MYCORRHIZA IN OPHIOGLOSSACEAE : I. MORPHOLOGY OF ENDOPHYTES *IN VIVO*

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ABSTRACT

Morphology of the endophytes *in vivo* was studied in *Ophioglossum reticulatum* L., *O. pedunculosum* Prantl *sensu non-* Desv., *O. vulgatum* L., *O. fibrosum* Schum., *O. aitchisoni* d'Alm., *O. nudicaule* L., *O. gramineum* Willd., *Botrychium virginianum* var. *daucifolium* Bedd. and *Helminthostachys zeylanica* L. It was observed that in the material of *Helminthostachys zeylanica* collected, no endophytes were present. In all other plants they were present. The mode of entry of fungus and three stages of endophytes that developed viz., mycelial, vesicular and arbuscular were found in succession in all of them. The paper discusses the mode of living of mycorrhiza in the host plant.

INTRODUCTION

The Eusporangiatae comprises two families Ophioglossaceae and Marattiaceae. Of these the family Ophioglossaceae consists of three genera: *Ophioglossum* which is cosmopolitan having about 30 species, *Botrychium* with about 12 species mainly in temperate regions, and *Helminthostachys* which is a tropical monotype. All of them are known to have mycorrhiza in their roots and prothalli.

Researches on the Ophioglossaceae date as far back as ROEPER (1826) and PRESL (1845). They have been reviewed by BOWER (1904) and CAMPBELL (1908). Important later authors who have worked on the family are FARMER AND FREEMAN (1899), CAMPBELL (1895, 1907, 1908, 1911, 1921, 1922), JEFFREY (1896-1897), LANG (1902, 1904), BRUCHMANN (1904, 1906), BOWER (1904), MAHESHWARI AND SINGH (1934), MAHABALE (1934, 1935, 1962), CLAUSSEN (1938), RAO (1939), NOZU (1954, 1955, 1956, 1958, 1961), NISHIDA (1956), HARLEY (1959), HEPDEN (1960), PANT AND KHARE (1972). All these authors mention endophytic fungus in the roots and or the prothalli in the members of Ophioglossaceae. But none of them had attempted to isolate the fungus either from the roots or from the prothalli except MAHABALE (1935) who had isolated a species of *Fusarium* from the roots of *Ophioglossum aitchisoni*. The present paper is a continuation of that work.

MATERIAL AND METHOD

Ophioglossum reticulatum was collected from Purandhar and Katraj in Poona District, Mahabaleshwar in Satara District (Maharashtra State), and at Trivandrum in Kerala State. *O. pedunculosum*, *O. itchisoni*, *O. nudicaule*, and *O. gramineum* were collected from the Poona University campus. *O. firbosum* was obtained from Khandala in Poona District and *O. vulgatum* from Darjeeling. The material of *Botrychium virginianum* var. *daucifolium* was collected at Ootacamand and Kodaikanal, and *Helminthostachys zeylanica* at Trivandrum.

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Roots and rhizomes were fixed in Randolph's Modified Navashin fluid and microtomed into 12-15 μ thick sections for the study of endophytes *in vivo*. Free-hand sections were taken and stained with cotton-blue-lactophenol which gave excellent results.

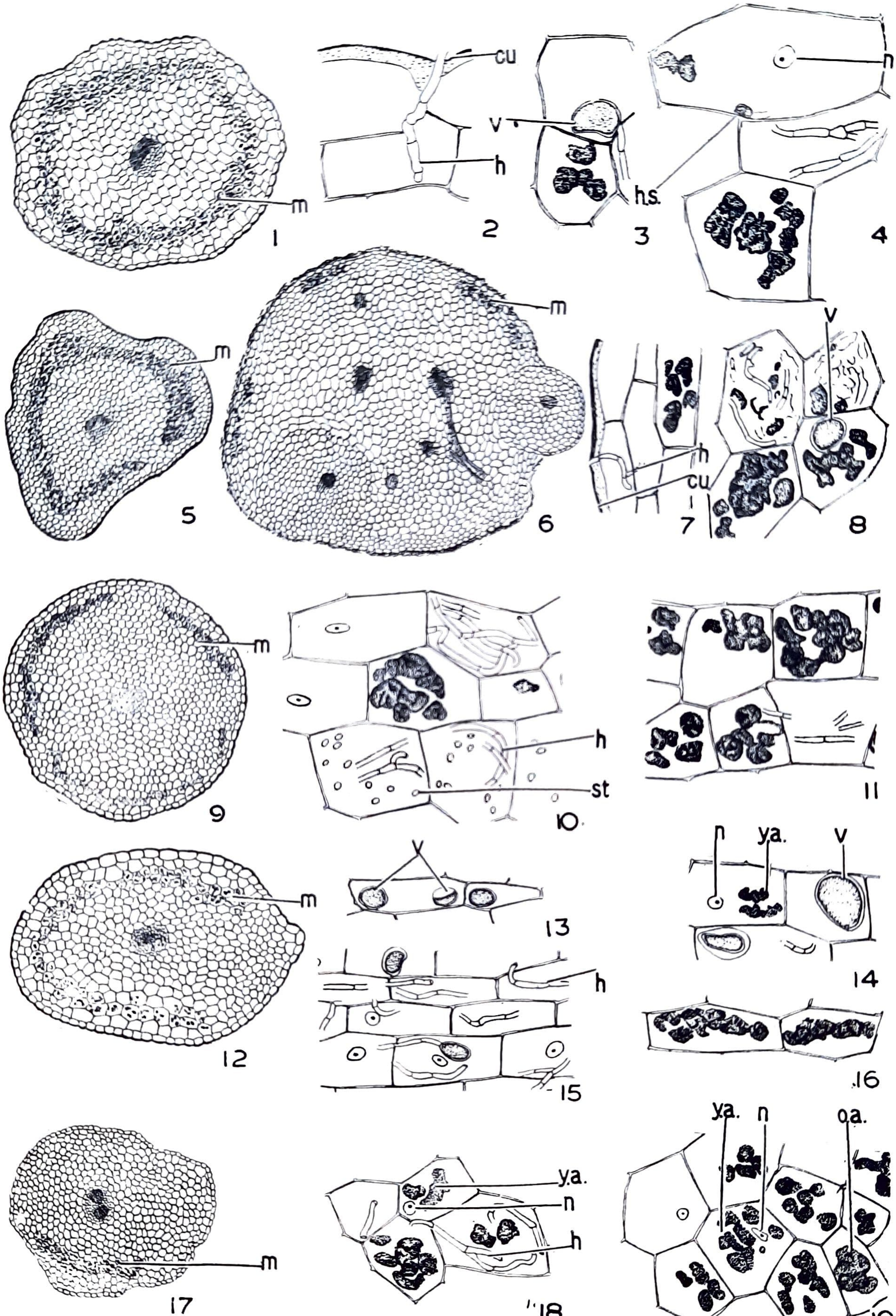
DESCRIPTION

1. Ophioglossum reticulatum L.

Observations were made on plants growing in different localities. Plants growing on sandy soil or on gravel were found to have poorly developed endophyte or they were nearly devoid of it. The plants growing in humus rich soil had abundant endophytes. It was further observed that in every plant all the roots were not necessarily occupied by the endophytic fungus, nor did it extend throughout the entire length of roots. In older roots endophyte was distributed in the cortical region throughout their length except at the root apex. Such roots with endophyte had uneven surface. Half the cortical region of the old roots had abundant fungus towards the proximal end and it was distributed in a definite zone in the roots. (Pl. 1, Figs. 1, 2; Text-fig. 1). The vascular region of the root was free from endophyte. In a T. S. of the root the fungus was found to occupy three or four outer layers of the cortex below epiblema (Text-fig. 1). The young roots, rhizome and aerial parts of the plants lack endophyte.

The entry of fungus was invariably through epiblemal layer. It entered the roots in mycelial stage (Pl. 1, Figs. 3, 4; Text-fig. 2). Hyphae pierce the cuticle of the epiblemal cells and passing through hypodermis spread intracellularly to the next three to four layers below cuticle and establish themselves there. Once fully established inside the root cells, connection of the hyphae in the cells with soil and outer layers, viz. epiblema and hypodermis is cut off and the inside mycelium gets cutinized. Such excised filaments cut off from outside are thick and dark brown. They were generally found in the proximal half of the root. From this it can be inferred that the fungus enters through the proximal half of the root and then moves towards its distal half. The region towards growing point thus appears to be secondarily infected and occupied by the endophyte. The cells of a root before the entry of fungus are packed with starch grains (Pl. 1, Fig. 6). Entry of the fungus and its establishment inside do not cause any abnormality in roots, except that the starch grains in the root cells disappear (Pl. 1, Figs. 7-8). No appressorial swellings were noticed at the point of entry of the fungus. Only a little unevenness of surface seen causes a slight depression in the cuticle as shown in Text-Fig. 2.

After the fungus enters root cells it establishes within its cells. Thus the mycelial stage is the first phase in the life cycle of endophyte (Pl. 1, Figs. 7-8). Once inside a root, mycelium grows actively and forms mycorrhizal zone within the host tissue. The hyphae within host tissue are septate and of uniform diameter (Text-fig. 4). They often contain oil drops. The septate intracellular hyphae pass from one cell to another by dissolving the cell wall at the point of contact (Text-fig. 2). They branch within the cell, and form entangled mass of hyphae in the host cell (Pl. 1, Fig. 7). Even the nucleus of the host cell remains intact, only the reserve food material in the host cell is consumed by it progressively, especially the starch grains. The external hyphae penetrating root from outside are stout and thicker than the ones established in the root tissues. They grow actively. Active phase of the mycelial growth coincides with the active phase of the host plant. They form amorphous masses inside its root cells known as "arbuscules" by Gallaud (1905). Hawker *et al.* (1957) did not observe them in the material they studied.



Text-figs. 1-19. Endophytes in the roots of *Ophioglossum in vivo.* (*m*—mycorrhizal zone, *cu.* cuticle, *h.* hypha, *v.* vesicle, *n.* host cell nucleus, *h.s.* hyphal swelling, *st.* starch grain, *y.a.* young arbuscule, *o.a.* old arbuscule).

Figs. 1—4. *Ophioglossum reticulatum* L. Fig. 1. T.S. of root showing mycorrhizal zone—m. ×13 Fig. 2. A hypha—h. piercing cuticle—cu. ×117. Fig. 3. Vesicle—v. ×117. Fig. 4. Hyphal swelling—h.s.: note host cell nucleus—n. intact ×117. Figs. 5-8. *Ophioglossum pedunculosum* Prantl. *sensu non*-Desv. Fig. 5. T. S. of root showing mycorrhizal zone—m. ×13. Fig. 6. T.S. of rhizome showing mycorrhiza—m. ×13. Fig. 7. A hypha—h. piercing the cuticle—cu. ×117. Fig. 8. Vesicle—v. and arbuscules ×117. Figs. 9-11.

The hyphae produced swellings within the host tissue in a number of specimens (Text-fig. 4). Such hyphal swellings were either intercalary or terminal and had dense cytoplasm. They were either single or in chains. Such hyphal swellings serve as an additional source for the propagation of fungus.

Second stage in the development of endophyte is "Vesicles". They are formed after the mycelia are firmly established in the host tissue. The hypha first enlarges and then forms vesicle. They have a distinct cell wall enclosing dense cytoplasm. They are spherical or ovoid structures within the host cells. Stages in the vesicle formations are illustrated in Text-figs. 32-35. Very few vesicles were noticeable in this species. All of them were terminal and only one in a cell (Text-fig. 3). To these intracellular swollen structures JANSE (1897) as HAWKER *et al.* (1957) state called "Vesicles". They mark second phase in the life cycle of endophyte. NISHIDA (1956) observed that vesicles discharge their contents inside the cell in which they develop as they grow old. But this was not observed here. The exact role of vesicles is not fully understood. They have been variously interpreted as storage organs by GALLAUD (1905), WEST (1917) and NISHIDA (1956) since they have dense granular contents rich in oil. Others consider them to be parenating or propagative branches. The formation of a germ tube by a vesicle in the cell was observed by us in *B. virginianum*. This suggests possibly that they are propagative branches.

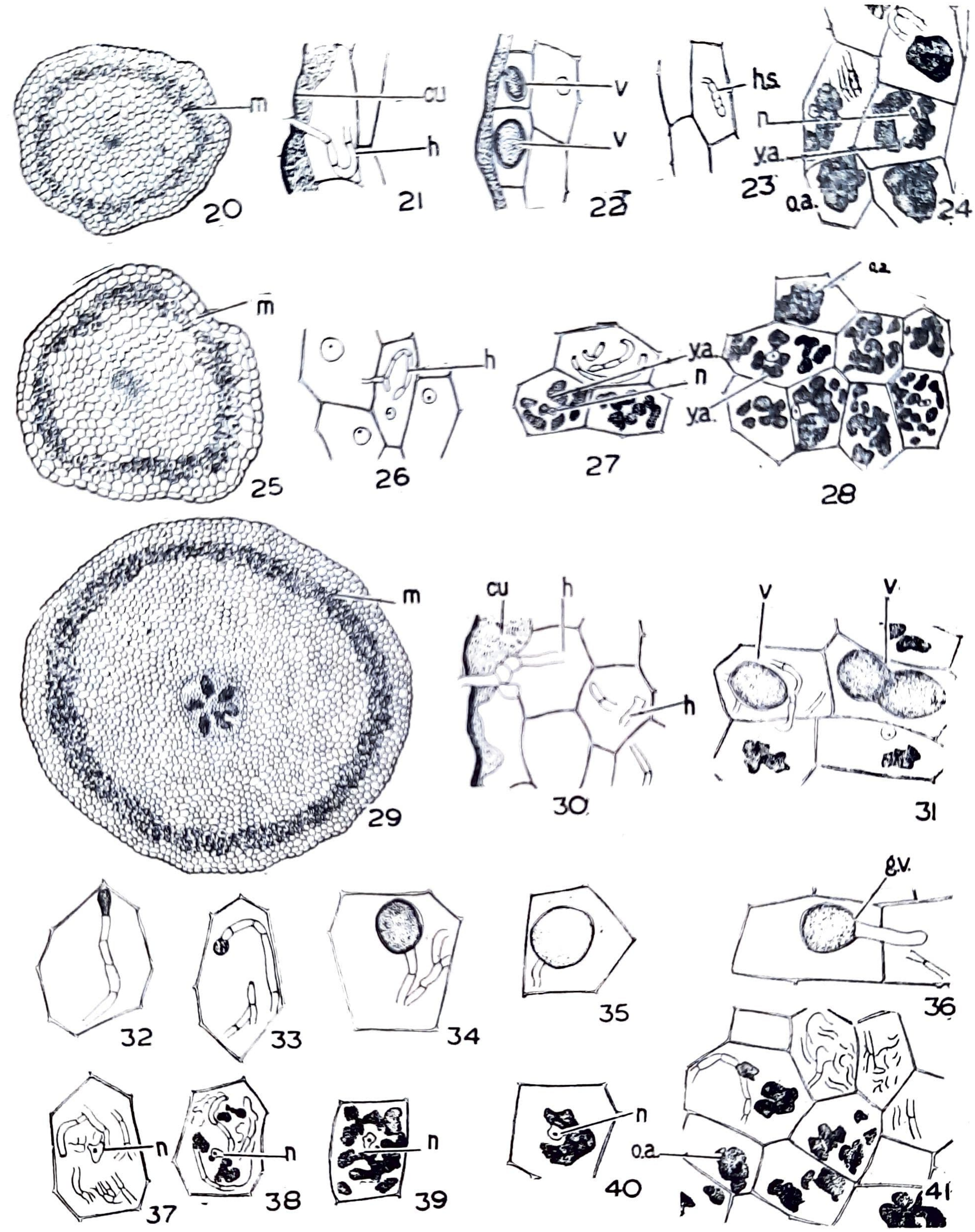
Besides vesicles, some dark irregular bodies are formed by the coalescence of old mycelial filaments in the host tissues and are called "Arbuscules". In the roots of this species there were abundant arbuscules, more than one in a cell (Text-fig. 4). They constitute the older stage of endophyte. They are quite abundant during aestivating period of the plant. They are considered to be the resting stage of the endophytic fungus, coincident with the resting stage of the host plant in dry season. They remain as dark shrivelled up bodies in the host cells throughout the summer. Exact function of the arbuscules is not known. They are not the haustorial bodies as BURGEFF (1938) thinks, because they remain quite amorphous in the host cells.

Old hyphae seem to lose their contents and form amorphous masses. The stages in their formation are shown in Text-figs., 37-40. The arbuscules seem to have been formed by the digestion of some of the mycelia while others remain intact. Cells in which the digestion takes place are called the "digestive cells". In the beginning arbuscules appear as small groups of compressed light coloured bodies in the cells which retain their nucleus. Later many of them unite and form large irregular dark masses. They encircle the host nucleus which disappears later completely (Text-figs. 4 & 40).

2. **O. pedunculosum** Prantl *sensu non*—Desv.

In most of the specimens of this species, collected from Poona University Campus, endophytic fungus was present. Unlike other species the roots of this species had slightly uneven surface suggesting abundance of endophyte. (Text-fig. 5). The endophytic fungus in this species was present in the rhizomes also (Text-fig. 6). The roots and rhizomes

Ophioglossum vulagatum L. Fig. 9. T.S. of root showing mycorrhizal zone—m. ×13. Fig. 10. Septate hyphae —h. : note the few starch grains—st. in the host cell ×117. Fig. 11. Arbuscules ×117. Figs. 12-16. *Ophioglossum fibrosum* Schum. Fig. 12. T.S. of root showing mycorrhizal zone—m. ×13. Fig. 13. Two vesicles —v. in one host cell ×117. Fig. 14. Vesicle—v. and young arbuscules—y.a. : note host cell nucleus—n. ×117. Fig. 15. Septate hyphae—h. and vesicles ×117. Fig. 16. Old arbuscules—o.a. ×117. Figs. 17-19 *Ophioglossum aitchisoni* d'Alm. Fig. 17. T.S. of root showing mycorrhizal zone—m. ×13. Fig. 18. Septate hyphae—h. and young arbuscules—y.*a.* : note that the host cell nucleus is disfiguerd ×117. Fig. 19. Young arbuscules with disfigured host cell nucleus and old arbuscules ×117.



Text-figs. 20-41. Endophytes in species of *Ophioglossum* and *Botrychium virginianum* L. *in vivo*. (*m.* mycorrhizal zone, *cu.* cuticle, *h.* hypba, *v.* vesicle, *h.* host cell nucleus, *h.s.* hyphal swelling, *g.v.* germinating vesicle, *y.a.* young arbuscule, *o.a.* old arbuscule).

Figs. 20-24. *Ophioglossum nudicaule* L. Fig. 20. T.S. of root showing mycorrhizal zone—m. × 13. Fig. 21. A hypha—h. piercing cuticle—cu. × 117. Fig. 22. Vesicles—v. in epiblemal cells × 117. Fig. 23- Hyphal swellings—h.s. × 117. Fig. 2 . Young—y.a. and old arbuscules—0.a. : note disfigured host cell nucleus—n. × 117. Figs. 25-28. *Ophioglossum gramineum* Willd. Fig. 25. T.S. of root showing mycorrhizal zone—m. × 13. Fig. 26. Septate hypha—h. × 117. Fig. 27. Young arbuscules y.a. : note that the host cell nucleus—n. is still present × 117. Fig. 28. Young—y.a. and old arbuscules—o.a. × 117. Figs. 29-41. *Botrychium virginianum* L. × Fig. 29. T.S. of root showing mycorrhizal zone—m. × 13. Fig. 30. Entry of hypha—h. through cuticle—cu. × 117. Fig. 31. Two vesicles—v. in a chain × 117. Figs. 32-35. Stages in vesicle formation × 117. Fig. 36. A vesicle germinating—g.v. × 117. Figs. 37-40. Stages in the formation of arbuscule: note disfigured host cell nucleus—n. × 117. Fig. 41. Old arbuscules—o.a. × 117.

had abundant mycelia and arbuscules (Text-fig. 7). The vesicles were very few (Text-fig. 8).

3. **O. vulgatum** L.

No fresh material of this species was available except the roots preserved in 4% formalin. Mycelial and arbuscular stages were noticed in them, but the vesicles were absent (Text-figs. 9-11).

4. **O. fibrosum** Schum.

A large number of vesicles were observed in the old roots of this species along with mycelial and arbuscular stages (Text-figs. 12-16).

5. **O. atichisoni** d'Alm.

Septate hyphae and arbuscules were abundant in old roots (Text-figs. 17-19). No vesicles were found.

6. **O. nudicaule** L.

There was a distinct zone of mycorrhiza in the cortex (Text-fig. 20). Hyphal and arbuscular stages were present (Text-figs. 21 & 24). Hyphal swellings of the fungus were also noticed (Text-fig. 23), but the vesicles were few (Text-fig. 22).

7. **O. gramineum** Willd.

Only hyphae and arbuscules were observed in this species (Text-figs. 25-28).

8. **Botrychium virginianum** var. **daucifolium** Bedd.

The endophyte showed the mycelial, vesicular and arbuscular stages in abundance (Plate 1, Figs. 9-18; Text-figs. 29-41). In some place a single host cell had two vesicles. One vesicle was found to produce a germ tube in the host cell.

9. **Helminthostachys zeylanica** L.

No endophyte was observed, either in the roots or the rhizome in our material.

The endophyte in *O. pedunculosum* was found to be *Fusarium solani* (Mart.) Appel & Wr. and that in *O. reticulatum* and *B. virginianum* var *daucifolium* Bedd. to be *Fusarium oxysporum* Schlect. The *in vitro* behaviour of these will be discussed in II Part of this work.

DISCUSSION

There exists a definite correlation between the active growth period of the host plant and of the endophyte. The endophyte develops mycelial and vesicular stages in host cells when the plant is actively growing. Plants growing in humus rich soil, .the endophytic fungus is abundant. Such plants are more vigorous and grow actively. This suggests that the presence of endophyte is beneficial to the host plants and helps to maintain nutritive balance.

The endophyte enters the roots from the surrounding soil in mycelial stage. It is one of the soil fungi from the rhizosphere of *Ophioglossum reticulatum*.

Ophioglossum plants begin to grow after the rains and so does the fungus. The mycelial stage is the first stage in the development of fungus. The septate, hyphae formed swellings within host tissue, had sickle-shaped conidia when cultured. Therefore the fungus appeared to belong to the genus *Fusarium*.

The next stage is vesicles formed by the swelling of fungal hyphae inside the host cells. They seem to be storage organs since they have dense cytoplasm and oil globules. GALLAUD (1905) and WEST (1917) also think so. One or more such vesicles are found in the host tissue as reported by BASS-BECKING (1921).

The endophyte is found to be restricted to 3 or 4 hypodermal layers of the host tissue. There is no malformation or hypertrophy of the root, but the fungus utilizes the reserve food material like starch in the cortical cells. This happens when the fungus is in its first or mycelial stage. The cells of the cortex adjacent to the ones having mycorrhiza have their reserve food contents intact. Probably there is a nutritive gradient set up by the mycorrhiza in the cortical cells.

Dark arbuscular bodies formed by the clumping of old hyphal walls are a sort of propagative bodies. They are mainly formed towards the closing period in a season. They are abundant when the plant is undergoing the resting period during summer. Probably they are not haustorial bodies as BURGEFF (1938) thinks, because they remain quite amorphous in the host cells. Moreover they do not produce haustoria. HAWKER *et al.* (1957) observed the formation of highly branched hyphae, the ultimate branches of which were considered to function as haustoria in the cells of grass roots. They were not observed in the present material. According to HAWKER *et al.* (1957) they cannot be called "sporangioles" as described by JANSE (1897) since they do not produce spores or spore masses. We agree with this view and prefer to call them as "arbuscules".

In conclusion, it must be stated that mycorrhiza in the Ophioglossaceae is definitely not a disease. On the contrary it is beneficial to the host plants as they grow more vigrously by the symbiotic action of the endophyte.

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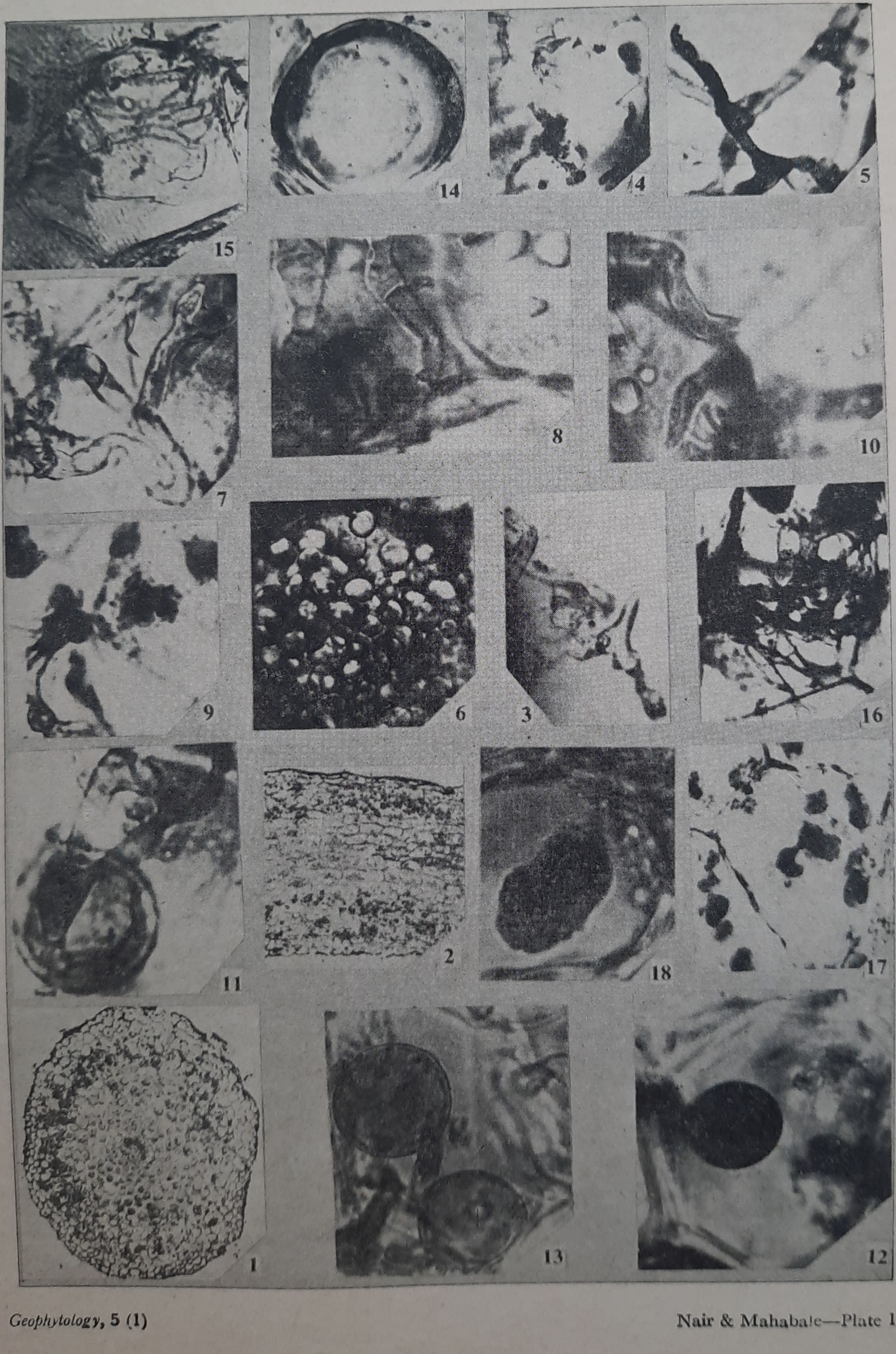
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EXPLANATION OF PLATE 1

Fig. 1. *Ophioglossum reticulatum* L. Root T.S. showing distribution of endophyte × 25. Fig. 2. L.S. of root showing mycorrhizal zone × 20. Fig. 3. Hyphae entering through outside of cuticle × 33. Fig. 4. Hyphae in epiblemal layer × 140. Fig. 5. Septate hyphae in the host cell × 140. Fig. 6. A cell of root completely packed with starch grains × 140. Fig. 7. Septate branched hyphae × 140. Fig. 8. Septate hyphae; note most of the starch grains disappeared × 140. Fig.s 9-18. *Botrychium virginianum* L. Fig. 9. Hyphal swellings × 140. Fig. 10. Hyphae passing from one cell to another × 140. Fig. 11. Vesicle germinating × 140. Fig. 12. Young vesicle × 140. Fig. 13. Two vesicles in one cell × 140. Fig. 14. Mature vesicle × 140. Fig. 15. Old hypahe in the cell × 140. Fig. 16. Clamping of hyphae × 140. Fig. 17 Young arbuscules; note disfigured host cell nucleus × 140. Fig. 18. Old arbuscule × 140.