MASS MULTIPLICATION OF ARBUSCULAR MYCORRHIZAL FUNGI OF *VETIVERA ZIZANIOIDES* (L.) NASH ON *ELEUSINA CORACANA* (L.) GAERTN

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ABSTRACT

A Mycorrhiza (fungus root) is a symbiotic association of a fungus and the roots of a vascular plant. These soil microorganisms enhance the plant's nutrient uptake by extending the root absorbing area. In return, the symbiotic receives plant carbohydrates for the completion of its life cycle. In this association, the fungus colonizes the host plant's roots, either intracellular as in Arbuscular mycorrhizal fungi or extracellular as in ectomycorrhizal fungi. The AM fungi are not host specific, any plant species can be infected by an AM fungal species. The Present work examined mass multiplication of Arbuscular mycorrhizal fungi of Vetivera zizanioides on Eleusina coracana. spores were isolated from rhizospheric soil of Vetivera zizanioides on Eleusina coracana by using Gerdmann and Nicolson method (1963). Mass multiplied spores were identified by using the manual of (Schenck and Perez, 1990). Glomus fasciculatum with subtending hyphae, rounded shaped Glomus reticulatum, Glomus species, Glomus fragilistatum, Glomus citricolla, ruptured wall of Glomus macrocarpum), Glomus globiforum, Glomus mosseae and Acaulospora laevis, Acaulospora sp, Scutellospora pellicida, Scutellospora auriglobosa and Scutellospora calspora, Gigaspora rosea all these spores were isolated from rhizospheric soil of Eleusina coracana. Coenocytic hyphae, Vesicles, Arbuscules were observed in the root of Eleusina coracana inoculated with pure and mix culture of Arbuscular Mycorrhizal fungi. The results evidenced that the rapid and efficient AM fungal root colonization and higher AM spores production was maximum observed in Eleusina coracana plants. Large quantities of the inoculums can be produced by pot culture technique. Therefore, the Eleusina coracana plant has been suggested as a suitable host for mass production of AM fungi.

Keywords: *Arbuscular Mycorrhizal spores, Percent root colonization and Mass Multiplication*

Introduction

German Botanist Frank (1885) coined the term Mycorrhizae for the first time to designate the symbiotic relationship between the fungi and plant roots. Since then scientists started exploiting them for the welfare of mankind. The term 'Mycorrhiza' in its broadest sense is the non-pathogenic association of fungi and the roots of higher plants. The root- fungus association is symbiotic and the whole association is being considered as a ''functionally distinct organ'' involved in mineral nutrient uptake from the soil. (Kar, 1993).Mycorrhizal fungi are having intimate association with roots of higher plants forming a symbiotic relationship providing nutrients to the plants. The Arbuscular Mycorrhizal diversity in herbaceous vegetation medicinal plants, in halophytes plants have been investigated by many workers (Bagyaraj, D. J. (2014) Kannan, K. and Lakshminarashiman, C. (1988) Kumar., *et. al* (2013). Mulla,R. M *et. al.*, (1994) Mulani., R. M *et. al.*, (2004) Mulani, R. M and Waghmare, S. S.(2012). Mulani, R. M and Prabhu, R. R. (2002). Parameswaran, P and Augustine, B. (1988). Isolation and identification of Arbuscular Mycorrhizal fungi from agricultural fields of Vietnam investigated by (Sasvari *et.al.*, 2012). Growth and biomass of *Piper longum* L was increased with inoculation of Arbuscular Mycorrhizal fungi. (Seema and Rajkumar, 2015). Essential oil production, nutrient uptake and root colonization in basil was increased with inoculation arbuscular mycorrhizal fungi. (Mirhassan *et.al.*, 2010). Arbuscular mycorrhiza fungi absorbs immobilized mineral nutrition such as phosphorus, zinc and copper from the soil. (Bagyaraj,2014).

Mass multiplication of Arbuscular Mycorrhizal fungi was observed in four plant species viz. *Hordeum vulgare, Triticum aestivum, Phaseolus vulgaris* and *Phaseolus mungo by* Chaurasia and Khare (2005). The advantageous of Arbuscular fungal inoculums used in agriculture field and nurseries (Smith and Read, 1997; Muthukumar *et al.*, 2001). Mass production of AM fungal inoculums by soil based pot culture was done by (Sadhna,2015).

Materials and Methods

1) Mass multiplication of Pure culture and Mix culture of Arbuscular Mycorrhizal fungi:

Open pot culture was used for the mass production of AM fungi. 3kg sterilized soil mixture was filled in plastic pot. 10 to 15 seeds were sowing in plastic pots. A layer of 100 g of collected soil samples (mycorrhizal inoculum) was spread over pot mixture) in plastic pots. Mass multiplication of mix culture and pure culture of arbuscular mycorrhizal fungi was done by using *Eleusina coracana* as host plants. *Eleusina coracana* seed surface disinfected with 0.01% (w/v) HgCl2 for 2 minutes and washed several (3–4) times with sterilized distilled water. These pots were used for the experimentation (Chaurasia and Khare, 2005, Prabhu, 2008).

2) Isolation of spores by using wet-sieving method. (Gerdman and Nicolson; 1963).

Spore extraction is involved in three sub steps such as wet-sieving, sedimentation, flotation. Mix 5 gm of soil in 250 ml of luck warm water in a beaker until all aggregates disperse to a uniform suspension. Allow the heavier particles to settle down. Filter the suspension through 710 µm sieve to remove large organic matter and roots. Then solution was sieved through series of sieves i.e710 µm, 210 µm 150 µm, 75 µm, 45 µm and 25 µm respectively. Content of each sieves i.e 210 µm 150 µm, 75 µm, 45 µm and 25 µm was taken separately on blotting paper in petriplate and This petriplate was observed under stereo zoom binocular microscope.

3)Percentage of root colonization. (Phillips and Hayman, 1970).

Young root segments were taken in test tube adding 10% KOH and it autoclaved at 15 lbs for 1 hr. After 10 minute 10% KOH was removed from test tube then root segments were washed under tap water with 2 to 3 times . Then 10 ml 1N HCL was added and were kept for 5 minute for neutralization of root tissue. Then HCL was removed and washed the root segments 2 to 3 times with tap water. After 30 minute root segments stained with cotton blue and kept for 24 hrs. After 24 hrs root segments mounted on slide with Acetic acid –glycerol (1:1v/v). Seal the corners of the cover slip with DPX, root colonization was observed under compound microscope. Then **%** of Arbuscular Mycorrhizal fungal colonization calculated by using this formula;

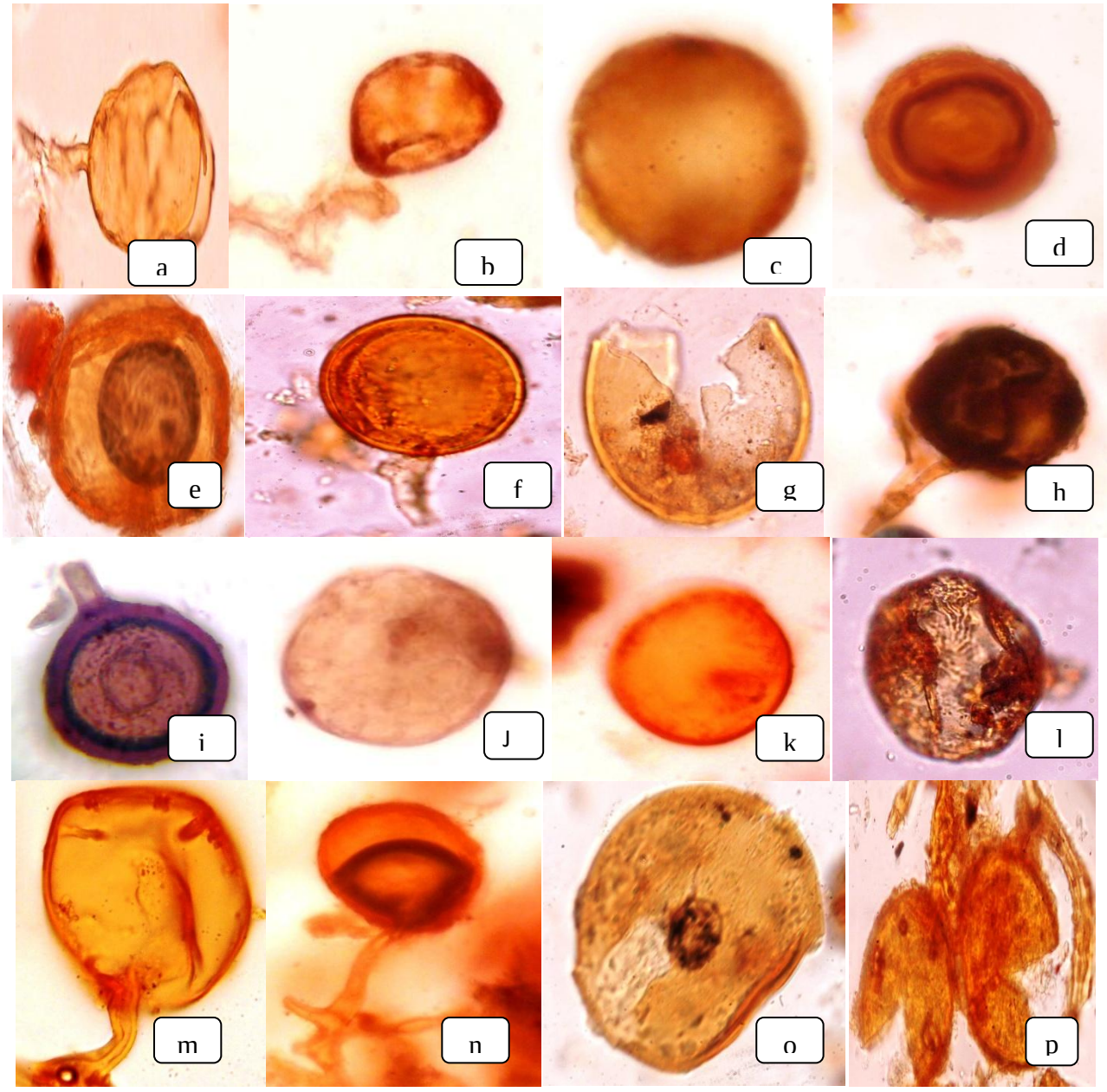
$$\textbf{Percent of mycorrhizal colonization} = \frac{\textbf{Number of root segments colonized}}{\textbf{Total number of root segments examined}} \times \mathbf{100}$$

Results and Discussion

Mass multiplication of mix culture and pure culture of Arbuscular mycorrhizal fungi of *Vetivera zizanioides* was done on *Eleusina coracana.* The Arbuscular mycorrhizal mix and pure culture was multiplied in pot condition using *Eleusina coracana* seeds as it is suitable host plant. Then the AM cultures were prepared from roots and rhizospheric soil of *Eleusina coracana* plants after harvesting Ragi and used for the experiments. The colonization in the root was observed on 16 to 22 days where as the sporulation was observed on 25 to 30 days.

After 30 days spores were isolated from rhizospheric soil of *Vetivera zizanioides* on *Eleusina coracana* by using Gerdmann and Nicolson method(1963). Mass multiplied spores were identified by using the manual of (Schenck and Perez, 1990) *Glomus fasciculatum* with *subtending hyphae(fig;a,b)* Rounded shaped *Glomus reticulatum (fig:c) Glomus sp.(fig;d) Glomus fragilistatum (fig:e), Glomus citricolla (fig;f), Ruptured wall of Glomus macrocarpum (fig;g) Glomus globiforum(fig;h), Glomus mosseae (fig;i), Acaulospora laevis(fig;j), Acaulospora sp.(fig;k,l), Scutellospora pellicida(fig;m) Scutellospora auriglobosa (fig;n)* and *Scutellospora calspora(fig;o), Gigaspora rosea (fig;p)* all these spores were isolated from rhizospheric soil of *Eleusina coracana* (Plate-I).

PLATE-I



Maximum number of spores and 90% root colonization was observed in host *Eleusina coracana* inoculated with pure culture of *Glomus mosseae*. Coenocytic hyphae, vesicles, elongated Vesicles, Arbuscles were observed in the root of *Eleusina coracana* inoculated with pure culture of *Glomus mosseae*. (Fig-a,b,c,d,e,f and Plate-II),(10X,40X and 100X). 92% percentage root colonization was observed in host *Eleusina coracana* inoculated with pure culture of *Acaulospora laevis*. Coenocytic hyphae, vesicles, elongated vesicles were observed in the root of *Eleusina coracana* inoculated with pure culture of *Acaulospora laevis*. (Fig-a, b, c, d, e, f and Plate-III,) (10X, 40X and 100X). 95% percentage root colonization was observed in host *Eleusina coracana* inoculated with mix culture of arbuscular mycorrhizal fungi. Coenocytic hyphae, vesicles. Arbuscles were observed in the root of *Eleusina coracana* inoculated with mix culture of arbuscular mycorrhizal fungi.

Mass multiplication of mix culture and pure culture of Arbuscular mycorrhizal fungi of *Vetivera zizanioides* was done on *Eleusina coracana***.** *Eleusina coracana* showed early

root infection and sporulation. Similar observation were made by Prabhu (2004), reported mass multiplication of mycorrhizal fungi was done by using Maize, Jowar, Rey grass, and Merigold. Mass multiplication of mycorrhizal fungi was done on Maize studied by Sadhana (2015). Alfa-Alfa, Mays, Bahiya, grass, Sweet potato, onion and hegari all these host were used for mass production of Arbuscular mycorrhizal fungi reported by Akhtar, *et. al*., (2014).

Mass production of Arbuscular mycorrhizal fungi using host *Eleusina coracana* plant showed significant effect in response morphological and biomass production as compared to control plant. Results of growth performance of *Eleusina coracana* plant after inoculation of mycorrhizal inoculums clearly indicated that increases fresh and dry biomass shoot and root length and percentage of root colonization as compared to control plant.

Current results showed that in *Eleusina coracana* colonization of AM fungi was highest in all the growth periods. Mass production of Arbuscular Mycorrhizal inoculums at large scale has been investigated by Sadhna (2015).The infectivity of the hypha network can be maintained in the absence of spores (Jasper *et al*., 1989; 1991). Simpson and Daft (1990) have reported that the growth stage and physiology of host plants have been postulated to influence spore production of endomycorrhizal fungi. The age of the crop and the harvest date greatly influence the size of the spore population and extent of root colonization of *Glomus mosseae* (Al-Raddad, 1991; Kapulnik and Koshnir, 1991).

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