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Full Length Research Paper

Mycorrhization effect on biomass of four rare millets of North Karnataka (INDIA)

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The present study was undertaken to elucidate the effect of different Arbuscular mycorrhizal fungi on growth, biomass and phosphorus uptake by rare millets. Glomus fasciculatum, Glomus macrocarpum Glomus bagyarajii and Sclerocystis dussii were used as AM fungal inocula. The millets inoculated with AM fungus Glomus fasciculatum showed increased value for growth, biomass and phosphorus uptake over the remaining treatments, but all the AM fungi inoculated rare millets had shown significantly greater values for the biomass growth over the non-inoculated ones. Mycorrhizal inoculation helped in enhancing the biomass of plant, per cent mycorrhizal colonization and spore number due to increased uptake of mineral nutrients. Throughout the present study, beneficial effect of arbuscular mycorrhizal fungi on four rare millets had been assessed.

Key Words: Rare millets, *Glomus fasciculatum,* Growth, Phosphorous, Beneficial effect.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are found in association with most of the crop plants and play a key role in the nutrition, water relations and disease resistance (*Kling and Jakobsen, 1998*), and thus increase the drought resistance (*Allen and Allen, 1987*), and increase mineral uptake, which reduces the use of chemical fertilizers. Therefore, AM fungal association is a significant component of tropical and temperate agricultural systems. During the past decade, interest in improving crop yields through low input, sustainable agriculture has been increased as a consequence of the environmental and economic constraints. Millets are some of the oldest of cultivated crops whose seeds are harvested for food or feed. There are many types of millets in the world. The millet grains are highly nutritious and even superior over rice and wheat. Millet food have low glycemic index thus helpful for diabetics. Millet grains are rich in vitamins viz., thiamine, riboflavin, folic acid and niacin. Incidence of cardiovascular diseases, duodenal ulcer and hyperglycemia are nil for the phytochemicals and pharmaco-nutrients present in the small millets are useful in health promoting activities such as protection against cataract, atherosclerosis, diabetics and cancer. They also know to possess antioxidant and antimicrobial properties. The chemo preventive substances present in small millets have positive impact on both physical and mental well being people. These millets have remained as the food for the people of lower socio-economic strata and traditional consumers. The production of these millets is subject to wide fluctuations and the area is declined from the last five decades. Krishna *et al.*, (1981) have reported anatomical changes in mycorrhizae inoculated finger millet over the non-mycorrhizal ones and increased growth and nutrient uptake in four finger millet varieties after AM fungal inoculation had been reported by Geeta and Lakshman (2005). Recently Channabasava and Lakshman (2011) have reported enhanced growth and nutrient uptake in Paspalum scrobiculatum L. (Kodo Millet) using single and multiple AM fungal inocula under polyhouse conditions. Therefore, the present investigation was undertaken to assess the effect of mycorrhizae as a biofertilizers on millets with special reference to growth responses and

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Table 1: Physicochemical properties of experimental soil.

Properties	Mean
Ph	6.89
EC	0.33
Clay	23.14%
Silt	25.79%
Coarse silt	38.50%
Fine sand	34.94%
Phosphorous	0.29%
Organic matter	0.65

phosphorous uptake, because AM fungi are known to improve plant growth and development under nutrient deficient conditions.

MATERIALS AND METHODS

Collection of seeds:

Paspalum scrobiculatum L. (Kodo millet), Panicum *miliaceum* L. (Proso millet), Panicum *miliare* Lamk. (Little millet), and *Setaria italica* (L.) Beauv. (Foxtail millet) seeds were collected from Agricultural research station, Hanumanamatti (UAS, Dharwad, India). The seeds were thoroughly washed in water and sterilized with 0.5% mercuric chloride solution before sowing.

Production of AM fungal Inoculum:

The AM fungal spores were recovered from the rhizospheric soils of millets by adapting wet sieving and decanting technique (*Gerdeman and Nicolson, 1963*). Among the recovered AM fungal spores *Glomus bagyarajii* Mehrotra. [LBGR], *Sclerocystis dussii* (Pat.) von Hohn. [SDSS] , *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker and Koske.[LFSC], and *Glomus macrocarpum* Tulasne & Tulasne [LMCC], (Codes given in parenthesis are according to VAM fungal identification manual by Schenck and Perez, 1990) were selected for inoculation based on their abundance in the rhizospheric soils. These AM fungal species were identified by using VAM fungal identification manual (Schenck and Perez, 1990). The inoculum of selected species were produced over a three month period on *Sorghum* under green house conditions using sterilized growth media (sterilized soil and sand mixture-3:1 v/v). The mixture of *Sorghum* root bits and soil containing mycelia and spores (250-300 spores/15g potting mixture) was used as inoculum.

Soil analysis:

The experimental soil was analyzed for various physico-chemical properties (*Jackson, 1973*) (Table 1).

Experimental design:

The experiment was conducted under green house conditions. Earthen pots containing unsterilized growth media were used. The AM fungal inoculum was placed just 5 cm below the surface of growth media and four surface sterilized seeds were sown in each pot. The experimental pots were arranged in completely randomized block design with triplicates per treatment. The different treatments are as follows. Per pot three seedlings were maintained.

T1: Non- mycorrhizal inoculum (control).
T2: Inoculum of AM fungus *Glomus bagyarajii*, Mehrotra.
T3: Inoculum of AM fungus *Sclerocystis dussii*, (Pat) Von Hohn.
T4: Inoculum of AM fungus *Glomus fasciculatum*, (Thaxter) Gerdemann and Trappe. emend.
Walker and Koske.
T5: Inoculum of AM fungus *Glomus macrocarpum*, Tulasne and Tulasne

The pots were watered as and when required and nutrient solution without phosphorous was given at every fortnight till harvest.

Biomass assessment and nutrient analysis:

At the harvest 60 days, total plant biomass (dry weight of shoot and root) and plant height were measured. Dried material from each pot was grounded with pestle and mortar. Then 0.5 g of grounded material was ashed at 55^0 c followed by dissolution in 3.5% HCl. The concentration of phosphorous was determined (Jackson, 1973).

Shoots were separated from roots at 0.5 cm above the soil surface at harvest. Roots were separated from the soil by washing under tap water and distilled water. Then they were placed in hot air oven for drying at 70^0 C for 48 hrs. Root samples collected from other pots with same treatments and preserved in a mixture of ethanol, glacial acetic acid and formalin (FAA), for the determination of mycorrhizal colonization (*Philips and Hyman, 1970*).

The stored root samples were thoroughly washed in tap water followed by distilled water thrice and they were chopped into approximately 1cm length. These chopped root bits were placed in 10% KOH solution on water both for about 15 min. Then root bits washed under tap water twice and placed in 0.1N HCl for 2-3

Table 2 : Effect of different AM fungi on Foxtail millet (*Setaria italica*) at 60 DAS.

Treatments	SL	RL	FWS	FWR	PC	SN	P
Control	48.77±0.22e	4.07±0.01e	3.98±0.04e	0.17±0.01e	0.00±0.00e	0.00±0.00e	0.05±0.006e
LBGJ	51.12±0.06d	6.34±0.07d	6.07±0.02d	0.85±0.02d	82.00±1.52c	191.00±0.11c	0.12±0.008d
SDSS	59.27±0.06c	10.14±0.04b	7.62±0.09c	1.62±0.03c	86.66±1.45b	217.66±0.34b	0.16±0.008c
LFSC	67.61±0.13a	13.21±0.15a	10.55±0.02a	2.27±0.02a	90.33±0.88a	272.00±0.70a	0.29±0.008a
LMCC	63.08±0.02b	9.81±0.04c	8.69±0.11b	2.03±0.03ab	79.66±0.88d	186.33±0.66d	0.24±0.02ab

Table 3: Effect of different AM fungi on Proso millet (*Panicum miliaceum*) at 60 DAS.

Treatments	SL	RL	FWS	FWR	PC (%)	SN	P
Control	42.18±0.01e	3.13±0.00e	2.66±0.08d	0.25±0.00e	0.00±0.00e	0.00±0.00e	0.03±0.26e
LBGJ	53.28±0.00d	6.14±0.01d	8.34±0.01c	0.8±0.01d	76.33±0.88d	170.66±0.00d	0.14±0.003cd
SDSS	64.56±0.01c	9.40±0.01c	8.92±0.02c	1.06±0.02bc	90.33±0.66b	182.66±0.26b	0.16±0.003bc
LFSC	71.52±0.01a	12.47±0.01a	13.26±0.01a	2.46±0.00a	94.66±0.12a	195.66±0.66a	0.27±0.003a
LMCC	68.13±0.00b	10.31±0.01b	12.06±0.01b	1.65±0.00b	87.66±0.66c	180.33±0.00bc	0.18±0.003b

Table 4: Effect of different AM fungi on Little millet (*Panicum miliare*) at 60 DAS.

Treatments	**SL**	**RL**	**FWS**	**FWR**	**PC**	**SN**	**P**
Control	36.24±0.01e	1.25±0.008e	2.00±0.01e	0.92±0.003d	0.00±0.00d	0.00±.00e	0.05±0.006e
LBGJ	39.56±0.003d	2.95±0.01d	3.36±0.01d	1.25±0.01c	74.33±0.66c	165.66±0.66d	0.08±0.003d
SDSS	45.29±0.005c	3.43±0.01c	4.91±0.01c	1.99±0.008b	79.33±1.45bc	170.00±1.00c	0.14±0.02b
LFSC	56.69±0.008a	5.27±0.006a	7.32±0.01a	2.34±1.00a	91.00±1.15a	177.00±1.15a	0.17±0.006a
LMCC	51.06±0.01b	4.53±0.01b	6.83±0.006b	2.25±1.00a	77.33±0.88b	173.00±1.15b	0.11±0.003c

*Note: **SL**- shoot length, **RL**- root length, **FWS**- fresh weight of shoot, **FWR**- fresh weight of root, **PC**- per cent of colonization, **SN**- spore number, **P**- phosphorus, **LBGJ**. - *Glomus bagyarajii*, **SDSS** - *Sclerocystis dussii*, **LFSC** – *Glomus fasciculatum*, and **LMCC** - *Glomus macrocarpum* (codes for AMF species referred here are according to Schenk and Perez, 1990). Each value represents the mean of three determinations. Mean values followed by the same letter within a column do not differ significantly at P = 0.05 according to DMRT.

min. and later root bits were transferred to the watch glass containing tryphan blue in lacto phenol. Then mount the stained root bits on micro slide for the assessment of mycorrhizal colonization. The per cent of mycorrhizal colonization was determined by using the following formula:

Statistical analysis:

All the data were subjected to one-way analysis of variance (ANOVA) by using the SPSS student version-9 software (SPSS Inc., Chicago, IL, USA). Within each variable, significant difference among the means were assessed with the Duncan's multiple Range Test (DMRT) (P<0.05).

$$\text{PMC} = \frac{\text{Number of root bits colonized}}{\text{Total number of root bits colonised}} \text{ X } 100$$

RESULT AND DISCUSSION

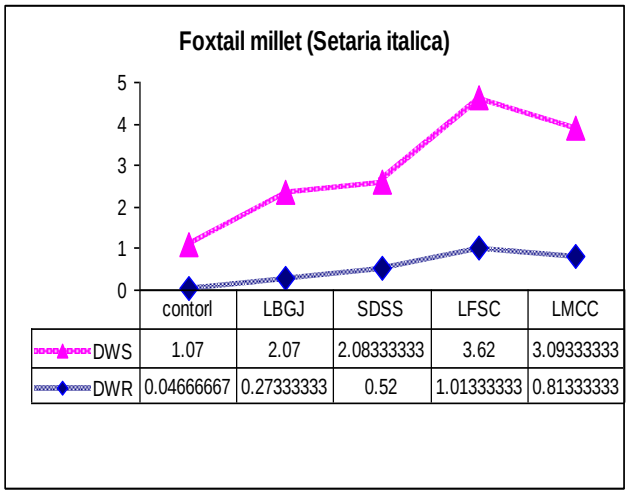
The plants inoculated with AM fungi showed significantly increased growth over the non-mycorrhizal plants. The maximum growth rate was observed in plants inoculated with *Glomus fasciculatum* and it was followed by *Glomus macrocarpum* and least was with *Glomus bagyarajii* (Table 2-5). Similarly the fresh and dry weight of both shoot and root (biomass) was more in plants treated with *Glomus fasciculatum* (Figure 1 and 2) and the least with *Glomus bagyarajii.* Similar results were reported by *Reena and Bagyaraj (1990 a&b) Lakshman and Srinivasalu, (2002)*. According to *Utkhede, (2006)* plant dry weight and per cent root colonization was more in mycorrhizal plants than in non-mycorrhizal ones.

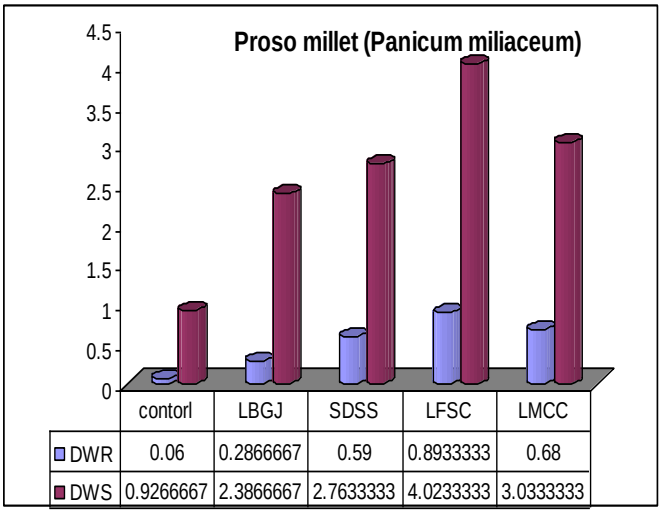
Table 5: Effect of different AM fungi on Kodo millet (*Paspalum scrobiculatum*) at 60 DAS.

Treatments	SL	RL	FWS	FWR	PC	SN	P
Control	44.33±0.01d	2.03±0.006d	1.74±0.006e	1.03±0.008e	0.00±0.00e	0.00±0.00e	0.04±0.01d
LBGJ	48.86±0.01c	3.2±0.008bc	3.46±0.003d	1.11±0.006d	74.33±0.33d	169.00±1.00d	0.18±0.006c
SDSS	48.25±0.008c	3.53±0.006bc	4.68±0.01c	1.44±0.003bc	79.33±0.88c	175.33±0.66c	0.23±0.01b
LFSC	59.66±0.01a	4.06±0.003a	7.44±0.006a	2.01±0.028a	96.00±1.52a	198.33±0.33a	0.29±0.008a
LMCC	52.14±0.003b	3.90±0.006b	6.95±0.01ab	1.65±0.008b	85.66±0.88b	182.33±0.66b	0.23±0.01b

*Note: **SL**- shoot length, **RL**- root length, **FWS**- fresh weight of shoot, **FWR**- fresh weight of root, **PC**- per cent of colonization, **SN**- spore number, **P**- phosphorus, **LBGJ**. - *Glomus bagyarajii*, **SDSS** - *Sclerocystis dussii*, **LFSC** – *Glomus fasciculatum*, and **LMCC** - *Glomus macrocarpum* (codes for AMF species referred here are according to Schenk and Perez, 1990). Each value represents the mean of three determinations. Mean values followed by the same letter within a column do not differ significantly at P = 0.05 according to DMRT.

Figure 1. Effect of different AM fungi on biomass Dry weight of root and shoot in Proso millet (*Panicum miliaceum*) and Foxtail millet (*Setaria italica*) at 60 DAS.



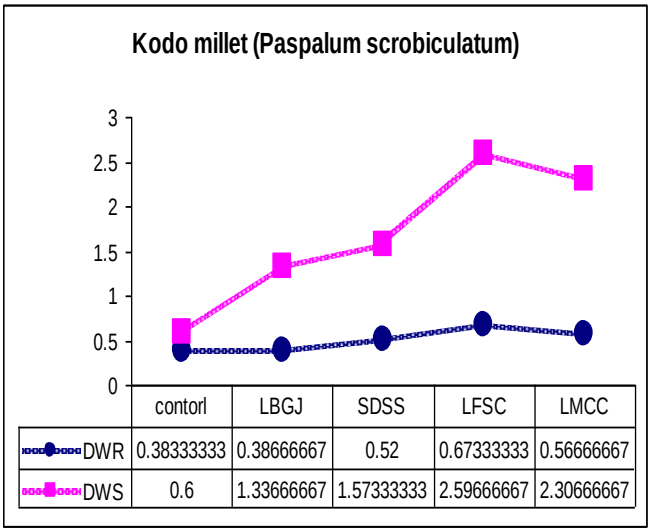


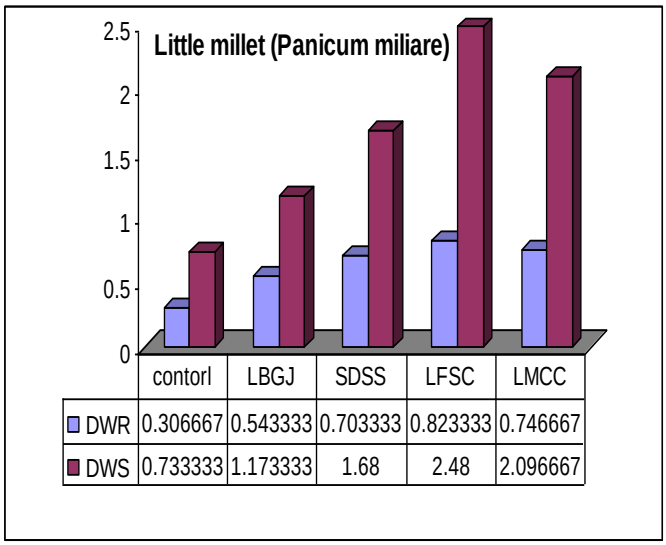
Mendeiros et al., (1994), reported that immobile minerals can increase shoot fresh and dry weight and this is due augmented water and nutrients absorption and increased photosynthesis activity of the mycorrhizal plants *(Jakobson 1998).*

The millets exhibited increased per cent mycorrhizal colonization (PMC) in all the inoculated plants over the non-mycorrhizal ones. The highest per cent mycorrhizal colonization was measured in plants inoculated with *Glomus fasciculatum* and least was with inoculation of *Glomus bagyarajii*. Similarly increased spore number in the rhizosphere of rare millets inoculated with *Glomus fasciculatum* over other AM fungal inoculated and non-mycorrhizal plants was recorded. *Hall et al., (1977)* and *Abbott and Robson (1991)*, found that, the per cent mycorrhizal colonization and number of AM fungal spores were inversely proportional to the amount of phosphorus in the rhizospheric soils. It has been found that, in the field soils, AM fungal spore number appears to reach a maximum value in the conditions where phosphorus status is less than that required for maximum growth and may then decrease with increasing phosphorus content in the soils. The association of AM fungi and variation in percent root colonization in the roots of fourteen grasses were reported by *Gupta and Mukerji, (1996)* and similar findings were also denoted by *Lakshminarasimhan and Vijaykumar (1994)*.

The increased phosphorous content of shoot in all the inoculated plants was recorded over the control plants (Table 2-5), but the extent of increase was varied among the plants with each AM fungus inoculation. The highest phosphorous content in shoot was observed in rare millets inoculated with *Glomus fasciculatum* over other inoculated rare millets and the moderate increase in *Glomus macrocarpumI* inoculated rare millets. According to *Safir et al., (1971)*, mycorrhizae can dramatically increase absorption of mineral nutrition, particularly immobile nutrients by host plants from the soil. There are

Figure 2. Effect of different AM fungi on biomass Dry weight of root and shoot in Little millet (*Panicum miliare*) and Kodo millet (*Paspalum scrobiculatum*) at 60 DAS.





*DWR- Dry Weight of Root and DWS- Dry Weight of Shoot. Each value represents the mean of three determinations.

indirect evidences that show mycorrhizal roots are more efficient in nutrient uptake than non-mycorrhizal roots. This evidence originates from the fact that mycorrhizal plants are frequently not only large but also contain higher concentration of phosphorous in their tissues than non-mycorrhizal plants (*Smith and Read, 1997*). The similar findings were encountered in the present investigation. The *Glomus fasciculatum* had more efficiency in uptake of phosphorous (*Kadam, and Lakshman, 2001*) and it also provides platform for the growth and development of plants.

Arbuscular mycorrhizal fungal species differ considerably in their efficiency to infect and influence plant growth (*Carling and Brown, 1980*). These are known to supply phosphorous through the large surface area of their hyphae, by their high affinity phosphorous uptake mechanism, by organic acid production and observed that different AMF species have different effects on growth of host plants in terms of dry mass. Host response also differs with fungal species and with geographic isolate within a species. The extent of response may also be due to changes in efficiency of different endophytes during the growing season (*Daft and Nicolson, 1966*), to varying uptake or exclusion capabilities of AM fungi for different element or a change in soil environment itself during the season (*Bazin et al., 1990*).

The best improved plant height because of AM fungal inoculation has been reported by many workers. Growth and mineral nutrition of plants are commonly enhanced by inoculation with AM fungi. (*Diop, 1996; Clark and Zeto, 2000*). The plant biomass is an important parameter for selecting a fungus for its symbiotic efficiency. Increased growth and biomass in rare millets was recorded. This increase in growth and biomass of inoculated rare millets strongly depends on their ability to access minerals from the soil. Therefore, positive effects of tested AM fungi on phosphorus content could be related to the ability of symbiotic fungi to enhance soil phosphorous depletion zones around roots (*Li, et al., 1991; Clark and Zeto, 2000; Smith et al., 2001*). Enhanced uptake of phosphorous is generally regarded as the most important benefit that AMF provide to their host plant, and plant phosphorous status is often the main controlling factor in the plant fungal relationship (*Thompson, 1987; Smith and Read, 1997; Graham, 2000*).

CONCLUSION

Inoculation with Arbuscular mycorrhizal fungi was significantly increases millets biomass and alters morphological structure of root. AM fungus *Glomus fasciculatum* may be effective and environmentally sustainable organism to develop and increase rare millets production. It indicates that the *Glomus fasciculatum* was found to be more efficient among all the AM fungal species used. The second best species was *Glomus macrocarpum*. Further research will provide a more detailed analysis of the grain nutritional status in the plants inoculated with different AM fungi.

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