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MYCORRHIZAL STUDIES ON SOME ORCHIDS FROM "ALEXANDRU BORZA" BOTANICAL GARDEN

Crina MOCAN[1], Mihai DRĂGAN-BULARDA[2]
[1]Universitatea "Babeş-Bolyai", Grădina Botanică "Alexandru Borza" Str. Republicii, nr. 42, **RO-400015 Cluj-Napoca**
[2]Universitatea "Babeş-Bolyai", Facultatea de Biologie şi Geologie,
str. M. Kogălniceanu, nr. 1, **RO-400084 Cluj-Napoca, România**
e-mail: cmocan@yahoo.com

Abstract: Because in Romania until now nobody has made studies of the relationship between fungi and the roots of orchids under greenhouse conditions, we set out to identify the presence of this sort of symbiosis. Having gathered the roots, in order to identify the presence of the mycorrhizal fungi we made transverse and longitudinal sections, which we examined by optical microscope. As a result of our observations, we were able to record the presence of the mycorrhizal fungi in the zone of the root cells and see that infection is not permanent, but is developed especially in the vegetative period of the plants. At the same time, among other observations we have emphasized thallophagy, which is specific to the mycorrhizal fungi of *Orchidaceae*.

Keywords: mycorrhizal fungi, orchids, symbiosis, pelotons, talyphophagy phenomenon.

Introduction

Taking into consideration that in Romania there have been only a few studies [14] of the genera and species of cultivated orchids, and few [11] on the symbiosis between these plants and specific mycorrhizal fungi, we aimed to determine the symbiosis between two species – one (a fungus) considered a 'lower plant', the other a 'higher plant', indeed one of the most evolved of plants (Angiosperms, Monocotyledons).

The purpose of our studies of three species of greenhouse orchids was to determine the nature of this symbiosis, by various methods, and to emphasize possible changes in the structure of the root.

Materials and Methods

The plant material used represented the roots of three species of orchid: *Anoectochilus regalis* Blume (*A. setaceus* Blume), *Epidendrum radicans* Pav.ex Lindl. and *Paphiopedilum insigne* (Wall. ex Lindl.) Pfitz.

Anoectochilus regalis, jewel orchid, native to S. Asia (India), belongs to a family of 50 species, most terrestrial but some lithophytic, which prefer well-shaded wet conditions. This orchid has short roots, unbranched with many very long hairs that join together the soil particles.

The second species, *Epidendrum radicans*, is an epiphytic orchid that occurs in Central America, from 900 m to 2500 m altitude. It develops numerous aerial roots with a typical structure for an epiphytic orchid.

The third species, *Paphiopedilum insigne*, a terrestrial orchid, occurs widely over the Himalaya mountains, in N. India, Nepal and China. It is listed on Appendix 1 of CITES, and has been declared Endangered [4].

The plant material was collected in summer, which is the flowering period, and we wished to observe if there were variations in the "infection" with mycorrhizal fungi, in accordance with the literature [1].

To make observations by optical microscope, we prepared sections of the fresh orchid roots [7, 8, 9]. Each root was washed under a jet of water, and brushed to remove as much as possible of the residual soil from the root surface. After washing, fragments of the main root and then the lateral roots were sectioned transversally and longitudinally.

Some sections were stained with fuchsin acid, for 2–3 minutes, and then washed twice with water. Other sections were observed through the microscope without being stained. For observation under the Olympus microscope, we prepared slide-lamella samples and made observations at magnifications x 10, x 20 , x 40 and x 100.

We sampled roots from five plants for each of the three species and made 25 sections per species.

Results and Discussions

In *Epidendrum radicans,* which has aerial roots typical for epiphytic orchids, we have not found mycorrhizal hyphae between or within the cortical cells. Because these aerial roots do not have immediate contact with soil, it is not possible for them to interact with mycorrhizal fungi, the orchid using only the nutritive substances available in the rain drops that trickle along the roots after watering.

Some researchers report that they have found mycorrhizal hyphae belonging to *Rhizoctonia* sp., in the roots of this type of orchid [10, 12, 13].

The above images (Figs. 1, 2) show a structure typical for an epiphytic orchid root. Thus, we can observe the stratified rhizodermis (velamen radicum) with five layers of rhomboidal cells and walls with suberin thickening. Within the velamen radicum are elongated cells, also with suberin-thickened walls – the exodermis, which surrounds the cortex, with some inclusions in the outer layers. This orchid seems to lack fungi, probably because of growing conditions in which the plant does not interact with soil and fungi, so that symbiosis does not occur, at least at this stage of the life cycle.

Figures 3 and 4 show existing mycorrhizal hyphae in the rhizodermis zone, and we can ascertain the presence of hyphae as abundant by comparison with other observations made during the dormant period (autumn). The first picture reveals that the hyphae penetrate via the root hairs. Some authors have specified that the presence of mycorrhizae in the plant causes a decrease in number of root hairs, until they are absent [3, 5, 6], but when the orchid has numerous root hairs the ‚infection' becomes stronger.

In the case of *Anoectochilus regalis* (Figs. 5, 6), it is revealed that the main means of penetration of the mycorhizal hyphae into the cortex zone of roots is represented by the root hairs.

In Figure 7, we have indicated with a yellow arrow the presence of pelotons (coiled hyphae in the cortex cells), which linger in the cell few days, after which the hyphae will be digested by phytoalexins (hircinol, orchinol) through the phenomenon of thallophagy (Fig. 7) [14] and the resulting compounds used by the orchid host cell [2].

Conclusions

We did not find mycorrhizal fungi in the epiphytic orchid *Epidendrum radicans*.

The non-epiphytic orchids *Anoectochilus regalis* and *Paphiopedilum insigne* exhibit mycorrhizae during their flowering cycle.

The means of penetration by the fungus into the zone of the cortical cells is mostly by the absorbent root hairs, when these are present.

Fig. 1: Transverse section through the root of *Epidendrum radicans* (x 10), no stain

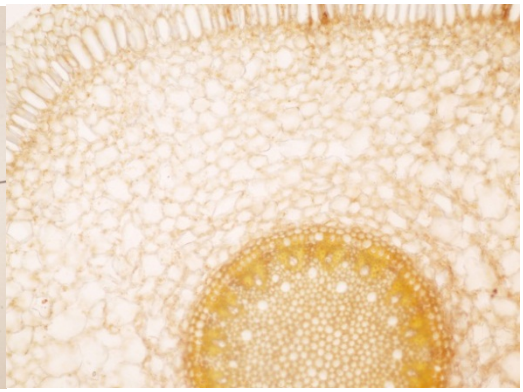
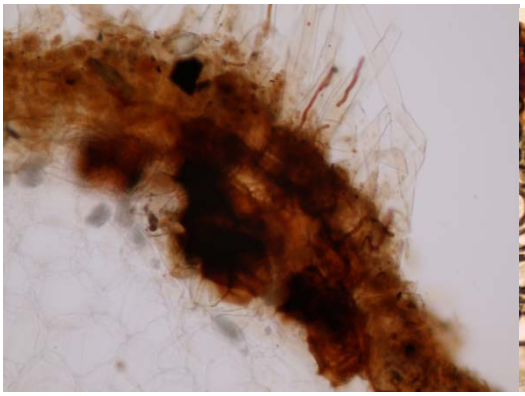


Fig. 2: Transverse section through the root of *Epidendrum radicans* (x 20), no stain



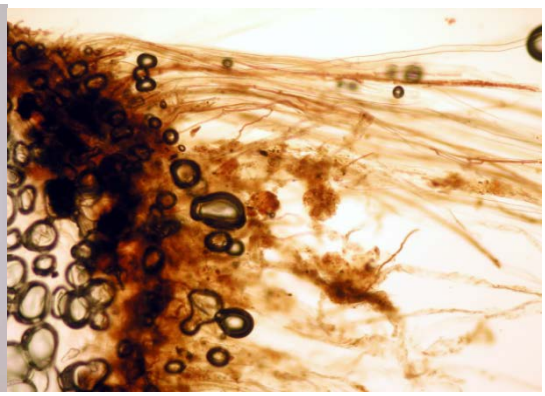


Fig. 3, 4: Transverse section through the root of *Paphiopedilum insigne* (x 40), no stain

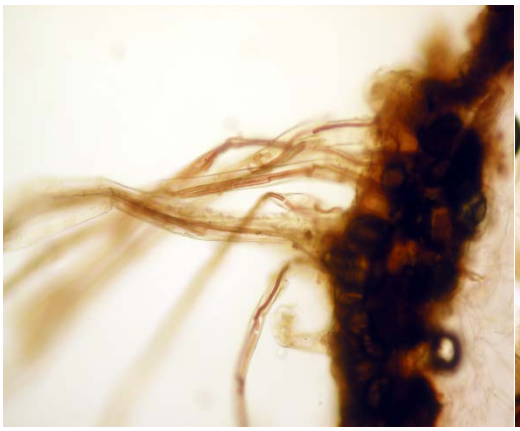


Fig. 5: Transverse section through the root of *Anoectochilus regalis* (x 40), no stain

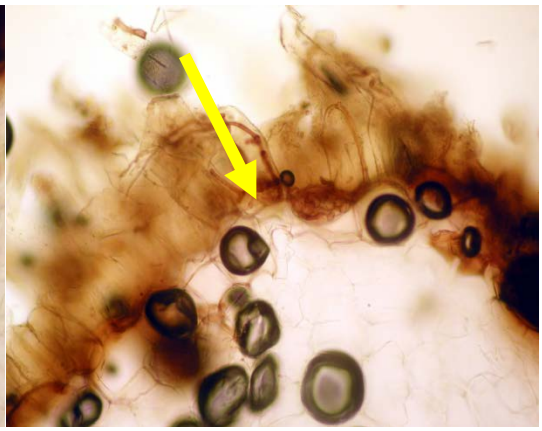


Fig. 6: Transverse section through the root of *Anoectochilus regalis* (x 100), no stain
Yellow arrow – pelotons

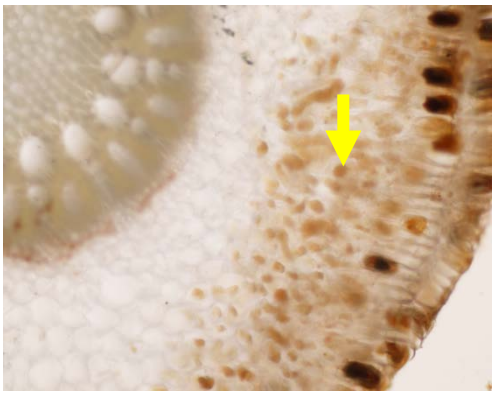


Fig. 7: Transverse section through the root of *Paphiopedilum insigne* (x 20), no stain
Yellow arrow – pelotons in talyphophagy phenomenon

Before flowering and at the time of flowering we found a great number of mycorrhizal hyphae.

We have emphasized the presence of pelotons in the cortical cells and the phenomenon of thallophagy.

In the sections where we observed numerous lateral roots, we found the presence of a greater number of fungal hyphae.

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STUDII ASUPRA MICORIZELOR LA CÂTEVA ORHIDEE CULTIVATE ÎN SERELE GRĂDINII BOTANICE "ALEXANDRU BORZA"

(Rezumat)

Deoarece în România, în condiţiile din seră, până la această dată nu au fost făcute studii asupra relaţiei de simbioză între fungii de micorize şi orhidee tropicale, prin lucrarea de faţă ne propunem să identificăm prezenţa acestei relaţii la trei specii de orhidee şi anume: *Anoectochilus regalis* Blume, *Epidendrum radicans* Pav.ex Lindl. şi *Paphiopedilum insigne* (Wall. ex Lindl.) Pfitz.

Primul pas a constat în colectarea materialului vegetal, din sere, în diferite sezoane ale anului.

Pentru identificarea prezenţei fungilor de micorize s-au realizat secţiuni transversale prin rădăcinile proaspete de orhidee, urmate de examinarea la microscopul optic.

Astfel s-a constatat prezenţa hifelor micorizante la nivelul celulelor radiculare şi faptul că g"infecţia" cu aceste hife nu este permanentă, ci se dezvoltă cu precădere în perioada vegetativă a plantei, iar specia epifită *Anoectochilus regalis* nu a prezentat fungi micorizanţi.

De asemenea, în unele preparate s-a pus în evidenţă fenomenul de talyphopagie, specific pentru micorizele de la orhidee.

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