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Arbuscular Mycorrhizal Fungal Diversity in Sugarcane Rhizosphere in Relation with Soil Properties

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Abstract

Arbuscular mycorrhizal (AM) species diversity and their root colonization patterns may vary in a plant species as influenced by soil environmental and biological factors. In the present study, sugarcane rhizospheric soils were collected from 41 main sugarcane producing tehsil places belonging to 10 districts from Maharashtra, India. Rhizospheric soil samples and roots were analyzed for spore density, relative abundance and frequency of AM spores at genus as well as at species level, extent of AM colonization in roots and various soil chemical properties. Soil sample from Jalgaon district possessed maximum spore density and AM root colonization. Genus *Glomus* exhibited highest relative abundance with maximum frequency of 32.55%. Species wise, *Glomus fasciculatum* possessed highest relative abundance and maximum frequency was observed in case of *Glomus fasciculatum*, *Glomus intraradices*, *Glomus mosseae* and *Glomus versiforme*. Maximum similarity of AM spores was recorded between Satara and Sangli districts which may be because of almost similar soil pH profile. Data obtained after cluster analysis represented the close relationship between spore density, AM root colonization and soil Cu, Zn and Fe concentrations. A statistically significant positive correlation was also found when AM spore density and root colonization was compared with soil Cu, Zn and Fe contents. This kind of data can be used to predict type of AM fungi to be used as bioinoculant in particular region.

Keywords: arbuscular mycorrhizal fungi, frequency, mycorrhizal root colonization, relative abundance, soil chemical properties

Introduction

Arbuscular mycorrhizal fungi (AMF) are considered as obligate biotrophic symbionts and are associated with the fine roots of over 80% terrestrial plant species (Smith and Read, 1997). In this plant-fungus association, fungus depends upon host plant for nutrition and reproduction and in return provides phosphate and essential mineral nutrients from soil to the host plant. AMF belongs to phylum *Glomeromycota* is characterized as formation of *Arum* type root colonization pattern whereas, *Gigasporaceae* form *Arum* type or intermediate types of AM with *Paris* type hyphal coil structure (Dickson *et al.*, 2007; Smith *et al.*, 2004).

The main function of AM fungi is of phosphorus transportation. Extra-radical mycelium of AM fungi easily access P from soil and deliver to root cortical cells as polyphosphate which finally translocate to host plant after solubilization and it is estimated that external hyphae deliver up to 80% of P requirement of the plant (Matamoros *et al.*, 1999). Other than P translocation, AM fungi provide protection to host plant roots from soil-borne pathogenic attack, they improve tolerance of plants to several abiotic stresses, including drought and saline stress condition, by producing plant growth hormones, maintain stability of soil aggregation, increase resistance to diseases etc (Evelin *et al.*, 2009). In addition, mycorrhizal association also enhances nitrogen uptake as well as utilization of several micro nutrients. AM fungi successfully colonize with a wide range of plant species and are considered as non-host specific (Evelin *et al.*, 2009). Population of AM fungi is highly influenced by several environmental factors including climatic conditions, soil physico-chemical status, age and variety of host plant and several agricultural practices etc. Additionally, different species of AM fungi differ in their tolerance to adverse physical and chemical condition in soil (Kumar and Ghose, 2008). It is already reported that, AMF is naturally occur in saline environment and their spore density is improved as saline stress stimulates sporulation (Sengupta and Chaudhuri, 1990; Tressner and Hayes, 1971). Mc Millen *et al.* (1998) reported that, salt stress inhibited AM spore germination as well as hyphal growth. It was observed that, plants growing in severe wet places seem to be non mycorrhizal but became mycorrhizal when water table is lowered. Likewise, rice in flooded soil is non mycorrhizal and became colonized when grown in non flooded soil. Also, winter wheat which usually is planted in autumn in temperate climates may not become mycorrhizal until spring (Asai, 1934). Moreover, plant species also vary in their symbiotic responsiveness to AM fungi with respect to plant growth, reproduction, abiotic stress, disease resistance etc (Danesh *et al.*, 2006).

Among a wide range of host species, plant belonging to Poaceae family is considered as one of the host of AM fungi (Powell, 1984). Sugarcane is one of the most important cash crop belonging to this family and West-

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ern Maharashtra is considered as the traditionally sugarcane growing areas in India and accounts for 60% of the national contribution. Ahmednagar, Satara and Kolhapur districts are recognized as sugarcane belt in Western Maharashtra. In Maharashtra, crop rotation in sugarcane cultivation is not used in regular practices. Crop rotation, with legumes planting has some positive effect on sugarcane productivity by improving soil fertility; reduce erosion etc (Ambrosano *et al*., 2010). Sugarcane production is mostly groundwater dependent as it requires high water availability at various growth stages (Shrivastava *et al.,* 2011). Since ground water table is getting reduced, new technique should be incorporated to sustain sugarcane productivity and it includes use of AM fungi as bioinoculant to tolerate drought stress, use of genetically modified sugarcane variety which requires less water for growth etc. Another major factor affecting sugarcane production is red rot disease caused by *Colletotrichum falcatum*. But, the severity of this disease is reduced by AM inoculation (Nasim *et al.,* 2008). Though several factors influence the population of AM fungi in rhizospheric soil but, species composition, quantification, identification of AM fungus in cultivated soils of different locations is necessary as well as impact of several soil factors on their colonization and symbiotic efficiency is also required. As there is no report, with regards to mycorrhizal status and diversity of AM fungi in sugarcane rhizospheric soil from sugarcane growing regions in Maharashtra and hence, the present study was aimed to investigate AM spore distribution in sugarcane rhizospheric soil and their root colonization profiling in relation to several soil properties.

Tab. 1. Study site with their geographical coordinates of various tehsil places belonging to surveyed 10 districts of Maharashtra

District	Tehsil	Coordinates
Dhule	Sindkheda	21°18'30"N and 74°43'18"E
	Shirpur	21°22'12"N and 74°57'10"E
Aurangabad	Aurangabad	19°54'2"N and 75°21'34"E
	Phulambri	20°7'34"N and 75°24'46"E
	Khuldabad	20°3'23"N and 75°12'33"E
Beed	Beed	18°59'13"N and 75°49'5"E
	Wadwani	18°59'7"N and 76°3'10"E
	Parli	18°51'1"N and 76°7'20"E
Ahmednagar	Ahmadnagar	19°6'9"N and 74°43'34"E
	Parner	19°0'29"N and 74°27'21"E
	Sangamner	19°34'25"N and 74°14'22"E
	Kopargaon	19°53'38"N and 74°29'43"E
	Shrirampur	19°7'10"N and 74°39' 37"E
	Rahuri	19°23'4"N and 74°39'12"E
	Karjat	18°33'44"N and 75°0'49"E
	Jamkhed	18°43'18"N and 75°19'26"E
Pune	Pune	18°32'6"N and 73°52'20"E
	Junnar	19°12'50"N and 73°53'23"E
	Mulshi	18°30'4"N and 73°30'50"E
	Sirur	18°50'7"N and 74°23'13"E
	Baramati	18°9'6"N and 74°35'43"E
	Daund	18°28'40"N and 74°36'13"E
Satara	Satara	17°41'10"N and 73°59'25"E
	Koregaon	17°43'28"N and 74°10'11"E
	Khatar	17°47'4"N and 74°11'48"E
	Patan	17°36'46"N and 73°9'18"E
	Karad	17°43'6"N and 74°10'19"E
	Phaltan	17°59'19"N and 74°26'29"E
Sangli	Miraj	16°50'22"N and 74°38'54"E
	Kavathe	17°0'32"N and 74°51'55"E
	Tasgaon	17°2'29"N and 74°36'33"E
	Islampur	17°3'54"N and 74°16'44"E
	Khanapur	17°16'29"N and 74°43'49"E
	Atpadi	17°25'11"N and 74°57'48"E
Jalgaon	Bhusawal	21°3'15"N and 75°46'21"E
Kolhapur	Kanvir	21°23'5"N and 75°49'29"E
	Panhala	16°49'24"N and 74°7'44"E
	Shirol	16°44'2"N and 74°36'48"E
	Bhudargad	15°49'22"N and 74°51'13"E
	Chandgad	15°56'12"N and 74°12'7"E
Osmanabad	Osmanabad	18°11'49"N and 76°3'31"E

Materials and methods

Study site and soil sampling

In Maharashtra, sugarcane crop is mainly cultivated in Western part and Ahmednagar, Satara and Kolhapur districts are known to be major sugarcane growing regions. In addition, sugarcane is also cultivated in some parts of Sangli, Pune, Osmanabad, Aurangabad, Beed, Dhule and Jalgaon. During August 2009 to December 2010, a total of 41 sugarcane rhizospheric soil samples were collected from various tehsils of 10 districts of Maharashtra with geographical, environmental and soil variations (Tab. 1). Soil samples from various surveyed places were collected only once and not seasonally.

Rhizospheric soil including sugarcane roots and stump were collected from 10 different fields belonging to each tehsil. For this purpose, 8-10 sub-samples (each of 1kg soil including roots) were collected to a depth of 5-20 cm from various sampling points (the distance between two sampling points was of about 5-8 meters) from each field. These sub-samples were brought to laboratory in sealed polythene bags and then thoroughly mixed. Finally, a composite sample was prepared by thorough mixing of samples from all fields belonging to a particular tehsil. Some representative roots from each composite sample were separated from adhering soil followed by proper rinsing under tap water and fixed in formalin : acetic acid : alcohol until AM root colonization was estimated. Rhizospheric soil samples were air dried in shade at room temperature and one-third part of each sample was used for determination of soil chemical properties, for development of trap culture and for spore counting.

Extraction, counting and identification of AM spores

One-third part of each collected soil sample was used for AM spore extraction using the method described by Gerdemann and Nicolson (1963). Spore extraction was done in two triplicates for individual soil sample and in each case 50 g of sample was mixed with 500 ml water followed by thorough stirring and then the suspension was sieved through a series of sieves (37 µ-500 µ). Each sieving was collected and was subjected to sucrose centrifugation (Daniels and Skipper, 1982). Spores were collected on grid-lined filter paper and then counted using trinocular compound microscope. During counting, sporocarp and spores in cluster were considered as one spore and spore density was expressed in terms of number of spores per 100 g of dry soil sample.

Relative abundance (RA) (%) and frequency (F) (%) of AM spore was calculated by the following formulae (Kumar and Ghose, 2008):

RA (%) = (number of AM spores of a genus or a species/total number of spores) X 100

F (%) = (number of samples in which genus or species of AM fungi was observed/total number of samples) X 100.

Another one-third part of each collected soil sample was used to establish trap culture in 10 L plastic pot. At this stage, trap culture was found necessary to obtain healthy, viable spores with prominent structural appearance which are important for identification of AM fungi at species level. Trap was developed by mixing field collected soil sample (including root pieces) and autoclaved river sand (autoclaved at 121°C, for three times at an interval of 4 days) (1:1, v/v), with maize as a host plant. Plants were allowed to grow for 6 months under green house conditions (temperature 30/20°C day/night, a relative humidity of 60-65% and at a photon flux intensity of around 280-350 µmol/m^2/s). At the end of growing cycle, AM spore counting was done using above mentioned method and trap culture was designated as positive, when AM spore counts (per 100 g dry soil) were increased as compared with field collected sample and detection of new fresh spores which were undetected in field collected samples. Fresh, healthy, morphologically similar spores (around 25-30 for each type) were extracted from trap culture followed by separation and fixed on glass slide in PVLG and PVLG with Melzer's reagent. Identification of each spore type was done on the basis of color, shape, size, surface ornamentation, spore wall structure and type of hyphal attachment as per the description provided in the database (www.amf-phylogeny.com) and as per the standard key features given in manual (Schenck and Perez, 1990). Identification of AM spores was done after trap culturing as propagation of field collected AM species sometimes require controlled environment.

Estimation of AM root colonization (%)

To estimate AM root colonization, root samples were removed from fixative and washed with deionized water followed by cutting into 1 cm pieces. The root pieces were thoroughly mixed and a sub-sample (0.5 g) was cleared in hot KOH solution (10% w/v, at 90°C) for 1 h. Cooled root samples were washed with deionized water and placed in HCl (10%, v/v) for 3 min and stained with trypan blue (0.05%, w/v) for 15 min at 90°C (Phillips and Hayman, 1970). Percentage of AM colonization in sugarcane root samples was estimated by gridline intersect method (Giovannetti and Mosse, 1980).

Soil chemical characteristics

Soil pH and EC (at 25°C) were analyzed from suspension of soil:water (1:5) and organic carbon (OC) was measured according to the method of Walkley and Black (1934). Concentration of Na, available P (Olsen P), K, Ca, Mg, Cu, Zn, Fe in soil samples were estimated according to the standard methods (Jackson, 1973; Kalra and Maynard, 1991).

Statistical analysis

Values for spore number and root colonization (%) were subjected to $\log_e(x+1)$ transformation and arcsine square root transformation respectively for normalization of the data (St. John and Koske, 1988; Zar, 1984). Pearson's correlation coefficient was calculated between spore number, root length colonization and soil properties. Statistical significant difference was set at $P<0.01$ level. Agglomerative hierarchical cluster analysis was done using average linkage within groups and the result of complex multivariate relationship among the variables was expressed as dendrogram. SPSS software version 9.0 was used for the statistical analysis.

Results

In the present investigation, individual dataset of spore density, root length colonization as well as soil chemical properties of each tehsil place are not illustrated rather, district wise mean values of spore density, root length colonization and soil chemical properties were calculated and mentioned. As certain species of AM fungi do not turn up in trap culture as well as some individual spore types in different trap cultures did not multiply at the same rate and hence to avoid ambiguity, spore density, frequency and relative abundance of AM spores were calculated from field collected soil samples.

AM spore density and Percentage of AM root colonization

Fig. 1 present district wise dataset of mean values of AM spore density and percent AM root colonization observed in sugarcane plant roots. Among the ten surveyed districts, rhizospheric soil from Jalgaon district had maximum spore density (395 spores /100 g soil) and Dhule had the least mean spore density (137 spores /100 g soil). The spore density of the three major sugarcane growing districts namely, Ahmednagar, Satara and Kolhapur ranged between 239-324 spores/100 g soil. Spore density from several districts was found in increasing order of Dhule>

Sangli> Beed> Ahmadnagar> Pune> Osmanabad> Satara> Kolhapur> Aurangabad> Jalgaon (Fig. 1). The average AM root colonization was observed within a range of 27.75-57.17% among ten districts. Highest AM root colonization was observed in root samples collected from Jalgaon district (57.17%) and least colonization was recorded in root samples collected from Dhule district (27.75%) (Fig. 1).

Frequency and relative abundance of AM spores

Totally five AM genera namely, *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and *Sclerocystis* including 32 species were identified from forty one soil samples after positive trap culturing. After species level identification, it was observed that, 15 species were belonging to genus *Glomus*, 4 species each of *Gigaspora* and *Acaulospora*, 6 species were of *Scutellospora* and 3 species of *Sclerocystis*.

Among the five genera of AM fungi observed, relative abundance of AM spores occurred in the *Glomus* (75.39%) followed by *Acaulospora* (8.62%) and *Scutellospora* (8.47%). Lowest relative abundance of AM spores was observed for genus *Gigaspora* (5.83%) and *Sclerocystis* (1.69%). Genus wise maximum frequency of spores was observed for *Glomus* followed by *Scutellospora*, *Acaulospora*, *Sclerocystis* and *Gigaspora* and ranged from 34.78% to 7.09% (Fig. 2).

When species wise relative abundance and frequency were calculated, it was observed that, *Glomus fasciculatum*, had highest RA value of 17.44% followed by *Glomus intraradices* and *Glomus mosseae* (14.16% and 9.67% respectively); whereas, *Scutellospora nigra* had the least RA value (0.25%) (Fig. 3). Species wise highest frequency was observed for four AM species such as *Glomus fasciculatum*, *Glomus intraradices*, *Glomus mosseae* and *Glomus versiforme* (each of 3.13%) and the lowest frequency occurred for *Scutellospora nigra* (0.76%) and *Scutellospora heterogama* (0.69%).

In the present study, several AM spore types observed in each tehsil place are presented in Tab. 2. It was observed that, four species of genus *Glomus* were present in all the soil samples collected from various districts. Whereas, other species of genus *Glomus* and the species belonging to genus *Gigaspora*, *Acaulospora*, *Scutellospora* and *Sclerocystis* were found in soils of some tehsil places only. Also, out of forty one soil samples from various tehsil places, *Scutellospora nigra* was observed at nine locations and *Scutellospora heterogama* at ten locations amongst the surveyed areas (Tab. 2).

Chemical properties of soil

District wise mean values of several soil chemical properties were obtained (Tab. 3). Among the ten districts selected for study, soil samples from Dhule, Beed, Ahmednagar, Pune and Jalgaon were of alkaline type while the remaining five districts Aurangabad, Satara, Sangli, Kolhapur and Osmanabad had slightly acidic soil (Tab. 3).

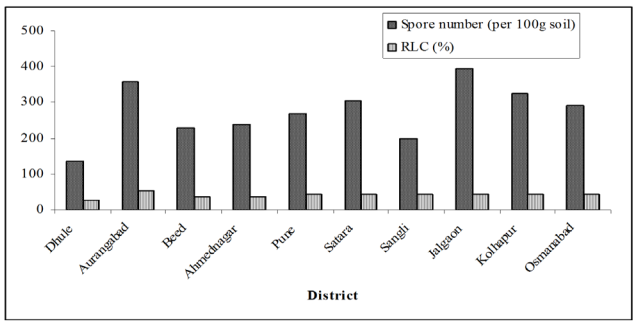

Fig. 1. Mean spore density (per 100 g dry soil) and AM root length colonization (RLC) (%) observed district wise. Mean values were calculated from the data obtained in all tehsil places belonging to particular district

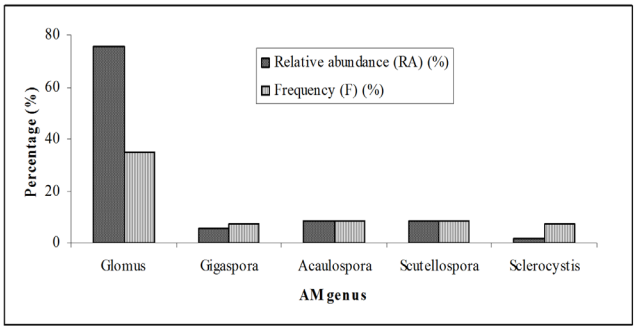

Fig. 2. Relative abundance (RA) (%) and frequency (F) (%) of AM spores identified at genus level from the soil samples. Mean values were calculated from the data obtained in all tehsil places belonging to the particular district

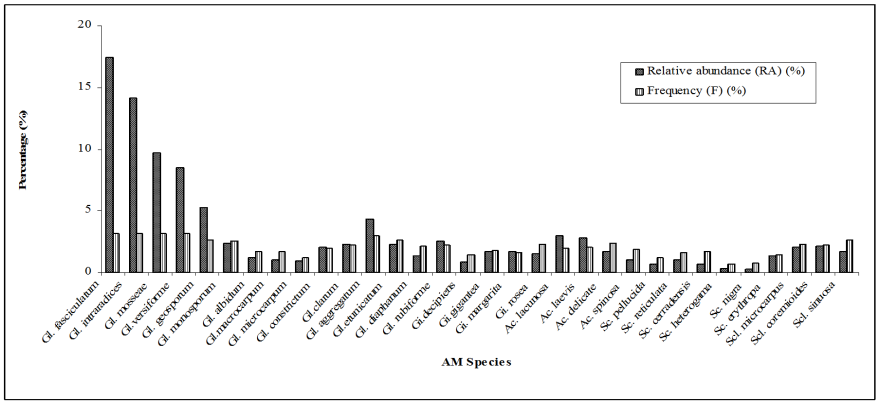

Fig. 3. Relative abundance (RA) (%) and frequency (F) (%) of AM spores identified at species level from the soil samples. Mean values were calculated from the data obtained in all tehsil places belonging to particular district. Note: Gl-*Glomus*, Gi-*Gigaspora*, Ac-*Acaulospora*, Sc-*Scutellospora*, Sc-*Sclerocystis*

Tab. 2. Distribution (presence or absence) of each AM spore type observed in surveyed tehsil places

Site	Gf	Gi	Gm	Gv	Gg	Gmo	Ga	Gma	Gmi	Gc	Gcl	Gag	Ge	Gd	Gr	Gid	Gig	Gim	Gir	Acl	Acla	Acd	Acs	Scp	Scr	Scc	Sch	Scn	Sce	Sclm	Sclc	Scls
Ds	+	+	+	+	-	-	+	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	-	+	+	-	-	-	-	-	+	+
Dsh	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+	-	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-
Aa	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	+	-	+	+	-
Ap	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+
Ak	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	-	+	+	+
Bb	+	+	+	+	-	+	-	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	-	+	+	-	-	-	-	-	+	+
Bw	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	+	+	-	-	-	+	+	+
Bp	+	+	+	+	+	+	+	-	-	-	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+
Aah	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+
Apa	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	-	-	-	+	+	-	+
As	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	-	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+	+
Ako	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	+
Ash	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	+	+
Ar	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	+	+
Aka	+	+	+	+	-	+	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	+	-	-	+	-	-	-	+	+	+
Aj	+	+	+	+	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	+	-	-	+	-	-	+	+	+	+
Pp	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	-	-	-	+	-	-	+	+	-	+	+	-	-	-	-	+	+
Pj	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	+
Pm	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	-	+	-	-	+	+	-	+
Ps	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	+	+	-	+	+	-	-	-	+	+	+
Pb	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	-	-	-	+	+
Pd	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+
Ss	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+	+	+
Sk	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-	+	-	+	+	+	+	+
Skh	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	-	-	+	-	-	+	-	-	+	+	+
Sp	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Ska	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Sph	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+	+	+	+	-	+	-	+	+	+
Sm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+
Skav	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-
St	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+
Si	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	-
Skha	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+
Sa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-
Jb	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	+
Kk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-
Kp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	-	+
Ks	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	+	-	-	+	-	-	+	+	+
Kb	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	-	+	-	-	+	-	+
Kc	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	-	-	+	+	-	-	+	+	+	+
Oo	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+

Note-Gf-*Glomus fasciculatum,* Gi-*Glomus intraradices*, Gm-*Glomus mosseae*, Gv-*Glomus versiforme*, Gg-*Glomus geosporum*, Gmo-*Glomus monosporum*, Ga-*Glomus albidum*, Gma-*Glomus macrocarpum*, Gmi-*Glomus microcarpum*, Gc-*Glomus constrictum*, Gcl-*Glomus clarum*, Gag-*Glomus aggregatum*, Ge-*Glomus etunicatum*, Gd-*Glomus diaphanum*, Gr-*Glomus rubiforme*, Gid-*Gigaspora decipiens*, Gig-*Gigaspora gigantea*, Gim-*Gigaspora margarita*, Gir-*Gigaspora rosea*, Acl-*Acaulospora lacunosa*, Acla-*Acaulospora laevis*, Acd-*Acaulospora delicata*, Acs-*Acaulospora spinosa*, Scp-*Scutellospora pellucida*, Scr-*Scutellospora reticulata*, Scc-*Scutellospora cerradensis*, Sch-*Scutellospora heterogama*, Scn-*Scutellospora nigra*, Sce-*Scutellospora erythropa*, Sclm-*Sclerocystis microcarpus*, Sclc-*Sclerocystis coremioides*, Scls-*Sclerocystis sinuosa*. Ds-Sindkheda (Dhule), Dsh-Shirpur (Dhule), Aa-Aurangabad, Ap-Phulambri (Aurangabad), Ak-Khuldabad (Aurangabad), Bb-Beed, Bw-Wadwani (Beed), Bp-Parli (Beed), Aah-Ahmadnagar, Apa-Parner (Ahmadnagar), As-Sangamner (Ahmadnagar), Ako-Kopargaon (Ahmadnagar), Ash-Shrirampur (Ahmadnagar), Ar-Rahuri (Ahmadnagar), Aka-Karjat (Ahmadnagar), Aj-Jamkhed (Ahmadnagar), Pp-Pune, Pj-Junnar (Pune), Pm-Mulshi (Pune), Ps-Shirur (Pune), Pb-Baramati (Pune), Pd-Daund (Pune), Ss-Satara, Sk-Koregaon (Satara), Skh-Khatar (Satara), Sp-Patan (Satara), Ska-Karad (Satara), Sph-Phaltan (Satara), Sm-Miraj (Sangli), Skav-Kavathe (Sangli), St-Tasgaon (Sangli), Si-Islampur (Sangli), Skha; Khanapur (Sangli), Sa-Atpadi (Sangli), Jb-Bhusawal (Jalgaon), Kk-Kanvir (Kolhapur), Kp-Panhala (Kolhapur), Ks-Shirol (Kolhapur), Kb-Bhudargad, Kc-Chandgad (Kolhapur), Oo-Osmanabad. '+'means Presence, '-' means Absence

Highest value of organic carbon and EC were observed in Dhule district, followed by Sangli and the lowest count was recorded in Jalgaon district. Data from Tab. 3 also indicated that, with increase in soil EC value, there was an increase in available Na, Ca and Mg concentrations in most of the districts.

Soils from Dhule district possessed highest concentration of available P, Na, Ca, Mg and K. But, this soil contained least amount of Zn, Cu and Fe. Concentrations of minor elements such as Zn, Cu and Fe were found superior in soils from Osmanabad and Aurangabad districts.

Jaccard index

Data of Jaccard similarity index is presented in Tab. 4, which gives the idea of presence of similar type of AM fungi between two sugarcane growing regions. The highest similarities of AM spores were found in Satara/Sangli and Ahmadnagar/Sangli and it may be due to similarity in pH of soil from these districts (Tab. 3).

Statistical correlation analysis and cluster analysis

Following Pearson's correlation analysis, it was observed that, spore density can be positively correlated with extent of root colonization and so, the percentage of AM root colonization was increased with increasing spore density. AM spore density showed a significantly positive correlation with concentrations of Zn, Cu and Fe whereas, a significant ($P<0.01$) negative correlation was observed when spore density was compared with other soil properties such as soil pH, EC, organic carbon, available P, Na, Ca, Mg and K concentrations (Tab. 5). A significant positive correlation existed between AM root colonization and concentrations of Zn, Cu and Fe. Except pH of soil samples, the remaining soil properties were negatively correlated ($P<0.01$) with AM root colonization (Tab. 5).

The dendrogram obtained after agglomerative hierarchical cluster analysis using average linkage within group, showed the close relationship among the variables namely spore density, AM root colonization, concentrations of Cu, Zn, Fe, soil pH and available P. But, relationship be-

Tab. 3. District wise mean soil chemical properties. Mean value of each parameter was calculated from the data obtained from different tehsil places respective to particular district

Site	pH	EC (dS/m)	OC (%)	P (kg/ac)	Na (meq/ 100g)	K (kg/ac)	Ca (meq/ 100g)	Mg (meq/ 100g)	Zn (ppm)	Cu (ppm)	Fe (ppm)
Dhule	7.91±0.4	1.86±0.2	2.74±0.0	22.49±0.1	29.9±0.9	2.7±0.1	9.55±0.2	6.37±0.6	0.73±0.1	0.66±0.1	18.25±5
Aurangabad	6.43±0.1	0.28±0.0	0.92±0.1	7.79±0.1	1.22±0.2	0.11±0.0	3.19±0.3	2.06±0.2	16.21±0.3	13.24±0.2	86.44±4
Beed	8.3±0.4	0.86±0.1	1.48±0.0	11.84±0.1	18.22±0.4	1.65±0.0	5.27±0.2	3.59±0.3	1.64±0.1	4.48±0.3	30.39±4
Ahmednagar	7.23±0.2	1.02±0.0	1.69±0.0	16.62±0.1	20.03±0.1	1.99±0.0	6.04±0.1	3.98±0.1	3.62±0.1	5.6±0.1	30.4±3
Pune	8.3±0.1	0.91±0.1	1.3±0.0	10.95±0.2	14.89±0.2	1.35±0.0	5.03±0.3	3.1±0.1	9.14±0.2	6.55±0.1	51.28±3
Satara	6.9±0.1	0.44±0.0	0.72±0.0	9.67±0.1	4.04±0.1	0.37±0.0	3.56±0.2	2.4±0.2	8.48±0.2	5.91±0.3	23.44±2
Sangli	6.98±0.1	1.39±0.1	1.94±0.0	8.9±0.1	21.08±0.3	1.91±0.0	6.83±0.2	4.32±0.3	5.57±0.3	1.78±0.1	28.42±2
Jalgaon	7.68±0.3	0.15±0.1	0.28±0.1	14.41±0.3	3.98±0.8	0.36±0.0	1.42±0.6	1.48±0.4	0.96±0.3	8.19±0.9	38.67±7
Kolhapur	6.53±0.0	0.42±0.0	0.63±0.0	10±0.0	3.61±0.2	0.33±0.0	3.87±0.3	2.45±0.2	11.57±0.2	7.3±0.1	57.47±2
Osmanabad	6.08±0.2	0.43±0.2	0.71±0.1	5.55±0.3	7.18±0.4	0.65±0.1	3.75±0.5	3.33±0.1	16.82±1	13.84±0.6	62±7

Note: Values are mean ± SE of two triplicates, OC-organic carbon

Tab. 4. Jaccard similarity index of AM spore type between sugarcane growing ten surveyed districts from Maharashtra

Compared District	Jaccard index	Compared District	Jaccard index	Compared District	Jaccard index
Dhule/Aurangabad	0.83	Aurangabad/Kolhapur	0.81	Pune/Satara	0.87
Dhule/Beed	0.79	Aurangabad/Osmanabad	0.80	Pune/Sangli	0.89
Dhule/Ahmadnagar	0.86	Beed/Ahmadnagar	0.85	Pune/Jalgaon	0.88
Dhule/Pune	0.90	Beed/Pune	0.89	Pune/Kolhapur	0.90
Dhule/Satara	0.85	Beed/Satara	0.84	Pune/Osmanabad	0.86
Dhule/Sangli	0.89	Beed/Sangli	0.87	Satara/Sangli	0.92
Dhule/Jalgaon	0.82	Beed/Jalgaon	0.80	Satara/Jalgaon	0.87
Dhule/Kolhapur	0.86	Beed/Kolhapur	0.85	Satara/Kolhapur	0.88
Dhule/Osmanabad	0.83	Beed/Osmanabad	0.82	Satara/Osmanabad	0.84
Aurangabad/Beed	0.81	Ahmadnagar/Pune	0.87	Sangli/Jalgaon	0.82
Aurangabad/Ahmadnagar	0.87	Ahmadnagar/Satara	0.84	Sangli/Kolhapur	0.84
Aurangabad/Pune	0.89	Ahmadnagar/Sangli	0.91	Sangli/Osmanabad	0.82
Aurangabad/Satara	0.83	Ahmadnagar/Jalgaon	0.88	Jalgaon/Kolhapur	0.74
Aurangabad/Sangli	0.85	Ahmadnagar/Kolhapur	0.90	Jalgaon/Osmanabad	0.70
Aurangabad/Jalgaon	0.78	Ahmadnagar/Osmanabad	0.87	Kolhapur/Osmanabad	0.74

Tab. 5. Pearson correlation coefficient between spore density and AM root colonization with several soil chemical properties

	Sp	RLC	pH	EC (dS/m)	OC (%)	P (kg/ac)	K (kg/ac)	Na (meq/100g)	Ca (meq/100g)	Mg (meq/100g)	Zn (ppm)	Cu (ppm)	Fe (ppm)
Sp	-	0.826*	-0.39*	-0.895*	-0.902*	-0.525*	-0.873*	-0.873*	-0.89*	-0.824*	0.672*	0.707*	0.461*
RLC	0.826*	-	0.006ns	-0.756*	-0.75*	-0.438*	0-.710*	-0.709*	-0.744*	-0.715*	0.567*	0.624*	0.479*

Note: * correlation is significant at P<0.01; ns-no significant, Sp-spore density, RLC-root length colonization

tween concentrations of other cations including Na, Ca, Mg, K and EC values and spore density as well as AM root colonization appeared furthest (Fig. 4).

Discussion

AM fungi are naturally associated with several plant species. Though they possess less host plant specificity, their occurrence and spore density is diverse and varies from place to place which may be related to physico-chemical properties of soil or due to variation in climatic changes etc (McGonigle and Miller, 1996; Sanders, 1990).

In the present study, variation of AM spore density and species diversity was observed in sugarcane rhizospheric soils of 41 different tehsil places belonging to 10 districts in Maharashtra, India. The lowest mean spore density observed in Dhule district was because of minimum spore count detected in Sindkheda tehsil place. It was observed that, spore density decreased significantly with increase in soil EC value and exactly the same trend of relationship was recorded in extent of AM root colonization with soil EC value. This finding was supported by the reports of several workers, where they have mentioned less spore density in saline soil (Barrow *et al.*, 1997; Carvalho *et al.*, 2004; Kim and Weber, 1985). The present results also support the previous findings where a negative correlation between spore density and concentrations of available P, Na, Ca, Mg etc have been reported (Aliasgharzadeh *et al.*, 2001). The decrease in spore density with an increase in soil available phosphorus observed in the study can be attributed to the fact that, available soil phosphorus inhibits AM root colonization as well as their density (Anderson, 1992).

In the present study, soil samples of most of the districts were near neutral to slightly alkaline and genus *Glomus* was dominant in this soil type and it is reported that *Glomus* is common in neutral and slightly alkaline soils (Mukerji *et al.*, 2002). Second dominant genus found in this study was *Acaulospora* and it was due to slightly acidity of soils present in some districts. Morton (1986) and Abbott and Robson (1991) have found better association of *Acaulospora* in acidic soil. It has already been reported that, pH regulates mycorrhizal status as it controls the nutrient availability from natural sources and difference in soil pH which actually determine the distribution of AM fungal species (Baylis, 1967; Moreira-Souza *et al.*, 2003).

Considerable spore diversity was observed in sugarcane rhizospheric soil samples of various districts under this study. Among the AM species, *Glomus fasciculatum*, *Glomus intraradices*, *Glomus mosseae* and *Glomus versiforme* were found to be abundant in various locations of ten districts. Though there are variations in soil chemical properties district wise but, the frequency of these four AM species was more as compared to others. It is possible that sugarcane root acts as suitable host for the formation of mutualistic symbiosis with these AM species.

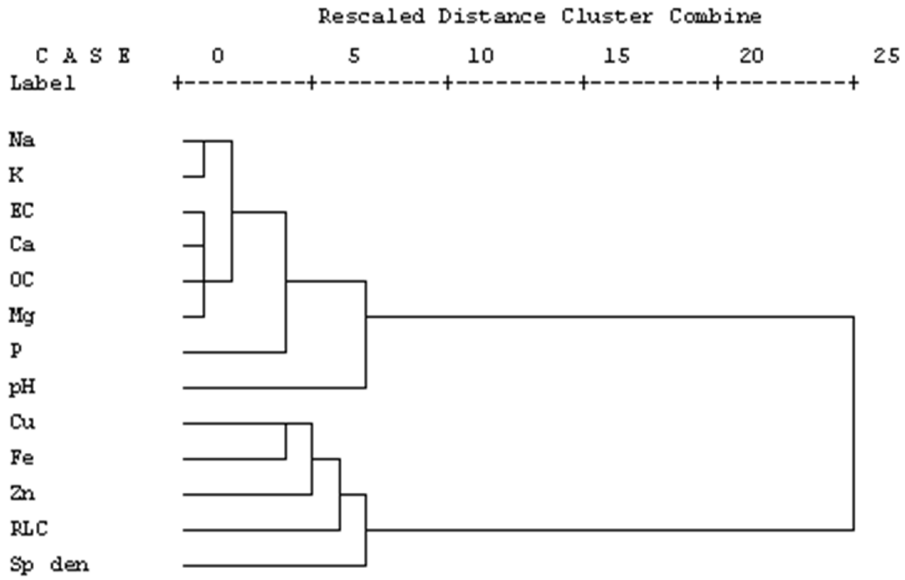

Fig. 4. Dendrogram obtained after agglomerative hierarchical cluster analysis among variables using Average Linkage.

Note: OC-organic carbon, RLC-root length colonization, Sp den-spore density

The diversity of AM fungi at the different sites may be influenced by several environmental and biological factors (Smith and Smith, 1996). Also, several researchers have pointed out the variation in AM spore density and their sporulation in association with particular host plant is because of their dormancy, seasonal sporulation and distribution patterns of AM spore in rhizospheric soil, age of host plants etc (Bever *et al.*, 1996; Gemma and Koske, 1988; Walker *et al.*, 1982). It is also reported that, intraradical development of AM fungi is highly influenced by plant species, soil pH, phosphorus contents etc (Carrenho *et al.*, 2007). The present study indicate spore density and species diversity and their extent of root colonization in sugarcane roots is also influenced by amount of Cu, Fe and Zn present in soils and this can be considered as an additional factors which supports AM spore diversity. This study would help to select appropriate AM bioinoculant for different types of soils in relation to the same host sugarcane.

Conclusions

The assessment of natural root colonization with AM fungi is essential to address the problem of nutritional management of stressed soil where crop rotation system is not followed for many years. In this study, considerable AM fungal diversity was observed under different soils with respect to the same host plant sugarcane. The variation in distribution can be correlated with Cu, Fe and Zn status of the soil. But, within this diverse distribution, the species *Glomus fasciculatum, Glomus intraradices, Glomus mosseae* and *Glomus versiforme* were the most dominant and can be used individually or as a consortium as bioinoculant for improvement of sugarcane productivity. This type of study would be useful for the enhancement of sugarcane productivity in stressed soil.

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