

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)

S. Merina PremKumari*, B. Jeberlin Prabina*

Department of Agricultural Microbiology, Agricultural College & Research Institute, Madurai, India.

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., *Gigasporasp.* 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 (Ilaget *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 *Giasporagigantia*. Some studies under pot culture conditions revealed that AMF increased grain and straw yields of wetland rice (Sivaprasadet *et al.*, 1990) and increased the grain yield and P and Zn content in rice

(Secilia and Bagyaraj, 1994a and 1994b). Gupta and Ali (1993) reported a significant increase in the grain yield by AMF colonization in wetland rice under both pot and field conditions. The inoculation of AMF directly into the flooded soil was not effective for wetland rice (Solaiman and Hirata, 1995). However, inoculation of seedlings under dry nursery conditions was effective for promotion of wetland rice growth and nutrient acquisition (Solaiman and Hirata, 1996). The present investigation was, therefore, undertaken to study whether the growth and yield of rice grown under the SRI could be enhanced through inoculation with AMF at the nursery and field conditions.

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.*, *Gigasporasp.* and *Acaulospora sp.* were used as mixed consortium of AMF inoculants. The nitrogen fixer, *Azospirillum lipoferum* (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 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 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 1.0g 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

range test (DMRT) as per the procedure described by Gomez and Gomez (1984).

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.*, *Gigasporasp.* and *Acaulosporasp.* (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.1; 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; Veluet *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.1). 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., 2011; Maitiet *al.*, 2011; Shukla *et al.*, 2013). Secilia and Bagyaraj (1992) evaluated 18 different inoculants of AMF on nursery seedlings for 15 days of growth under dry and then up to 28 days of growth under wet conditions. *Acaulosporasp.*, *Glomus etunicatum* and *Scutellosporasp.* exhibited stimulation of wetland rice growth. In our experiment, the indigenous AMF isolated are *Glomus sp.*, *Gigasporasp.* and *Acaulosporasp.* which were significantly effective for increasing the yield when inoculated at the nursery stage and also applied in the main field.

IV. CONCLUSION

The effect of inoculation of arbuscular mycorrhizal fungi (AMF) along with other microbial inoculants viz., *Azospirillum* and phosphobacteria 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 AC & RI, Madurai. The indigenous AMF was isolated from rice fields of Agricultural College & Research Institute, Madurai and were identified as *Glomus sp.*, *Gigasporasp.* and *Acaulospora sp.* These AMF were mass multiplied in maize plants using vermiculite as substrate. The mat nursery was prepared and AMF inoculated at the rate of 100g/m². Also seed 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 also application of 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 the 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. The increase in yield was 11.8% in the treatment with AMF, *Azospirillum* and 75% RDF over the uninoculated.

REFERENCES

- [1] Ashok Kumar., Sharma. and Gera. 2011. Arbuscular mycorrhizae (*Glomus mosseae*) symbiosis for increasing the yield and quality of wheat (*Triticum aestivum*). *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 IIKyung. and Lee SangChul. 2006. Effects of biofertilizer on growth and yield of rice. *Korean Journal of Crop Science*, 51(4) : 282-286.

- [4] **Chinnusamy.,Muthukumaravel., Kaushik., Brahma., Prasanna.andRadha. 2006.**Growth, Nutritional, and Yield Parameters of Wetland Rice as Influenced by Microbial Consortia Under Controlled Conditions. *Journal of Plant Nutrition*, 29(5) :857-871.
- [5] **Daniels, B. A. and Skipper, H. D. 1984.**Methods for the recovery and quantitative estimation of propagules in soil.*In* Methods and Principles of Mycorrhizal Research.Ed. N C Schenck.pp 29–35.*The American Phytopathological Society*, St. Paul, MN.
- [6] **Dhillon, S. S. and Ampornpan, L. A. 1992.**The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant rice (*Oryzasativa*L.) plants.*Biol. Fertil. Soils*,13 :85–91.
- [7] **Gao, X., Kuyper, T.W., Zou, C., Zhang F. and Hoffland, E. 2007.** Mycorrhizal responsiveness of aerobic rice genotypes is negatively correlated with their zinc uptake when nonmycorrhizal. *Plant and Soil*, 290: 283-291.
- [8] **Gederman, J.W. 1975.** Vesicular-arbuscularmycorrhizae.pp 575-591. In: J.C. Torey and D.P Clarkson (eds). The development and function of roots. Academic Press, NY, USA
- [9] **Gerdemann, J. W., and Nicolson, Y. H.1963.** Spores of mycorrhizae *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc*,46: 235-244.
- [10] **Gredemann, J.W. and Nicolson, T.H.1975.** Spores of Mycorrhizalendogone species extracted from soil wet sieving and decanting. *Trans. Brit. Mycol. Soc*, 46 :235-244.
- [11] **Giovannetti, M, and Mosse, B. 1980.**An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots.*New Phytol*, 84 : 489–500.
- [12] **Gupta, N. and Ali, S. S. 1993.**VAM inoculation for wetland rice.*Mycorrhiza News*, 5 : 5–6.
- [13] **Ilag, L.L., Rosales, A.M., Elazegvi, F.V and Mew T.W. 1987.** Changes in the population of infective endomycorrhizal fungi in a rice based cropping system. *Plant and Soil*, 103: 67-73.
- [14] **Koide, R.T. andScheiner, R.P. 1992.**Regulation of the vesicular–arbuscular mycorrhizal symbiosis.*Annul Rev of Plant Physiol. and Plant Molecular Biol*, 43:557-5891.
- [15] **Kormanik, P. and McGraw.A.C.1984.** Quantification of vesicular arbuscular mycorrhizae in plant roots. *In* Methods and Principles of Mycorrhizal Research.Ed. N C Schenck. pp 29–35. *The American Phytopathological Society*, St. Paul, MN.
- [16] **Maiti, D., Toppo, N.N.andVariar, M. 2011.** Integration of crop rotation and arbuscular mycorrhiza (AM) inoculum application for enhancing AM activity to improve phosphorus nutrition and yield of upland rice (*Oryza sativa* L.). *Mycorrhiza*,21(8):659-67.
- [17] **Mosse, B. 1973.**Advances in the study of VA-mycorrhiza .*Ann. Rev. Phytopathol*, 11:1711-194.
- [18] **Purakayastha, T.J.Chhonkar, P.K. 2001.** Influence of vesicular-arbuscular mycorrhizal fungi (*Glomus etunicatum*L.) on mobilization of Zn in wetland rice (*Oryza sativa* L.). *Biology and Fertility of Soils*, 33: 323-327.
- [19] **Sanni, S.O. 1976.** VA-mycorrhiza in some Nigerian soils .the effects of *Gisporagigantea*on the growth of rice .*New Phytol*, 77: 673-674.
- [20] **Schenck, N.C. and Perez, Y. 1988.**Manual for identification of VA Mycorrhizal fungi. 2d ed. Gainesville (Florida): International Culture Collection of [Vesicular] Arbuscular Mycorrhizal Fungi.
- [21] **Secilia, J. and Bagyaraj, D J. 1992.** Selection of efficient vesicular arbuscular mycorrhizal fungi for wetland rice (*Oryzasativa*L.) plants. *Biol. Fertil. Soils*, 13 : 108–111.
- [22] **Secilia, J. and Bagyaraj, D. J. 1994a.**Selection of efficient vesicular arbuscular mycorrhizal fungi for wetland rice - a preliminary screen.*Mycorrhiza*,4 : 265–268.
- [23] **Secilia, J. and Bagyaraj, D. J. 1994b.** Evaluation and first-year field testing of efficient vesicular arbuscular mycorrhizal fungi for inoculation of wetland rice seedlings.*World J. Microbiol.Biotechnol*, 10 : 381–384.
- [24] **Shukla, L. S.,Tyagi, P.,Manjunath, R., Jeetender. andSaxena, A. K. 2013.** Effect of vermicompost and microbial inoculants on soil health , growth and yield of HD 2687 wheat (*Triticumaestivum*). *The Indian Journal of Agricultural Scienses*, 83(3).
- [25] **Sivaprasad, P., Sulochana, K. K. and Salam, M. A. 1990.**Vesicular arbuscular mycorrhizae (VAM) colonization in lowland rice roots and its effect on growth and yield.*Int. Rice Res. Newslett*,15 : 14–15.
- [26] **Solaiman, M. Z. and Hirata, H. 1995.**Effect of indigenous arbuscular mycorrhizal fungi in paddy fields on rice growth and N, P, K nutrition under different water regimes.*Soil Sci. Plant Nutr*,41 : 505–514.
- [27] **Solaiman, M. Z. and Hirata, H. 1996.** Effectiveness of arbuscular mycorrhizal colonization at nursery-stage on growth and nutrition in wetland rice (*Oryzasativa*L.) after transplanting under

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., Sumathi.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 DMcLaren. pp 83–122. Marcel Dekker, New York.



Glomus sp.



Gigasporasp



Acaulosporasp

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

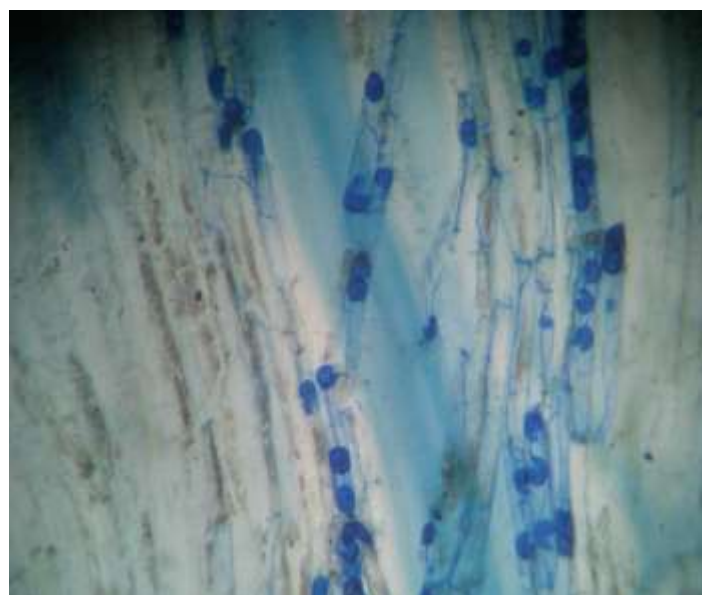


Fig.2: AMF root colonization in the rice field

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

Treatments	AMF colonization (%)			Spore count (nos./100g)			Dry weight (g/plant)			Yield of rice plants at harvest (110days)	
	Transplanting stage	Tillering stage	Flowering stage	Transplanting stage	Tillering stage	Flowering stage	Transplanting stage	Tillering stage	Flowering stage	Yield (t/acre)	
T1	18.6	26.0	26.3	5.5	12.3	10.2	9.26	15.7	66.9	2.12	8.7 %
T2	21.3	31.3	30.0	8.2	15.9	12.5	9.80	18.1	70.2	2.18	11.8 %
T3	18.0	23.6	23.3	5.3	11.5	9.4	8.96	12.3	62.6	2.05	5.1 %
T4	16.6	26.0	23.0	4.7	10.0	8.6	8.96	11.0	55.5	2.02	3.6 %
T5	3.3	2.3	2.3	1.0	2.2	2.0	9.16	12.0	62.3	2.06	5.6 %
T6	4.0	2.3	3.3	1.0	2.5	2.0	8.03	10.8	54.7	1.99	2.1 %
T7	2.6	2.6	2.3	0.0	1.5	1.2	7.36	7.8	38.5	1.92	-1.5 %
T8	1.3	2.6	2.6	0.0	1.0	1.0	8.06	9.2	45.4	1.95	8.7 %
CD(P=0.05)	3.21	3.74	2.99	2.12	2.50	1.90	0.55	0.84	3.26	0.05	