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STUDY ON DISTRIBUTION AND CHARACTERIZATION OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI IN DIFFERENT CROPS

Vanthala Arun Kumar[1], A. Vijaya Gopal[2], R. Subhash Reddy[3], S. Triveni[4] and K. Damodara Chari[5]

[1]Department of Agricultural Microbiology & Bioenergy, College of Agriculture,

[2]Department of Agricultural Microbiology, Advanced P.G. Research centre, LAM, ANGRAU, Andhra Pradesh,India

[3]Dept. of Agricultural Microbiology & Bioenergy, College of Agriculture,

[4]Dept. of Agricultural Microbiology & Bioenergy, College of Agriculture

[5]Dept. of Agricultural Microbiology & Bioenergy

College of Agriculture, Professor Jayashankar Telangana State Agricultural University, R'nagar, Hyd-30.

ABSTRACT

In the present investigation five rhizospheric soils of different crops were selected such as Maize, Sorghum, Greengram, Sunflower and Soybean for isolation of VAM spores by spores extraction by wet sieving and decanting. These spores were identified and characterized. In almost all soil samples *Glomus* spp., *Gigaspora* spp. and *Acaulospora* spp. were present. Further these characterized spores were used in development of AM culture by trap crop method. Dominant spores of VAM fungi were treated with selected five crops such as Maize, Sorghum, Greengram, Sunflower and Soybean by the multiplied culture with pot culture treatment with Complete Randomized Block Design experiment. In all the crops *Glomus* is recorded as the dominant genus when compared to other three types. The reason might be due to its high adaptability in the soil environment

Key words : *VAM, Glomus spp, wet sieving & decanting, Maize, Sorghum, Greengram, Sunflower and Soybean.*

The green revolution in agriculture with high input of energy and chemical fertilizers and the use of high yielding varieties have enabled us to face the challenges of self-reliance in the production of food grains. Productivity improvement achieved so far has been accompanied by pollution and an exponential increase in the consumption of non-renewable forms of energy. For economic and environmental reasons, the National Research Council (NRC, 1989) recommended sustainable farming options to reduce costs, protect health and environmental quality and enhance beneficial biological interactions and natural processes. Sustainable agriculture is a farming of low energy costs and lower fertilizer inputs causing no pollution of soil, air and water. The major strategy of this sustainable farming system is integrated nutrient supply by using judicious combination of chemical fertilizers, organic manures and bio fertilizers. The symbiotic fungal association with the root tissues of higher plants known as Vesicular Arbuscular Mycorrhizae (VAM) have been evaluated as stimulants or bio-fertilizers or synergists and proved to be effective in many crop plants (George *et al*., 1998 and Hazarika *et al*., 2000). Among the biofertilizers especially Mycorrhizae are ubiquitous beneficial biofertilizer that must have immense potential in the designing of sustainable systems.

Vesicular Arbuscular Mycorrhizal (VAM) fungi are a group of obligate biotrophs, to the extent that they develop a close symbiotic association with the roots of a living host plant in order to grow and complete their life cycle (Parniske, 2008). The term "Mycorrhiza" literally derives from the Greek *mykes*and*rhiza,* meaning fungus and root respectively. Vesicular Arbuscular mycorrhizal fungi have a high relevance in many ecosystem processes. Since they can be found in many different plant species, they can provide their favourable services to almost all terrestrial ecosystems from grasslands to forests, deserts and agro ecosystems(Opik*et al*., 2006). VAM fungi can play several roles in such environments. The most agriculturally significant and frequently investigated one from both the ecological and physiological points of view (Smith and Smith, 2011) is their positive effect on plant nutrition and consequently on plant fitness. The VAM fungi helps the plant in uptake of P, N, Zn, K, Cu, Sr, S and other mineral elements (Powel and Bagyaraj, 1984). The benefit of VAM fungi is due to an increased zone of nutrient depletion by mycorrhizal roots as compared to non mycorrhizalones.VAM fungi have been shown to have a pivotal role in plant community ecology by altering plant productivity and diversity (Klironomos*et al*., 2000) changing the course of succession and affecting plant competition(Hartnett and Wilson., 1999).

It is well established that VAM can improve phosphorus uptake and growth of plants (Hayman *et al.* 1980) and is prevalent also in field crops under optimal growth conditions .In annual crops phosphorus uptake may probably be affected significantly by VAM only if infection is well established shortly after seedling emergence.

These fungi are the major component of soil microbial communities in terrestrial ecosystems, which can form mutually beneficial symbiosis with 90% of vascular plants (Liu *et al*., 2007). On global basis, mycorrhizae occur in 83% of dicots and 79% of monocots, whereas all gymnosperms are mycorrhizal. The importance of mycorrhizae to crop production is well

Table-1 : Initial Spore counts of VAM fungi in the rhizospheric soils of different crops at 30, 60, 90 and 120 DAS.

crop intervals	Spore count (No./10 gm of soil)				
	Maize	Sorghum	Sunflower	Greengram	Soybean
30 DAS	43.67	47.00	33.00	123.00	71.22
60 DAS	109.00	175.67	109.67	109.67	131.44
90 DAS	233.33	279.33	86.33	-	256.33
120 DAS	217.67	255.00	-	-	236.33
Mean	150.92	189.25	76.33	116.33	173.83
CD	7.096	24.41	10.323	10.126	18.428
SE(d)	2.845	9.786	3.621	2.186	7.387
SE(m)	2.012	6.92	2.56	1.546	5.224
CV	2.309	6.333	5.81	2.301	9.458

Table-2 : Root colonization (%) of VAM fungi in different crops at 30, 60, 90 and 120 DAS.

crop intervals	Root colonization (%)				
	Maize	Sorghum	Sunflower	Greengram	Soybean
30 DAS	19.33	28.00	12.33	48.67	15.67
60 DAS	68.33	75.33	35.33	38.33	47.67
90 DAS	79.00	87.67	31.00	-	57.00
120 DAS	58.67	73.00	-	-	50.00
Mean	56.33	66.00	26.22	43.50	42.58
CD	5.081	6.591	7.216	1.544	10.605
SE(d)	2.037	2.642	2.531	0.333	4.251
SE(m)	1.44	1.868	1.79	0.236	3.006
CV	4.428	4.903	11.823	0.939	12.227

established, yet little is understood about how cultural practices influence mycorrhizae. Indeed, little is understood with respect to basic biology and ecology of VAM fungi.

The potential for employing VAM fungi on a wide scale in agriculture is dependent on the development of crop growth promoting strains of VAMF, which are superior to native soil population of VAM fungi (Menge, 1983). The beneficial effect of indigenous VAM fungi on the nutrition of agricultural plants depends on both the abundance and type of fungi present in the soil (Abbott and Robson, 1982). So studies on occurrence of VAM in diversified environments brought out that the distribution of VAM species and numbers vary with climatic and edaphic environments as well as with land use.

The physicochemical properties like soil, temperature, moisture, pH, phosphorous, heavy metals and pesticides significantly affect the VAM propagules in soil. Of the various pesticides studied, the fungicide captan and nematicidecarbofuran had no adverse effect on VAM symbiosis. Application of fertilizers at the recommended level for different crops decreased the mycorrhizalpropagules, while application of farmyard manure stimulated VAM fungi.

There have been large reports on the nature of redistribution and diversity of mycorrhizal fungi in the disturbed soil environments.However, no such reports are available from this hot semi-arid climatic region where cultivation with intensive use of agro chemicals is widely practiced as a dominant agricultural system. Hence there is a need for studying thedistribution and characterization pattern of VAM fungi in hot semi-arid climatic regions. Therefore, the present study wasundertaken to investigate the distribution of VAM fungi in different crops grown in farm lands.

MATERIALS AND METHODS

Collection of soil samples and root samples from the field

Collection of soil samples : Soil samples were collected from fields of five crop plants listed (table 3.2) at Rajendranagar, Hyderabad region to study the natural distribution of VAM fungi. Samples were collected from 10 different places in each field. Soil was dug out with a trowel to a depth of 30 cm after scrapping away the top litter layer. Then the samples from each place within the each field were pooled and homogenized. These representative soil samples were kept in the polythene bags,labeled and stored at 20ºC until they were processed further. From this sample 50 g soil was used for wet sieving to take spore count.

Collection of root samples : The root samples of five crop plants listed in (Table-3.2) were collected from farm lands of Rajendranagar, Hyderabad. Reasonable care was taken while taking the roots such that it contained sufficient amount of feeder roots. To obtain representative samples of entire root systems, roots were taken from four or five different portions of the root system and combined. These roots were freed from adhering soil, gently washed and cut into 1cm long segments. Root samples were then placed in plastic bags, vials or plastic capsules with formalin-acetic acid-alcohol (FAA) for preservation. The standard FAA solution was prepared with 50 per cent alcohol with v/v/v ratio of 90:5:5 in adequate quantity. Maize, Sorghum, Green gram, Soybean, Sunflower.

VAM enumeration from the soil : Vesicular arbuscular mycorrhizal population was estimated in the rhizospheric soil sample preserved for the bioassay by wet sieving and decanting method of Gerdemann and Nicolson (1963) shown in plate 3.1. 50 g of rhizospheric soil sample was mixed in 200 ml of distilled water in a large beaker. After 1 hr the contents of the beaker were decanted through the sieves (710, 425, 250 and 45 μm MIC mesh sizes) which were arranged in a descending order from 710 μm to 45 μm size. The process was repeated for thrice. The procedure was repeated until the upper layer of soil suspension is transparent. Circular filter paper was taken and folded into four equal quadrants. The paper was re-opened, two lines were drawn along the two folds to divide the filter paper into four equal quadrants. Vertical lines were drawn on one half of the filter paper so as to divide it into approximately 20 columns about 0.5 cm apart. Each column was then numbered and the direction of counting was marked by an arrow. The filter paper was then folded in such a way that the marked portion becomes the receiving surface for the sample during filtration. This filter paper along with sample spores was spread in a bigger petridish. The petridish was observed under stereo binocular microscope. Two lines were focused in the field and by moving the petridish, the spores were counted in every space between the two lines and since the lines were numbered and the direction was set, it was easy to keep track of each spore on the filter paper. Shriveled and desiccated spores were omitted. Only spores that appeared to be viable (based on colour, shape, surface conditions and examination of spore contents) were counted (Eom**,** *et al.,* 2001).

Identification of Spores : The separated spores were maintained in sterile distilled water in a refrigerator after surface sterilizing with 200 ppm streptomycin for 15 min. The spores were then identified based on the characters for different taxa of order Glomales (Schenck& Perez, 1988).

Assessment of % root colonization : Clearing and staining of root specimens was done following the method of Phillip and Hayman (1970). Root samples, collected from field were washed under running tap water thoroughly, placed in glass vials containing 10 % KOH solution and heated at 90°C for about 1 hour. The KOH solution clears the host cytoplasm and nuclei and readily allows stain penetration. After heating, KOH solution was poured off and the root samples were washed using at least three complete changes of tap water or until no brown colour appeared in the rinse water. Washed roots were placed in alkaline H_2O_2 at room temperature for 1 hour or until roots were bleached. Then the roots were washed with tap water thoroughly using three changes. The alkaline H_2O_2 solution was made as per need as it loses its effectiveness on storage. After H_2O_2 treatment, the samples were treated with 1.0 % HC1 for 30 minutes and then the solution was poured off. The roots were not rinsed with water after this step because these must remain acidified for proper staining. The root samples were kept in 0.01 % staining solution (acid fuchsin) after HCl treatment and kept at 90°C for 1 hour. After removing fuchsin solution the root specimens were placed in de-staining solution for mycorrhizal assay. The specimens were not washed with water after staining because the stain is readily removed from the fungal structures. The de-staining solution was the standard staining solution as mentioned above, without the stain. Mycorrhizal colonization percentage was determined by grid line intersection method of Giovannetti and Mosse (1980). Root segments, each approximately 1 cm long, were selected at random from stained samples and mounted on microscopic slides in groups of 10. Twenty root segments from each sample were used for assessing length of cortical colonization in millimeters, at 100 X. Average of the readings from a sample, were expressed as percentage of root length colonization. Mycorrhizal colonization was expressed using the following formula.

Root colonization **(%)**

$$= \frac{\textbf{Number of VAM positive segments}}{\textbf{Total number of segments observed}} \quad \textbf{100}$$

RESULTS AND DISCUSSION

Isolation and distribution of VAM Fungi : The results of the spore count of VAM fungi in the rhizospheric soil samples of Maize field at 30, 60, 90 and 120 DAS were recorded, data were analyzed and presented in Table-1. It was observed that the mean value of spore count ranging from 43.67 to 233.33 No./10 gm of soil at 30 and 120 DAS. The maximum spore count of VAM fungi was recorded at 90 DAS (233.33 No./10 gm of soil) followed by 120 DAS (217.67 No./10 gm of soil), 60 DAS (109.00 No./10 gm of soil) and the minimum at 30 DAS (43.67 No./10 gm of soil).

In Sorghum field the data showed that the mean value of spore count ranging from 47.00 to 767.00 No./10 gm of soil. The maximum spore count of VAM fungi was recorded at 90 DAS(279.33 No./10 gm of soil) followed by 120 DAS (255.00 No./10 gm of soil). 60 DAS (175.67 No./10 gm of soil) and the minimum at 30 DAS (47.00 No./10 gm of soil). In Sunflower, it was observed that the mean value of spore count ranging from 33.00 to 109.67 No./10 gm of soil. The maximum spore count of VAM fungi was recorded at 60 DAS (109.67 No./10 gm of soil) followed by 90 DAS (86.33 No./10 gm of soil) and the minimum at 30 DAS (33.00 No./10 gm of soil). In Greengram field, the data showed that the mean value of spore count ranging from 109.67 to 123.00 No./10 gm of soil. The maximum spore count of VAM fungi was recorded at 30 DAS (123.00 No./10 gm of soil) and the minimum at 60 DAS 109.67 No./10 gm of soil.

In Soybean,thedata showed that the mean value of spore count ranging from 71.22 to256.33 No./10 gm of soil. The maximum spore count of VAM fungi was recorded in at 90 DAS (256.33 No./10 gm of soil) followed by 120 DAS (236.33 No./10 gm of soil) 60 DAS (131.44

No./10 gm of soil),) and the minimum at 30 DAS (71.22 No./10 gm of soil).

Root colonization (%) of VAM fungi in the root samples of different crops : The results of the % root colonization of VAM fungi in roots of Maize,Sorghum, Green gram, Soybean, Sunflower. crops at 30, 60, 90 and 120 DAS were recorded, data were analyzed and presented in table no 2. It was observed that the mean value of the % root colonization ranging from 19.33 to 88.00 percentage. The maximum % root colonization of VAM fungi was recorded in maize roots at followed by 90 DAS (79.00%), 60 DAS (68.33%), 120 DAS (58.67%) and the minimum at 30 DAS (19.33%).The maximum % root colonization of VAM fungi was recorded in sorghum roots at 90 DAS (87.67%) followed by 60 DAS (75.33%) 120 DAS (73.00%) and the minimum at 30 DAS (28.00%). In Sunflower crop,it was observed that the mean value of the % root colonization ranging from 53.33 to 12.33 percentage. The maximum spore count of VAM fungi was recorded at 60 DAS (35.33%) followed by 90 DAS (31.00 %) and the minimum at 30 DAS (12.33%). In Greengram crop, It was observed that the mean value of the % root colonization ranging from 38.33 to 48.67 %. The maximum spore count of VAM fungi was recorded at 30 DAS (48.67 %) and the minimum at 60 DAS (38.33%).

In soyabean crop, itwas observed that the mean value of the % root colonization ranging from 61.33 to 15.67 percentage. The maximum % root colonization of VAM fungi was recorded in soybean roots at 90 DAS (57.00 %) followed by 120 DAS (50.00%) 60 DAS (47.67 %) and the minimum at 30 DAS (15.67%).

From all the above mentioned results it can be inferred that the % root colonization of VAM fungi in the crops was low in the initial days and increased with the advancing in crop growth period and declined at 120 DAS in all the crops and also the % root colonization of VAM fungi was highest in sorghum roots. This might be due to more abundance of active spores in the rhizospheric soil due to multiplication of spores and also on the surface of the roots. These findings are in accordance with the findings of Ocampo and Hayman (1980), who all reported varied % of root colonization in different crops.

The difference in % root colonization varied with different crops. This might be due to the levels of P availability. These findings are similar to the findings of (Bagyaraj *et al.*, 1999) who reported that P availability is critical to AM symbiosis, and that percentage colonization among different species varies widely at a fixed level of P). In this study, where P content was high (>25 kg ha^{-1}), the highest percentage of colonization, and spore counts were observed. Similar observations were reported by Hayman (1983) and Sylvia and Neal (1990).

The difference in percentage colonization on roots of different crops may be due to the difference in symbiosis relation or colonizing capability of mycorrhizal fungi inhabiting the crop roots. These observations are in accordance with the observations of Neeraj and Chauhan (2006) who reported that different AM fungi differ in the ability to colonize roots of a host plant.

Morphological characterization of the isolated VAM fungi : The characterization studies and identification of spores of VAM fungi were done as per the procedure given by Schenck and Perez (1988). The spore observations, Hyphal observations, colour of spore wall and spore count percentage were done in all the soils of five crops. In this study totally of four genus of VAM fungi have been isolated from five different crops grown in farmlands of Rajendranagar. The isolated VAM spores are *Glomussp., Acaulospora sp., Gigaspora sp., and Sclerocystis sp.*

It was observed that in all the five soil samples the highest spore count percentage was registered by the genus *Glomus* only , it was followed by *Acaulospora* and *Gigaspora*the least by *Sclerocystis*. Therefore it is concluded that the predominant genus among VAM fungi are *Glomus*only, compared to all other genera. Similar observations were also reported by Schwarzott (2001) and Deepak and Anuradha (2004) stated that the largest predominant and accepted genus is *Glomus*among various genus of other VAM fungi.

In all the crops *Glomus* is recorded as the dominant genus when compared to other three types. The reason might be due to its high adaptability in the soil environment. This study corroborate to the work undertaken by Rosendahl and Stukenbrock (2004). They reported that environmental variables shaped AMF communities especially disturbance tolerant *Glomus* spp. The dominant of *Glomus* in all the rhizospheric soils may due to the abundance of *Glomus* spp. distribution in soils of Rajendranagar. Similar findings were observed by Rani and Mukerji (1987) while studying the species distribution of VAMfungi found that Indian soils are rich in *Glomus* spp.

The dominance of *Glomus sp.* may also be due to low pH of the soils. These findings are in accordance with the findings of Kendrick and Berch (1985)who reported that species of *Glomus* is linked to acid soils, in conformity with the present study. Jha *et al.* (1988) also observed that *Glomus spp*. was most abundant in tropical conditions. Joseph (1997) studied VAMspecies distribution in acid soils of South India and found maximum spore count of *Glomus spp*. which were similar with the present research findings.The nutrient tolerance also may be one reason for the dominance of the genus *Glomus* which is similar to the observations of (Valsalakumar*et al*., 2007) who reported that wide range of nutrient tolerance may be one reason for the wide spread in occurrence of the genus *Glomus.*

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