Callus Induction from Yam, 
*Dioscorea opposita* Thunb., and The Subculture

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The yam tuber is a high class vegetable in Japan. We have found chitinase in yam tubers, and electrophoretically purified the enzyme in high yield. Yam chitinase is useful for preparation of protoplasts from some basidiomycetes such as *Tricholoma matsutake, Lentinus edodes,* and *Lyophyllum shimeji.* Recently, the physiological role of plant chitinase has been proposed to be self defense against plant pathogens. To study the induction of chitinase from yam tissue by treatment with plant pathogens or elicitors, the callus is required. In this study, we induced calli from yam tubers and established subcultures.

Bud shoots from tubers of Yam (*Dioscorea opposita* Thunb.) was sterilized with 1% Brij 3.5 for 10 sec, 70% ethanol for 5 to 6 sec, and 0.1% mercuric bromide for 10 min, and placed on Murashige–Skoog's agar (1%) medium containing 10 μM 2,4-dichlorophenoxyacetic acid. 

Fig. 1. Callus of Yam, *Dioscorea opposita* Thunb.
The callus was induced from a bud of yam tuber on Murashige–Skoog's agar (1%) medium containing 10 μM 2,4-D, 0.1 μM kinetin, and 5% sucrose in the presence of 0.1% activated charcoal in the dark at 27°C for 8 weeks. The induced callus (about 0.3 g) was then transferred to the same medium in the absence (A) or presence (B) of 0.1% activated charcoal, and cultured in the dark at 27°C for 8 weeks. The diameter of the test tube is 2.5 cm.

Fig. 2. Effects of 2,4-D and Kinetin on the Growth of Yam Callus.
The callus (about 0.2 g) was transferred onto Murashige–Skoog's agar (1%) medium containing various concentrations of 2,4-D and kinetin in the presence of 0.1% activated charcoal for 8 weeks in the dark at 27°C. The callus was harvested and weighted. Mean values, n = 3.
The callus (about 0.2 g) was cultured on Murashige-Skoog's agar (1%) medium containing 100 \( \mu \text{M} \) 2,4-D and 10 \( \mu \text{M} \) kinetin in the presence of 0.1% activated charcoal in the dark at 27°C. After the given time, the callus was harvested and weighted. Mean values ± SE, \( n=2 \).

The callus was induced within 4 weeks. The induced callus was then transferred to the same medium in the presence or absence of 0.1% activated charcoal, and cultured in the dark at 27°C for 8 weeks. The results are shown in Fig. 1. The presence of activated charcoal in the medium was necessary for the growth of yam callus. The optimum conditions for subculture of yam callus were further investigated using various concentrations of the plant hormones 2,4-D and kinetin up to 100 \( \mu \text{M} \) in the same medium. The results are shown in Fig. 2. The growth was strongly influenced by the amounts of the plant hormones. The optimum concentrations of 2,4-D and kinetin were 100 \( \mu \text{M} \) and 10 \( \mu \text{M} \), respectively. One hundred \( \mu \text{M} \) 2,4-D is a high concentration for culture of the usual plant calli. We furthermore compared the effects of 100 and 200 \( \mu \text{M} \) 2,4-D on growth under the same conditions in the presence of 10 \( \mu \text{M} \) kinetin. However, no significant difference was observed between both cases. The growth curve of the yam callus in the optimum medium using 100 \( \mu \text{M} \) 2,4-D and 10 \( \mu \text{M} \) kinetin is shown in Fig. 3. The growth reached a plateau within 50 days.

Callus cultures of some other Dioscorea species were reported. However, induction of callus from Dioscorea opposita Thunb. and the subculture had not been done. One of the reasons is browning of the callus. The callus turned brown and died in the MS medium containing only 2,4-D and kinetin. In this study, we succeeded in the callus culture in the presence of 0.1% activated charcoal. The phenomenon of browning seems to be a result of lignification of plant cell walls for self defense. However, if such a response is excessive, phenolic compounds would accumulate too much, lignification would proceed, and the callus would die. Activated charcoal seems to adsorb some phenolic compounds and prevent such excessive lignification. On the other hand, relatively high concentrations of plant hormone, especially 2,4-D (100 \( \mu \text{M} \)), were required for the optimal subculture of yam callus in the presence of activated charcoal, compared with the usual conditions (about 1 \( \mu \text{M} \)). 2,4-D may also be adsorbed to activated charcoal, and the available amount in the medium decreased.

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References