World Journal of Agricultural Sciences 6 (6): 728-734, 2010
ISSN 1817-3047

Diversity of Arbuscular Mycorrhizal Fungi in Agricultural Fields of Hassan District

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Abstract: The objective of the present study was to investigate arbuscular mycorrhizal status of five samples collected from agricultural fields of Hassan district, Karnataka. Root and rhizospheric soil samples of the five sampling sites HSN-1 (*Elusine coracana*), ARSK-1 (*Helianthus annus*), H.N.P-1 (*Zea mays*), CRP-1 (*Sorghum bicolor*) and CRP-2 (*Cajanus cajana*) were collected. Physico-chemical parameters of soil like pH, phosphorous and potassium was greatly varied. AM root colonization was more in the CRP-1 sample and very low in the HSN-1 sample. Higher spore number was recorded in the CRP-2 sample although its pH was low (5.5) and less number of spores were recovered from ARSK-1 sample as its pH was normal (7.7). Maximum number of species was recovered from ARSK-1 and HNP-1 sample and minimum number of species was recovered from CRP-1 sample. AM fungi belonging to the genera *Glomus, Acaulospora, Scutellospora* and *Gigaspora* were recovered and identified. Genus *Glomus* and *Acaulospora* were found to be more dominant in all sampling sites.

Key words: Arbuscular mycorrhizal fungi · Sunflower · Ragi · Sorghum · Maize · Pigeon pea

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are recognized as an essential component of sustainable agricultural ecosystems [1. 2]. They colonize the roots of the vast majority of the plants, including most crop plants [3]. The AM association increases uptake of immobile nutrients, especially Phosphorous and micronutrients [4]. AMF have also been shown to affect N uptake from soil [5. 6]. AMF have been shown to improve both water and nutritional status [7] of the host plants by forming an extended intricate hyphal network, which efficiently absorb mineral nutrients from soil and deliver them to their host plants in exchange for carbohydrates. The beneficial effect of indigenous AM fungi is most important in stressed environments and circumstances termed as ecological crunches [8] is now widely accepted that climatic and edaphic factors can substantially influence AM association and their population. Rapid changes in soil nutrients may affect AM and spore numbers [9]. It has been noted by the authors that [10] that the diversity of the spores in the soil does not correlate with the mycorrhization rate. AMF can also enhance tolerance of or resistance to root pathogens. [11], or abiotic stresses such as drought and metal toxicity [12].

Diversity of AM association in different crops is currently of great interest. Crop plants are almost always colonized by AMF in agricultural ecosystems. However the composition of AMF communities and the abundance of fungal inoculum vary substantially among sites and, in general mycorrhizal colonization of crop plants is limited by the availability of the fungal inoculum in agricultural soils [13]. Conventional agricultural practices such as fertilization and tillage tend to decrease AMF abundance and alter community composition [14. 15], similarly crop rotation can disrupt the long term crop-AMF associations and the order of crop rotation affects AMF community composition [16]. Normally mycorrhizal fungi have not received much attention with respect to high-input agriculture because they are considered to provide little benefit to heavily fertilized and irrigated crops in conventional agricultural systems [17].

Factors known to influence AMF distribution include phosphorus levels [18] and soil pH [19]. It is well established that AM symbiosis improves phosphorus nutrition, but since the work of Sanders and Tinker [20], numerous experiments have also shown that increasing phosphate availability decreases the mycorrhization level, suggesting that AMF might play a minor role in natural ecosystems or agriculture fields with high 'P' availability

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[21]. From all these beneficial effects on plant performance and soil health it is evident that AMF are crucial for the functioning of terrestrial ecosystems. We under took a study of the diversity of the AM fungi in agricultural fields to assess the occurrence and distribution of AM fungi in Hassan District, Karnataka state, India.

MATERIALS AND METHODS

Study Site: The present study sites situated in Hassan district, Karnataka state, India. All the sampling sites are agricultural lands of conventional farming types. Geographically Hassan district lying between 12° 13' and 13° 33' North latitudes and 75° 33' and 76° 30' East longitude and elevated at 934 (3189 ft) MSL. Average rainfall is about 1031mm/annum. Hassan district subdivided into 7 Taluks, among them 2 taluks are completely lies in Western Ghats, remaining five districts are plain lands and major food crops grown here are Potato, Maize, Ragi (Finger millet), Sorghum, Sunflower, Pigeon pea and some vegetable crops. Tube well irrigation is a major type of irrigation in this district. Almost all the sampling sites are non irrigated lands except Channaraya Patna (C.R. Patna) area sampling site. Rainfall could be variable in some regions, as Arasiekere lies north to the district recorded very low rainfall in the district.

Chemical Properties of Soil Samples: Soil sampling was done in the month of February 2009. Soil samples were collected in sterile polyethylene bags using soil auger, at a depth of 0-30 cm from 5 different locations in each replication plot. The samples were collected from rhizosphere of crop plants and finally mixed to get a homogenous mixture of soil for each replicate pot. Soil physico-chemical parameters were analyzed at Central Sericulture Research and Training Institute, Mysore, Karnataka. Different parameters of soil like pH, Electrical conductivity, Organic carbon, Phosphorus, Potassium were analyzed (Table 1).

Collection of Soil and Root Samples: A totally of 5 sampling sites were selected to collect rhizospheric soil and root samples from the standing crop plants of Hassan district (Table 2). The samples were taken from a particular plant block covering an area of 100m^2 randomly from a depth of 10-30cm. soil particles attached to fine roots were removed by generous shaking and the root samples stored in standard FAA solution. These samples combined to make the composite samples, which were air dried and stored at 4°C.

Assessment of AM Fungal Colonization: The root samples were stained with 0.05% trypan blue prepared in lactophenol [22]. The assessment of mycorrhizal infection was done by using slide method [23]. 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 colonization by AM fungi was calculated using the following formula,

$$\text{Percent colonization (\%)} = \frac{\text{Total number of root segments colonized} \times 100}{\text{Total number of root segments studied}}$$

Isolation and Identification of AM Fungal Spores: Extraction of AM fungal spores was carried out using wet sieving and decanting method [24]. 100gms of soil was suspended in tap water and mixture was decanted through stacked sieves. The sieve sizes ranged from 32-420µm. spores from bottom sieve and middle were collected on

Table 1: Soil analysis of samples collected from Hassan district

Sl. No.	Sample	pH	EC Mmhos/cm	OC (%)	Available P (kg/ha)	Available K (kg/ha)
1	HNP-1	7.72	0.06	0.57	9.5	403
2	HSN-1	4.87	0.02	0.34	33.3	403
3	CRP-1	6.58	0.02	0.28	2.0	224
4	CRP-2	5.45	0.40	0.68	70.7	717
5	ARSK-1	6.12	0.03	0.40	26.0	358

Table 2: Principal agricultural management practices and standing crop at sampling date for field sites

Site name.	Sample	Farming system, crop rotation	Irrigation and type of irrigation	Standing crop
Hassan-Arasiekere	(ARSK-1)	Conventional.Sunflower, Maize.	No.	Sunflower *(Helianths annuas)*
Hassan-Hassan	(HSN-1)	Conventional.Ragi, Maize, Potato.	No.	Ragi *(Elusine coracana)*
Hassan-C.R.patna1	(CRP-1)	Conventional.Sorghum, Ragi	No.	Sorghum *(Sorghum bicolor)*
Hassan-C.R.patna2	(CRP-2)	Conventional.Pulse crop.	Yes. Tube well irrigation	Pulse crop. (*Cajanus cajana*)
Hassan-H.N.pura	(HNP-1)	Conventional.Maize, Tobacco.	No.	Maize (*Zea mays*)

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 identifications were based on identification manuals and current species descriptions [25, 26].

Statistical Analysis: Spore density, species richness, isolation frequency (IF), relative abundance (RA) and Shannon-Wiener index of diversity were conducted as follows.

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; IF= (the number of samples in which a given species was isolated/ the total number of samples) x100%; RA=(the number of given species spore/ total number of spores) x 100; Shannon-Wiener index of diversity $H = -\Sigma p_i \ln (p_i)$; where p_i is the proportion of total number of species made up of the ith species [27]. 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

Total 5 soil samples were collected from 5 sampling sites throughout the Hassan. Twenty two taxa of AMF were detected and identified. There were 13 taxa from the genus *Glomus*, 4 from *Acaulospora,* 3 from *Scutellospora* and 2 Spp. belonging to *Gigaspora* (Table 6). Among 22 taxa 1 species belonging to *Acaulospora* was not identified to species level. AMF spore density varied among sampling sites, although percent colonization not varied too much as much as spore density (Fig. 1) (Table 4). The spore density was higher in CRP-2, followed by CRP-1 and HSN-1. In ARSK-1 sample 12 species were recovered and 11 species recovered in each of HSN-1, HNP-1 and CRP-2 samples (Table 6).

Glomus and *Acaulospora* species occurred most frequently, followed by *Gigaspora* and *Scutellospora. 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 exhibit >50% IF and >5% RA (Table 6). *Glomus albidum, Glomus verruculosum, Scutellospora heterogama and Gigaspora albida* were found very less frequent in all the samples and also in the view of RA. Among 23 species of AM fungi identified *Glomus mossae, Glomus fasciculatum* and *Glomus ambisporum* were found to be very dominant.

The diversity of AM fungi expressed by Shannon-Wiener index, were presented in Table 3. The maximum diversity index observed in the HNP-1 which has 2.28, HSN-1 has 2.11, CRP-2 has 2.24 and ARSK-1 exhibited 2.2. Occurrence of maximum number of species would results to higher index of diversity in the samples.

Table 3: Shannon-Wiener index of diversity of sampling sites of Hassan district

Sample	Shannon-Wiener index (H')
HNP-1	2.28
HSN-1	2.11
CRP-1	1.82
CRP-2	2.24
ARSK-1	2.20

Table 4: Percent colonization and spore population of all the 5 samples

Sl. No.	Sample Name	Percent (%) association	Spore density/ 100gm dry soil
1	HNP-1	68	174
2	HSN-1	40	320
3	CRP-1	72	341
4	CRP-2	64	367
5	ARSK-1	50	247

Table 5: Results of correlation studies. (Hassan District)

	% colonization	Spore count	Soil pH	Soil 'P'	Soil 'K'
% colonization	1	-0.043(0.946)	0.697(0.191)	-0.260(0.672)	-0.034(0.956)
Spore count	-0.043(0.946)	1	-0.731(0.161)	0.516(0.373)	0.299(0.625)
Soil pH	0.697(0.191)	-0.731(0.161)	1	-0.656(0.230)	-0.373(0.536)
Soil 'P'	-0.260(0.672)	0.516(0.373)	-0.656(0.230)	1	0.934*(0.020)
Soil 'K'	-0.034(0.956)	0.299(0.625)	-0.373(0.536)	0.934*(0.020)	1

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

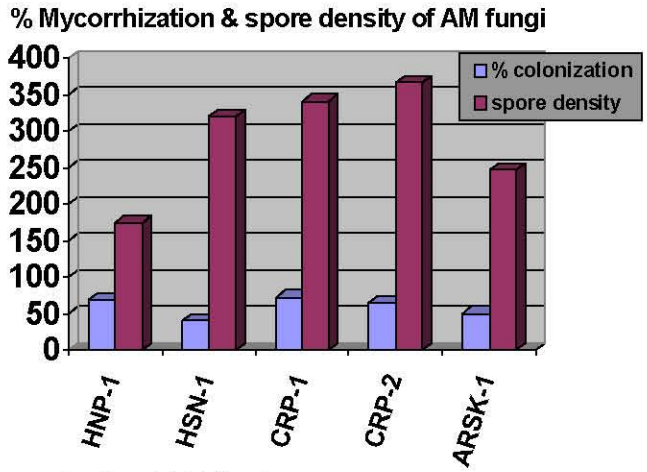

Fig. 1: Percent colonization and spore density of AM fungi

Table 6: Abundance of spores of different AM fungal species, 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	Total	IF (%)	RA (%)
Acaulospora Spp.1	64	-	04	-	24	92	60	6.3
Acaulospora denticulate Sieverding and Toro	-	11	-	22	-	33	40	2.2
Acaulospora myriocarpa Spain, Sieverd. and N.C. Schenck	73	19	03	-	13	108	80	7.4
Acaulospora bireticulata F.M. Rothwell and Trappe	41	-	06	15	19	81	80	5.5
Glomus albidum Walker and Rhodes	-	-	-	-	21	21	20	1.4
Glomus ambisporum G.S. Sm. and N.C. Schenck	23	-	-	63	-	86	40	5.9
Glomus clarum Nicolson and Schenck	18	15	-	-	-	33	40	2.2
Glomus manihotis R.H. Howeler, Sieverd. and N.C. Schenck	25	-	22	-	13	60	60	4.1
Glomus coronatum Giovann.	-	10	-	-	33	43	40	2.9
Glomus fasciculatum (Thaxter) Gerd. and Trappe emend. Walker and Koske	15	13	38	77	57	200	100	13.8
Glomus intraradices Schenck and Smith	-	33	-	-	49	82	40	5.6
Glomus luteum	09	-	42	-	-	51	40	3.5
Glomus mossae (Nicol. and Gerd.) Gerd. and Trappe	-	19	-	84	69	172	60	11.8
Glomus sinuosum (Gerd. and B.K. Bakshi) R.T. Almeida and N.C. Schenck	-	11	21	-	29	61	60	4.2
Glomus tortuosum Schenck and Smith	39	-	-	-	40	79	40	5.4
Glomus verruculosum B³aszk.	-	15	-	-	-	15	20	1.03
Glomus versiforme (P. Karsten) S.M. Berch	09	23	40	-	-	72	60	4.9
Scutellospora armeniaca Blaszk.	-	-	17	-	-	17	20	1.1
Scutellospora cerradiensis Spain and J. Miranda	-	-	33	80	-	113	40	7.7
Scutellospora heterogama (Schenck and Nicol.) Walker and Sanders	04	-	18	-	-	22	40	1.5
Gigaspora dicepens Hall and Abbott	-	05	-	-	-	05	40	0.3
Gigaspora albida Schenck and Smith	-	-	03	-	-	03	20	0.2

SD= 320 174 247 341 367 = 1449

SR= 11 11 12 6 11

SD-Spore density, SR-Species richness

* Species absence indicated by (-).

The results of correlation studies between percent AM colonization, spore count, soil pH, soil 'P' and soil 'K' is summarized in Table 5. Correlation analysis showed that there is no significant correlation was observed except in soil 'K' which is significantly correlated with soil 'P' (r=0.934, $p<0.05$). Where as soil pH positively correlated with percent colonization (r= 0.697) and spore count is positively correlated with Potassium (K) and Phosphorus (P).

DISCUSSION

In the present study we under took field survey to assess the diversity of arbuscular mycorrhizal fungi in non irrigated agricultural fields of Hassan district. The results showed that AMF percent root colonization and spore number varied from different sites. Soil physico-chemical parameters were also shown huge variations. Samples from HSN-1 and CRP-2 are acidic with pH 4.87 and 5.45 respectively. Here the available phosphorous and potassium is comparatively very high. Others samples possess pH of around 6-7 and available phosphorous is comparatively very low (Table 1).

Variation in AM colonization and spore density for different agro ecological zones might be in response to various causes including wide range of environmental, plant and fungal factors [28]. The stimulating effects of organic matter on AM colonization and sporulation have earlier reported [29].

The highest percent colonization was observed in CRP-1 sample (72%) of sorghum, here spore density was also high (320 spores/100gm of soil), followed by HNP-1 sample (68%) of maize, but spore density is low compared to others (174 spores/ 100gm of soil). Then CRP-2 sample (64%) of pulse crop, here spore density was higher (367 spores/100gm of soil) compared to others. The lowest percent colonization was recorded in HSN-1 sample (40%) of ragi, here spore density (320 spores/100gm of soil) was found to be normal (Table 4) (Fig. 1).

As the study areas are semiarid, we predict the irrigation and soil chemical properties would be the strong determinant of AMF occurrence and diversity. Very low soil pH was observed in HSN-1 sample, here percent colonization is very low. Correlation studies shows there is positive correlation between soil pH and percent colonization (Table 5) and also available phosphorous (P) is higher in three samples namely CRP-2 (70.7 kg/ha), HSN-1(33.3 kg/ha) and ARSK-1 (26.0 kg/ha). But there was no significant correlation between soil P and spore density. Correlation between soil P and potassium (K) shows significant correlation (0.934), but it cannot be attributed for the decrease or increase of the percent colonization and spore number of AM fungi.). It is almost impossible to distinguish between biotic and abiotic factors affecting spore abundance and percent root colonization of AMF in natural conditions [30]. But some of the results show that soil pH influences both spore density [31] and species composition [32].

In cultivated lands AMF population, species composition and diversity are often decreased compared to natural ecosystem [33. 34]. Because when a soil is put to agricultural use it undergoes a series of physical, chemical and morphological changes, which can affect the root inhabiting micro organisms and poor plant growth [35, 36]. Miller [37] also reported that when soil is disturbed or is partially removed, a decrease in the number of mycorrhizal propagules occurs. Remarkably AMF community differed not only in diversity but also in functional aspects (rates of root colonization and spore formation). The dependence of spore population on pH of the soil was also reflected in the present study. Decline in the percent colonization was observed in the acidic soils, while spore numbers were not affected with the declining soil pH.

Although the genus *Glomus* has been found to dominate in the rhizosphere soil of crop plants, the species composition of AM fungi varied in different crop plants. Highest species composition was recorded in ARSK-1 (12 species) followed by HSN-1 (11 species), CRP-2 (11 species) and HNP-1 (11 species) (Table-6). Very low species composition was observed in CRP-1, where only 6 species were recovered, although this sample exhibited high percent colonization and spore density. This was indicated by Shannon-wiener index, where CRP-1 shows low index (1.82), where as other samples shows relatively higher index (>2). This can be attributed to the occurrence of higher number of species in these samples (Table 3).

The number of AMF spores in soils did not significantly decrease with increasing soil salinity. It has been suggested that sporulation by AMF is stimulated under salt stress conditions [38. 39]. Renker *et al.* [40] observed that despite high soil phosphate contents in field sites, they were able to reveal almost all investigated plant species to be mycorrhizal, while plants in green house experiments supplied with even lower amount of Phosphate are known to lose their ability to form their AM. Plant species, soil pH, Phosphorus and organic matter levels had been influenced the intra-radical development of these fungi and has reinforced the significance of these factors on the association [41].

More than 150 species are described based on their spore morphology [42], but spore morphotyping requires considerable experience [43] and spore count may not reflect the in-plant composition of AMF communities [44]. AMF may also increase or decrease the production of indirect defenses by crop. For example, inoculation with AMF significantly reduces the extrafloral nectaries (EFN's) produced by faba beans (*Vicia faba*) compared with non mycorrhizal controls [45]. So there is need to study the occurrence and composition of AM fungi in agricultural fields and need to develop AMF inoculum strategies, as they play major role in the supply of nutrients from soil to roots of the host plants and also they act as determinant factors in the sustainable agriculture.

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