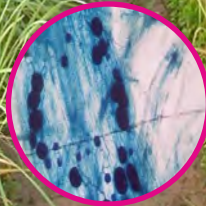


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# ARBUSCULAR MYCORRHIZAE (AM) - INOCULUM PRODUCTION FOR UPLAND CROPS UNDER RAINFED DROUGHT-PRONE ECOLOGY



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Arbuscular mycorrhiza fungi (AMF) are soil borne fungi forming a mutualistic symbiosis with many terrestrial plants. Spores germinate in soil, infect the root system and form an intercellular hyphal network, intracellular arbuscules (specialized structures) inside the host root, and extra-radicular hyphal networks outside the host root. Arbuscules are the site of nutrients exchange between colonized plants and fungi. The extra-radicular hyphal network around the root system extends several centimetre beyond the rhizosphere which promotes the acquisition of less mobile nutrients like phosphorus (P) by (i) intercepting labile P pool beyond P depletion zone and (ii) synergistic interactions of other P-solubilizing micro-organisms with mycorrhizal plants (AM-plants). It helps in the translocation of P by increasing its mobility as well as reducing its fixation by the soil matrix. Upland soils mostly maintain aerobic soil conditions even in rice fields during the rainy season, favour arbuscular-mycorrhizal fungal activities. Therefore, biofertilizers in the form of microbial inoculants especially AM based inoculums are very suitable complementary source to chemical fertilizers particularly for P sources. Moreover, P nutrition is one of the limiting factors in rainfed upland ecology owing to its less mobile nature and high fixation rate, particularly in rainfed, moisture limiting environments.

### **Native AM-fungi in rainfed uplands**

Among the common six genera of AMF, *Glomus* followed by *Acaulospora* in *unbanded* (unfavorable) uplands with aerobic soil conditions and *Glomus* followed by *Gigaspora* in *banded* (favorable) uplands are predominant. Usually, the native AMF population increases during the rainy season (*kharif*) due to the presence of crop and declines during winter and summer fallows.



## The basic protocol for mass inoculums production involves following steps.

### 1<sup>st</sup> phase:

1

• Production of Starter inoculum of native AMF (consortium of native flora) on *Sorghum* plant roots, grown in sterilized soil:sand:FYM mixture (1:1:1; v:v:v) under controlled glass-house condition for 30-45 days following the standard procedure (**mid-April to mid-May**)

2

• Multiplication of Starter inoculum on *Sorghum* roots, grown in micro-plots (minimum 4m x 4m) which are partially pre-sterilized by soil-solarization using transparent, thin (1-2 mm), LDPE (Low Density Poly-Ethylene) film as mulching on moist soil for 30 days during peak summer (**mid-April to mid-May**)

3

• The LDPE cover is removed after 30 days (**mid-May**) and furrows (10-15 cm depth) are opened manually at 10 cm apart. The starter inoculum (@ 400 gm<sup>-2</sup>) is band placed in furrows followed by dense sowing of *Sorghum* seed (20-24 kg ha<sup>-1</sup>) and covering by the plot soil.

4

• The micro-plots are watered regularly until germination and the plants are grown with life-saving irrigation for 30 days during **mid-May to mid-June** to ensure that the mass-produced inoculum is ready prior to sowing of upland rice (normally 3<sup>rd</sup> to 4<sup>th</sup> week of June depending on the onset of monsoon).

5

• After 30 days' growth of *Sorghum* in the micro-plots, aerial plant parts are removed (for fodder use), soil and roots are harvested from upto 15 cm depth, roots are cut into small pieces, mixed thoroughly, air dried under shade and used as mass-produced inoculum.

6

• Mass-produced inoculum containing 60.8-80.7 AMF propagules g<sup>-1</sup> is applied at a rate of 1.0-1.25 t ha<sup>-1</sup> in furrows opened for sowing seeds and placed beneath the seeds.

### On-farm production of native AMF inoculum

AMF inoculums developed from native sources (consortia of existing genus) are considered to be more efficient, cost effective (particularly for field crops like upland rice), adapted to the target ecology and have less



negative ecological consequences in terms of chances of invasive species introduction as unintended contaminants. Farmer friendly on-farm multiplication method of soil-root based AMF starter inoculums on *Sorghum* roots in partially sterilized (by soil solarization during summer) micro-plots was standardized. Application of these inocula enhanced (28.4%) P uptake by upland rice with the concomitant increase in grain yield (24.5%). The protocol of mass inoculum production developed for rainfed upland ecology was refined in three phases reducing soil-root based inoculum dose from 1.0-1.25 t ha<sup>-1</sup> to 0.5-0.75 t ha<sup>-1</sup> and finally to 0.25 t ha<sup>-1</sup>, thus reducing inconveniences like handling, transportation and application.



**(a) Nucleus/starter inoculum (NI) under preparation**



**(b) Soil solarization in micro-plots**



**(c). Multiplication of nucleus inoculum in solarized plots**

### **Fine tuning nucleus inoculums production protocol for developing improved mass inoculums of native AM fungal consortium (2<sup>nd</sup> phase)**

Results of the next (second) phase of fine tuning of the protocol revealed that mass inoculums (MI) produced by multiplying starter/nucleus inoculums (NI) developed on substrate mixture of vermiculite: soil: FYM; 75: 25: 5 (w/w/w) fortified with Hogland solution @ 10 ml/100 g/week (for 4 weeks) substantially improved efficacy of MI in terms of reducing the effective dose by half (1 t ha<sup>-1</sup> to 0.5 t ha<sup>-1</sup>). An attempt was initiated for further improvement (3<sup>rd</sup> phase) in the efficacy of MI by increasing AMF population in NI. Standard initial inoculation rate for NI substrate with the native AMF spore (1 spore per gram substrate) was compared with that of 2, 3, 4 & 5 spores per gram to ascertain a proportional increase of multiplied population of AMF (in mass inoculum) after standard



incubation (multiplication) of 30 days with sorghum plant in solarized micro-plots.

### **Field evaluation of improved mass inoculums in upland rice and other crops (3<sup>rd</sup> phase)**

Progressive, proportionate increases in spore and infective propagule population (in MI) with an increase in initial spore inoculation dose (for NI production) were evident. MI developed from improved NI inoculated with 2, 3, 4 & 5 spores g<sup>-1</sup> were compared with standard MI (prepared from NI inoculated with 1 spore g<sup>-1</sup>) under field conditions and found to be 0.25 t ha<sup>-1</sup> for direct sown upland rice.

Further, field experiments were conducted for confirmation of effective dose of improved mass inoculums of AMF in non-rice upland crops *viz.* maize, horse gram and pigeon pea which are recommended for rice based cropping systems. From this experiment, it was observed that, by increasing dose of improved mass inoculums (MI) the yield of pigeon pea did not significantly increase. However, significant increases in yields of maize and horse gram were recorded with 0.3 and 0.6 t ha<sup>-1</sup> MI respectively. This validation trial proved the efficiency of improved MI at the lower dose of 0.3 to 0.6 t ha<sup>-1</sup> for non rice upland crops as compared to that of 0.25 t ha<sup>-1</sup> as standardized for upland rice (Table 1)

**Table 1. Yield of different crops under the MI (AMF) treatments**

MI (t ha <sup>-1</sup> )	Yield (t ha <sup>-1</sup> )		
	Maize	Horse gram	Pigeon pea
5.0	3.85 <sup>a</sup>	0.52 <sup>c</sup>	1.31
2.5	3.33 <sup>ab</sup>	0.80 <sup>ab</sup>	1.62
1.2	3.34 <sup>ab</sup>	0.51 <sup>c</sup>	1.73
0.6	2.97 <sup>b</sup>	0.93 <sup>a</sup>	1.55
0.3	3.64 <sup>a</sup>	0.72 <sup>b</sup>	1.33
0	3.54 <sup>ab</sup>	0.86 <sup>ab</sup>	1.78

**\*Figures with different letters are significantly different at 5% probability level**



## Enhancing efficacy of improved mass inoculum

The AM-supportive crop culture components identified were fine-tuned and validated through farmers participatory on-farm trials in villages of Jharkhand State. Based on validation, following recommendations were made.

Application of on-farm produced AM-inoculum (improved mass inoculums @  $0.25 \text{ t ha}^{-1}$  for direct seeded rice;  $0.3 \text{ t ha}^{-1}$  for maize and  $0.6 \text{ t ha}^{-1}$  for horse gram) should be integrated with;

1. Two consequent off-season tillage operations should be spaced minimum by 13 weeks. The best option may be; one initial tillage (after rice harvest) followed by one summer tillage (if remains fallow).
2. Following rice-based cropping system options should be practiced;
  - a. Two years rotation of maize relay cropped by horse gram in the first year followed by upland rice in the second year
  - b. Rice-pigeon pea intercropping or crop rotation in alternate years. Intercropping with pulses should not be continued in same plot to avoid sick-plot (soil borne diseases) development.
3. Under above AM-supportive cropping systems, an optimum P dose of  $20 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  in compared to the recommended dose of  $30 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  may be applied to rice





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