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Research Article

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Screening of efficient AM fungus for *Brassica juncea* (L.) Czern & Coss to improve biomass yield and seeds number

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ABSTRACT

Greenhouse pot experiments were carried out to Know the effect of four Arbuscular mycorrhizal (AM) fungi. Gigaspora margarita, Scutellospora nigra, Glomus mosseae and Glomus macrocarpum. The results revealed variedly with different AM fungi, experimental pots were maintained in sterile soil. The mycorrhizal inoculation greatly influence on plant growth, root length, fresh and dry weight of shoot and root, the dependency of Brassica plants to AM fungi and per cent root colonization and spore number was increased, after the inoculation of Glomus macrocarpum over the three other AM fungal strains. Thus Glomus macrocarpum the best microbial consortium for inoculating Brassica juncea seeds before sowing to get better seedling vigour and seed number.

Key words: *Brassica juncea (L.) Czern & Coss. Arbuscular mycorrhizal (AM) fungi, Glomus macrocarpum, biomass production, Per cent root colonization.*

INTRODUCTION

The importance of AM fungi for plant sustainable growth now has been fully appreciated. They form mutualistic associations with herbs shrubs and trees. The AM fungal symbionts becomes a major interface and connection between the plant and soil, plays an important role in the uptake of nutrients and water. In fact, it is generally accepted that as few as 10% of vascular plant species belong to families that do not form such a symbiosis[10,19,26]. Among the disputed angiosperms, Brassicaceae is one, where the Brassica juncea have been selected in the present investigation. Generally available P concentration is low then the positive growth responses of the AM fungui was documented, especially in nutrient poor soils[18,26].

The biological potential of AMF to promote plant growth and nutrition in many disciplines of plant biology[11] could also be extended to the cultivation of disputed plant members. *Brassica juncea*. is one of the important oil yielding plant, ranks third among the oil yielding crops. Its cultivation extends to tropical and temperate regions. Studies on screening of efficient AM fungi are very meager on this plant. The purpose of this study was to select better AM Strain for its improvement growth biomass yield and to understand Phosphorus uptake in shoots of mycorrhiza inoculated and non inoculated plants.

MATERIALS AND METHODS

Collection and surface sterilization of seeds.

Seeds of *Brassica juncea* were procured from oil yielding collection centre at University of Agricultural Science Dharwad -580005 India. Seeds surface was sterilized by keeping them 1% sodium hypochlorite for 5 minutes. Then these seeds were sown in the earthen pots measuring 15×20 cm (length × breadth) diameter containing 4kg growth media (sand: soil FYM = 1:2:1 ratio v/v) per each pot.

AM Fungal inoculum (15 g) was placed just 4 cm below the surface of the growth media. The control treatment was not provided with any AM Fungal inoculum. Single seedling of *Brassica juncea* was maintained in each pot and placed in green house conditions. Pots are arranged in randomized block design with 4 replicates per each treatment. Plants were watered on alternate day. 10 ml of Hoagland's nutrient solution minus P was added to each experimental pot in a interval of 15 days.

Source of AM Fungal inoculum:

Four different AM fungal species were selected for the experiment, namely *Gigaspora margarita*, *Scutellospora nigra*, *Glomus mosseae* 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 polyhouse in the Department of Botany, Karnatak University, Dharwad – 580003. as a source of inoculum.

Harvest and analysis of growth parameters:

Plants were harvested once in 30 days interval to understand the effect of different AM Fungal inoculation on growth. First harvest was done at 30 days after sowing and second harvest was done after 60 days of sowing and third harvest after 90 days. The growth parameters such as shoot length, root length, number of leaves, number of root seeds, and fresh weight of both root and shoot. Dry weight of root and shoot was determined after drying at 70^0C for 48 hrs under hot air oven.

Recovery and estimation of Mycorrhizal spores:

AM fungal spores were recovered from the rhizosphere soil of *B. juncea*. inoculated with different AM fungi, by adopting wet sieving and decanting method described by Gerdemann and Nicolson[9]. Mycorrhizal spore number/50g of rhizospheric soil were estimated by using the procedure described by Gionvannetti and Mossae[12] were recorded for all AM fungal inoculated *Brassica juncea.*

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[21]. The following formula was used to calculate the root colonization according to Giovannetti and Mosse[12].

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

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 *Gigaspora margarita* Becker & Hall.
3. AM Fungus *Scutellospora nigra* (Redhead.) Walker & Sanders.
4. AM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe.
5. AM Fungus *Glomus macrocarpum* Tul. & Tul.

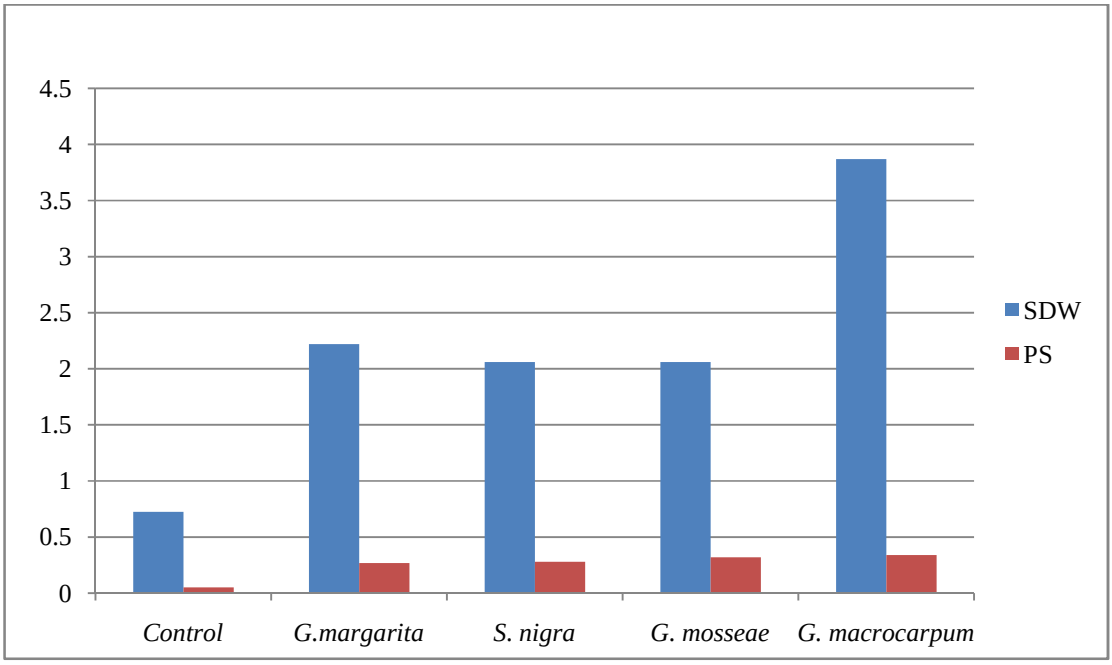
Table 1. Effect of different AM fungi on growth parameters of *Brassica juncea* inoculated with four different AM fungi at 60 days

Parameter	Control (NM)	*Gigaspora margarita*	*Scutellospora nigra*	*Glomus mosseae*	*Glomus macrocarpum*
SH	121.33±0.88e	161.66±0.66a	169.00±10.26c	178.33±8.56b	184.33 ± 3.52d
SFW	2.37 ± 0.13e	8.17 ± 0.81a	9.24 ± 0.71c	9.47 ± 0.81b	11.26 ± 0.42
SDW	0.724±0.03e	2.22±0.39a	2.06±0.23c	2.06±0.16b	3.87±0.12d
RFW	0.28±0.01e	1.50±0.21a	0.75±0.07c	1.11±0.06b	1.61±0.04d
RDW	0.11±0.00e	0.431±0.01a	0.26±0.01c	0.33±0.01b	0.52±0.01d
NL	14.00±0.57e	42.66±2.18a	29.00±1.73c	32.00±1.15b	30.00±0.57d
PMC	0.00±0.00e	53.40±0.28a	51.79±2.78c	52.49±3.47b	68.55±0.35d
MSN	0.00±0.00e	102.00±1.52a	151.33±0.88c	105.00±1.00b	161.1±0.33d
NSC	108.4±6.2e	1493.4±3.12a	1646.3±5.40c	1178.5±5.20b	1931.7±5.20d
PS	0.05±0.00e	0.27±0.00a	0.28±0.00c	0.32±0.00b	0.34±2.0d

SH = shoot height, SFW = shoot fresh weight, RFW = Root fresh weight, NL= number of leaves, NM non mycorrhizal, SDW = shoot dry weight, RDW = Root dry weight, PMC = per cent mycorrhizal colonization, MSN = Mycorrhizal Spore number. NSC=number of seed count per plant; PS=phosphorous content in shoot.

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

Fig. 1: Showing symbiotic response of AM fungal strains of *Gigaspora margarita* Becker & Hall. *Scutellospora nigra* (Redhead.) Walker & Sanders. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. *Glomus macrocarpum* Tul. & Tul. On shoot dry weight and phosphorus content in shoot of *Brassica juncea* (L.) Czern & Coss.



RESULTS

Brassica juncea plants showed positive growth response to AM fungal inoculation over the (control) plants, but the rate of increased growth was varied with each AM fungal inocula (Table -1). Experimental results showed that the *Brassica juncea* (L) Merr. inoculated with *Glomus macrocarpum* showed the significantly increased plant height i.e. root and shoot length when compared to the experimental plant treated with other three AM fungal inoculum (fig.1). The second best AM fungus for *Brassica juncea* L. was the *Glomus mosseae*, as because plants showed significant growth i.e. shoot and root length when compared to the plant inoculated with AM fungi *Gigaspora margarita*, *Scutellospora nigra*. The maximum value for fresh weight of root and shoot was recorded for the *Brassica juncea* with the inoculation of AM fungus *Glomus macrocarpum* when compared to the other AM fungal treated plants. Minimum value was recorded with *Scutellospora nigra* (fig.1). But this is significantly higher over the non-mycorrhizal plants. Maximum dry weight was recorded with *Brassica juncea*. inoculated with AM fungus *Glomus macrocarpum* over the remaining three was also increased in *Brassica juncea* plants with inoculation of AM fungal treatments. Percent root colonization and spore number was also noted down with increase in *Brassica juncea* inoculation *Glomus macrocarpum*, *Glomus mosseae*, *Gigaspora margarita* 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 *Brassica juncea* inoculated with AM fungus *Glomus macrocarpum* when compared to other three AM fungal inoculated plant. Minimum value for mycorrhizal growth responsiveness (MGR) was recorded with *Brassica juncea* inoculated with AM fungus *Scutellospora nigra*. Plants inoculated with four AM fungi were subjected to determine mycorrhizal growth dependency. *Brassica juncea* showed positive mycorrhizal growth dependency. Maximum MGD was recorded in *Brassica juncea* inoculated with AM fungus *Glomus macrocarpum*.

DISCUSSION

Our studies on *Brassica juncea* 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[13]. There are indirect evidences that shows mycorrhizal roots are more efficient in nutrient acquisition than non-mycorrhizal roots. This evidence originates from the fact that mycorrhizal plants are frequently not only larger but also contain higher concentration of "P" in their tissue than non-mycorrhizal plants[2,26]. Mycorrhizal symbiosis in terrestrial ecosystems has effect on organic and inorganic plant nutrition acquisition, plant water relation and carbon cycle in plants[7]. 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 *Brassica juncea* inoculated with *Glomus macrocarpum*. Performance of AM fungus inoculation is in agreement with the contribution of Roy *et al.*,[24]; Sohn *et al.*,[27]; Channabasava, *et al.*,[3].

Host preferences among arbuscular mycorrhizal fungi have been reported by earlier workers[20]. Hence, there is a need for selecting efficient AM fungi that can be used for inoculating different mycotropic plants. 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[4]. 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 selected four AM fungi for the inoculation influenced early establishment mycorrhizal colonization, AM fungal spore population in the rhizosphere of the experimental plant. Increased per cent mycorrhizal colonization was responsible for the improved plant growth parameters such as plant height, number of leaves. Similar observations were made by Bagyaraj *et al.*. In all the growth phases, non-mycorrhizal *Brassica junceaL.*, showed lesser value for all the growth parameters over the mycorrhizal plant and similar observations of Vinayak and Bagyaraj[31]; Gianazzi and Vosatka[11].

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. There was no relationship between biomass production and per cent of mycorrhizal colonization. *Brassica juncea* showed maximum mycorrhizal colonization with *Glomus macrocarpum* followed by *Glomus mosseae*. In conclusion, the AM fungus *Glomus macrocarpum* was the most potential and efficient AM fungus for *Brassica juncea*. 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 *Brassica juncea* to increase biomass yield and seed production.

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