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Production of Sporophytic Plants of *Cyathea lepifera*, a Tree Fern, from in vitro Cultured Gametophyte

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Summary

Spores of *Cyathea lepifera* were cultured aseptically on half strength Murashige and Skoog's medium (MS medium) without sucrose. Germinated spores grew to form heart-shaped gametophytes after about 30 days of culture that initiated both antheridia and archegonia. Mature gametophytic tissues can be subcultured on the same medium. The addition of sucrose to the culture medium enhanced the growth rate significantly, but it did not induce the production of a sporophytic plant. When MS medium without sucrose was diluted to 1/10, 1/20, 1/40 or 1/80, sporophytic plants were produced. Sporophytic plants can be propagated on full strength of MS medium with or without sucrose by excising and culturing a part of a shoot tip.

Key Words: *Cyathea lepifera*, in vitro culture, micropropagation, tree fern.

Introduction

Many species of ferns belonging to some genera, such as *Nephrolepis, Adiantum* or *Asplenium* are grown commercially as ornamental plants. Sporophytic plants of ornamental ferns are often propagated by in vitro culture technique (Amaki and Higuchi, 1991; Caponetti, 1978; George, 1996; Higuchi et al., 1987; Murashige, 1974). A tropical tree fern, *Cyathea lepifera* (Fig. 1A), is rarely grown in the mainland of Japan except in conservatories, although *C. lepifera* is widely grown and circinate leaves of this fern are eaten in the Ryukyu Islands. Recently, sporophytic plants of this fern, grown in pots, are commercially produced as ornamentals.

In this study, we attempted to produce sporophytic plants of *C. lepifera* through a gametophyte originating from a spore. In vitro-produced sporophytic plants were propagated by excising and culturing a part of the shoot tip.

Materials and Methods

In vitro culture of spores, gametophytes and sporophytes

Spores of *C. lepifera* that were obtained from the National Museum of Tsukuba, were sterilized with 70% (v/v) ethanol for 1 min and 0.5% sodium hypochlolite solution for 1-2 min, followed by rinsing in sterilized water. To obtain gametophytes, sterilized spores were sown on half strength of MS medium (Murashige and Skoog, 1962) without sugar (1/2 MS medium), solidified with 0.2% (w/v) Gellan Gum (Wako-junyaku, Osaka, Japan). Gametophytic mass that formed from a germinated spore was divided into pieces, 5 mm long and wide. Individual pieces of gametophytic tissue were cultured on 10 ml of 1/2 MS medium without sucrose in a test tube (20 mm × 150 mm) with an aluminium foil cap. Thereafter, the gametophytic tissues were subcultured at about 40-day intervals. To produce sporophytic plants, gametophytic tissues that were subcultured for 1 month, were transplanted 1, 1/2, 1/4, 1/10, 1/20, 1/40 and 1/80 MS medium without sucrose. When MS basal medium was diluted to less than 1/4, agar was used in stead of Gellun Gum as the solidifying agent. Effect of the addition of 1, 2 and 3% sucrose to MS and 1/2 MS medium on the production of sporophyte was also examined. No growth regulator was tested in this study. All media were adjusted to pH 5.7-5.8 prior to the addition of solidifying agent and autoclaved at 121℃ for 15 min. All cultures were incubated at 26℃ under continuous fluorescent light at an intensity of 20 μmol·m^{-2}·s^{-1} PPFD.

Microscopic observation

Microscopic observations were carried out with an inverted microscope (Olympus CK-40) and photo-

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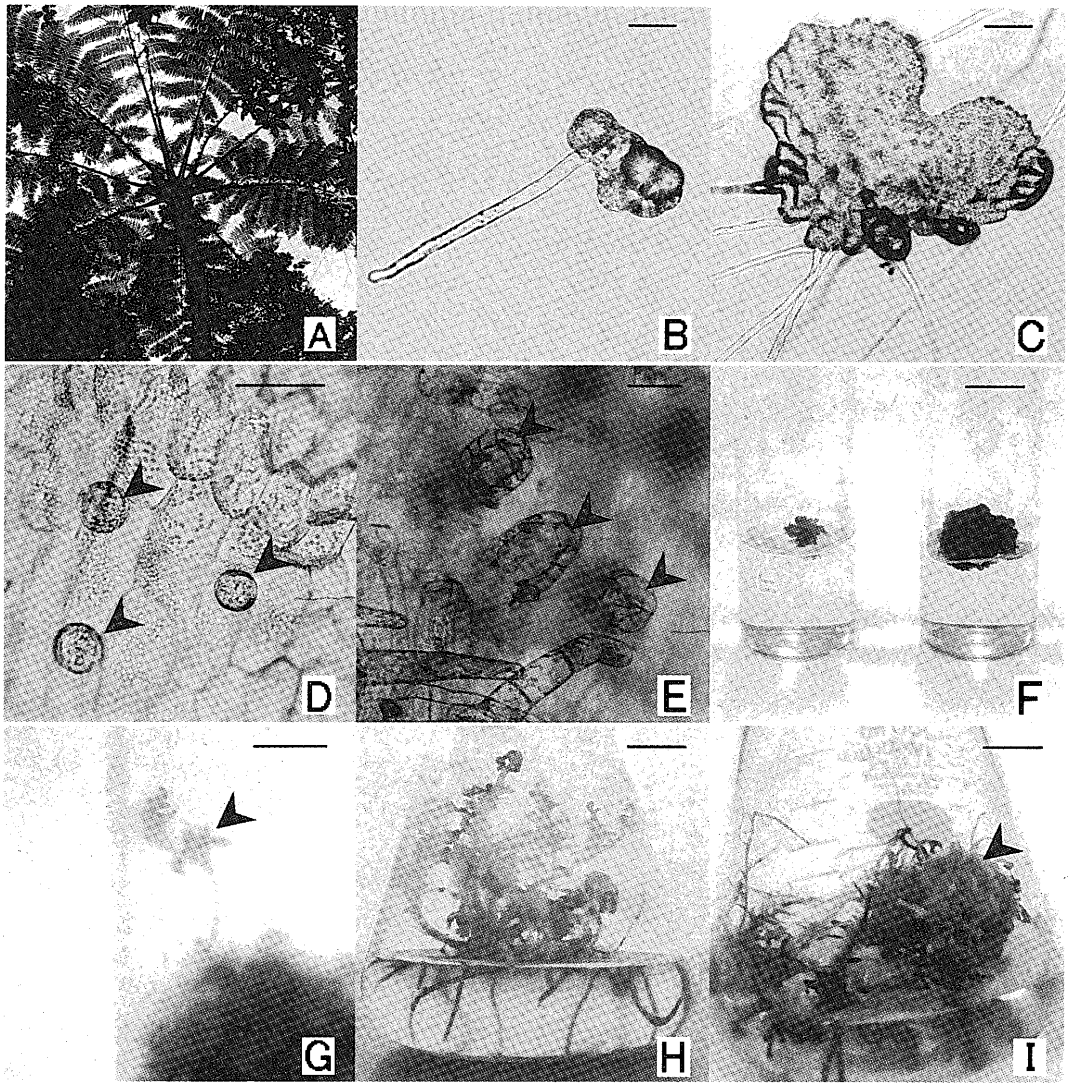

Fig. 1. *Cyathea lepifera*. A: A sporophytic plant grown in the main island of Ryukyu. B: Germinated spore, cultured for 6 days. Bar = 50 μm. C: Heart-shaped gametophyte formed after 30 days of culture. Bar = 100 μm. D: Antheridia (indicated by arrows) formed on a subcultured gametophyte. Bar = 100 μm. E: Archegonia (indicated by arrows) formed on a subcultured gametophyte. Bar = 50 μm. F: Difference in growth rate of cultured gametophyes. Gametophytes cultured on MS medium with 3% sucrose (right) and without sucrose (left). Bar = 1 cm. G: Juvenile sporophyte (a primary leaf is indicated by an arrow) formed on gametophyte that was cultured on 1/10 MS medium without sucrose for about 60 days. Bar = 5 mm. H: A developed sporophyte that was detached from gametophyte and cultured for about 2 months. Bar = 1 cm. I: A gmetophyte mass (indicated by an arrow) induced aposporously from a segment of rhizome cultured on MS medium with 3% sucrose. Bar = 1 cm.

graphs were taken by an attached camera (Olympus PM-35DX).

Results and Discussion

Spores sown on 1/2 MS medium without sucrose germinated in 5-7 days (Fig. 1B) and grew to heart-shaped gametophytes after 1 month of culture (Fig. 1C). After 2 month, the heart-shaped tissue grew into mass that consisted of several sheets of gametophytes. The gametophytic tissue that was subcultured repeatedly on 1/2 MS medium without sucrose did not produced sporophytic plants, but formed antheridia and archegonia (Fig. 1D and 1E). The addition of sucrose to MS and 1/2 MS medium enhanced the growth of gametophyte (Fig. 1F), but had no effect on the production of a sporophytic plant. When gametophytes were cultured on 1/10, 1/20, 1/40 or 1/80 MS medium, sporophytic plants were produced (Fig. 1G). The average number of sporophytic plants per test tube was as follows; 0.14 (1/10 MS), 0.28 (1/20 MS), 0.57 (1/40 MS) and 1.43 (1/80 MS). Therefore, 1/80 MS was selected for the production of sporophytic plants in this study. When sporophytes developed to 1-2 cm long, they were detached from the gametophyte with a knife and forceps and transplanted to a fresh medium. Detached sporophytes that were cultured on MS medium with or without

sucrose grew to initiate new roots and rhizomes (Fig. 1H). For the propagation of sporophytic plants, a shoot tip with a few of circinately vernate leaves was divided into two or three pieces and each was subcultured on a fresh medium. In an attempt to propagate sporophytes via adventitious shoots, segments (5 mm) of rhizome and leaf were excised and cultured on MS medium with 3% (w/v) sucrose. These segments produced no adventitious shoot but only aposporous gametophytes (Fig. 1I).

Although many species of ferns have been successfully propagated by tissue culture technique (Caponetti, 1978; George, 1996; Murashige, 1974), only a few studies on the genera *Cyathea* were made (Finnie and Van Staden, 1987; Padhya, 1987) and none on *Cyathea* lepifera. Results of this study indicated that sporophytic plants of *C. lepifera*, a tropical tree fern, can be obtained through in vitro culture of gametophytes and that propagation of this fern was possible, independent of the season and climate.

The addition of sucrose to the culture medium produces apogamous sporophytes in the gametophyte culture of *Pteridium aquilinum* (Whittier, 1964; Whittier and Steeves, 1962). However, in this study, sucrose appeared to be ineffective for the production of sporophytic plants from the subcultured gametophyte, although the sugar remarkably enhanced its growth rate. A similar result was obtained when the gametophyte of *Equisetum arvense* was cultured (Kuriyama et al., 1989). When sugar-free MS basal medium (mineral nutritions and organic compounds) was diluted to 1/10, 1/20, 1/40 or 1/80 strength, sporophytic plants were readily produced. These sporophytic plants could be clonally propagated by dividing their shoot tips, but not from their rhizome or leaf. These organs generated aposporous gametophytes.

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無菌培養したヒカゲヘゴ前葉体からの胞子体植物の形成

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摘　要

ヒカゲヘゴ (*Cyathea lepifera*) の胞子を無菌的に培養した．発芽した胞子は前葉体を形成し，発達した前葉体上には造精器，造卵器ともに観察できた．ショ糖は前葉体の増殖を飛躍的に高める効果はあったものの，胞子体植物の形成にはまったく効果がなかった．基本培地 (MS 培地) を 1/10，1/20，1/40，1/80 と希釈することにより，胞子体が形成された．幼若なゼンマイ状の葉を 2，3 つけた胞子体の茎頂部分をナイフで 2，3 に切り分け，それぞれを培養することにより胞子体植物を増殖させることができた．しかし，根茎や葉の切片から不定芽を誘導することはできなかった．根茎や葉の切片からは無胞子生殖的に前葉体が分化した．