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Development of Indigenous Am Fungi for Inoculum Production

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Abstract: ***AMF is the most important mycorrhiza in agriculture and ecosystems due to the fact that they colonize the majority of crop plants AMF are obligetive biotrops. All mycelium produced inside or outside of the host root (Ecto-Endo). AMF spores are important as a source of inoculums has been used in field experiments. Infected roots contain internal fungal mycelium. Gramineae (Sorghum) family plants are used as host plants, field experiments were conducted at 3 sites for AMF inoculum production under natural conditions. For single spore production Hoagland's method has been followed. The identified spores belongs to Glomus sps., There is no significant difference in diversity of AMF spores while increasing in number in all 3 sites.***

Keywords: AMF, Gramineae family, Hoagland's Method, *Glomus* sps

1. Introduction

AMF are highly capable in fixing N, P, K by showing oblique symbiotic association with the roots of higher plants. They restore plant growth by increasing they mobilizes the nutrients from the soil and helps to overcome drought and salinity stress and considered as universal symbionts. There are two opportunities for the utilization of AM fungi. The first is to manage effectively the indigenous population already present on the farm. The second is to inoculate your plants with effective isolates of AM fungi. Arbuscular mycorrhizal fungi are believed to be obligate symbionts, that is they must colonize plant roots to grow and reproduce. Only the phase of the fungus inside the root ("intraradical hyphae") can absorb sugar and express certain metabolic pathways necessary for growth, such as the synthesis of fats. Therefore, the fungus has a very limited ability to grow asymbiotically, i. e. without living in symbiosis. Failure by researchers to overcome these limitations has prohibited the growth of these organisms in pure culture on Petri dishes or in fermenters for inoculum production.

2. Methods and Methodology

Mass Multiplication of Vam Fungi
Vasicular arbusclar mycorrizal fungi are obligate symbionts and are ubiquitous. The adaptability of VAM symbiosis depends upon the host plant and the soil. We used *Sorghum vulgare* as a host plant for mass multiplication of VAM fungi. (Fig.1). The VAM fungi were picked up from the indigenous soil in the form of spores and sporocarps. They were identified, among them *G. mosseae* and *G. aggregatum* were selected for the mass multiplication.

Preparation of Starter Inoculum:
Single spore pure inoculums of each VAM fungi were elevated by using funnel technique (Menge and Timmer 1982). *Sorghum vulgare* as a host plant. The funnel was filled with 1: 1 ratio of sterilized soil and sand mixture. Single spore VAM fungal spores were added to this mixture. Then seeds of *Sorghum vulgare* were sown in the funnel and watered at regular intervals. And the funnel submerged in a conical flask which has Hoagland's nutrient solution. With the help of absorbent cotton plugs the transportation of Hoagland's solution was supplied. The preparation of Hoagland's solution was given below. After 30 days seedling, roots were analyzed for VAM colonization and abundance of VAM fungal spores. Then these pure inoculum was transferred to field for the mass multiplication. Field beds were prepared at Agricultural Research field at Telangana University for the mass multiplication of VAM fungi.

The field level VAM multiplication bed measurements:
- 3-6.5 kg of inoculum.
- 13 X 3m of plastisheet.
- Requires 12 weeks of period to increase number of spores.
- In 1st Bed 68% VAM propagules were calculated.
- In 2nd Bed 97% VAM propogules were calculated.
- In 3rd Bed 54 VAM propogules were calculated.

Preparation of Hoagland's Solution (Hoagland 1950)

Solution A
KH_2PO_4-0.136 g
KNO_3-0.020 g
Ca (NO3) $_2$-0.492 g
$MgSO_4.7H_2O$-0.490 g
Distilled water-1000 ml

Solution B
H_3BO_3-2.86 mg
$MnCl_2.4H_2O$-1.81 mg
$ZnSO_4.7H_2O$-0.22 mg
$CuSO_4.5H_2O$-0.08 mg
$H3MoO_4.4H_2O$-0.09 mg
Distilled water-1000 ml

Solution C
EDTA-26.1 g
KOH (1N)-268 ml
$FeSO_4.7H_2O$-24.9 g
pH-5.5
Distilled water-1000 ml

For the preparation of Hoagland's solution, the Solutions were mixed in given compositions, and taken in conical flask for 100 ml solution.
Solution A-10 ml
Solution B-1 ml
Solution C-1 ml
And pH adjusted to 6.

Mass multiplication of VAM spores (Wood 1987, Menge and tTimmer, 1982): (fig.1).

Preparation of starter inoculum of *G. aggrigatum* and *G. mosseae* through Hoagland's solution (1950). (Fig.2).

Maintenance of VAM cultures on *Sorghum vulgare* under glass house conditions (Fig.3).

Utilization of inoculum in Agricultural research field at Telangana University:

The research plant inoculated with AM fungi one of two ways. Plants were inoculated in the field at planting and at the time of seedlings production in the research field we utilized this inoculum in both pot and field experienments to enumarate the growth of the plant by taking growth parameters in both inoculated and non-inoculated plants. We have targeted in two cultivars of *Chenopodium quinoa* (INIA 431, INIA 427) and produced our own seedlings for out planting because we feel the inoculum can be easily mixed into potting media. Potential benefits to crop growth arise from the advantage the plant receives from having a pre-established symbiosis upon entering the field rather than experiencing the lag time before being colonized by the indigenous AM fungi.

Utilizing the inoculum now requires three steps and a little decision making: harvest of the inoculum, mixing into the potting media, and finally, potentially modifying one's

greenhouse nutrient regime. The VAM inoculum mixed to research field at intervals.

3. Results and Conclusion

Crop/ cultivar	Treatment	Height (cm)	Shoot Wt. (g)	Colonization (% root length)
INIA 431 cultivar				
	Glomus sps.,	30.3	0.876	3.1
	Glomus sps.,	23.2	0.701	6.4
	Glomus sps.,	28.5	0.809	4.1
INIA 427 cultivar				
	Glomus sps.,	20.9	1.041	0.9
	Glomus sps.,	19.9	0.746	4.6
	Glomus sps.,	23.4	0.882	5.6

In developing a system for the on-farm production of AM fungus inoculum (*Glomus* sps,), the goal of our research was to make a potent, effective, species diverse inoculum that was also very inexpensive. Our research examining the yield response of crops inoculated with AM fungi, in high P soils under both conventional and organic management, has shown highly significant increases some years, and little or no response in other years (Douds and Reider, 2003; Douds et al., 2007). However, routine use of this inoculum should not be an economic burden during years of optimal conditions in which inoculation gives no response yet positions one to take advantage of the potential for a significant yield response when the symbiosis alleviates conditions that depress yield. workers found beneficial effects in terms of growth and yield due to VAM mycorrhizal inoculation in legumes (McKey 1994), ground nut (Copetta et al. (2006) Increase in biomass, dry matter production, plant height and number of leaves resulted due to VAM infection in Soya been variety of JS – 335 with *G. fasciculatum* and *G. mosseae* were reported with significance increase in dry weight Pods weight and yield per plant in pot experiments. (Shashank Ashokrao Tidke 2018)

In the present study root colonization was 90% with G. aggregatum and 95% with G. mosseae in the cultivar INIA – 427 over INIA-431 over 80% root colonization was recorded by both fungi at 90days of crop growth. Significant increase in growth parameters such as plant height, root and shoot (fresh and dry) and biomass was observed in the present study in both the cultivars pot and field experiments.

Performance of *G. mosseae* was better than *G. aggregatum* while *P. aeruginosa* also significantly enhanced plant growth with *G. mosseae* than *G. aggregatum*. Combination treatments in general and triple combination treatments in particular exhibited more growth over control and individual treatments. The present results confirm the earlier findings on other protein seed crops and suggest a beneficial activity of VAM fungi on experimental plant.

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