Impact of the Mixed Consortium of Indigenous Arbuscular Mycorrhizal Fungi (AMF) on the Growth and Yield of Rice (*ORYZA SATIVA* L.) under the system of Rice Intensification (SRI)

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Abstract—The effect of inoculation of indigenous arbuscular mycorrhizal fungi (AMF) co-inoculated with *Azospirillum lipoferum* (strain Az204) and phosphobacteria (*Bacillus megaterium* - strain PB2) on the growth and yield of rice under the System of Rice Intensification (SRI) in the nursery and field was studied by conducting a field trial at Agricultural College & Research Institute, Madurai. The indigenous AMF was isolated from rice fields of this Institute and were identified as *Glomus sp.*, *Gigaspora* sp., and *Acaulospora* sp. These AMF were mass multiplied in maize plants using vermiculite as substrate and used as mixed consortium AMF. The mat nursery was prepared and AMF inoculated at the rate of 100g/m². Also treatment was done using *Azospirillum* and phosphobacteria on treatment-wise. At the time of transplanting seedling dip was done for the 8-day old rice seedlings using the same microbial inoculants. In the main field seed also application of mixed consortium AMF along with *Azospirillum* and phosphobacteria was carried out based on the treatment schedule. The results of the field trial revealed that the seedlings in the nursery showed vigorous growth and AMF colonization and spore count were recorded the maximum in the treatment with AMF, *Azospirillum* and 75% RDF of N and P. In the main field also there was increased growth and yield of rice plant in the same treatment due to the inoculation of mixed consortium AMF co-inoculated with *Azospirillum* on rice variety, ADT43 in the presence of 75% N and P. The yield of rice in this treatment recorded 11.8% higher than with 100% NP alone, besides saving 25% NP. We conclude that the mixed consortium of indigenous AMF inoculation at the nursery and main field under SRI increased growth and grain yield of rice.

Keywords—arbuscular mycorrhizal fungi, *Azospirillum*, phosphobacteria, rice, growth, yield, System of Rice Intensification.

I. INTRODUCTION

Rice in India is cultivated as an irrigated enterprise in which farmers face major constraints that can adversely affect production levels. The yields of rice (*Oryza sativa*) in India are low because of a gradual decline in soil fertility. In order to tackle the situation farmers have intensified their tillage and cropping practices without making the necessary organic inputs to restore and maintain soil fertility. The use of AMF that maintain a type of mutualistic association between crop and fungus may contribute to reducing chemical fertilizer inputs and sustaining crop productivity. In contrast to other crop species, there is little experimental evidence about the role of mycorrhizal colonization in rice plants (Purakayastha and Chhonkar, 2001; Gao et al., 2007). It was reported that rice plants readily form mycorrhizal associations under upland conditions, but under submerged conditions infection is rare due to the anoxic environment (Ilag et al., 1987). Barea (1991) reported, however, that AMF can survive in waterlogged conditions, and this is supported by the fact that *Glomus etunicatum*, showed fairly high colonization in rice roots and best survival under submerged conditions (Purakayastha and Chhonkar, 2001). In a work on six aerobic rice genotypes, relatively high colonization of roots, 28-57% depending on genotypes was observed (Gao et al., 2007). However, there is a paucity of information available on the involvement of AMF in rice particularly under waterlogged conditions.

Arbuscular mycorrhizal fungi have their greatest effect when a host plant associated with them is of deficient in phosphorus (Koide, 1992). It is a fact that mycorrhizal fungus is able to increase growth in a number of agricultural crops (Mosse, 1973; Gredemenn, 1975; Tinker, 1975). Sanni (1976) demonstrated the increase in the growth of rice plants after inoculation with *Gia sporagigantia*. Some studies under pot culture conditions revealed that AMF increased grain and straw yields of wetland rice (Sivaprasad et al., 1990) and increased the grain yield and P and Zn content in rice.
II. MATERIALS AND METHODS

2.1. Experimental Setup
The experiment was conducted in a field at Agricultural College & Research Institute, Madurai, Tamil Nadu, India with 8 treatments and 3 replications. The indigenous AMF cultures isolated from rice fields of this Institute and identified as Glomus sp., Gigaspora sp., and Acaulospora sp. were used as mixed consortium of AMF inoculants. The nitrogen fixer, Azospirillum lipofeferum (Az204) and phosphobacteria (Bacillus megaterium- PB2) were also included in the treatments as biological inputs for N and P. The replications were made in a random throughout the plot. Also the recommended dose of N:P:K (120:38:38 kg/ha) at various levels was added to the treatments. The Statistical Design adopted was RBD. The various treatments were as follows:

T1 Azospirillum + Concentrated AMF + 75% N and P
T2 Azospirillum + Normal AMF + 75% N and P
T3 Azospirillum + Concentrated AMF + 100% N and P
T4 Azospirillum + Normal AMF + 100% N and P
T5 Azospirillum + phosphobacteria + 75% N and P
T6 Azospirillum + phosphobacteria + 100% N and P
T7 75% N and P
T8 100% N and P (120:38:38 kg/ha)

2.2. Arbuscular mycorrhizal fungi (AMF) isolation and multiplication
Soil samples were collected from different locations of rice fields of Agricultural College & Research Institute, Madurai and were examined for the presence of AMF spores by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and examined under a stereo zoom microscope for their shape, colour and the hyphal attachment to spores. Based on the taxonomic keys of Schenck and Perez (1988) and through INVAM web based identification (http://invam.caf.wvu.edu/cultures/cultsearch.htm), the AMF isolates from rice field soil were identified and mass multiplied in maize plants using vermiculite as substrate and used as mixed consortium AMF. The mycorrhizal inoculum consisting of spores in vermiculite substrate, and infected root fragments is Normal AMF. The AMF colonized maize root bits alone is Concentrated AMF.

2.3. SRI rice nursery preparation and transplantation to main field
Raised nursery bed is formed and mixed consortium of indigenous arbuscular mycorrhizal fungi is inoculated @100g/m² and is spread in the nursery bed to a depth of 2-3 cm. Also rice seeds were treated with Azospirillum (Az.204) and phosphobacteria (Bacillus megaterium-PB2) as per the treatment and germinated in the dark. Germinated seeds were sown in the nursery treatment wise. At the time of transplanting seedling dip was done for the 8-day old rice seedlings using the same microbial inoculants. In the main field also application of mixed consortium AMF along with Azospirillum and phosphobacteria was carried out based on the treatment schedule.

2.4. Mycorrhizal assays
Spores of AM fungi in the soil were estimated by the wet-sieving and decanting method described by Daniels and Skipper (1984). The roots in each treatment plot were washed free of soil particles and organic debris on a 2 mm sieve under a jet of tap water. A 0.5 g sample of fresh roots at the nursery stage or a 1.0 g sample of the roots was excised from each hill from field samples, to assess the percentage of AMF colonization. The root samples were preserved in formalin-aceto-alcohol (FAA) to fix the roots and the standard procedures for clearing and staining of roots as modified from Kormanik and McGraw (1984) were used. Percentage colonization of roots was estimated by visual observations of stained root segments mounted in lactoglycerol, counting the number of root bits colonized to that of the total number of root bits observed (Giovannetti and Mosse, 1980).

2.5. Dry matter production
Three plants were randomly selected from each treatment, washed and dried in an oven at 80°C till constant weight was observed. The plants were weighed and dry weight was expressed in g/plant during transplanting, tillering, flowering and harvest stages.

2.6. Grain yield
The grains harvested from each treatment plots were weighed and the mean value was expressed in tones /acre.

2.7. Statistical analysis
The experimental results were statistically analyzed in randomized block design (RBD) and in Duncan’s multiple
III. RESULTS AND DISCUSSION

3.1. Arbuscular mycorrhizal fungi (AMF) isolation and multiplication

AMF spores isolated from rice fields were examined under a stereozoom microscope for their shape, colour and the hyphal attachment to spores. Based on the taxonomic keys of Schenck and Perez (1988) and through INVAM web based identification (http://invam.caf.wvu.edu/cultures/cultsearch.htm), the AMF isolates from rice field soil were identified as *Glomus sp.*, *Gigaspora sp.* and *Acaulospora sp.* (Fig.1). These AMF were mass multiplied in maize plants using vermiculite as substrate and used as mixed consortium AMF.

3.2. AMF colonization and sporulation

Colonization of rice roots increased from nursery to tillering stage and decreased after the flowering stage. Colonization was negligible in rice roots when the soil was not inoculated with AMF. Sporulation also increased till tillering stage and decreased thereafter. AMF colonization and sporulation was maximum in the T2 treatment, *Azospirillum* + Normal AMF + 75% N and P (Table.I;Fig.2). A unique characteristic of rice roots that overcomes these reduced conditions in soil is the presence of large air spaces in mature roots (Yoshida, 1975; Velue et al., 2009). Thus, the aerated region around the rice roots may provide a suitable environment for rhizosphere microorganisms including mycorrhizal fungi.

3.3. Dry matter production

Dry weight of the rice plant was significantly higher in the T2 treatment, *Azospirillum* + Normal AMF + 75% N and P followed by T1 in all the stages of sampling (Table.I). There are few reports to elucidate the essential role of AMF on rice plants at the nursery-stage and its function after transplanting to the field. The inoculated seedlings had a higher total biomass than uninoculated seedlings at transplanting to the field. This indicates that seedlings benefited from mycorrhizal colonization prior to transplanting as already reported (Dhillion and Ampornpan, 1992; Solaiman and Hirata, 1996).

3.4. Grain yield

The grain yield after harvest, at 110th day was recorded the highest in T2 treatment, *Azospirillum* + Normal AMF + 75% N and P of 2.18 t/acre and it is 11.8% increase over the control, T8 treatment with 100% N and P (Table.1). Mycorrhizal inoculation with *Glomus fasciculatum* in dry nursery-stage seedlings increased grain and straw yields (Sivaprasad et al., 1990; Chinnusamy et al., 2006; Bhuiyan et al., 2006; Ashok Kumar., Sharma.and Gera. 2011.Arbuscular mycorrhizae (*Glomus mosseae*) symbiosis for increasing the yield and quality of wheat (*Triticumaestivum*). Indian Journal of Agricultural Sciences, 81(5): 478–80.

REFERENCES

[1] Ashok Kumar., Sharma.and Gera. 2011.Arbuscular mycorrhizae (*Glomus mosseae*) symbiosis for increasing the yield and quality of wheat (*Triticumaestivum*). Indian Journal of Agricultural Sciences, 81(5): 478–80.

[2] Barea, J.M. 1991.Vesicular-Arbuscular Mycorrhizae as Modifiers of Soil Fertility. *Advances in Soil Science*, 15: 1-40.

[3] Bhuiyan, M. K. I., Rico, C. M., Mintah, L. O., Kim ManKeun., Shon TaeKwon., Chung IlKyung.and Lee SangChul.2006. Effects of biofertilizer on growth and yield of rice. *Korean Journal of Crop Science*, 51(4) : 282-286.
Mycorrhiza – Effect of Oryza sativa, 41:

Growth

Oryza sativa Vesicular arbuscular mycorrhizae (VAM) –

In Oryza sativa, The effects of Vermicompost and microbial inoculants on soil health, growth and yield. Int. Rice Res. Newsletter, 15: 14–15.

Effect of arbuscular mycorrhizal fungi for inoculation of lowland rice (Oryza sativa L.). Plant and Soil, 2013:

Effect of plant and microbial inoculants on soil health , growth and yield of HD 2687 wheat (Triticum aestivum L.) after transplanting under

The American Phytopathological Society, St. Paul, MN.

Manual for New Phytol.

Quantification of vesicular arbuscular mycorrhizae in plant roots. In Methods and Principles of Mycorrhizal Research.Ed. N C Schenck. pp 29–35.
different soil fertility and water regimes. Soil Sci. Plant Nutr, 42: 561–571.

[28] Tinker, P.B. 1975. In: Symbiosis. 29th Symp: Soc. Exp. Biol. (Ed.) D.G. Jennings and D.L. Les. Cambridge University Press, p. 325.

[29] Velu, R., Chettipalayam, S., and Sellamuthu, M. 2009. Arbuscular Mycorrhizal Fungi Colonization in Upland Rice as Influenced by Agrochemical Application. Rice Science, 16(4): 307-313.

[30] Yoshida, T. 1975. Microbial metabolism of flooded soils. In: Soil Biology and Biochemistry, Vol. 3. Eds. E.A. Paul and A. McLaren. pp 83–122. Marcel Dekker, New York.

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**Table 1:** AMF colonization (%), Spore count (nos./100g), Dry weight (g/plant) and Yield of rice under SRI

| Treatments | Transplanting stage | Tiller stage | Flowering stage | Transplanting stage | Tiller stage | Flowering stage | Transplanting stage | Tiller stage | Flowering stage | Yield of rice plants at harvest (110 days) |
|------------|---------------------|-------------|-----------------|---------------------|-------------|-----------------|---------------------|-------------|-----------------|------------------------------------------|
|            | AMF colonization (%) | Spore count (nos./100g) | Dry weight (g/plant) |                      |             |                 |                     |             |                 |                                          |
| T1         | 18.6                | 26.0        | 26.3            | 5.5                  | 12.3        | 10.2            | 9.26                | 15.7        | 66.9            | 2.12                                     |
| T2         | 21.3                | 31.3        | 30.0            | 8.2                  | 15.9        | 12.5            | 8.90                | 18.1        | 70.2            | 2.18                                     |
| T3         | 18.0                | 23.6        | 23.3            | 5.3                  | 11.5        | 9.4             | 8.96                | 12.3        | 62.6            | 2.05                                     |
| T4         | 16.6                | 26.0        | 23.0            | 4.7                  | 10.0        | 8.6             | 8.96                | 11.0        | 55.5            | 2.02                                     |
| T5         | 3.3                 | 2.3         | 2.3             | 1.0                  | 2.2         | 2.0             | 9.16                | 12.0        | 62.3            | 2.06                                     |
| T6         | 4.0                 | 2.3         | 3.3             | 1.0                  | 2.5         | 2.0             | 8.03                | 10.8        | 54.7            | 1.99                                     |
| T7         | 2.6                 | 2.6         | 2.3             | 0.0                  | 1.5         | 1.2             | 7.36                | 7.8         | 38.5            | 1.92                                     |
| T8         | 1.3                 | 2.6         | 2.6             | 0.0                  | 1.0         | 1.0             | 8.06                | 9.2         | 45.4            | 1.95                                     |
| CD (P=0.05)| 3.21                | 3.74        | 2.99            | 2.12                 | 2.50        | 1.90            | 0.55                | 0.84        | 3.26            | 0.05                                     |

Fig. 1: AM fungal spores isolated from the rhizosphere soil of rice.

Fig. 2: AM root colonization in the rice field.