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# Occurrence and distribution of arbuscular mycorrhizal fungi in finger millets growing tracts of Tamil Nadu, India

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**Abstract**
Arbuscular mycorrhizal fungi (AMF) in soil are a diverse group of organisms that play a vital role in ecosystem balance. However, little research has focused on the AMF diversity of finger millet. In the present study the AM fungi in the finger millet rhizosphere were extracted and diversity and distribution pattern were assessed. Result shows that AMF established a good symbiotic relationship with finger millet. Morphological identification of spores showed existence of 21 AM species in the rhizosphere of finger millet crop. Spore density in samples ranged from 47±0.73 to 132±2.4 spores 100 $g^{-1}$ soil. Species richness of AMF ranged from 4±0.04 to 10±0.15. Shannon and Simpson diversity index ranged from 1.25±0.01 to 2.1±0.003 and 0.71±0.02 to 0.87±0.01. The 21 species recorded from the samples were belongs to 11 genera, viz., *Glomus, Claroideoglomus, Paraglomus, Rhizophagus, Funneliformis, Gigaspora, Entrophosphora, Acaulospora, Ambispora, Scutellospora* and *Septoglomus.* The genus *Glomus* was the most dominant genera and *Funneliformis mosseae* was dominant species in our study. This research implies that native AM inoculum could be used in the respective environments to enhance plant growth and productivity.

**Keywords:** finger millet, AM fungi, colonization, diversity

## 1. Introduction
Minor millets or small millets are small seeded crops, cultivated throughout the world for food and fodder. They have ability to flourish in harsh environments. They are mostly grown in areas where major cereals would fail to give sustainable yields. But currently, attention on minor millets are increasing due to 5 to 6 times higher nutritious than major millets. Finger millet (*Eleusine coracana*) is grown worldwide and is the staple food for millions of people in Africa and Asia. It is rich in calcium, phosphorus, iron, and amino acids like tyrosine, tryptophan, cysteine and methionine, which are essential for human health. This plant is mostly grown in semiarid and tropical regions. It is a highly productive crop that can thrive under various environmental stresses. It can be cultivated on low fertile soils and is independent on use of chemical fertilizers, hence, it is a boon for the arid and semi-arid regions (Gull *et al*., 2014) [7].

One of the important components of soil microorganisms is Arbuscular mycorrhizal fungi, form symbiotic association with 80% of the terrestrial plants thereby playing a key role in plant nutrition and soil fertility. The intraradical mycelium is comprised of hyphae and other structures like arbuscules and vesicles, whereas the extra radical mycelium produces spores, explores new areas for colonization and consumes nutrients (Tommerup and Sivasithamparam, 1990) [22]. The relationship between plants and AMF are bidirectional. The host plants supply carbon compounds to AMF and in turn the plants get benefitted through AMF by increasing absorption of inorganic nutrients (Lee *et al.*, 2013) [9], tolerance to stresses created by salinity, drought, nutrient deficiency, heavy metals or high temperature (Mosbah *et al.*, 2018) [13], improve soil structure through soil aggregation by producing a glycoprotein called glomalin (Wright and Upadhyaya, 1998) [25]. As these fungi are obligatory symbionts, the plant species present in the ecosystem might determine their population and diversity (Doss and Bagyaraj, 2001) [5]. Therefore, it is essential to understand the diversity and distribution of AMF to maintain and improve the sustainability of the ecosystem. In this contest, we evaluated AMF diversity in different finger millet growing areas of Tamil Nadu.

## 2. Materials and Methods

### 2.1. Sampling sites

The samples for the present study were selected based on the finger millet cultivated districts of Tamil Nadu viz., Krishnagiri (KRG 1 & KRG 2), Coimbatore (CBE 1 & CBE 2), Namakkal (NMK 1 & NMK 2) and Thiruvannamalai (TVM 1 & TVM 2). Eight samples (two samples per district) were collected from the finger millet growing fields. Soils samples at a depth of 5 – 20 cm from 5 different positions in the field were collected in a sterile polyethylene bag and transferred to the laboratory. The root samples were separated from the adhering soil by gently washing under tap water and fixed in FAA (Formalin – Acetic acid – Alcohol) solution and were used for estimation of AMF colonization.

### 2.2. Soil properties

The soil samples were air dried ground and passed through a 2 mm sieve. The air dried soil samples were used to analyze physicochemical properties. Soil pH and Electrical conductivity were determined using pH meter and conductivity meter. We quantified soil available nitrogen, phosphorous and potassium following standard protocols (Subbiah and Asija, 1956; Olsen *et al*., 1954: Toth and Prince, 1949) [21, 14, 23]. Soil organic carbon content was estimated by volumetric method (Walkley and Black, 1934) [24].

### 2.3. Assessment of AMF colonization

Roots were cut into 1 cm pieces, cleared in 10% KOH at 90 °C for 30 min and washed three times in tap water. The root samples were acidified in 5% HCl for 5 min and then stained with 0.05% trypan blue in lactoglycerol. The stained root fragments were placed on glass slides and observed under compound microscope. Hundred root bits were examined and colonization percentage was calculated as explained by (Phillips and Hayman, 1970) [16].

### 2.4. Extraction of AMF spores

AMF spores were isolated from the soil samples by wet sieving and decantation method (Gerdemann and Nicolson, 1963). Intact and crushed spores were mounted in polyvinyl lacto-glycerol and observed under a microscope. The shape, color, spore wall and type of hyphal attachment were observed and photographed. Morphological identification was carried out using culture database established using INVAM (http;//invam.wvu.edu/) and other related studies about diversity of AMF species.

### 2.5. Diversity studies and statistical analysis

To assess the AMF diversity, species richness, relative abundance, isolation frequency, Simpson's diversity index and Shannon-weiner diversity index were determined. Species richness was defined as number of AM fungal species recorded in each study site. The relative abundance (RA) was calculated according to the equation: RA = A / $\sum a$ X 100, where A = abundance of the species i, / $\sum a$ = sum of abundances of all species. The isolation frequency was defined as the percentage of samples from which particular species were isolated. Shannon-weiner index was calculated using the formula: H' = -$\sum pi \ln pi$, where $p_i$ = $n_i$/N, $n_i$ = number of individuals of the species i and N = total number of individuals of all species (Shannon and Weaver, 1949) [18]. Simpsons's diversity index was calculated using the equation: D = 1 – $\sum n(n-1)$/N (N-1) where, n = number of individuals of particular species and N = total number of individual of all species (Simpson, 1949). To examine the relationship between soil available phosphorous and AMF diversity indices, Principal component analysis was carried out using XLSTAT software. Linear Pearson correlation was performed to study the effect of available phosphorus on spore density and root colonization.

## 3. Result

### 3.1. Soil physico chemical analysis

Roots and rhizosphere soil samples from the minor millet growing areas were collected from Tamil Nadu. The physico chemical properties of eight samples were presented in Table 1. Soils of the samples were slightly acidic to moderately neutral with pH value range from 6.5±0.08 to 8.5±0.15 and EC value range from 0.22 to 0.37±0.003 ds/m. The available nitrogen, phosphorous and potassium were ranged from 207.62±5.29 to 304.56±2.94 kg $ha^{-1}$, 22.31±0.38 to 53.63±0.5 kg $ha^{-1}$ and 160.36±1.33 to 401±4.78 kg $ha^{-1}$, respectively. The samples recorded organic carbon content from 0.35±0.01 to 0.79±0.003%.

**Table 1:** Physico-chemical properties of soil samples

| Sample name | pH | EC (ds/m) | N (Kg $ha^{-1}$) | P (Kg $ha^{-1}$) | K (Kg $ha^{-1}$) | Organic carbon (%) |
|---|---|---|---|---|---|---|
| KRG 1 | 6.7±0.08 | 0.28±0.003 | 304.56±2.94 | 48.39±0.14 | 393.53±0.8 | 0.43±0.003 |
| KRG 2 | 6.5±0.08 | 0.30±0.003 | 302.35±7.37 | 53.63±0.5 | 401±4.78 | 0.57±0.01 |
| CBE 1 | 8.5±0.15 | 0.33±0.003 | 241.69±4.51 | 46.67±0.74 | 290.39±7.25 | 0.78±0.01 |
| CBE 2 | 8.1±0.08 | 0.29±0.003 | 261.32±2.58 | 41.37±0.74 | 288.64±3.60 | 0.65±0.01 |
| NMK 1 | 8.2±0.12 | 0.30 ±0.01 | 207.62±5.29 | 25.34±0.66 | 396.49±4.33 | 0.79±0.003 |
| NMK 2 | 7.3±0.17 | 0.33±0.01 | 221.3±4.66 | 41.2±0.29 | 313.8±4.81 | 0.72±0.01 |
| TVM 1 | 7.0 ±0.08 | 0.22 | 230.34±4.28 | 22.31±0.38 | 160.36±1.33 | 0.53±0.01 |
| TVM 2 | 8.3±0.08 | 0.37±0.003 | 254.94±1.84 | 29.8±0.48 | 271.37±4.33 | 0.35±0.01 |

### 3.2. AMF root colonization

AM fungal root colonization was recorded for finger millets growing in the eight different locations. The colonization potential ranged from 69±1.4 to 91±1.79% (Fig. 1). The highest level of colonization (91±1.79%) of was observed in TVM 1 followed by KRG 2 and the lowest (69±1.4) was found in CBE 2. Correlation studies showed that AMF root colonization was negatively correlated with soil available phosphorous ($R^2$ = -0.24). Belay *et al.,* (2013) [2] reported a negative correlation between available phosphorous and AM colonization. It is known that increasing soil P will arrest the formation of AM fungi, which may be either due to the direct reaction of P on external hyphal development or indirect reaction connected with the P content of the plant (Songachan *et al.,* 2011) [20].

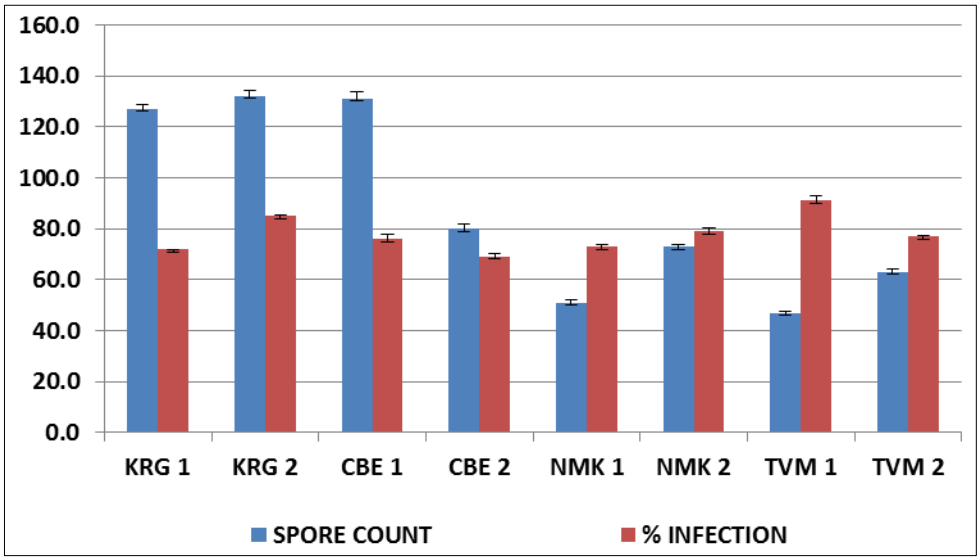


**Fig 1:** Spore density and root colonization in different samples

### 3.3. AM Fungal spore density in soil samples

In natural environment, uneven distribution of AM fungal species is common. Many studies have reported wide variations in spore density and species richness of AM fungi in different rhizosphere and root samples (Radhika & Rogrigues, 2010) [17]. AMF spores were extracted and morphologically identified from eight different soil samples. There were 47±0.73 to 132±2.4 spores in 100 g soil collected from different finger millet soil samples (Fig. 1). Among the eight soil samples, the maximum spore density (132±2.4 spores) was observed in KRG 2 and minimum (47±0.73 spores) in TVM 1. The correlation analysis found that spore density was positively correlated with soil available phosphorous ($R^2$ = 0.93) and negatively correlated with AMF colonization rate ($R^2$ = -0.151).

### 3.4. AMF diversity indices

A total of 704 AM fungal spores were extracted using wet sieving and decantation method from the eight different field samples. 21 species belonged to 11 genera were detected. The genera with highest number of species were: *Glomus* (7 species), *Claroideoglomus* (2 species), *Gigaspora* (2 species), *Funneliformis* (2 species), *Acaulospora* (2 species), while one species were *Rhizophagus*, *Paraglomus*, *Entrophosphora*, *Ambispora*, *Scutellospora* and *Septoglomus.* The study revealed that *Glomus* was the most dominant genus in our samples. The previous studies (Manoharachary *et al.*, 2005, Ambili *et al.*, 2012) [10, 1] revealed that the most dominant AMF genera were *Glomus*, *Gigaspora* and *Acaulospora*. The ability of *Glomus* to dominate the rhizosphere soil revealed that the genus had a broad host range and has a wide range of germination temperature and pH preferences (Ming-Yuan *et al.*, 2007) [12]. Among the AM fungal genera, *Glomus* and *Acaulospora* are the smallest spores so they can easily multiply and produce a large number of spores quickly (Hepper, 1984) [8]. The relative abundance of *Funneliformis mosseae* and *Rhizophagus intraradices* was higher than that of other AMF species. The most frequently occurring AMF species in our study is *Funneliformis mosseae*, recovered from 7 samples, *Rhizophagus intraradices* was recovered from 6 samples and the species *Glomus fascicultum* and *Claroideoglomus etunicatum* were recovered from 5 samples (Table 2). This is comparable with the results of Pal *et al.*, (2017) [15]. Blaszkowski *et al.*, (2001) [3] reported that *F.mosseae* is a frequently isolated species of AM fungi connected with plants of different locations.

**Table 2:** Distribution of AMF in finger millet growing areas

| AMF species | KRG 1 | KRG 2 | CBE 1 | CBE 2 | NMK 1 | NMK 2 | TVM 1 | TVM 2 | Isolation frequency (%) |
|---|---|---|---|---|---|---|---|---|---|
| *Glomus fascicultum* | + | + | + | + | - | + | - | - | 62.5 |
| *Glomus macrocarpum* | - | - | - | + | - | + | + | - | 37.5 |
| *Glomus aggregatum* | - | + | - | - | - | - | - | - | 12.5 |
| *Glomus clarum* | + | + | - | - | - | - | - | + | 37.5 |
| *Glomus microcarpusum* | - | - | + | - | - | - | - | - | 12.5 |
| *Glomus hoi* | - | - | - | - | - | - | - | + | 12.5 |
| *Glomus albidum* | - | - | - | + | - | - | - | - | 12.5 |
| *Claroideoglomus etunicatum* | + | + | - | + | + | - | + | - | 62.5 |
| *Claroideoglomus claroideum* | - | - | + | - | - | - | - | - | 12.5 |
| *Paraglomus brasilianum* | - | + | - | + | - | - | + | - | 37.5 |
| *Rhizophagus intraradices* | + | + | + | - | + | + | + | - | 75.0 |
| *Funneliformis mosseae* | + | + | - | + | + | + | + | + | 87.5 |
| *Funneliformis geosporum* | - | - | + | - | - | - | - | - | 12.5 |
| *Gigaspora margarita* | - | + | + | - | - | - | - | - | 25.0 |
| *Gigaspora gigantean* | - | + | - | - | - | - | - | + | 25.0 |
| *Entrophosphora colombiana* | - | - | + | - | - | - | - | - | 12.5 |
| *Acaulospora denticulate* | + | - | - | - | - | + | - | - | 25 |
| *Acaulospora delicate* | + | - | + | - | + | - | - | - | 37.5 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Ambispora lepoticha* | + | - | + | + | - | + | - | - | 50.0 |
| *Scutellospora erythropha* | - | - | - | - | - | - | - | + | 12.5 |
| *Septoglomus constrictum* | - | + | + | - | - | - | - | + | 37.5 |

Average AMF species richness ranged from 4±0.04 to 10±0.15 species (Table 3). Maximum species richness (10±0.15) was recorded in KRG 2 and CBE 1 and minimum (4±0.04) was recorded in NMK 1. Chaurasia and Khare (2005) reported 15 AMF species in *Taxus baccata* rhizosphere soil. Diversity was calculated using Shannon and Simpson diversity index for eight samples. The Shannon and the Simpson diversity index ranged from 1.25±0.01 to 2.1±0.003 and 0.71±0.02 to 0.87±0.01. Maximum AM fungal diversity was recorded in KRG 2. Presence of maximum number of species results in higher diversity index. The lowest diversity indicates dominance of few genera (Martins and Rodrigues, 2020) [11].

**Table 3:** Diversity measurement of AMF community

| Diversity studies | KRG 1 | KRG 2 | CBE 1 | CBE 2 | NMK 1 | NMK 2 | TVM 1 | TVM 2 |
|---|---|---|---|---|---|---|---|---|
| Species richness | 8±0.03 | 10±0.15 | 10±0.21 | 7±0.15 | 4±0.04 | 6±0.12 | 5±0.1 | 6±0.03 |
| Shannon diversity index | 1.89±0.03 | 2.1±0.003 | 1.98±0.01 | 1.82±0.02 | 1.25±0.01 | 1.67±0.03 | 1.49±0.04 | 1.62±0.04 |
| Simpson diversity index | 0.82±0.001 | 0.87±0.01 | 0.84±0.02 | 0.84±0.002 | 0.71±0.02 | 0.81±0.02 | 0.78±0.01 | 0.79±0.01 |

Principle Component Analysis (PCA) was carried out to understand the influence of available phosphorous on AMF diversity indices. The results showed that first, second and third components accounted 97.12 of total variance. Cumulative Variability of PC1 (77.93%) and PC2 (15.20%) was 93.14% (Fig. 2). The variables, spore density, species richness, Shannon and Simpson diversity index were positively correlated with available phosphorous whereas root colonization percentage was negatively correlated with phosphorous. Factor loading of more than 0.9 was observed in F1 variables available phosphorous (0.968), spore density (0.938), species richness (0.964), Shannon index (0.987) and Simpson index (0.926) and the factor loading for root colonization percentage was -0.113. In F1, contribution of all the variables ranged from 0.232 to 17.843%, the highest contribution was from Shannon diversity index and the lowest was from root colonization percentage.

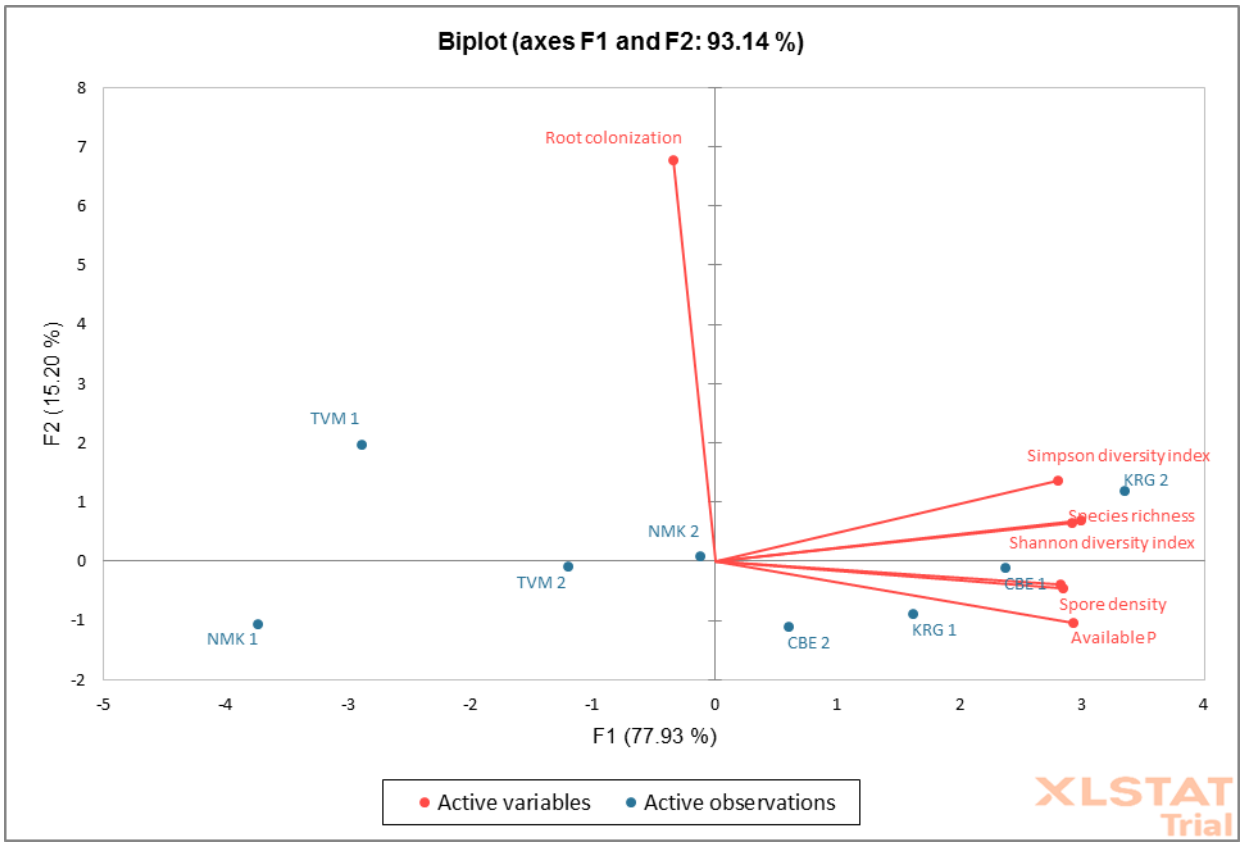


**Fig 2:** Principle component analysis of variables of soil available phosphorous with AMF diversity

## 4. Conclusion

The difference in the finger millet ecosystem can cause significant change in the diversity of AM fungi. As AM fungal colonization from the native soil is better in efficiency, cost effectiveness and easy adaptation to the environment, they can be employed as inoculum for different ecosystems. The AMF association with finger millet was further studied to improve plant stress resistance.

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