponding author:** Songachan, L. S.; **Email:** mima_hdy@yahoo.com

programs, as the efficiency of mycorrhizal inoculation on the plant growth depend on the AMF isolate (Duponnois *et al*., 2001). Thus, it is essential to screen for an efficient AMF for a particular host in order to harness the maximum benefit from the fungus (Bagyaraj and Varma, 1995). While the field-efficient colonizers are crucial under the natural conditions, it is also important to have isolates that are easily multiplied and sporulate under laboratory conditions in order to produce well-defined inoculum for practical applications. The current research is concentrated on developing techniques for enhancing crop production along with producing of high number of AMF propagules (Gaur and Adholeya, 2002). Thus, mycorrhizal research and its practical use as a low-input technology for managing soil fertility and plant nutrition has been the subject of increasing interest.

Flemingia vestita Benth. ex Baker (Fabaceae) is an indigenous plant of Meghalaya, Northeast India. It is a weak climber that produces an edible root tuber and has a high local market value. Its root-tuber peel is use as curative against worm infection in traditional medicine among the natives of Meghalaya. Anthelmintic efficacy of this plant derived materials has provided evidences that support and authenticate the usage of the tuberous root of this plant as vermifuge and vermicide (Das *et al*., 2004). Songachan and Kayang (2011) reported that *F. vestita* is highly associated with AMF and it harbors a relatively high AMF community. Many researchers have generally indicated that AMF can enhance plant growth and yield; however, no information is available on the effects of different AMF isolates on growth and yield of *F. vestita.*Therefore, in the present study, selected native and commercial AMF isolates were inoculated on *F. vestita* plants to determine its effect on growth and yield of *F. vestita*.

Materials and methods

Establishment of AMF monospecific cultures

Monospecific AMF spore isolation

Native as well as commercial AMF species were used to inoculate *Flemingia vestita* plants. Three native AMF species (*Acaulospora scrobiculata*, *Glomus aggregatum* and *Glomus luteum*) were isolated from rhizosphere soils of *F. vestita* using wet-sieving and decanting method of Gerdemann and Nicolson (1963). Native AMF species were isolated and identified using the identification keys from International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi i.e., INVAM (http://invam.caf.wvu.edu). Healthy and morphologically identical spores of each target species were

carefully isolated manually and stored at 4℃. Commercial AMF species (*Acaulospora laevis*, *Glomus fasciculatum* and *Glomus macrocarpum*) were also procured from Centre for Natural Biological Resources and Community Development (CNBRCD), Bangalore, India.

Seedling preparation and AMF inoculation and propagation for bulk production

AMF propagation was done by following the methods of INVAM. Two weeks before setting up a bulk production of monospecific culture, seeds of *Paspalum notatum* Flügge were surface-sterilized with 1% sodium hypochlorite. It was then evenly sown on 25cm diameter plastic pot using a sterilized sand-soil substrate in (1:1 v/v). After two weeks, seedlings of *P. notatum* were collected and a gentle stream of tap water was allowed to pass over their roots so they stick together. Each target native spores are placed and allowed to adhere along the length of the intertwined roots, so they are in contact with the maximum range of root physiological states. Seedlings were immediately transplanted into the 200ml disposable plastic containers filled with sterilized sand-soil substrate. All disposable plastic containers were placed in a room with indirect lighting for 24 h and then moved to greenhouse. It was then watered gently from time to time as required. Monospecific AMF inoculated seedlings of *P. notatum* were grown for five months, allowing them to sporulate and propagate after which, it was left to dry undisturbed in a shade at room temperature. This AMF monospecific cultures were stored in air tight gallon zip-loc plastic bags at 4℃. The bagged material containing growth medium with AMF spores were used as inoculum for each individual target AMF species.

Plant materials and growth conditions

Seeds of *F. vestita* were collected from natural and cultivated sites during September, 2011. Before plantation, seeds were disinfected with 1% sodium hypochlorite. In the month of March, 2012, individual seed was placed on sterilized sand-soil substrate (1:1 v/v) in 200ml disposable plastic containers. The germination set up were kept in B.O.D. incubator at 25℃ under white fluorescent tubes (photoperiod 12h) and watered whenever required to keep the soil mixture moist. After one month, germinated seedlings of *F. vestita* were transferred in sterilized plastic pots containing sterilized sand-soil substrate (1:1 v/v) with 10g each of six different AMF inoculants containing 250-300 spores approximately. Uninoculated (control) plants were also maintained. Each treatment was maintained in ten replicates in greenhouse. It was watered

whenever required. Every month, number of leaves, leaf area, plant heights and rate of AMF colonization were assessed. In the month of October 2012, plants were harvested for assessment of tuber yield, root and shoot weight and its ratio. Phosphorus content of soil was also estimated by following the molybdenum blue method of Allen *et al.*, (1974).

Data analysis

Means and standard errors were calculated for the plant growth parameters, and also for mycorrhizal colonization in AMF inoculated plants. The relationships among plant growth parameters were analyzed by calculating Pearson's correlation coefficients (*r*) values. One way ANOVA was conducted to analyze the variation between plant growth parameters in AMF inoculated and uninoculated plants.

Results

Growth parameters of AMF inoculated and uninoculated *Flemingia vestita* plants are given in Table 1. The growth of AMF inoculated plants was higher than that of uninoculated plants.The phosphorus content in the roots ranged from 0.11% to 15.60%. It was lowest in *G. luteum* inoculated plants and highest in *G. macrocarpum* inoculated plants. The shoot P content ranged from 1.24% to 6.40% and soil P content ranged from 0.12% to 1.44%. Both shoot and soil P content was lowest in uninoculated plants and highest in *G. macrocarpum* and *G. fasciculatum* inoculated plants. The rate of AMF colonization in *F. vestita* inoculated with six different AMF species are given in Figure1. It was lowest in *G. Aggregatum* inoculated plants (19.65%) and highest in *G. luteum* inoculated plants (32.13%). The mycorrhizal colonization in the form of arbuscules, hyphae and vesicles were observed in all AMF inoculated plants (Table 2).

Table 1. Growth parameters and phosphorus content (%) of AMF inoculated and uninoculated *Flemingia vestita* plants

Treatment	Number of leaves	Plant height (cm)	Leaf area (cm^2)	Root dry weight (g)	Shoot dry weight (g)	Root:shoot dry weight (g)	Tuber wt (g)	Soil phosphorus
A. scrobiculata	131.75 ±7.19	33.13 ±3.27	3.01 ±0.13[a]	0.19 ±0.02	1.58 ±0.07	0.11 ±0.02	113.34 ±3.49[a]	0.08 ±0.005
G. aggregatum	91.00 ±5.25	27.70 ±2.99	2.13 ±0.13[c]	0.21 ±0.02	0.88 ±0.06	0.24 ±0.03	108.37 ±4.03[a]	0.09 ±0.010
G. luteum	104.60 ±2.26	30.74 ±2.73	2.43 ±0.11[b]	0.23 ±0.01	1.13 ±0.03	0.20 ±0.04	99.06 ±3.98[b]	0.01 ±0.003
A. laevis	78.97 ±4.53	30.12 ±3.10	2.93 ±0.19	0.24 ±0.02	1.16 ±0.08	0.20 ±0.02	118.08 ±5.87[b]	0.07 ±0.006
G. fasciculatum	99.77 ±8.58	30.17 ±2.99	2.05 ±0.20	0.28 ±0.03	0.91 ±0.13	0.30 ±0.04	104.78 ±2.38[b]	0.14 ±0.036
G. macrocarpum	81.10 ±9.72	28.00 ±3.77	1.18 ±0.09	0.31 ±0.03	1.45 ±0.14	0.21 ±0.01	134.98 ±3.46[c]	0.08 ±0.000
Control	71.00 ±4.02	24.98 ±3.69	2.14 ±0.05	0.18 ±0.04	0.86 ±0.12	0.22 ±0.02	67.08 ±2.98	0.01 ±0.008

Note: The results were analyzed by one-way ANOVA. Fisher's least significant difference (LSD) at $p<0.05$ is indicated by different letters.

Table 2. Mycorrhizal structural colonization (%) in *Flemingia vestita* under six different treatments

Treatment	Arbuscules	Vesicles	Hyphae
Acaulospora scrobiculata	16.01 ± 0.09	0.08 ± 0.00	8.87 ± 0.06
Glomus aggregatum	10.77 ± 0.04	0.34 ± 0.01	8.54 ± 0.01
Glomus luteum	20.23 ± 0.00	2.97 ± 0.00	8.93 ± 0.03
Acaulospora laevis	21.09 ± 0.06	0.88 ± 0.02	6.78 ± 0.40
Glomus fasciculatum	13.08 ± 0.03	0.65 ± 0.02	5.96 ± 0.01
Glomus macrocarpum	23.98 ± 0.08	0.08 ± 0.02	7.38 ± 0.04

Table 3. Pearson's correlation analysis between growth parameters in AMF inoculated and uninoculated *F. vestita* plants

Sources of variation	LA	PH	SDW	RDW	RSDW	TW	SP
NL	0.48[a]	0.85[c]	-	-	-	-	-
LA		-	-0.47[a]	-0.72[b]	-	-	-
PH			-	-	-	0.47a	-
SDW				-	-	0.55[a]	-
RDW					-	0.60[a]	0.45[a]
RSDW						-	-
TW							0.53[a]
SP							

Note: NL = Number of leaves, LA = Leaf area, PH = Plant height (cm), SDW = Shoot dry weight (g), RDW = Root dry weight (g), RSDW = Root: shoot dry weight (g), TW = Tuber weight (g), SP = Soil P. Values marked with a, b, and c are significant at $p < 0.05$, $p < 0.01$and $p < 0.001$; insignificant values are marked with '–'.

Pearson's correlation coefficient among different growth parameters are depicted in Table 3. The number of leaves was positively correlated with leaf area ($p < 0.05$) and plant height ($p < 0.001$). Leaf area also has a positive correlation with plant height ($p < 0.05$) while a negative correlation ($p < 0.05$) with shoot dry weight, root and dry weight. Plant height also shows a positive correlation ($p < 0.05$) with tuber weight. Shoot dry weight has a positive correlation with tuber weight at $p < 0.05$. Root dry weight has a positive correlation ($p < 0.05$) with tuber weight and soil P. Root: shoot dry weight ratio was positively correlated with soil P content at $p < 0.05$. Tuber weight was positively correlated with soil P at $p < 0.05$, ANOVA shows that there was no significant variation ($p < 0.05$) in number of leaves among different treatments

of AMF inoculated and uninoculated plants. Likewise, plant height, shoot dry weight, root dry weight, root: shoot dry weight and tuber weight does not show any significant variation among different treatments of AMF inoculated and uninoculated plants. Significant variations ($p< 0.05$) were observed in leaf area of uninoculated plants and *A. scrobiculata*, *G. aggregatum* and *G. luteum* inoculated plants. An uninoculated plant shows a variation ($p< 0.05$) in tuber weight with all the AMF inoculated plants.

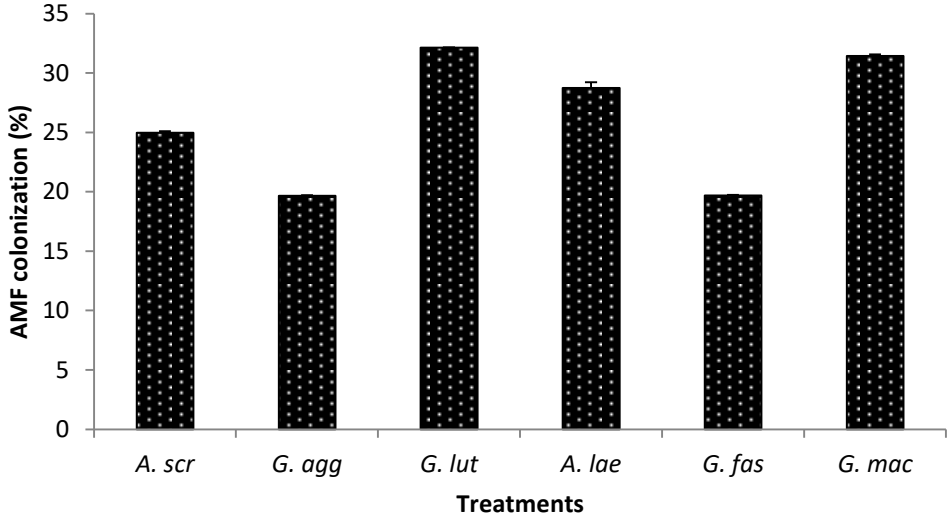

Figure 1. AMF colonization in *Flemingia vestita* with eight different treatments. (Note: *A. scr = Acaulospora scrobiculata, G. agg = Glomus aggregatum, G. lut = Glomus luteum, A. lae= Acaulospora laevis, G. fas = Glomus fasciculatum and G. mac = Glomus macrocarpum*).

Discussion

AMF inoculation has a positive effect on the growth and yield of *F. vestita*. Growth and yield was higher in inoculated plants as compared to uninoculated plants. The root: shoot ratio is generally an important parameter when evaluating responses in AMF associations, and are often altered in the presence of AMF due to a change in the uptake capability of AMF roots (Gaur and Adholeya, 2002; Singh *et al*., 2008). In the present investigation, commercial inoculum, *G. macrocarpum* inoculated plants showed highest root dry weight accompanied by increased in shoot dry weight, (though shoot dry weight was highest in *A. scrobiculata* inoculated plants), thus indicates the presence of an efficient symbiotic mechanism (Redente and Reeves, 1981; Gazey *et al*., 2004). Although none of the native as well as commercial AMF

showed high AMF colonization rate, all AMF isolates colonized *F. vestita* roots. It was postulated that the degree of colonization and functioning of the AMF symbiosis can depend on the AMF ability to colonize a specific host. Several recent studies have also revealed that at least some host preference may exist in the AMF symbiosis (Bever *et al*., 2001; Sýkorová *et al*., 2007). Even though *G. luteum* has the highest colonization rate, it does not show any remarkable effect on the plant growth parameters. Quantitatively high or similar infection levels in the roots may not necessarily produce a similar physiological response to the host plant, as mycorrhizal colonization rate does not always control effectiveness (Gianinazzi-Pearson *et al.*, 1985); different species and ecotypes of AMF elicit different effects on plant growth (Caravaca *et al.*, 2004). Beneficial responses have been reported with only 0.4% mycorrhizal colonization (Niemira *et al.*, 1995).

When compared with uninoculated plants, all the mycorrhizal inoculated plants showed increased in tuber weight, confirming that AMF species promoted tuber growth of *F. vestita* to varying degrees. Commercial species (*G. macrocarpum* and *A. laevis*) had a greater effect on *F. vestita* tuber yield than native species. Among the native isolates, *A. scrobiculata* was effective in promoting *F. vestita* tuber yield. These results contrasted with those of Rowe *et al*., (2007), who described poor performance by commercial inoculum relative to field soil inoculum in a greenhouse study. Tchabi *et al*.,(2010) also reported that native AMF isolates led to increased yam tuber weights, compared to the commercial isolates.Various other studies have also found variable effects of different AMF species on plant growth growth (Sieverding, 1991; Frey and Schüepp, 1993; Tchabi *et al*., 2010), which can be related to specific compatibility between host plant and AMF species that has been reported in a number of crops such as maize (Khalil *et al*., 1994), onion (Yao, 1996), potatoes (Yao *et al*., 2002; Diop *et al*., 2003) and sweet potato (Gai *et al*., 2006). Recent studies also strongly indicated that such effects may vary intra-specifically at a high level (Munkvold *et al*., 2004; Koch *et al*., 2006).

Shenpagam and Selvaraj (2010) reported that *G. aggregatum* was the best among 7 indigenous AMF species for inoculating *Solanum viarum*in the nursery in order to obtain healthy, vigorously growing seedlings. Ndiaye *et al*., (2011) also reported that G. *fasciculatum* was the most efficient fungus in terms of *Acacia Senegal* plant performance. Several inoculation studies have reported different improvement of host plant growth by different AMF (Dixon *et al.*, 1984; Qu *et al.*, 2004). In this study, growth enhancement by AMF varied widely, confirming different ability of mycorrhizal fungi to enhance plant growth. Different AMF species might differ in their effects on plant growth and it is possible that in this study, the strain of commercial inoculum as compared

to native inoculum might have interacted better with the host plants, and subsequently resulted in a better growth and yield. An on-farm experiment is required to evaluate whether the positive inoculation effects can be reproduced under field conditions. Such experiments should include possible measurements to manage the inoculum of AMF in the field, to establish when AMF inoculation is most effective and how beneficial AMF populations with a high infection level can be maintained (Larsen *et al.,* 2007, Sieverding 1991, Sørensen *et al.,* 2005 and 2008).

Growth and mineral nutrition of plants are commonly enhanced by inoculation with AMF (Clark and Zeto, 2000). Increase in growth and biomass of AMF inoculated plants strongly depends on their ability to access minerals from the soil. Therefore, positive effects of tested AMF on P content could be related to the ability of symbiotic fungi to enhance soil P depletion zones around roots (Smith *et al*., 2001). Higher P content in AMF inoculated plants is probably due to more efficient uptake of available P from the soil and possibly to mineralization of organic phosphorus due to a higher phosphatase production by AMF plants (Tarafdar and Marschner, 1994).

From the present investigation, it was observed that *F. vestita* responds positively to AMF inoculation. The level of response however, depends on AMF species, and consequently on overall host-AMF compatibility. To optimize the potential of such an inoculation practice it is necessary to consider and carefully select an appropriate AMF isolates. Moreover, on-farm experiments are required to evaluate whether the positive inoculation effects can be reproduced under field conditions. The use of native as well as commercial AMF inocula might represent a convenient alternative to chemical fertilizers, and offer economically and ecologically important advantages in sustainable or organic cropping systems.

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