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Symbiotic response of fodder cowpea (*Vigna unguiculata* L.) and field bean (*Lablab purpureus* L.) with different arbuscular mycorrhizal fungi

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ABSTRACT

Fodder cowpea and field bean are the minor legumes cultivated mainly in arid and semi-arid tracts of India. Species of arbuscular mycorrhizal (AM) fungi known to improve plant growth may not have the same effect on all the plants due to host preference. Hence, screening and selecting the efficient AM fungus for each crop is important for maximum symbiotic response. The information available on the response of fodder legumes to AM fungal inoculation is meager. Hence a pot culture experiment was conducted to screen and select the efficient AM fungi for inoculating cowpea and field bean. The screening was done with ten different species of AM fungi (*Acaulospora laevis, Gigaspora margarita, Glomus bagyarajii, Claroideoglomus etunicatum, Rhizophagus fasciculatus, Rhizophagus intraradices, Ambispora leptoticha, Glomus macrocarpum, Funneliformis caledonius* and *Funneliformis mosseae*). Plant parameters like height, stem girth, bio-volume index, the biomass of shoot and root, and the percent mycorrhizal root colonization were recorded according to the standard procedures. In the present study, the two legumes differed slightly in their response to inoculation with different AM fungi. Giving weightage to shoot biomass, being fodder legumes, it was concluded that *Funneliformis caledonius* is the best AM fungus for inoculating cowpea and field bean.

Keywords: AM fungi, Mycorrhizae, *Glomus*, Fodder legumes, Symbiosis

INTRODUCTION

Cowpea is also known as black-eyed pea or Southern pea, and field bean is popularly known as Dolichos bean and hyacinth bean in other parts of the world. These fodder crops are known for grazing, haymaking, and ensiling with grasses (Samireddypalle *et al.,* 2017). The average nutrient content of these legumes is 20-32% protein, 50-60% carbohydrate, and about 1% fat on a dry weight basis (Jayathilake *et al.,* 2018). In India, some varieties of these legumes are grown as minor fodder crops cultivated mainly in arid and semi-arid tracts of different states as a mono-crop or intercrop along with forage grasses. In the rainfed areas, these are a valuable source of fodder during the lean winter months (Shekar *et al.,* 2019).

It is well known that adding chemical fertilizers to soil is detrimental to microbial growth and deteriorates soil quality (Sharma *et al.,* 2019). Microorganisms like bacteria, fungi, and actinomycetes in the soil play a major role in plant growth and conserving the environment. It is essential to reduce the use of chemical fertilizers by introducing microbial inoculants like mycorrhizal fungi, N fixers, etc., to sustain plant productivity and maintain soil health (Alori and Babalola, 2018).

AM fungi are ubiquitous and can live symbiotically in plant roots (Bagyaraj *et al.,* 2015). The contribution of AM fungi in agricultural land may reduce the input of chemical fertilizers and help sustain plant productivity and retain soil quality (Raghavendra *et al.,* 2016). AM fungi are important in ecological agriculture because they act as biofertilizers, bio-protectors, and biocontrol agents (Mathimaran *et al.,* 2017; Bhantana *et al.,* 2021). These symbiotic fungi facilitate the uptake of diffusion-limited nutrients, particularly phosphorus (P), and protect the plants against biotic and abiotic stresses, thus increasing crop productivity (Thirkell *et al.,* 2017; Begum *et al.,* 2019). AM fungi are not host-specific but exhibit host preference (Thilagar and Bagyaraj, 2015); thus, it was envisaged to screen and select the efficient AM fungi for inoculating fodder varieties of cowpea and field bean.

MATERIALS AND METHODS

The pot experiment was conducted to screen and select the efficient AM fungi for inoculating fodder cowpea and field bean based on the symbiotic response. The AM fungi used in the study were *Acaulospora laevis, Gigaspora margarita, Glomus bagyarajii, Claroideoglomus etunicatum, Rhizophagus fasciculatus, Rhizophagus intraradices, Ambispora leptoticha, Glomus macrocarpum, Funneliformis caledonius* and *Funneliformis mosseae*. These fungi maintained in the culture collection of CNBRCD were selected based on earlier studies on other crop plants (Thilagar and Bagyaraj, 2015; Ashwin *et al.,* 2018). The AM fungi were multiplied in pots under polyhouse conditions using vermiculite, perlite, and soilrite in 1:1:1 (v/v/v basis) and Rhodes grass (*Chloris gayana*) as the host. After 75 days of growth, shoots of Rhodes grass were severed, and the substrates containing spores, hyphae, and root bits (cut into about 1 cm pieces) were air-dried and used as inoculum.

The soil used in this experiment was collected from an uncultivated field from 0-15 cm depth and has been classified as fine, kaolinitic isohyperthermic kanhaplustalfs. The pH of the soil was 6.0 (1:10 soil to water extract ratio) with an available P of 5.2 ppm (NH4F+HCl extractable) and an indigenous mycorrhizal spore count of 107 in 50 g soil. The forage cowpea variety MFC-08-14 and field bean local variety used in the experiment were procured from the All India Co-ordinated Research Project (AICRP) on arid legumes, University of Agricultural Sciences (UAS), Bengaluru, and AICRP on pigeon pea, UAS, Bengaluru

respectively. The polybags of the size 27×14 cm were filled with around 4 kg soil, sand, and vermicompost in the ratio of 1:1:0.25 (v/v/v) as substrate, and each treatment was replicated six times. One gram of mycorrhizal inoculum was added to respective polybags as per the treatment. The control treatment received 10 ml of the washings of the all AM fungal inoculum mix used in the study, which was passed through a 45 µm sieve containing only associated microorganisms and no AM fungi. Initially, three seeds were sown to ensure germination in all treatments and thinned out to one after ten days of sowing. The pots were maintained in a polyhouse and watered as and when needed.

Seventy days after sowing (DAS), plant height was measured from the soil surface to the growing tip of the plant; stem girth was measured 1 cm above the soil surface using digital vernier calipers and number of leaves per plant were enumerated. Plants were harvested after the 70 DAS. The shoot and root of the plants were separated, and their dry biomass was determined after drying at 60°C in an oven to a constant weight. The biovolume index was calculated using the appropriate formula (Hatchell *et al.,* 1985). The AM colonization in roots was determined by staining the fine roots with Trypan blue (Philips and Hayman, 1970) and estimated by adopting the gridline intersect method outlined by Giovannetti and Mosse (1980). The data were analyzed using a completely randomized design with the help of a computer (Microvex System VAX/VMS, version 5.4, Digital Equipment Corporation, USA). The means were compared by Duncan's multiple range test at 5% level (Gomez and Gomez, 1984).

RESULTS

The present study was conducted with the objective of screening and selecting efficient AM fungi for inoculating fodder cowpea and field bean crops. The plants showed varied plant growth responses to different AM fungal inoculation. In general, mycorrhizal inoculation significantly improved plant growth parameters compared to uninoculated plants in both crops.

In cowpea, the plant height was significantly higher in all the inoculated plants compared to the uninoculated plants, the highest being in *F. mosseae* treated plants. The stem girth was highest in plants inoculated with *A. laevis*. In field bean, inoculation with *F. mosseae*, *A. laevis*, and *Gi. margarita* showed maximum plant height compared to control and many other inoculated treatments. Still, the stem girth was highest in the plants inoculated with *R. fasciculatus* but statistically on par with the treatments *F. mosseae, F. caledonius, C. etunicatum,* and *Gi. Margarita*. The increased biovolume index of cowpea was observed in all plants inoculated with different AM fungal cultures. Among them, *A. laevis* exhibited the highest BI, but statistically not differing from the treatments are *R. fasciculatus, F. mosseae, G. bagyarajii, F. caledonius, C. etunicatum,* and *G. macrocarpum*. The BI in field bean was significantly more and on par in treatments *R. fasciculatus, F. mosseae, A. laevis,* and *Gi. margarita* compared to uninoculated and few inoculated treatments. The uninoculated cowpea and field bean plants had the least plant height, stem girth, and BI (**Table 1 & 2**).

There was a significant increase in the total number of field bean leaves per plant inoculated with AM fungi, except *R. fasciculatus*, compared to uninoculated plants. Among the 10 AM fungi, *G. macrocarpum* inoculated field bean plants resulted in the highest number of leaves (42 per plant). In cowpea plants, there was no significant difference with respect to the total number of leaves per plant.

Shoot dry biomass of cowpea was significantly higher in the plants inoculated with *G. caledonius, G. macrocarpum,* and *F. mosseae* than in uninoculated plants and plants inoculated with other AM fungi. The root dry biomass of cowpea differed significantly in plants inoculated with *R. fasciculatus, F. caledonius,* and *G. bagyarajii* over uninoculated plants. The total dry biomass was maximum in plants inoculated with *F. caledonius* but not significantly differing from plants inoculated with *G. macrocarpum, R. fasciculatus, G. bagyarajii,* and *F. mosseae* (**Table 1**). In field bean highest shoot dry biomass was recorded in *F. caledonius* inoculated plants, not differing significantly from plants inoculated with *R. fasciculatus, R. intraradices, G. bagyarajii,* and *Gi. margarita.* The root dry biomass was significantly more in the treatment *F. caledonius* and statistically on par with the treatments *G. bagyarajii, A. laevis,* and *G. margarita* compared to uninoculated and few inoculated treatments. The total dry biomass was highest in plants treated with F. caledonius but did not differ statistically from the treatments of *R. fasciculatus, R. intraradices, G. bagyarajii,* and *Gi. margarita.* Uninoculated plants had the least total plant dry biomass (**Table 2**).

The percent root colonization was significantly higher in all the inoculated plants than in uninoculated plants. The highest root colonization was observed in the roots of cowpea inoculated with *F. caledonius* (100%) and *G. macrocarpum* (96%), followed by *F. mosseae* (86%) (table 1). The field bean plants inoculated with *F. caledonius* and *G. bagyarajii* had 100% mycorrhizal root colonization, which was at par with *Gi. margarita* (97%) and *R. intraradices* (96 %) and *R. fasciculatus* (92%) (**Table 2**).

DISCUSSION

Different symbiotic efficiency among AM fungi for a particular host has been reported by earlier workers (Soram *et al.,* 2012). Hence the need for selecting efficient AM fungi that can be used for inoculating the different mycotrophic plants has been emphasized (Bagyaraj and Kehri, 2012; Sale

Table 1: Influence of soil inoculation of different AM fungi on plant growth parameters of cowpea (*Vigna unguiculata* L.)

Treatment	Plant height (cm)	Stem girth (mm/plant)	Bio volume index (BVI)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)	Total dry biomass (g/plant)	Average number of leaves/plant*	Mycorrhizal root colonization (%)
Uninoculated	17.00^{d}	4.85^{d}	82.47^{c}	5.21^{d}	1.62^{cd}	6.83^{d}	18	25^{g}
R. fasciculatus	35.08^{c}	7.12^{ab}	249.68^{b}	5.5^{cd}	2.65^{a}	8.22^{abc}	20	80^{bcd}
F. caledonius	43.00^{ab}	7.20^{ab}	309.74^{a}	6.93^{a}	2.58^{a}	9.51^{a}	22	100^{a}
R.intraradices	35.42^{c}	6.78^{ab}	240.07^{bc}	5.75^{cd}	2.04^{bc}	7.80^{abcd}	19	75^{cde}
G.bagyarajii	42.42^{abc}	7.34^{ab}	311.41^{a}	5.9^{bcd}	2.21^{ab}	8.19^{abc}	22	82^{bc}
F. mosseae	46.75^{a}	6.66^{ab}	311.36^{a}	6.70^{ab}	1.72^{cd}	8.42^{ab}	20	86^{b}
A. laevis	40.58^{abc}	7.68^{a}	311.95^{a}	5.53^{cd}	1.65^{cd}	7.18^{bcd}	22	70^{ef}
C. etunicatum	36.95^{bc}	6.58^{bc}	243.13^{bc}	5.51^{cd}	1.36^{d}	6.88^{cd}	18	65^{f}
A.leptoticha	41.28^{abc}	5.55^{cd}	228.98^{bc}	5.18^{d}	1.61^{cd}	6.80^{d}	21	65^{f}
G. macrocarpum	41.67^{abc}	6.25^{bc}	260.56^{b}	6.34^{abc}	1.98^{bc}	8.32^{abc}	22	96^{a}
Gi. margarita	41.15^{abc}	4.86^{d}	199.98^{c}	5.99^{bcd}	1.64^{cd}	7.64^{abcd}	23	72^{def}
SEd	3.78	0.72	12.67	0.42	0.24	0.51	NS	5.42
CD (0.05)	1.092	1.43	43.71	0.85	0.48	1.33	NS	9.07

Note: Values in each column followed by the same letter are not significantly different at P≤0.05; *Not significant

Table 2: Influence of soil inoculation of different AM fungi on plant growth parameters of field bean (*Lablab purpureus* L.)

Treatment	Plant height (cm)	Stem girth (mm/plant)	Bio volume index (BVI)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)	Total dry biomass (g/plant)	Average number of leaves/plant	Mycorrhizal root colonization (%)
Uninoculated	24.58^{c}	4.33^{d}	106.53^{e}	6.77^{e}	1.45^{ef}	8.21^{e}	27^{c}	30^{g}
R. fasciculatus	33.95^{abc}	6.07^{a}	205.96^{a}	10.07^{ab}	1.90^{bcd}	11.98^{abc}	28^{c}	92^{abc}
F. caledonius	26.283^{c}	5.05^{ab}	132.60^{de}	11.12^{a}	2.37^{a}	13.49^{a}	38^{ab}	100^{a}
R.intraradices	32.16^{abc}	4.90^{bc}	157.4^{cd}	10.35^{ab}	1.79^{cd}	12.14^{ab}	41^{a}	96^{ab}
G.bagyarajii	32.66^{abc}	4.39^{cd}	143.24^{cd}	10.95^{ab}	2.19^{ab}	13.14^{ab}	38^{ab}	100^{a}
F. mosseae	38.55^{a}	5.52^{a}	212.80^{a}	7.75^{de}	1.83^{cd}	9.59^{de}	37^{ab}	70^{f}
A. laevis	40.16^{a}	4.88^{bc}	195.95^{a}	7.51^{de}	2.15^{ab}	9.66^{de}	34^{b}	78^{def}
C. etunicatum	27.41^{bc}	5.05^{ab}	138.45^{cd}	8.48^{cd}	1.65^{def}	10.13^{cde}	35^{b}	81^{cde}
A.leptoticha	27.91^{bc}	4.85^{bcd}	135.49^{d}	8.17^{cde}	1.44^{f}	9.61^{de}	38^{ab}	74^{ef}
G. macrocarpum	36.50^{ab}	4.54^{cd}	165.8^{bc}	9.51^{bc}	1.76^{de}	11.27^{bcd}	42^{a}	88^{bcd}
Gi. margarita	38.16^{a}	5.05^{ab}	192.55^{ab}	10.51^{ab}	2.06^{abc}	12.62^{ab}	37^{ab}	97^{ab}
SEd	4.95	0.51	6.12	1.22	0.35	1.38	3.14	4.3
CD (0.05)	9.92	1.01	28.07	1.56	0.33	1.91	6.3	10.01

Note: Values in each column followed by the same letter are not significantly different at P ≤0.05; *Not significant

et al., 2021). In the present study, inoculation with AM fungi significantly enhanced the growth of cowpea and field bean compared to uninoculated plants. Improved plant growth because of AM fungal inoculation is well documented in many forage crops, mostly grasses (Sun *et al.,* 2017; Flores-Juárez *et al.,* 2020) and a few legumes (Puschel *et al.,* 2017). Of the various AM treatments, inoculation of cowpea with *F. caledonius* enhanced all the plant growth parameters studied, viz., plant height, stem girth, BI, shoot, and total plant dry biomass. In field bean *G. mosseae, R. fasciculatus* and *Gi. margarita* improved three plant growth parameters, viz., plant height, stem girth, and biovolume index. The other three plant growth parameters, viz., shoot, root, and total plant dry biomass, were enhanced by *F. caledonius, G. bagyarajii,* and *Gi. margarita*.

Improved plant height and stem diameter due to AM fungal inoculation have been reported earlier in other fodder crops like maize, sorghum, and alfalfa (Gaur *et al.,* 2002), and cowpea (Haro *et al.,* 2020). Similar results were reported by Sun *et al.* (2017), where inoculation with AM fungi enhanced plant height, number of leaves, and total forage yield of forage sorghum. This was further confirmed by reports by Flores-Juárez *et al.* (2020) on forage oat and by Heydarzadeh *et al.* (2022) on smooth vetch. Shockley *et al.* (2004) reported significantly increased plant height, leaf and stem dry weight of the forage legume panicled tick clover (*Desmodium paniculatum*) grown under an agroforestry system.

Enhanced shoot, root, and total plant dry biomass observed in the present study uphold the observations made by earlier workers in other forage legumes. Zarea *et al.* (2011) reported increased forage yield of berseem clover and Persian clover because of inoculation with AM fungi. Significantly enhanced plant dry biomass and P uptake of fodder legumes *Medicago truncatula* and *M. sativa* due to AM fungal inoculation was reported by Puschel *et al.* (2017), confirming the observation made in the present study. Several workers around the globe have proven that AM fungi improve plant growth by supplying diffusion-limited nutrients like P, Zn, and Cu, increasing the water uptake, protecting the plants from pathogens, and producing plant-growth-promoting hormones (Begum *et al.,* 2019; Noceto *et al.,* 2021). Being fodder legumes, giving weightage to shoot dry biomass, *G. caledonius* appears to be the best AM fungus for inoculating both the legumes. However, host preference by AM fungi has been reported by earlier workers (Bagyaraj and Kehri, 2012; Sale *et al.,* 2021). In this case, the same fungus resulted in a significant growth response in both the legumes studied.

Inoculated plants showed a higher percent of mycorrhizal root colonization, agreeing with the reports of earlier workers in other forage legumes (Shockley *et al.,* 2004; Puschel *et al.,* 2017; Zarea *et al.,* 2011). Higher mycorrhizal root colonization allows more fungal host contact, more uptake of nutrients, and hence better plant growth. Further, in the present study, there was a positive correlation between percent mycorrhizal root colonization and plant growth parameters supporting the observations made by earlier workers (Thilagar and Bagyaraj, 2015; Bhale, 2018).

CONCLUSION

In the present study, the plant growth parameters of the two legumes varied slightly to inoculation with different AM fungi. Giving weightage to shoot biomass (being fodder legumes), it is concluded that *F. caledonius* is the best AM fungus for inoculating both the fodder legumes, cowpea and field bean.

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