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Protocorm or rhizome? The morphology of seed germination in *Cymbidium dayanum* Reichb.

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Abstract. Seeds of *Cymbidium dayanum* Reichb. were sown in vitro and germinated to produce protocorms one month later. Protocorms elongated, and the unicellular cell absorbing hairs were initiated at the base. The anterior of the protocorm produced the sheath leaf primordium. After two months, the protocorms grew, with the structure similar to that of rhizomes with nodes and lateral buds. The terminal buds spontaneously developed into shoots. According to the morphological pattern in vitro, we inferred that the seed germination of *C. dayanum* involved a fast transition from protocorm to plantlet by rhizome.

Keywords: *Cymbidium dayanum* Reichb.; Gravitropism; Protocorm; Rhizome.

Introduction

Under natural conditions, the seeds of all orchids germinate only after they are infected by their mycorrhizal fungi. In the laboratory, seeds of tropical epiphytic orchids germinate with relative ease on appropriate media. Seeds of terrestrial orchids from temperate regions are much more difficult to germinate in vitro (Arditti, 1992).

The structures that form between the germination of seeds and the establishment of seedlings have been denominated as protocorms or rhizomes. The term "protocorm" was first proposed by Melchior Treub in 1890 to describe the early stages in the germination of lycopods. Orchid protocorms resemble those of lycopods—according to Bermard's use of the term—and it has been used extensively (Arditti, 1992). Protocorms have round or elliptical shapes with some unicellular absorbing hairs on the basal part and an apex meristem on the tip. The protocorms of most of tropical epiphytic orchids show the capacity to develop shoots directly. In many terrestrial orchids, the apices of protocorms elongate to form rhizomes that continue to grow and branch. The rhizome has several nodes, and the apex shows the physiology of gravitropism in vitro. After the elongation of the rhizome, the terminal bud grows upward and differentiates into shoots and roots.

Seedling establishment of the genus *Cymbidium* is distinguished by protocorm or by rhizome (Shimasaki and Uemoto, 1987). In epiphytic *Cymbidium*, protocorm directly develops into a shoot as, for example, *C. dayanum* Reichb., (Lu and Lee, 1990). *Cymbidium aloifolium* (L.) Sw. exhibits a fast transition from protocorm to plantlet by rhizome (Nayak et al., 1998). In terrestrial *Cymbidium*, a rhizome needs 1-2 year to differentiate to a plantlet as, for example, *C. ensifolium* (L.) Sw. (Chung et al., 1985; Lu et al., 1992), *C. forrestii* Rolfe (Paek and Yeung, 1991), *C. goeringii* (Rchb.) Rchb. (Nagashima, 1982; Duan and Xie, 1983; Shimasaki and Uemoto, 1991), and *C. sinense* Willd. (Chiou and Wang, 1985; Chang and Chang, 2000).

This report describes the outer morphological changes during the seed germination of *Cymbidium dayanum*, an epiphytic *Cymbidium*, and indicates the morphology of intermediate structures between seed and plantlet.

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Materials and Methods

Seed Germination

Capsules of *Cymbidium dayanum* were harvested 12 months after pollination and then sterilized with 2% NaOCl supplemented by one drop of Tween 20 in 15 min. The seeds were sown in 20×150 mm pyrex tubes each with 9 ml gelrite-medium that contained 1/4 MS salts (Murashige and Skoog, 1962), 0.5 mg l^{-1} niacin, 0.5 mg l^{-1} pyridoxine HCl, 0.1 mg l^{-1} thiamine HCl, 100 mg l^{-1} myo-inositol, 1 g l^{-1} peptone, 20 g l^{-1} sucrose, 2 g l^{-1} activated charcoal, 50 g l^{-1} banana pulp, and 4 g l^{-1} gelrite with a pH of 5.5. The cultures were exposed to artificial light of 1000 lux with a light/dark cycle of 16/8 h at 25°C±1°C.

SEM

Samples of seeds germination were fixed in 2.5% glutaradehyde in a 0.1 M phosphate buffer for 4 h, dehydrated through an ethanol series, dried in a critical-point dryer (Hitachi, HCP-2), and coated with gold in an ion coater (Hitachi, E1010). A Hitachi S-3000N scanning electron microscope was used to examine the samples.

Result and Discussion

Seeds of *C. dayanum* are minute, dust-like, and fusiform in shape (Figure 1a). They are 650-900 μm long, 200-300 μm wide, and have an aperture in the posterior (Figure 1b). One month after sowing, the embryo swelled (Figure 1c), then broke out of the testa, and the morphology resembled a protocorm (Figure 1d). This protocorm elongated, and the anterior cells proliferated (Figure 1e), and the absorbing hairs originated from a single cell at the base of the structure (Figure 1f, g). The anterior of the protocorm produced the sheath leaf primordium (Figure 1h), and the absorbing hairs became elongated (Figure 1i).

After two months, the structure of seed germination grew and produced several lateral buds covered by cataphyll (Figure 2). The elongated structure was similar to that of rhizome with nodes and lateral buds, otherwise the terminal buds spontaneously developed into shoot. According to the morphological pattern in vitro, we inferred that the seed germination of *C. dayanum* was associated with a transition from protocorm to plantlet by rhizome.

Lu and Lee (1990) mentioned the seed germination process of *C. dayanum* directly through protocorm into plantlet. In our germination process, we observed the appearance of rhizome formation that was transited from protocorm. The distinctive characteristic of rhizome is that it has lateral buds covered by cataphyll.

Nayak et al. (1998) metioned that *Cymbidium aloifolium* seeds germinated within 15-18 days to form protocorms. The protocorms were transferred to a fresh medium, in which rhizomes developed within 2-3 months, and they studied plant growth regulators on the seed-derived rhizomes for promotion of shoot formation and plantlets established after 8 weeks of culture.

The morphological pattern of *Cymbidium aloifolium* seed germination was associated with a fast transition from protocorm to plantlet by rhizome. In this paper, the *Cymbidium dayanum* had the same pattern as *Cymbidium aloifolium*, and the difference with terrestrial *Cymbidium* was that rhizomes needs 1-2 year to differentiate to a plantlet.

Chinese *Cymbidium* is a valuable and beautiful floriculture crop in Eastern Asia, but its proliferation rate is very

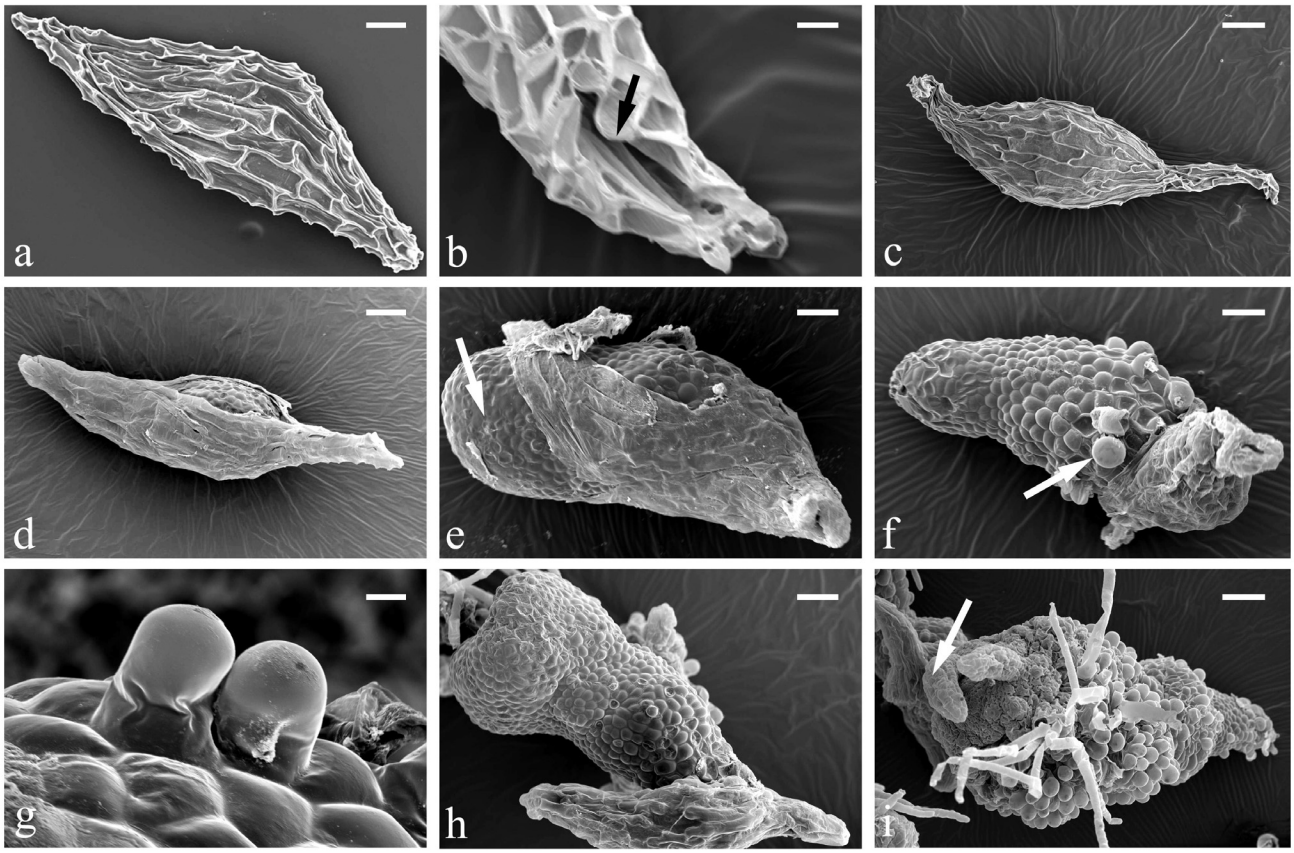

Figure 1. The morphology of the seeds in *C. dayanum* during the germination by scanning electron microscopy. a: The seed of *Cymbidium dayanum* (bar = 60 μm); b: The aperture (➔) in the posterior end of the seed. (bar = 15 μm); c: Swelled seed one month after sowing. (bar = 60 μm); d: The protocorm broken out the testa after cultured. (bar = 75 μm); e: The anterior (➔) cells of protocorm elongation. (bar = 60 μm); f: The absorbing hairs (➔) were formed at the base. (bar = 60 μm); g: Absorbing hairs were originated from single cell of the surface. (bar = 15 μm); h: The anterior of the protocorm produced the sheath leaf primordium. (bar = 60 μm); i: The sheath leaf (➔) formed in the front and the absorbing hairs became elongated (bar = 150 μm).

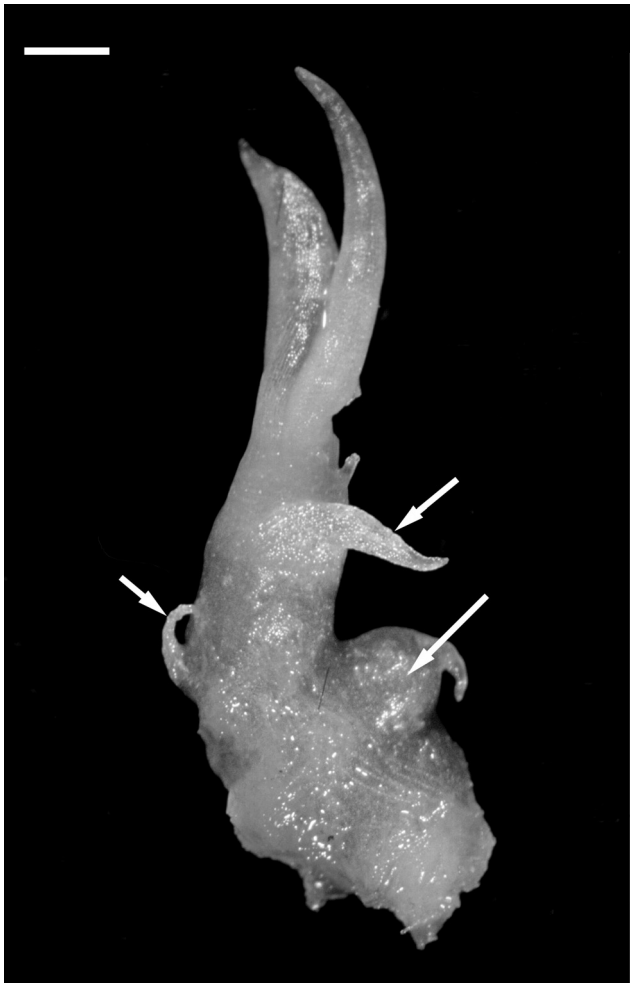

Figure 2. The apex shoot formation and subsequent the lateral bud (➔) developed of rhizome of *C. dayanum*. (bar = 400 μm)

low. Because of the long period from seed germination to rhizome formation to shoot development, propagation of terrestrial *Cymbidium* through rhizome culture was considered difficult and time-consuming. Shoots that develop spontaneously from the rhizome of *C. dayanum* are an exception and may be useful in a program seeking to breed Chinese *Cymbidium*.

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原球體或根莖？鳳蘭種子發芽之形態研究

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鳳蘭為蘭科蕙蘭屬附生性植物，將種子播種在試管內一個月後發芽，胚先膨大突破種皮形成原球體，在原球體的下半部形成吸收毛。接著原球體形成頂芽，發育成長條形，鞘葉在頂端形成，並產生側芽及鱗片葉。鳳蘭的種子發芽是先形成原球體，再過渡根莖構造，頂芽無向地性，具直接形成莖葉的能力。

關鍵詞：鳳蘭；向地性；原球體；根莖。