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SEASONAL DYNAMICS OF AM FUNGI IN SUGARCANE (*SACCHARUM OFFICINARUM* L. CV. SPF-213) IN RELATION TO RED ROT (*COLLETOTRICHUM FALCATUM*) DISEASE FROM PUNJAB, PAKISTAN

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Abstract

In this study we have observed the seasonal spore dynamics of 15 species of AM fungi prevalent in (cv. SPF-213) sugarcane fields in and around District Jhang, Punjab, Pakistan through out the growing season. Maximum number of spores per 10g sample soil was recorded for *G. mosseae, G. fasciculatum* and *G. monosporum* towards the end of growth period. However the pattern for highest values of propagule number in rhizosphere soil was variable for rest of the species. Maximum spore abundance for *G. mosseae, G. fasciculatum* and *G. monosporum* was noticed at the time of crop harvest. However figures close to the peak values were observed even during the growth period. The presence of 2 *Gigispora* spp. (*Gi. nigra* and *Gi. minuta*,) was recorded only in February. Lowest propagule number for *G. mosseae*, *G. monosporum* and *G. fasciculatum* was in October, September and November respectively. The difference between highest and lowest spore densities was statistically significant for *G. mosseae* and *G. monosporum* and *Gi. nigra* and *Gi. minuta,* at 5% level and insignificant for *G. fascicultum* and *G. mosseae*. There was a positive correlation between average number of spores and average percentage frequency of *G. mosseae, G. fasciculatum and G. monosporum*, while this relationship was not apparent for rest of the species. In interspecific interactions it was observed that in a particular sample higher number of propagules of one species was associated with significantly lower values of spore number of some other species.

In order to assess the AM colonization of sugarcane plants in relation to Red Rot, 4 categories of plants for disease incidence were identified as healthy, partially diseased, diseased and severely diseased. A significant change in pattern of AM colonization was recorded. Percentage frequencies of arbuscules, vesicles and intra-matricle mycelium exhibited a gradual increase from slightly diseased to severely diseased. Various AM structures showed a significant variation with the passage of time in the extent of infection. Alongwith AM and pathogenic fungi, Dark septate endophytic fungi were also observed in 80% of the samples particularly in the severely diseased specimen. Heavy colonization suggest a significant role of these fungi as biocontrol agent

Introduction

Arbuscular mycorrhizal fungi play a pivotal role in plant growth enhancement in natural and managed ecosystems (Muthukumar *et al.,* 2006; Li & Guan, 2007). The significance of these soil fungi for improving the growth of crop plants has been reported variously. These fungal structures have been reported to colonize plant portions other than roots. These organs include sheathing leaf bases and decaying stumps as in the case of wheat, maize and rice (Nasim *et al.,* 1998). Some of the relatively uncommon species of these fungi have been reported to sporulate in these decaying plant portions (Nasim & Zahoor 1995, 1996, 1997).

Seasonal variation in the activity of AMF in temperate soil is poorly understood and generally based on few observations (Sutton and Barron, 1972; Saif & Khan, 1975; Nicolson & Johnston, 1979; Muthukumar *et al.,* 2006). Although the importance of AMF to the growth and betterment of many plants is well known (Smith and Read, 1997) but the presence of propagules through the growing season has not been investigated. Availability of AM spores may restrict the extent and time of colonization of plants.

Accurate mathematical studies of seasonal spore abundance and interspecific interactions are prerequisites for constructing a picture to predict seasonal dynamics of AMF. Most of the studies of seasonal dynamics of AMF spore abundance have reported maximum populations at the end of growing season (Mason, 1964; Hyman, 1970; Sutton & Barron; 1972; Saif & Khan, 1975; Douds & Chaney, 1986). But this procedure has not been statistically documented. A common difficulty in detecting statistically significant seasonal trends results from an aggregated type of spore distribution of AMF spores in the soil (Anderson *et al*., 1983; Fries, 1984; Sylvia, 1986).

The AM fungi afford host plant greater resistance to environmental stresses like osmotic stress (Ruiz-Lozano, 2003), salinity (Feng *et al*., 2002, Cho *et al*., 2006) and pollution (Shetty *et al*., 1995). Safir (1968) presented the first report about the interaction of pathogenic fungi and AM fungi. Root colonization by arbuscular mycorrhizal fungi has been frequently reported to reduce root infection by various root borne pathogens (Fritz *et al*., 2006, Azcon-Aguilar & Barea, 1996; Smith & Read, 1997). The mechanism involved in this biocontrol are not clear, but localized and systemic induced resistance (Cordier *et al*., 1998) as well as increase in plant P status in response to mycorhiza formation (Graham, 2000) appear to be involved.

Sugarcane is an important cash crop of Pakistan. It is suffering from a number of diseases, which cause severe economic losses. Red rot caused by *Colletotrichum falcatum* Wint is one of them. The causal pathogen is soil as well as seed borne (Agrios, 2002). In this disease, upper leaves of shoot begin to loose color and droop slightly, the entire tip wither and withering progresses down the margins. In the latter stages, the cane becomes shriveled, the rink shrinks and become longitudinally wrinkled (Panday, 1997). Most of the sugarcane growing districts of Punjab in Pakistan especially districts of Jhang, Faisalabad, Sargodha are now under severe attack of this disease. In the present investigation an attempt has been made to address the following hypothesis:

a. The dynamics of AM propagules changes through out the growth season of sugarcane.
b. The interspecific interaction of AM species affects the structure of AMF communities in the sugarcane rhizosphere.
c. Disease severity is correlated to extent of AM root colonization.

Materials and Methods

Estimates of AM colonization: Sugarcane roots, stumps with decaying leaf bases and rhizospheric soil were sampled from fields around Jhang city, by regular intervals of 15 days each from October 1998 to April 1999 (Plate 1&2). In the laboratory these roots and stumps were washed with tap water and cut into 1.0 cm pieces before fixed in FAA (formaline acetic acid alcohol in 5:5: 90 ratio). FAA fixed roots were washed, cleared in 2.5% KOH at 90°C (Koske & Gemma 1989), acidified with 5N HCl and stained with trypan blue (0.05% in lactoglycerol). Roots that remained dark after clearing were bleached with 3% H_2O_2 before staining. The roots were left overnight in trypan blue-lactoglycerol for staining.The clearing and staining of roots was carried out following Philips & Hayman, (1970).

Fifty 1 cm long stained or unstained root samples were mounted on microscope slides in lactoglycerol and examined for AM fungal structures. Root length colonization by AM fungi was estimated according to the intersect method of McGonigle *et al*., (1990). Extent of mycelium was recorded with already calibrated ocular micrometer. Number of vesicles and arbuscules was recorded by random focusing the plant material and counting them. The sheathing leaf pieces were stained and mounted in the same fashion except the time for clearing and bleaching step was variable depending upon the stain present in the scales.

Collection, enumeration and isolation of AM fungal spores: This study was conducted in sugarcane fields within the District Jhang, Punjab, Pakistan. Sampling was carried out in transects in three adjacent fields throughout the growth season. Twenty samples of approximately 100g soil per sample of root zone and rhizosphere of healthy and diseased plants were collected per field, then each of the category was mixed to give ten final samples per field. Regular sampling was done after an interval of 30 days. These soil samples were colleted from root zone (5-10cm in depth) from each collection site. Samples were place in plastic bags and brought back to the laboratory for further processing.

Extraction of AM fungal spores from soil was done by following Gerdeman & Nicolson (1963) and soil paste method of Nasim & Iqbal (1991). For the former, 100 gram of soil was dispersed in 1 l water and decanted through a series of 710- to 38-µm sieves. Residues were filtered through gridded filter papers and all intact spores (noncollapsed spores with cytoplasmic contents, free from parasitic attack) were counted using a dissection microscope at ×40 magnification. Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were mounted in polyvinyl alcohol–lactoglycerol with or without Melzers reagent for identification using keys of Morton (1988) and Schenck & Perez (1990) and INVAM (http://www.invam.caf.wvu.edu). Because of the generally poor state of field material and the low abundance of certain morphotypes, species identification was performed only with sufficient spores (minimum 25) in good condition (no sign of degradation or parasitism).

Study of red rot status: Infected plant portions were inoculated in PDA for identification and verification of pathogen. These cultures were identified following manual of Agnihotri (1990).

Statistical analysis: Standard Error (SE), Standard Deviation (SD), Student's T test, analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) were performed to analyze the data following Steel & Torrie (1980) and Rosner (2000) through computer software SPSS 10.0 (Carver & Nash, 2000).

Results

Part I: This study was conducted in Sugarcane fields, in rural Punjab, Pakistan. Regular sampling was done after an interval of 15 days from October 1998 to April, 1999. These soil samples were collected from root zone (5-10 cm in depth) of plants from each collection site. Ten grams of soil from each of the sample was processed for the screening and identification of glomalean spores.

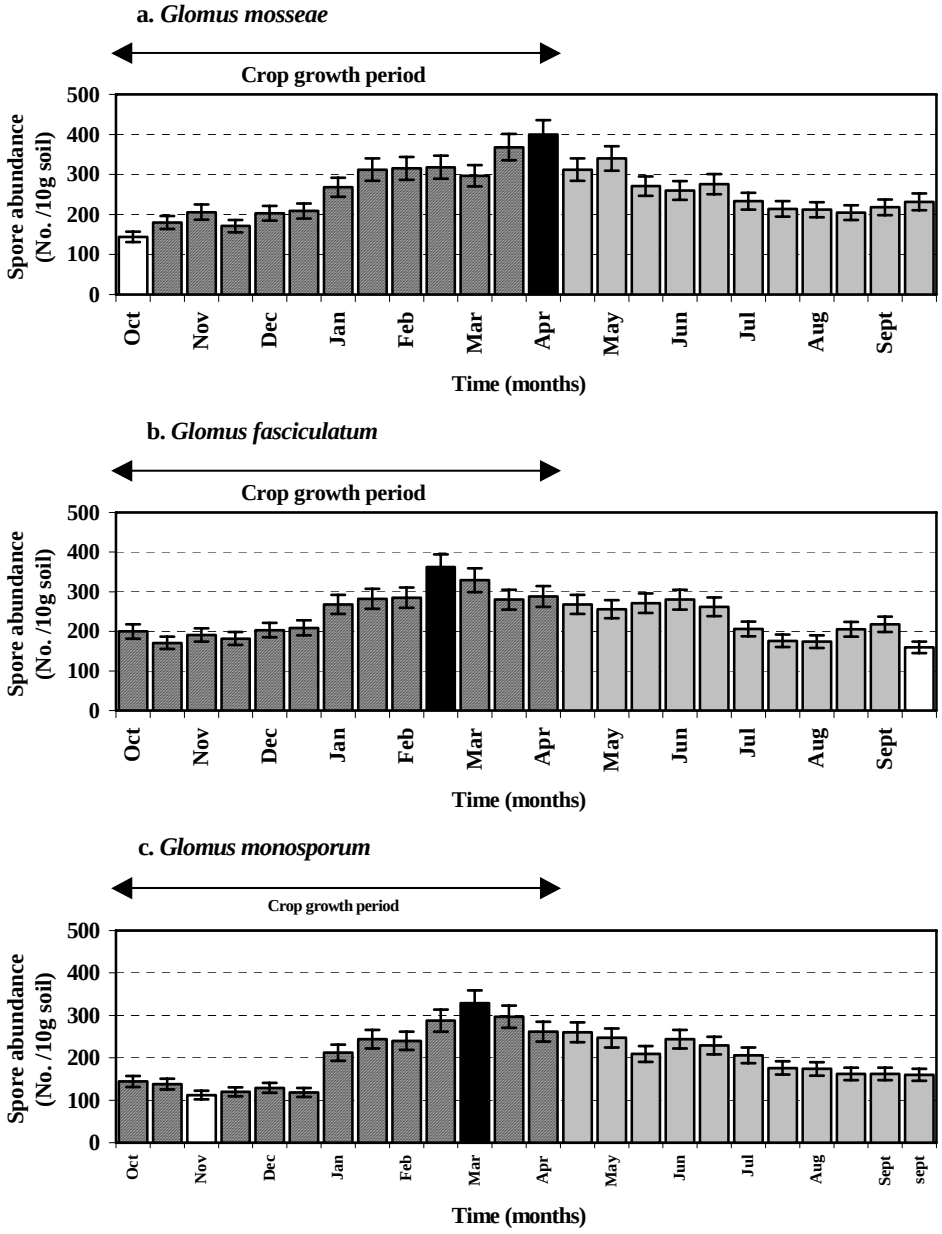

Fig. 1. A-C. Spore dynamics of three major species of *Glomus* in the sugar cane rhizosphere from October 1998 to September 1999. In each figure the black bar shows the abundance of the species and white bar shows minimum spore abundance. The line on data bars shows the SE values.

For *Glomus mosseae*, the spore counts in the rhizosphere soil were maximum in April, 1999 while lowest abundance was recorded in October 1998. The period of maximum spore abundance spanned between January till April and continued till June after the crop harvest (Fig. 1a).

The results of the present study indicate that the greatest overall spore abundance of AMF propagules occurred in March, towards the end of growth season of sugarcane crop (Fig. 2a). However different seasonal pattern were observed when individual species were analysed.

Comparison of seasonal spore dynamics of *G. fasciculatum* (Fig. 1b) through out the study period indicated highest spore population in late February 1999 and lowest in September 1999 when the crop had been harvested but difference was not significant statistically at 5% level (p=0.05) as compared to the lowes values of spores of *G. mosseae*.

For *Glomus monosporum*, spore abundance was found greatest between end of February 1999 and end of March 1999. Lowest abundance was in November 1999. The period of maximum spore abundance was between January to April 1996 for *G. monosporum* (Fig. 1c). In early sampling period (October and Decemberr 1998) spore abundance /10 g of soil for this particular species was close to minimum, it increased to a maximum in March 1998 (Fig. 1c). In case of *G. aggregatum* spore abundance was also bimodal but was very low throughout the sampling period. While few spore types were only detected in March 1999. Statistically significant seasonal differences were noted in data for *G. monosporum* and *G. mosseae*. Frequency of occurrence of *G. monosporum, G. mosseae* and *G. fasciculatum* closely paralleled their seasonal spore dynamics. The maximum frequency of occurrence for a particular species coincided with the corresponding highest spore populations in a given period and *vice versa*. This relation was not apparent in case of *G. aggregatum*, *Gi. erythropa Gi. Margarita* (Table 1).

Spores collected during March and April were uniform in outline and light coloured with subtending hyphae intact and appeared to be freshly formed. In contrast spores collected during November-December 1998 and August-September 1999 were somewhat thick walled dark brown to reddish brown in colour and without subtending hyphae.

Interspecific competition was also recorded in this study. For this purpose, samples in which one or more species occurred in high densities were collected. For each of the two co-dominant species occurred (*G. mosseae* and *G. fasciculatum*) each sample in which spore abundance equaled or was greater than 300 spores/10g of soil was claimed as high density sample for that species. For the three species (*Glomus mosseae, G. fasciculatum* and *G. monosporum*) high density sample was 300-400 spores/10g of soil but in samples where another species was in high density, average abundance of *G. mossseae* was much lower ranging from 200-300/10g. High levels of sporulation of other species studied in the present investigation in a sample was associated with significantly low levels of sporulation of other species in that particular sample (Fig. 1a-c).

Part II: Seasonal variation in the degree of occurrence of general mycelial infection had significant variations through out the growth period in case of healthy sugarcane plant. The general trend for AM hyphal colonization was the same (Fig. 3c). It was low in the beginning and increased in February and March and declined towards the end of the growth period. The stumps remaining after the harvest also had scanty AM hyphae in the attached roots. In all categories of red rot affected plants the level of colonization were low as compared to the healthy plants. Reduction was up to 50% in partially diseased plants and was maximum in severely diseased ones at the peak growth period (Fig. 3c). The pattern for number of vesicles and arbuscules followed the same trend (Fig. 3b &c). However the vesicles were present in roots of stumps even after the crop harvest while the arbusclues were totally absent in the later samples. Abuscular infection was present on all the studied samples. However, severely diseased plants showed 100% infection in first and final sample and 86.25% in third sample. So, this category was considered as having significant variation with respect to season. Variation within category was more significant in sample 3 i.e., 100% in healthy and 86.25% in severely diseased plants.

Table 1. Frequency of occurrence of Glomalean spores per 10 g of soil sample from sugarcane fields. Shaded cells for each species are showing the abundance points.

S. No.	AMF spores	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
1.	*G. mosseae*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2.	*G. fasciculatum*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
3.	*G. monosporum*	++++	++++	++++	++++	++++	++++	++++	++	++++	++++	++	+++
4.	*G. aggegatum*	++	++	++	++	+++	++++	+++	++	++++	+++	++	++
5.	*G. macrocarpum*	++	++	++	++	+++	++	++	++	+++	+++	++	++
6.	*G. halonatum*	++	++	++	++	+++	++	+	+++	+++	+++	++	+
7.	*G. microcarpum*	+	+	++	++	++	++	++	++	+++	+++	++	+
8.	*G. claroitum*	++	++	++	++	++	++	+++	++	+++	+++	++	++
9.	*G. convolutum*	++	+	+	++	++	++	++	++	+++	++	+	+
10.	*Gigaspora aurigloba*	-	-	-	+	++	+	++	-	-	-	-	-
11.	*Gi. heterogama*	-		+	+	+	+++	+	+	-	-	-	-
12.	*Gi. nigra*	-	-	-	-	+	-	-	++	-	-	-	-
13.	*Gi. minuta*	-	-	-	+	++	+	++	-	-	-	-	-
14.	*Gi. erythropa*	-	-	-	-	-	+	-	-	-	-	-	-
15.	*Gi. margarita*	-	-	-	-	-	++	-	-	-	-	-	-

Key: + = Rare (25%); ++ =Frequent (50%)t, +++ = Abundant (75%) & ++++ = Highly abundant species (100%).

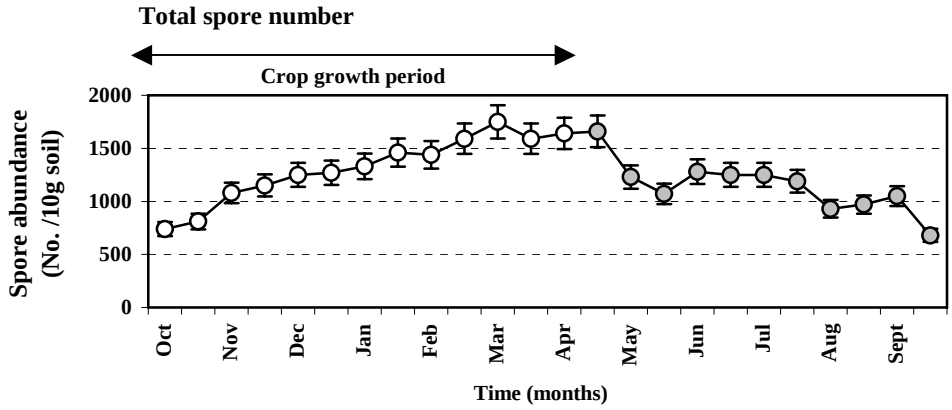

Fig. 2. Total Spore abundance of fifteen Glomalean spore species in the sugar cane rhizosphere from October 1998 to September 1999. In each figure the black bar shows the abundance of the species and white bar shows minimum spore abundance. The line on data bars shows the SE values.

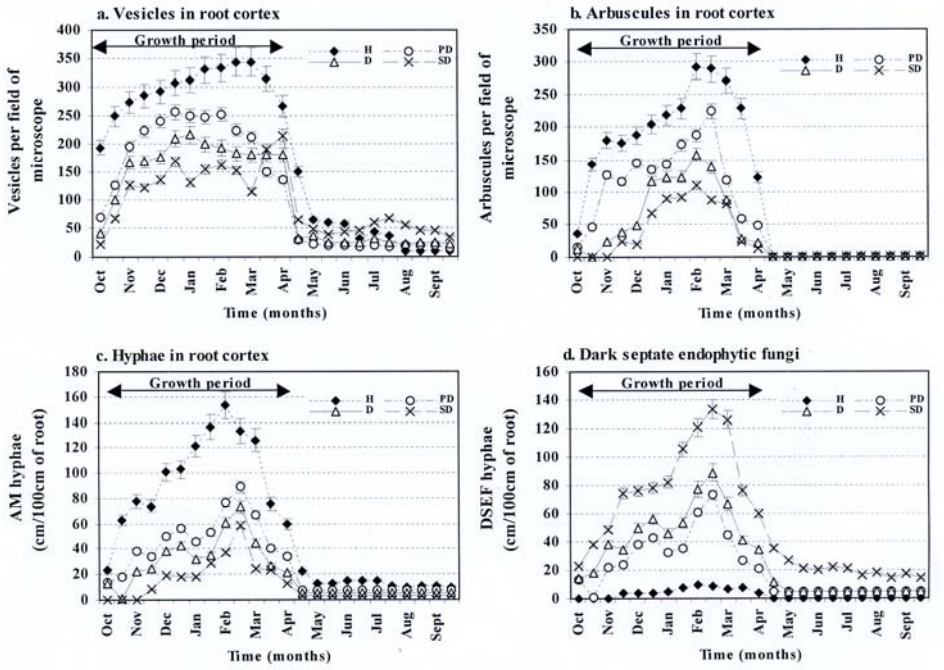

Fig. 3a-d. AM colonization and Dark septate endophytic fungi in sugarcane plants. Vertical bars show standard error.

Values with different letters in each disease category show significant difference (p= 0.05) as determined by DMR Test H: Healthy PD: Partially diseased D: Diseased SD: Severely diseased.

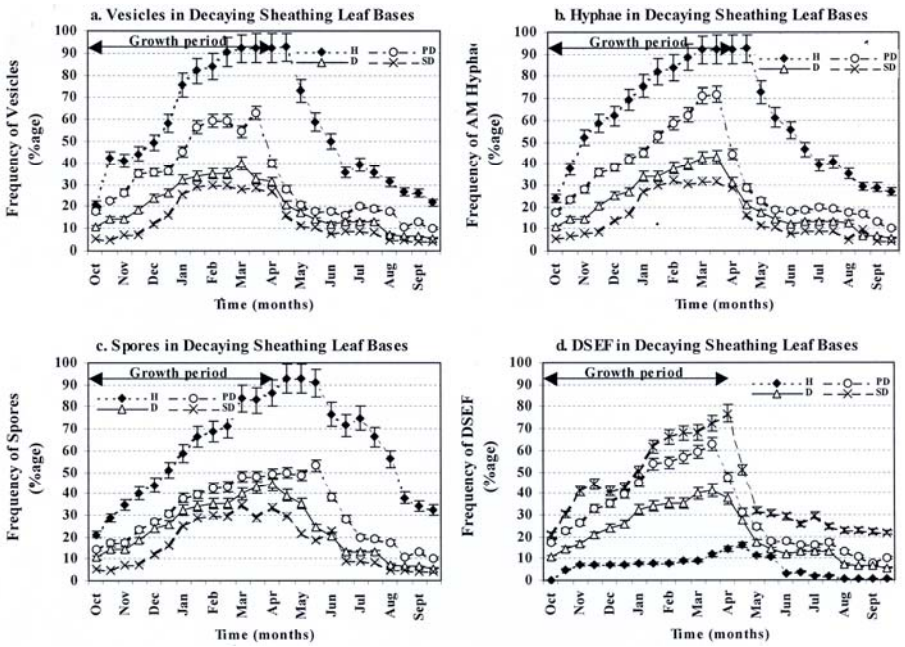

Fig. 4a-d. AM colonization and Dark septate endophytic fungi in decaying sheathing leaf basis of sugarcane plants.
Vertical bars show standard error. H: Healthy PD: Partially diseased D: Diseased SD: Severely diseased

Vesicles showed significant variation with respect to season and stage of disease. AM fungi form vesicles when plant is maturing or conditions are unfavorable. In all the categories of disease plants, the percentage of vesicles first decreased and then again increased at later stages. However, it showed highest percentage (99% in sample 1) in severely diseased plants while lowest (45%) was recorded in sample 4 (Fig 3 a). Vesicle formation increased in later stages of growth. The variation was significant as regards the health of plant. Variation in size and shape of vesicles is indicative of specificity of these fungi.

Appreciable variation in degree of presence was shown by rhizospheric soil spores in between the samples and four categories of diseased plants. Extreme values (21%- 95%) were exhibited by severely diseased plants. In healthy plants their frequency increased initially in sample 3 (55%) then decreased (42%) after this again increased to 60% in final sample.

Dark septate endophytic fungi were also recorded in all root samples (Fig. 1b). It had a strong positive correlation with the disease severity. Although this frequency and extent of occurrence was significantly low in healthy plants but it was very high in all categories of diseased plants (Fig. 3d). But variation within sample and four stages was highly significant. Within sample it was maximum in severely diseased plants then it stared decreasing reaching 1% in healthy plant in last sample. As regards the variation within four stages of disease, it was significant in all the samples i.e., in first sample it was 13%, 16%, 17% and 21% in healthy plants, partially diseased plants, diseased and severely diseased plants, respectively (Fig. 2d).

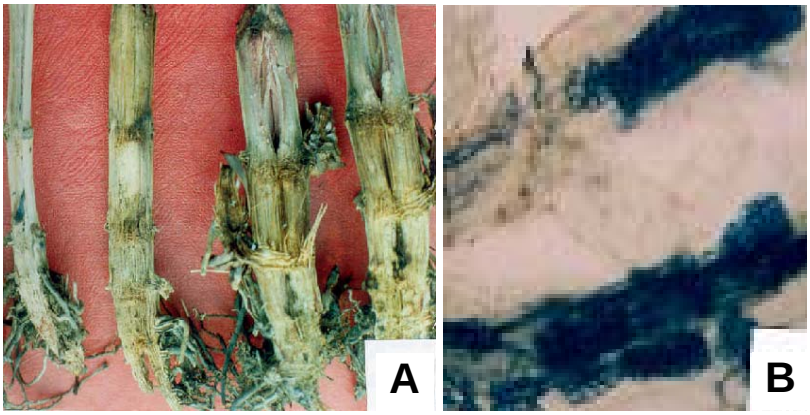

Plate 1. A. Sugarcane plants showing various stages of disease. B. Dark septateendophytic fungi (Bar = 25 µm).

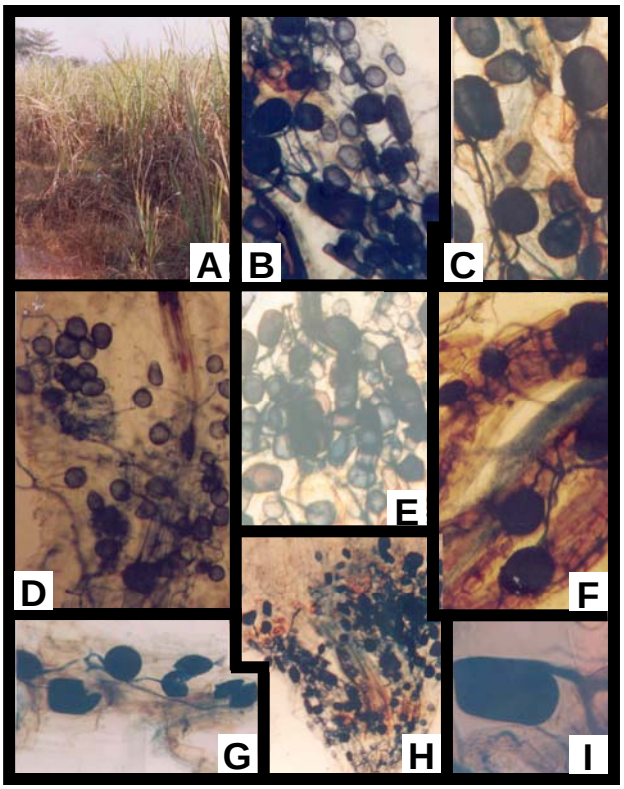

Plate 2. A: Sugarcane plantation in Jhang area of Pakistan. B-G: Vesicles in the roots of sugarcane. H: Vesicles in the decaying sheathing leaves of sugarcane (bar=50µm).

There was found an increasing trend in %age and extent of all AM mycorrhizal structure (except arbuscules) was observed in dead decaying sheathing leaf bases in all samples (Fig. 4a-d) 10% and 90% vesicular colonization was recorded in 1st and last sample respectively. Presence of AM spores and mycelia in decaying leaf bases of sugarcane stumps have been reported for the first time.

Discussion

Spore abundance of three *Glomus* species investigated in the present study was influenced by the season. Different response of AMF propagules to this variable may in order be to minimize or lessen their direct competition. This interspecific competition appears to be a major factor in determining the spore abundance/densities of these *Glomus* species in experimental sugarcane field.

Higher spore abundance of a particular species in a sample was typically associated with reduces spore densities of other AMF spores in that sample. In several previous studies high spores abundance in relation to host phenology in agriculture crops has been reported at the end of growing season (Sutton & Barron, 1972; Saif & Khan, 1975). Present results are in line with those of Sylvia (1986) who reported that as plant matured overall populations of AMF propagules increased but the sporulation was not synchronous.

Although maximum spore densities of 3 *Glomus* species (*G. mosseae*, *G. fascicultum* and *G. monosporum*) occurred towards the end of growing season but abundance of three of these raised almost to the maximum during vegetative growth also. High values of sporulation of *G. mosseae* in January followed by a fall at the time of maturity of host. In case of *Glomus fascicultum* and *G. monosporum*, maximal sporulation coincided with the beginning of maturation period, unlike other *Glomus* species, which were not producing significant number of spores. The variations in the sporulation pattern noticed among these *Glomus* species could be attributed to the ability of individual species to out compete other species at certain times for host cortical cells or to lessen the degree of simultaneous competition for substrate from host at sporulation.

The presence of senescing or dead roots has also been proposed as a possible stimulus for the onset of sporulation that occurs at the end of growing season (Baylis, 1969; Sutton & Barron, 1972; Saif & Khan, 1975). It has also been reported that root growth dynamics may differ between host species. Root turnover/root death during the growing season also exceeds that of which occurs at the end of growing season/growth cycle of host. Sylvia (1986) and Gemma *et al*., (1989) suggested that stimulation other than accumulation of dead and senescing roots is required for sporulation.

Abiotic factors such as temperature and light (Furlan & Fortin, 1973) and biotic factors i.e., changes in amount of photosynthates production (Wallen, 1980), quality and quantity of root exudates (Fluck, 1963) and fluctuations in root hormone level occurring during flowering/maturation and growth cessation (Torry, 1976) are the primary non genetic determinants of AMF sporulation.

Importance of competition in determining the composition of AMF community in a particular area of root zone has been provided by species interactions. The phenomenon of one species sporulating at the expense of other species or in the absence of sporulation of other species as observed in the present study may be due to many factors such as interspecific competition, spatial restriction and stability of host.

Previously differential sporulation has been used to investigate interspecific competition/interaction among AMF (Daft & Hogarth, 1983) and its importance in determining the AM community. Koske (1981) reported that vegetational characters are more important indetermining the extent of sporulation by individual species than competition with other AM propagules.

The results from previous studies and present work suggest that the individual AMF spores compete for resources by combination of strategies, which result in the maintenance of a diverse AMF community. This finding is in line with Nasim *et al*., 1998. They found wheat stumps to harbor fungal structures particularly spores. These structures were also reported in decaying stumps, leaves of rice and maize (Nasim *et al.,* 1999a,b). Reports like these suggest that AM fungi are capable of independent growth.

This fluctuation occurs due to effects of *Colletotrichum falcatum* invasion on host physiology. Disease causing organisms tend to reduce the rate of disease incidence (Agrios, 2002). Reduced availability of photosynthates may be responsible for a decrease in mycorrhizal infection. At later stages the infection increased due to the onset of unfavorable conditions like senescence of plant parts and formation of storage structures.

Bioprotection of AM-colonized plants against soil borne pests like nematodes and various root diseases is commonly observed (Cordier *et al*., 1996; Boedker *et al*., 1998). Studies have shown mycorrhizal protection of plants against, for example, the root pathogens *Phytophthora parasitica, Erwinia carotovora* and *Pseudomonas syringae* (Garcia-Garrido & Ocampo 1988; Cordier *et al*., 1996, 1998; Iqbal *et al*., 1990; Zahoor *et al*., 1992). Dehne (1982) suggested that the systemic influence of AM fungi may be attributed to enhanced nutrition, plant growth and physiological activity of mycorrhizal plants and therefore with increased level of assimilation, such plants can serve as improved nutrient sources for plant parasitic organisms. Shaul *et al*., (1999) proved an alternate mechanism by explaining the increased disease severity by the suppression of the plant defense responses by AM fungi shortly after the early events of root colonization. However mycorrhiza may induce qualitative or quantitative changes in plant performance that could compensate higher disease susceptibility as reported in the case of barley leaf infection by *Blumeria graminis* f.sp. *hordei* (Gernns *et al*., 2001).

Though an early mycorrhizal inoculation, previous to pathogen attack has been shown to be a successful practice to increase disease tolerance in a number of economically important species however no one has tried it with sugar cane as yet. In the present study, which is a survey work, a strong correlation has been found in the percent colonization of the sugarcane roots by AMF and the severity of the disease. It may therefore be concluded that presence of mycorhiza in roots led to significantly lower infection levels of *C. falcatum* than observed in non-mycorrhizal plants. Results of this investigation suggest that screening of AM flora should be done to select the best and most efficient AM endophyte suited for sugarcane crop as well as different aspects of interaction of AM fungi and pathogen should be known. Dark septate hyphae are thought to belong to some asco or basidiomycetous fungi. The true affiliation of these fungi is yet to be verified. However the evidence is accumulating that the presence of these fungi in root systems is of ecological significance (Li & Guan, 2007; Muthukumar *et al*., 2006).

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References

Agnihotri, V.P. 1990. *Diseases sugarcane and sugarbeet*. Oxford and IBH Pub. Co. Pvt. Ltd. New Dehli, pp. 483.

Agrios, G.N. 2002. *Plant Pathology 5th Edition. Academic Press, London.* pp. 388.

Anderson, R.C., A.E. Liberta, L.A. Dickman and A.J. Katz. 1983. Spatial variation in vesicular arbuscular mycorrhiza spore density. *Bulletin Toori Botanical Club*, 110: 519-525.

Azcon-Aguilar, C. and J.M. Barea. 1996. Arbuscular mycorrhizas and biological control of soil borne plant pathogens-an overview of mechanisms involved. *Mycorrhiza,* 6: 457-464.

Baylis, G.T.S. 1969. Host treatment and spore production by *Endogone*. *New Zaeland Journal of Botany*, 7: 173-174.

Boedker L., R. Kjoeller and S. Rosendahl. 1998. Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. *Mycorrhiza,* 8: 169-174.

Carver, R.H. and J.G. Nash. 2000. Doing data analysis with SPSS 10.0. Brooks/Cole, Duxbury, UK.

Cho, K., H.D. Toler, J. Lee, B.H. Ownley, C. Jean, J.C. Stutz, J.L. Moore and R.M. Augé. 2006. Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. *Journal of Plant Physiology,* 163: 517-528.

Cordier, C., M.J. Pozo, J.M. Barea, S. Gianinazzi and V. Gianinazzi-Pearson. 1998. Cell defence responses associated with localized and systemic resitance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. *Mol. Plant Microbe Interaction*, 1071-1028.

Cordier, C., S. Gianinazzi and V. Gianinazzi-Pearson. 1996. Colonization patern of root tissue by *Phytophthora nicotianae* var., Parasitica related to reduces disease in mycorrhizal tomato. *Plant & Soil*., 185: 223-232.

Daft, M.J. and B.G. Hogarth. 1983. Competitive interaction amongst four species of *Glomus* on maize and corn. *Transactions of British Mycological Society*, 80: 399-345.

Dehne, H.W. 1982. Interaction between vesicular arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology*, 72: 1115-1119.

Douds, D.D.Jr. and W.R. Chaney. 1986. The effect of high nutrient addition upon seasonal patterns associated with Uniola paniculata in Florida foredunes. *Mycologia*, 78: 728-734.

Feng G., F.S. Zhang., XL. Li, Tian of mycorrhizal development, host growth and root phosphorus and carbohydrate content in *Fraxinus pennsylvanica* Marsh. *New Phytologist,* 103: 91-106.

Fluck, H. 1963. *Chemical Plant taxonomy*, (Ed.) T. Swain. Academic press, New York, 543p.

Friese, C.F. 1984. *The distribution and dispersal of spores of VA mycorrhizal fungi in a sand dune*. M.S. Thesis, University of Rhode Island, Kingston.

Fritz, M., I. Jakobsen, M.F. Lyngkjaer, H. Thordal-christensen and J. Pons-Kuhnemann. 2006. Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani. Mycorrhiza*, 16: 413-419.

Furlan, V. and J.A. Fortin. 1973. Formation of endomycorrhizae by *Endogone calospora* on *Allium cepa* under three treatment regimes. *Naturaliste Canadian,* 100: 467-477.

Garcia-Garrido, J.M. And J.A. Ocampo. 1988. Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. *Soil Biology and Biochemistry*, 21: 165-167.

Gemma, J.W., R.E. Koske and M. Casseiro. 1989. Seasonal dynamics of selected species of VA mycorrhizal fungi in a sand dune. *Mycological Research*, 92 (3): 317-321.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal "Endogone' extracted from soil by wet sieving and decanting. *Transactions of British Mycological Society*, 84: 679-684.

Gernns, H., H. von Alton and H.M. Poehling. 2001. Arbuscular Mycorrhiza increased the activity of biotrophic leaf pathogen-is a compensation possible? *Mycorrhiza,* 11: 237-243.

Graham, J.H. 2000. Assessing costs of arbuscular mycorrhizal symbiosis in agroecolosystems. In: *Current Advances in mycorrhizae research*. (Eds.): G.K. Podila, D.D.J. Douds. APS Press., St. Paul., Minnesota, pp 127-140.

INVAM (http://www.invam.caf.wvu.edu).

Iqbal, S.H, R. Zahoor, G. Nasim, A.N. Khalid. 1990. Occurrence of vesicular arbuscular mycorrhizae in chillies infected with root born pathogen. *Science International (Lahore)*, 3(2): 183-194.

Koske, R.E. 1981. Multiple germination by spores of *Gigaspora gigentea. Transactions of the British Mycological Society,* 76: 328-330.

Koske, R.E. and J.N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas, *Mycological Research*, 92: 486-488.

Li, A.R. and K.Y. Guan. 2007. Mycorrhizal and dark septate endophytic fungi of *Pedicularis* species from northwest of Yunnan Province, China. *Mycorrhiza*., 17: 103-109.

Locations in Central Iowa. *Canadian Journal of Botany*, 60: 2518-2529.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild and J.A. Swan. 1990 A method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytol.,* 115: 495–501

Morton, J.B. 1988. Taxonomy of mycorrhizal fungi. Classification, nomenclature and Identification. *Mycotaxicon*, 32: 267-324.

Muthukumar, T., M. Senthilkumar, M. Rajangam and K. Udaiyan. 2006. Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India, Mycorrhiza, 17(1): 11-24.

Nasim, G. and R Zahoor. 1996. Seasonal spore dynamics of six *Glomus* species and their interspecific interactions in a wheat field. *Scientific Sindh*, 3: 73-82.

Nasim, G. and R. Zahoor. 1995. Comparison of spore morphology of *Glomus monosporum* from Pakistan and USA. *Scientific Khyber*, 8(1): 49-55.

Nasim, G. and R. Zahoor. 1997. Ontogeny of sporocarps of *Glomus monosporum*. *Sarhad Journal of Agriculture*, 13(2): 181-18.

Nasim, G. and S.H. Iqbal. 1991. Species of *Glomus* associated with non-root portions of some rhizomatous plants and characteristics of their mycorrhizae. *Transactions of the Mycological Society of Japan*, 32: 541-545.

Nasim, G., S. Riaz and S.W. Mian. 1999b. VAM structures in senescing roots and sheathing bases of maize left after crop harvest. *Scientific Khyber*, 11(2): 43-50.

Nasim, G., S. Riaz, S.W. Mian. 1999a. VAM structures in senescing roots and sheathing bases of rice left after crop harvest (In press).

Nasim, G., Z. Naqvi, S. Sheikh, S. Saeed and M. Shaheen. 1998. Wheat stumps a source of VAM inoculum for the incoming crop. *Scientific Khyber*, 11(2): 43-50.

Panday, B.P. 1997. *Diseases of sugarcane*. *A text book of plant pathology*, pp. 466-480.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic VAM fungi for rapid assessment. *Transaction of British Mycological Society*, 5: 158-161.

Rosner, R. 2000. *Fundamentals of Biostatistics*, Fifth edition, Brooks/Cole, Duxbury, UK.

Ruiz-Lozano, J.M. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza*, 13: 309-317.

Safir, G. 1968. *The influence of vesicular ans arbuscular mycorrhizae on the resistence of onion to Pyrenochaeta terrestris*, M.S. Thesis, University of Illionis, Urbana.1968.

Saif, S.R. and A.G. Khan. 1975. The influence of season and stage of development of plant on *Endogone* mycorrhiza of field grown wheat. *Canadian Journal of Microbiology*.

Schenck, N.C. and Y. Perez. 1990. *Manual for the identification of VA mycorrhizal fungi*. In: VAM 1453. Fairfield Hall, University of Florida, 32611, pp245.

Shaul, O., S. Galili, H. Volpin, I. Ginzberg, Y. Elad, I. Chet and Y. Kapulnik. 1999. Mycorrhiza induced changes in disease severity and PR protein expression in tobacco leaves. *Mol Plant Microbe Interaction,* 12: 1000-1007.

Shetty, K.G., Herick BAD and A.P. Schwab. 1995. Effects of mycorrhizae fertilizer amendments on zinc tolerance of plants*. Environmental Pollution*, 88: 307-314.

Smith, S.E. and D.J. Read. 1997. *Mycorrhizal symbiosis*. Academic, London.

Steel, R.G.D. and V.J.H. Torrei. 1980. *Principles and procedures of statistics*. McGraw Hill,

Sutton, J. and G.L. Barron. 1972. Population dynamics of *Endogone* spores in soil. *Canadian Journal of Botany*, 50: 1909-1940.

Sylvia, D.M. 1986. Spatial and temporal distribution of vesicular arbuscular mycorrhizal fungi, *Oikos*, 34: 227-238.

Tang C.Y. and Z. Rengel. 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza*, 12: 185-190.

Torry, J.G. 1976. Root hormone and plant growth. *Annual Review of Plant Physiology*, 27: 435-459.

Wallen, B. 1980. Changes in structure and function of *Ammophila* during primary succession. *Oikos*, 34: 227-238.

Zahoor, R, Ikram-ul-Haq and S.H. Iqbal. 1992. VAM as detterent to pathogenic infection caused by *Macrophomina phaseolina* (Tassi). Goid in *Capsium annum* L., at different soil moisture regimes. *Science International (Lahore)*, 4(1): 103-107.

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