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Seasonal variation of AM fungal colonization in Sugarcane (*Saccharum officinarum* L.) plants suffering from Ratta Roag (Red rot) disease.

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Abstract

In a survey conducted to assess the AM infections of sugarcane plants suffering from Ratta Roag (Red Rot), from various sugarcane-fields in and around District Jhang four categories of plants for disease incidence were identified as healthy, partially diseased, diseased, and severely diseased. A significant change in pattern of AM infection was recorded. Percentage frequencies of arbuscules, vesicles, intra-matricular mycelium and external spores exhibited a gradual increase from initial to final stages of the studies. Various AM structures showed a significant variation with the passage of time in the extent of infection. The diseased plants showed a significant difference in AM infection as compared to normal plants.

Introduction

Arbuscular mycorrhizal fungi play a pivotal role in plant growth enhancement. These fungi afford host plant greater resistance to environmental stresses like, osmotic stress (Ruiz-Lozano, 2003), salinity (Feng *et al.*, 2002) and pollution (Shetty *et al*., 1995). Safir (1968) presented the first report about the interaction of pathogenic fungi and AM fungi. Most of the studies of this AM fungal and pathogenic fungal interaction suggest that AM fungi decrease the severity of disease while few reports indicated that there was either no effect or there was an increase in disease severity due to AM mycorrhizal infection, Davis *et al.*, (1979) reported higher disease incidence in cotton plants colonized by *Glomus fasciculatum* than non-mycorrhizal plants. While mycorrhizal infection was found insignificant against disease incidence of *Phytophthora palmivora* in Papaya (Ramierz, (1974) and Thielaviopsis root rot of citrus (Davis, 1980). But most of the investigations indicate that mycorrhizal fungi have potential to decrease the disease severity. Krishna (1983) found that inoclation with *Glomus fasciculatum* against root rot fungus *sclerotium rolfsii* reduced the severity of disease. Iqbal *et al.*, (1987) proved that mycorrhizal *Brassica oleracea* exposed to pathogenic infection of *Rhizoctonia solani* resisted the pathogenic stress while non-mycorrhizal plants poorly survived. AM fungi were also found suppressing pathogenic infection when tested against damping off at various temperature regimes Iqbal and Nasim, (1988).

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 relate the incidence of disease severity to AM colonization in root systems of sugarcane.

Materials and Methods

Sugarcane roots, stumps along with rhizospheric soil were sampled from fields around Jhang city, by regular intervals of 30 days each from Oct.98 to Apr.99. In laboratory these roots and stumps were washed with tap water and cut into 1.0 cm pieces. The clearing and staining of roots was carried out following Philips and Hayman, 1970. Twenty root pieces of each sample were mounted in lactic acid on glass slides and observed under microscope for AM fungal infection. 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.

While extraction of AM fungal spores from soil was done by following Gerdeman and Nicolson, 1963. Infected plant portions were inoculated in PDA for identification of pathogen. These cultures were identified following manual of Agnihotri, 1990.

Results and Discussion

Seasonal variation in the degree of occurrence of general mycelial infection was almost negligible through out the study period in case of healthy sugarcane plant. Severely diseased plants showed significant variation i e., 100% at the start and end while 89.75% at the middle of the experiment at middle of the season as compared to other three categories (Fig 1 A). This fluctuation occurs due to effects of pathogenic 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.

Abuscular infection was present on all the studied samples. However, severely diseased palnts showed 100 % infection in first and final sample and 86.25% in third sample (Fig 1 B). 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 (Fig 1 B). Reason may again be attributed to the effect of disease causing pathogen on host plant physiology.

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 1 C). Vesicle formation increased in later stages of growth. The variation was significant as regards the health of plant. Variation in size and shape of vesicals is indicative of specificity of these fungi (Morton, 1988).

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 (Fig 2 A).

As regards the variety of AM fungal spore species it was observed that species of genus *Glomus* were abundant as compared to species of *Gigaspora*. This finding was parallel with previous study by Morton (1988). Common species of *Glomus* were *G. aggegatum, G. albidum, G. fasciculatum, G. monosporum, G. macrocarpum*, *G. halonatum, G. microcarpum, G. claroitum* and *G. convolutum.* While following species of genus *Gigaspora* were found, i e., *G. aurigloba, G. heterogama, G. nigra, G. minuta, G. erythropa* and *G. margarita.*

Beaded mycelium was also recorded in all tested root samples. Although this frequency of occurrence was very low (Fig 2 B). But variation within sample and four stages was highly significant. Within sample it was maximum (21% in severely diseased plant) 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 2 B). Its supposed that beaded mycelium is formed by certain AM fungi in place of arbuscules. The function of the two may be the same (Nasim *et al.*, 1998).

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 3). 10% and 90% vesicular infection was recorded in 1st and last sample respectively. Presence of AM spores and mycelia in decaying leaf bases of sugarcane stumps have been reported first time. 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.,* 1999 a, b). Reports like these suggest that AM fungi are capable of independent growth.

AM fungi occurring reported in diseased plants are known to reduce the disease severity by acting as biocontrol agents (Krishna and Bagyaraj, 1983). Many investigations have shown that precolonization of host roots by AM fungi enhanced the host resistance against the pathogenic fungi (Iqbal *et al.,* 1990*;* Zahoor *et al.,* 1992).

The present study was a survey work. 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.

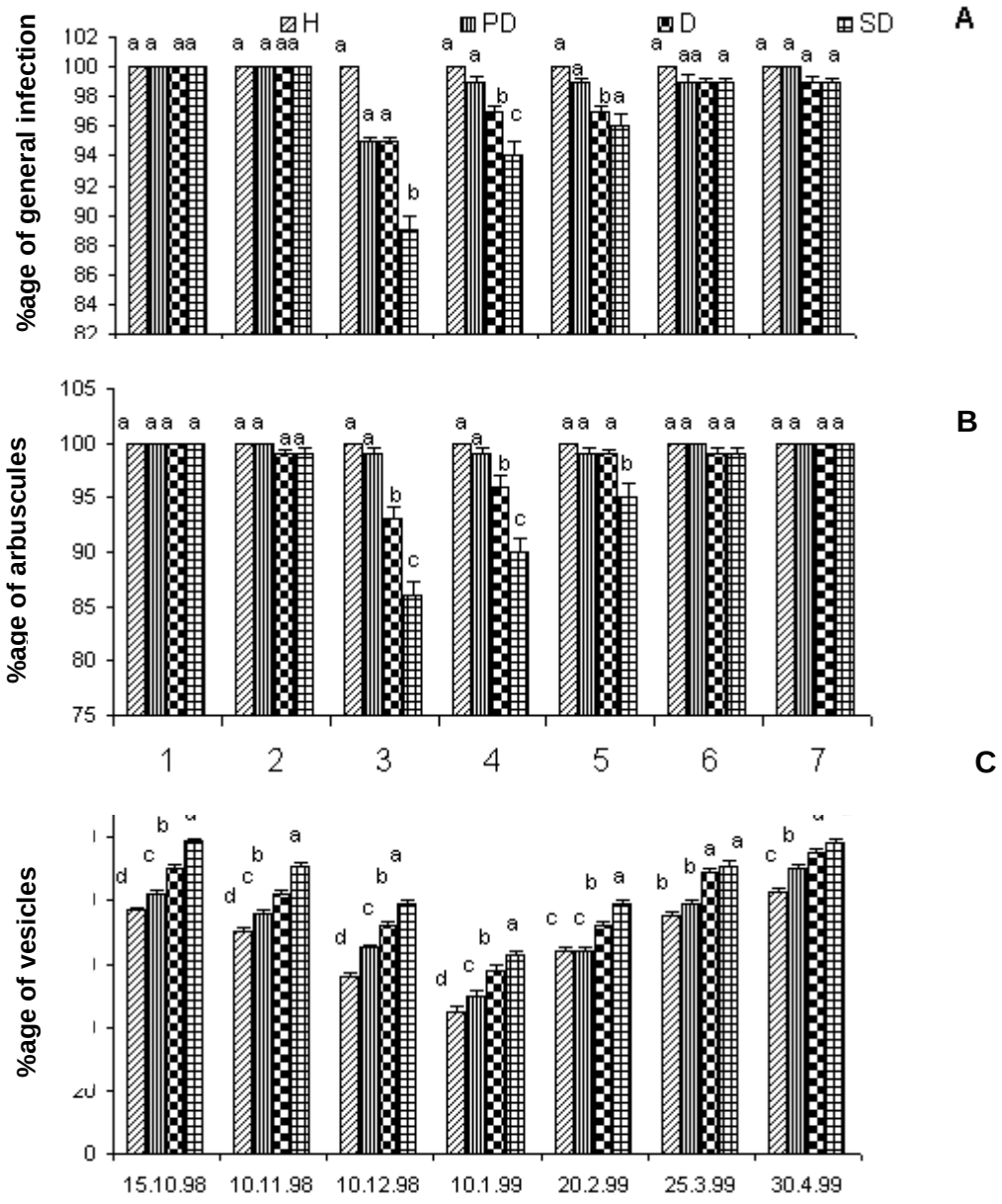

Fig 1.A-C: AM colonization in sugarcane plants.
Verticle bars show standard error.
Values with different letters in each disease category show significant difference (P = 0.05) as determined by DMR Test
H: Healthy **PD:**Partially disaesed **D:** Diseased **SD:** Severly diseased

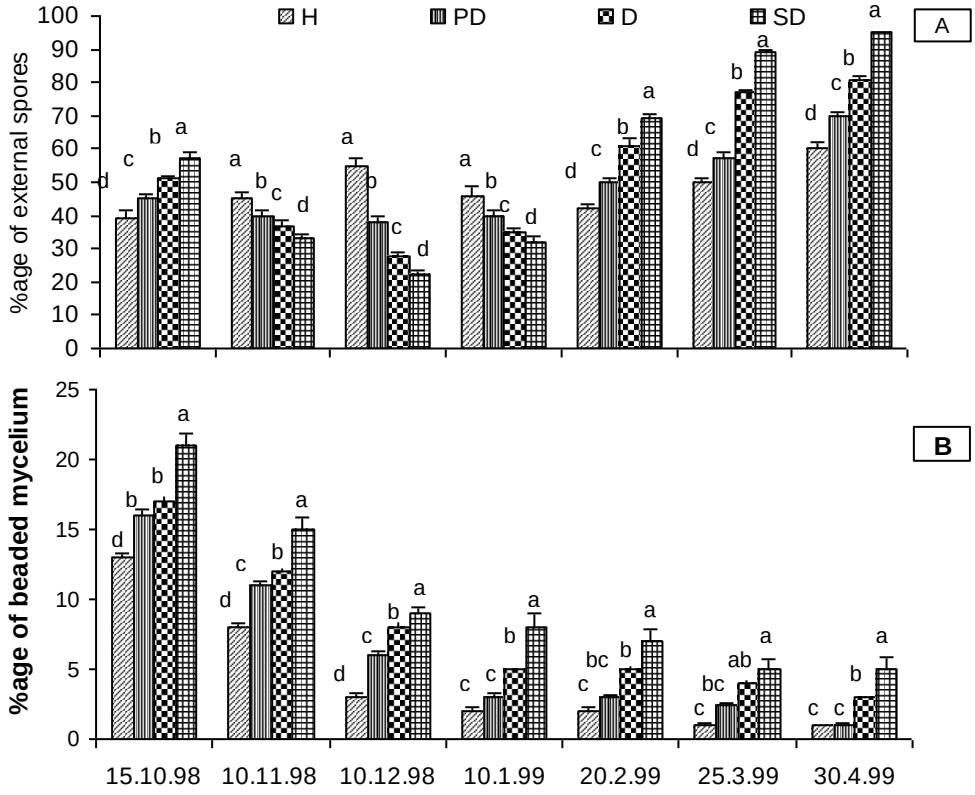

Fig 2.A: Percentage occurrence of external AM fungal spores in rhizospheric soil of sampled sugarcane plants. (B). Percentage occurrence of beaded mycelium in all sampled sugarcane plants.

Vertical bars show standard error.

Values with different letters in each sampling date show significant difference (P = 0.05) as determined by DMR Test

H: Healthy PD: Partially diseased D: Diseaed SD: Severly diseased

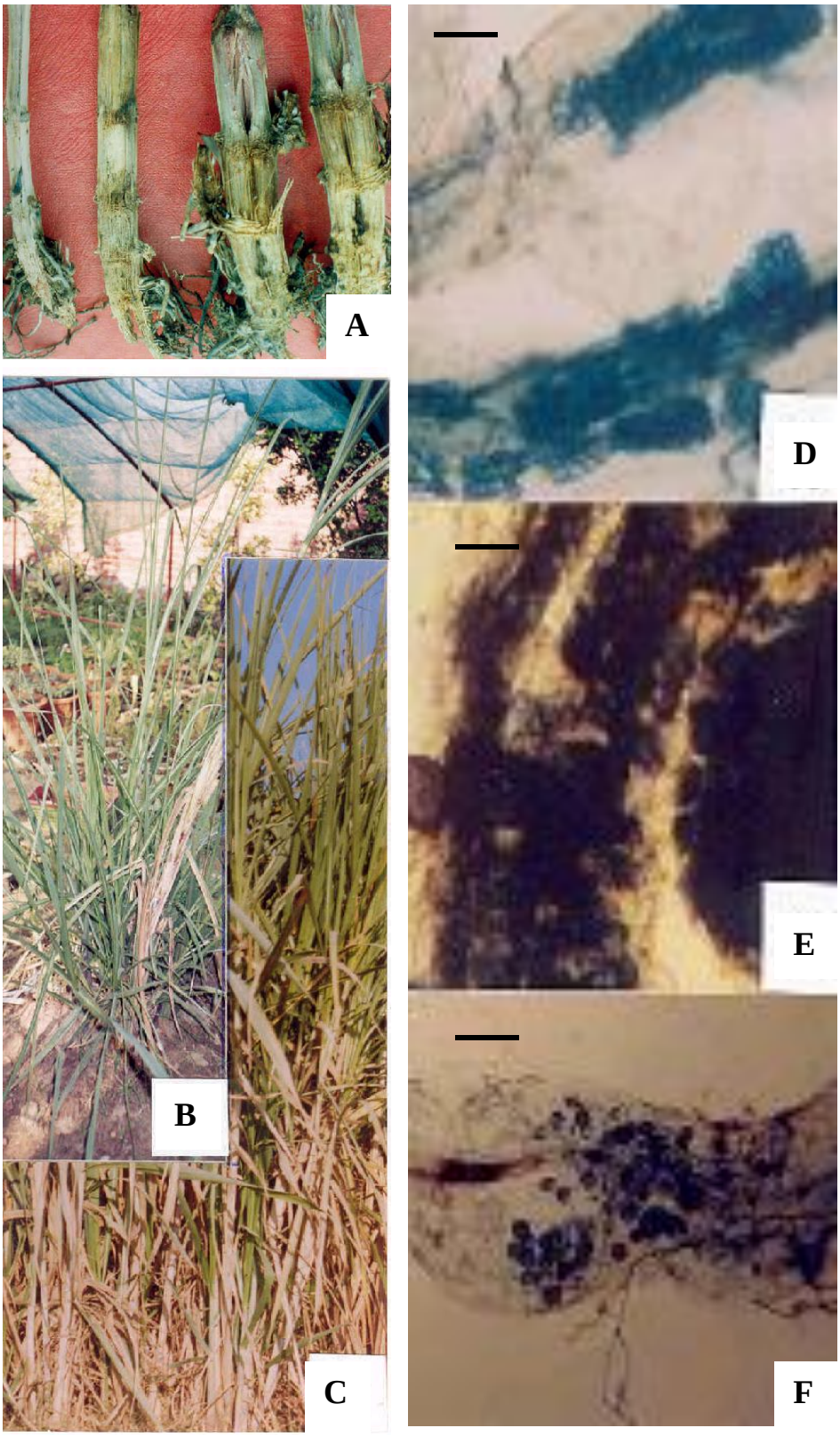

Plate A-C: Sugarcane plants showing various stages of disease.
D-F: Sugarcane roots showing various arbuscular vesicular structures
Bar = 25 µm.

References

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

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

Davis RM, 1980. Influence of *Glomus fasciculatum* in Theilaviopsis basicola root rot of citrus. *Plant Diseases*, **64**: 839.

Davis RM, Merge JA, Erwin DC, 1979. Influence of *Glomus fasciculatum* and soil phosphorus on *Verticillium* wilt of cotton. *Phytophthology*, **69**: 453.

Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z, 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.

Gerdemann JW, Nicolson TH, 1963. Spores of mycorrhizal "Endogone' extracted from soil by wet sieving and decanting. *Transactions of British Mycological Society*, **84**: 679-684.

Iqbal SH, Nasim G, 1988. VA mycorrhiza as deterrent to damping off caused by *Rhizoctonia solani* at different temperature regimes. *Biologia*, **34**: 215-219.

Iqbal SH, Nasim G, Niaz M., 1987b. Role of VA mycorrhizae as deterrent against pathogenic infections caused by *Rhizoctonia solani* in *Brassica oleraccea. Biologia*, **34**: 79-84.

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

Krihsna KJ, Bagyaraj DJ, 1983. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in Peanut. *Canadian Journal of Botany*, **61**: 23-49.

Morton JB, 1988. Taxonomy of mycorrhizal fungi. Classification, nomenclature and Identification. *Mycotaxicon*, **XXXII**: 267-324.

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

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

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

Ross JP, 1972. Influence of endogone mycorrhizae on *Phytophthora* rot of soyabean. *Phytophthology*, **62**: 896.

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.

Shetty KG, Herick BAD, Schwab AP, 1995. Effects of mycorrhizae fertilizer amendments on zinc tolerance of plants. *Environmental Pollution.* **88**: 307-314.

Subramanian KS, Charest C, Dwyer LM, Hamilton RI, 1995. Arbuscular mycorrhizae and water relations in maize under drought stress at tasseling. *New Phytol.* **129**: 643-650.

Ramirez BN, 1974. Influence of endomycorrhizae on the relationship of inoculum density of *Phytophthora plamivora* in soil to infection of papaya roots, M.S. thesis, University of Florida, Gainesville.

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

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

Phillips JM, Hayman DS, 1970. Improved procedures for clearing roots and staining parasitic VAM fungi for rapid assessment. Transection of British Mycological Society, 5: 158-161.

Zahoor R, Ikram-ul-Haq, Iqbal SH, 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.