Pak. J. Bot., 42(2): 1313-1316, 2010.

DECAYING LEAF SHEATHS OF WHEAT-AN UNUSUAL NICHE FOR *GLOMUS MONOSPORUM*

GHAZALA NASIM

Institute of Mycology and Plant Pathology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.

Abstract

The sporocarp formation by a species of arbuscular mycorrhizal fungus, *Glomus monosporum* (Gerd.) Trappe has been reported in sheathing leaf bases of decaying wheat stumps. For this purpose wheat stumps left after crop harvest were sampled periodically with an interval of 5 days from a cultivated field along with rhizosphere soil. All stages of sporocarp development of *G. monosporum* and changes taking place after dispersal were studied in senescing leaf bases and roots of these stumps. In pot cultures developing sporocarps were occasionally observed while large sized, thin-walled vesicles were observed in roots of wheat plants inoculated with leaf bases of wheat colonized with *G. monosporum*.

Introduction

Arbuscular mycorrhizal fungi are most ubiquitous soil fungi and play major role in establishment of plant communities in natural or managed ecosystems (Siddiqui & Pichtel, 2008; Nasim *et al*., 2008; Barea *et al*., 2002). Mycorrhizal fungi are known for nutrients acquisition, their microscopic pipelines transports carbon and minerals to and away from the plant (Jalaluddin *et al*., 2008; Barrow, 2004). There are 60 fungi belonging to Glomeromycota and form association with 90% of land plants (Krishna, 2005) belonging to Angiosperms, Gymnosperms, Pteridophytes, Bryophytes and Algae. These fungi reproduce asexually through formation of spores and sporocarps in the soil. However, some fungi form propagules in rather unusual niches. The sporulation sites may be the scale leaves and epidermis of underground portions (corms and rhizomes) or decaying sheathing leaf bases of grasses (Iqbal & Nasim, 1991; Nasim & Iqbal, 1991a; Nasim & Iqbal 1991b; Nasim *et al*., 1998; Nasim & Bajwa, 2009). The present study reports that *G. monosporum* forms spores in the decaying leaf sheath bases of wheat and has mycorrhiza forming potential while enhancing the growth of wheat. The inoculum potential of *G. monosporum* colonized leaf sheaths has also been evaluated.

Materials and Methods

Samples in triplicates of wheat stumps were collected at random 10 days after crop harvest from the University Farm. The stumps were washed carefully and fixed in formaline acetic acid alcohol (FAA) in 5:5:90 ratio. These samples were cleared in 10% KOH for 2-3 minutes by autoclaving. The dark coloured samples were bleached in 30% H_2O_2, while varying the time interval for treatment depending upon the intensity of colour. Samples were washed under tap water and then stained in 0.05% trypan blue in lactophenol after acidifying them with 0.01N HCl.

Slides of 1x1cm pieces of leaf sheaths and 1cm long piece @ 25 pieces per slide of stained roots were prepared for sporocarp counting and determination of AM colonization. The spore counts in the soil were made by wet sieving and decanting technique of Gerdemann & Nicolson (1963) and our soil paste method (Nasim & Iqbal, 1991b). Spores were identified with the help of keys by Trappe (1982), Morton (1988) and Schenck & Perez (1987).

E-mail: ghazalanasim@hotmail.com

The colonized leaf sheaths were surface sterilized in a mixture of H_2O_2 and spirit in 1:1 ratio and 25cc of these were used as inoculum to grow wheat seeds in aseptic conditions in plastic growth tubes (250cc). The inoculum potential was evaluated harvesting wheat seedling after 6 weeks. Care was taken not to disturb fine roots. Roots and sheathing leaf bases were stained following the methods described above.

Results and Discussion

Sporocarps collected in association with decaying wheat stumps resembled to those of *Glomus monosporum*. Sporocarps are defined as aggregate of many spores with loose or highly organized structures which do not separate into individual spores upon extraction from soil (Morton, 1988).

Glomus monosporum Gerd. & Trappe has been reported from North West Oregon to North West Washington from near the cost, in forest, fields and the greenhouses from August to March but probably present throughout the year (Gerdemann & Trappe, 1974). *G. monosporum* forms globose to ellipsoid sporocarp (80x100-95x12 μm) sporocarps in association with wheat and other plants [*Bellis perennis*, *Chamaecyparis lawsonia*, *Hypochaeris redicata* (Gerdemann & Trappe, 1974)]. However there is no information regarding the sporulation of this AM species, because many studies were done on *Glomus intraradices*, *Glomus mosseae*, *Glomus fasciculatum*, *Gigaspora margarita* etc. (Auge *et al*., 2008). Previous studies have indicated the significance of left over wheat straw as AM inoculum for the succeeding crop (Nasim *et al*., 1998) and the crop straw burning threatens the associated AM fungal communities in field soil in which crop straw is set on fire (Nasim, 2009).

This study elucidated that the spore bank in the soil is maintained due to the yearly addition of spores in the soil and the spore and sporocarp development is affected significantly by rice cultivation system than wheat monoculture (Nasim, 2008).

Sporocarps of *G. monosporum* were always observed forming in the senescing leaves of decaying wheat stumps in the field. However in pot cultures sporocarps were occasionally seen. Other structures like hyphae, vesicles and arbuscules were observed more frequently. Vesicles formed were thin walled and large sized. In the field sporocarps developed on leaf bases and then dispersed and mixed in the soil at maturity (Fig. 1). The development of sporocarp started hundreds of thick walled hyphae (8-10 μm in dia) lying parallel to each other in leaf tissue (Fig. 1A). In this hyphal mat, thin walled vesicles differentiated and increased in size (20-30 μm in dia) to form spores which remained covered with thickly interwoven hyphal network. There was then a simultaneous growth of thick walled-hyphae from the parallel hyphal mat. These thick walled hyphae then grew and covered the whole spore as it matured. There was usually only one spore in each hyphal mat but occasionally there were 2-3 spores per sporocarp, the hyphal network grew thicker and thicker to form a thick peridium like structure. According to Ainsworth (1971) a true peridium is defined as the wall of a sporangium or other fruit body. However for most mycorrhizal species a hyphal network covering small clustures of spores (*Glomus mosseae* and *G. monosporum*) or a sporocarp has been interpreted as a peridium. Interwoven hyphae encircling individual spores have been termed as hyphal mantle although differences in origin and development are not clear cut (Morton 1988).

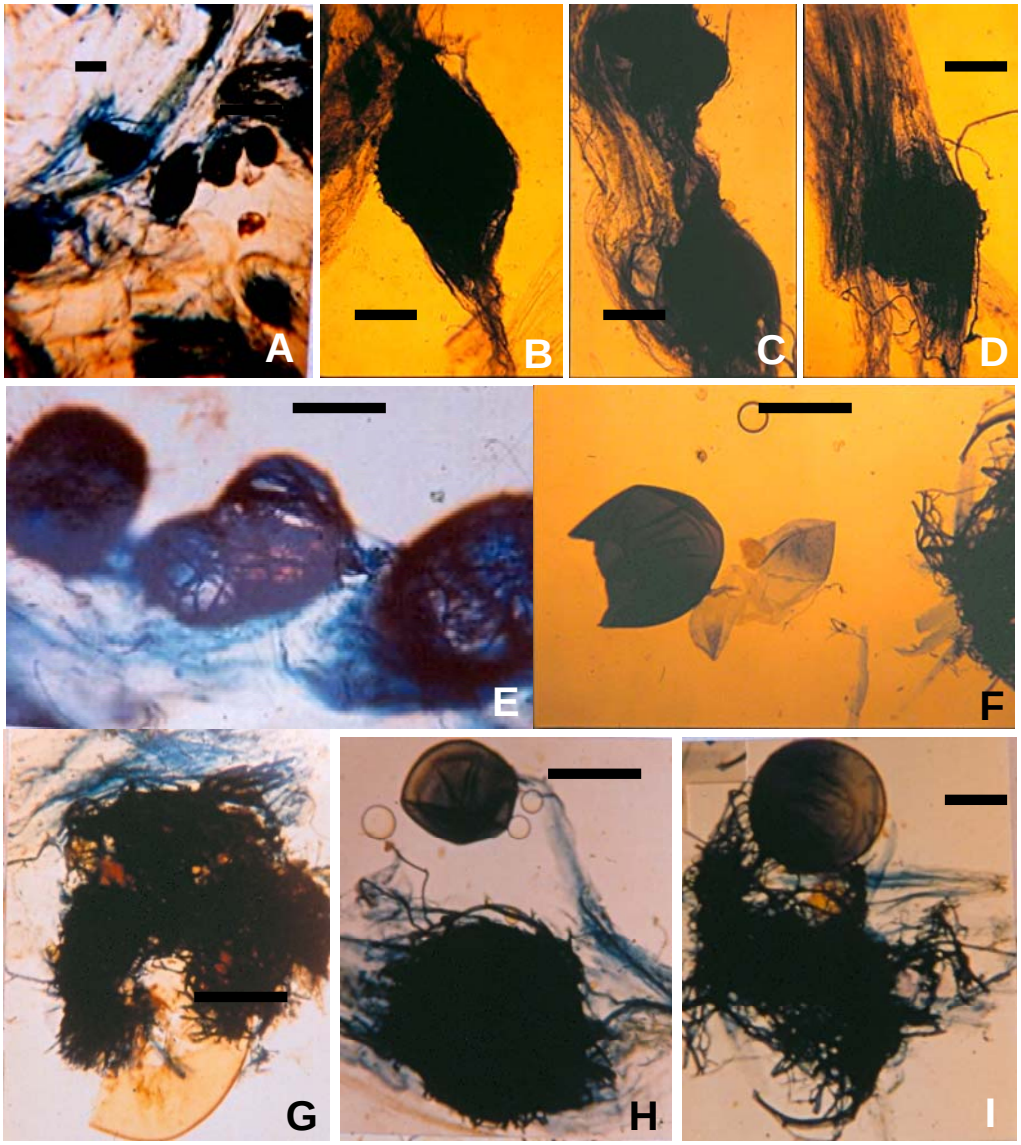

Fig. 1. Stages of sporocarp formation and release of spore by *Glomus monosporum* in decaying leaf sheaths of wheat. **A-D:** Sporocarps as seen in the decaying leaf sheaths; **E:** Mature sporocarps; **F-I:** Stages in the liberation of spore from sporocarp at maturity, (Bar=50μm).

Sporocarp development started two weeks before the crop was harvested and these matured within one month after harvest. As the sporocarp matures and dispersed into the soil, the peridium became denser and thicker and the identity of the hyphae forming hyphal mantle could not be recognized. Eventually the peridium dissolves away liberating light brown-coloured spores into the soil. During slide preparation a slight pressure on the cover slip caused mature spore to pop out. The sporocarp releases spores (60-70 μm dia., globose to sub-globose) either detached in the soil or released from the sheathing leaves. The subtending hyphae (8-13 μm dia) was slightly swollen, strongly recurved and appressed to spore wall.

The specificity of *G. monosporum* to grow on such kind of very specialized niche like left over wheat crop is highly threatening its existence. Farmers in this part of the world burn the left over crop straw. Some earlier studies have indicated that burning sterilized the top soil layer killing all living organism and their propagules. It is therefore indicated with concern that if the burning practice continues, it may lead to complete extinction os at least one species of AM fungi from the field soils of this region.

References

Auge, R.M., H.D. Toler, C.E. Sams and G. Nasim. 2008. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza induced increases in stomatal conductance. *Mycorrhiza,* 18: 115-121

Barea, J.M., M. Gryndlelr., P. Lemanceau, H. Schuepp and R. Azcon. 2002. The rhizosphere of mycorrhizal plants. In: *Mycorrhizal Technology in Agriculture-From genes to Bioproducts,* (Eds.): S. Gianinazzi, H. Schuepp. J.M. Barea and K. Haselwandter. Birkhauser Verlag, Berlin, Germany.

Barrow, J.R. 2004. Unique characteristics of a systemeic fungal endophyte of native grasses in arid southwestern rangelands. *Proceedings USDA Forest Service*. Las Cruces, pp. 54-56.

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

Iqbal, S.H. and G. Nasim. 1991. Are under ground non-root portions of tropical plants vesicular arbuscular mycorrhizal? *Transactions Mycological Society of Japan,* 32: 467-476.

Jalaluddin, M., M. Hamid and S.E. Mohammad. 2008. Selection ans application of a VAM-fungus for promoting growth and resistance to charcoal rot disease of sunflower var. Helico-250. *Pakistan Journal of Botany*, 40(3): 1313-1318.

Krishna, K.R. 2005. *Mycorrhizas*: *A Molecular Analysis.* Science Publishers, Inc. Enfield, New Hampshire, USA.

Morton, J.B. 1988. Taxonomy of VA mycorrhizal fungi: Classification, nomenculature and identification. *Mycotaxon*, 32: 267-324.

Nasim, G. 2008. Influence of rice allelopathy on sporocarp formation by *Glomus monosporum* in leaf sheath bases of wheat. *Allelopathy Journal*, 22(2): 403-408.

Nasim, G. 2009. Crop straw burning Practice- A threat to arbuscular mycorrhizal biodiversity. *Bangladesh Journal of Botany*, In Press.

Nasim, G. and R. Bajwa. 2009. *Glomaromycetous Fungi of Pakistan*. Higher Education Commission of Pakistan. (In press).

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

Nasim, G. and S.H. Iqbal. 1991b. Fate of Endogonaceous spores in soil. *Transactions Mycological society of Japan*, 32: 517-522.

Nasim, G., A. Ali, A. Munawar and R. Bajwa 2008. Seasonal dynamics of AM fungi in sugarcane (*Saccharum officinarum* L. cv. SPF-213) in Punjab, *Pakistan. Pakistan Journal of Botany* 40(6): 2587-2600.

Nasim, G., S. Saeed, M. Shaheen, Z.H. Naqvi and S. Sheikh. 1998. Wheat stumps, a source of VAM inoculum for the incoming crop. *Scientific Khyber,* 11: 43-56.

Phillips, T.H. and D.S. Hayman. 1970. Improved procedures for clearing root parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infections. *Transactions British Mycological Society*, 55: 158-160.

Schenck, N.C. and Y. Perez. 1987. *Manual for identification of VA mycorrhizal fungi*. Field Hall, University of Florida, Gainesville, Florida.

Siddiqui, Z.A. and J. Pichtel. 2008. Mycorrhizae : An overview. In *Mycorrhizae: Sustainable Agriculture and Forestry*, (Eds.): Z.A. Siddiqui, M.S. Akhtar and K. Futai. 1-35. Springer Verlag, Germany.

Trappe, J.M. 1992. Synoptic key to the genera and species of Zygomycetous mycorrhizal fungi. *Phytopathology*, 72(8): 1102-1108.

(Received for publication 21 January 2009)