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EFFECT OF ARBUSCULAR MYCORRHIZAL (AM) INOCULATION ON UPLAND RICE ROOT SYSTEM

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ABSTRACT

This paper provides a brief assessment of the effect of AM (arbuscular mycorrhizal) inoculation on certain root morphological characters (root length, root diameter, root surface area, root volume, root length density) and branching pattern (fractal dimension) of upland rice (*Oryza sativa* L.) an important food grain crop. The uplands in India being rainfed is drought prone and crop productivity is severely crippled due to major constraint of inefficient phosphorus (P) acquisition. The effects of AM fungi (AMF) on enhancing rice plant growth, P uptake and yield are well-acknowledged. The present investigation will aid in generating information on influence of AMF association on rice root growth which would provide further clues for possible exploitation of AMF activities in favor of rice crop. For this, three upland varieties, Sathi 34-36 (highly AM-responsive), Jonga (AM non-responsive) and Vandana (moderately AM-responsive) were selected. AM-responsiveness of these three varieties was measured as a change in total dry matter production as a result of AM-symbiosis (on inoculation with AM fungi). P uptake at 40 DAE (days of emergence) under un-inoculated low P conditions for the three rice varieties was also evaluated with Sathi 34-36 having the highest tissue P content. Root morphological attributes like root length density, total root length, root diameter, root volume and root surface area were increased on AMF inoculation in both responsive (Sathi 34-36) and non-responsive (Jonga) variety with higher magnitude only in non-responsive variety (Jonga). Thus, AM-responsive variety (Sathi 34-36) having higher P-demand (due to increased P-uptake) was more dependent on mycorrhizal pathway for P acquisition than non-responsive (Jonga) variety. Between responsive varieties, Sathi 34-36 along with its inherent better P demand compared to Vandana was altered significantly in root morphology suggesting that varieties with high P uptake ability under low P conditions would be more responsive to AM inoculation and thus would be more likely to undergo root morphological changes.

INTRODUCTION

Spatial deployment of the root system largely determines the ability of a plant to exploit the soil nutrient resources. Root activity (in terms of nutrient acquisition) creates strong gradients in surrounding soil through depletion of immobile nutrients such as phosphorus (P) (Lynch, 1995). AM fungi aid in going beyond this depletion zone to reach a new pool of soluble phosphate (Marschner, 1995; Smith, 1997) via its extra-radical mycelial network extending the absorbing area (Johanson *et al.,* 1993, Li *et al.,* 1991) for increased P uptake. Along with facilitating nutrient uptake this AM association is also important in terms of root architecture as they are known to alter root topology (Schellenbaum *et al*., 1991). Thus mycorrhizae could complement the root architecture in terms of improving or altering root morphological and branching pattern for better P acquisition which is a major concern for rice productivity in the upland acidic soil conditions, due to high fixation rate of P on application as fertilizers. Similar studies on grapevine (Aguín et al 2004) and *Prunus cerasifera* (Berta *et al*., 1995) on AM inoculation has shown to increase branching of roots especially the first order lateral roots. Effects of AM fungal inoculation on root morphology has also been observed only two weeks after transplanting of micro-propagated plants (Schellenbaum et al., 1991) and colonization effects have been shown to affect root morphology in several plant species (Chatzistathis et al., 2013; Citernesi et al., 1998; Malusa et al., 2007). Assessing the effect of AM fungi on the root system of upland rice varieties could help select varieties highly AM-responsive for enhanced P acquisition suitable for this ecology under low soil P conditions. The present investigation was undertaken with an objective to determine the effect of AM inoculation on root morphology and branching pattern of three upland varieties under low soil P conditions typical of the upland eco-system.

MATERIALS AND METHODS

The study was performed using three upland rice varieties-Sathi 34-36 (selection from land-race), Jonga (-indigenous land-race) and Vandana (modern high yielding, semi-tall variety). Each variety (single plant) was grown in black tubes (20 X 12 cm) containing 1 kg low phosphorus soil (3.89 ppm soil P) in 15 replications. The tubes were inoculated with AMF inoculum (1g/kg soil) containing- *Glomus intraradices* (AM+) with a population of 1066.39 MPN/g soil. Control tubes (AM-), without any inoculation were also maintained. The inoculum was procured from 'The Energy and Resources Institute' (TERI), New Delhi, India. The seeds were incubated in the oven at 48°C for 24 hours before direct seeding in the tubes for breaking possible dormancy. The inoculum was applied 2 inches below the seeds. The planted tubes were incubated in glass house with regular watering to maintain 25% of water holding capacity of test soil by weighing method. Glasshouse temperature was maintained at 35±1°C. Whole plants were harvested on 21 days after emergence (DAE).

Roots were stored in FAA (Formaldehyde-acetic acid-alcohol) and were scanned for measuring root characters/parameters (root length, root diameter, root surface area, root volume and root length density-RLD) and fractal dimension (FD) referred to as an index for the complexity of root branching pattern (Tatsumi *et al*., 1989, Fitter and Stickland, 1992). A *WinRHIZO Pro* 2012b (Régent Instrument Canada Inc.) system was used, connected to a EPSON Perfection V700 scanner equipped with an additional light unit (TPU). A 600 dpi (minimum dpi for reliable diameter measurements of fine rooted species) resolution was used for measuring roots morphology. The roots were arranged in a 22 cm wide and 30 cm long acrylic container, containing approximately 1 cm of water. With this device, three dimensional images were acquired and superposition of the roots was avoided.

Total dry weight was considered for growth rate analysis and mycorrhiza responsiveness (%MR) was calculated using the formula:

$$MR(\%) = \frac{(AM+)-(AM-)}{(AM-)} \times 100 \quad \text{(Hetrick et al. 1992).}$$

A separate experiment was performed for computing the tissue P content which was used as a measure of P acquisition capacity/P-demand of the variety in low soil P conditions. Each variety was grown in plastic tubes (40 X 12 cm) containing 2kg soil with the soil P maintained at 10ppm with the same moisture levels and temperature conditions as in the AM-responsive experiment mentioned above. Ten replications of each variety was maintained and the shoot harvested at 40 DAE. Tissue P content was calculated by digesting the dried and ground aerial matter in tri-acid mixture (Jackson, 1962).The P in the digest was estimated colorimetrically (Murphy and Riley, 1962).

Analysis of variance (ANOVA) was performed for all root morphological parameters at $p<0.05$ probability level.

RESULTS

The effect of mycorrhizal inoculation on the three varieties was measured in terms of %MR. Sathi 34-36 showed the highest %MR and was the only variety, among the three varieties tested, showing a significant difference in total dry matter production on AM inoculation suggesting the variety to be highly mycorrhiza responsive (Table 1). Vandana with a lower %MR was moderately responsive to mycorrhiza inoculation. Jonga, on the other hand, was non-responsive with a negative %MR. The difference in total dry matter production was non-significant for Vandana and Jonga. Growth rate (dry matter production in g/day/plant) of all the three test

Table 1: Mycorrhizal responsiveness based on total dry matter production (TDM) at 21 DAE, (n=5)

Parameters	Variety Sathi 34-36	Jonga	Vandana
AM- (TDM; g/plant)	0.064	0.111	0.058
AM+(TDM; g/plant))	0.075	0.101	0.061
Pr>F(T test)	0.046	NS	NS
MR%	17.19	-9.00	5.172

Table 2: Influence of AM inoculation on plant growth rate and Shoot-to-root-ratio at 21 DAE

Variety	Treatment	Growth rate (g/day/plant)	Shoot/root ratio (g/g)
Jonga	AM+	0.0048^{bc}	5.066^{a}
	AM-	0.0053^{c}	5.326^{ab}
Sathi 34-36	AM+	0.0036^{abc}	7.876^{c}
	AM-	0.0037^{abc}	7.958^{c}
Vandana	AM+	0.0029^{ab}	7.383^{c}
	AM-	0.0028^{a}	7.278^{bc}

*different letters within the treatments of each species indicate significant differences at the $p<0.05$ level to the LSD test.

Table 3: Root length density (RLD) at 21 DAE

Variety	Treatment	Root lengthdensity
Jonga	AM+	0.2037
	AM-	0.1926
Sathi 34-36	AM+	0.1799
	AM-	0.1741
Vandana	AM+	0.1021
	AM-	0.1622

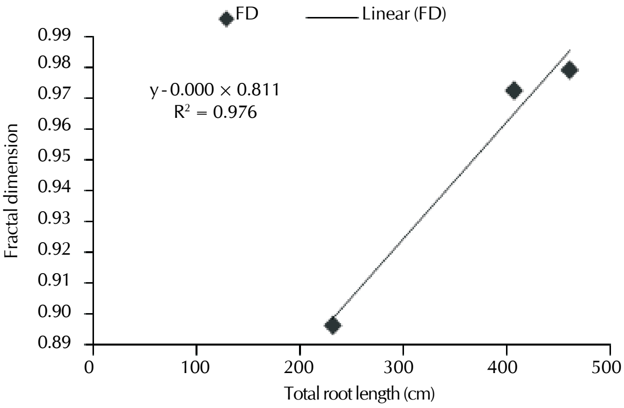

Figure: 1: Correlation between FD and Total root length (TLR) on AM in

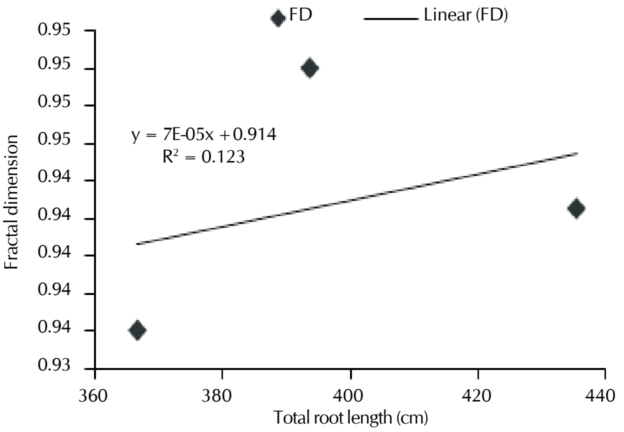

Figure 2: Correlation between FD and Total root length (TLR) without AM inoculation for all the three varieties

Table 4: Root diameter, root length, root volume, root surface area and root fractal dimension of the three varieties with AM inoculation (AM+) and control (AM-)

Variety	Treatment	Root dia. (cm)	Root length (cm)	Root volume (cm^3)	Root surface area (cm^2)	Root fractal dimension
Jonga	AM+	0.252 c	460.49 b	0.232 c	36.551 d	0.98 ns
	AM-	0.211 ab	435.37ab	0.180 bc	25.342abc	0.94
Sathi34-36	AM+	0.243 c	406.68b	0.183 c	30.434bcd	0.97
	AM-	0.247 c	393.67ab	0.182 c	29.970bcd	0.95
Vandana	AM+	0.188 a	230.85 a	0.075 a	14.571 a	0.90
	AM-	0.202 ab	366.73ab	0.119 ab	23.463 ab	0.94

varieties however, was not significantly influenced by AM inoculation though the shoot:root ratio of the two responsive varieties was significantly different to the non-responsive variety(Table 2).

RLD (Table 3), irrespective of AM inoculation, was highest for Jonga followed by Sathi 34-36 and Vandana. AM inoculation increased RLD of the former two but had a retarding effect on Vandana.

Root fractal dimension (Table 4), did not show significant differences between the treatments (AM+ and AM-) but tended to be higher in AM+ within varieties except Vandana. The FD values, on the other hand, showed significant correlation with the total root length of the three genotypes on AM inoculation as against the control (Fig 1).

Among the root parameters tested, AM inoculation significantly enhanced root diameter and thereby root surface area only in non-AM-responsive variety Jonga with figurative increase (in all root parameters including that of root diameter and surface area) in moderately (Vandana) to highly (Sathi 34-36) AM-responsive variety (Table 3). The highly responsive (Sathi 34-36) and non-responsive (Jonga) varieties followed the same pattern as of improved FD and RLD on AM inoculation with decrease in moderately responsive (Vandana) variety for all root morphological parameters.

Tissue P content (%) was highest for Sathi 34-36 (0.362 ppm), mediocre for Vandana (0.305 ppm) and lowest for Jonga (0.286 ppm).

DISCUSSION

It is well-established that plants with small root systems have high positive AM-response (AMR) and plants with extensive root system have low or no AMR when soil P availability is low (Smith and Smith, 2011). This was corroborated in our studies where Sathi 34-36 and Vandana with lower values of RLD, FD and other root morphological parameters were positively AM-responsive as compared to Jonga on AM inoculation which is AM non-responsive variety. AMF inoculation, however, increased all the root parameters (root diameter, root length, root volume and root surface area) in both AM-responsive (Sathi 34-36) and non-responsive (Jonga) with higher magnitutde in non-reponsive variety. The FD values were significantly correlated ($R^2 = 0.9767$) with the total root length for all the three varieties under AM inoculation as compared to that of no inoculation ($R^2 = 0.1235$). This conforms with studies already done in upland rice where FD and root length traits have showed significant correlation (Hong *et al*., 2008; Wang *et al*., 2009).This was mainly due to the trend that both values showed a decrease/increase in the presence or absence of inoculation. Variation between varieties (Sathi 34-36 vs. Vandana and Jonga vs Vandana) on inoculation being significant for root length, root diameter, root volume and root surface area suggest that arbuscular mycorrhizae improve the root system. Positive effect on improvement of root volume by *Glomus intraradices* inoculation has been demonstrated in sesame (Boureima *et al*., 2008) and alteration in root length, surface area and volume as a result of mycorrhization has also been reported in *Citrus* and maize. (Wu *et al*., 2012; Sheng *et al*., 2009)

Among the two AM-responsive varieties, Sathi 34-36 being highly responsive showed significantly greater variation in root morphological parameters compared to Vandana (moderately responsive), though the branching pattern was not significantly affected. This suggests that a higher response of a variety to mycorrhizal inoculation may partially be due to improved root characters as a consequence of the symbiotic association. Tissue P concentration was highest for Sathi 34-36 suggesting that it had an inherently higher P demand for supporting optimum growth and hence, is more responsive to AM inoculation for P acquisition. Jonga on the other hand with low P demand (low tissue P concentration) and higher soil exploration capability (high RLD) is not AM responsive. Similarly, Vandana having moderate P demand and RLD is moderately AM responsive and it has already been reported that it is the rate of P uptake which affects root branching pattern (Amijee et al 1989) even in the presence of mycorrhiza which are known to increase tissue P but have no positive effect on the dry weight (Smith and Smith, 2011).

Consistent higher values of root parameter in the control (AM-) compared to inoculation in moderately responsive variety Vandana suggested that even though it is responsive to inoculation, the mycorrhiza did not alter the root system in any way. Jonga on the other hand, inspite of being AM-non-responsive did benefit in terms of root morphology from AM-inoculation with a significant increase in root diameter and root surface area suggesting that mycorrhiza do alter the root system but might not always elicit a positive response in terms of increased dry matter production because of its (Jonga) inherent capability to meet lower P demand for attaining optimum growth. This was also supported by the fact that Vandana and Sathi 34-36 have a significantly higher shoot/root ratio than Jonga on AM inoculation. Generally, there are many cases where moderate, or even negative, effects under AMF inoculation occur (White *et al*., 2008; Detloff, 2011) as is in the case of these two varieties (Vandana and Jonga) but a majority of researchers refer AM inoculation to have a positive effect on a number of plant species(Lynch, 2007; Tawaraya *et al*., 2001).

The investigation, therefore, confirms that upland rice variety with lower soil exploration capability (low RLD) and high P demand (Sathi 34-36) are likely to be more arbuscular mycorrhiza responsive by means of AM induced improvement of root morphology and therefore liable to better acquire P in low soil P conditions typical of the upland ecology. Further studies via field experimentation still needs to be carried out.

REFERENCES

Aguín, O., Mansilla, J.P., Vilarino, A., and Sainz, M.J.2004. Effects of Mycorrhizal Inoculation on Root Morphology and Nursery Production of Three Grapevine Rootstocks. *American Society for Enology and Viticulture*. **55**:1-9.

Berta, G., Trotta, A., Fusconi, A., Hooker, J.E., Munro, M., Atkinson, D., Giovannetti, M., Morini, S., Fortuna, P., Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S.1995. Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera*. *Tree Physiology*. **15(5):**281-93.

Boureima, S., Diouf, M., Diop, TA., Diatta, ., Leye, E.M., Ndiaye, F. and Seck, D. 2008. Effects of arbuscular mycorrhizal inoculation on the growth and the development of sesame (*Sesamum indicum* L.). *African J. Agri. Research*. **3(3):**234-238

Chatzistathis, T., Orfanoudakis, M., Alofragis, D., Therios, I. 2012. Colonization of Greek olive cultivars' root system by arbuscular mycorrhiza fungus: root morphology, growth, and mineral nutrition of olive plants. *Scientia Agricola*. **70(3):**185-194.

Citernesi, A.S., Vitagliano, C., Giovannetti, M. 1998. Plant growth and root system morphology of Olea europaea L. rooted cuttings as influenced by arbuscular mycorrhizas. *J. of Horti. Sci. Biotech*. **73**: 647-654.

Detloff, S.J. 2010. The Effects of Mycorrhizae on the Growth of the Radish. *Raphanus sativus* L. *ESSAI*. **8**:Article 15.

Fitter, A.H. and Stickland, T.R. 1992. Fractal characterization of root-system architecture. *Functional Ecology*. **6**:632-635

Hetrick, B.A.D., Wilson, G.W.T. and Cox, T.S. 1992. Mycorrhizal dependence of modern wheat varieties, landraces and ancestors. *Can. J. Botany*. **70**:2032-2040

Hong, W., Ji-Yun, J. and Akira, Y. 2008. Fractal analysis of root system architecture by box-counting method and its relationship with Zn accumulation in Rice (*Oryza sativa* L.). *Acta Agronomica Sinica*. **34(9)**:1637-1643.

Jackson, M.L. 1962. *Soil Chemical Analysis* (1st edition),1962, Asia Publ. House, New Delhi, India. pp. 29-32.

Johansen, A., Jakobsen, I. and Jensen, E.S.1993. External hyphae of vesicular arbuscular mycorrhizal fungi associated with *Trifolium subterranean* L. III. Hyphal transport of ^{32}P and ^{15}N. *New Phytologist*, **7**:365-386.

Li, X.L., George, E. and Marschner, H. 1991. Extension of the Phosphorus depletion zone in VA-mycorrhizal white clover in calcareous soil. *Plant and Soil*. **136**:41-48.

Lynch, J. 1995. Root architecture and Plant productivity. *Plant Physiology*. **109**:7-13

Lynch, J.P. 2007.Roots of the second green revolution. *Austrian Journal of Botany*. **55**:491-501.

Marschner, H. *Ed*. **1995** Mineral nutrition of higher plants. *Academic Press*.

Malusa, E., Sas-Paszt, L., Popinska, W. and Zurawich, E. 2007.The effect of a substrate, containing arbuscular mycorrhizal fungi, and rhizosphere microorganisms (Trichoderma*, Bacillus, Pseudomonas and Streptomyces*), and foliar fertilization on growth response and rhizosphere pH of three strawberry cultivars. *Internat. J. Fruit Science*. **6**: 25-41.

Murphy, J. and Riley, J.P. 1962. A modified single solution method for determination of phosphorus in natural water. *Anaytica Chim. Acta*.27:31-36.

Schellenbaum, L., Berta, G., Ravolanirina, F., Tisserant, B., Gianinazzi, S., Gianinazzi-Pearson, V. and Fitter, A.H. 1991. Influence of endomycorrhizal infection on root morphology in a micropropagated woody plant species (*Vitis vinifera* L.) *Annals of Botany* **68**:135-141

Sheng, M., Tang, M., Chen, H., Yang, B., Zhang, F. and Huang Y. 2009. Influence of arbuscular mycorrhizae on root system of maize plants under salt stress. *Can. J. Microbiology*. **55(7):**879-886.

Smith, F.A. and Smith, S.E. 1997. Structural Diversity in (vesicular) arbuscular mycorrhizal symbiosis. *New Phytologist* **137**:373-388

Smith, F.A. and Smith, S.E. 2011. What is the significance of the arbuscular mycorrhizal colonization of many economically important crop plants? *Plant soil* **348**:63-79

Tatsumi, J., Yamauchi, A. and Kono, Y. 1989. Fractal analysis of plant root systems. *Annals of Botany* **64**:499-503

Wang, H., Siopongco, J., Wade, L.J., and Yamauchi, A. 2009. Fractal analysis on root systems of rice plants in response to drought stress. *Environ. Experimental Botany*. **65**:338-344.

White, J.A., Tallaksan, J.,and Charvat,I. 2008. The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. *Mycologia*. **100(1):**6-11.

Wu, Q.S., He, X.H., Zu, Y.N., Liu C.Y., Xiao, J. and Li, Y. 2012. Arbuscular mycorrhizas alter root system architecture of *Citrus tangerine* through regulatory metabolism if endogenous polyamines. *Plant Growth Regulation*. **68(1)**: 27-35.