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Vesicular arbuscular mycorrhiza in subtropical aquatic and marshy plant communities

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Abstract. Occurrence of vesicular arbuscular mycorrhiza in five subtropical ponds, i.e., eutrophic (*P*1, *P*2 and *P*3), running water (*P*4), oligotrophic lake (*P*5) and marshy plant community (*M*) was studied. It was observed that the plants growing in *P*1, *P*5 and *M* habitats exhibited the vesicular arbuscular mycorrhizal association, whereas the fungal association was lacking in plants of *P*2, *P*3 and *P*4 ponds. The endogonaceous spore population was estimated from water and sediments of the different ponds and it was found that endophytes in sediments are less in terrestrial habitats and completely absent from water samples. The bioassay studies revealed that plants without mycorrhizal association grew poorly and all the endophytes isolated could establish vesicular arbuscular mycorrhizal associations in pot cultures.

Keywords. Vesicular arbuscular mycorrhiza ; subtropical aquatic community ; endophytes ; bioassay.

1. Introduction

Vesicular arbuscular mycorrhiza (VAM) is universal in occurrence (Nicolson 1967; Mosse 1973) and is useful to the host plant in various ways ; in enhancing the uptake of nutrients (Hayman 1975) and water (Safir *et al* 1972), in resisting against pathogen (Marx 1975) and in increasing the effective absorption surface of roots (Hayman and Mosse 1971). Most of the rushes and sedges, however, are reported to be non-mycorrhizal (Powell 1974 ; Khan 1974 ; Harley 1969 ; Gerdemann 1975). Recently, a few temperate (Sondergåard and Laegåard 1977) and tropical (Bagyaraj *et al* 1979) aquatic species were reported to be mycorrhizal. In the present study five aquatic and one marshy sub-tropical plant community were examined for occurrence of VAM and endophytic fungi. To test the ability and efficiency of these endophytes a bioassay was also developed to test its potential use in propagation of VAM in successiona communities of aquatic systems.

2. Materials and methods

2.1. *Site selection*

Five fresh water bodies and one marshy habitat in Shillong (altitude 1450 m, latitude 25·34° N and longitude 91·56° E) were selected for the present study.

The ponds are designated as *P*1, *P*2, *P*3, *P*4, *P*5 and *M* (marshy habitat). *P*1, *P*2 and *P*3 are eutrophic ponds, *P*4 is a running stream and *P*5 is an oligotrophic lake. *P*1 remains dry in summer and receives water during the rainy season.

2.2. *Collection of root samples and assessment of VAM*

Intact plants with roots from different localities were collected in containers. The roots were washed with tap water and cut into segments of approximately 1 cm in length (100 segments from five plants). Further, the root segments were processed and stained for VAM infection by the Phillips and Hayman (1970) technique. Percentage of root infection was calculated in the presence of either vesicle, arbuscules or both by counting the infected segments by the slide method (Mishra *et al* 1981).

2.3. *Estimation of endogonaceous spores*

Fifty ml of water and 30 g of sediment were collected by water sampler from each site in five replicates. The sediment was wet sieved and decanted (Gerdemann and Nicolson 1963) and water was filtered through Whatman No. 1. filter paper. The spores retained on the sieves and filter paper were examined under a binocular microscope.

2.4. *Bioassay study for infection efficiency of endogonaceous spores in pot cultures*

Sterilized maize seeds were germinated in sterilized moist chambers. Five seedlings (2 cm radicle stage) were transplanted to pots (11 × 10″) containing sterilized soil (soil + sand in equal amount w/w). The plants were inoculated with endophytes isolated from the sediments ; uninoculated pots received soil with microflora but were devoid of mycorrhizal propagules. All the pots were regularly watered. Plants were harvested 15, 30 and 45 days after transplanting. Root infection, shoot height, dry weight and leaf production were recorded at each harvesting.

2.5. *Physico-chemical analysis of water and soil*

pH of soil and water was measured by electronic digital pH meter. Organic carbon (Walkey method), nitrate (phenol disulphonic method) and phosphorus (Bray's method) were estimated as outlined by Jackson (1967).

3. Results

Five subtropical aquatic species, *viz. Rotala rotundifolia, Paspalum dilatatum, Polygonum hydropiper, Nymphaea alba* and *Hydrilla verticellata* have been observed for the first time as mycorrhizal (table 1, figures 1-4). Vesicular arbuscular mycorrhiza (VAM) was observed in *P*1, *P*5 and *M* plant communities. Plants growing in *P*2, *P*3 and *P*4 ponds were devoid of VAM infection. Percentage infection was highest in *P*5 plants followed by *P*1 and was least in plants from the marshy (*M*) habitat (table 2). Vesicles and hyphae were regularly observed. The population of endogonaceous spores in general was low in all the sediments from different sites and the number did not differ significantly (table 2). No

Table 1. Percentage occurrence of vesicular arbuscular mycorrhiza in roots of different plant species of sub-tropical aquatic and marshy communities.

Aquatic community	Plant species	VAM (%)	Marshy community	Plant species	VAM % (root)
P_1	*Rotala rotundifolia*	10·00		*Impatiens chinensis*	100·00
	Paspalum dilatatum	60·00		*Drosera* sp.	64·00
	Polygonum hydropiper	56·00		*Utricularia* sp.	73·00
	Cyperus distans	0·00		*Sonchus* sp.	52·00
				Polygonum capitatum	51·00
P_2	*Cardamine hirsuta*	0·00		*Drymaria cordata*	33·00
	C. macrophylla	0·00		*Plantago major*	19·00
	Scirpus articulatus	0·00		*Nasturtium indica*	0·00
	S. juncoides	0·00		*Anemone rivalaris*	0·00
	Eleocharis congesta	0·00		*Steudnera colocasioides*	0·00
P_3	*Rotala rotundifolia*	0·00		*Oenothera javinaca*	0·00
	Cyperus distans	0·00		*Brassica juncea*	12·00
	Eleocharis congesta	0·00		*Rumex nepalensis*	28·00
	Spargonium ramosum	0·00		*Galium rotundifolium*	16·00
				Panicum brevifolium	46·00
P_4	*Hydrilla verticillata*	0·00			
	Alternanthera philoxeroides	0·00			
	Monochoria hastata	0·00			
	Hydrocotyle sibthorpioides	0·00			
	Lasia spinosa	0·00			
P_5	*Rotala rotundifolia*	3·00			
	Hydrilla verticillata	16·00			
	Nymphaea alba	12·00			

Table 2. Endogonaceous spore population and frequency of VAM mycorrhizal plants in different localities.

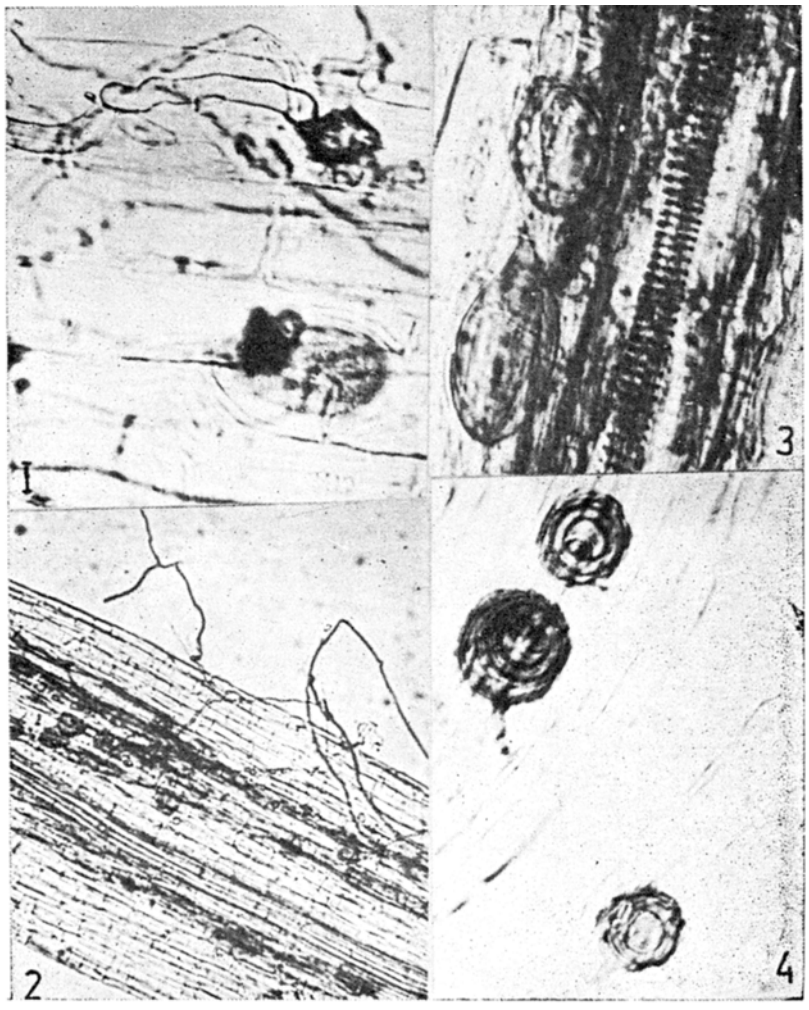
Sites	Mean spore population/30 g/ml soil/water		Frequency (%) of mycorrhiza
	Water (per 30 ml)	Soil (per 30 g)	
P_1	0	27	75
P_2	0	33	0
P_3	0	6	0
P_4	0	7	0
P_5	0	11	100
M_6	0	34	71·3

endogonaceous spores were found in water. The pot culture studies on infection efficiency of endogonaceous spores from different sites revealed that they may infect and establish in roots growing in soil and that their efficiency may differ (table 3). Uninoculated seedlings did not grow well, whereas the seedlings with mycorrhizal association grew better.

Soil and water from all the sites were acidic in nature. Organic carbon was very low in water samples and highest in soil of marshy land. Nitrate and phosphate were highest in *P*2 and *P*1 and lowest in *P*5 and *M* sites (table 4).

4. Discussion

The study reveals that VAM occur rarely in aquatic subtropical plant communities, but they are not completely absent. The results are, therefore, contrary to the views of Harley (1969), Gerdemann (1975), Powell (1974) and Khan (1974), but support the findings of Sondergåard and Laegåard (1977) and Bagyaraj *et al* (1979). Apart from environmental factors like light, temperature and aeration, the occurrence of VAM and its intensity may be regulated by the nutrient status of the aquatic system. Gerdemann (1968) observed that mycorrhiza may be low in rich soil. The amount of infection in a few species, *i.e.*, *Rotala rotundifolia* and *Hydrilla verticellata* differed insignificantly in different water systems depending on the nutrient status and other physical factors of these systems. In general, *P*1, *P*5 and *M* sites favoured VAM establishment but *P*2, *P*3 and *P*4 ponds did not possess any mycorrhizal association. It seems that temporary drying of *P*1 and *M* habitats in summer may initiate the VAM establishment. A similar trend was also observed in other environmental conditions (Read *et al* 1976). The high percentage occurrence of VAM in *P*5 may be attributed to its oligotrophic nature (Sondergåard and Laegåard 1977), as *Hydrilla verticellata* was mycorrhizal in *P*5 community but not in *P*4. Besides the root system, the shoot also helps the aquatic plants in absorbing the nutrients from water and this may be one of the reasons for the absence or less frequent mycorrhizal association in certain plants. Sutcliffe (1962) suggested that in aquatic plants, the roots primarily act as anchors ; on the other hand, Denny (1972) concluded that the nutrients may enter through roots and shoots. Therefore, it seems that phosphorus uptake may depend on the efficiency of the root system, *i.e.*, some root systems may be adaptive enough to draw the phosphate at low level even in the absence of VAM association (Powell 1975). VAM may not be of much importance in plants growing in rich medium (*P*2, *P*3 and *P*4 system) but it may help the hosts in absorption of the nutrients growing in low level nutrient systems (*P*5). Therefore, two conditions, *i.e.*, temporary drying of the aquatic system and the oligotrophic nature seem to be more favourable for VAM development. The presence of endophyte spores in all aquatic systems suggests that these spores probably enter into the water system through run off from terrestrial ecosystem, their subsequent development, however, is governed by water regime, light, aeration and other factors (Mosse 1973). The presence of endophyte spore in *P*2 community may further indicate that either these species are not capable of causing infection or plants may not be susceptible in such systems (Reeves *et al* 1979 ; Miller 1979). The bio-



Figures 1–4. 1. Spore, *Glomus* sp. infecting root tissue of *Panicum brevifolium* (× 1,000), 2. Vesicles and hyphae in root tissue of *Impatiens balsamia* (× 100), 3. Obovate vesicles of *Glomus* sp. in the root tissue of *Rotala rotundifolia* (× 400), 4. Round vesicles with oil globule and degenerating stage of hypha in root tissue of *Nymphaea alba* (× 400).

Table 3. Development and effect of VAM in maize inoculated with endophytes from different habitats.

Endophyte inoculation from different sites	15 days				30 days				45 days			
	Leaf No.	Stem height (cm)	Shoot dry weight (g)	VAM	Leaf No.	Stem height (cm)	Shoot dry weight	VAM	Leaf No.	Stem height (cm)	Shoot dry weight (g)	VAM
P_1	5	5·7	80·0	..	5	16·9	120·0	+	6	20·4	160·0	+
P_2	5	5·0	125·0	..	7	19·5	200·0	+	8	27·5	245·0	+
P_3	4	4·1	65·0	..	5	15·5	80·0	—	5	17·6	92·0	+
P_4	5	5·3	90·0	..	6	17·8	180·0	—	6	22·8	108·0	+
P_5	4	3·9	53·0	..	5	16·2	96·0	—	7	24·4	102·0	+
M_6	5	5·6	130·0	..	7	18·9	210·9	+	8	28·6	265·0	+
Uninoculated	3	3·6	45·0	..	4	12·0	60·0	—	4	14·2	70·1	—

Table 4. Physico-chemical characters of soil and water in different water bodies.

Site	pH		Org. Carbon (%)		Nitrate (ppm)		Phosphate (ppm)	
	water	soil	water	soil	soil	water	water	soil
P_1	6·5	8·3	0·05	4·9	2·2	0·20	3·6	6·62
P_2	6·4	6·3	0·07	7·6	1·6	0·17	3·8	7·8
P_3	6·0	6·3	0·05	5·3	1·6	0·07	1·07	3·18
P_4	6·6	6·5	0·06	5·6	1·8	0·09	0·1	2·01
P_5	6·1	6·3	0·04	3·9	1·4	0·05	0·01	1·96
M	..	6·2	0·00	8·0	0·1	..	..	1·42

assay studies using maize (*Zea mays*) as test plant suggested that these endophytes may develop VAM and enhance the growth of plants, when placed into sterilized soil. It is of great ecological importance to study the establishment, germination and entrance of these endophytes into the hosts under diverse environmental conditions, in understanding of the developmental pattern of aquatic and terrestrial communities. Baylis (1959) suggested that VAM play a significant role in the evolution of plant communities. Therefore, studies in controlled conditions in aquatic habitats on the esta lishment of VAM will provide information towards further understanding of succession in aquatic systems and would aid management of aquacultural systems.

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