Morphological study on symbiotically germinated seedlings in *Arundina graminifolia* (Orchidaceae)

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Summary

Morphological study was conducted on cymbiotically germinated seedlings in *Arundina graminifolia* with special reference to the cotyledon morphology. The embryo in the matured seed had a rudimentary cotyledon. After seeds were placed in the medium, the cotyledon developed into a leaf-like structure and a vascular bundle differentiated in it. The fungus penetrated the basal cells of the hypocotyl and formed fungal clots. In some seedlings the cotyledon did not develop and remained as a small protrusion.

Introduction

Two groups can be distinguished structurally in the orchid embryos. A rudimentary cotyledon is recognised in a few orchid species, and in numerous other species the embryo has no cotyledon (Veyret 1974). The auther is studying the cotyledon morphologies among the cotyledonous species (Nishimura 1981). In my study all seeds were sown in Knudson C culture medium (Knudson 1946), and cotyledon structures were observed. After the seeds were placed in the culture medium, (1) the cotyledon in *Bletilla striata* develops into a leaf-like structure and a vascular bundle differentiates in it, (2) it becomes a leaf-like structure but fails to differentiate a vascular bundle in *Sobralia macrantha* and *Sobralia xantholeuca*, and (3) it does not develop and remains as a small protrusion in *Arundina graminifolia* (Nishimura unpublished).

Orchid seeds germinate symbiotically in the field. The embryo gets nutrients from the fungus (Arditti 1967). Detailed observations have not been conducted on the cotyledon development in symbiotically germinated seedlings. This study was undertaken to get the morphological information on cotyledon development in symbiotically germinated *Arundina graminifolia* seedlings.

Material and methods

Seeds, taken from an undehiscent capsel, of *Arundina graminifolia,* and fungus, isolated from roots of *Liparis nervosa* and identified as *Rhizoctonia repens,* were placed on a filter paper (3 cm square) laied on the surface of an oat agar medium (Kimoto 1987) prepared in glass tubes (6.5 cm in diameter and 10.5 cm in depth). The glass tubes were placed at 25 C in the dark for the first three weeks, and then maintained at 22-25 C and 16-h photoperiods produced by fluorescent tubes. The light intensity was 2,000-3,000 Lux. Seedlings were fixed weekly in FAA containing 50% ethanol and observed under a dissecting microscope.

After that the seedlings were dehydrated through a n-butyl alcohol series and embedded in paraffin. Sectins, 8μ thick, were cut and stained with Delafield's hematoxylin.

Results

The seed averages 1.3 mm long and 0.2 mm wide, and the embryo averages 0.4 mm long and 0.15 mm wide. The embryo possesses a small cotyledonal protrusion at the charazal end (Fig. 1).

After the seeds are placed in the culture medium, the cotyledon starts to elongate and becomes a leaf-like structure (Fig. 2, 3, 9, 10). The hypocotyl (embryonal portion below the cotyledon) develops first into a pear-shaped (Fig. 2, 9) and then into a globular body (Fig.

3). A vascular bundle differentiates at the basal region of the cotyledon and extends into the cotyledon and hypocotyl (Fig. 10, 11). The fungus penetrates the basal cells of the hypocotyl and forms clots (Fig. 10). The fungal infection does not take place during the first two weeks and the fungal clots were first observed in the 21-day-old seedings (Fig. 10). In some seedlings the cotyledon does not develop and remains as a protrusion (Fig. 5). In this case the leaf arrangement between the cotyledon and the first foliage leaf is not always distichous. A new protrusion differentiates near the apical meristem opposite the cotyledon (Fig. 4, 11). It develops into the first foliage leaf (Fig. 6, 12). The second foliage leaf differentiates in the same manner as the first one (Fig. 7, 12). The following leaves and the adventitious roots differentiate (Fig. 8). When mature the cotyledon is much smaller than the foliage leaves.

Discussion

Two cotyledon types were observed in the symbiotic germination of *Arundina graminifolia;* in most seedlings the cotyledon develops into a leaf-like structure and a vascular bundle differentiates in it, and in some seedlings it does not develop and remains as a protrusion. When the seeds of *Arundina* were sown in Knudson C medium, in the most seedlings the cotyledon does not develop and remains as a protrusion (Nishimura unpublished). Knudson C culture medium contains only some minerals and sugar, and the nutrient condition is rather poor. On the other hand, the seedlings, germinated in the symbiotical condition, are surrounded by prenty hyphae, and seemed to be supplied with abundunt nutrient from the fungi. These facts my suggest that the morphology of the cotyledon in *Arundina graminifolia* may be affected by the nutrient condition of the culture medium; it becomes a leaf-like structure and

differentiates a vascular bundle in a nutrient rich medium, but it does not develop and remains as a protrusion in a nutrient poor medium.

The cotyledons of *Bletilla striata, Sobralia macrantha,* and *Sobralia xantholeuca* develop into a leaf-like sructure even in a nutrient poor Knudson C medium, and in *Bletilla* it differentiates a vascular bundle (Nishimura unpublished). This indicates that the cotyledons of these orchids have more vigor than the one in *Arundina,* and the one in *Bletilla* is the most vigorous, in other words, it is the most well developed one.

Acknowledgement

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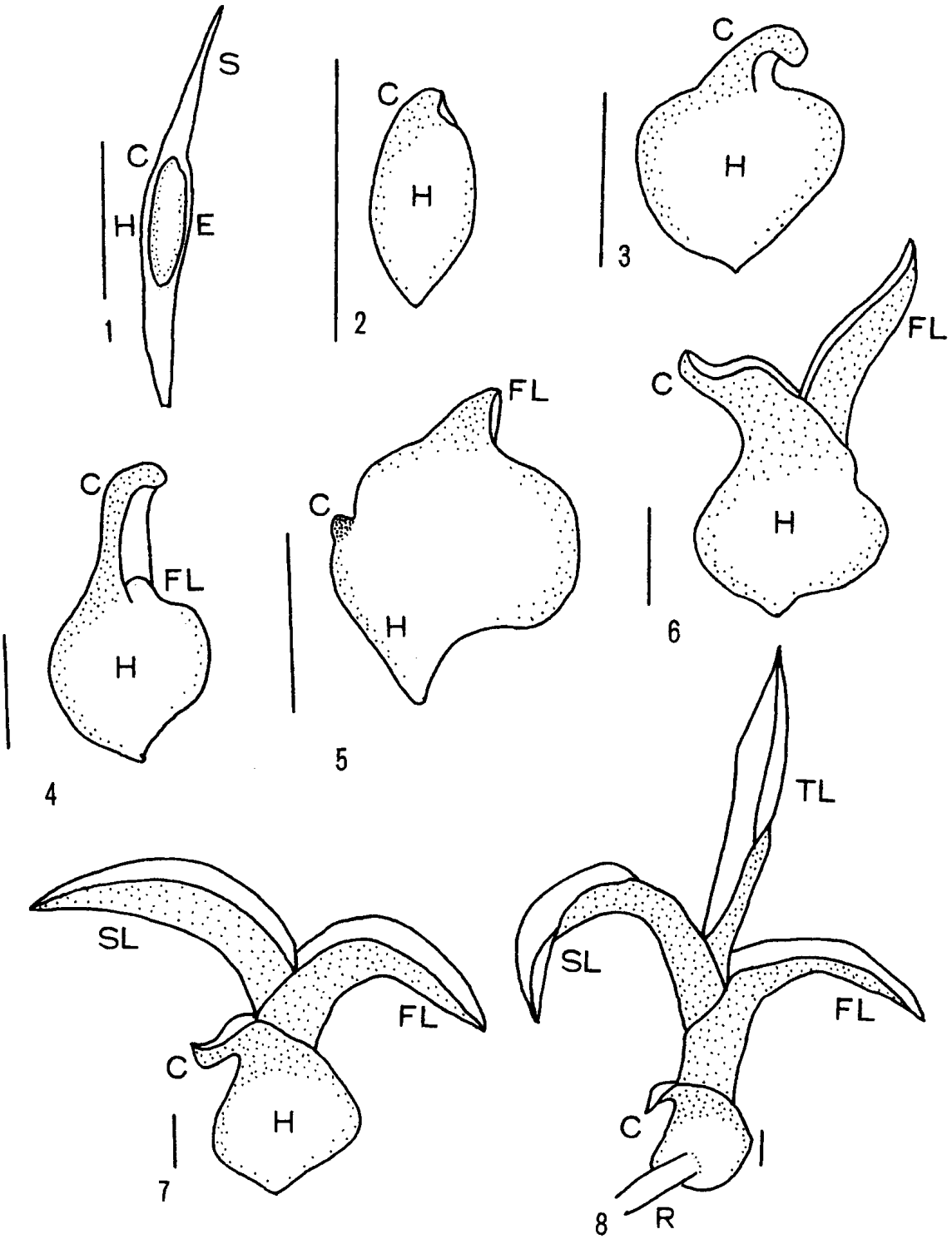
Figs. 1-12. — Seedling development in *Arundina graminifolia* in a symbiotic germination. Fig.1, A seed consists of a seed coat and an embryo. The embryo consists of a rudimentary cotyledon and a hypocotyl. Fig.2, A 14-day-old seedling with a swollen cotyledon and a pear-shaped hypocotyl. Fig.3, A 21-day-old seedling with aleaf-like cotyledon and a globular hypocotyl. Fig.4, A 28-day-old seedling with a young first foliage leaf. Fig.5, A 35-day-old seedling with cotyledonary protrusion. The leaf arrangement between the cotyledon and the first foliage leaf is not distichous. Fig.6, A 42-day-old seedling with a developing foliage leaf. Fig.7, A 70-day-old seedling with the second foliage leaf. Fig.8, A 116-day-old seedling with a matured cotyledon, several foliage leaves and an adventitious root. Fig.9, Longitudinal section of 14-day-old seedling. The fungus has not been found in the hypocotyl cells.x165. Fig.10. Longitudinal section of 21-day-old seedling. A vascular bundle and fungal clots can be seen.x113. Fig.11, Longitudinal section of 28-day-old seedling. The first foliage leaf can be seen.x98. Fig.12, Longitudinal section of 42-day-old seedling. The primordium of the second foliage leaf can be seen. x97. C, cotyledon ; E, embryo ; F, fungal clots ; FL, first foliage leaf ; H, hypocotyl ; R, root ; S, seed coat ; SL, second foliage leaf ; TL, third foliage leaf ; V, vascular bundle. Bar indicates 0.5 mm.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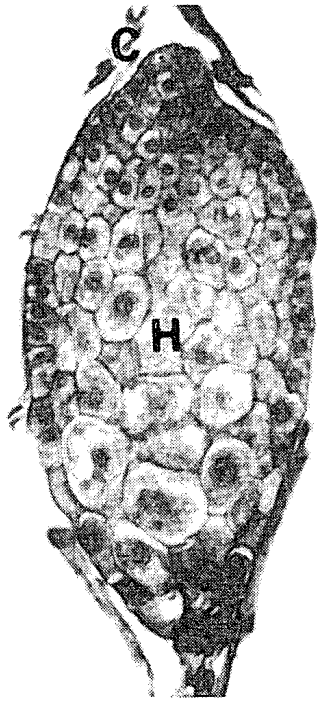

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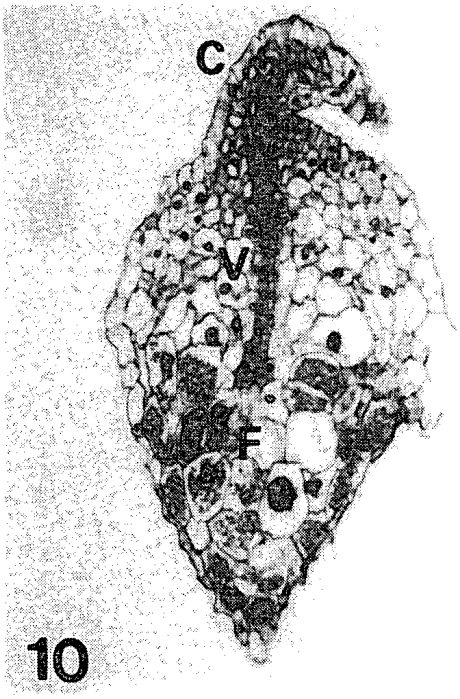

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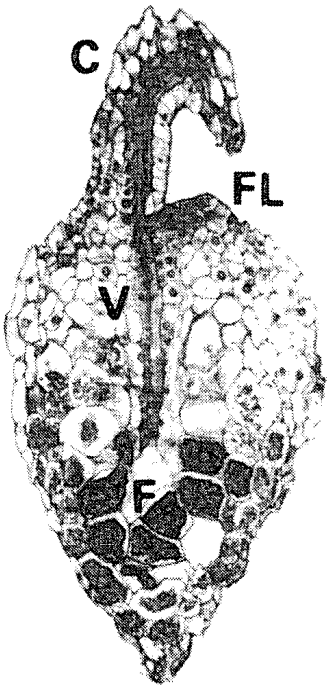

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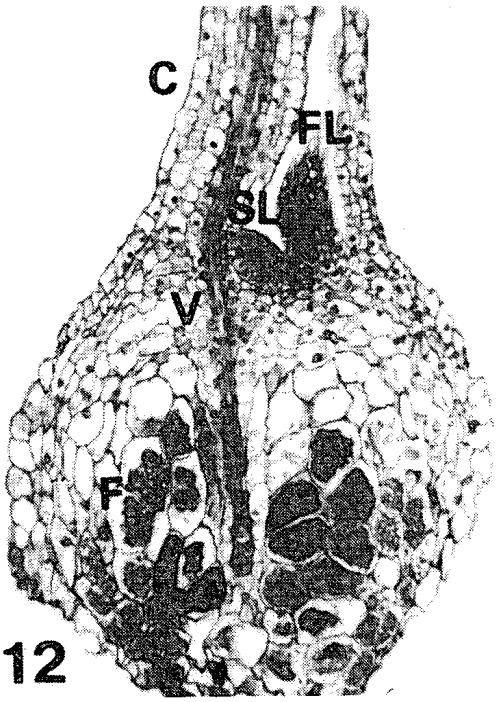

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