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Diversity of arbuscular mycorrhizal fungi in irrigated and non-irrigated fields of southern Karnataka, India

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Abstract

The two different agro-ecosystems were selected to study the spore density, species abundance, and diversity of arbuscular mycorrhizal fungi (AMF) in irrigated (Mandya district) and non-irrigated (Hassan district) agricultural fields in southern Karnataka region, India. A total of 22 AMF species were recorded during the study. Out of which 13 sp. were of *Glomus*, 4 sp. of *Acaulospora*, 1 sp. of *Cetraspora,* 1 sp. of *Dentiscutata* and 3 sp. of *Gigaspora*. The difference in species richness of AMF species in irrigated fields ranged from 5-12 sp. as compared to non-irrigated fields (5-11 sp.) and the difference may be attributed to the nutritional status of the soil. We also assumed that lower AMF colonization and abundance would be affected by water stress. Highest spore number and percent colonization of AM fungi were recorded in irrigated sites, showing 356-748 spore density and 70-92 % colonization. Whereas, in non-irrigated sites, 174-341 spore density and 40-72 % colonization was recorded. Different agro-climatic conditions like irrigation, soil pH, soil organic carbon, phosphorous correlated with the abundance and colonization of AM fungi.

Key words

Arbuscular mycorrhizal fungi (AMF), Spore density, Diversity

Introduction

Arbuscular mycorrhizal fungi (AMF), which are ubiquitous in nature, forms associations with the majority of terrestrial plant species and have been shown to improve the growth and nutrition of individual plants (Smith and Read, 1997). Until recently, AMF species were generally assumed to be functionally similar, so there was little focus on AMF diversity in natural habitats. However, some researchers (VanderHeijden *et al*., 1998) have demonstrated that plant diversity and ecosystem variability and productivity (Hart and Klironomos, 2002) are directly influenced by AMF diversity, making an accurate assessment of species richness and community composition crucial to understanding the role of AMF in ecosystem functioning. It is now widely accepted that climatic and edaphic factors can substantially influence AMF association and their population (Karthikeyan and Selvaraj, 2009). AMF can play a significant role in increasing phosphorus uptake (Koide *et al.,* 2000), which may be associated with increased growth and yield crop plants (Vosatka, 1995). AMF have also been shown to improve both water status and nutritional status of the host plants (Auge, 2001).

The major factors affecting the diversity, abundance and distribution of AM fungi in agro-ecosystems are soil pH, phosphorous (P), nitrogen (N), organic matter, water stress etc. These factors could also affect the crop production in different agro-ecosystems (Porras-Soriano *et al*., 2009). Water availability has been recognized as the most critical determinant of crop productivity in agro-ecosystems. Water deficit and soil nutrients imbalance are the most common stresses affecting the diversity, abundance of AMF in arid and semi-arid regions (Cartmill *et al.,* 2008).However, there are examples where crops fail to respond to colonization by native AMF (Ryan *et al.,* 2002) and in many cases, may be due to a high concentration of available soil P (Bethlenfalvay and Barea, 1994). Similarly

some modern agricultural practices such as continuous monoculture, non host crop in rotation, tillage can also impact on the AM association, both directly, by damaging or killing AMF and indirectly, by creating conditions either favorable or unfavorable to AMF. Conversion from natural habitats to agricultural lands has been referred as one of the leading causes for loss of biodiversity worldwide. In cultivated lands, AMF population, species composition, and diversity are often decreased compared to natural ecosystem (Helagson *et al.,* 1998).

The majority of research on AMF has focused on AMF-plant symbiosis, diversity in natural conditions like forest, grasslands. In the present study, an effort was made to evaluate the diversity of AM fungi in irrigated and non-irrigated agricultural fields of Mandya and Hassan district of Karnataka State, India.

Materials and Methods

Study site : Two geographical regions, *viz*., Hassan district (Non-irrigated) lying between 12.13º and 13.33º N latitudes and 75.33º and 76.30º E longitude and Mandya district (Irrigated) lying between 12.52^{0}N and 76.9^{0}E.Mandya district is well facilitated with channel irrigation of Krishnarajasagara Reservoir. Total 10 sampling fields (5 from Mandya and 5 from Hassan district) were selected for the study. The fields of Hassan districts were Arasiekere (ARSK-1) were the standing crop was sunflower *(Helianthus annuus)*, Hassan (HSN -1) were the standing crop was Finger millet *(Elusine coracana*), Channarayapatna-1 (CRP-1) were the standing crop was Sorghum *(Sorghum bicolor*), Channarayapatna-2 (CRP-2) were the standing crop was Red Gram (*Cajanus cajana*) and Holenarasipura (HNP- 1) were the standing crop was maize (*Zea mays*). The fileds of Mandya were Bannur (BNR-1) were the standing crop was Finger Millet *(Elusine coracana)*, Malavalli (MLV-1) were the standing crop was Brinjal *(Solanum melongena)*, Maddur (MADD-1) were the standing crop was Maize (*Zea mays*), Mandya (MND- 1) were the standing crop was tomato (*Lycopersicon esculentum)* and Pandavpura (PDP- 1) were the standing crop was black gram *(Vigna mungo)*. All the agricultural fields surveyed were practicing conventional farming methods.

Collection and analysis of soil : The rhizospheric soil samples from the depth of 10-30 cm below soil surface from all the 10 fields were collected randomly for analysis of physico-chemical parameters individually as per the method of Chaturvedi and Sankar (2006).

Identification of AM fungi : Root samples from the standing crop of irrigated and non-irrigated fields were stored in FAA solution. The cleaned roots were cut into 1cm long pieces and stained with trypan blue, according to the procedure described by Philips and Hayman (1970). The roots were boiled in 10% KOH for 1hour, acidified with 5N HCl and stained overnight with 0.5% trypan blue. The roots were then destained with 70% glycerol. The assessment of mycorrhizal infection was done by the slide method; root segments were selected randomly from the stained samples and observed for the presence or absence of functional structures (mycelium, arbuscules and vesicles) of AM fungi. A minimum of 100 root segments were used for this enumeration and the percent colonization of AM fungi was calculated using the following formula (Giovanetti and Mosse, 1980);

Extraction of AM fungal spores was carried out using wet sieving and decanting method (Gerdemann and Nicolson, 1963). 100 g of soil was suspended in tap water and mixture was decanted through stacked sieves. The sieve sizes ranged from 32-420mm and the spores from bottom sieve and middle were collected on Petri dish and counted under stereo zoom microscope (Labomed-CZM6). Spores were then mounted with PVLG (polyvinyl alcohol + Lactic acid + Glycerol), for identification of AM fungal species. Species were photographed and identified up to species level according to Schenck and Perez (1990).

Spore density was defined as the number of AMF spores and sporocarps in 100gm soil; species richness was measured as the number of AMF species occurred per soil sample; Isolation frequency = (the number of samples in which a given species was isolated / the total number of samples) x100%; Relative abundance = (the number of a given species spore/the total number of spores) x 100%; Shannon-Wiener index of diversity (H) = $- \sum p_i \ln[p_i]$ where, p_i is the proportion of total number of species made up of the $_i$th species (Li *et al.,* 2007). Pearson's correlation coefficient [*r*] was calculated between percent colonization, spore population, soil pH, soil 'P' and soil 'K', using SPSS software (version 17.0).

Results and Discussion

AMF spore density varied greatly among irrigated sites and non-irrigated sites. Although percent colonization did not vary too much as compared to spore density. The spore density was higher in samples collected from site BNR-1of Mandya district (743 spores 100 g^{-1} of soil) followed by PDP-1 (599 spores 100 g of soil), MLV-1 (543 spores 100 g^{-1} of soil) and MADD-1 (418 spores 100 g^{-1} of soil). Low spore density was observed in samples collected from site ARSK-1 of Hassan district (247 spores 100 g^{-1} of soil) followed by HNP-1 (174 spores 100 g^{-1} of soil) (Table 1). Contrary to spore density, the maximum species richness occurred in Hassan district samples, in which 11 species were recovered each from ARSK-1, HSN-1, HNP-1 and CRP-

Table 1 : Percent colonization,spore population, Shannon-Wiener index and species richness of AM fungi in samples collected from Hassan and Mandya districts

Sl. No.	Sample Name	Percent (%) colonization	Spore density $100g^{-1}$ m dry soil	Shannon-Wiener index (H)	Species richness
1	HNP-1	68	174	2.28	11
2	HSN-1	40	320	2.115	11
3	CRP-1	72	341	1.549	5
4	CRP-2	64	367	2.24	11
5	ARSK-1	50	247	2.007	11
6	BNR-1	92	743	2.39	12
7	MND-1	70	356	1.364	5
8	MADD-1	89	418	1.61	8
9	PDP-1	72	599	1.91	9
10	MLV-1	76	543	1.84	7

2 samples. But in Mandya district, only the samples of BNR-1 showed highest number (12) of species abundance when compare to others, where it was comparatively less (Table1).

Twenty two taxa of AMF were isolated from the 10 sampling sites. Among the 22 taxa, 13 species belonged to genus *Glomus* and 3 each from *Acaulospora* and *Gigaspora* and 1 sp. each of *Cetraspora* and *Dentiscutata* (Table 3). One species of *Acaulospora* and one species of *Gigaspora* were not identified to species level.

Glomus and *Acaulospora* species occurred most frequently, followed by *Gigaspora, Cetraspora* and *Dentiscutata. Glomus fasciculatum, Glomus ambisporum, Glomus mossae, Glomus versiforme, Glomus intraradices, Glomus coronatum* and *Acaulospora myriocarpa* were identified as dominant species based on the isolation frequency and relative abundance, as these species exhibited >50% IF and >5% RA. *Glomus albidum, Glomus verruculosum, Dentiscutata armeniaca* and *Gigaspora albida* were found less frequently in all the samples and also in the view of RA. Among 22 species of AM fungi identified *Glomus mossae, Glomus fasciculatum* and *Glomus ambisporum* were found to be dominant (Table 1).

The diversity of AM fungi expressed by Shannon-Wiener index is presented in Table-1. The samples collected from Mandya district exhibited less index of diversity when compared to Hassan district. In Mandya district, the maximum diversity index observed in the BNR-1 sample which exhibited 2.39, whereas all other samples showed diversity index < 2. In Hassan district almost all the samples exhibited diversity index > 2, except CRP-1 which exhibited 1.54 index of diversity. Occurrence of maximum number of species would results to higher index of diversity in the Hassan samples.

The results of soil physico-chemical analysis were displayed varied parameters among different sampling sites. Soil pH of irrigated fields varied from 6.25 to 7.46, whereas in non-irrigated filed it ranged from 4.87-7.72. Electrical conductivity in irrigated fields was ranged from 0.07 to 0.30 mmhos cm^{-1}, whereas, in non-irrigated fields it was 0.02-0.40 mmhos cm^{-1}. The soil phosphorous was recorded 54.7 to 142.8 kg ha^{-1} and 2.0 to 70.7 kg ha^{-1} respectively in irrigated and non-irrigated fields. Available phosphorus ranged from 90 to 296 kg ha^{-1} and 244 to 717 kg ha^{-1} in irrigated and non-irrigated fields kg ha^{-1} respectively. Organic carbon content of all the soil samples were ranges from 0.28-0.88%.

The correlation studies between percent AM colonization, spore count, soil pH, soil P and K is summarized in Table-2. Correlation analysis of non-irrigated sites revealed that soil K significantly correlated with soil P (r=0.934, $p<0.05$). Whereas, soil pH positively correlated with percent colonization (r= 0.697) and spore count positively correlated with the amount of K and P in the soil (Table2). In irrigated soil samples, percent colonization, spore numbers were not significantly correlated with either total P or total K or pH of the soil.

The occurrence and distribution of arbuscular mycorrhizal fungi in two different agro-climatic conditions were carried out to study community composition and species diversity. Although 22 AMF species recovered in the present study, we acknowledge that this number does not represent the total diversity of AM fungi occurring in these regions. It is evident from the earlier reports that agricultural soils have a low diversity of AMF compared with natural ecosystems. When a soil is put to agricultural use, it undergoes a series of physical, chemical and morphological changes, which can affect the root inhabiting microorganisms and plant growth (Bellgard, 1994). The dependence of spore population on soil pH was also reflected in the present study. Although genus *Glomus* has been

found to be abundant in the rhizosphere soil of crop plants, the species composition of AM fungi varied in different sampling sites. The present findings are correlated with the results of Bhattacharjee and Sharma (2011) that the species of *Glomus* are dominant in Indian soils. The study also revealed that, there is not much difference in the percent association of AM fungi in irrigated sites when compared to non-irrigated sites, but statistically it is an important observation. Further, the spore count was found to be high in irrigated soils when compared to non-irrigated soils. However, the study recorded a huge variation in spore density between irrigated and non-irrigated sites. This may reflect the positive impact of irrigation on AMF abundance.

Spore density was higher in irrigated samples as compare to non-irrigated. But, there was no correlation between spore numbers found in the soil and percent colonization in standing crops in both the conditions. In contrast to spore density, the species richness and Shannon-Wiener index of diversity were found to be the highest in non-irrigated samples when compared to irrigated samples. Spore count in non-irrigated samples were positively correlated with P (0.782) and K (0.617) and negatively correlated with percent colonization (-0.226) and soil pH (-0.836) (Table-2). Whereas in irrigated samples, there is no significant correlation among the different parameters except spore count, which was positively correlated with most of the factors.

The physico-chemical parameters of soil from the study sites revealed that there is considerable variation in soil pH from place to place. Soil chemical parameters could be the determinant factor for AM fungi colonization in the crop plants under study. One of the factors known to influence spore numbers is soil pH (Michel-Rosales and Valdes, 1996). The samples from non-irrigated soil were

Table 2 : Results of correlation studies of non-irrigated fields of Hassan district

	% colonization	Spore count	Soil pH	Soil 'P'	Soil 'K'
% colonization	1	-0.226(0.714)	0.697(0.191)	-0.260(0.672)	-0.034(0.956)
Spore count	-0.226(0.714)	1	-0.836(0.078)	0.782(0.118)	0.617(0.267)
Soil pH	0.697(0.191)	-0.836(0.078)	1	-0.656(0.230)	-0.373(0.536)
Soil 'P'	-0.260(0.672)	0.782(0.118)	-0.656(0.230)	1	0.934*(0.020)
Soil 'K'	-0.034(0.956)	0.617(0.267)	-0.373(0.536)	0.934*(0.020)	1

* Correlation is significant at the 0.05 level (2-tailed)

Table 3 : Isolation frequency, Relative abundance and Species richness of the identified AMF species in the different samples

Species	HSN-1	HNP-1	ARSK-1	CRP-1	CRP-2	BNR-1	MLV-1	MADD-1	MND-1	PDP-1
Acaulospora sps1	+	-	+	-	+	-	-	-	-	+
Acaulospora denticulate	-	+	-	+	-	+	-	+	-	-
Acaulospora myriocarpa	+	+	+	-	+	-	+	-	+	-
Acaulospora bireticulata	+	-	+	+	+	+	-	+	-	+
Glomus albidum	-	-	-	-	+	+	-	+	-	-
Glomus ambisporum	+	-	-	+	-	-	+	-	-	+
Glomus clarum	+	+	-	-	-	+	-	-	-	-
Glomus manihotis	+	-	+	-	+	+	-	-	+	-
Glomus coronatum	-	+	-	-	+	+	-	+	+	-
Glomus fasciculatum	+	+	+	+	+	+	+	+	-	+
Glomus intraradices	-	+	-	-	+	-	-	+	-	+
Glomus luteum	+	-	+	-	-	+	-	-	-	-
Glomus mossae	-	+	-	+	+	+	+	-	+	+
Glomus sinuosum	-	+	+	-	+	+	-	-	-	-
Glomus tortuosum	+	-	-	-	+	-	+	-	-	-
Glomus verruculosum	-	+	-	-	-	+	-	-	-	-
Glomus versiforme	+	+	+	-	-	-	-	+	-	+
Cetraspora armeniaca	-	-	+	-	-	-	+	-	-	-
Dentiscutata heterogama	+	-	+	-	-	-	-	-	-	-
Gigaspora sps1	-	-	-	-	-	+	+	-	+	-
Gigaspora dicepens	-	+	-	-	-	-	-	+	-	+
Gigaspora albida	-	-	+	-	-	-	-	-	-	+

* Presence indicated by '+' and absence as '-'

slightly acidic in nature and the pH ranged from 4.87 to 7.72 as compared to irrigated soil (6.25 to 7.46). The AM spore number varied from site to site depending upon the type of agricultural practices. It is well established that, AM root colonization and spore population may vary greatly in different plant species grown in different types of soils (Jakobsen and Nielsen, 1983). Spores recovered from irrigated soil samples were high in number when compared to non-irrigated (Table 1). This could be attributed to the availability of soil nutrient and water. Use of other readily soluble fertilizers, particularly N fertilizers (Miller and Jackson, 1998) and degree of management practices like tillage (Mirás-Avalos *et al.,* 2011) has also been reported to have a negative impact on AM colonization and/or diversity in some cases. But, in the present exploration percent association of AM fungi in non-irrigated samples were significantly reduced which had low levels of phosphorous as compared to irrigated samples which had high level of phosphorous. Similarly, spore number also showed the same observation correlating with the percent association (Table 1).

However, the species composition varied in both the conditions. The soil samples of non-irrigated sites showed higher species diversity when compared to irrigated sites. In non-irrigated soil samples, 22 species of AMF were recovered and all the samples were rich in species except in the sample of CRP-1, whereas irrigated samples possessed less than 9 species in all the sites except in BNR-1 sample which possessed 12 species (Table 1). Absence of irrigation and increased soil acidity may have influenced negatively on sporulation and percent colonization, but could not able to affect the species composition. In non-irrigated samples, *Acaulospora myriocarpa, Acaulospora* sp1, *Glomus fasciculatum*, *Glomus mossae,* and *Glomus tortuosum* were the dominant species, whereas in irrigated samples, spore density is high but species richness was low having *Glomus fasciculatum*, *G. intraradices, G. mossae, G. versiforme* and *G. ambisporum* as dominant species (Table 3).

These findings could be supplemented as total root colonization potential of *Glomus* sps. which seems to be unaffected by duration of irrigation, and genus *Glomus* tend to dominate over other genera as irrigation proceeds (Ortega-Larrocea *et al.,* 2007). Presence of high phosphorus content in the soil samples in present study would also have negative impact on species richness. Isobe *et al.* (2011) studied the AMF community structure in soybean roots and concluded that temperature and phosphate absorption coefficient were the determining factors of AMF community structure in agriculture fields.

As the study sites are semi-arid in nature, we predict that the irrigation and soil chemical properties would be the strong determinants of AMF occurrence and diversity. It is almost impossible to distinguish between biotic and abiotic factors affecting spore abundance and percent root colonization of AMF in natural conditions. Based on these results we concluded that the different agro-ecosystem conditions like, irrigation and non-irrigation patterns have strongly influenced the spore population and percent association of AM fungi in these study sites.

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