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ROOT COLONIZATION STUDIES ON TOMATO, BAJRA AND MAIZE BY *GLOMUS MOSSEAE*

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Abstract

A mycorrhizal fungus infects the plant roots to form mutualistic associations, whereby the fungi give nutrients, water and protection to the plant in exchange for food. Mycorrhizal diversity is important to enhance the crop yield and soil fertility. Arbuscular mycorrhizal(AM) fungi are useful to maintains the intimate link between the plant roots and soil. The aim of present work is to grow and pure culture of *Glomus mosseae* on different host plants like Tomato, Maize and Bajara in order to multiply it on mass scale. The experimental results showed that a root of these plants showed positive colonization for *G. mosseae.* The percentage of root colonization in Tomato roots (97%) followed by Bajara (92%) and Maize (76%) were reported. Microscopic observations of root segments showed presence of mycelial fragments, vesicles and arbuscules.

Keywords: Arbuscular mycorrhiza, root colonization, *Glomus mosseae.*

Introduction:

Arbuscular mycorrhizas are the symbiotic association present between the plants and fungi (Frank, 1885).From few decades, Arbuscular mycorrhizal (AM) fungi have emerged as potential biofertilizers, a cheap, environmental friendly alternative to expensive chemical fertilizers (Srivastava *et. al*., 1996).This root- fungus association is mutualistic and is being considered as a functionally distinct which is involved in mineral nutrient uptake from the soil (Kar, 1993). The work on survey, isolation and identification of arbuscular mycorrhizal fungi is continuously going in different parts of world. Vesicular arbuscular mycorrhizal fungi have the potential to influence the ecosystem processes, it has great potential to determine the plant communities and its ability to induce a wide variety of growth responses in coexisting plant

species (Hartnett and Wilson, 1999; Heijden *et. al*., 1998a; 1998b; Klironomos *et. al*., 2000; Sanders *et al*., 1996).

The key benefit of mycorrhizal association is enhanced soil exploration with increasing mineral uptake of N, P, K, S, Mg, Ca, and Cu (Algunde *et. al*., 2018). Due to presence of arbuscular mycorrhiza, the host plant forms highly branched root system and the hyphae of arbuscular mycorrhiza grow from root to soil which enables the roots to remains in contact with increased area of soil surface. The arbuscular mycorrhizal plants have ability to enhances the tolerance capacity to drought, salinity, production of growth promoters of plants etc. In addition to this successful AM fungal colonization will induce disease resistance in plants against variety of infections (Jan *et. al*. 2007).By considering its multivalent benefits to plants, the present investigation was carried out to study the root colonization of Tomato, Bajra and Maize by *Glomus mosseae* which enables to utilize these plants for potential mass production of mycorrhiza for cost effective biofertilizer production.

Materials and Methods:

Isolation of Mycorrhizal Spores:

In laboratory isolation of mycorrhizal spore was done as per method described by Gerdemann and Nicolson (1963). Hundred gram of soil sample was weighed and thoroughly mixed with water (500 ml) by stirring with the glass rod. The mixture was allowed to stand till soil particles settled down. The suspension was then passed through series of sieves stacked in descending order of their mesh sizes viz.500 to 45µm from top to bottom. This procedure was repeated for three to four times. The contents of sieves were washed with the running tap water and washings were collected in a separate dish for each sieve. Collected contents in each dish were observed for presence of mycorrhiza spores under the stereo zoom Microscope CZM-6.

Identification of Mycorrhizal Spores:

Mycorrhizal spores isolated from soil samples were collected separately on the basis of their morphological features like colour, shape, hyphal attachments etc. Intact as well as broken spores were isolated using capillary tube and hydraulic micropipette (1.0 to 50 microliter) and mounted on microscopic slides using lacto glycerol and observed by putting cover slip over it using Labomed made binocular microscope. Each spore was photographed through Camera. Identification of AM species was done using manual given by Schenk and Perez (1989), by referring dedicated research papers in the field of mycorrhiza and also by using authentic web resources available on mycorrhiza specific web link INVAM worksheet (http://invam.caf.wvu.edu).

Pure culturing of *Glomus mosseae* for mass multiplication:

Almost 35 different mycorrhizal fungal spores were identified in present study. Among all *Glomus mosseae* was found to very common in all sample studied. Therefore, by considering its common occurrence in

diversified habitats we decided to pure culture and multiply the same on different herbaceous host as per method described by Gerdemann (1955) Gerdman and Nicolson (1963) in glass funnel. The mycorrhizal spores were collected by wet sieving and decantation method. Later spores of *Glomus mosseae*are picked by hydraulic micropipette in a small watch glass. The spores were surface sterilized by washing for three to four times with sterile distilled water using small brush under stereo zoom microscope. All the required materials were properly sterilized using autoclave. Cotton plug was fixed in the neck of big sized funnel. The funnel is filled with mixture of soil and sand in 1:1 ratio and is warped with aluminium foil and sterilized for 45 min at 121^0 C and 15 lbs pressure. The surface sterilized spores are transferred in the centre of funnel by making little whole using micropipette. In the whole later added 4-5 surface sterilized and washed seeds of Maize, Bajara, and Tomato. After that funnel were kept on conical flask containing sterile distilled water. Later on, after emergence of seedlings the tiny plants were transferred to the pot containing sterilized sand-soil mixture.

Transfer of Content of funnel culture into pots:

After seven days of seedling growth in funnel culture, the content was later transferred in to pots containing sterilized mixture of sand and soil for further establishment of mycorrhiza in root.

Root Colonization Studies:

After four weeks of successful growth in pots, the plants were uprooted and roots of Tomato, Bajara and Maize were washed thoroughly in tap water and then distilled water. Roots samples in laboratory were gently washed and immediately fixed in Formalin acetic acid alcohol (FAA) solution (5:5:90) in the field (Kormanik *et. al*.1980, Bhale *et. al*.2011.) Fixed roots were rewashed again by using tap water and then distilled water. Roots were segmented into1cm length and later placed in 10% KOH, autoclaved for 15 to 20 minutes and cleaned in distilled water. Root segments later neutralized with 2% HCL and stained with lactophenol along with Trypan blue (0.05%) (Phillips and Hymen,1970). Root segment were mounted on glass slide with lactophenol and observed under compound microscope (Labomed). About 100 segments of roots of each plant were observed thrice for the assessment of percentage of colonization of AM Fungi using following formula (Selvaraj *et. al*. 2011; Poonal Verma, *et. al*. 2019) and mean percentage of root colonization was determined. As per following formula percentage of colonization of AM Fungi is calculated

$$\% \text{ Root colonization} = \frac{\text{Number of root bits showing AM fungi}}{\text{Total number of root bits infected}} \text{ X } 100$$

Results and Discussion:

It is now well established that AMF acts as good symbionts in promoting growth productivity of host plants (Shahabivand *et. al*., 2012.) The major positive effect on plant growth by root colonization by AM fungi derives from their interaction with the host root system (Gutjahr *et. al*., 2009; Vos *et. al*., 2013). Mycorrhiza increases surface area the roots during nutrient transfer (Akhtar *et. al*., 2011).During the formation of AM fungal association root exudates plays an important role in attracting and colonizing fungi on root system. Exudates stimulate AM hyphal growth towards root (Hepper and Mosse, 1975).*G. mosseae* is one of the widely occurring AM fungi, which can enter easily in to host cortical and epidermal cells (Brundrett, 2002). The roots of plants species selected in the current study such as *Solanum lycopersicum*, *Zea mays* and *Pennisetum glaucum* showed successful colonization of *G. mosseae.* Tomato roots (*S. lycopersicum*) showed 97% of root colonization by *G. mosseae.* Microscopic observation of root segments showed presence of arbuscles, vesicle and mycelial filaments in side the roots (Table 1; Fig 1). In previous studies it was observed that tomato roots easily support growth and colonization of *G. mosseae*. It was also observed that *G. mosseae* induces systemic resistance against pathogenic fungus *Phytophthora parasitica* in tomato tissue. The cellular and molecular plant defense reactions are associated with this resistance as well as that of arbuscules formation in cortical cells of tomato roots (Cordier *et. al*., 1998).

Followed by tomato 92% root colonization was observed in Bajara (*Pennisetum glaucum*). The results are presented in Table 1.The mycelial filaments, vesicle and arbuscules were observed in the root fragments under microscopes after staining (Fig 2). *G. mosseae* is known to be colonized by roots of Bajara.Inearlier studies it was reported that mycorrhizal spores of *G. mosseae* can be pure cultured and multiplied by using funnel culture technique (Ajay and Sonali, 2017). Arbuscular mycorrhizal fungi including *G. mosseae* induces plant growth in *P. glaucum* by enhancing photosynthetic activity, plant biochemical content and alsonutrient uptake under abiotic stress conditions (Borde *et. al*. 2011).Similarly, maize (*Zea mays*) shows 76% of root colonization by *G. mosseae.* Arbuscule, vesicle and mycelial filaments were also observed in root segments under the microscope (Fig 3). Abdelmoneim *et. al*. (2013) found that *G. mosseae* can be grown on roots of maize which will provide resistance against draught stress to plant.The increase in such tolerance of *Z. mays* is due to the extra radical growth of fungal mycelia of *G. mosseae* which extends the root surface area and improve the uptake of water and nutrients by the roots (Bethlenfalvay *et al.,* 1988). Due to obligate nature of Mycorrhiza it becomes very difficult to cultivate it on artificial media in laboratory. By using substrate based technique such as root trap culture method in controlled condition it becomes possible to propagate the mycorrhizal species on mass scale. In current piece of work, we have made an attempt to isolate *G. mosseae* and tried to culture it on different hosts like Tomato, Maize and Bajara. The experimental results clearly shows that all these three host plants supports growth of *G. mosseae* and therefore roots of these plants can be used as substrate for mass multiplication.

Sr. No	Name of Plant Species	Percentage of root colonization by *Glomus mosseae*	Mean Percentage Colonization	*Glomus mosseae* colonization		
				Mycelium	Vesicle	Arbuscle
1	*Solanum lycopersicum*	95	97%	+	+	+
		99		+	+	+
		97		+	+	+
2	*Zea mays*	69	76%	+	+	+
		78		+	+	+
		81		+	+	+
3	*Pennisetum glaucum*	89	92%	+	+	+
		96		+	+	+
		91		+	+	+

Table 1. Table showing Percentage root colonization of Tomato, Maize and Bajara by *Glomus mosseae* and Presence of Mycelium, Vesicle and Arbuscles respectively.

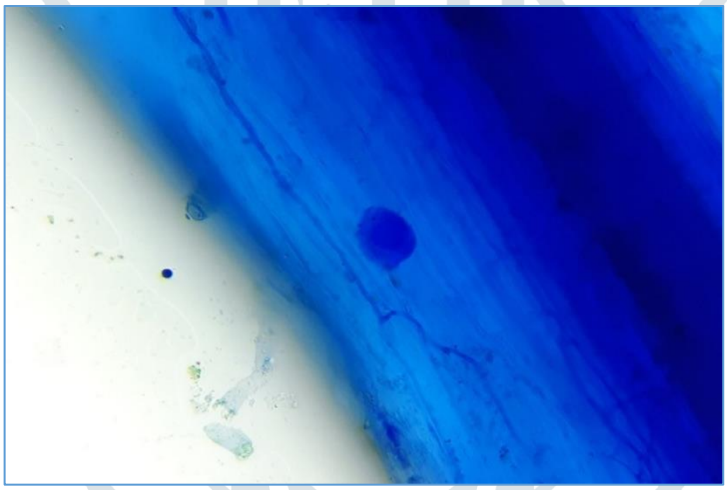

Fig 1. T. S. of Tomato roots showing presence of Vesicles and Mycelium

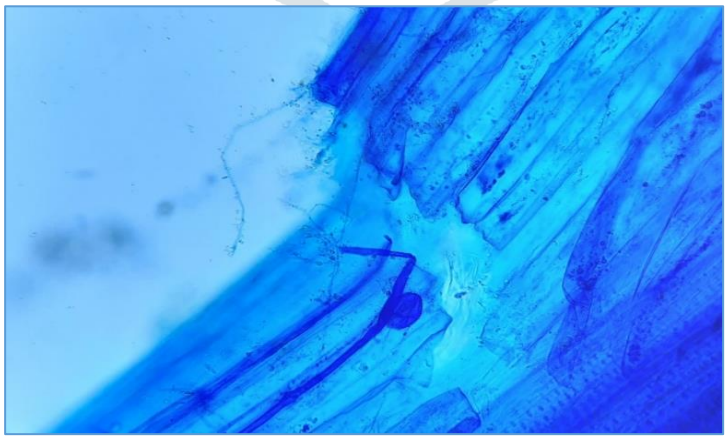

Fig 2. T. S. of Bajara roots showing presence of Vesicle and Mycelium

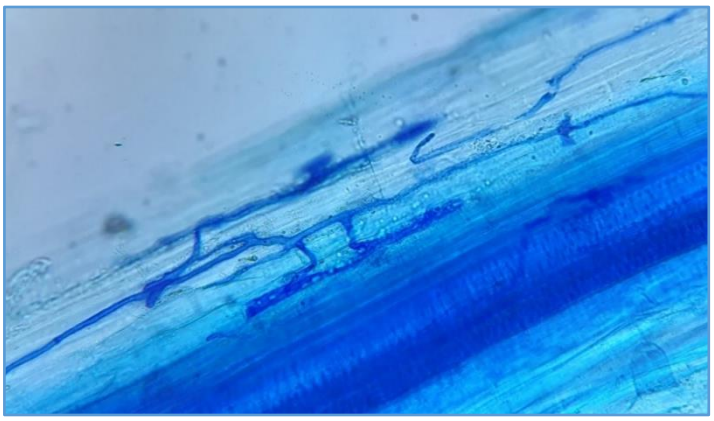

Fig 3. T. S. of Maize Root showing presence of Coenocytic Mycelium, Arbuscules

Conclusion: It is possible to use substrate dependent root trap culture technique for growth and multiplication of *Glomus mosseae*. Three selected host plants shows successful colonization of *G. mosseae* and therefor for large scale production of bio fertilizer containing *G. mosseae*the technique discussed above can be employed.

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