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Cotyledon Induction from Juice Vesicles of *Citrus hassaku* Cultured *in Vitro*

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Introduction

In vitro culture of mature juice vesicles of lemons^{1,3)} and oranges⁴⁾ has been tried. Calluses were induced in these trials, but not developed into shoots nor roots. This paper reports a case of cotyledon induction from pseudobulbils, differentiated from callus obtained from immature juice vesicles of 'Hassaku' fruit, *Citrus hassaku* Hort. ex Tanaka.

Materials and Methods

Hassaku fruit in a younger stage, 2.4 cm in diameter, collected five weeks after pollination with *Poncirus trifoliata* (L.) Raf., was surface-sterilized with 80% ethanol. Juice vesicles, two to three mm in length, were excised aseptically, and placed in the 50 ml Erlenmeyer flasks containing Murashige and Skoog's basal medium²⁾ supplemented with 30 g/liter sucrose, 10 g/liter agar, 400 mg/liter malt extracts, 0.5 mg/liter indoleacetic acid (IAA) and 0.1 mg/liter kinetin. The pH was adjusted to 5.8 with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide. Twenty vesicles were placed in each flask, replicating five times. Flasks were placed in a growth chamber with 16 hr light (30°C) and 8 hr dark (19°C). Infrared light of 3000 Lux was used as the light source.

Results and Discussion

After one week's culture, callus began to differentiate from the base of the juice vesicles. This callus grew rapidly and then turned into green color (Fig. 1). Some juice vesicles grew up to two to three times of their initial size after the callus differentiation. Callus developed only at the base of the vesicles. Among all the juice vesicles cultured, only two produced pseudobulbils in calluses. The pseudobulbils eventually developed cotyledon-like structures in about two to three weeks (Fig. 2). Each structure consisted of two cotyledons.

Kordan¹⁾ cultured mature juice vesicles of lemon fruit on an agar medium of Hoagland solution supplemented with several micronutrients. He reported the induction of callus which developed into lamina-like masses of tissue as a result of horizontal growth. Unger and Feng⁴⁾ cultured mature juice vesicles of orange fruit in Murashige and Skoog's medium containing IAA and kinetin. They obtained callus which was rough and elevated, and observed the differentiation of vascular bundles.

However, the induction of shoot and root differentiation has not been reported in these cases. The lack of differentiation may reasonably be due to the large amount of organic acids and low

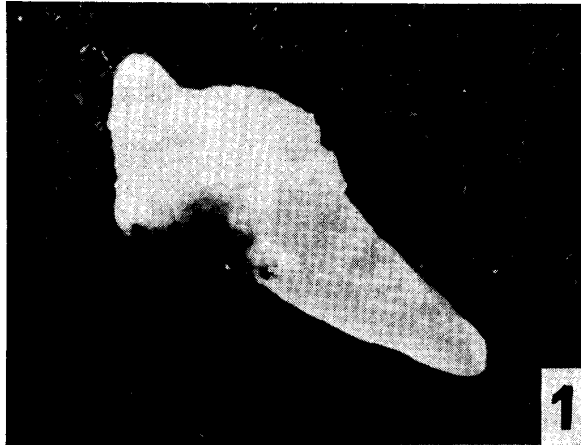


Fig. 1. Callus developed at the base of a juice vesicle.

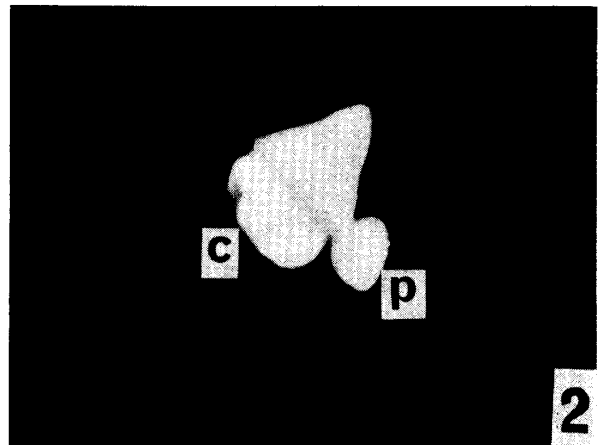


Fig. 2. Cotyledon-like structure differentiated from a pseudobulbil.

c : cotyledon-like structure
p : pseudobulbil

pH in mature juice vesicles. The accumulation of organic acids was not set in the immature juice vesicles used in the study. It may also be probable that those vesicles are in possession of a higher meristematic activity. Further investigation is needed on what developmental stage the juice vesicles lose their totipotency. Trials are under way to get an entire plant from the pseudobulbils with cotyledon-like structures.

Summary

Cultured *in vitro* with Murashige and Skoog's medium supplemented with IAA and kinetin, immature juice vesicles of *Citrus hassaku* Hort. ex Tanaka produced callus, which developed itself into pseudobulbils. The pseudobulbils eventually developed cotyledon-like structures.

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