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Short Communication

A Method for the Rapid Growth in Culture of Gametophytes of *Equisetum arvense* with Antheridia

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Rapid growth in culture of *Equisetum arvense* gametophytes was obtained using Murashige-Skoog's medium plus 3% (w/v) sucrose and continuous illumination. In darkness, growth was reduced and chlorophyll synthesis markedly inhibited. Antheridia formed on the top margin of gametophytes in light and darkness but archegonia were not observed in either case.

Key words: Antheridium formation — *Equisetum arvense* — Gametophyte — Light-dependent growth — Pteridophytes — Sex organ differentiation.

The gametophytes of pteridophytes can be cultured in vitro on simple defined media in a fully controlled environment (Brandes 1973), and they have been used for studies of growth, development and differentiation (Bopp 1968, Miller 1968, Sheffield and Bell 1987). However, because of difficulties of obtaining large amount of tissue from gametophytes, some research e.g., biochemical analysis, has been limited (Miller 1968). In the case of *Equisetum* gametophytes, large quantities of tissue have also not been available although successful culture in vitro was reported by Sporne (1964), Duckett (1970a, 1970b, 1972) and Hauke (1971).

In this report, we describe the establishement of a culture system for the rapid growth of *Equisetum arvense* gametophytes and some features of the system, as well as the characteristics of the differentiation of the sex organs.

To initiate the culture of gametophytes, spores of *Equisetum arvense* were harvested from strobili of sporophyte plants grown near Saitama University. Collected strobili that had not yet dehisced were washed with tap water and sterilized by immersion in 70% ethanol for 1 min, and in a 1% solution of sodium hypochloride for 10 min. The strobili were rinsed with sterile distilled water and placed on filter paper in a petri dish. To desiccate the strobili, the petri dish was put into a desiccator and kept in the shade at room temperature. After 7 days, collected spores were sowed on one-fifth strength Murashige-Skoog's agar medium (Murashige and Skoog 1962) (MS medium) without sugar and cultured at 26°C in continuous light at 1,000 lux (Toshiba fluorescent lamp FL 40s W and 40s BFL, Toshiba Co. Ltd., Tokyo Japan). Sowed spores germinated in 2 days and formed colonies of young gametophytes within 2–3 weeks. Gametophytes derived from a single spore were transferred to petri dishes that contained full strength MS medium supplemented with various concentrations (0–5% (w/v)) of sucrose. The gametophytes showed the highest growth rate in 3% (w/v) sucrose, and one line of gametophytes was chosen for subculturing. Subculturing was carried out as follows. A mass of gametophytes was cut in pieces and each piece was transplanted to fresh MS medium supplemented with 3% (w/v) sucrose and cultured at 26°C in continuous light, with transfer to fresh medium at intervals of 1 month.

In order to examine growth characteristics of the gametophytes in established clutures, fresh weight, dry weight, and chlorophyll content were determined periodically. Dry weight was measured by drying the gametophyte tissues in an oven at 100°C overnight. Chlorophyll was extracted by 5 or 6 treatments with 80% (v/v) acetone and determined by measurements of optical density at 645 nm and 663 nm (Arnon 1949).

Fig. 1 shows the fresh and dry weights of gametophytes cultured in the light for 40 days with different

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concentrations of sucrose. As was expected, the optimal growth rate was obtained from the medium supplemented with 3% (w/v) sucrose. At higher concentrations of sucrose (4 and 5% (w/v)) growth was inhibited and reductions in fresh weights were particularly apparent. On sucrose-free medium, growth of gametophytes was as poor as that on Knop's medium (Knop 1865).

Illumination was also necessary for the rapid growth of gametophytes. Fig. 2 and 3 show the growth curves and changes in chlorophyll content of *Equisetum arvense* gametophytes cultured on MS medium supplemented with 3% (w/v) sucrose in light or darkness. Gametophytes in the light grew exponentially from the start of culture for approximately 20 days and reached a stationary phase after about 30 days. As growth proceeded, total chlorophyll content also increased. By contrast, in cultures in the dark, green gametophyte tissue changed to yellow or white and growth was markedly inhibited. Any increase in chlorophyll content was barely recognizable.

The morphology of the gametophytes was stable through subculturing under continuous light and a number of antheridia formed successively on the top margin of the gametophyte tissue (Fig. 4). When gametophyte tissue was placed in water, numerous sperm were liberated (Fig. 5). Even in darkness, the formation of antheridia was observed but archegonia were seen neither in light- nor in dark-grown cultures.

A method for the rapid growth in culture of *Equisetum arvense* gametophytes has been established using MS medium supplemented with 3% (w/v) sucrose. This culture system provides experimental material constantly and throughout the year, as to callus cultures or suspension cultures of cells of higher plants. However, the growth characteristics of *Equisetum arvense* gametophytes

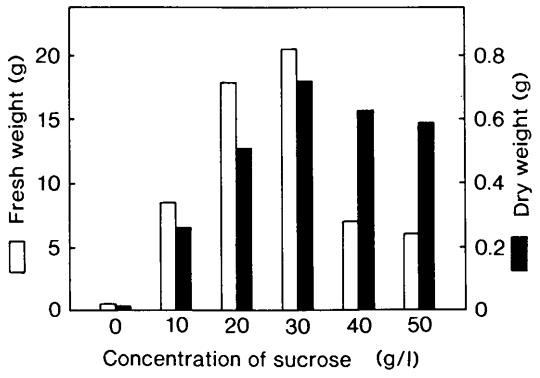

Fig. 1 Fresh and dry weights of *Equisetum arvense* gametophytes cultured on Murashige and Skoog's medium with different concentraions of sucrose under continuous illumination for 40 days. Samples of 0.1 mg (fr wt) of gametophyte tissue, cultured for 14–18 days, were placed on 40 ml of agar medium in 100-ml Erlenmeyer Flasks and cultured for 40 days.

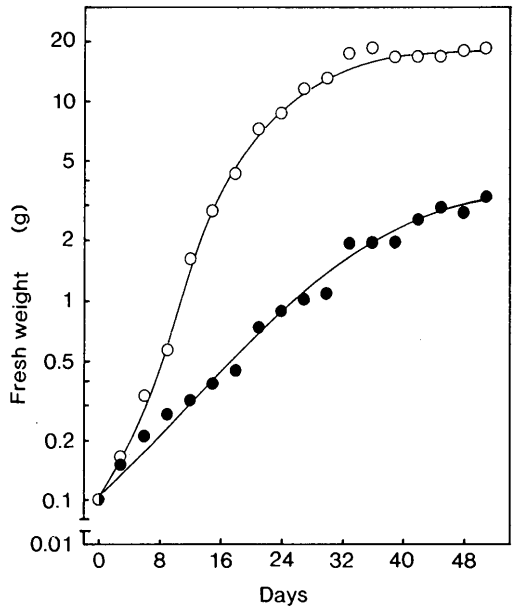

Fig. 2 Growth curves for *Equisetum arvense* gametophytes cultured on Murashige and Skoog's medium with 3% (w/v) sucrose under continous illumination (○) and in darkness (●). Samples of 0.1 mg (fr wt) of gametophyte tissue, cultured for 14–18 days, were placed on 40 ml of agar medium in 100-ml Erlenmeyer Flasks and cultured.

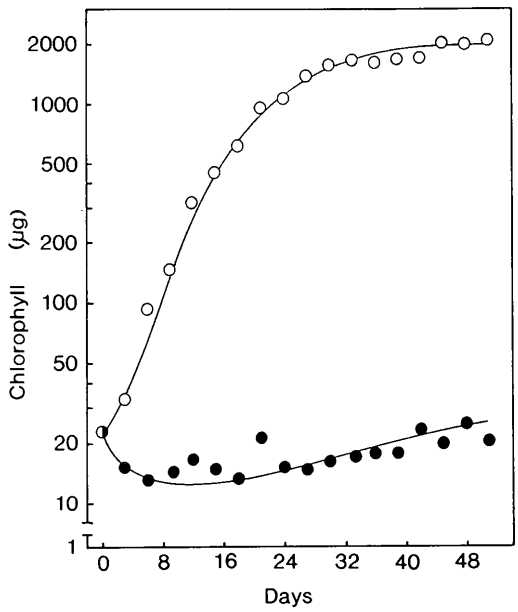

Fig. 3 Increases in chlorophyll content of *Equisetum arvense* gametophytes cultured on Murashige and Skoog's medium with 3% (w/v) sucrose under continuous illumination (○) and in darkness (●). Samples of 0.1 mg (fr wt) of gametophyte tissue, cultured for 14–18 days, were placed on 40 ml of agar medium in 100-ml Erlenmeyer Flasks and cultured.

Fig. 4 Subculturing of *Equisetum arvense* gametophytes on Murashige and Skoog's medium supplemented with 3% (w/v) sucrose. Arrows show antheridia. Bar represents 500 μm.

were different from those of cultured cells of higher plants. For a higher growth rate, *Equisetum arvense* gametophytes have an absolute requirement for illumination, while cultured cells of higher plants can usually grow at a reasonable rate without illumination if a suitable carbon source is provided in the medium. Light-dependent growth was reported in the case of *Marchantia polymorpha* cells in suspension culture in the presence of glucose as a carbon source (Ohta et al. 1977), and further studies of photosynthesis and photoassimilation of glucose revealed that *Marchantia polymorpha* cells grow photomixotrophically (Katoh 1983). The results of the present study suggest the possibility that growth of *Equisetum arvense* gametophytes is photomixotrophic or photoheterotrophic under our culture conditions. Further investigations are needed to resolve this issue.

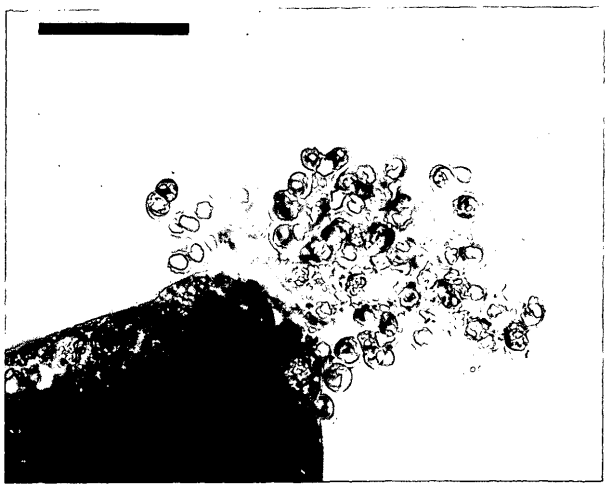

Fig. 5 Liberation of sperm from an antheridium of a gametophyte of *Equisetum arvense*. Bar represents 100 μm.

Antheridia differentiated on the top margin of gametophytes in clutures, both in light and in darkness, through successive subculturing but formation of archegonia was not observed when MS medium and sucrose were used. The quality and intensity of light regulates the diffentiation of sex organs in *Equisetum* gametophytes (Hauke 1971). In addition to light conditions, temperature and duration of culture have been shown to be involved in the control of the diffentiation of sex organs (Duckett 1970b, 1972). An examination of the factors that control such differentiation is being carried out using our system for the culture of gametophytes.

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