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Medium Composition for the Production of Sporophytes of the Fern *Adiantum capillus-veneris*

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Summary

The medium composition for the production of sporophytes from gametophytes by sexual reproduction in *Adiantum capillus-veneris* was investigated. The addition of 3% sucrose to Murashige and Skoog's medium (MS medium) significantly enhanced the growth of gametophyte. However, sucrose did not induce the production of a sporophyte. The elimination of NH_4NO_3 from the culture medium induced the production of sporophytes, although elimination of KNO_3 was not effective. Reduction of total nitrogen to 25% of the original MS medium effectively promoted sporophyte yield, indicating that the nitrogenous component is one of the most important factors for the production of sporophytes by sexual reproduction in *Adiantum capillus-veneris*.

Key Words: *Adiantum capillus-veneris*, in vitro culture, nitrogen source, ornamental fern.

Introduction

As with micropropagation of higher plants, in vitro culture techniques were used for the propagation of ornamental ferns (Amaki and Higuchi, 1991; Caponetti, 1978; Murashige, 1974; Padhya, 1987). In *Adiantum*, many cultivars have been produced and propagated by in vitro culture (Amaki and Higuchi, 1991; Pais and Casal, 1987; Wetmore and Morel, 1949). In these studies, sporophyte tissues-for example, shoot apex, young leaf or rhizome segment were used as the source explants for culture. We have subcultured gametophytes of *Adiantum capillus-veneris* for more than 5 years. Antheridia and archegonia continually formed on the gametophytes. However, the growth rate of gametophytes was very slow with the culture medium used; it took more than 3 months for the formation of sporophytes by sexual reproduction. In this study, we examined the medium composition for an efficient production of sporophytes in *Adiantum capillus-veneris* by sexual reproduction.

Materials and Methods

Gametophytes of *Adiantum capillus-veneris* were routinely subcultured at 27℃ every one month on half strength of MS medium (Murashige and Skoog, 1962) containing 1/2 vitamins and minor elements without sugar (1/2 MS medium), solidified with 0.2% Gellan Gum (Wako-junyaku, Osaka).

To examine the effect of sucrose concentration on the gametophyte growth, a thin spatulate gametophyte was cut into pieces, 5 mm long and wide, and the explants were cultured on 10 ml of full strength MS medium with different concentrations (0, 1, 2, 3, and 4% (w/v)) of sucrose in a culture tube (20 mm × 150 mm) with an aluminium foil cap. Gametophyte growth was determined by its fresh weight after 50 days of culture.

To examine the effect of nitrogen sources on the production of sporophytes, NH_4NO_3 or KNO_3 was eliminated from MS medium. To examine the effect of nitrogen level, NH_4NO_3 and KNO_3 of MS medium was diluted to 0, 25, 50 or 75% of the original concentration with the sucrose adjusted to 3%. The average number of sporophytes, longer than 5 mm, was counted after 50 days of culture.

No growth regulator was tested in this study. All media were adjusted to pH 5.7-5.8 with 0.1N NaOH or HCl prior to the addition of solidifier (0.2% Gellan Gum) and autoclaved at 121℃ for 15 min. All cultures were incubated at 27℃ under continuous fluorescent light (FL40S-W MITSUBISHI/OSRAM) at 20 μmol·m^{-2}·s^{-1} PPFD.

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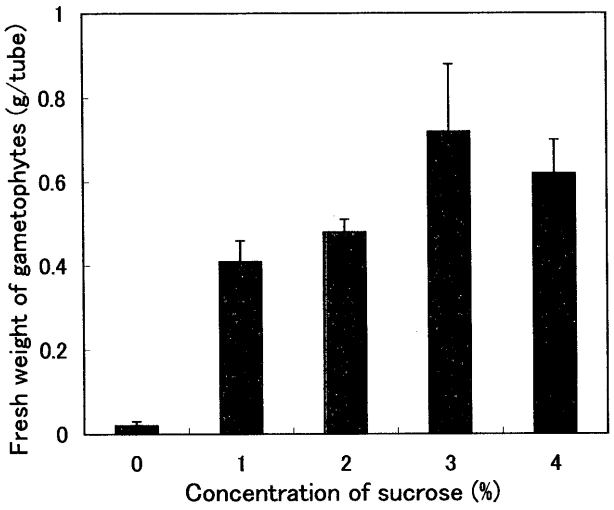

Fig. 1. Effect of sucrose concentrations on the gametophyte growth of *Adiantum capillus-veneris*. Gametophytes were cultured on MS medium containing different concentrations of sucrose for 50 days. Vertical bars indicate SD (n = 5).

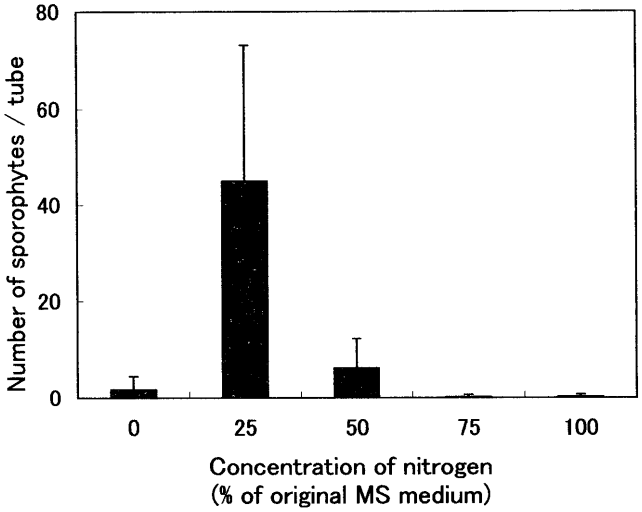

Fig. 2. Effect of different concentrations of nitrogen source on the production of sporophytes of *Adiantum capillus-veneris*. Nitrogen source (KNO_3 and NH_4NO_3) of MS medium was adjusted to 0, 25, 50, 75 or 100% of the original concentration. Gametophytes were cultured for 50 days. Vertical bars indicate SD (n = 5).

Table 1. Number of sporophytes of *Adiantum capillus-veneris* produced from gametophytes cultured with different nitrogen source.

Nitrogen source [z]		Number of sporophytes / tube [y]
NH_4NO_3	KNO_3	
+	-	0
-	+	24.5 ± 9.8

[z] KNO_3 or NH_4NO_3 was eliminated from MS medium containing 3% sucrose.

[y] Mean ± SD (n =10).

Results

The fresh weights of gametophytes, cultured on the MS medium containing various concentrations of sucrose, revealed that the addition of sucrose considerably elevated their growth rate (Fig. 1). The growth rate peaked with 3% sucrose, while it decreased at higher concentrations. Although the growth rate was hastened by the addition of sucrose, no sporophytes were formed. Likewise, no sporophyte was formed on the medium without KNO_3, whereas many sporophytes were produced on the medium without NH_4NO_3 (Table 1).

The number of sporophytes formed on the media containing various concentrations of nitrogen sources differed (Fig. 2). The nitrogen level in the original MS medium was unsuitable for the formation of sporophytes, but 0.25-fold nitrogen level maximized the number of sporophytes. Complete elimination of the nitrogen sources did not favor the production of sporophytes.

Discussion

Addition of sucrose markedly increased the growth rate of gametophytes of *Adiantum capillus-veneris* in this study as was reported for *Equisetum arvense* (Kuriyama et al., 1989). The fern gametophytes might also grow photoheterotrophically or photomixotrophically (Katoh, 1983; Kuriyama et al., 1989), as its growth depended on the illumination (data not shown). In *Pteridium aquilinum*, sporophytes were formed apogamously if gametophytes were cultured on sugar-added media (Whittier, 1964). In *Adiantum capillus-veneris*, sporophyte formation was not induced even though the gametophyte growth was enhanced on the sugar-added media. This result suggested that other factor was involved in the formation of sporophytes.

In *Adiantum capillus-veneris*, apogamous production of sporophyte did not occurr by culturing gametophytes in MS or 1/2MS medium. However, elimination of NH_4NO_3 from the culture medium or reduction of total nitrogen content induced the production of sporophytes by sexual reproduction. Antheridia and archegonia appeared on the gametophytes cultured in any media used in this study. Therefore, the original nitrogen content of MS medium might be inhibitory to fertilization or the developmental process of fertilized eggs.

In plant tissue culture, the nitrogen source as well as plant hormones is important factor for differentiation and morphogenesis (Thorpe, 1980). In the leaf culture of *Adiantum capillus-veneris* sporophyte, higher concentrations of nitrogen source promoted the formation of abnormal leaves (Pais and Casal, 1987). In apogamous sporophyte formation of *Equisetum arvense*, nitrogen source and level regulated sporophyte formation

(Kuriyama et al., 1992). In this experiment, the importance of nitrogen source and its level for the sporophyte formation by sexual reproduction was demonstrated.

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ホウライシダ胞子体植物の生産のための培地の検討

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摘 要

ホウライシダ (*Adiantum capillus-veneris*) 配偶体 (前葉体) から有性生殖で胞子体植物を形成させるための培地を検討した. MS培地にショ糖を加えることにより配偶体の増殖速度は高まり, 3%の添加が最も効果的であった. しかしショ糖の添加は胞子体形成には有効ではなかった. MS培地からNH_4NO_3を除去した場合, 胞子体が形成されたが, KNO_3の除去では胞子体の形成はみられなかった. また, MS培地の全窒素量を25%に希釈することによっても胞子体誘導は可能であった. これらの結果より, ホウライシダの有性生殖による胞子体形成にとって, 培地の窒素源は重要な制御要因であると推定された.