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Arbuscular mycorrhizal fungal diversity in *Oryza sativa* (rice) varieties cultivated in *Khazan* lands in Goa

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ABSTRACT

This study was conducted to assess arbuscular mycorrhizal (AM) fungal diversity associated with rice (*Oryza sativa* L.) cultivated in the *Khazan* lands in Goa. AM fungi (*Glomeromycota*) are vital components of almost all terrestrial ecosystems, forming a mutualistic symbiosis with roots of more than 80% of vascular plants including agronomically important species. Roots of rice varieties from six different agricultural sites were found to be colonized, with AM fungi ranging from 18.0% to 98.0%. Variety Korgut showed the least mycorrhizal colonization while maximum colonization was recorded in variety Jyoti. AM fungal species belonging to four genera *viz., Acaulospora, Glomus, Funneliformis* and *Entrophospora* were recorded from the rhizosphere soils and *Acaulospora* being the dominant genus.

Key words: Root colonization, spore density, endomycorrhiza

INTRODUCTION

Arbuscular mycorrhiza is a mutualistic association between fungi and plant roots. In this association the fungus receives photosynthetically derived carbon compounds from the green plants and the plants have an increased access to mineral nutrients especially phosphorus (P) (Rivera *et al.*, 2005) and other minerals like K, Fe, Cu, Ca, Mg and Zn (George, 2000;Yaseen *et al.*, 2011). The association also helps to improve the tolerance of the host plant towards biotic (Singh *et al.*, 2000) and abiotic stress (Gaur and Adholeya, 2004).

Goa is the smallest state of the Republic of India, its position is marked by 15º 48' 00" N and 14° 53' 54" N Latitude and 74° 20' 13" E and 73° 40' 33" E Longitude having a total geographical area of 3,61,113 hectares covering both north and south Goa districts (Gune, 1979). Rice (*Oryza sativa* L.) is the predominant staple food crop of Goa. Rice fields here are called differently, depending on the soil, rainfall conditions and nearness to the riverside. They have been distinguished into *Morod* (Upland), *Ker* (Midland) and *Khazan* (Lowland).

Khazans is a konkani term in Goa for its coastal saline lowland soils. They are integrated agro-aqua ecosystems which are traditionally managed. They have been reclaimed over centuries from marshy mangrove swamps with an intricate system of bunds and sluice gates. The gates protect the fields from inundation and control the water flow in and out of the rivulets. In Goa these lowlands were originally used for paddy cultivation, traditional farming, pisciculture and salt extraction. Paddy fields have been cultivated by using bunds to keep the sea water away and sluice gates to control the inflow of saline water.

Agricultural lands are artificial ecosystems and are subjected to human intervention. Nature's diversity, due to agriculture, is replaced with a small number of cultivated plants. With the change of natural ecosystem to agro-ecosystem and increase in the intensity of agricultural inputs there is a decrease in AM fungal diversity (Oehl *et al.*, 2003; Jefwa *et al.*, 2012). Rice is grown in different ecosystem, when cultivated in the uplands readily forming mycorrhizal association has been reported by Ilag *et al.* (1987). Barea (1991) has reported that AM fungi can survive in water logged condition. Wetland rice was previously considered to be non mycorrhizal but a positive response to AM fungal inoculation has been observed (Sharma *et al.*, 1988). AM fungi are important in organic and sustainable farming system that relies on biological process rather than agrochemicals (Harrier and Watson, 2004), thus offering a great potential for sustainable agricultural system (Khalil *et al.*, 1992). A better understanding of the field study, based on AM fungal diversity associated with agronomic crops is necessary. Hence, in the present paper, an effort was made to study the AM fungal association in the different varieties of rice cultivated in different *Khazan* lands of Goa.

MATERIALS AND METHODS

Collection of rhizosphere soil samples: Field visits were conducted during flowering stage in rice dominated *Khazan* areas of six different talukas of Goa. The mean maximum and minimum temperature recorded during that period were 32.11º C and 23.4º C, respectively with relative humidity ranging from 46 to 95.68%, the seasonal total rainfall was 2595.1 mm as obtained from the Meteorological Department, of ICAR (Central Coastal Agricultural Research Institute, Goa). Three healthy plants of each of the 11 varieties *viz.*, Jyoti, Jaya, Assgo, Bello, Damgo, Kalo korgut, Kalo novan, Khochri, Korgut, Muno and Shiedi (**Table 1**) were collected randomly from different parts of the *Khazan* lands at each site. While sampling rhizosphere soil was collected along with the roots. Samples were collected within 0-25 cm depth and then mixed thoroughly to obtain a composite sample of approximately 500 g of soil from June 2015 to November 2015 and brought to the laboratory for further analyses.

Soil Analyses: From the composite sample three sub samples were drawn and analyzed separately. Soil pH was measured in 1:1 water solution suspension using a pH meter (LI 120 Elico, India). Electrical conductivity (EC) was measured using conductivity meter (CM 180 Elico, India). Walkley and Black (1934) rapid titration method was used to estimate organic carbon content. Nitrogen was assessed by micro-Kjeldahl method (Jackson, 1971). Available P was estimated using Bray and Kurtz method (1945). Potassium (K) was estimated by ammonium acetate method (Hanway and Heidal, 1952).

Estimation of AM fungal root colonization: The root samples were processed for AM fungal colonization using Phillips and Hayman (1970) method. Three samples were considered per variety per site. The roots were cleared in 10% KOH heated at 90ºC, acidified in 5N HCl and stained with Trypan blue. The stained roots were examined using an Olympus research compound microscope (100x 1000x) for AM fungal structures. Percentage of root colonization was determined by root slide method (Read *et al.*, 1976).

Spore density, abundance and taxonomic identification: Wet sieving and decanting method by Gerdemann and Nicolson (1963) was used to isolate AM spores. Estimation of AM fungal spore density was carried out following the method of Gaur and Adholeya (1994). To identify AM morphotypes, intact and unparasitized spores were used. Spores were identified by comparing them to the descriptions in Schenck and Perez (1990), Almeida and Schenck (1990), Rodrigues and Muthukumar (2009) and International Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf.wuv.edu). Species richness (SR) is the number of AM fungal species recovered from each site per sample collection. Relative abundance (%) for each area was calculated by using the following formula (Beena *et al.*, 2000).

$$\text{Relative Abundance (\%)} = \frac{\text{Number of spores of particular AM species}}{\text{Total spore number of all the AM Species}} \times 100$$

Diversity Studies: Diversity studies were conducted for each site separately by calculating Simpson's Index of Diversity 1-D (Simpson, 1949) where $D = 1 - \Sigma (P_i)^2$, $(P_{i=} n_i / N)$. n_i the relative abundance of the species is calculated as the proportion of individuals of a given species (n_i) to the total number of individuals in a community (N) and Shannon Wiener diversity index (H) by Shannon and Wiener (1949), which was used to characterize species diversity in a community, accounting for both abundance and evenness of the species present using the formula: $H = -\Sigma (Pi \ln (P_i))$.

Statistical Analyses: Data of AM fungal colonization and spore density were statistically analyzed for standard deviation. Relationship of AM fungal root colonization to spore density was determined by Pearson's correlation coefficient using WASP (Web based Agricultural Package) 2.0($P \leq 0.05$) significance level.

RESULTS AND DISCUSSION

Rice is a staple food in Goa. It is cultivated only once a year, during the rainy season, in the *Khazan* lands **(Table 1)**. Results of soil analysis of the different agricultural sites are shown in **Table 2**. From the results, it was observed that the *Khazan* soil is acidic, and the pH ranged from 4.8 to 6.4, EC ranged from 0.07 to 0.50 dS/m. Available P ranged from 4.48 to 67.2 kg/ha, while available K ranged from 54.90 to 269.00 kg/ha. Such variation can be attributed to the constant flushing and washing of salt water into the area, which leads to the deposition of salt at different region (Rodrigues and Anuradha, 2009)

Rice has a shallow, fibrous rooting system. All 11 rice varieties cultivated in the six different *Khazan* lands showed

Table 1: Geographical location and rice varieties cultivated at the study sites.

Taluka	Site	Rice Variety	Geographical coordinates		
			Latitude	Longitude	Altitude
Pernem	Tuem	Jyoti, Jaya, Shiedi, Korgut	15° 30' 22'' N	73° 48' 12''E	3 m
Bicholim	Sikeri	Jyoti, Khonchri, Shiedi, Muno, Kalo Novan, Kalo Korgut, Bello, Damgo, Assgo	15° 35' 18'' N	73° 53' 20''E	7 m
Salcette	Chinchinim	Jyoti, Jaya, Korgut	15° 11' 29'' N	73° 58' 22''E	10 m
Ponda	Shiroda	Jyoti, Jaya Assgo	15° 18' 31'' N	74° 01' 18''E	11 m
Tiswadi	Neura	Jyoti	15° 26' 25'' N	73° 54' 30''E	12 m
Bardez	Salvador do Mondo	Korgut	15° 53' 78'' N	73° 84' 38''E	20 m

Table 2: Soil Chemical analysis of the agricultural soils in each site.

Soil characteristics	Agricultural Study sites in Goa					
	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo
pH	5.20	6.40	5.30	5.10	5.00	4.80
E.C. dS/m	0.19	0.07	0.50	0.30	0.40	0.30
Organic carbon %	0.49	1.54	1.21	5.11	1.63	1.10
Nitrogen kg/ha	220.50	423.50	350.90	204.00	80.00	55.00
Phosphorus kg/ha	22.40	11.86	67.20	4.48	71.68	10.23
Potassium kg/ha	150.30	156.80	213.00	269.00	123.00	54.90

Table 3: AM root colonization of different rice varieties cultivated in *Khazan lands.*

Rice Variety	Agricultural Study sites in Goa					
	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo
Assgo	nd	79.33 ± 0.57	nd	31.70 ± 1.55	nd	nd
Bello	nd	28.33 ± 1.52	nd	nd	nd	nd
Damgo	nd	26.00 ± 2.00	nd	nd	nd	nd
Kalo Korgut	nd	52.00 ± 1.73	nd	nd	nd	nd
Korgut	66.53 ± 1.50	nd	18.00 ± 2.00	nd	nd	nd
Kalo Novan	nd	24.00 ± 1.00	nd	nd	nd	nd
Khonchri	nd	27.33 ± 2.08	nd	nd	nd	nd
Shiedi	76.23 ± 1.36	nd	nd	nd	nd	nd
Muno	nd	28.00 ± 1.00	nd	nd	nd	nd
Jyoti	88.33 ± 1.52	98.33 ± 0.57	42.86 ± 3.38	76.33 ± 1.85	48.66 ± 1.52	28.00 ± 1.15
Jaya	81.00 ± 1.00	nd	23.33 ± 3.51	27.68 ± 2.51	nd	nd

Legend: nd = Rice variety not detected in study site; Data presented is the mean of three readings ± SD

AM colonization (**Table 3**). Percent A roMot colonization in different rice varieties varied from site to site. Maximum root colonization was observed in variety Jyoti (98%) at Sikeri and minimum was in variety Korgut (18%) at Chinchinim.

The rhizosphere soils showed variation in AM spore number (**Table 4**). Maximum spore density was observed at Sikeri (203 spores) and minimum was observed in Salvador do Mondo (18 spores). The highest number of spores was observed in the variety Assgo (52 spores $100g^{-1}$ of soil) from Shiroda and the least was recorded in variety Jyoti (5 spores $100g^{-1}$ of soil) at Sikeri. There was no significant correlation between root colonization and spore density at any of the study sites. This finding is in agreement with Miller (2000) and D' Souza and Rodrigues (2013). Variation in AM fungal association and spore number are known to be affected by rapid changes in soil nutrients (Abbott and Robson, 1991), environmental factors, soil fertility (Brundrett, 1991) or soil disturbances in the sites (Jasper *et. al.*,1991; Boddington and Dodd, 2000).

In the present study, the rhizosphere soils of different varieties of rice cultivated in the *Khazans* showed variation in AM fungal diversity. A total of 14 AM fungal species were recorded from six agricultural study sites **(Table 5).** *Acaulospora* was the dominant genus, represented by eight species and this may be due to the fact that *Acaulospora*

Table 4: AM fungal species and spore density in agricultural study sites

Rice Variety	Agricultural Study sites in Goa					
	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo
Assgo	nd	*A. di., A. so., A. la., F. mo.* 38.33 ± 2.88	nd	*A. di., R. fa.* 52.33 ± 2.08	nd	nd
Bello	nd	*A. di., A. la.* 16.66 ± 1.20	nd	nd	nd	nd
Damgo	nd	*R. fa.* 31.33 ± 0.57	nd	nd	nd	nd
Kalo Korgut	nd	*F. mo. , A. tu., E. ne.* 25.00 ± 0.34	nd	nd	nd	nd
Korgut	*A. sc., A. de.* 20.00 ± 0.57	nd	*A. de., A.la.* 36.66 ± 0.66	nd	nd	nd
Kalo Novan	nd	*E. in., R. fa.* 26.00 ± 1.15	nd	nd	nd	nd
Khonchri	nd	*A. my., R. fa.* 35.00 ± 1.15	nd	nd	nd	nd
Shiedi	*A. de. , A. di , A. la.* 25.00 ± 0.54	nd	nd	nd	nd	nd
Muno	nd	*G. fa.* 25.00 ± 0.52	nd	nd	nd	nd
Jyoti	*A. sc., A. de., A. bi., E. ne.* 15.33 ± 0.50	*R. fa., F. mo., E. ne., G. mi.* 5.33 ± 0.53	*A. de., A. la.* 26.66 ± 0.57	*A. di., F. mo.* 27.00 ± 2.00	*A. bi., F. mo.* 25.33 ± 1.45	*F. mo., A. di., A. de.* 17.66 ± 1.51
Jaya	*A. de. , A. di., A. sc.* 39.00 ± 0.50	nd	*A. de., G. ag.* 24.66 ± 1.52	*A. di., F. mo., R. fa.* 48.33 ± 4.16	nd	nd
Total No. of spores	99.98	202.98	87.98	127.66	25.33	17.66

Legend: Rice variety not detected in study site; data presented is the mean of three readings; ± SD

Table 5: Spore Abundance of AM fungal species in agricultural study sites.

AM species	Spore abundance at study sites					
	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo
Acaulospora bireticulata	5.2	nd	nd	nd	59.4	nd
Acaulospora delicata	29.8	nd	61.6	nd	nd	33.9
Acaulospora dilatata	22.6	10.3	nd	67.1	nd	39.6
Acaulospora laevis	7.2	11.3	33.7	nd	nd	nd
Acaulospora scrobiculata	34.0	nd	nd	nd	nd	nd
Acaulospora soloidea	nd	1.9	nd	nd	nd	nd
Acaulospora tuberculata	nd	6.4	nd	nd	nd	nd
Acaulospora myriocarpa	nd	11.3	nd	nd	nd	nd
Entrophospora infrequens	nd	4.9	nd	nd	nd	nd
Entrophospora nevadensis	1.0	2.9	nd	nd	nd	nd
Funneliformis mosseae	nd	7.4	nd	12.5	40.5	26.4
Glomus aggregatum	nd	nd	4.6	nd	nd	nd
Glomus microcarpum	nd	0.4	nd	nd	nd	nd
Rhizoglomus fasciculatum	nd	42.5	nd	20.3	nd	nd

Legend: nd = AM species not detected in study site .

species are often associated with acidic soils (Morton, 1986; Abbot and Robson, 1991). The other genera included *Glomus* (2 spp.), *Entrophospora* (2 spp.), *Funneliformis* (1 sp.) and *Rhizoglomus* (1 sp.). Species of *Acaulospora* are identified mainly in low input farming system and are considered as facultative symbionts adapted to a wide array of soils and host species, appearing in soils of widely different pH and nutrient availability (Sieverding, 1991; Shepherd *et al.*, 1996; Straker *et al.*, 2010). The highest numbers of AM fungal species recovered were from variety Jyoti (4) at Tuem, and Assgo (4) and Jyoti (4) at Sikeri. Variation in AM fungal diversity in rhizosphere soils in the different sites may be due to factors such as pH, available P or other nutrients in the soil (Chetan *et al.*, 2008).

Variation in abundance of AM species was observed in all the study sites. Similar observations have been reported in earlier studies (Schenk and Kinlock, 1980; Chetan *et al.*, 2008). Our study revealed that *A. scrobiculata* was the most abundant species in Tuem, *R. fasciculatum* in Sikeri, *A. delicata* in Chinchinim, *A. bireticulata* in Neura, and *A. dilatata* in Shiroda and Salvador do Mondo (**Table 5**). *Gigaspora* and *Scutellospora* species were not detected in any of the study sites. Bever *et al.* (1996) reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment due to their smaller spore size, and require less time to sporulate (Hepper, 1984).

Table 6: Diversity of AMF community at different agricultural study sites.

Ecological parameters	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo
Simpson's Index of diversity	0.734	0.769	0.504	0.492	0.482	0.658
Shannon-Weiner Index (*H*)	1.454	1.820	0.880	0.851	0.675	1.085
AMF species richness (SR)	6	10	3	3	2	3

Species richness (**Table 6**) was maximum in Sikeri (10) and the minimum in Neura (2). Simpson's Index of diversity was maximum at Sikeri (0.769) and least in Neura (0.482) which indicates shared dominance of AM fungal species. Shannon-Weiner diversity Index was higher in Sikeri (1.82) suggesting greater diversity. The higher the diversity, the greater the benefits crops gain, as the AM community will span a broader range of functions (Koide, 2000). Hence there is a need to identify AM fungal species in agricultural sites, study their effects on agricultural practices to develop best regime suited for the crop.

CONCLUSION

The present work documents the diversity of AM fungi with rice cultivated in the K*hazan* lands of Goa. However, further studies need to be carried out to understand the association of AM fungi at different growth stages of rice in K*hazans* and other land types.

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