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# Screening of an efficient AM fungi isolate in rice

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**Abstract**
The response of the wetland rice to inoculation with ten arbuscular mycorrhizal fungi (AMF) from rice rhizosphere were studied in a pot experiment under puddled condition to select the most efficient AMF for inoculating rice. The experiment was conducted during the year 2018-19 at Agriculture Research Station, Amaravathi. A sandy clay loam soil was used. The AMF was inoculated @ 30 spores per plant at time of transplanting in root zone and crop response was studied. Plant growth parameters plant height (95.80 cm), number of productive tillers (12.00) and grain yield (4520.57 kg $ha^{-1}$), available soil nutrients (N,P,K) (127.06 kg $h^{-1}$, 29.66 kg $ha^{-1}$ and 261.83 kg $ha^{-1}$ respectively), nutrient uptake (N,P,K) in stover (25.30 kg $h^{-1}$, 7.96 kg $ha^{-1}$ and 93.60 kg $ha^{-1}$ respectively) and grain (32.60 kg $h^{-1}$, 10.10 kg $ha^{-1}$ and 13.70 kg $ha^{-1}$ respectively), enzyme activity(dehydrogenase 78.79 µg TPF $g^{-1}$ soil $day^{-1}$, acid phosphatase 38.70 µg pNP $g^{-1}$ soil $h^{-1}$ and alkaline phosphatase 61.21 µg pNP $g^{-1}$ soil $h^{-1}$) and AMF activity (spore load of 31.33 spores per 10 g soil and per cent root colonization 47.51%) were highest in the treatment T8 with isolate 28. The above results clearly showed that the isolate 28 can be better utilized for rice cultivation to enhance plant growth and yield.

**Keywords:** Arbuscular mycorrhizal fungi (AMF), rice, plant growth, nutrients and AMF activity

## Introduction
*Oryza sativa* L. is the second most important cereal in the world after wheat and the principal staple crop in Asia, serving food for 50% of the world's population. India is the second largest producer of rice next to China with an area of 42.94 m ha annually and a production of 112.90 MT which accounts for about 45% of food grain production in the country (www.indiastat.com) [11].

The demand for food is rising day by day because of increased population and changing dietary habits. Therefore to meet this demand, the global food production should be increased by over 40% at the end of 2030 and 70% by 2050 (FAO, 2009) [7]. However, there are certain obstacles which are responsible for decreased productivity need to be addressed. Increased application of fertilizers in large quantities to enhance yield in HVY resulted in negative effects in soil such as leaching, pollution of water, acidification, reduced availability of trace elements and alkalization of soil. Increased use of chemical fertilizers also destroy decomposers, soil organisms, and also hazardous to the soil environment. This creates problems not only to the soil health but also to the human health and environment (Chen, 2006) [5]. However, application of high amount of fertilizers not only affects soil health also increase the cost of cultivation (Miransari and Mackenzie, 2012) [14]. Increased fertilizer usage continues, in future, there will be chances of crisis of phosphorus fertilizers as the phosphorus reserves get depleted. To overcome this problem one should think of alternate ways of supplementing nutrients and one among them is use of biofertilizers.

Integrated nutrient management with different kinds of biological fertilizers can improve rice (*Oryza sativa* L.) productivity, soil health and fertility. In order to overcome the future phosphorus crisis and mitigate drought for sustainable rice cultivation, Arbuscular Mycorrhizal Fungi (AMF) can be tested as a "saviour for rice" against various problems irrespective of different cultivations. AMF colonize majority of the plants but it is having preference where it impart more benefits to the preferred crop species. Hence it is important to find out the most preferential for rice. There are many AMF types are available in Agricultural research Station, Amaravathi where it is necessary to select most suitable AMF for rice. In this context, this study was taken up to screen the available AMF types for selecting the most suitable one for inoculating rice to derive maximum benefits.

## Materials and Methods

The soil used in the experiment was sterilized. MTU - 1001, a cultivar of wetland rice, was used in the present study. The isolates used in this study were procured from ARS Amaravathi which are isolated from rice rhizosphere soil of different agro climatic zones of Andhra Pradesh. These AMF isolates were multiplied by using suitable host (sorghum). Spore load and AMF per cent root colonization of these isolates was determined by Wet Sieving and Decantation procedure as outlined by Gerdemann and Nicholson (1963) [8] and staining of root segments was carried out as per the procedure proposed by Phillips and Hayman (1970) [18] respectively. Ten best isolates were selected based on the AMF spore load and per cent root colonization. Based on these 10 isolates the following treatments were imposed. Inoculums with 30 spores were added per pot and transplanting was taken under puddled conditions in glass house conditions. Watering was done regularly. Control was grown without inoculating AMF inoculum.

The plant height, number of productive tillers per hill were recorded at 30 DAT, flowering and harvest. The grain yield was recorded after harvest. The available N was determined by the alkaline potassium permangante method outlined by Subbaiah and Asija (1960) [23]. Available P in soil was determined by the method described by Olsen *et al*. (1954) [16]. Available potassium content from soil was extracted by using 1N $NH_4OAC$ as described by Jackson (1973) [13]. Dehydrogenase was estimated as described by Casida *et al*. (1964) [4]. Acid phosphatases were determined by the procedure given by Tabatabai and Bremner (1969) [24] and Alkaline phosphatases by the procedure given by Eivazi and Tabatabai (1977) [6]. Mycorrhizal spore numbers in the soil were estimated by the wet sieving and decantation method described by Gerdemann and Nicolson (1963) [8]. The percentage mycorrhizal colonization of roots was determined by clearing the roots with KOH and staining them with trypan blue (Phillips and Hayman 1970) [18]. Plant samples collected at harvest stage were oven dried at 60 $^0$C. The dried samples were powdered and analyzed for total N, P and K contents by adopting the standard procedures. The total N, P and K uptake by the plant samples at harvest were calculated by using the NPK contents. The percent nitrogen content of the plant samples was estimated by the modified micro Kjeldahl method as described by Piper (1960) [19]. The di-acid digested plant samples were analyzed for phosphorus content by Vanado - molybdate phosphoric acid method as described by Jackson (1967) [12]. Potassium content in the di-acid extract was determined by using flame photometer by as described by Jackson (1973) [13].

The data obtained were statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1968) [21].

## Results and Discussion

In this study, the growth and yield of rice was enhanced with the inoculation of AMF (Table 1). Highest plant height was recorded in the plants of the pots inoculated with treatment $T_8$ (Isolate number-28) (95.80 cm). It is very well documented in the earlier studies that the inoculation of AMF enhances the mitotic activity of stem cells which result in taller plants and more availability of phosphorus for absorption by roots (Bucher *et al.,* 2009)[3]. Similar results of plant height increases with *Glomus fasciculatum* due to synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients was also reported by Ayub *et al.,* (2002) [2]. The numbers of productive tillers per hill were recorded more in the plants of the pots inoculated with treatment $T_8$ (Isolate number-28) (12.00). Similar results reported by Wangiyana *et al.* (2018) [25] who reported that AMF application combined with reduced doses of N-P-K fertilizers, significantly increased the productive tillers number.

The highest grain yield was recorded in the plants of the pots inoculated with treatment $T_8$ (Isolate number-28) (4520.57 kg $ha^{-1}$). AMF inoculation improves overall growth of crop plants with increased uptake of nutrients and production of growth hormones and finally enhances the yield. There are various research reports indicating increased yield due to AMF inoculation in various crops. Similar results reported by Sharma *et al*., (2011) [20] who reported that AMF plus 75% RDF inoculated plots obtained higher yield than the conventional system in wheat. Zhang *et al.* (2014) [26] reported that AMF inoculation resulted in greater allocation of shoot biomass to panicles further increased grain yield by stimulating N and P redistribution to panicles.

The highest available NPK content in soil was observed in the treatment $T_8$ (Isolate number-28) (127.06 kg $h^{-1}$, 29.66 kg $ha^{-1}$ and 261.83 kg $ha^{-1}$ respectively) (Table 2). AMF activity in root zone enhances the availability of P to plant roots and encourages the activity of N fixers, P solubilizers and K solubilizers. Further, extraradical hyphae in soil enhances nutrient uptake. Similar results reported by Oliveira *et al.*, (2016) [15] stated that AMF inoculated to wheat plants through soil application under zero fertilization increased phosphorous (40%) levels in soil than the control plants. Thus AMF can play an indirect stimulatory role in enhancing organic and inorganic sources of N in soil (Hodge *et al*., 2010) [10].

Highest dehydrogenase activity was reported in the treatment $T_8$ (Isolate number-28) (78.79 µg TPF $g^{-1}$ soil $day^{-1}$) (Table 3). It reflects the oxidative activity of microflora in soil. AMF colonization influence root exudation and it enhances rhizosphere microflora. Some bacteria survive for a longer time under mycorrhizal plant rhizosphere than in non-mycorrhizal plant rhizosphere. Similar findings reported by Sharma *et al.* (2011) [20] he reported that higher dehydrogenase activity in mycorrhizal plant rhizosphere than non mycorrhizal plant rhizosphere.

Highest acid and alkaline phosphatase activity was reported in the treatment $T_8$ (Isolate number-28) (38.70 µg pNP $g^{-1}$ soil $h^{-1}$ and 61.21 µg pNP $g^{-1}$ soil $h^{-1}$) (Table 3). Increase in phosphatase activities and growth responses of the plants to AMF symbiosis were directly proportional to the levels of the mycorrhizal colonization. These results were in agreement with the previous observations of Gosling *et al*., (2013) [9] who stated that mycorrhiza-specific phosphatase was detected only in the root extract colonized with mycorrhizal fungi as compared to nonmycorrhizal root extract.

Highest AMF spore load and per cent root colonization was noticed in treatment $T_8$ (Isolate number-28) (31.33 spores per 10 g soil and 47.51% respectively) (Table 4). Similar results reported by Abdullahi *et al.* (2014) [1] who reported that AM colonization in un-inoculated plants that arise later during crop growth might also produce chlamydospores which contribute to higher AM spore count at the time of harvesting. Most of the research findings have clearly proved that application of AMF significantly improved mycorrhizal colonization and plant growth (Panneerselvam *et al*., 2012) [17].

The highest a plant NPK content in stover (25.30 kg $h^{-1}$, 7.96 kg $ha^{-1}$ and 93.60 kg $ha^{-1}$ respectively) and grain (32.60 kg $h^{-1}$,

10.10 kg ha$^{-1}$ and 13.70 kg ha$^{-1}$ respectively) was observed in the treatment $T_8$ (Isolate number-28) (Table 5). AMF activity in root zone enhances the availability of P to plant roots and encourages the activity of N fixers, P solubilizers and K solubilizers. Further, extraradical hyphae in soil enhances nutrient uptake. These are the processes which might responsible for accumulation of nutrients in shoot and grains in the form of proteins, amino acids, nucleic acids and phospholipids. There many reports which are in agreement with the results of the present investigation. Similar results reported by Solaiman and Hirata (1997) [22] who reported that increase in shoot nitrogen and phosphorous concentration in AMF inoculated plants under both field and green house conditions.

**Table 1:** Influence of AMF isolates on plant height, number of productive tillers per hill and grain yield in rice

| Treatments | Plant height (cm) | Number of productive tillers per hill | Grain yield (kg ha$^{-1}$) |
|---|---|---|---|
| $T_1$ | 73.97 | 8.67 | 3253.74 |
| $T_2$ | 78.13 | 9.00 | 3606.80 |
| $T_3$ | 72.50 | 8.00 | 3136.90 |
| $T_4$ | 81.13 | 9.33 | 3843.66 |
| $T_5$ | 86.57 | 10.33 | 4005.58 |
| $T_6$ | 88.13 | 10.67 | 4135.76 |
| $T_7$ | 89.67 | 12.00 | 4266.57 |
| $T_8$ | 95.80 | 97.00 | 4520.57 |
| $T_9$ | 73.45 | 7.00 | 3077.85 |
| $T_{10}$ | 75.52 | 8.33 | 3409.95 |
| $T_{11}$ | 70.75 | 6.67 | 3002.92 |

**Table 2:** Influence of AMF isolates on available soil nutrients (N, P, k) content in rice

| Treatments | Available soil nitrogen (kg ha$^{-1}$) | Available soil phosphorus (kg ha$^{-1}$) | Available soil potassium (kg ha$^{-1}$) |
|---|---|---|---|
| $T_1$ | 114.70 | 25.47 | 242.23 |
| $T_2$ | 116.81 | 27.37 | 243.06 |
| $T_3$ | 114.24 | 25.21 | 242.15 |
| $T_4$ | 120.71 | 27.00 | 245.15 |
| $T_5$ | 121.81 | 27.44 | 247.46 |
| $T_6$ | 118.31 | 27.81 | 250.28 |
| $T_7$ | 124.77 | 28.01 | 252.01 |
| $T_8$ | 127.06 | 29.66 | 261.83 |
| $T_9$ | 113.39 | 25.10 | 241.95 |
| $T_{10}$ | 117.47 | 25.53 | 242.66 |
| $T_{11}$ | 108.59 | 23.77 | 235.83 |

**Table 3:** Influence of AMF isolates on dehydrogenase, acid and alkaline phosphatase activity in rice

| Treatments | Dehydrogenase (µg TPF g$^{-1}$ soil day$^{-1}$) | Acid phosphatase (µg pNP g$^{-1}$ soil h$^{-1}$) | Alkaline phosphatase (µg pNP g$^{-1}$ soil h$^{-1}$) |
|---|---|---|---|
| $T_1$ | 69.49 | 34.69 | 54.19 |
| $T_2$ | 71.36 | 33.44 | 56.00 |
| $T_3$ | 66.87 | 33.66 | 53.22 |
| $T_4$ | 72.20 | 34.15 | 58.50 |
| $T_5$ | 72.83 | 34.50 | 59.21 |
| $T_6$ | 73.43 | 34.87 | 58.47 |
| $T_7$ | 75.28 | 36.58 | 59.67 |
| $T_8$ | 78.79 | 38.70 | 61.21 |
| $T_9$ | 67.07 | 33.31 | 54.11 |
| $T_{10}$ | 71.19 | 33.51 | 57.44 |
| $T_{11}$ | 63.44 | 31.28 | 50.27 |

**Table 4:** Influence of AMF isolates on AMF spore load and per cent root colonization in rice

| Treatments | AMF spore load (Number of spores/10 g soil) | AMF per cent root colonization (%) |
|---|---|---|
| $T_1$ | 19.67 | 32.35 |
| $T_2$ | 21.00 | 36.07 |
| $T_3$ | 19.00 | 33.49 |
| $T_4$ | 21.67 | 38.94 |
| $T_5$ | 22.00 | 39.14 |
| $T_6$ | 27.00 | 40.85 |
| $T_7$ | 27.67 | 41.13 |
| $T_8$ | 31.33 | 47.51 |
| $T_9$ | 18.33 | 31.54 |
| $T_{10}$ | 20.33 | 34.82 |
| $T_{11}$ | 0.00 | 0.00 |

**Table 5:** Influence of AMF isolates on nitrogen, phosphorus and potassium uptake in rice

| Treatments | Nutrient uptake (kg ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Nitrogen | | Phosphorus | | Potassium | |
| | Stover | Grain | Stover | Grain | Stover | Grain |
| $T_1$ | 9.61 | 14.64 | 3.62 | 4.34 | 48.40 | 7.70 |
| $T_2$ | 12.72 | 20.68 | 4.84 | 5.53 | 61.41 | 8.90 |
| $T_3$ | 8.66 | 13.70 | 2.89 | 3.76 | 43.90 | 7.00 |
| $T_4$ | 14.58 | 21.13 | 5.59 | 6.53 | 66.73 | 10.25 |
| $T_5$ | 16.59 | 23.64 | 6.29 | 7.34 | 72.78 | 10.95 |
| $T_6$ | 19.65 | 25.50 | 6.64 | 7.72 | 78.00 | 11.72 |
| $T_7$ | 22.08 | 28.58 | 6.87 | 8.39 | 84.23 | 12.38 |
| $T_8$ | 25.30 | 32.60 | 7.96 | 10.10 | 93.60 | 13.70 |
| $T_9$ | 7.56 | 12.51 | 2.71 | 3.28 | 39.91 | 6.16 |
| $T_{10}$ | 1.099 | 16.72 | 4.19 | 4.89 | 53.87 | 8.29 |
| $T_{11}$ | 6.45 | 11.12 | 1.84 | 2.57 | 36.96 | 5.00 |
| SEm± | 0.382 | 0.513 | 0.120 | 0.165 | 1.586 | 0.244 |
| CD | 1.203 | 1.616 | 0.378 | 0.519 | 4.996 | 0.769 |
| CV | 4.720 | 4.424 | 4.279 | 4.871 | 4.444 | 4.553 |

## Conclusion

From the findings of this study, it can be concluded that treatment inoculated with Isolate number-28 improved growth, grain yield, nutrient uptake, enzyme activity and AMF spore load and percent root colonization compared to treatments inoculated with other AMF isolates. Isolate-28 can be further used in standardizing the method of application of AMF in rice.

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