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THE SPECIES COMPOSITION AND DISPERSION OF ARBUSCULAR MYCORRHIZAL FUNGAL (AMF) SPORES IN WHEAT FIELDS OF EIGHT DISTRICTS OF SINDH, PAKISTAN

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ABSTRACT

The species composition and dispersion of arbuscular mycorrhizal fungi (AMF) spores belonging to 5 genera varied significantly ($p<0.001$) over a 3 successive wheat crop years (WCY1 to WCY3) in 8 districts of Sindh, Pakistan. WCY1 showed significantly higher number of AMF species than WCY2 and WCY3. The district of Nawabshah showed higher dispersion rate (R=1232) with significantly ($p< 0.001$) greater number of AMF species comparatively higher organic matter, soil moisture, slightly basic p^H and low percentage of soluble salts (SS) whereas the Jacobabad district showed least dispersion (R= 560) with lowest number of AMF species having low organic matter, soil moisture but higher soil p^H and higher percentage of SS. The genus *Glomus* was found to be the most predominant and genus *Acaulospora* was the least frequent. Among the 22 species, *Glomus mosseae* was the most prevalent whereas *Acaulospora gdanskensis* was the least occurring species.

Key words: Species composition of AMF spores, wheat fields and wheat crop year (WCY)

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ubiquitously found throughout the world forming symbiotic association with a wide range of vascular plants. In symbiotic associations, the absorptive area of host roots greatly extended that consequently increases the mobility of available phosphorus and other minerals from soil into host plants, which results in the increase of their growth, and yield many folds (Harley, 1989; Yocom and Boosalis, 1991). The study on species composition of AMF in different agro-ecological areas in relation to specific plant is important, as it is known that there is a wide variation in their ability to infect host roots, which ultimately increase nutrient uptake and plant growth (Sanders *et al* 1977; Habte, 1990). The AMF species composition may indicate the relative number of AMF spores species in rhizosphere region of particular plant in a given area and reflect their preference (susceptibility) towards the host. Khan (1971) identified 8 types of AMF spores in Pakistan soils on the basis of their shapes and colours viz. yellow vaculate, non endosporic, yellow vaculate endosporic, red brown laminate, white reticulate, funnel shaped honey colour, bulbous reticulate and paired bulbous reticulate from the soil of northern areas of Pakistan. On the same basis Khan (1974) further reported 5 types of AMF spores from the soil of Islamabad. Iqbal *et al* (1978) reported 6 types of AMF spores from the cereal crops of Pakistan. These early reports merely based on the descriptions of morphology of AMF spores extracted from different ecological zones of Pakistan. Saif *et al* (1977) first time identified AMF spores up to the level of species. They described three species of AMF spores viz., *Glomus mosseae*, *Glomus macrocarpum* and *Acaulospora laevis* from the soil of wheat fields of northern areas of Pakistan. Jalaluddin and Anwar (1991) then identified 13 AMF species belonging to 3 genera from wheat and rice fields of Sindh, Pakistan. Anwar and Jalaluddin (1993) described the quantitative distribution of AMF spores in the soil of 8 districts of Sindh. Here, comprehensive, statistically well planned, and methodical studies on AMF species composition and their spore dispersion in soils of wheat (*Triticum aestivum* L.) fields in the 8 districts of Sindh, Pakistan during three crops years have been carried out.

MATERIALS AND METHODS

1. **Collection of Soil samples**:

Soil samples were collected from the rhizospheres of wheat plants at their flowering stage in 8 districts of Sindh viz. Badin, Hyderabad, Jaccobabad, Mirpurkhas, Nawabshah, Sanghar, Sukkur and Thatta during three successive wheat crop years (WCY1-3). The collections were made by using multistage stratified random sampling design (Sokal and Rholf, 1987) in which the 8 districts were divided into 8 regions / strata and from each region, at least 3 wheat fields were selected randomly.

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Each field of a region in a district then divided into 3 blocks and 20 sub-samples of 200 g each collected from each block in such a way that sub-samples were located at least 30 meters apart. The sub-samples of each region of a district were then thoroughly mixed to make a composite sample. The samples were collected in transparent polyethylene bags of 30 cm^2 size and brought to the laboratory for further processing.

2. **Extraction and counting of viable AMF spores**

AMF spores from composite soil samples of a district were extracted using wet sieving and decanting method (Gerdemann and Nicolson, 1963) followed by sucrose centrifugation method (Jenkins, 1964) to obtain healthy and viable spores. Viability of the extracted spores was confirmed by vital stain method (Menge and Timmer, 1982). The extracted viable AMF spores were then studied under compound microscope (Nikon, Japan) using Polyvinyl Alcohol Lectophenol−PVAL as mounting medium on glass slide to avoid discolouration of spores. Only viable AMF spores were identified based on their macro and micro morphological characters (Schanck and Perez, 1990; Morton and Benny, 1990) and counted / 200 g soil on eelworm counting dish (Southy, 1985). The identification of AMF spores was further confirmed by their culturing, purification and recovery with the same characteristics of species.

3. **Pot culturing of AMF spores**

The identified viable AMF spores were surface disinfected using the methods of Lida (1982) and Menge (1984). At least five surface disinfected and viable spores of each species were separately and aseptically placed in close proximity with fine feeder roots of 6 days old live seedlings of wheat, grown on water agar slant (@ 1 seedling / slant) using the method of Heeper (1981). Sixteen days old AMF infected wheat seedlings from the water agar slant aseptically transplanted in well washed and surface disinfected (with $NaOCl_2$ 2 % v/v) backed clay pot @ 3 seedlings / pot of 15 cm height with 12 / 8 centimeters top and bottoms diameters respectively containing 2 Kg steam sterilized (under 1.1 Kg / cm^2 at 121 ^oC for 2 separate 1-hours periods) sandy clay loam soil (true density 2.66 g / cc, pore space 43 % and pH level 7.2) supplemented with vermiculite @ 1.5 g / 100 g soil. True density, pore space and pH of soil samples were determined by the method of Singh (1988). In this way pot of each species with 3 replicas were placed in screen house under natural condition along with control series by complete randomized design method. The seedlings were then regularly watered with sterilized distilled water. During early 3 months of growth ½ strength of Hogland solution (Hogland and Arnon, 1938) excluding "P" ingredients was added to each pot @ 250 ml / pot at 6 days interval for better growth of plants and multiplication of AMF spores (Menge, 1984).

Recovery of AMF spores

On maturity, the inoculated plants were harvested from soil level of the pots. The substrata of the pots were separately collected as mother culture. The multiplied AMF spores were then recovered from the substrata using a sieve mesh of 100 µm pore size to retain the spores of all sizes–wet sieving and decanting method (Gerdemann and Nicolson, 1963) followed by sucrose centrifugation method (Jenkins, 1964) to obtain only healthy and viable spores. The recovered spores were further studied, identified and compared with the characters of spores of pre-inoculated species to strengthen the confirmation.

RESULTS

A great deal of variation was observed in the range of dispersion (R) of AMF spores in wheat fields of 8 districts of Sindh (Fig. 1 A to H). The factorial Analysis of variance (FANOVA) of the data (Table 1) showed that the species composition of AMF spores also differed significantly ($p<0.001$) in the 8 districts of Sindh during 3 successive wheat crop years (WCY1-3). The soil of Nawabshah district showed higher range of dispersion of AMF spores (R= 1232) with organic matter (4.32 %), soil moisture (0.85 %), slight basic soil (pH 7.20) and low Soluble Salts–SS (0.12 %) with significantly higher number of AMF spores of *Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe (Fig. 1E and Table 2). The soil of Jaccobabad showed lower range of dispersion of AMF spores (R=560) with least organic matter (0.35 %), low moisture (0.35 %), basic soil (pH 8.10) higher SS (3.15 %) and lesser number of AMF species (Fig. 1C and Table 2). The dispersion of AMF spores and its species in remaining 6 districts were found in between the two streams (Fig. 1 A, B, D, F, G and H). The physico-chemical characteristics of the soil samples collected from the 8 districts of Sindh for the extraction of AMF spores are given in Table 2.

Soil samples from wheat fields of 8 districts of Sindh (Pakistan) showed the presence of AMF spores composed of 22 species belonging to 5 genera viz. *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. Of the 5 genera, the genus *Glomus* was in highly abundant than other genera and the genus *Acaulospora* was least frequent

(Fig. 1A to H). Of the 22 species, *Glomus macrocarpum* Tulasne and Tulasne was prominent species in the districts of Hyderabad, Mirpurkhas, Sanghar and Thatta (Fig. 1 B, D, F and H) whereas *Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe prevailed prominently in the districts of Badin, Jaccobabad, Nawabshah and Sukkur (Fig. 1A, C, E and G). A great deal of variation was also found in the least distribution of AMF species in the 8 districts. *Acaulospora mellea* Spain and Schenck occurred as least frequent species in Badin (Fig. 1A), *Acaulospora gdanskensis* Blaszkowski in Hyderabad, Mirpurkhas, Nawabshah (Fig. 1B, D and E), *Acaulospora foveata* Trappe and Janos in Jaccobabad and Thatta (Fig. 1C and H), *Acaulospora gerdemanii* Schenck and Nicolson in Sanghar and Sukkur (Fig. 1F and G). During three years studies, the crop year WCY2 showed higher number of AMF species in which *Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe were predominant as compared to WCY1 and WCY3 whereas *Acaulospora gdanskensis* Blaszkowski was least occurring species in the same year (Fig. 1A to 3C).

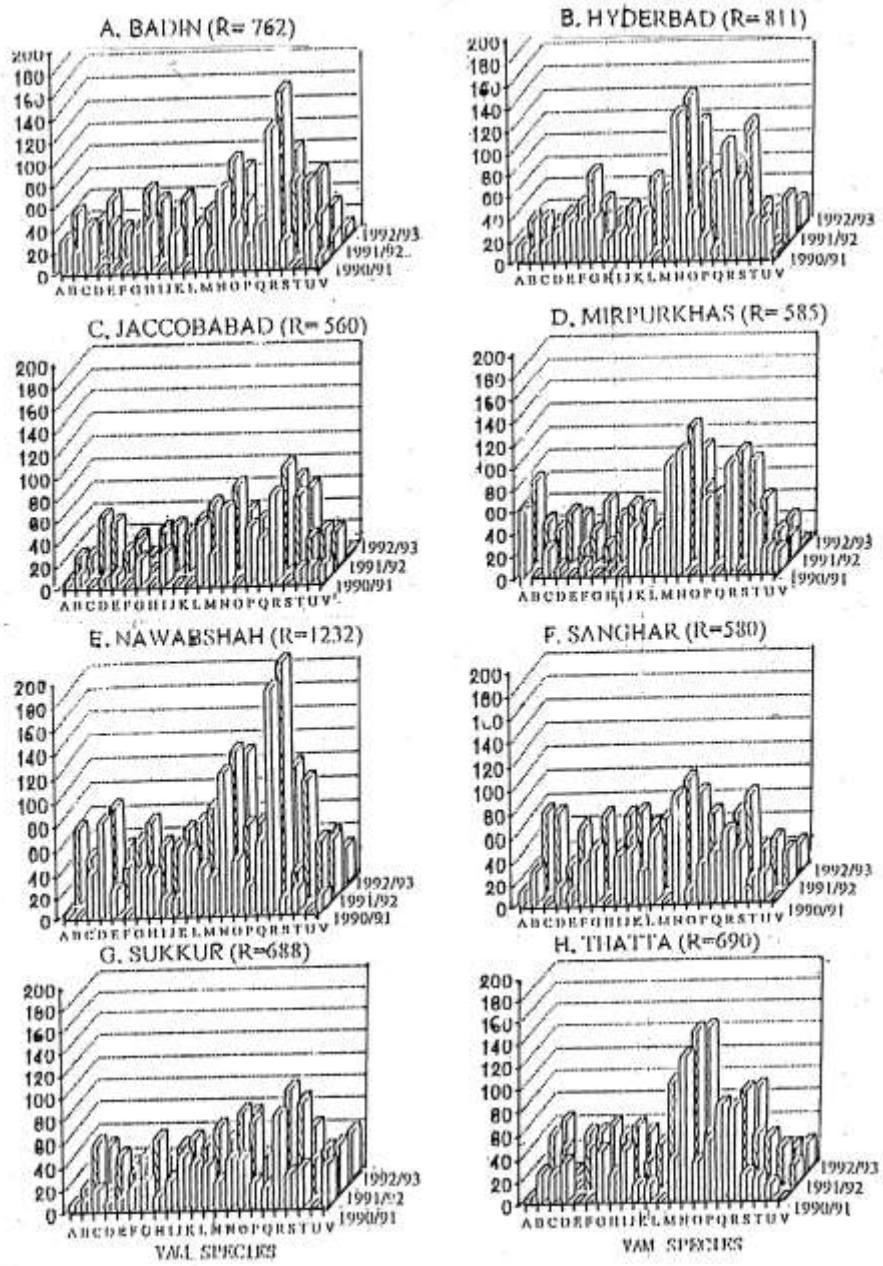

Fig.1A-H. Spores of AMF fungi in 8 districts of Sindh.

Table 1. Factorial analysis of variance (fanova) for species composition and dispersion of amf fungi in 8 districts of Sindh.

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY LEVEL
Species	1650347.14	21	30968.91	186.91	< 0.001
Districts	140568.95	07	5795.56	34.97	< 0.001
Years	4328.58	02	2164.29	13.06	< 0.001
Interactions					
Species x Districts	219709.28	147	1494.62	9.02	< 0.001
Species x Years	120056.50	42	2858.48	17.25	< 0.001
Districts x Years	6458.29	14	461.30	2.78	< 0.001
Species x Districts x Years	337913.50	294	1149.36	6.93	< 0.001
Error	174966	1056	165.68		
Total	1554348.24	1583			

Table 2. Physico chemical characteristics of soil in eight districts of Sindh, Pakistan.

	PHYSICAL PROPERTIES					CHEMICAL PROPERTIES				
DISTRICTS	**Soil color**	**Soil Texture**	**Soil composition % Sand:**	**Silt:**	**Clay**	**Moisture**	**Loss on ignition %**	**Organic matter %**	**Soil pH**	**Total Soluble Salts– TSS (%)**
1.Badin	Grey	Sandy loam	56	27	17	0.68	3.77	2.19	6.40	0.53
2.Hyderabad	Grey black	Sandy loam	57	18	55	0.76	6.67	3.08	7.20	0.18
3.Jaccobabad	Light grey	Clay loam	18	14	68	0.34	2.10	0.35	8.10	0.48
4.Mirpurkhas	Grey black	Sandy clay	40	29	31	0.58	7.73	2.90	7.10	0.24
5.Nawabshah	Black grey	Clay loam	20	28	52	0.85	10.45	4.32	7.20	0.12
6.Sanghar	Grey	Sandy loam	48	28	24	0.73	5.54	1.19	7.00	3.15
7.Sukkur	Grey	Clay loam	18	40	42	0.53	4.78	2.87	7.80	0.25
8. Thatta	Grey	Silty loam	38	41	42	0.46	3.49	2.98	8.00	0.46

DISCUSSION

The data was obtained on AMF spores dispersion and their species composition in the wheat fields of 8 districts of Sindh (Pakistan) since prior to this investigation such data were not available from this part of the world.

The identification of AMF species was carried out on the basis of macro and micro morphological characteristics (Schenck and Perez, 1990; Morton and Benny, 1990). We have found this method of identification easy and convenient as compared to the techniques given by Marx (1974), Advell *et al* (1985) and Jabaji-Hare (1988). During studies we observed that morphological features of AMF spores still form the good basis of identification, as they are easy to observe under microscope and thus practical means of distinguishing these organisms. Morton *et al* (1995) also realized the complexities for AMF spores identification at different levels. The incredulity in specificity between extracted AMF spores and the host was eliminated by performing their inoculations into the roots of wheat followed by the recovery of multiplied spores of each species from its host subsequently in the form of separate pot cultures.

Our findings showed a good deal of variation in AMF species composition (15 genera and 22 species) and their dispersion pattern in wheat fields of the 8 districts of Sindh. Our studies showed that the variation of AMF species in soil depend in multi factors including soil moisture, pH, organic contents and available soluble salts and minerals (Table 2). Abbott and Robson (1985) also observed AMF spores varied greatly in wide range of soil p^H (5.3 to 7.5).

Spore populations belonging to four genera: *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* were also isolated and analyzed by Pagano *et al*. (2010). They observed influence of different elements sequestered by AMF spores also caused variations in the population. However, our results showed the variation in species of AMF species due to organic matter, soil moisture and percentage of soluble salts (SS).

A number of environmental and soil factors might have influenced the dispersion and composition of AMF species. In addition to other factors, the physico-chemical properties of soil (Table 2) might have played the crucial role in causing variation in composition and dispersion of AMF. Such an assertion lend support to the findings of Porter *et al.* (1987), Jefferies *et al.* (1988), Abbot and Robson (1991) also reported variations in AMF population, composition and dispersion pattern which was considered due to physico-chemical properties of soil along with other external factors. Land and Schonbeck (1991) also reported that soil with different nutrient contents display a characteristic distribution pattern in the population of AMF species within a population. Joshi and Singh (1995) further reported that AMF population in soil have a direct correlation with soil properties such as available carbon, available salts, sand contents and soil pH influence the population. The previously mentioned reports corroborate our findings. Duponnois *et al* (2001) also worked out the influence of physico-chemical components of soils on the distribution of endomycorrhizal fungal spores and the mycorrhizal soil infectivity. It was concluded that relationships between abundance of mycorrhizal spores and the physico-chemical characteristics of the soils were markedly variable among species of mycorrhizal fungi. High concentrations of heavy metals have been shown to adversely affect the size, diversity, and activity of microbial populations in soil (Del Val *et al.*, 1999). Relative densities of most AMF species were also significantly influenced by soil treatments. In the case of present studies variations in the quality of the AMF population also observed in the 8 districts of Sindh, the district of Nawabshah showed higher dispersion rate (R=1232) with significantly ($p< 0.001$) greater number of AMF species comparatively higher organic matter, soil moisture and low percentage of soluble salts (SS). This result corroborated with the work of Duponnois *et al* (2001) as discussed above. Whereas the Jacobabad district showed least dispersion (R= 560) with lowest number of AMF species having low organic matter, soil moisture but higher pH and percentage of SS. It clearly indicates that species of VAMF vary in soil relation to the presence of various factors including the soil moisture, organic matters and soluble salts. Our studies showed that of the 5 genera, the genus *Glomus* was found to be most predominant and genus *Acaulospora* was least frequent. Among the 22 species, *Glomus mosseae* was most prevalent whereas *Acaulospora gdanskensis* was the least occurring species.

Fungal spore diversity of arbuscular mycorrhizal fungi associated with spring wheat was also determined by Schalamuk *et al*. (2006). They found six genera of AMF: *Acaulospora Archaeospora, Entrophospora, Gigaspora, Glomus* and *Scutellospora*. The variation in AMF species composition and its dispersion in rhizosphere soil can be explained in light of the investigation of Hall (1981) who described that there are more than 100 different AMF species. According to him, each AMF species differs in stimulating the growth of particular host due to the enhancement of phosphorus uptake and sporulating in the host roots. Therefore, such a possibility cannot be ruled out altogether and it may also be of the many factors which influences on the number of AMF spores and species composition in a rhizospheric region of particular plant species. The AMF species present in wheat field soil might have rapidly grown and sporulated to produce a higher number of spores as was found in Nawabshah (Fig. 1E). Ross & Ruttencutter (1977) correlated the higher number of AMF species in a rhizospheric soil with a particular plant species under favourable soil condition. The favourable soil condition might be in the physico-chemical properties of soil that favour AMF growth resulting in the increase of AMF spores population as was found Nawabshah (Fig, 1E). The soil of Nawabshah district in addition of containing high organic matter and soil moisture, low soluble salt (SS) as opposed to the low organic matter, soil moisture and high SS in the soil of Jaccobabad revealed a higher species composition and dispersion of AMF spores than Jaccobabad. Diedrichs & Moawad (1993) reported that some AMF species could be specific to a cultivated wheat variety. Harinkumar & Bagyaraj (1989) have reported the presence of higher number of AMF spores in intensively cultivated agricultural land with higher organic matter. The information on the prevalence of various AMF species in wheat fields of Sindh (Pakistan) should be of great importance for future research.

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