RESEARCH PAPER | Microbiology | Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

Selection of Suitable Efficient Am Fungus for Soybean [Glycine Max (L.) Merr.]

KEYWORDS: Glycine max (L.) Merr. Arbuscular mycorrhizal (AM) fungi, Acaulospora laevis, Biomass production, Per cent root colonization, spore number.

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ABSTRACT *Experiments on selection of suitable Arbuscular mycorrhizal fungi (AM) under green house conditions were carried out by using four Arbuscular mycorrhizal (AM) fungi viz; Acaulospora laevis, Sclerocystis dussii, Rhizophagus fasciculatus and Glomus macrocarpum. AM fungi inoculated plants in sterile soil showed, significantly increased plant growth, root length, fresh and dry weight of shoot and per cent root colonization and spore number and P content of shoots over the non inoculated (content) plants. Among four AM fungi Acaulospora leavis considered to be the most efficient indigenous fungus for Glycine max (L.) Merr. Therefore, the Acaulospora leavis, the best microbial consortium for inoculating soybean seeds before sowing compared to Sclerocystis dussii, Rhizophagus fasciculatus and Glomus macrocarpum. The screening of suitable AM fungus for Soybean plants has been discussed.*

INTRODUCTION

Since the early mycorrhiza screening trails of Mosse (1973), become more and more obvious that had wide host range, and host preference and their efficiency of improving the nutrition is varying with hosts (Wilson, 1984; Lakshman, 1996). Therefore, the microbial populations in the rhizosphere, some groups of microorganisms form symbiotic association and others form symbiotic associations with plant roots. Among these Arbuscular Mycorrhizal fungal (AMF) symbiosis, certainly one of the most prevalent symbiotic associations found in nature in a wide range of ecosystem are Arbuscular mycorrhizal fungi. These are known for a broad range of functions, but are characterized by two major aspects firstly, AMF colonize roots, improving plant nutrition by transferring poorly available nutrients, mainly phosphate (P) from the soil to the plant, whereas the plants provide essential carbohydrates to the fungi in order to complete their life cycle (Smith and Read, 1997). Other macronutrients such as nitrogen (N) can also be more easily acquired through the AM Fungi (Johansen *et al*., 1994; Tobar *et al*., 1994; Lakshman and Inamdar, 2005). Generally, this results in positive growth responses of the AM fungus inoculated plants, especially in nutrient poor soils (Smith and Read, 1997). Secondly, plants colonized by AMF may directly or indirectly acquire protection against pathogens (Kothari *et al*., 1991). However, the mechanisms involved in bio-protection have not been clearly identified (Azcon-Aguilar and Berea, 1996; Cordier *et al*., 1998). Other functions also well known to this symbiotic association and possibly related to an improved nutrition are drought resistant (Cui and Nobel, 1992; Subramanian and Charest, 1997; Hodge, 2000). The biological potential of AMF to promote plant growth and nutrition in many disciplines of plant biology (Gianiazzi and Vosatka, 2004) could also be extended to the cultivation of Legumes also.

Glycine max (L.) Merr. is one of the important food plant, ranks high among the leguminous crops in its nutritional value owing to a high protein content. Its cultivation extends to tropical and temperate regions. Studies on selection of suitable AM fungi are very meager on this plant. The purpose of this study was to select better AM strain for its improvement, growth, biomass and yield to understand Nitrogen uptake in mycorrhiza inoculated plants.

MATERIALS AND METHODS

Collection and surface sterilization of seeds.

Seeds of soyabean *Glycine max* (L.) Merr. were procured from Soyabean cultivation centre, University of Agricultural Science, Dharwad-580005, India. Seeds were washed under running water, surface sterilization of seeds was done by keeping them in 0.5% gibberellin to ensure the early breakdown of seed dormancy. Then these seeds were sown in the earthen pots measuring about 15× 20 cm (length × breadth) containing 4 kg growth media (sand: soil FYM = 1:2:1 ratio v/v) were used for each pot. AM Fungal inoculum (15g) was placed just 2 cm below the surface of the growth media. The control treatment was not provided with any AM Fungal inoculum. All the experimental pots were watered on alternate day. Watering was stopped after 90 days. All the inoculated and non inoculated plants were harvested for further analysis.

Source of AM Fungal inoculum:

Four different AM fungal species were selected for the experiment, namely *Acaulopora leavis, Sclerocystis dussii*, *Rhizophagus fasciculatus,* and *Glomus macrocarpum.* The soil based inoculum containing chlamydospores, infected roots, rhizospheric soil of *Sorghum vulgare* L. (i.e., host plant used for the mass multiplication of all the AM fungal species) having mycelia was served as AM fungal inoculum. Host plants were maintained in separate earthen pots measuring 30 × 35 cm (length × breadth) and care has been taken (to avoid contamination) in the poly house of the Botany Department, Karnatak University, Dharwad, as a source of inoculum.

Harvest and analysis of growth parameters:

All the experimental plants were harvested to analyze the effect of different AM Fungal inoculum on growth. First harvest was done at 30 days after sowing, second harvest was done after 60 days of sowing and third harvest

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was done after 90 days after sowing. The harvested plants were subjected for analysis of growth parameter such as shoot length, root length, number of leaves, fresh weight of both root and shoot. Dry weight of root and shoot was determined after drying at 70° c for 48 hrs under hot air oven. Nitrogen content of shoot was determined by Micro-kjeldahl method (Jackson, 1973). Shoot P concentration was determined by vanadomolybdate phosphoric yellow colour method of (Jackson, 1973).

Recovery and estimation of Mycorrhizal spores:
AM fungal spores were recovered from the rhizosphere soil of Soybean. inoculated with different AM fungi, by adopting wet-sieving and decanting method described by (Gerdemann and Nicolson, 1963). Mycorrhizal spore number/50 g of rhizospheric soil was estimated by using the procedure described by (Gionvannetti and Mossae, 1980). The readings were recorded for all AM fungal inoculated Soybean (*Glycine max* (L.) Merr.) plants.

Root Colonization
The per cent root colonization was evaluated microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described by Phillips and Hayman (1970). The following formula was used to calculate the root colonization according to Giovannetti and Mosse (1980).

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

Treatments:
Five treatments were maintained at experimental garden with triplicates per treatment. The treatments were as follows:

1. Control or non-Mycorrhizal
2. AM Fungus *Acaulospora laevis* Gerdemann & Trappe
3. AM Fungus *Sclerocystis dussii* (Pat.) von Hohn.
4. AM fungus *Rhizophagus fasciculatus* (Thaxt.) C.Walker & A. Schüßler
5. AM Fungus *Glomus macrocarpum* Tul. & Tul.

RESULTS
Plants showed the positive growth response to AM fungal inoculation over the control treatment, but the rate of increased growth was varied with each AM fungal inoculums (Table. 1). Experimental results showed that the *Glycine max* (L.) Merr. inoculated with *Acaulospora laevis* had significantly increased plant height i.e. root and shoot length when compared to the experimental plant treated with other three AM fungal inoculation *Sclerocystis dussii*, *Rhizophagus fasciculatus,* and *Glomus macrocarpum* (Table.1). Minimum value was recorded for the *Glycine max* (L.) Merr. inoculated with AM fungus *Sclerocystis dussii* when compared to experimental plant inoculated with *Glomus macrocarpum* and *Rhizophagus fasciculatus*. The second best AM fungus for *Glycine max* (L.) Merr. was *Rhizophagus fasciculatus*, as because the plants inoculated with this fungus showed significant growth in length of shoot and root when compared to the plant inoculated with AM fungi *Sclerocystis dussii* and *Glomus macrocarpum*. The maximum value for fresh weight of root and shoot was recorded for the *Glycine max* (L.) Merr. inoculated with AM fungus *Acaulospora laevis* when compared to the other AM fungal treated plant. But this is significantly higher over the non-mycorrhizal plants. Maximum dry weight was recorded with *Glycine max* (L.) Merr. inoculated with AM fungus *Acaulospora laevis* over the remaining three was also increased in soybean plants with inoculation of AM fungal treatments. Percent root colonization, spore number, percentage of P in shoot and percentage of N in shoot were also noted down with increase in soybean inoculation *Acaulopora leavis, Rhizophagus fasciculatus, Glomus macrocarpum and Sclerocystis dussii* respectively.

All the AM fungal inoculated plants showed positive mycorrhizal growth responsiveness but the extent of positive responsiveness was varied with each AM fungal inocula (Table 1). Maximum value for MGR was recorded with *Glycine max* (L.) Merr. inoculated with AM fungus *Acaulospora laevis* when compared to other three AM fungal inoculated plant. Minimum value for mycorrhizal growth responsiveness (MGR) was recorded with *Glycine max* (L.) Merr. inoculated with AM fungus *Sclerocystis dussii* (Fig. 1). Plants inoculated with four AM fungi were subjected to determine mycorrhizal growth dependency. *Glycine max* (L.) Merr. showed positive mycorrhizal growth dependency. Maximum MGR (mycorrhizal growth responsiveness) was recorded in *Glycine max* (L.) Merr. inoculated with AM fungus *Acaulospora laevis* (Fig. 1). Plants inoculated with four AM fungi were also subjected to determine the percentage of P in shoot, percentage of N in shoot and spore number. *Glycine max* (L.) Merr. showed increased spore number (Fig. 2), with uptake of N and P inoculated with AM fungus *Acaulospora laevis* (Fig. 3&4) compared with other three AM fungal inoculation (*Sclerocystis dussii*, *Rhizophagus fasciculatus,* and *Glomus macrocarpum*).

Table 1. Effect of different AM fungi on growth parameters of *Glycine max* (L.) Merr. inoculated with four different AM fungi at 90 days.

Parameter /treatment	Control	Acaulospora laevis	Sclerocystis dussii	Rhizophagus fasciculatus	Glomus macrocarpum
SH	80.33±0.88e	115.66±0.66a	95.00±10.26c	102.33±8.56b	106.33 ± 3.52d
SFW	3.37 ± 0.13e	12.17 ± 0.81a	7.24 ± 0.71c	9.47 ± 0.81b	8.26 ± 0.42d
SDW	0.824±0.03e	4.22±0.39a	1.26±0.23c	2.08±0.16b	2.87±0.12d
RFW	0.27±0.01e	1.54±0.21a	0.75±0.07c	1.11±0.06b	0.91±0.04d
RDW	0.10±0.00e	0.438±0.01a	0.26±0.01c	0.31±0.01b	0.32±0.01d
NL	13.00±0.57e	42.66±2.18a	29.00±1.73c	32.00±1.15b	30.00±0.57d
PMC	0.00±0.00e	86.40±0.28a	53.79±2.78c	65.49±3.47b	57.55±0.35d
MSN	0.00±0.00e	192.00±1.52a	60.33±0.88c	125.00±1.00b	105.34±0.33d
PPS	0.05±0.02e	0.39±0.04a	0.28±0.02c	0.34±0.05b	0.31±0.04d
PNS	1.203±1.00e	2.19±0.04a	2.14±2.07c	2.17±2.10b	2.15±1.02d

Means sharing a letter in columns are not significantly different according to Duncan's test P <0.05.

SH = Shoot height, SFW = Shoot fresh weight, SDW = Shoot dry weight, RFW = Root fresh weight, RDW = Root dry weight, NL= Number of leaves, PMC = Per cent mycorrhizal colonization, MSN = Mycorrhizal Spore number, PPS = Percentage of P in Shoot and PNS = Percentage of N in Shoot.

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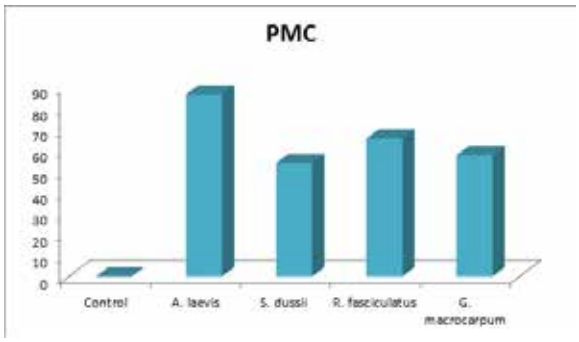
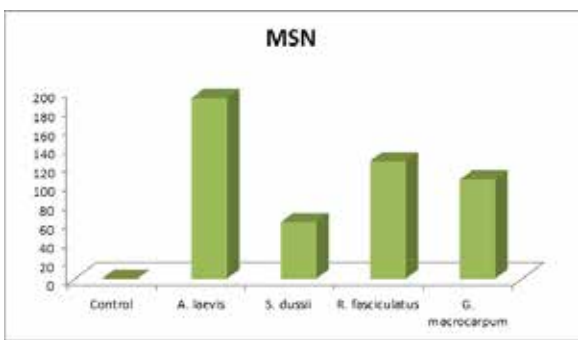
Figure 1. Showing effect of different AM fungi on per cent mycorrhizal colonization (PMC)



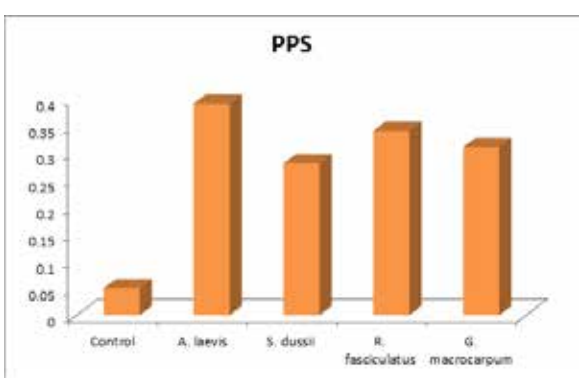
in *Glycine max* (L.) Merr. (Soybean).

Figure 2. Showing effect of different AM fungi on per cent Mycorrhizal spore number (MSN)



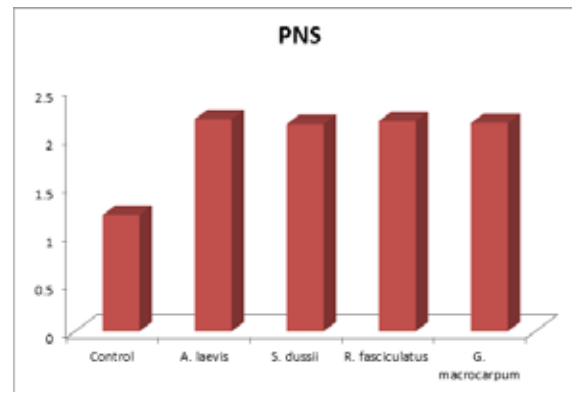
in *Glycine max* (L.) Merr. (Soybean).

Figure 3: Showing effect of different AM fungi on Percentage of P in Shoot (PPS) in



Glycine max (L.) Merr. (Soybean).

Figure 4: Showing effect of different AM fungi on Percentage of N in Shoot (PNS) in



Glycine max (L.) Merr. (Soybean).

DISCUSSION

The experimental plant *Glycine max* (L.) Merr. showed positive responses to AM fungal inoculation irrespective of AM fungal species. But the extent significant improvement was varied with each AM fungus. This increased growth of mycorrhizal plants is due to dramatically increased absorption of mineral nutrition, particularly immobile nutrients by host plant from the soil (Herrera *et al.*, 1993). There are indirect evidences that shows mycorrhizal roots are more efficient in nutrient acquisition than non-mycorrhizal roots (Roopa and Lakshman, 2008). This evidence originates from the fact that mycorrhizal plants are frequently not only larger but also contain higher concentration of P and N in their tissue than non-mycorrhizal plants (Smith and Read, 1997). Mycorrhizal symbiosis in terrestrial ecosystems has effect on organic and inorganic plant nutrition acquisition, plant water relation and carbon cycle in plants (Cui and Nobel, 1992). Experiments were conducted under poly house conditions with inoculation of four different AM fungi. Experimental results revealed that, there was significantly increased biomass production in *Glycine max* (L.) Merr. inoculated with *Acaulospora laevis.* Performance of AM fungus inoculation is in agreement with the contribution of Roy *et al.*, (2002) and Sohn *et al.*, (2003).

Host preferences among arbuscular mycorrhizal fungi have been reported by earlier workers (Vasanthkrishnan *et al.*, 1995; Manjunath *et al.*, 2001). Hence, there is a need for selecting efficient AM fungi that can be used for inoculating different mycotropic plants. *Glycine max* (L.) Merr. showed maximum mycorrhizal colonization with Acaulospora laevis followed by *Rhizophagus fasciculatus*. Mycorrhizal dependency is the results of morphological and physiological plant traits modulated by the effectiveness of the mycorrhizal fungus involved. Present experimental results showed that, all the mycorrhizae inoculated plants have higher mycorrhizal dependency. These results are in consistence with the results of (Channabasava and Lakshman, 2010). The present findings supported the view, that such dependence was affected also by associated microorganisms which many enhance the mycorrhizal effect under limiting conditions. The greater growth rates achieved in mycorrhizal plants is important, not only for contributions to the re-establishment of vegetation, but also for protection against soil erosion (Pushpa and Lakshman, 2009). The selected four AM fungi for the inoculation influenced early establishment mycorrhizal colonization, AM fungal spore population in the rhizosphere of the experimental plant. In-

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creased per cent mycorrhizal colonization was responsible for the improved plant growth parameters such as plant height, number of leaves, etc. Similar observations were made by (Bagyaraj *et al.,* 1982; Shwetha and Lakshman, 2012). In all the growth phases, non-mycorrhizal *Glycine max* L., showed lesser value for all the growth parameters over the mycorrhizal plant and similar observations were noted by (Vinayak and Bagyaraj, 1990; Gianazzi and Vosatka, 2004; Geeta and Lakshman, 2012).

The present work clearly indicated that the pre-inoculation with AM fungi had significant role in promoting seedling growth and establishment of plants under experimental conditions. In conclusion the AM fungus *Acaulospora laevis* was the most potential and efficient AM fungus for *Glycine max* (L.) Merr. Based on its influences and the efficiency of AM fungal species seems to be their external or extra radical hyphae or mycelium, and thus selection of most effective AM fungal pre inoculation needed for *Glycine max* (L.) Merr. However, the present work does not dealt with the number of nodules, nodules dry weight and N content of each nodule.

ACKNOWLEDGMENT
First author is thankful to UGC New Delhi for the selection of teacher fellow under FDP XI plan scheme with financial support and also thankful to Honorable Guide and Chairman P.G. Department of Botany, Karnatak University, Dharwad, JSS College management and Principal for supporting to continue Ph.D programme.

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