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Prevalence of AM Fungal association in Some Weeds Growing in University Botanical Garden

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Abstract:
Screening of AM fungal association on fifty five weeds belonging to nineteen families were studied by selecting university Botanical garden. It was observed that all weeds were established with AM Fungal association. However per cent root colonization and spore number do not correlated with each other. AM fungal spores represented seven species of four genera, of which Glomus was most predominated one, which is followed by Acaulospora and Scutellospora species. Very meager number of spores of sclerocystis species was found in all the selected ten sites. Abiotic factors were found to be influenced in the distribution of fungal spores. The increase in pH and decrease in phosphate results increase in spore population. Families such as Chenopodiaceae, Cyperaceae, Amaranthaceae, Polygonaceae and Zygophyllaceae were found with mycorrhization with lower percentage, though these families were disputed by early workers. In conclusion, the importance of AM fungal association with weeds community has been discussed.

Keywords: *Weeds, Botanical garden, Arbuscular Mycorrhzal (AM) fungi, per cent root colonization, spore number, Glomus species*

1. Introduction

The AM fungal symbiont becomes a major interface and connection between the soil and the plant. And they play an important role in the uptake of nutrients and water. Most of the benefits of the host plant have been obtained in soils where the available P concentration to the plant is low. They are known to enhance plant biomass through better uptake of nutrients, water relations, resistant to drought and increased tolerance to invading plant pathogens (Koide and Li, 1989; Doss and Bagyaraj, 2001; Rilling and Mummey, 2006; Lakshman, 2009). The occurrence of AMF association in natural ecosystems is currently of great interest because of the role played by AM Fungi in plant establishment and survival (Brundett, 1991; Francis and Read, 1994; Lakshman, 1996; Smith and Read, 1997). Mycorrhiza has a vital role in wasteland development programmes (Thatoi *et al.*, 1993; Chandra *et al.*, 2000b). These fungi stimulate plant growth through enhanced nutrient and water uptake in wasteland is now widely recognized. The objective of this study was to survey and identify various Arbuscular Mycorrhzal Fungi (AMF), which are prevalent in some weeds in the Botanical Garden of Karnatak University and the studies of screening on weeds are very meager. Therefore, this study was undertaken to screen AM fungi in weeds, so that cultures of indigenous AM Fungal species could be established for future studies.

2. Materials and Methods

The selected study sites were in the Botanical garden of Karnatak University. It is one of the best known Botanical garden in south western part of India. It covers 4000 square meters. Its geographical location is lying in between 14˚15' and 15˚5' North longitude and 74˚ 49' and 79˚ 21' east latitude. There is marked diurnal temperature differences. The temperature can be as below as 20.2˚C in June and high as 38. 42˚ in March. The annual rain fall is 600-850 mm, with humid or semihumid climatic conditions. Soil is covered with a hard, compact crust having dark brown colour.

Screening of weeds in Botanical garden was carried out during June 2012 to May 2013. Roots and rhizosphere soil samples were collected from 55 plants belonging to fifteen dicot and three monocot families (Table 2). Plant roots were dug out from spade, washed free of soil, and stored in formalin-aceto-alcohol (FAA) prior to staining. The cleaned roots were transferred into 10% KOH solution and autoclaved at 90ºC degree for one hour and the time period was adjusted according to root bit delicacy. 10% KOH was poured off and roots were rinsed with tap water. Root bits were taken out and acidified by placing in 2% N HCl solution washed with distilled water, stained in 0.05% tryphan blue in lactophenol, the method is proposed by (Phillips and Hayman, 1970). The per cent root colonization was estimated by the magnified interaction method (Giovennetti and mosse, 1980).

AM Fungal spores were recovered by the wet sieving and decanting technique (Gerdeman and Nicolson, 1963). Selected AM fungal spores were mounted in polyvinyl alcohol lacto phenol and identified using Schenck, Perez's manual (1990).The stained root bits were arranged on the slide and observed under microscope to study the mycelia, vesicles and arbuscules. The stained root pieces of 1 cm length were selected randomly, arranged on a slide in groups of ten. The length of infection was assessed in mm or cm for each root piece and averaged for ten pieces and expressed in percentage of colonization. The presence or absence of infection was also recorded in groups of ten each and results expressed in percentage (on the basis of slide method) 100 root bits were observed in each replicate sample. The percentage colonization was calculated by the following formula,

$$\text{Percentage of root colonization} = \frac{\text{Number of root bits showing AMF colonization}}{\text{Total number of root bits observed}} \times 100$$

The rhizosphere soil samples of individual plants within a species were mixed and one part was used for the analysis of AM Fungal spore enumeration and the other for the analysis of soil physico-chemical characters. Ten soil variables were measured (Table 1) and, the nutrients estimated according to Jackson (1967) and the percentage of organic matter according to Piper (1950). Electrical conductivity was measured using a bridge meter and pH by 1:1 soil to water ratio. The soil texture was determined by the sieving and the Bouyoucos methods(1962).

3. Results and Discussion

Rhizosphere soil sample from ten different locations in the garden were subjected for recovery of AM Fungal spores. The soil samples of ten different location showed varied spores (Table 3). All the recovered AM fungal spores are represented seven species belonging to four generas namely *Glomus, Acaulospora, Sclerocystis* and *Scutellospora*. Significantly higher number of AM fungal spore population in each soil sample recorded in Table (2). AM fungal spore number at different locations range from 52 to 340 per 50 gram of soil. Whereas, the percentage of colonization was observed from 12.3% to 94.2%. During the study period a wide variation in AM fungal species and percentage of root colonization was observed. The *Glomus* was found dominant spore genera than the other three genera in most of the regions as showed in the Table (3). Significantly, least, number of spores belonging to the genera sclerocystis was found in all locations. In the present investigation optimum numbers of fungal spores were isolated. However, there was significant co-relation between percentage of colonization and spore number.

Maceration and anatomical studies followed by tryphan blue staining revealed different stages with distinct components of AM fungi. Microscopic measurements provided an assessment of the relative abundance of mycelia in roots, the density and wall thickness, etc. The coarse aseptate hyphal coils were often seen from initial penetration points. Mycorrhizal colonization and spreading of hyphae was seen more in the young terminal roots. The external hyphae showed thick irregular wall and aseptatic condition.

The highest per cent root colonization was observed i.e. from 83% to 94.2% (Table. 2) in the following plants - *Chloris barabata* Sw. (91.3%), *Eleusine indica* (L.) Gaertn. (94.2%), *Chloris gayana* Kuntch.(90.3%), *Ergostris pilosa* Beaur.(83.5), *Setaria intermedia* Roem.(92.4), *Vicoa indica* (L.) DC.(89.8%), *Ocimum bacilicum* L.(92.6%), *Sonchus oleraceus* L.(84.5%) and *Tridax procumbens* L. (86.1%). *Ocimum sanctum* L. (85.4%)

The moderate per cent root colonization was observed i.e. from 50% to 83% (Table. 2) in the following plants - *Ergrostis gangetica* (Roxb.) Steud.(71.4%), *Andropagon halepense* Pers.(69.6%), *Abutilon indicum* (Linn.)(62.5%), *Sida rhomboidea* Roxb.(53.2%), *Croton sparsiflorus* L.(78.3%), *Acalypha indica* L.(73.1%), *Euphorbia hirta* L.(61.6%), *Euphorbia prostrata* Roxb.(66.1%), *Euphorbia geniculata* Orteg.(53.1%), *Ageratum conyzoides* L.(76.4%), *Bidens pilosa* L.(69.2%), *Eclipta alba* (L.) Hassk.(74.2%), *Eupatorium odoratum* L.(66.5%), *Asteracantha longifolia* (Nees.)(54.5%), *Borreria hispida* (Linn.) K. Schum.(67.5%), *Cassia tora* L.(52%), *Crotalaria juncea* L.(55.3%), *Cassia siamea* Linn.(53.4%), *Cassia occidentalis* Linn.67.6%), *Mimosa pudica* Linn.(58.4%), *Desmodium triflorum* (L.) DC.(56.5%), *Datura stramonium* L.(63.4%), *Centella asiatica* (Linn.) (57.5%), *Oxalis corniculata* Linn.(54.4%), *Lactuca runcinata* DC.(81.9%), *Sonchus aspera (L.)* Hill (74.2%), *Vernonia cinerea* (L.) Less.(79.0%), *Evolvulus alsinoides* (L.) (61.2%), *Leucas aspera* Willd.(75.7%) and *Cleome viscosa* (L.) DC. (66.3%).

The lowest per cent root colonization was observed i.e. below 50% (Table. 2) in the following plants - *Cynodon dactylon* (L.) Pers.(37.5%), *Tribulus terestris* Linn.(23.6%), *Alternanthera sessilis* L.(19.7%), *Amaranthus spinosus* L.(14.5%), *Amaranthus viridis* L.(17.5%), *Achyranthes aspera* L.(12.3%) *Amaranthus tricolor* L.(19.8%), *Gompherena serrata* L.(21.2%), *Commelina diffusa* Burm.(13.8%), *Commelina bengalensis* Linn.(14.7%), *Commelina communis* L.(15.3%), *Polygonum glubrum* Wild.(17.2%) *Cyperus articulatus* L.(12.7%), *Cyperus kysoor* Roxb.(13.4%) and *Cyperus rotundus* Linn.(27.3%).

4. Discussion

In the present investigation most of the weeds were found in association with AM fungi. Weeds rhizosphere and roots revealed the varied number of spores and per cent root colonization. This could provide an estimation of the AM Fungal status of the weeds (Chellamuthu and Poonguzhalan, 2008), as well as measurement of the mycorrhizal colonization of each contributing species. And thus there is a possibility of an interconnected link between weeds and other plants growing in the field (Read *et al*., 1976; Khan, *et al*., 1988; Lakshman, 1994, 1996; Bowen and Rovira, 1999). In a disturbed or undisturbed plant community most of the plants will be colonized with AM Fungi in one way or the other. Low availability of spore in aquatic conditions would be directly attributed to soil aeration (Saif, 1981; Smith and Read, 1997). The present findings are in concurrence with the reported findings of early workers contribution. Abiotic factors were found to be influence mycorrizal spore population and per cent root colonization at ten localities in this study. Increase in soil pH with decrease in soil phosphorus and nitrogen results in increase

spore population and root colonization by AM fungi. However, organic matter and soil moisture do not affect these two parameters (Johnson, 1993; Lakshman, 1996; Dodd, 2000; Bagyraj, 2006).

The agroecosystems with high and intensive agronomic inputs, the number of fungal species, in comparison with undisturbed soils decreases by more than the high inputs agronomic means planting few species in monoculture, field of weeds suppressed by weedicide application, etc., can reduce the fungal distribution. The judicial application of pesticides, fungicides and fertilizers can also alter the environment and can lead the crop species almost independent of fungi or can even eliminate certain strains of AM fungal population from the rhizosphere. The elimination can occur either 1) by the AM fungus which do not tolerate the new edaphic conditions or 2) they are not able to infect the host plant under the changed conditions or 3) they are not able to complete with other AM fungal species which have become dominant due to the new growth situation (Lakshman, 2009).

Heavy colonization may be the result of early colonization. Vesicles and extensive hyphae were found in the sloughing cortical tissue of the primary roots of many plants, indicating that VAM formed soon after germination. Similarly in the present study, members of Asteraceae, Poaceae, Compositae, Euphorbiaceae and Lamiaceae had significantly higher colonization with optimum spore count was documented in (Table 2). Similar observation was reported by early workers (Harley and Harley, 1987; Brundett, 1991; Lakshman, 1996). Heavy colonization may result from the rapid colonization which is likely to occur when large numbers of propagules are present in the soil (Wilson and Trinick 1983; Johnson *et al*., 1992; Bever *et al*., 2001). Roots of weeds collected in this study were often full of vesicles and spores of *Glomus.* It was found most dominant compared to the other three genera which were recorded. Such propagules colonize more rapidly than other spores (Biermann and Linderman, 1983).

Some families like Chenopodiaceae, Cruciferae and Cyperaceae that were earlier reported to be non-mycorrhizal (Khan, 1978; Smith and Read, 1983; Francis and Read, 1995). The members of Cyperaceae, Polygonaceae, Zygophyllaceae, Amaranthaceae and Commelinaceae were found to be mycorrhizal in the present study though to a lesser content.

5. Conclusion

Screening of AM fungi on weeds in the university Botanical garden seems to be first of its kind to our knowledge. However, this investigation needs detailed seasonal study and more on physico-chemical characteristics to be know in all the selected sites to understand AM fungal distribution and its contribution.

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Characteristics	Mean value
Sand (%)	73.19
Silt (%)	16.51
Clay (%)	5.69
Organic matter (%)	0.84
pH	6.8
EC (mmohs/cm)	0.96
P (mg/kg)	0.29
K (mg/kg)	2.43
N (mg/kg)	1.44
Zn (mg/kg)	2.02
Cu (mg/kg)	1.07
Mg (mg/kg)	1.42
Pb (mg/kg)	0.83

Table 1: Mean value (SEM) of soil characteristics studied at single site

Scientific name	Family	% Root colonization	AMF spores / 50 g.of soil
Chloris barabata Sw.	Poaceae	91.3	320
Cynodon dactylon (L.) Pers.	Poaceae	97.5	98
Eleusine indica (L.) Gaertn.	Poaceae	94.2	331
Eragrostis gangetica (Roxb.) Steud.	Poaceae	81.4	214
Andropagon halepense (L.)Pers.	Poaceae	79.6	234
Chloris gayana Kuntch.	Poaceae	90.3	302
Eragostris pilosa Beaur.	Poaceae	83.5	314
Setaria intermedia Roem.	Poaceae	92.4	338
Abutilon indicum Linn.	Malvaceae	62.5	205
Sida rhomboidea Roxb.	Malvaceae	53.2	170
Croton sparsiflorus L.	Euphorbiaceae	78.3	290
Acalypha indica L.	Euphorbiaceae	73.1	216
Euphorbia hirta L.	Euphorbiaceae	61.6	235
Euphorbia prostrata Roxb.	Euphorbiaceae	66.1	299
Euphorbia geniculata Orteg.	Euphorbiaceae	53.1	143
Ageratum conyzoides L.	Asteraceae	76.4	194
Bidens pilosa L.	Asteraceae	69.2	219
Eclipta alba (L.) Hassk.	Asteraceae	74.2	262
Eupatorium odoratum L.	Asteraceae	66.5	207
Amaranthus spinosus L.	Amaranthaceae	14.5	72
Alternanthera sessilis L.	Amaranthaceae	19.7	92
Amaranthus viridis L.	Amaranthaceae	17.5	89
Achyranthes aspera L.	Amaranthaceae	12.3	52
Amaranthus tricolor L.	Amaranthaceae	19.8	96
Gompherena serrata L.	Amaranthaceae	21.2	101
Asteracantha longifolia (Nees.)	Acanthaceae	54.5	179
Borreria hispida (Linn.) K. Schum.	Rubiaceae	67.5	225
Cassia tora L.	Fabaceae	52.1	169
Crotalaria juncea L.	Fabaceae	55.3	172
Cassia siamea Linn.	Fabaceae	53.4	159
Cassia occidentalis Linn.	Fabaceae	67.6	283
Mimosa pudica Linn.	Fabaceae	58.4	207
Desmodium triflorum (L.) DC.	Fabaceae	56.5	189
Commelina diffusa Burm.	Commelinaceae	13.8	62
Commelina bengalensis Linn.	Commelinaceae	14.7	75
Commelina communis L.	Commelinaceae	15.3	81
Datura stramonium L.	Solanaceae	63.4	256
Polygonum glubrum Wild	Polygonaceae	17.2	78
Centella asiatica Linn.	Apiaceae	57.5	197
Oxalis corniculata Linn.	Oxalidaceae	54.4	169

Tribulus terestris Linn.	Zygophyllaceae	23.6	252
Vicoa indica (L.) DC.	Compositae	89.8	331
Lactuca runcinata DC	Compositae	81.9	307
Sonchus aspera (L.) Hill	Compositae	74.2	193
Sonchus oleraceus L.	Compositae	84.5	318
Tridax procumbens L.	Compositae	86.1	329
Vernonia cinerea (L.) Less.	Compositae	79.0	303
Cyperus articulatus L.	Cyperaceaeceae	12.7	56
Cyperus kysoor Roxb.	Cyperaceaeceae	13.4	64
Cyperus rotundus Linn.	Cyperaceaeceae	27.3	107
Evolvulus alsinoides L.	Convolvulaceae	61.2	201
Leucas aspera Willd.	Lamiaceae	75.7	207
Ocimum bacilicum L.	Lamiaceae	92.6	230
Ocimum sanctum L.	Lamiaceae	85.4	218
Cleome viscosa (L.) DC.	Capparidaceae	66.3	195

Table 2: Showing the presence of AM fungi in Fifty five weeds, of their root per cent colonization and spore number

Sl no	Locations	Glomus bagyarajii	Glomus geosporum	Glomus mosseae	Acaulospora trappei	Sclerocystis clavispora	Scutellospora minuta	Scutellospora calospora
1	Site 1	+	+	+	+	-	+	+
2	Site 2	+	+	+	-	-	+	-
3	Site 3	+	+	+	+	+	-	+
4	Site 4	+	-	+	+	+	-	-
5	Site 5	-	+	+	-	-	+	-
6	Site 6	+	+	+	-	+	-	-
7	Site 7	+	+	-	+	-	+	+
8	Site 8	-	+	+	+	-	+	-
9	Site 9	+	+	+	-	-	-	+
10	Site 10	+	+	+	-	-	-	-

Table 3: The distribution of different AM fungal spore genera of rhizospheric soil of weeds at different locations in Botanical Garden of Karnatak university, Dharwad
Note: + : Present ; - : Absent

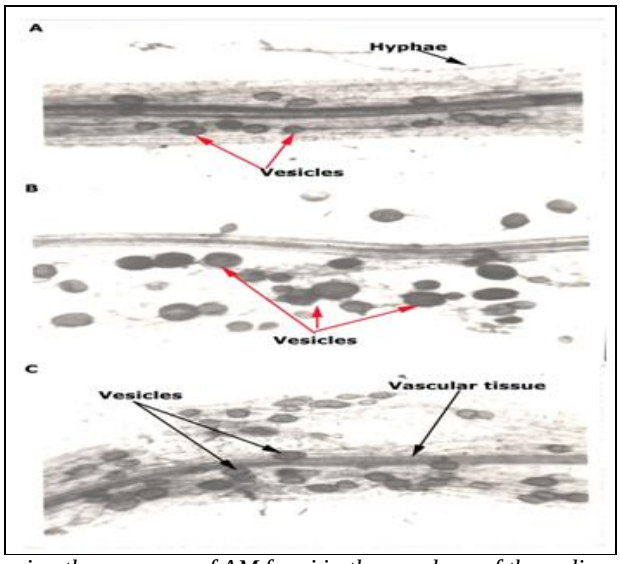

Plate 1: Showing the presence of AM fungi in the members of three disputed families

- **A** = Tribulus terestris Linn. (Zygophyllaceae): Macerated and stained root section showing hyphae entering at approsorium region, vesicles, arbuscules and central cylinder.
- **B** = Polygonum glubrum Wild (Polygonaceae): Macerated and stained root section showing globular vesicles, arbuscules and central cylinder.
- **C** = Alternanthera sessilis L. (Amaranthaceae): Macerated and stained root section showing sub globular vesicles, arbuscules and central cylinder.

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