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Seasonal dynamics and relative abundance of AM fungi in rhizosphere of rice (*Oryza sativa* L. cv. Basmati Supper)

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Abstract

Seasonal spore dynamics and relative abundance of five *Glomus* species (*Glomus mosseae, G. fasciculatum, G. monosporum, G., aggregatum, G. microaggregatum*) and one *Acaulospora* species (*A. bireticulata*) was studies in the field through out the growing season of rice. Maximum number of spores per 10g sample soil was recorded for *G. faciculatum* at the end of growth period. The pattern for highest values of propagule number in rhizosphere soil was variable for rest of the AM species. Maximum spore abundance for *G. mosseae, G. microaggregatum* and *G. aggregatum* 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 *A. bireticulata* was recorded only rarely. Lowest propagule number for *G. microaggregatum, G. mosseae, G. fascicultum* and *G. aggregatum* was in the months of June to July. The difference between highest and lowest spore densities was statistically significant for all the AM species observed at 5% level. In relative abundance pattern it was observed that for a particular *Glomus* sample higher number of propagules of one species was associated with significantly lower values of spore number of some other species. The AM hyphae, number of arbuscules, and vesicles decreased with crop maturation while the spores increased dramatically. Spores covering a wide size range were recorded in degenerating roots and sheathing leaf bases which were lying buried in the soil. Hyphal mats and clumps were extensively observed. Mycelium often became beaded in the sheathing leaf bases. Arbuscules were not seen in the moribund roots. An innumerable number of other non-AM Dark Septate Endophytic Fungi were occasionally seen.

Keywords: Relative abundance, sheathing leaf basis, dark septate endophytic fungi, arbuscular mycorrhiza.

Introduction

Glomeromycete fungi (arbuscular mycorrhizal fungi-AMF) are ubiquitous soil organisms that often proliferate with in patches of soil organic material (Nasim and Zahoor, 1996; Kapulnik and Dauds, 2000; Mehrotra, 2005). These fungi commonly grow in living plant tissues other than roots (e.g rhizome and corm scales-Brundret & Kendrich, 1988, Nasim, 1990, 1991; Nasim and Iqbal., 1991a). They also occupy dead soil animals and spores of other AM fungi, presumably to acquire nutrients or avoid predation, or perhaps as mycoparasites (Nasim *et al.*, 1997).

These fungi play a vital role in growth and development of important crop plants (Krishana, 2005; Auge *et al.,* 2007). Their ecology in a cultivated field is not known enough. The benefits to host plants in association with AMF especially in nutrient deficient soils are well established (Cho *et al.,* 2006). Although the importance of AMF to the growth and betterment of many plants is well known 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. Seasonal variation in the activity of AMF in temperate soil is poorly understood and generally based on few observations (Hayman, 1970; Suton and Barron, 1972; Saif and Khan, 1975; Giovannetti, 2000, Mehrotra, 2005).

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 (Douds and 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 (Nasim and Zahoor 1984; Sylvia, 1986; Koske and Gemma, 1989).

The purpose of the present study is to investigate the seasonal dynamics of some AM spp., in a cultivated field throughout the growing season. The effects of variable pattern of relative abundance on the structure of AMF community are also determined. In this study, we wanted to check two hypotheses, firstly that the variation exists in arbuscular mycorrhizal colonization with crop maturity and secondly that the major species of AM fungi have specific abundance pattern in the rhizosphere of the crop.

Materials and Methods

This study was conducted in a rice (*Oryza sativa* L. Supper Basmati) fields with in the premises of University of the Punjab, Lahore, Pakistan. Sampling was carried out in transects in three adjacent fields of rice during its growth seasons of 1999. Twenty samples of 100g soil each of root zone and rhizosphere were collected per field, then mixed to give ten final samples per field. Regular sampling was done after an interval of 15 days from June to December.

Isolation and identification of spores:

Soil samples were collected from root zone (5-10cm in depth) of rice from each collection site. Ten grams of soil was processed to recover AM spores and a final list of spores was prepared by examination of the roots of mature plants for the presence of mycorrhizae, direct examination by soil past method (Nasim and Iqbal, 1991b) of soil samples for the presence of AM forming propagules and wet sieving and decanting technique of Gerdemann and Nicolson (1963).

Intact spores were collected by pinpointing spores on a filter paper through a binocular at 10 and 40x, picking them up with a sharpened toothpick or a hypodermal needle was used for the purpose. After washing several times, spores were either mounted in a drop of sterile water or these were stained in a drop of trypan blue in lactophenol and examined under the microscope. Identification of spores was done with the help of keys by Trappe (1982), Hall (1984), Morton (1988), Schenck and Perez, 1992). Five species of *Glomus* and one of Acualospora were selected because they were the most abundant at certain periods of crop growth.

Statistical analysis:

Standard error (SE), analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were performed to analyze the data following Steel and Torrie (1980) and Rosner (2000) using SPSS 10.0 (Carver and Nash 2000).

Clearing and staining of roots and stump portions:

Decaying root pieces and plant debris were also picked up and observed under the microscope after giving several washing with sterile water. The plant portions were stained following the method of Phillips and Hayman (1970) with some modifications (Iqbal and Nasim, 1986).

Different plant portions were processed separately. Plant parts like roots and leaf bases were sorted out. Each of the sample was washed under tap water. Clearing was done in 10% KOH by autoclaving for 2-3 minutes. Samples that remained dark coloured after clearing in KOH were bleached in alkaline Hydrogen peroxide (Koske and Gemma, 1989). These bleached plant portions were washed with 0.01N HCl to neutralise. Staining was done in acidic glycerophenole containing 0.05% trypan blue.

Stained materials were examined under a dissecting microscope. Portions carrying mycorrhizal structures were gently picked up with the help of a hypodermal needle and were mounted in lactophenol. Care was taken while transferring these pieces that not to disturb intact extramatrical mycelia, spores and auxilliary cells. Unless otherwise stated all microscopic examinations were made with mounts in lactophenol.

For the assessment of mycorrhizal extent and frequency of various AM structures, stained root materials of each sample were cut up into one cm long pieces. Ten such root pieces were mounted paralleled to each other on a microscope slide. Such slides were prepared in triplicates.

Prepared slides were observed and mycorrhizal colonization were assessed under light microscope. Slides with characteristic structures were photographed with a Minolta X700 Camera with microscope adapter tube.

Results

The stumps of rice were collected with an interval of fifteen days for 8 months. These soil samples were collected from root zone (5-10 cm in depth) of crop plants from each collection site. Ten grams of soil from each of the sample was processed for the screening and identification of endogonaceous spores.

Dynamics of AM structures

There was a significant difference among samples as regards the number of arbuscules, vesicles and spores in decaying roots. The percentage of occurrence of various types of fungal structures and extent of mycelium in root

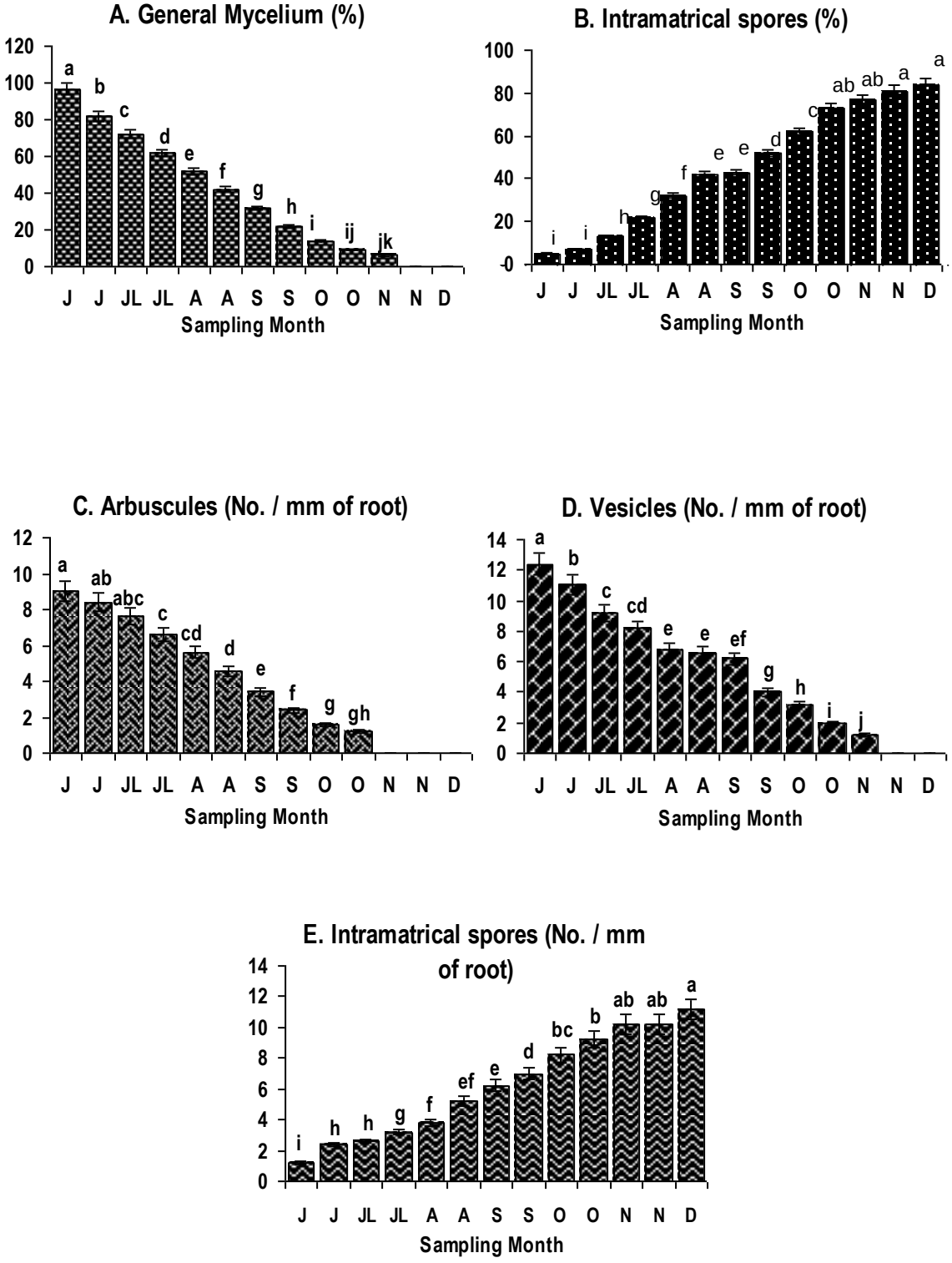

Fig. 1: Seasonal pattern of mycorrhizal structures in senescing roots of rice stumps. Line on data bars show standard error. Data bars with different letters are significantly different at P<0.05 according to DMRT.

cortex varies markedly from one sample to the other (Fig. 1).

The percentage of general infections decreased with the passage of time and so was the case with vesicles in the roots. On the other hand the incidence of Intramatrical spore formation increased as the stage of decay of the sumps progressed, (Fig 1).

Spores of different sizes were often seen intramatrically and in the root cortex. Some large sized spores were composed of thin outer walls and a compartmented inner contents (Plate 1) some spores with translucent inner contents were darkly stained. These were mostly borne on subtending hyphae, which in certain cases were slightly swollen at the point of attachment. Individual spores and clusters of spores with attachments were recorded along and around the steler portion of decaying roots. Unlike all other parameters the occurrence of spores found inside the root showed an increasing tendency. The values for percentage of spores increased with the passage of time. These were minimum (5%) in the very first sample which increased to 84% in the last sample. The change in the number of these spores per mm length of the root was synchronous i.e. steadily increasing towards the end of the sampling period, (Fig. 1).

Senescing leaf bases also harboured AM infections. Mycelium was often observed traversing the parenchymatous tissue of the leaf bases. General infection and vesicles decreased while the Intramatrical spores increased as the stumps underwent decay. Appreciable values were reported in the case of number of spores at the end of the study period, (Fig. 2).

Seasonal pattern and relative abundance of six AM species

The Results of the present study indicate that the greatest overall spore abundance of AMF propagules occurred in the fall i.e. at the end of growth season of rice crop. However different seasonal; pattern were observed when individual species were analysed.

The period of maximum spore abundance was between June and July and then November-December for *G. mosseae* (Fig. 4a). In early sampling period (June and July) spore abundance /10 g of soil for this particular species was close to maximum, it decreased to a minimum in July (Fig. 4a).

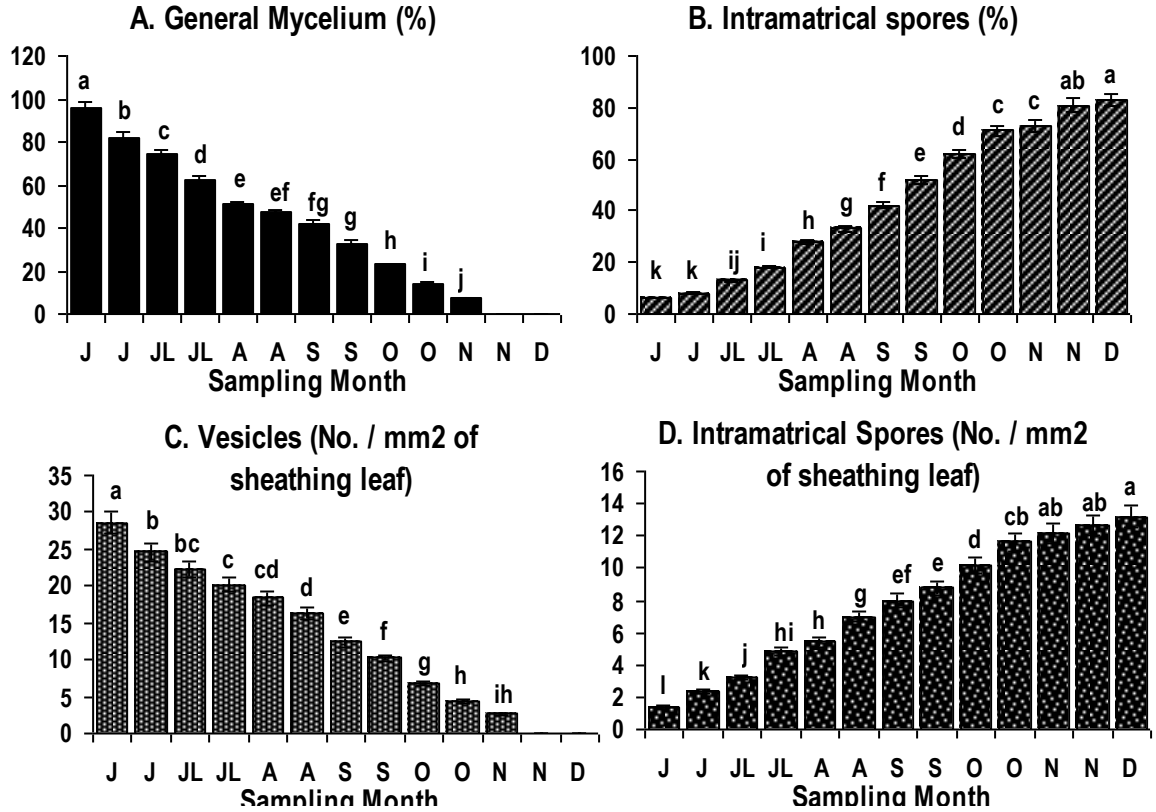

Fig. 2. Seasonal occurrence of mycorrhizal structures in senescing sheathing leaf bases of rice stumps left after crop harvest. Lines on data bars show standard error. Data bars with different letters are significantly different at P<0.05 according to DMRT.

Comparison of seasonal spore dynamics of *G. fasciculatum* (Fig. 4b) through out the study period indicated highest spore population in June and lowest in July and these difference were significant statistically at 5% level ($P<0.05$). For *G. microaggregatum* the number of spores increased gradually till December. In case of *G. aggregatum* spore abundance was also bimodal but was generally low throughout the sampling period. For *Glomus monosporum*, spore abundance was greatest between September and November. Lowest abundance was in August. *Acaulospora bireticulata* was detected in June, September and November.

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, G. microaggragatum* and *A. bireticulata*.

Spores collected during November-December were uniform in outline and light coloured with subtending hyphae intact and appeared to be freshly formed. In contrast to this, spores collected during and June and July were somewhat thick walled dark brown to reddish brown in colour and without subtending hyphae.

Interspecific competition was also studied 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 120 spores/10g of soil was claimed as high density sample for that species. For the four species (*G. monosporum, G. aggregatum, G. microaggregatum* and *A. reticulata*) high density sample was 65-130 spores/10g of soil but in samples where another species was in high density, average abundance of *G. mossseae* was much lower ranging from 35-80 spores/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. 3).

Discussion

As a result of our study addressing two specific hypothesis, it was found that the AM structures exhibited variations in response to time. In the time course studies of root/sheathing leaf samples the attentions was focused on the colonization patterns of general mycelium, Arbuscules. Intramatrical spores. Rice being a submerged crop during early phases of growth, is usually thought of poorly mycorrhizal. In the present study the colonization was reported right from the beginning though the figures were not highly appreciable. These findings are very much inline sith those of some earlier workers (Iqbal et al., pers comm.). Both vesicles and Arbuscules decreased in intensity with the progress in crop maturity replaced by an increase in number of intramatrical and extrametrical spore formation. Pandey and Singh (1990) and Rachel et al., (1990) have also presented same trend in the result of their study. The report of the presence of vesicles and spores in the sheathing leaves is a noval finding for rice. This finding however is supported by a number of previous findings (Nasim et al., 1998) from Punjab University stating the same in a number of plants including wheat. These authors have drew attention to three aspects when addressing the question of AM fungal biotrophy: i) nutrition, since the growth of the fungus may depend on specific nutrients supplied by the host ii) physical aspects, since some growth conditions may be essential for invitro growth; and iii) genetics, since the fungus may have lost a part of its genetic material or may have repressed its genome repressed. All cytochemical, chemical , metabolic and genomic evidence suggest that VAF resemble saprotrophs in presymbiotic or post symbiotic phase, however it must interact with its host to fully express its potential (Hepper, 1987; Bago and Becard, 2002).

Spore abundance of six AM species investigated in the present study was influenced by the season. This interspecific competition appears to be a major factor in determining the spore abundance/densities of these AM species in experimental wheat field.

Higher spore abundance of a particular species in a sample was typically associated with reduced spore densities of other AMF species 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 (Nasim and Zahoor, 1996). Present results are in line with those of Pandey and Tarafdar (1999), who demonstrated/reported that as plant matured overall populations of AMF propagules increased but the sporulation was not synchronous.

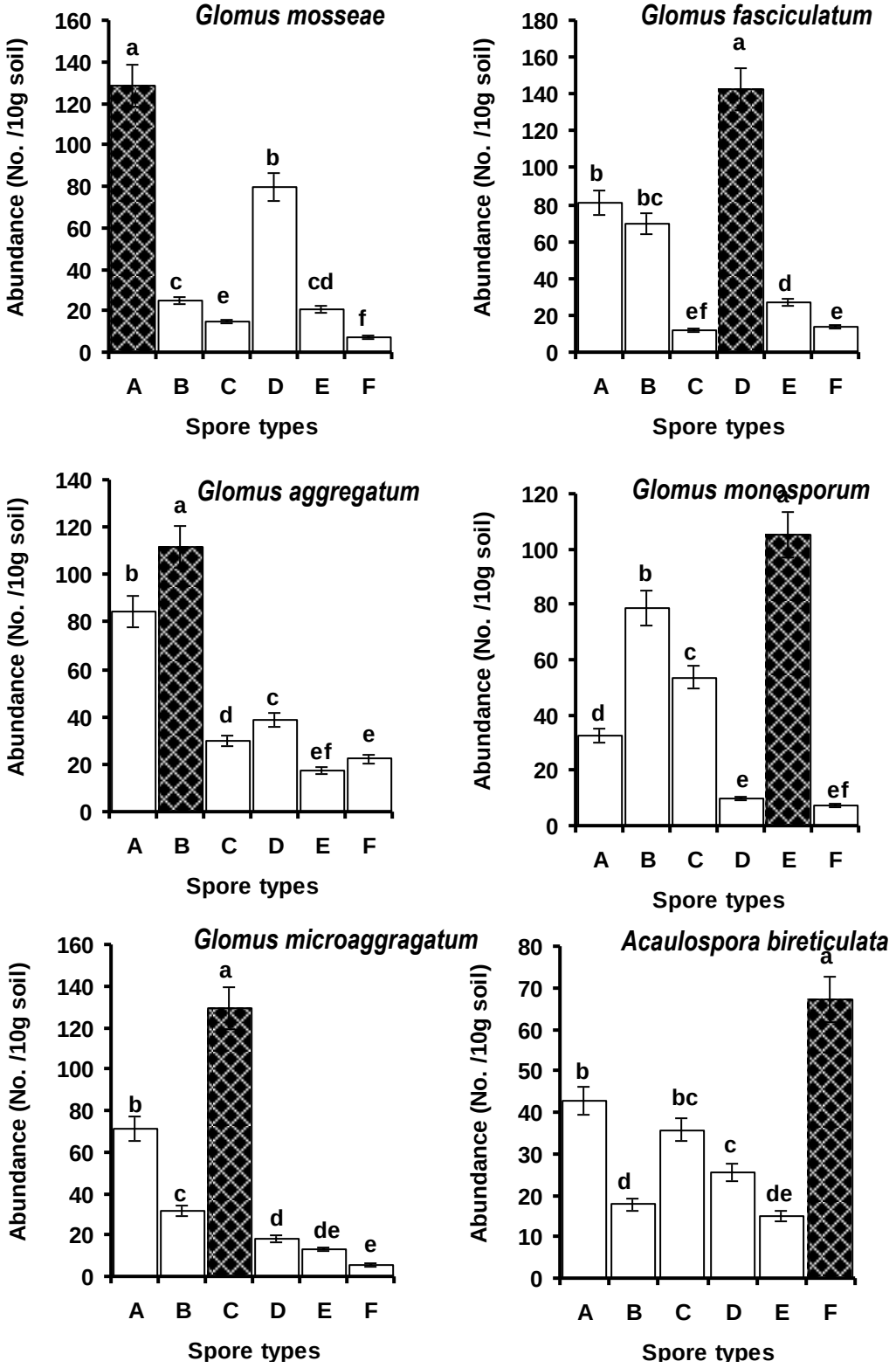

Fig. 3: Spore abundance in individual soil samples of AM species collected from a rice field. Line on data bars show standard error. Data bars with different letters are significantly different at $P<0.05$ according to DMRT.

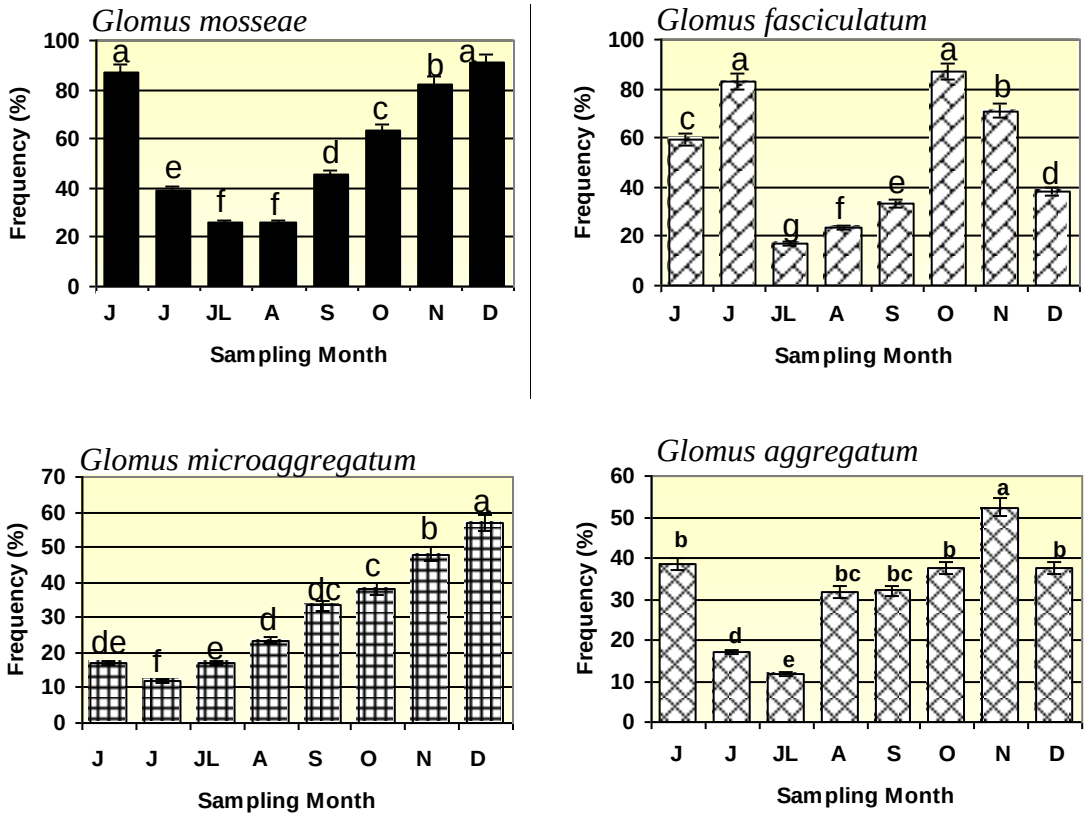

Fig. 4: Frequency of occurrence of four major AM species in the rhizosphere during rice growth period. Line on data bars show standard error. Data bars with different letters are significantly different at P<0.05 according to DMRT.

Key for spore types: A: *Glomus mosseae*; B: *G. aggregatum*; C: *G. microaggregatum*; D: *G. fasciculatum*; *G. monosporum*; and F: *Acaulospora bireticulata*

Although maximum spore densities of four *Glomus* species (*G. mosseae*, *G. monosporum*, *G. Fascicultum* and *G. agregatum*) occurred at 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 June followed by a sharp fall at the time of flowering of host (wheat). In case of *Glomus monosporum*, G. microaggragatum and *A. reticulate*, maximal sporulation coincided with the beginning of flowering period, unlike other *Glomus* species, which were not producing significant number of spores. The variations in the sporualtion pattern noticed among these six AM 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. 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 (Pandey and Tarafdar, 1999).

Abiotic factors such as temperature and light and biotic factors i.e. changes in amount of photosynthates production, quality and quantity of root exudates and fluctuations in root hormone level occurring during flowering and growth cessation 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 and its importance in determining the AM community. It has been reported that vegetational characters are more important indetermining the extent of sporulation by individual species than competition with other AM propagules (Chandra and Kehri, 2006).

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 highly diverse AMF community even in a managed ecosystem. Looking at the present results this would be suggested that the stumps should not be burnt after the crop is harvested. Instead these should be ploughed back into the soil preferably after crushing and chopping. This would act as an excellent source of AM inoculum for the incoming crop.

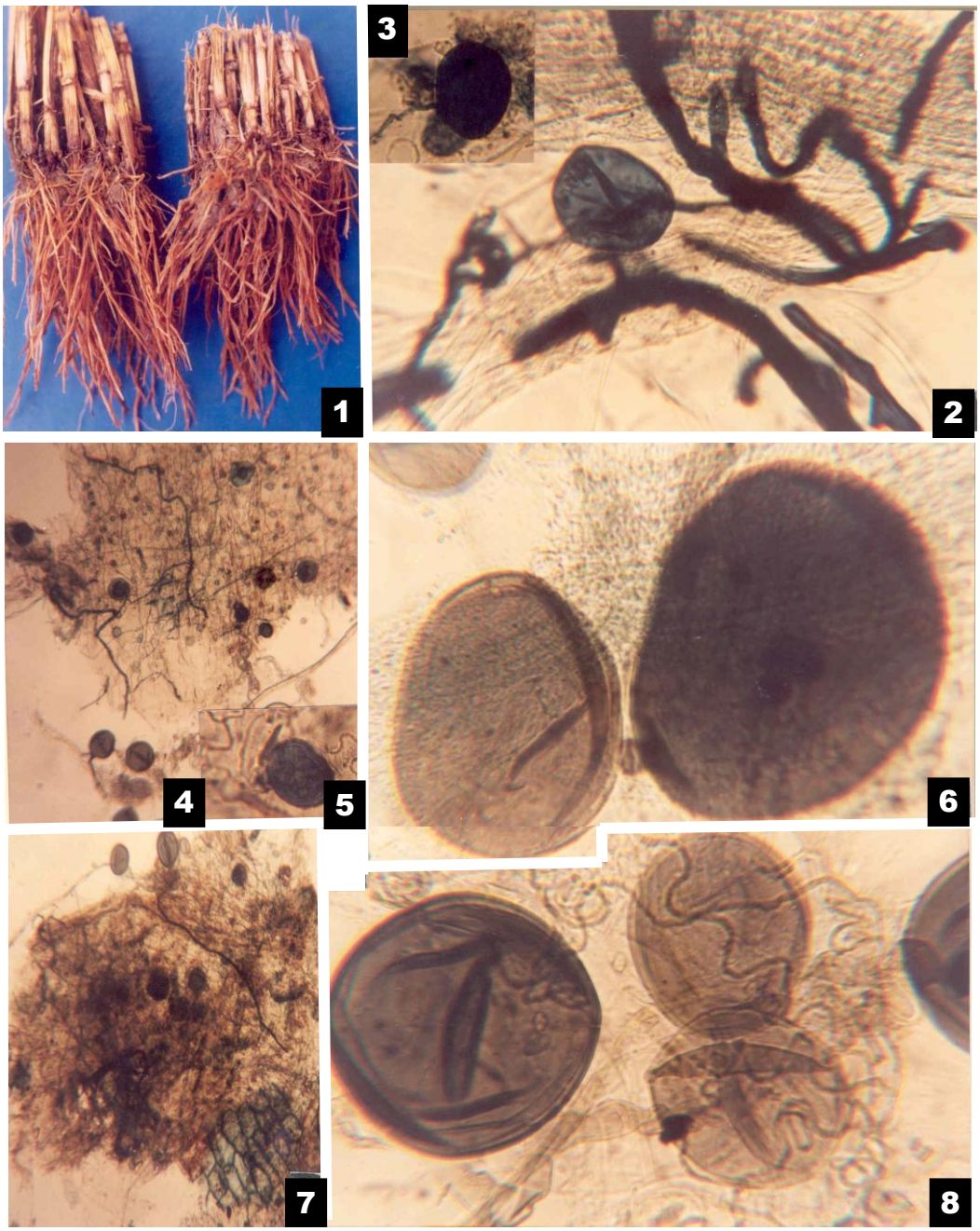

Plate: 1: Rice stumps collected from a cultivated field after crop harvest (bar=5cm). 2-8: vesicles and spores in decaying sheathing leaf bases (bar=20μm).

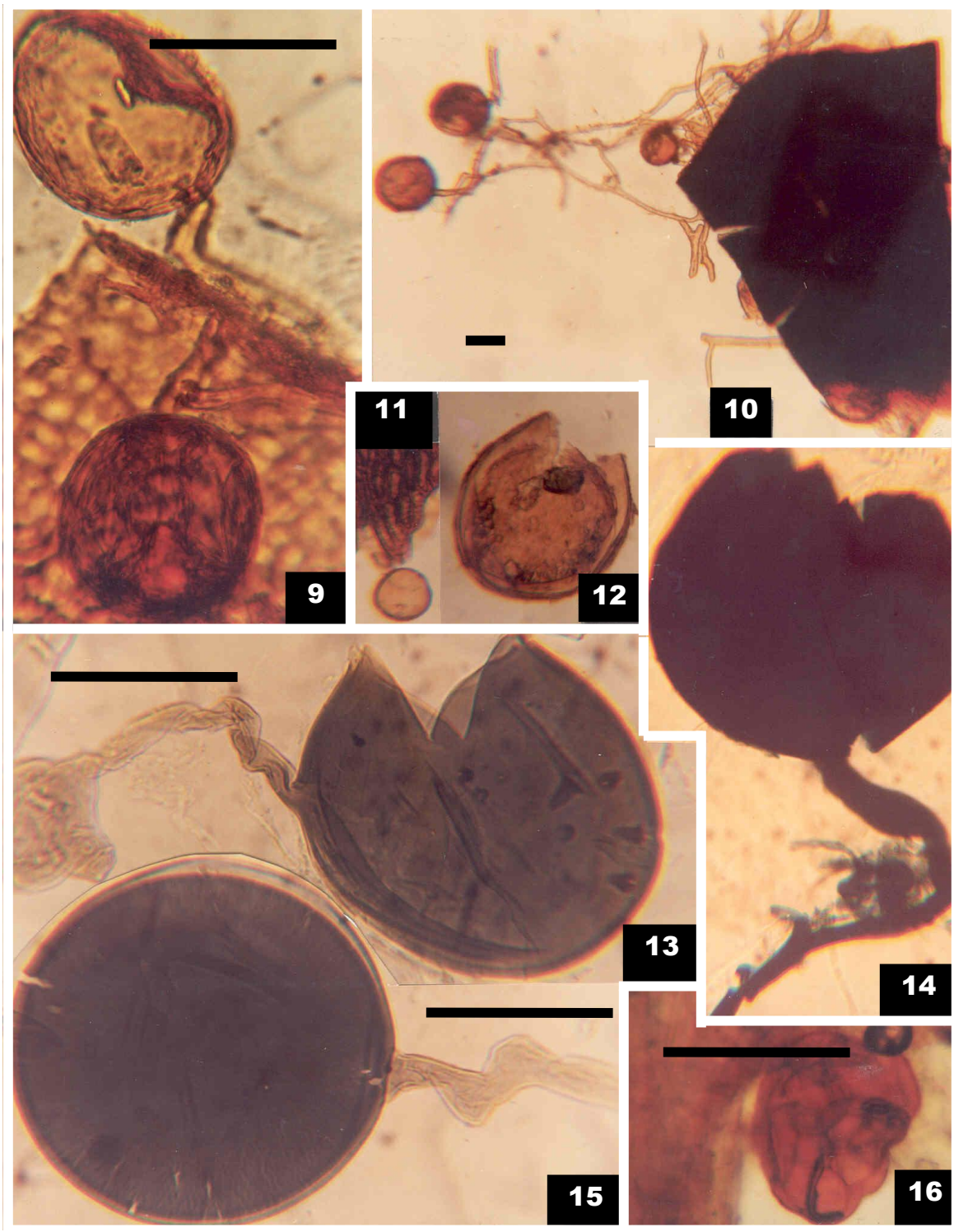

Plate 11-16 showing arbuscular fungi formed in association with senescing sheathing leaf basis of rice stumps. 9 & 10: Spores of *Glomus mosseae*; 11: Spore of *Glomus microagregatum*; 12: Spore of *Glomus aggragatum*; 13-15: Developing spores of *Glomus fasciculatum*; 16 Spore of *Acaulospora bireticulata*. (bar=30μm).

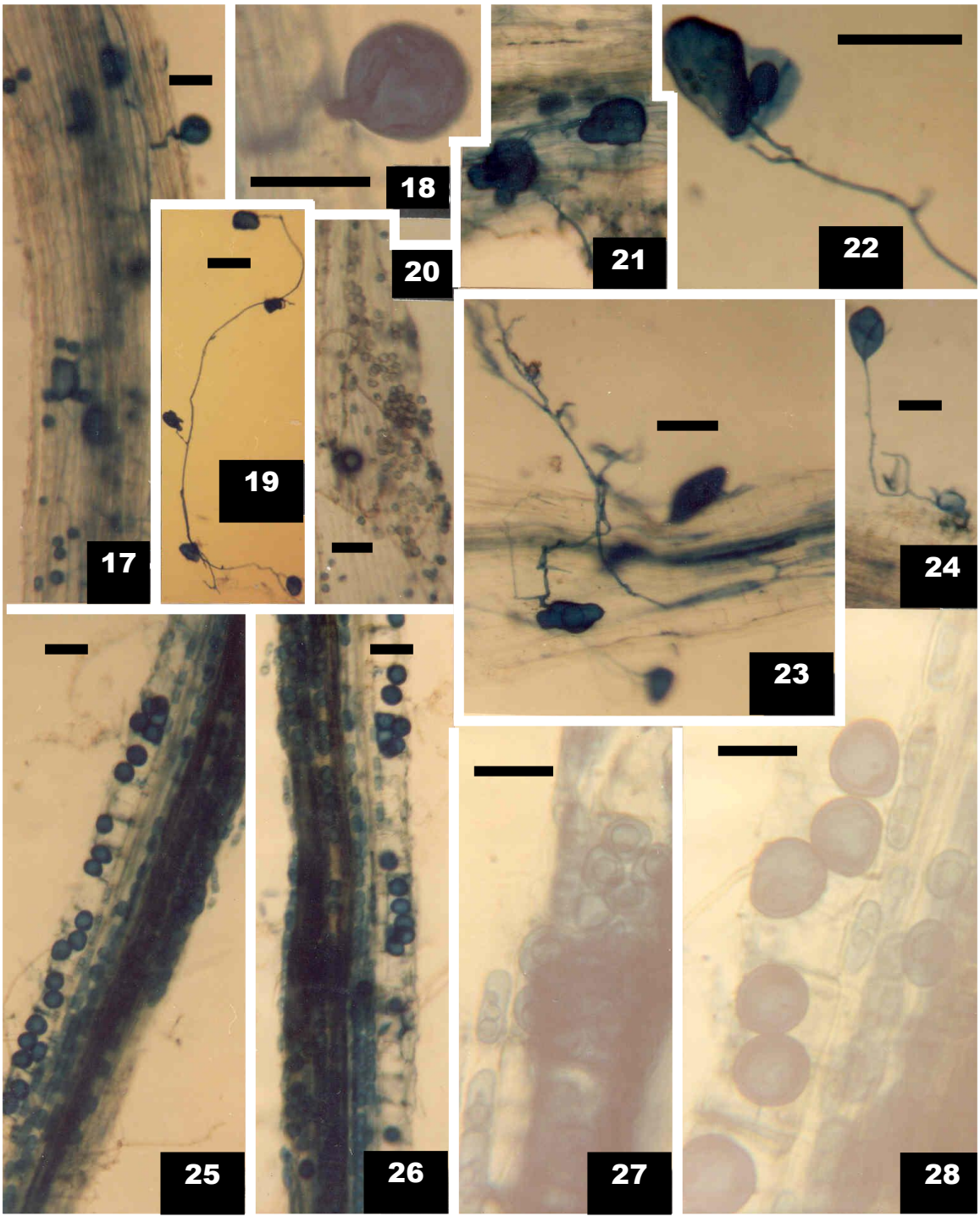

Plate 17-28: Intramatrical and extrametrical spores observed in association with senescing roots of rice (bar=30μm).

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References

Auge RM, Toler HD, Moore JL, Cho K, and Saxton AM, 2007. Comparing contributions of soil versus root colonization to variations in stomatal behavious and soil drying in mycorrhizal Sorghum bicolour and Cucurbita pepo. *J. Plant Physiol*, 1289-1299.

Bago B and Becards G, 2002. Bases of the obligate biotrophy of arbuscular mycorrhizal fungi. In Mycorrhizal techn-ology in agriculture-From genes to bioproduct (eds.): S. Gianinzzi, H. Schuepp, JM Barea and K. Haselwandter, Birkhauser, Germany. pp. 33-48.

Brundrett M, and Kendrick B, 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest *Canadian J. Bot.*, ***66:*** 1153-1173.

Carver RH, and Nash JG, 2000. Doing data analysis with SPSS 10.0. Brooks/Cole, Duxbury, UK.

Chandra and Kehri, 2006. Chandra S, and Kehri HK, 2006. Biotechnology of VA mycorrhiza: Indian Scenario, New India Publishing Agency, Pitam Pura, New Delhi, India.

Cho K, Toler HD, Lee J, Ownley BH, Jean C, Stutz JC, Moore JL, Augé RM, 2006. Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. *J. Plant Physiol*., **163**: 517-528.

Douds DD, Jr., and Chaney WR, 1986. The effect of high nutrient addition upon seasonal patterns of mycorrhizal development, host growth, and root phosphorus and carbohydrate content in Fraxinus pennsylvanica Marsh. New Phytologist **103**: 91-106.

Gerdemann JW, and Nicolson TH, 1963. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. soc*., **84**: 679-684.

Giovannetti M, 2000. Spore germination and pre-symbiotic mycelial growth, pp. 47-68. In: *Arbuscular mycorrhizas: physiology and function.* Eds: Y Kapulnick and DD Douds Jr. Kluwer Academic Press.

Hall IR, 1984. Taxonomy of VA mycorrhizal fungi. In: VA Mycorrhiza. Eds. C. L. Powell and D. J. Bagayaraj. C. R. C. Press Inc., Boca Ratoon, Florida. Pp. 57-74.

Hayman DS, 1970. Endogone spore number in soil and vesicular arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Trans. Brit. Mycol. Soc.*, ***54:*** 53-63.

Hepper CM, 1987. VAM spore germination and hyphal growth in invitro prospects for exenic cultures, p. 172-174 In DM Sylvia, LL Hung, JH Graham (eds) 7th NACOM. Mycorrhizae in the next decade-Practical applications and research priorities, University of Florida, Gainsville.

Iqbal SH, and Nasim G, 1986. Vesicular arbuscular mycorrhiza in roots and other underground portion of *Curcuma longa*. Biologia, **32**: 223-228.

Kapulnik Y, and Dauds DD, Jr. 2000. Arbuscular Mycorrhizas: Physiology and Functions. Kluwer Academic Publishers, London, UK.

Koske RE, and Gemma JN (1989). Ecology of mycorrhizal Zygomycetes. In: Biology of Zygomycetes. Ed. T. Hammil, *Mycolo. Mem.*, ***12:*** 1-54.

Krishana KR, 2005. Mycorrhizas: A molecular analysis. Science Publishers, Inc. Plymouth, UK.

Mahrotra VS, 2005. Mycorrhiza: Role and applications, Allied Publishers Pvt. Ltd., Mumbai, India.

Morton JB, (1988) Taxonomy of VA mycorrhizal fungi: classification, nome-nclature, and identification. *Mycotaxon*, ***32:*** 267-324.

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

Nasim G, and Iqbal SH, 1991a. Species of *Glomus* associated with non-root portions of some rhizomatous plants and characteristics of their mycorrhizae. *Trans. Mycol. Socy. Japan*, **32**: 541-545.

Nasim G and SH Iqbal 1991b Fate of Endogonaceous spores in soil. *Trans. Mycol. Socy. Japan*, **32**: 517-522.

Nasim G, 1990. Vesicular arbuscular mycorrhizal endophyte in roots, scale leaves and epidermal cells of the rhizomatous tissues of *Colocasia antiqourum* (L.) Schott. *Scient. Khyber*, **3(2)**: 183-194.

Nasim G, 1991. Vesicular arbuscular mycorrhizae in two *Curcuma* Species (*C. zeodaria and C. longa*) of medicinal importance. *Pak. J. Forest.*, **41(4):** 194-201.

Nasim GR, Zahoor and Shaheen M, 1997. Endogonaceous Sopre Flora Of Pakistan. VIII. VAM fungi sporulating inside spores of other VAM fungi. *Scient. Khyber*, **10(1):** 21-31.

Nasim GS, Saeed, Shaheen M, Naqvi ZH and Sheikh S, 1998. Wheat stumps, a source of VAM inoculum for the incoming crop. *Scient. Khyber*: 43-56.

Pandey S, and Singh VK, 1990. Ecophysiological responses of litchi cultivars to vesicular arbuscular mycorrhizal colonization. In Mycorrhizal Symbiosis and Plant Growth (Eds. D. J. Bagyaraj and A Manjunath, UAS, Bagalore: 49-50.

Pandey and Tarafdar, 1999. Pandey M, and Tarafdar JC, 1999. Critical level of VAM of fungal propagules in different arid crops. Agrochimica **43(3/4)**: 187-192.

Phillips JM, and Hayman DS, 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.*, ***55:*** 158-160.

Rachel EK, Reddy SR, and Reddy SM, 1990. Seasonal variations in the colonization of different angiospermic plant species in the semiarid soils of A.P. National academy of Science Letters. **13(6)**: 219-223.

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

Saif, S.R. and Khan, A.G. (1975). The influence of season and stage of development of plant on Endogone mycorrhiza of field grown wheat. *Canadean J. Microbiol*.

Schenck NC, and Perez Y, 1992. Methods and Manual for VA Mycorrhizal Research. Synergistic-Publications 250p.

Sutton J, and Barron GL, 1972. Population dynamics of *Endogone* spores in soil. *Canadian J. Bot.*, **50**: 1909-1940.

Sylvia DM, 1986, Spatial and temporal distribution of vesicular arbuscular mycorrhizal fungi associated with Uniola paniculata in Florida foredunes. *Mycologia*, **78**: 728-734.