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University of Calcutta, Kolkata 700 019, India

Seasonal and spatial influence on dynamics of Arbuscular Mycorrhizal Fungal (AMF) diversity and their colonization potentials in *Andrographis paniculata*

SOURAV DE AND NANDLAL MANDAL*
Department of Botany, Visva-Bharati University, Santiniketan- 731235, West Bengal

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Present study investigates the impact of seasonal and spatial variations in arbuscular mycorrhizal fungal (AMF) root colonization potential and diversity in terms of spore density and species richness, in relation to different soil physical properties in *Andrographis paniculata* from Ballavpur Wildlife Sanctuary. AMF root colonization was significantly higher in the rainy season compared to the summer season in both central and peripheral zones. Similarly, arbuscule formation in root cortical cells was more prominent during the rainy season, indicating active symbiosis, whereas vesicular structures increased during the summer season, suggesting survival strategies of AMF under water-limited conditions. AMF spore density and species richness peaked in the summer peripheral zone, followed by the summer central zone, while comparatively lower values were recorded during the rainy season. Among all six isolated AMF genera, *Glomus*, *Acaulospora* and *Scutellospora* were commonly observed in both zones, irrespective of the season, although their RA values showed significant variations. Overall, higher AMF species diversity was observed in the summer season as compared to the rainy season. Among all isolated species, only five, viz., *G. intraradices*, *G. mosseae*, *A. mellea*, *A. nicolsonii,* and *S. pellucida,* were commonly detected throughout the seasons and zones and were considered as dominant AMF species. Principal Component Analysis (PCA) and Pearson correlation analysis clearly demonstrated that seasonal and spatial variations strongly influenced the dynamics of AMF communities in different soil properties. Higher moisture content and WHC during rainy season favored arbuscule formation and hence active colonization by AMF, whereas elevated soil pH and reduced moisture content during summer season promoted AMF spore density and species richness.

Keywords : *Andrographis paniculata*, AMF root colonization, Ballavpur Wildlife Sanctuary, spore diversity, seasonal and spatial variation, soil properties

INTRODUCTION

Arbuscular mycorrhizal fungi are obligate symbionts and signify a mutualistic association between the fungal partner and roots of most terrestrial plants (Read *et al*. 2000). It is a type of endomycorrhiza belonging to the phylum Glomeromycota and plays a key role in plant nutrition, particularly phosphorus, in water uptake and in enhancing soil fertility and ecosystem sustainability (Schüâler *et al*. 2001).

The solubilization of different minerals by AMF is mainly achieved through the secretion of various enzymes and their mobilization via its extensive hyphal networks beyond root areas (Martin *et al*. 2017; Franco-Ramirez *et al*. 2021). Characteristically, AMF produces a unique finger-like structure inside the root cortical cells, termed arbuscules and a globose-like structure, termed vesicles, which serve for various purposes. Arbuscules are mainly known for nutrient transfer between the endosymbiont and plant partner in exchange for photosynthates. In contrast, vesicles are primarily used to store nutrients during adverse climatic conditions. Among these two structures, arbuscules are more common than vesicles; hence, this endomycorrhizal association is termed Arbuscular Mycorrhizal Fungi (AMF) rather than Vesicular Arbuscular Mycorrhizae (VAM) (Brundrett and Tedersoo, 2018).

Andrographis paniculata (Burm.f.) Nees (Acanthaceae) is an important medicinal plant known for its traditional medicinal properties to treat several respiratory, gastrointestinal,

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*Correspondence : nandlal.mandal@visva-bharati.ac.in

cardiovascular and other metabolic disorders, including skin and liver diseases. This plant is rich in diverse phytochemicals, including diterpenoids, flavonoids, phenolics and the most medicinally important andrographolide, in its leaves and stems, resulting in high demand from pharmaceutical companies (Chakraborty *et al*. 2019). Although this plant is naturally found in different agroclimatic conditions, including India, it is now cultivated on a large scale to ensure sufficient supply (Suhartono *et al*. 2020). The luxuriant growth of *A. paniculata* across diverse agroclimatic conditions is mainly due to its dependence on various rhizosphere plant growth-promoting microorganisms, including AMF. Several earlier workers reported that these AMF have immense capacity to provide mineral nutrition and water beyond the root surface, promoting growth and supporting soil aggregation and ecosystem sustainability (Lutz *et al*. 2023).

AMF root colonization and spore diversity vary from plant to plant, soil properties, geographical location and seasonal variation, resulting in differences in plant growth, survival and variable biochemical constituents. Ballavpur Wildlife Sanctuary in West Bengal is a unique protected area that represents a dry deciduous ecosystem, with clearly demarcated central and peripheral zones for anthropogenic activity (Ganguli *et al*. 2016). Being a high-value medicinal plant and strongly dependent on AMF for growth, nutrient acquisition and amelioration of soil stress, *A. paniculata* was selected as a model plant to investigate seasonal and spatial variation in AMF diversity across different ecological zones within Ballavpur Wildlife Sanctuary. Furthermore, the ubiquitous nature of *A. paniculata* as a wild plant in this sanctuary throughout the year in both zones, along with variations in soil properties and limited information on AMF associations with this plant in such lateritic soil, provides an excellent opportunity to explore more visible AMF diversity. Considering these facts, this study aims to explore how spatio-seasonal variations and soil properties influence the dynamics of AMF associations and spore diversity to support better plant growth and survival strategies.

MATERIALS AND METHODS

Study area and sampling

Ballavpur Wildlife Sanctuary (BWS) (latitude- 23.685011°N, longitude- 87.653021°E) is in Birbhum district, West Bengal and is one of the oldest sanctuaries spanning about 200 hectares and demarcated as a protected area by fencing. It has a tropical dry deciduous vegetation type with different soil types and topography in the central and peripheral zones. Red alluvial soil with low moisture content, and more woody plants and some herbaceous plant species are some characteristics of the central zone of BWS. In contrast, fine soil with comparatively high soil moisture and more herbaceous plant species in the northeast area of this sanctuary is considered the peripheral zone. Based on seasonal and spatial variations, the entire area of this sanctuary was categorized into four zones, i.e., rainy central, rainy peripheral, summer central and summer peripheral. For sampling, approximately 50 g of rhizospheric soil and fine rootlets (about 1cm) of *Andrographis paniculata* were collected in triplicate from all four zones at the maturity stage. Rhizospheric soils were collected in sterile polythene zipper bags and brought to the laboratory, where they were stored at 4°C in a refrigerator for further study. Collected fine rootlets were washed in water and preserved in formalin-acetic acid solution (FAA) with a ratio of 12.5% formaldehyde, 12.5% glacial acetic acid and 200 ml of ethanol and similarly stored at 4°C for further study (Yang *et al*. 2019).

Assessment of AMF root colonization

Following the trypan blue staining technique of Phillips and Hayman (1970), root colonization by AMF was determined as total root colonization (TRC), arbuscules and vesicles. For this, previously preserved rootlets in FAA solution were removed and washed twice with distilled water. Thereafter, they were boiled in a 10% KOH solution at 90°C in a water bath for approximately 45 minutes, or until the roots became softened. After cooling, the KOH solution was decanted, and the rootlets were acidified with 1 N HCl for about 15 minutes. After the acid treatment for the specified time, rootlets were washed again in distilled water. Next, rootlets were stained with trypan blue (0.05%) to visualize fungal structures within the root cortex cells and were left overnight to allow proper fixation. Following fixation, stained rootlets were washed to remove excess stain and using a compound microscope (ZEISS Primo Star binocular microscope), finally, rootlets were examined for the occurrence of one or more types of AMF root associations in the form of different diagnostic structures, such as arbuscules, vesicles and hyphae inside the root cortical region. Finally, using the formula given below, root colonization percentage (%RC) was calculated.

$$\%\,\mathrm{RC} = \frac{\text{No. of infected rootlets}}{\text{Total no.of rootlets observed}} \times 100$$

Isolation of AMF spores and their identification

The wet-sieving and decanting method of Gerdemann and Nicolson (1963) was used to isolate AMF spores from previously collected rhizosphere soil samples. For this, 25 g of collected rhizosphere soil samples was air-dried and taken in 500 ml beakers. Next, about 300 ml of lukewarm water was added to each beaker containing the sample soil, and the solutions were mixed thoroughly using a magnetic stirrer. Each solution was then poured into different sieves (500 to 40 ìm) arranged from bottom to top and allowed to pass through for the separation of AMF spores based on their sizes. Separated AMF spores were collected from each of the different-sized sieves and kept in respective culture tubes. These isolated AMF spores were then centrifuged at a relatively low speed (3,000 rpm) in a 10% sucrose solution for 5 minutes to remove debris and achieve a debris-free separation. Again, the centrifuged suspension containing AMF spores was passed through a series of sieves, and the residues from each sieve were collected separately. Species identification was based solely on morphological characteristics, and isolated AMF spores were transferred to a prepared solution of PVLG (Poly-Vinyl-Lacto-Glycerol), spread on a slide, gently pressed and then placed under a compound microscope to observe their characteristics. For identification of AMF spores, various morphological characteristics, including spore shape, size, colour, surface, hyphal attachment and cell wall structures were considered. Based on observations regarding the above-mentioned criteria, the final identification was made following the manuals for AMF spore identification given by Schenck and Perez (1990) and Schüâler *et al*., (2001) and using information available at Glomeromycota PHYLOGENY, ZOR Virtual Glomeromycota Herbarium and the www.invam.caf.wvu.edu website.

Determination of AMF diversity

The following formulae were used to determine AMF spore density (SD), species richness (SR), and relative abundance (RA). Similarly, different diversity indices such as Shannon-Wiener index (H'), Simpson index (D) and evenness (E) were calculated by using the following formula and methods (Gao and Guo, 2010; Kavitha and Nelson, 2013; Chahar and Belose, 2018; Verma and Verma, 2017).

Spore density (SD) = number of VAM spores in a 25 g soil sample

Species richness (SR) = number of VAM species isolated in a 25 g soil sample

$$\mathrm{RA} = \frac{\text{No. of VAM spores of a particular genus or species present}}{\text{Total no. of VAM spores isolated}} \times 100$$

Shannon-Wiener index (H')' = – ∑□□ln□□
Simpson index (D) = (*ni* -1)/*N*(*N*-1)
Evenness (E) = H'/H'max

Where *pi* represents the proportion of individuals belonging to the i^{th} species, *ni* is the number of individuals of that taxon (*i*), N refers to the total number of individuals recorded and H'max = ln S, where S is the total number of species present.

Determination of physical properties of rhizosphere soil

Soil pH and electrical conductivity (EC)

Using a digital meter, the pH value of rhizosphere soil was measured. For this, a sample of about

10 g was weighed and placed in a 100 ml beaker, then mixed with 20 ml of double-distilled water, gently stirred for 1-2 minutes with a glass rod, and the pH reading was noted (Rowell, 1994). To measure electrical conductivity (EC), an additional 30 ml of distilled water was added to the same soil-water mixture. This solution was allowed to stand overnight at room temperature, and then EC was measured in a conductivity meter (Rhoades *et al.* 1999).

Water holding capacity (WHC) and moisture content (MC)

To measure soil water-holding capacity and moisture content, a 100 g soil sample was air-dried and transferred to a perforated container lined with filter paper. This setup was then placed in a tray filled with water, allowing the soil to absorb as much moisture as possible gradually. The soil was left undisturbed for approximately 24 hours, until it became fully saturated. Wet soil, along with the container, was weighed to obtain the saturated soil weight (Gupta and Dakshinamoorthy, 1981; Black, 1965). Afterward, the moist soil was transferred to a pre-weighed moisture box and placed in a hot-air oven at 95°C for 24 hours to remove all moisture. After cooling, the oven-dried soil was weighed again to determine its dry weight. Finally, soil water-holding capacity (%) was calculated using the standard formula:

$$\begin{array}{l}\text{Water Holding}\\ \text{Capacity (\%)}\end{array} = \frac{\begin{array}{c}\text{Weight of saturated soil} -\\ \text{Weight of oven-dry soil}\end{array}}{\text{Weight of oven-dry soil}} \times 100$$

$$\text{Moisture content (\%)} = \frac{\begin{array}{c}\text{Weight of moist soil} -\\ \text{Weight of oven-dry soil}\end{array}}{\text{Weight of oven-dry soil}} \times 100$$

Statistical analysis

To determine relationships among different AMF colonization characters and spore density, all triplet data were statistically analyzed. Principal Component Analysis (PCA) and Pearson correlation were performed at the $p < 0.05$ significance level using OriginPro 2026.

RESULTS AND DISCUSSION

AMF Root Colonization

Spatial and seasonal influences on total root colonization (TRC) in *A. paniculata* were determined by the presence of any one or more fungal structures (arbuscules, vesicles and internal hyphae) inside root cortex cells in all four sampling plants (Table 1). The highest TRC% was observed in the rainy central zone (92), followed by the rainy peripheral (86), summer central (82), and the lowest (79) was observed in the summer peripheral. Similarly, Arbuscule % was highest during rainy seasons, with values of about 68% in the central zone and 60% in the peripheral zone. In contrast, arbuscule % was much lower in the summer peripheral (32%) and lowest in the summer central (30%). This result clearly indicates that variations in TRC and arbuscule formation across different zones and seasons were mainly due to differences in soil moisture content. High soil moisture content during rainy seasons creates a favorable environment for spore germination, hyphal growth, cell wall penetration and, finally, the establishment of functional symbiosis through arbuscule formation, resulting in increased percentages of TRC and arbuscules compared to summer seasons (Zhang *et al.* 2022a). Vesicle formation in the cortical cell was reversed to TRC and arbuscule formation. It was always higher (43% central and 35% peripheral) in the summer season, whereas comparatively lower values (17% central and 15% peripheral) were observed in the rainy season. That could mainly be due to water scarcity, which drives a shift in functional AMF from the active to the survival stage (Chandra, 2018).

AMF Spore Density and Species Richness

Variable spore density and species richness were observed in the rhizosphere soil of selected plants growing in different seasons and zones (Table1). Maximum SD in 25 g of soil was found in the summer peripheral zone (148), followed by the summer central zone (120). In contrast, during the rainy season, it was (114) in the peripheral rainy zone and (98) in the central rainy zone. During the study of species richness, the maximum SR value was observed in the summer

Table 1: AMF association and spore diversity in different seasons and zones in *A. paniculata*

Isolated AMF Genera	AMF association and spore diversity in different seasons and zones			
	Rainy Central	Rainy Peripheral	Summer Central	Summer Peripheral
TRC %	92 ± 2.65	86 ± 3.05	82 ± 4.16	79 ± 3.21
Arbuscule %	68 ± 3.61	60 ± 4.93	30 ± 2.65	32 ± 4.58
Vesicle %	17 ± 2.31	15 ± 2.08	43 ± 1.73	35 ± 2.65
Spore Density	98? ±3.78	114 ± 3.35	120 ± 4.19	148 ± 4.73
Species Richness	8	10	13	21

Table 2: AMF relative abundance of associated isolated AMF genera in *A. paniculata*

AMF Genus	Relative abundance (RA) of AM genera			
	Rainy Central	Rainy Peripheral	Summer Central	Summer Peripheral
Glomus	55.1	65.8	56.6	63.5
Acaulospora	32.6	28.0	25.0	22.3
Gigaspora	-	-	10.0	5.4
Scutellospora	9.2	4.3	5.0	4.7
Claroideoglomus	-	-	3.3	2.0
Diversispora	3.0	1.8	-	2.0

peripheral zone (21), followed by the summer central zone (13). In contrast, during the rainy season, it was much lower (10) in the peripheral rainy zone and reached its minimum (8) in the central rainy zone. The value of SR was higher in summer than in rainy seasons, and its number was directly associated with the SD. When AMF diversity is maximized, it can improve plants' adaptation to fluctuating environmental conditions. This study clearly reflects that the summer season in both zones showed the highest SD and SR than the rainy season, and this was mainly due to limited water availability and high temperature, which influenced AM fungi to adopt a survival mode by means of sporulation and shifted from an active growth state (Aloud *et al.* 2025).

Relative Abundance of isolated AMF genera

Altogether six AMF genera, viz., *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora*, *Claroideoglomus* and *Diversispora* were invariably isolated with different RA (Table 2 & Fig. 1). Among all *Glomus*, *Acaulospora* and *Scutellospora* were commonly present at both sites and seasons and were considered frequently occurring AMF genera; however, their RA values varied significantly. Maximum (65.8) RA of *Glomus* was recorded in the rainy peripheral zone, followed by summer peripheral (63.5), summer central zone (56.6), and minimum was noticed in rainy central (55.1) zone. RA value of *Acaulospora* and *Scutellospora* was comparatively much lower than that of *Glomus*.

Table 3 : Isolated AMF Genera and their species in different season and zone *A. paniculata*

Isolated AMF Genera and total no. of species	Isolated AMF species in different season and zone			
	Rainy Central	Rainy Peripheral	Summer Central	Summer Peripheral
Glomus (10)	*G. intraradices* *G. mosseae* *G. macrocarpum*	*G. intraradices* *G. mosseae* *G. hoi* *G. clarum*	*G. intraradices,* *G. mosseae* *G. macrocarpum* *G. fasciculatum* *G. hoi* *G.* sp1	*G. intraradices,* *G. mosseae* *G. macrocarpum* *G. fasciculatum* *G. hoi* *G.* sp1 *G. multicaulis* *G. clarum* *G.* sp2
Acaulospora (5)	*A. elegans,* *A. mellea* *A. nicolsonii*	*A. elegans,* *A. mellea* *A. nicolsonii*	*A. mellea* *A. nicolsonii* *A. laevis*	*A. elegans* *A. mellea* *A. nicolsonii* *A. laevis* *A.*sp1
Gigaspora (2)	-	-	*G. margarita* *G.* sp1	*G. margarita* *G.* sp1
Scutellospora (2)	*S. pellucida*	*S. pellucida* *S. rubra*	*S. pellucida*	*S. pellucida* *S. rubra*
Claroideoglomus (2)	-	-	*C. claroideum*	*C. claroideum* *C. etunicatum*
Diversispora (2)	*D. epigaea*	*D. epigaea*	-	*D. gibbosa*

NB: The former names of *Glomus intraradices, Glomus clarum*, *Glomus claroideum, Glomus epigaeus* and *Glomus gibbosum* is now renamed with *Rhizophagus intraradices, Rhizophagus clarus*,*Claroideoglomus claroideum*, *Diversispora epigaea* and *Diversispora gibbosa* respectively.

Table 4: Different soil physical properties in different season and zone

Rhizosphere soil properties	Season / Zone			
	Rainy Central	Rainy Peripheral	Summer Central	Summer Peripheral
pH	4.81	5.17	5.01	5.93
Moisture content (%)	17.62	32.48	5.94	14.51
Water holding capacity (WHC) %	41	59	38	54
Electrical Conductivity (EC) mS/m	0.67	0.29	0.59	0.16

The genus *Acaulospora* showed a maximum (32.6) RA value in the rainy central zone, and a minimum (22.3) was recorded in the summer peripheral zone. In contrast, *Scutellospora* showed a maximum (9.2) RA in the rainy central zone and a minimum (4.3) in the rainy peripheral zone. The genus *Diversispora* was present at all three sites, except summer central. In contrast, the genera *Gigaspora* and *Claroideoglomus* were present only in the summer central and summer peripheral zones and were absent in rainy seasons. These variations in RA values were mainly due to differences in soil physical properties (Sivakumar, 2013). During the study of the overall % RA, *Glomus* showed maximum (60.3), followed by *Acaulospora* (27), and the remaining four genera show RA below (6), indicating *Glomus* and *Acaulospora* are the most dominating genera among all other isolated genera (Fig.2). The ubiquitous dominance of

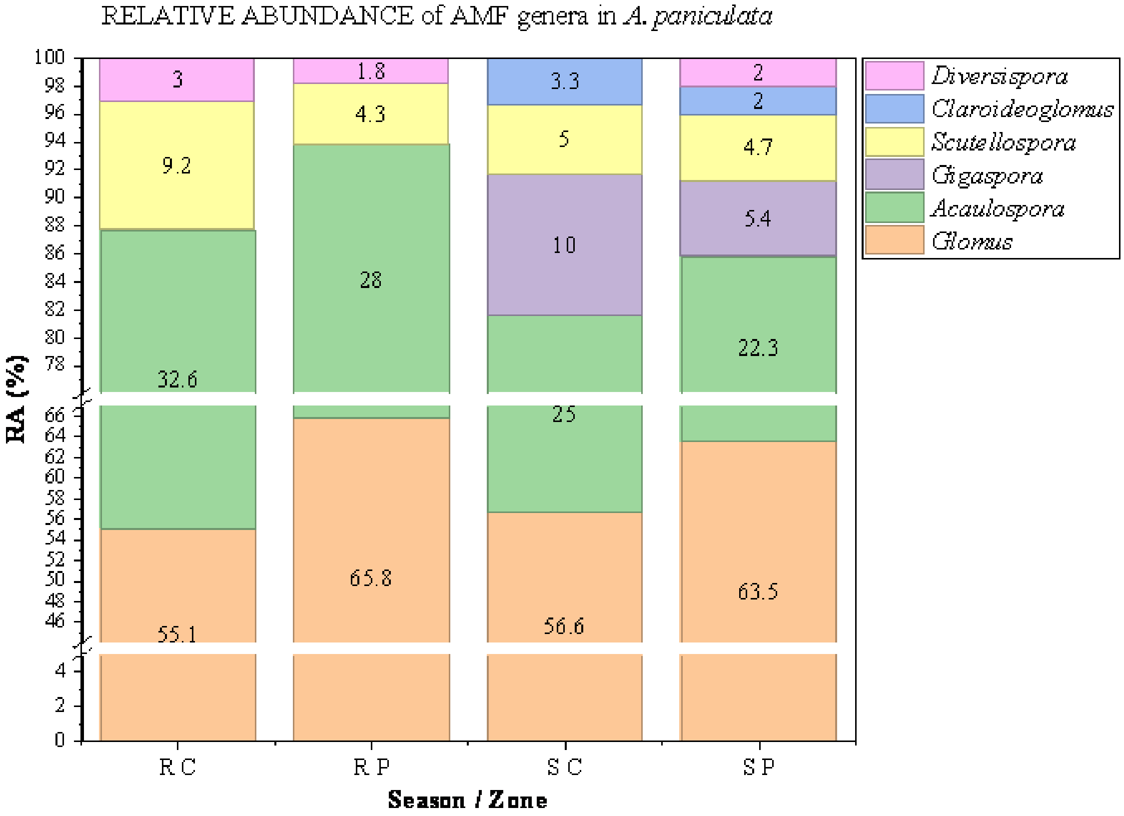

Fig.1: Relative Abundance of associated AMF genera in different seasons and zones in *A.paniculata*. RP= Rainy Peripheral, RC= Rainy Central, SP= Summer Peripheral, SC=Summer Central

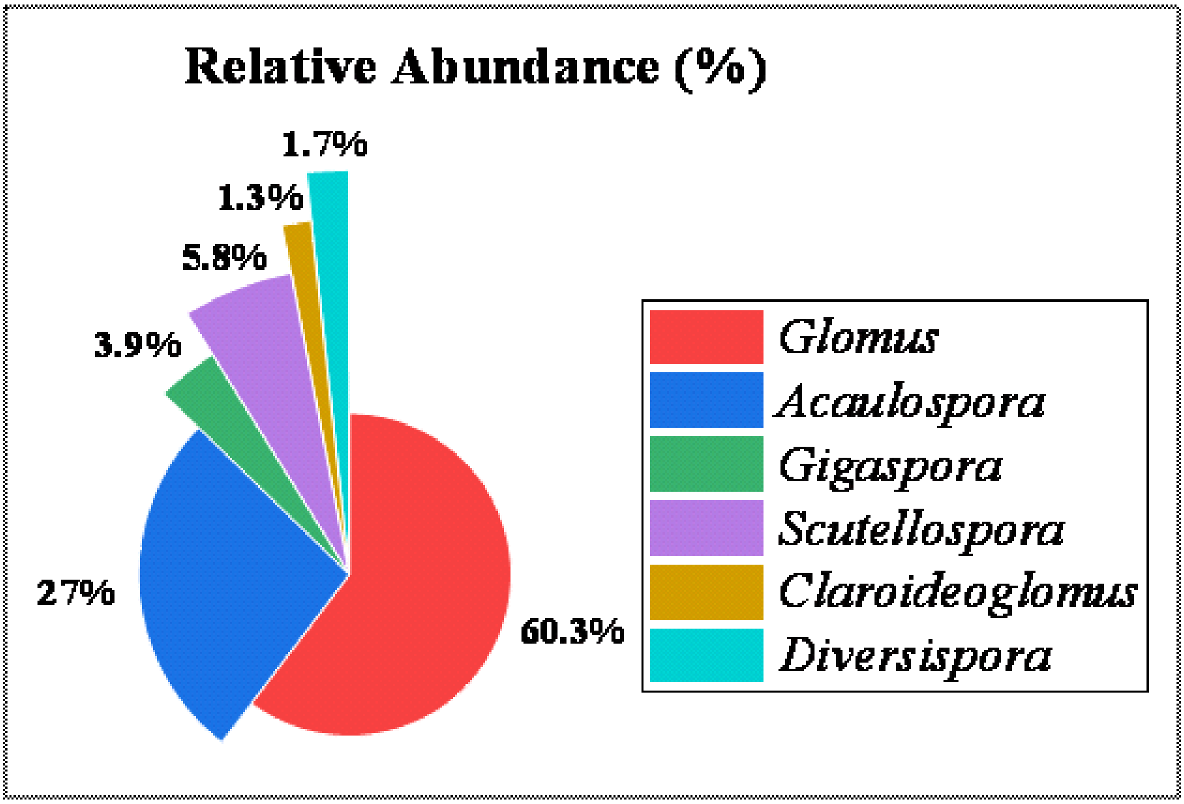

Fig 2: Overall Relative Abundance of isolated AMF generain *A. paniculata*

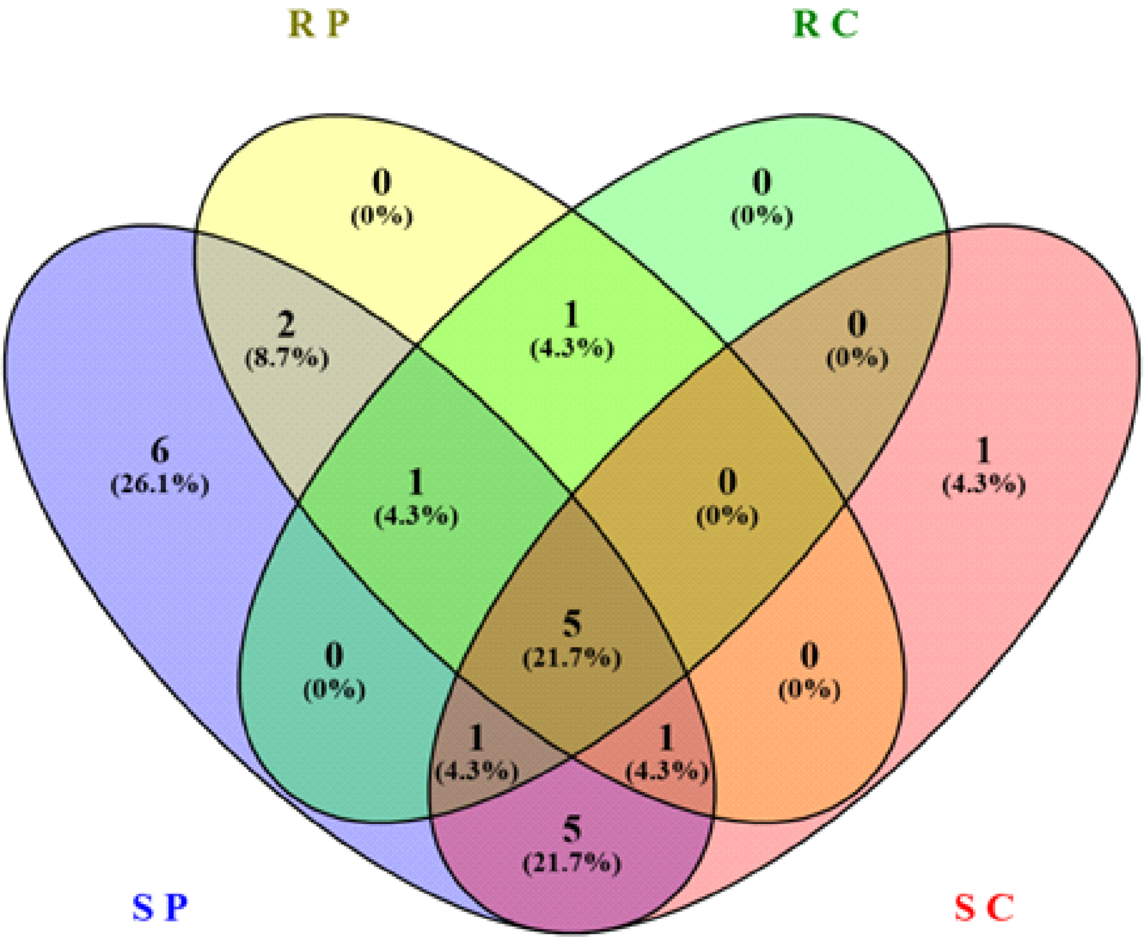

Fig 3 : Common and exclusive AMF Species in different seasons and zones in *A. paniculata* RP= Rainy Peripheral, RC= Rainy Central, SP= Summer Peripheral, SC= Summer Central

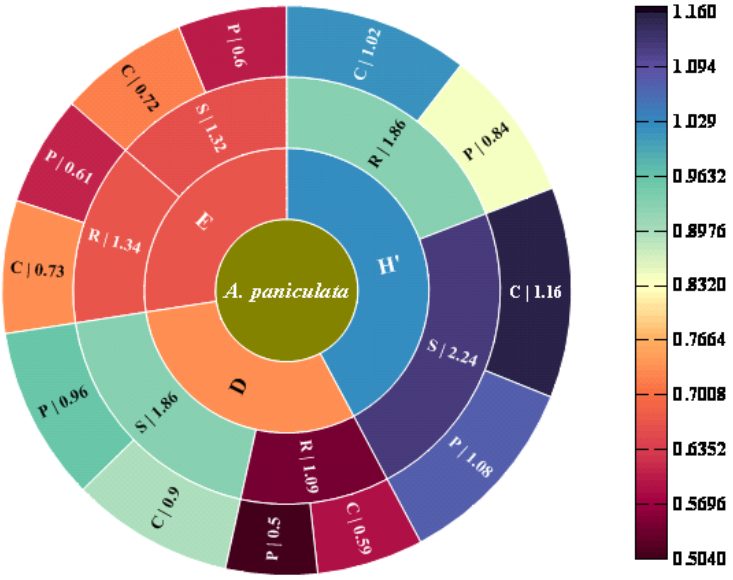

Fig 4: AMF spore diversity indices (Shannon-Wiener index (H'), Simpson index (D) and evenness (E)) in different seasons and zones in *A. paniculata*

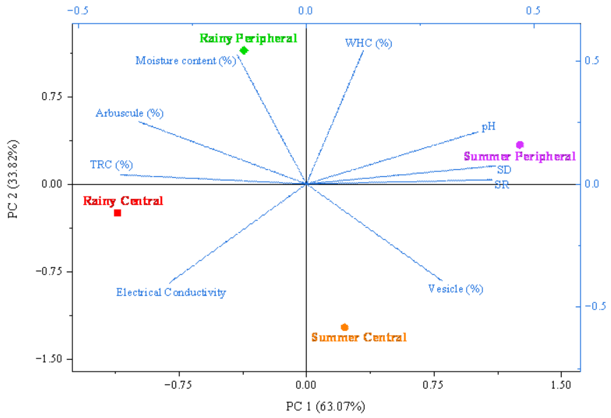

Fig 5: Principal Component Analysis among different AMF characters (TRC, Arbuscule, Vesicle, SD and SR) and soil physical parameters

Fig 6 : Correlation matrix among different AMF characters (TRC, Arbuscule, Vesicle, SD and SR) and soil physical parameters.

Glomus and *Acaulospora* over other AMF genera indicates their strong infective and adaptive abilities and is reported as the most frequent AMF genus in different climatic conditions (Halder *et al.* 2015).

AMF Species Diversity

A variable number of AMF genera and their species were observed in selected plants across different seasons and zones (Table 3). Among all sites, the summer peripheral zone contains the highest AMF genera (6) with a maximum of 21 species, followed by the summer central zone (5 genera and 13 species), indicating the highest AMF species diversity in comparison to the rainy peripheral zone (4 genera and 10 species) and rainy central zone (4 genera and 8 species). The genus *Glomus* had the most AM species (10), followed by *Acaulospora* (5), and the remaining four AMF genera (*Gigaspora*, *Scutellospora*, *Claroideoglomus* and *Diversispora*) each contained only 2 species. A Venn diagram clearly demonstrated that, across all four sampling conditions, only five AMF species, viz. *G. intraradices*, *G. mosseae*, *A. mellea*, *A. nicolsonii,* and *S. pellucida* were commonly found in all the seasons and zones, and the rest of the other species were present in variable ways in both seasons and zones (Fig. 3).

Diversity Indices

Different AMF diversity indices associated with *A. paniculata* varied seasonally and spatially across the study sites (Fig.4). Shannon-Wiener diversity index (H'), which reflects both species richness and distribution, ranged from 0.84 in the rainy peripheral zone to 1.16 in the summer central zone. The highest value recorded in the (1.16) indicates a comparatively richer and more complex AMF community. High values in Simpson's index of dominance (D) in summer peripheral (0.959) and summer central zone (0.896) indicated that only a limited number of AMF species were highly dominant in this season. Higher evenness index (E) values in the rainy central zone (0.733) suggested a relatively stable and equitable distribution of AMF species under conditions of adequate moisture availability. These findings align with earlier studies demonstrating that AMF diversity is strongly influenced by seasonal changes, particularly soil temperature, moisture, and nutrient availability, which regulate fungal sporulation and root colonization dynamics (van der Heijden *et al.* 2015; Zhang *et al.* 2022b).

Soil Physical Properties

Soil physical properties varied across zones and seasons (Table 4). Soil pH ranged from 4.81 in the rainy central zone to 5.93 in the summer peripheral zone, reflecting a slightly acidic nature. Moisture content was always higher in the rainy season than in the summer season in both zones, ranging from 5.94 in the summer central zone to 32.48 in the rainy peripheral zone. Similarly, WHC ranged from (38%) in the summer central zone to a maximum (59%) in the rainy peripheral zone. Comparatively higher (0.59-0.63 mS/m) EC was

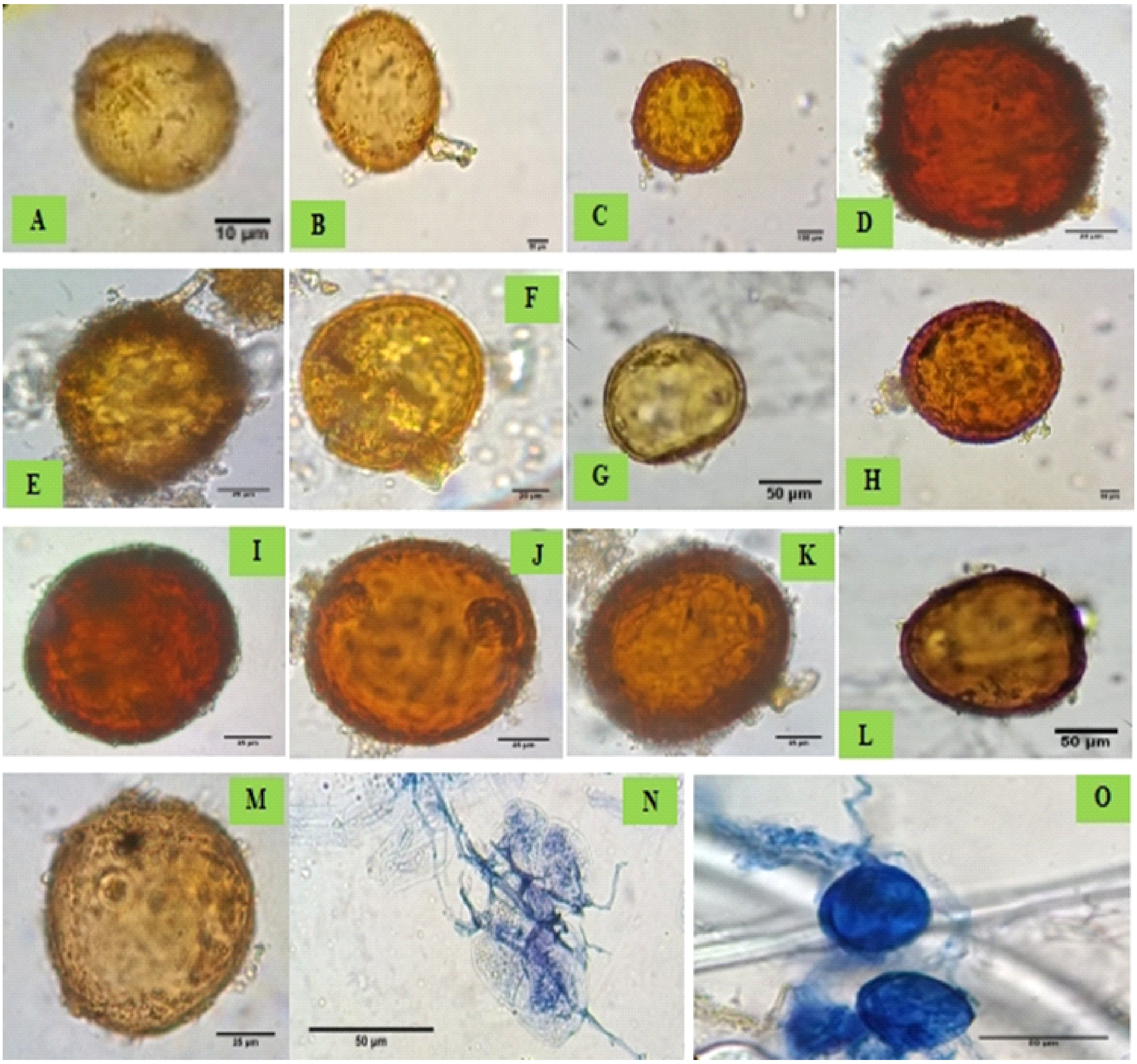

Fig 7 : Microphotograph of some dominant AM species: A) *Glomus intraradices,* B) *Glomus hoi,* C) *Glomus clarum,* D) *Glomus macrocarpum,* E) *Glomus fasciculatum,* F) *Glomus sp.,* G)*Gigaspora sp.,* H)*Scutellospora rubra,* I)*Acaulospora mellea,* J)*Acaulospora laevis,* K) *Acaulospora nicolsonii,* L) *Diversispora epigaea,* M) *Claroideoglomus claroideum* isolated from rhizosphere soil and N and O represent Arbuscules and Vesicles respectively inside the cortical cell of *A. paniculata*.

observed in the central zone, and much lower (0.29-0.16 units) in the peripheral zone. These Varied soil physical properties act as major drivers of AMF community structure and function, often determining plant-AMF-soil interactions (Wu *et al*. 2024).

Statistical analysis

Principal Component Analysis (PCA) study clearly demonstrated that rainy-season samples, particularly those from the rainy peripheral, were closely aligned with moisture content, water-holding capacity (WHC), arbuscule (%) and total root colonization (TRC), indicating that water availability was a key factor regulating AMF activity (Fig.5). Recent studies have confirmed that AMF significantly improve plant water uptake and nutrient acquisition under favorable moisture conditions, thereby strengthening the plant-fungus symbiosis (Wu *et al*., 2024). In contrast, summer samples show a clear shift toward stress-associated traits. Positioning of summer peripheral along vectors representing pH, spore density (SD), and species richness (SR) suggests that AMF communities respond to drier conditions by enhancing reproductive output and, consequently, diversity. Recent multidimensional analyses have shown that seasonal changes significantly influence AMF community composition, with spore density and diversity often increasing during warmer or drier periods (Zhang *et al*., 2022a). Summer central, positioned in the lower quadrant, shows a strong association with vesicle (%) and electrical conductivity (EC). The association with EC may reflect changes in soil ionic balance, which, in turn, can influence fungal physiology and colonization efficiency. Another important feature of the plot is the clear separation between colonization-related traits (TRC, arbuscule) and reproduction-related traits (spores, diversity). Such trade-offs, in which AMF distribution and function are mainly shaped by both climatic factors and soil physicochemical properties (Jerbi *et al*. 2022; Formenti *et al*. 2026).

Pearson correlation heatmap showed a clear trend in the matrix: strong positive correlations between parameters such as symbiotic activity (total colonization percentage) and arbuscule formation, with correlation coefficients close to +1 (Fig.6). This indicates that these variables were tightly linked and tend to increase simultaneously under favorable environmental conditions. Recent studies have emphasized that such coordinated responses are indicative of optimal plant-fungus interactions, particularly under conditions of adequate moisture and nutrients (Zhang *et al*. 2022a). In contrast, spore density, species richness, and vesicle formation show strong negative correlations with colonization traits with values approaching -0.9 or lower. This suggests a clear inverse relationship and a functional trade-off, in which AMF shift from active symbiosis to survival strategies under adverse conditions (Wu *et al*., 2024).

Microphotograph of some of the isolated AMF has been given Fig.7.

CONCLUSION

The variable AMF association between plant roots and fungi and spore diversity in BWS provides a unique ecological gradient between the central and peripheral zones and supports enhanced plant nutrient uptake, stress tolerance, and soil structure. Seasonal variation, soil properties and habitat heterogeneity significantly influence AMF colonization and diversity. Present findings clearly indicate that the rainy season promoted root colonization, whereas the summer season enhanced spore diversity and species richness. Variation in soil physical properties played an important role in shaping AMF communities and in aiding the survival of *A. paniculata.* The current study clearly indicates that AMF community structure in *A. paniculata* is shaped by a complex interaction between seasonal factors and habitat heterogeneity, underscoring the importance of microenvironmental conditions in regulating below-ground AMF dynamics.

DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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