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Influence of Soil properties on Native Arbuscular Mycorrhizal Fungi and their Distribution in Soybean (*Glycine max* (L.) Merr.) Cultivation

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ABSTRACT: Insufficient availability of comprehensive soil property data and the difficulty in establishing causality present significant impediments to researching the influence of soil properties on the incidence and distribution patterns of native arbuscular mycorrhizal fungi (AMF) associated with soybean (*Glycine max* (L.) Merr.). Furthermore, the intricate interrelationships between soil properties, spatial and temporal variabilities, and the taxonomic identification of native AMF species introduce additional complexities, hampering the comprehension of their associations and the formulation of practical strategies for promoting sustainable soybean cultivation.

Arbuscular mycorrhizal (AM) fungi are essential constituents of soil microbiota and exhibit interactions with other microorganisms in the rhizosphere, which is the region of influence of plant roots on microbial populations and other soil constituents. AM fungal interactions are influenced by general and annual fluctuations in addition to abiotic factors such as environmental and climatic conditions. This study aimed to evaluate the population dynamics of AM fungal spores in the rhizosphere soil of soybean crops in relation to physico-chemical properties. Soil and root samples were collected from three sites in the Satara district of Maharashtra State, India, and analyzed for AM spore numbers and physico-chemical properties. Results showed well-distributed AM fungal species with numbers ranging from 55 to 78 spores per 10 g of soil. A total of 28 AM fungal species representing four genera were isolated, with Glomus being the predominant genus represented by 13 species, followed by Gigaspora (4), Scutellispora (7), and Acaulospora (4). The quantity and type of AM spores found were affected by the variable soil profile, with alkaline soil attributed to an increase in the population of *Glomus* species.

Keywords: AM Fungi, Soil properties, *Glomus* spp., Soybean, Soil Profile, Rhizosphere.

INTRODUCTION

Mycorrhizae, which are plant roots that associate with symbiotic fungi, serve as functionally distinct organs that aid in the uptake of mineral nutrients from soil. This symbiotic behavior has gained significant attention in recent years due to the potential benefits it offers in agriculture, forestry, wasteland development, and the revegetation of damaged ecosystems. Furthermore, the occurrence of arbuscular mycorrhizal (AM) fungi is widespread, and they substantially enhance water and nutrient absorption in the root surface area, leading to improved plant growth (Rouphael *et al*., 2015; Bowles *et al*., 2016). Arbuscular mycorrhizal (AM) fungi enable the transfer of a variety of macro- and microelements, such as phosphorus (P), nitrogen (N), sulfur (S), potassium (K), calcium (Ca), copper (Cu), and zinc (Zn), from the soil to host plant roots in exchange for valuable photosynthates (Porras-Soriano *et al*., 2009; Briccoli *et al*., 2015; Wang *et al*., 2017). A significant number of soils across the globe are rapidly deteriorating. This decline in soil fertility is due to multiple factors, including climate change and certain agricultural management practices such as excessive chemical use and tillage (Fall *et al*., 2022). Therefore, arbuscular mycorrhizal (AM) fungi play a crucial role in enhancing both plant productivity and the sustainability of ecosystems (Gianinazzi *et al*., 2010). Among various factors, soil chemical parameters predominantly influence the dynamics of spore population, colonization, distribution, and diversity of arbuscular mycorrhizal (AM) fungal species. (Song *et al*., 2019; Šmilauer *et al*., 2020) especially the availability of mineral elements (Johnson *et al*., 2010) variations in pH (Dumbrell *et al*., 2011), and electrical conductivity (EC) (Giri *et al*., 2007; Sheng *et al*., 2008). A positive correlation exists between the availability of nitrogen (N) and the spore population as well as colonization of arbuscular mycorrhizal (AM) fungi (Egerton-Warburton *et al*., 2007; Silvana *et al*., 2020).

Soybean (*Glycine* max) is a primary source of protein, carbohydrates, vitamins, minerals, and other essential nutrients for millions of people worldwide. Approximately 85% of the global soybean crop is processed annually by crushing, resulting in soybean meal and oil production. Of the soybean meal produced, 98% is further processed for animal feed preparation. The edible oil derived from soybean accounts for around 95% of the total oil production, while the remaining 5% is used for industrial applications, such as the production of fatty acids, soaps, and biodiesel. In recent times, farmers have resorted to the use of chemical pesticides and foliar sprays to increase

soybean yield and productivity. However, the extensive use of these chemicals has led to residual toxic effects on the soil, ultimately affecting productivity. In order to employ arbuscular mycorrhizal (AM) fungi as biofertilizers within field environments, it is imperative to investigate the variables that impact their population dynamics, spatial distribution, and compositional makeup within distinct agro-climatic zones. This would enable the selection of functionally significant species and their combinations. Given the changing soil status, the objective of this research is to investigate the interrelationship of AM fungi in the occurrence and variation of soil properties in soybean fields. The study aims to isolate and identify native AM fungal species from the rhizosphere of soybean crops from selected sites in Satara district and evaluate the impact of various chemical parameters of soil on their occurrence, distribution, and spore density under field conditions.

MATERIALS AND METHODS

A. Study Sites and Duration

The investigation was conducted in the cultivated soybean fields of Satara district, Maharashtra, India, during the kharif season of 2021. Rhizosphere soil samples were collected 90 days after soybean transplantation. The experiment was carried out under a maximum relative humidity range of 6.47% - 8.61%. Table1 displays the names of the villages and the locations of the three chosen sites.

B. Collection of Roots and Soil Samples

During the luxuriant flowering and fruiting stage of soybean plants, rhizosphere soil and roots were collected from three fields of the selected site, 90 days after transplantation. Rhizosphere soil samples, weighing about 1,000 g each, were randomly collected from selected plants in each field, at a depth of 15-30 cm, and placed in polythene bags. Three replicates of each soil sample were transported to the laboratory and stored at 5°C-10°C. The physico-chemical characteristics of each replicate of soil sample, such as pH, electrical conductivity (EC), and major and minor element content, were analyzed. Additionally, the soil samples were used for the isolation, quantification, and identification of arbuscular mycorrhizal (AM) fungal spores.

C. Estimation of physico-chemical Properties of soil

A subset of the soil samples obtained from each site was utilized to determine their chemical properties. Soil analysis was conducted to assess the pH, electrical conductivity (EC), organic carbon content (OC, %), major elements (N, P, and K), and trace elements (Ca and Mg). Soil pH was determined using a pH meter, while electrical conductivity (dS/m), which is a measure of the concentration of soluble salts in the soil, was measured using a conductivity meter in 1:5 (W/V) soil-water suspensions at 25°C. The chromic acid titration method was employed to estimate OC (Walldey and Black 1934). The available nitrogen content was estimated using the Kjeldahl method, with alkaline permanganate (Subbiah, 1956) as the reagent. Meanwhile, the available phosphorus content in the soil was determined by Olsen's method, which involved extraction with sodium bicarbonate and spectrophotometric analysis (Olsen *et al.*, 1954). The total exchangeable potassium was measured using the ammonium acetate method, which utilized a flame photometer for analysis (Hanway and Heidel 1952).

D. Isolation and Estimation of AM Fungal Spore Density

The density of AM fungal spores was determined using the wet sieving and decanting method, with 100 g of rhizosphere soil as the sample (Gerdemann and Nicolson 1963). For spore isolation, the composite soil sample was used in three replicates. Approximately 100 g of soil was taken from each replicate, mixed with 1000 mL of water, and allowed to settle. After some time, the supernatant was poured through a stack of sieves with different sizes of 250, 210, 150, and 75 µm from top to bottom. The spores were collected on Whatman filter paper No. 1, and their quantification was carried out using a bino-head stereo zoom microscope. Distinguished spores/sporocarps were picked up and mounted on slides using polyvinyl alcohol lactoglycerol (PVLG).

E. Identification of Native AM Fungal Species

The PVLG-mounted intact spores and sporocarps were morphologically identified by color, size, shape, hyphal attachment, bulbous suspensor, wall structure, number of wall layers, and wall thickness using taxonomic keys. The spores were visually documented and their morphological features were assessed using the Trinocular Research Microscope with a high-definition digital camera and Biowizard imaging software. Identification of AM fungal spores was performed by following the Manual for Identification of Vesicular Arbuscular Mycorrhiza Fungi (Schenck and Pérez 1988).

F. Statistical Analysis

The Statistical Package was used to calculate Pearson's correlation coefficients between the various chemical parameters of the soil and the density and occurrence of AM fungal spores associated with soybean plants.

RESULTS AND DISCUSSION

The research was conducted in three distinct locations of soybean fields situated in Satara District, Maharashtra, India, with the purpose of investigating the influence of soil physico-chemical characteristics on the abundance, distribution, composition, and spore density of AM fungi associated with soybean cultivation.

Table 1: Names of location of three selected sites from study area (Satara District).

Sites	S1	S2	S3
Site name	UMBRAJ	PATAN	KARAD
Location	17.4006° N 74.1010° E	17.372704° N 73.9027254°E	17.2722° N 74.1844° E

Physico-chemical Properties of Soil. Table 2 presents the mean values and standard deviation (SD) of the physico-chemical properties of soil samples. The pH of soil ranged from low (7.35) to high (8.42), while the electrical conductivity (EC) ranged from 0.59 to0.72 mmhos/cm. The S3 site had the highest organic carbon (OC) content (0.67%), whereas the S2 site had the lowest (0.52%). The maximum concentrations of available nitrogen (258.7 kg/ha), potassium (1101 kg/ha), and available phosphorus (176.1 kg/ha) were found at the S1 and S2 sites, respectively. On the other hand, the minimum concentrations of available nitrogen (255.5 kg/ha), potassium (687 kg/ha), and phosphorus (32.6 kg/ha at S3) were recorded at the S3 site.

Table 2: Physico-chemical properties of soil of soybean fields.

Sr. No.	Physico-chemical properties	S1	S2	S3
1.	pH	7.35±2.71	8.28±2.87	8.42±2.91
2.	Electrical conductivity (mmhos/cm)	0.72±0.84	0.59±0.76	0.45±0.67
3.	Moisture %	6.46±2.54	8.60±2.93	8.61±2.93
4.	Organic carbon %	0.66±0.81	0.52±0.72	0.67±0.81
5.	Ca (Meql)	20.58±4.53	21.16±4.6	17.64±4.2
6.	Mg (Meql)	13.37±3.65	9.93±3.15	14.43±3.79
7.	P_2O_5 (Kg ha^{-1})	154.9±12.44	176.1±13.27	32.6±5.70
8.	K_2O (Kg ha^{-1})	1101±33.18	687±26.21	923±30.38
9.	N (Kg ha^{-1})	258.7±16.08	257.1±16.03	255.5±15.98

S1, S2 & S3: Sites of soybean crop from study area. Data are presented as mean ± SD. The means were obtained from three replicates (n = 3). EC = electrical conductivity; OC = organic carbon; N = available nitrogen; P = available phosphorous; K = available potassium; Ca = Calcium; Mg = Magnesium

Table 3: Number and FO of species of AM fungi in rhizosphere soils of soybean.

Sr. No.	AM Fungal species	RS1	RS2	RS3	FO (%)
1.	*Acaulospora laevis* Gerd. & Trappe	-	+	+	66.66
2.	*Acaulospora scorbiculata* Trappe	+	-	-	16.66
3.	*Acaulosporaspinosa* Walker & Trappe	-	-	+	16.66
4.	*Acaulospora delicata* Walker, Pfeiffer & Bloss	-	+	-	16.66
5.	*Gigaspora albida* Schenck & G.S. Sm.	-	-	+	16.66
6.	*Gigaspora decipiens* Hall & Abbott	-	+	-	16.66
7.	*Gigaspora gigantea* (Nicolson & Gerd.) Gerd. & Trappe	+	-	-	16.66
8.	*Gigaspora margarita* Becker & Hall	+	-	+	66.66
9.	*Glomus aggregatum* Schenck & Smith, Emend. Koske	+	+	+	100.0
10.	*Glomus austrae* (Berk.) Berch & Fortin	-	+	+	66.66
11.	*Glomus callosum* Sieverding	+	-	+	66.66
12.	*Glomus clarum* Nicolson & Schenck	+	-	+	66.66
13.	*Glomus constrictum* Trappe	+	+	+	100.0
14.	*Glomus fasciculatum* (Thaxt.) Gerd. & Trappe emend. Walker & Koske	+	+	+	100.0
15.	*Glomus geosporum* (Nicol. & Gerd.) C. Walker	+	+	+	100.0
16.	*Glomus halon* Rose & Trappe	+	+	+	100.0
17.	*Glomus hoi* Berch & Trappe	+	+	+	100.0
18.	*Glomus intraradices* Schenck & Smith	-	+	+	66.66
19.	*Glomus microcarpum* Tul. &Tul.	+	+	-	66.66
20.	*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe	+	+	+	100.0
21.	*Glomus versiforme* (Karsten). Berch	-	+	+	66.66
22.	*Scutellisporaauri globosa* Walker, C Hall, I.R.	+	+	-	66.66
23.	*Scutellispora calospora* (Nicol. & Gerd.) Walker & Sanders	+	-	+	66.66
24.	*Scutellispora dipapillosa* (Walker & Koske) Walker & Sanders	+	-	+	66.66
25.	*Scutellispora dipurpurascens* Morton & Koske	+	+	+	100.0
26.	*Scutellispora gilmorei* Gerd. & Trappe	-	+	+	66.66
27.	*Scutellispora heterogama* (Nicol. & Gerd.) Walker & Sanders	+	+	+	100.0
28.	*Scutellispora minuta* (Ferrer & Herrera) Walker & Sanders	+	-	+	66.66
	Total no. of species at each site	**19**	**18**	**22**	

RS= Rhizosphere field, + =Present, - = Absent

Table 4: Distribution of different species belonging to four genera of AM fungi and FO at three selected sites.

Sr. No.	Name of genus	S1	S2	S3	Total no. of AM species	FO (%)	AM spore density
1.	*Glomus*	10	11	12	33	55.93	11.00
2.	*Acaulospora*	01	02	02	05	8.47	01.66
3.	*Scutellispora*	06	04	06	16	27.11	04.33
4.	*Gigaspora*	02	01	02	05	8.47	02.00
Total no. of species at each site		**19**	**18**	**22**	**59**		

Occurrence, distribution and spore density of native AM fungal species. The aim of the present study was to investigate the distribution and occurrence of arbuscular mycorrhizal (AM) fungal species associated with soybean in Satara district, Maharashtra, India. A total of 28 AM fungal species were isolated from the rhizosphere soil of soybean plants, and their identification was based on various morphological and diagnostic characteristics (Table 3). The genus Glomus was found to be the most diverse, with a total of 13 species, followed by Scutellispora with 7species, while Acaulospora and Gigaspora represented 4 species each.

The frequency of occurrence (FO) of each species was also determined (Table 3). Results indicated that *Glomus agreegatum*, *Glomus constrictum*, *Glomus fasciculatum*, *Glomus geosporum*, *Glomus halon*, *Glomus hoi* and *Glomus mosseae* were the most abundant species, with a FO of 55.93% across all selected sites. The study aimed to investigate the distribution and occurrence of AM fungal species associated with soybean plants in selected sites of Satara district, Maharashtra, India. A total of 28 species of AM fungi were identified using morphological and other diagnostic characters. The genus Glomus was the most frequently occurring genus (55.93%), followed by Scutellispora (27.11%), and Acaulospora and Gigaspora (8.47% each). The highest values of pH, EC, OC, and N were observed in S1 and S3 sites, while the lowest values were observed in S2 site. The spore density of AM fungal species was positively correlated with these values. The highest number (22) and diversity of AM species were observed in S3 site, while the lowest number (18) of species were observed in S2 site. The findings were consistent with previous reports, indicating that the genus Glomus is widely distributed and commonly occurring in cultivated lands. The study reported maximum spore density per 100 g soil (733.33) at S3 site and minimum spore density per 100 g soil at S2 site (600.0) (Table 4). The findings indicate that the physico-chemical characteristics of soil influence the occurrence, distribution, and diversity of native AM fungal species. The specific composition of soil chemical parameters in agriculture lands determines the composition of native AM fungal species, making them bioindicators (Baltruschat *et al.*, 2019). Our study reveals that soil chemical parameters such as higher pH, EC, OC, and N values can drive the occurrence, diversity, and distribution of native AM fungal species in selected areas. Our results are consistent with previous research (Wang *et al.*, 2019; Zhu *et al*., 2020).

In the present investigation, a correlation analysis was performed to determine the relationship between soil pH and mycorrhizal status in the rhizosphere. The findings demonstrated that soil pH was the main contributing factor and had a positive influence on the mycorrhizal status of the rhizosphere. Moreover, the study revealed that soil pH was a crucial factor that influenced the composition of arbuscular mycorrhizal fungi (AMF) in the rhizosphere. This could be attributed to the fact that soil pH played a vital role in spore germination and hyphal growth (Namdas *et al*., 2007; Hindumathi and Reddy 2011). The other soil properties such as NO_3 −-N content and available K content also contributed towards mycorrhizal development in rhizosphere (Namdas *et al*., 2007). In the literature, it has been reported that the population of arbuscular mycorrhizal fungi (AMF) is influenced by various factors such as sampling time, climatic environment, and soil physico-chemical properties (Guo *et al*., 2014; Jing-Ping *et al.*, 2004; Singh *et al*., 2008). However, the current study only involved sampling at a single time point and not repeated in subsequent years. Therefore, it is recommended that future studies should consider multiple and successive samplings to assess the temporal and spatial diversity of AMF. In addition, the growth of AMF can be influenced by soil properties, particularly soil pH, NO_3−N, and available K, which may vary with soil depth. These findings provide a scientific basis for further research on the role of AMF in soybean field management to enhance sustainable productivity.

CONCLUSIONS

Mycorrhiza is a mutualistic symbiotic association between fungi and plant roots. In this association, mycorrhizal fungi facilitate the uptake of nutrients, especially phosphorus, and enhance the growth of crop plants and trees. The present study demonstrates that the population of arbuscular mycorrhizal fungi (AMF) can be influenced by various factors, including soil properties. Specifically, soil properties can impact the occurrence and distribution of AMF in soil. These findings provide a basis for future research in the field of mycorrhiza.

FUTURE SCOPE

Arbuscular mycorrhizal fungi (AMF) are beneficial fungi that establish a mutualistic symbiosis with plant roots, promoting the absorption of water, phosphorus, and other nutrients from the soil. This enhances plant growth, development, and productivity. AMF are commonly used as soil microbe-based biofertilizers that contain fungal spores. One of the key functions of AMF is to absorb, store, and transport phosphate through their hyphae, which they release into plant root tissues, thus contributing to the plant's nutrient uptake and overall health.

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Conflict of Interest. None.

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