Effect of edaphic factors on root colonization and spore population of arbuscular mycorrhizal fungi

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Abstract

Arbuscular mycorrhizal (AM) fungi in agricultural crops grown under AEZ-28 (CARS, Joydebpur), AEZ-9 (RARS, Jamalpur), AEZ-11 (RARS, Ishurdi) and AEZ-23 (RARS, Hathazari) in Bangladesh were assessed during 1999 and 2000 to study the effect of edaphic factors on AM colonization and spore population. Mainly cereals, pulses, oilseeds, vegetables and spices were selected for the assessment. The average root colonization of mycorrhizal fungi in all the selected crops during two years differed with the location. Average colonization in 1999 was the highest (43.0%) at AEZ-9 (Jamalpur) and the lowest (37.0%) at AEZ-23 (Hathzari) but in 2000, the highest colonization (45.0) was found at AEZ-11 (Ishurdi) and the lowest (39.0%) at AEZ-28 (Joydebpur). Considerable variation was also observed in average spore number recorded in the 4 AEZs. Maximum average spore numbers (148.0 and 167.0 per 100g soil) were recorded at AEZ-23 (Hathazari) and the minimum (92.0 and 106.0 per 100g soil) at AEZ-28 (Joydebpur) during 1999 and 2000. The spore number varied within and between sites. Edapho-climatic factors played an important role causing variation in AM colonization and spore population. Soil moisture, pH and nutrient levels influenced colonization and spore number. Soil moisture, OM, total N and soil potassium were insignificantly and positively correlated to root colonization. A negative and insignificant correlation of root colonization was observed only with soil phosphorus. A positive but insignificant correlation existed between spore number and edaphic factors.

Keywords: Edaphic factors, AM root Colonization, AP Spore population

Introduction

The role of arbuscular mycorrhizal (AM) fungi in enhancing plant growth and yield, resistance to drought and salinity and tolerance to pathogens is well documented (Smith and Gianinazzi-Pearson, 1988). However, wide diversity exists within the group of fungi responsible for the formation of AM by most plants in the majority of terrestrial ecosystems. The selection of the most specific appropriate plant-fungus association for each specific environmental and ecological situation is one of the main challenges in current research on AM. Therefore, knowledge of the different factors influencing the

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population biology of AM fungi is essential in any attempt to use them in environmental conservation (Allen, 1991), biotechnology (Mulongoy *et al*., 1992) or in sustainable agriculture (Bethlenfalvay and Linderman, 1992).

The identification of indigenous AM fungi is a fundamental requirement to understand biodiversity and essential for monitoring changes in natural, managed or disturbed ecosystems. Diversity in AM fungi can be explored at this level by studying spore characteristics, ultrastructural features and infection patterns in different agricultural crops including different varieties of the same crop. It is also important to study the diversity of AM fungi in relation to different kinds of fertilizer treatments in important cereal, legume, vegetable and other crops. Many researchers observed that excessive chemical fertilizers had adverse effects on colonization and spore population (Carling *et al*., 1996). The AM fungi are abundant and ecologically very important in the tropics and have been recognized as a promising alternative for reducing fertilizer requirements of major crop species (Mosse, 1981). Soils in the tropics are either poor in P and other essential nutrients or have an immobile form of P (Menge, 1983). Hence, AM fungal inoculum could be added to the soil for better uptake of P to enhance crop production in tropical countries.

Diversity in the occurrence of many AM fungi is probably related to their edaphic requirements but there is a lack of data on the ranges of soil variables under which specific AM fungal species occur. Several edaphic factors viz. textural class, soil pH, organic matter, soil moisture and nutrient levels have been shown to affect spore germination, root colonization and efficiency of AM fungi (Khalil *et al*., 1992). Distribution and diversity of AM fungi in different plant species of a particular agro-ecological zone are important in order to evaluate the natural status of AM fungi in that region. The beneficial influence of indigenous AM fungi is most important in stressed environments and circumstances, but few studies have attempted to explain how plant species and their mycorrhizal status are related to varying environmental factors in natural ecosystem. It is now widely accepted that climatic and edaphic factors can substantially influence AM fungi and their populations, rapid changes in soil nutrients may affect AM association and spore numbers (Abbott and Robson, 1991).

In addition, no work has been done to elucidate any correlation between edaphic factors and either mycorrhizal infection or spore density. It is very much essential to determine the effect of edaphic factors on AM colonization and spore population. Hence, the present work was undertaken to study the dynamics of AM fungal root colonization and spore population in relation to edapho-climatic factors.

Materials and Methods

Selection of sites

This study was conducted at four different sites situated in 4 Agro Ecological Zones (AEZs) in Bangladesh. The selected sites were: (i) AEZ-28: Central Agricultural Research Station (CARS), Joydebpur, (ii) AEZ-9: Regional Agricultural Research Station (RARS), Jamalpur, (iii) AEZ-11: RARS, Ishurdi and (iv) AEZ-23: RARS, Hathazari.

Selection of crops

Important available agricultural crops grown in different AEZs were assessed for AM colonization and spore population in rhizosphere soil. Studies included a number of crops comprising cereal, legume, vegetable and spices.

Climate and weather conditions

The 4 selected AEZs have different climatic conditions. Total annual rainfall, humidity and temperature during the study period were recorded. In general, with a few exceptions, heavy rainfall was recorded in May to August in both years in the 4 AEZs. Occasionally the rainfall was heavy during the months of April, September and October. No or little rainfall was recorded in the months of January to April and September to December. October to March prevailed as a cool dry winter season with low humidity. Temperature was comparatively low and rainfall hardly occurred during this period.

Collection of samples

Roots and rhizosphere soil samples were collected from the 4 AEZs during December to February (1998-99 and 1999-2000) when abundant plants were available in the fields. Five plants for each crop were sampled. Plant roots were dug up, washed thoroughly with water to remove the adhering soil particles and then cut into 1cm long segments. The root samples were then preserved in screw cap test tubes with 50% ethanol for future use. Soil samples were collected up to a depth of 0-15 cm with roots (3 samples/crop). Then, the collected samples were air-dried, packed in airtight polyethylene bags and stored at 4oC for assessing spore density.

Assessment of root colonization and spore population

The percentage of AM infection was estimated by root slide technique (Read *et al*., 1976). A root segment was considered as positively infected if it showed mycelium, vesicles and arbuscules or any other combination of these structural characteristics of AM infection. The presence or absence of infection in the root pieces were recorded and the-per cent of infection was calculated as follows:

$$\% \text{ root infection} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments scored}} \times 100$$

Spore population was determined by following the wet sieving and decanting method (Gerdemann and Nicolson, 1963).

Counting of AM spores

All the AM spores were isolated from the extract using fine forceps and placed into a watch glass with a small quantity of water. The extract, with AM spores, was observed under stereomicroscope and the number of spores was counted. Spore numbers from the three replicates per sample were averaged and the result was expressed as a number per 100g of dry soil.

Results and Discussion

Average root colonization and spore population varied considerably in different locations (Tables 1 and 2). In 1999, the highest average per-cent of root colonization (42.6%) was recorded at AEZ-9 (Jamalpur) but the highest average spore number (147.7 per 100 g soil) was recorded at AEZ-23 (Hathazari). In 2000, the average highest percentage (44.5%) of root colonization was found at AEZ-11 (Ishurdi) but the average highest spore population (167.1 per 100 g soil) was counted at AEZ-23 (Hathazari).

Variables such as soil pH, total soil P, available P, type of soil, soil moisture and cropping season influence on AM population in the natural ecosystem and spore population are abundant in sandy soils as compared to loamy sands (Bhardwaj *et al*., 1997). Soil phosphorus content of Jamalpur and Ishurdi sites was comparatively low (Tables 1 and 2), which might have favored higher mycorrhizal colonization. The AM fungi are often important in root colonization especially in soils with limited phosphorus (Allen, 1991). Arbuscular mycorrhizal fungi could help to improve plant productivity (Habte and Soedarijo, 1996) through increased absorption of mineral nutrients from soil including phosphorus. Soil texture, optimum moisture, neutral pH, high organic matter, comparatively high nutrient level (N and P) all might have created a favourable condition for maximum sporulation of AM fungi at AEZ-23 (Hathazari).

Table 1. Colonization and spore population of AM fungi and physico-chemical properties of soil at 4 AEZs (1999)

AEZ and Locations	Percent colonization	Spore population	Textural class	Moisture %	pH	OM %	Total N%	Pμg/g	K meq/100g
AEZ-28 (Joydebpur)	38.78 ± 4.59	92.16 ± 15.98	Silty clay loam	17.5	6.0	1.02	0.09	11.5	0.22
AEZ-9 (Jamalpur)	42.57 ± 6.00	114.03 ± 22.97	Sandy loam	40.0	6.5	1.67	0.11	10.4	0.16
AEZ-11 (Ishurdi)	40.24 ± 5.19	123.73 ± 23.79	Silt loam	30.2	7.0	1.65	0.10	10.7	0.19
AEZ-23 (Hathazari)	37.39 ± 7.31	147.72 ± 38.12	Sandy clay loam	35.3	6.8	1.70	0.10	15.1	0.12

Table 2. Colonization and spore population of AM fungi and physico-chemical properties of soil at 4 AEZs (2000)

AEZ and Locations	Average percent of root colonization	Average no. of spore	Textural class	Moisture %	pH	OM %	Total N%	Pμg/g	K meq/100g
AEZ-28 (Joydebpur)	38.72 ± 4.40	105.54 ± 17.68	Silty clay loam	21.4	6.2	1.05	0.08	11.0	0.20
AEZ-9 (Jamalpur)	43.99 ± 5.58	128.22 ± 24.55	Sandy loam	38.8	6.8	1.50	0.10	10.0	0.17
AEZ-11 (Ishurdi)	44.50 ± 5.31	129.07 ± 25.04	Silt loam	33.5	7.2	1.60	0.11	10.0	0.25
AEZ-23 (Hathazari)	40.80 ± 6.75	167.05 ± 40.59	Sandy clay loam	34.7	7.0	1.75	0.11	16.0	0.12

It was, however, not clear why AM colonization and sporulation were favoured by the root habitats of those plant species and what environmental or host factors influenced their dominance. The diversity of root infection recorded among the locations and crop species might be due to a variety of causes including infective propagules quantity, host plant physiology, host plant specificity, soil aeration, soil moisture and the soil's physico-chemical properties in that particular field (Hamel, 1996).

Correlation between edaphic factors and per-cent of root colonization including spore number, are shown in Tables 3 and 4. The 4 selected AEZs have different climatic conditions. Total annual rainfall, humidity and temperature during the study period were recorded. In general, with few exceptions, heavy rainfall was recorded in May to August in both years in the 4 AEZs. Occasionally the rainfall was heavy during the months of April, September and October. No or little rainfall was recorded in the months of January to April and September to December. October to March prevailed as a cool dry winter season with low humidity. Temperature was comparatively low and rainfall hardly occurred during this period.

A negative and insignificant correlation was observed between the percent of root colonization and spore number in 1999 (Figure 1) but it was positively correlated in 2000 (Figure 2). Soil moisture, pH and nutrient levels influenced root colonization and spore density. In 1999, soil moisture, OM, total N and soil potassium were insignificantly and positively correlated to root colonization (Figure 3). In 2000, soil moisture, soil pH, OM, total N and potassium were insignificantly and positively correlated to root colonization (Figure 4). A negative correlation of root colonization was observed only with soil phosphorus in both years (Figures 3 and 4). A positive correlation existed between spore numbers and soil moisture, soil pH, OM, total N and phosphorus, which were statistically insignificant in both years (Figures 5 and 6).

Table 3. Correlation studies of AM colonization, spore population and physico-chemical properties of soil at 4 AEZs (1999)

Variables	Colonization (%)	Spore number	Moisture (%)	pH	OM %	Total N	P	K
Colonization (%)		-0.349^{ns}	0.434^{ns}	-0.334^{ns}	0.264^{ns}	0.699^{ns}	-0.907^{ns}	0.180^{ns}
Spore number			0.653^{ns}	0.766^{ns}	0.740^{ns}	0.388^{ns}	0.519^{ns}	-0.894^{ns}

Table 4. Correlation studies of AM colonization, spore population and physico-chemical properties of soil at 4 AEZs (2000)

Variables	Colonization (%)	Spore number	Moisture (%)	pH	OM %	Total N	P	K
Colonization (%)		0.143^{ns}	0.795^{ns}	0.740^{ns}	0.577^{ns}	0.678^{ns}	-0.575^{ns}	0.362^{ns}
Spore number			0.602^{ns}	0.625^{ns}	0.886^{ns}	0.786^{ns}	0.700^{ns}	-0.674^{ns}

ns = Not significant
* = significant at 5% level.

In this study, both positive and negative correlation between AM colonization and spore population were observed. This relationship was found to be positive by Blaszkowski (1994) but negative by others (Louis and Lim, 1987). Some researchers have also found no relationship to exist between mycorrhizal colonization and spore density (Diaz and Honrubia, 1994). Hetrick and Bloom (1986) reported that AM fungal spore number in natural ecosystems is not necessarily related to the abundance of AM colonization.

Soil moisture, pH and available nutrients (N and P) had varied influence on AM fungal colonization and spore number. Generally, AM fungi are sensitive to soil moisture and the optimum moisture for plant growth is suitable for AM colonization and sporulation (Redhead, 1975). In addition, the survival of plants in natural ecosystems depends upon the ability of plants to take up water under fluctuating soil moisture conditions (Sala and Lauenroth, 1982). The role of AM fungi in enhancing the water uptake by plants under these fluctuating soil moisture conditions is well-documented (Sylvia *et al*., 1993). However, information on the effects of soil moisture on mycorrhization and spore abundance is very limited. In the present study, soil moisture and AM fungal colonization were positively correlated, which is in agreement with Allen and Allen (1984).

Spore populations were positively correlated with soil moisture in both years. A similar relationship between soil moisture and spore abundance was reported by Dickman *et al*. (1984). Soil pH had a negative influence on root colonization in 1999 but positive influence in 2000. This is not in conformity with the earlier report (Abbott and Robson, 1991). Variation in soil pH may affect the development and functioning of arbuscular mycorrhizae (Hayman and Tavares, 1985) by altering the concentration of many nutrients and toxic ions in a soil solution as well as hydrogen ions. Conversely, spore number was positively influenced by soil pH in both years. The response of AM fungi to soil pH may depend on the species and strains constituting the indigenous AM flora (Robson and Abbott, 1989). The variation could also be attributed to the host's mediated changes in rhizosphere pH. The nitrate reduction process of the mycorrhizal host changes the pH of the root exudate, which in turn alters the rhizosphere pH, affecting pH sensitive microorganisms including AM fungi (Smith and Gianinazzi-Pearson, 1988). Both root colonization and spore number were positively correlated with organic matter in both years. Boddington and Dodd (2000) observed the development of indigenous AM fungi by adding organic matter.

Nitrogen plays an important role in influencing the mycorrhizal formation and function mainly through changes in soil pH. Soil N was positively correlated with root colonization and spore numbers, which corroborates with the findings of Aziz and Habte (1989) who reported the stimulation of root colonization by soil N. However, the effect of N on AM fungal spore abundance is related to other soil factors and to the host with which they are associated. There are reports that N can either stimulate or suppress root colonization and spore production through modifications of soil pH (Sylvia and Neal, 1990).

Soil P in the present study was negatively correlated with root colonization in both years. Previous studies showed a negative association between the amount of extractable soil phosphate and the abundance of AM colonization (Morita and Konishi, 1989). Soil P was positively correlated with spore density in our present observations, which is in agreement with Bhardwaj *et al*. (1997). They reported that the number of AM spores was highly correlated with total soil P. However, it is well established that higher soil P can reduce AM formation and the inhibition may be due to a direct effect on the exter-

nal hyphal growth or be indirectly associated with host P status (Sander, 1975). Roy *et al*. (1997) did not find any correlation between P content of the soil and the density of AM fungi in the rhizosphere soil of Kodo (*Paspalum scrobiculatum*) in Southern Bihar, India. However, the effect of P on spore density varied with host species and P status in the soil. The low soil P in the study soils and the characteristics of host species might explain the cause of such a positive correlation between soil P and spore density in the present study. Variations in the response of root colonization and spore number to soil P could be attributed to a number of factors such as:

a) arbuscular mycorrhizal fungi species colonizing the roots, since species and strains vary in their sensitivity to phosphorus (Trouvelot *et al*., 1987).
b) the varied host root growth response to changing P levels (Smith, 1982) or
c) changes in the cell membrane permeability to varying cellular P concentrations, which affect the degree of AM colonization and sporulation (Daniels Hetrick, 1984).

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