

THE OCCURRENCE OF VESICULAR—ARBUSCULAR MYCORRHIZA IN ROOTS OF SOME LEPTOSPORANGIATE FERNS AND ITS EFFECT ON NUTRIENT UPTAKE

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The occurrence and intensity of VA-mycorrhizal association in several species of leptosporangiate ferns growing on three different substrata have been investigated and the role of such association in uptake of phosphorus from substrata has also been studied. Out of ten species of ferns studied *Pteris vittata*, *Ampelopteris prolifera* and *Nephrolepis cordifolia* growing in the field showed the presence of mycorrhizae in their root systems. Species growing on brick walls or tree branches failed to develop mycorrhizal association. The relative abundance of endophytic structures formed, their shape and size varied in the three mycorrhizal fern species. The members of these three species growing in field soil absorbed more phosphorus from soil as compared to the nonmycorrhizal members of these species growing on brick walls.

INTRODUCTION

Vesicular-arbuscular mycorrhiza (VAM) or endotrophic mycorrhiza is of common occurrence in angiosperm roots, but they have also been reported to occur in bryophytes, pteridophytes and gymnosperms (Gerdemann, 1968). The recent stress in studies on VAM is probably due to reports made by several authors that this mycorrhizal association can improve the growth of plants, specially in soil of low fertility (Sanders and Tinker, 1971 ; Bowen *et al.*, 1975). Mosse (1973) reported that this improved growth is caused by an increased uptake of phosphate from soil mediated through the fungal partner which has been supported by several authors (Gray and Gerdemann, 1969 ; Sanders and Tinker, 1973 ; Hatting *et al.*, 1973 ; Cox and Tinker, 1976).

VA-mycorrhizal association in roots of ferns have been reported from different countries of the world. Boullard (1957) in France, Hepden (1960) in U. K., Cooper (1975) in New Zealand, Iqbal *et al.* (1981) in Pakistan and Berch and Kendrik (1982) in Canada made extensive surveys of ferns in their countries for the occurrence of VA-mycorrhizae in their roots. Some of them identified the VA fungi forming the mycorrhizal association and studied the development of root-fungus association in artificially inoculated plants.

In India, though some amount of work has been done on VAM in angiosperms (Bagyaraj *et al.*, 1979 ; Jalali, 1979), ferns have received little attention with

respect to this type of fungus-root symbiotic association. Therefore the present investigation was undertaken to conduct a survey on ferns growing naturally at Kalyani (West Bengal) for the occurrence and intensity of VA-mycorrhizae in them. Besides the survey work, the present paper deals with some preliminary work on the effect of this mycorrhizal association on the uptake of phosphorus and nitrogen from soil.

MATERIAL AND METHODS

Entire plants of ferns growing naturally in four different habitats i. e. on open fields, old walls of abandoned buildings, tree branches and in pots kept in verandah were collected just after the rainy season in September when plants were in mature condition. Plants were collected carefully with adjacent soil or substrata to avoid injury to roots. Roots were then rinsed with water to remove adhering soil particles, and fixed in formalin-acetic acid-alcohol. They were processed for microscopic observation following the method of Phillips and Hayman (1970). Fixed root pieces were washed and then cleared by boiling in 10% KOH solution, after which they were treated in an alkaline solution of H_2O_2 (pH 8–8.5) until bleached. They were washed, acidified with 0.5 N HCL, cut into 1 cm pieces and stained with 0.05% trypan blue in lactophenol. After washing them in lactophenol to remove excess stain, the root pieces were placed on glass slides and examined microscopically at X 100 for the presence and frequency of vesicles (V) or arbuscles (A) or both (VA) which are the diagnostic structures of Vesicular-arbuscular fungi. The presence of endotrophic mycelium inside roots was also noted.

Mycorrhizal infection was estimated by observing root pieces (100 segments taken at random from 3 plants of each habitat) for the presence of mycorrhizal structures (V or A or VA) and the percentage of root infection was calculated. The phosphorus content of plant materials was estimated colorimetrically following the method of Fiske and Subbarow (1925) after drying the fronds in oven and subsequently digesting a known quantity of the dried tissue. Oven dried plant material was ashed at 450°C for 6 hours. 100 mg of ash was taken in a long neck flask to which were added 2 ml of conc. HNO_3 and 0.5 ml of conc. H_2SO_4 . The flask was placed on a hot plate and the contents were boiled until white fumes appeared. The flask was cooled, 10 ml of glass distilled water was added to its contents and the solution was boiled for another 30 minutes. The solution was filtered and the filtrate was made upto a volume of 50 ml with water and this served as the stock solution for phosphorus estimation.

Nitrogen content in plant tissue was estimated using Kjeldahl's method.

Available phosphorus of substrata (soil and wall material) on which plants were growing was determined colorimetrically by phosphomolybdate method follow-

ing Jacobs *et al.* (1971). Dry matter was estimated by drying freshly collected fronds in oven at 80°C for two days before weighing.

RESULTS AND DISCUSSION

Among the ten species of ferns studied, which were growing under four different habitats (open field, old wall, tree branch and earthenware pots) VA-mycorrhizae were observed only in three species growing in the field, namely *Pteris vittata*, *Ampelopteris proliferata* and *Nephrolepis cordifolia*.

Results on the incidence and frequency of VA-mycorrhizae in species of ferns growing in four habitats have been presented in Table 1. It is evident from the table that out of 10 species of ferns studied the three species growing in the field only showed mycorrhizal association. The fact that the mycorrhizal association developed only in those growing in the field, indicates that

TABLE 1. Occurrence and the extent of mycorrhizal infection in ferns growing in different habitats

Fern species	Habitats			
	Field	Wall	Tree branch	Pot
	Figures in parenthesis indicate % infection in mycorrhizal species			
<i>Pteris vittata</i>	M(50)	NM(0)	—	—
<i>Adiantum cordatum</i>	—	NM(0)	—	—
<i>Adiantum lunulatum</i>	—	NM(0)	—	—
<i>Ampelopteris proliferata</i>	M(100)	NM(0)	—	—
<i>Christella dentata</i>	—	NM(0)	—	—
<i>Nephrolepis cordifolia</i>	(M100)	NM(0)	—	—
<i>Drynaria quercifolia</i>	—	—	NM(0)	—
<i>Pyrrosia lanceolata</i>	—	—	NM(0)	—
<i>Nephrolepis species</i>	—	—	—	NM(0)
<i>Atherium species</i>	—	—	—	NM(0)

M = Mycorrhizal

NM = Non-mycorrhizal

— = Plants were not found growing in these habitats

mycorrhizal fungi are normally present in soil wherefrom they invade the root system. Limited dispersal of VA-fungi may occur with windblown soil particles (Hayman, 1982) containing propagules of VA-fungi but in case of these fern species, root infection by these windblown propagules did not occur since roots were always found to be non-mycorrhizal. However the efficiency of the VA-fungi in forming the association in the three mycorrhizal species varied; in

two species infection was 100% and in one it was 50%. The two species growing in pots were also non-mycorrhizal. This can be attributed to several factors such as regular watering, low light intensity (since pots were kept in shade) which adversely affect the development of VA-mycorrhizae. Other factors which may also be involved in unresponsiveness to infection, are unknown host factor or some adverse soil condition which did not support good growth of the VA-fungi in pots.

The type of endophytic structures, their shape and size that were formed in mycorrhizal species have been presented in Table 2. In *Pteris vittata* only vesicles were formed and arbuscules were totally absent. But in *A. prolifera* and *N. cordifolia* both vesicles and arbuscules were formed. It was further observed that species which contained both vesicles and arbuscules in their tissues, differed

TABLE 2. Types of endophyte structures, their relative abundance and dimensions of vesicles in mycorrhizal fern species

Name of the plant	Occurrence of Vesicles (No/mm root length)	Occurrence of Arbuscules	Shape of Vesicles	Dimension of Vesicles
<i>Pteris vittata</i>	20	None	Spherical	22.5 μ in diameter
<i>Ampelopteris prolifera</i>	4	35*%	Spherical, ovate to elliptical	25 μ 28-30 μ X 45-47 μ
<i>Nephrolepis cordifolia</i>	10	30*%	Spherical, ovate to elliptical	24 μ 39-41 μ X 58-61 μ

*Average percentage of cells containing arbuscules counted from 3 microscopic fields (X 200) per root segment.

also with respect to the relative abundance of these two diagnostic structures. For example in *A. prolifera* nearly 4 vesicles were present per 1 mm root length while 35% of cells contained arbuscules in them. In *N. cordifolia* the corresponding figures were 10 and 30%. The shape and dimension of vesicles formed in different species also showed variation.

The three fern species growing in the field and having distinct VA-mycorrhizal association contained nearly two fold amount of phosphorus as compared to the amount present in non-mycorrhizal plants of the same species. Mycorrhizal

plants also showed some increase in growth response, though not remarkable as compared to their non-mycorrhizal counterparts. Results on these aspects have been presented in Table 3. Data on nitrogen content of plant tissue indicate that VA-mycorrhiza in ferns under study did not help the plants to absorb more nitrogen from soil and accumulate in fronds, although such increase in nitrogen content of shoots and seeds of certain mycorrhizal angiosperms have been reported (Rose, 1971 ; Schenck and Hinson, 1973).

TABLE 3. Nitrogen and phosphorus content and dry weight of fern fronds in mycorrhizal fern in species growing in two different habitats

Species	Phosphate content in habitats (ppm)		Phosphorus content in fronds (% dry wt.)		Nitrogen content in fronds (% dry wt.)		Frond dry wt./plant (g)	
	Soil	Wall	Soil	Wall	Soil	Wall	Soil	Wall
<i>Pteris vittata</i>	4.5	4.0	0.90	0.45	1.178	1.176	1.5	1.4
<i>Ampelopteris prolifera</i>	4.4	4.2	0.85	0.41	1.23	1.34	2.1	1.9
<i>Nephrolepis cordifolia</i>	4.3	4.2	0.92	0.43	1.18	1.17	1.7	1.5

Thus the data obtained in the present investigation support the findings of the previous authors who established that endomycorrhizal association in plants causes enhanced phosphate uptake. Schoknecht and Hatting (1976) observed cells having arbuscles in them contained more phosphorus than cells without them. Cox and Tinker (1976) also showed greater uptake of phosphorus by VA-fungi from soil and its translocation to host roots. Sahni (1976) too demonstrated positive correlation between mycorrhizal infection and the amount of phosphorus and nitrogen present in tissues.

In the present studies, although phosphorus content in tissues of mycorrhizal ferns was more compared to non-mycorrhizal plants of the same species, this was not so for the amount of nitrogen present. Results presented in the paper support Hayman (1982) who expressed the opinion that VA-mycorrhiza do not appear to increase the uptake of nitrogen and further, VA-fungi cannot fix nitrogen. Mosse (1973) also expressed the opinion that differences in nitrogen concentration of mycorrhizal and non-mycorrhizal plants were inconsistent in many studies and were subject to criticism and therefore the role of VAM in increased uptake of nitrogen is questionable. From the data on quantitative estimation of available phosphate near the root zone of ferns growing on soil and wall, it is evident that the amounts in the two different habitats were nearly same. However, since the plants on wall did not establish the mycorrhizal association in their roots, they showed lesser amount of phosphorus in their

tissues. In conclusion it may be said that some ferns at least, growing in the field in nature get infected with indigenous VA-fungi, develop the mycorrhizal association in their roots, absorb and accumulate more phosphate from soil, than those plants which fail to develop such an association.

It has been planned to follow the course of establishment of VA-fungi in host roots by artificially growing fern sporophytes in aseptic conditions and thereafter to investigate whether they help the plants in absorbing more phosphate and some other elements from soil.

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