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Enhanced Production of Ethylene from *Equisetum arvense* Tissue during Sporophytic Shoot Formation

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All plant tissues are capable of producing ethylene, although the production rate is normally low[1]. Rates of ethylene production vary in different organs and tissues and are dependent on growth and developmental stages[2]. Gametophytes of *Equisetum arvense* cultured in vitro can grow rapidly on Murashige and Skoog's[3] medium that contains 3% (w/v) sucrose but no growth regulators (MS medium) under continuous light[4], while cytokinin supplemented to the culture medium shift their growth from gametophytic to sporophytic without fertilization[5,6]. It is hypothesized that rates of ethylene production vary in these different developmental states.

The gametophyte culture was initiated from a single spore of *E. arvense* and subcultured every 30 days on 40ml MS agar (0.8%) medium in a 100ml flask under continuous light as described previously[4]. Gametophyte tissues cultured for 14-18 days were cut in pieces (2-3mm in length and thickness) by razor and transplanted onto 10ml fresh MS agar medium with or without Benzylaminopurine (BA, 1×10^{-6}M) in test tubes (20mm×150mm). Explanted tissues in test tubes capped with aluminium foil were cultured at 26°C under continuous light (1000 lux). For ethylene measurements, test tubes were newly capped with silicon rubber septum. After 24 hr, a 2m*l* gas sample was withdrawn from the head space of the test tube with a hypodermic syringe and the ethylene content was measured by a gas chromatographic apparatus (Hitachi 163), at 80°C, equipped with a hydrogen flame ionization detector with activated alumina as column packing[7].

Gametophyte tissues cultured on MS medium without BA grew vigorously and formed a large

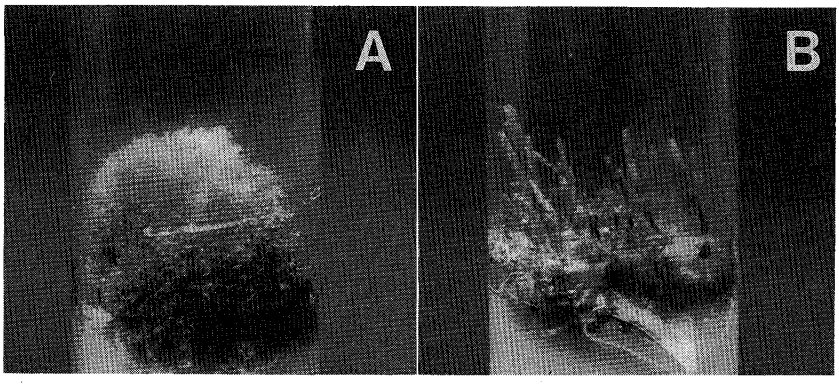

Fig. 1A : Mass of gametophytes formed after 40 days of culture on MS medium.
Fig. 1B : Sporophytic shoots formed after 40 days of culture on MS medium supplemented with 1×10^{-6}M BA.

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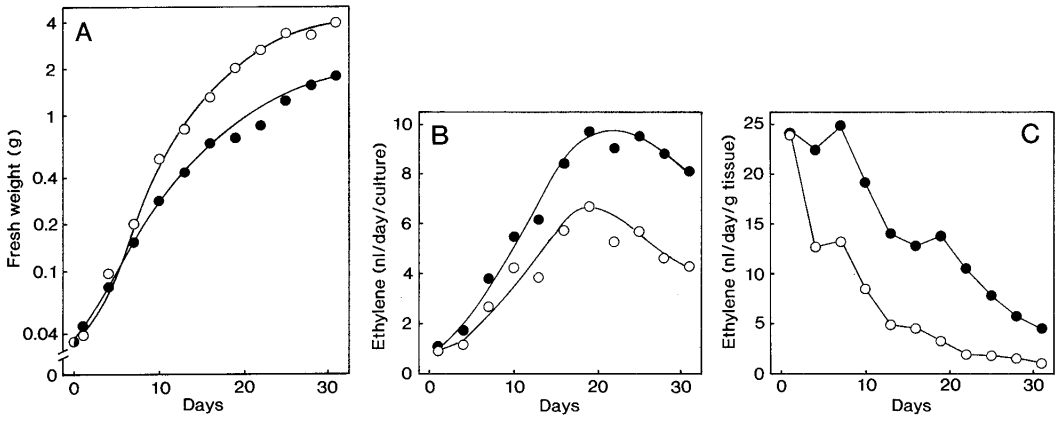

Fig. 2: Effect of BA on growth (A), ethylene production (B and C) of *Equisetum arvense* cultured in vitro. Gametophyte tissues 2-3mm in thickness and length were transplanted to MS medium with (●) or without (○) 1×10^{-6}M BA and cultured in light. Ethylene production/day/culture (B) and ethylene production/day/gtissue (C) are shown. Each value shows average of three measurements.

gametophytic mass after 40 days of culture (**Fig. 1A**). On the other hand, a number of sporophytic shoots were produced from explanted gametophyte tissues, which were cultured on MS medium with 1×10^{-6}M BA (**Fig. 1B**). The average number of shoots formed after 40 days of culture per a test tube was 86. 1±31. 2. Growth curves were determined in the respective two cultures by periodic measurements of fresh weights. As shown in **Fig. 2A**, rate of gametophytic growth was higher than that of sporophytic growth. In contrast with growth rate, more ethylene was produced from cultures of sporophytic growth than from ones of gametophytic growth. In both types of cultures, ethylene production per culture increased and reached optimal values and then began to decrease at around the 20th day (**Fig. 2B**). This culture stage corresponded to the late exponential phase or early stationary phase. As shown in **Fig. 2C**, the capacity of a gram tissue to produce ethylene was highest immediately after transplant of experimental material to fresh medium in both types types of cultures. After that, ethylene produced from a gram tissue decreased rapidly in the culture without BA. In the culture with BA, the capacity of a gram tissue to produce ethylene was sustained until the 7th day and then began to decease.

Sporophytic shoots were produced from *E. arvense* gametophytes in response to exogenously supplied cytokinin. Total ethylene production was higher in cultures of sporophytic growth than in ones of gametophytic growth while the growth rate shown by fresh weight reduced along with the sporophytic shoot formation by cytokinin. As a matter of course, there were significant differences in the rate of ethylene production per a gram tissue between the cultures with and without cytokinin except for the initial 24 hr in culture. Probably, higher values of initial 24 hr depend on the cut and transplant of experimental materials to fresh medium which becomes in itself a stress to induce ethylene production. Cytokinin is known to be involved in the induction of ethylene production[1]. Indeed, it can be considered that exogenously supplied cytokin is a mere enhancer of ethylene production in the culture of *E. arvense*. However, we must pay attention to the relation between sporophytic shoot formation and ethylene production. It is well known in higher plants that ethylene produced from cultured cells or tissues enhances[8-10] or inhibits[11-13] the adventitious shoot formation. Now, it is impossible to conclude whether ethylene produced from *E. arvense* tissue is promotive to the production of a number of sporophytic shoots or not. However, there is the possibility that also in pteridophyte *E. arvense* ethylene may be involved in shoot formation.

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《和文要約》

スギナ (*Equisetum arvense*) 胞子体植物形成時におけるエチレン生成量の増大について

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スギナ (*Equisetum arvense*) 配偶体組織は MS 培地上，連続光下で培養すると配偶体の体制のまま活発に増殖する．一方，MS 培地にさらにサイトカイニン (BA) を加えた培地で培養すると，多くの胞子体 Shoot の形成がみられる．それぞれの条件下で培養した組織からのエチレン生成量を比較したところ，胞子体 Shoot 形成に伴ってエチレン生成量が増大することが明らかになった．