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武庫川女子大紀要（自然科学）

Plant Regeneration from Hypocotyl-derived Calli of *Gardenia jasminoides*

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The fruits of *Gardenia jasminoides* are used as the coloring agent of various foods. In this paper, we report successful results in the induction of callus and plant regeneration from flesh and leaf cultures. The high frequency of embryogenic callus formation from fruit was obtained with the medium containing 0.01μM of IAA and 0.01μM of kinetin. On the other hand, the better results were obtained with the medium containing more than 0.1μM of 2,4-D.

Introduction

The fruits of *Gardenia jasminoides* Ellis f. *grandiflora*(Lour.)Makino(Rubiaceae)are used as an antiphlogistic and cholagogue under the name of Shan-zhi-i(in Chinese)or San-shi-shi(in Japanese)[1]. And also, the fruits are used as the coloring agent of various foods.

In this paper, we report the induction of callus and plant regeneration from flesh and leaf cultures of *Gardenia jasminoides*.

Materials and Methods

Induction and culture of callus

A herb garden grown *G. jasminoides* plants of approximately ten years of age were used (Fig.1.). Fruits of this plant were rinsed in 70%(v/v)ethanol for 30℃ seconds, sterilized by immersion for 5 minutes in 5% sodium hypochlorite solution containing 0.01 ml/L of Tween 80, and rinsed three times in sterilized distilled water. The sterilized fruits were cut into 1cm diameter x 1cm diameter blocks with a surgical knife and placed on Murashige and Skoog medium(MS medium)[2] supplemented with 3% sucrose, various concentration(0, 0.01, 0.1, 1, 10μM)of 2,4-dichlorophenoxyacetic acid(2,4-D), 1-naphthaleneacetic acid(NAA), indole-3-acetic acid(IAA), 6-benzyladenine(BA) and kinetin. The medium was solidified by 0.2% Gelrite. The culture were incubated in dark at 25℃.

Plant regeneration from somatic embryo

After 30 days of culturing on MS growth regulator-free medium, somatic embryos formed in fruit calli were transferred to the plant regeneration medium containing half-strength MS salts and vitamins, 5% sucrose and 0.2% Gelrite. Plantlets derived from somatic embryos were transferred to the growth medium containing one-half strength MS medium and 0.2% Gelrite. Somatic embryos and regenerated plantlets were incubated at 25℃ under cool white fluorescent light(6,000 lux)with a 16 hour-photoperiod.

Results and Discussion

Induction and culture of callus

Callus formation from fruit explants was observed within 8-36 days of culture as shown in Fig.2. The media containing IAA and BA effectively induced callus.

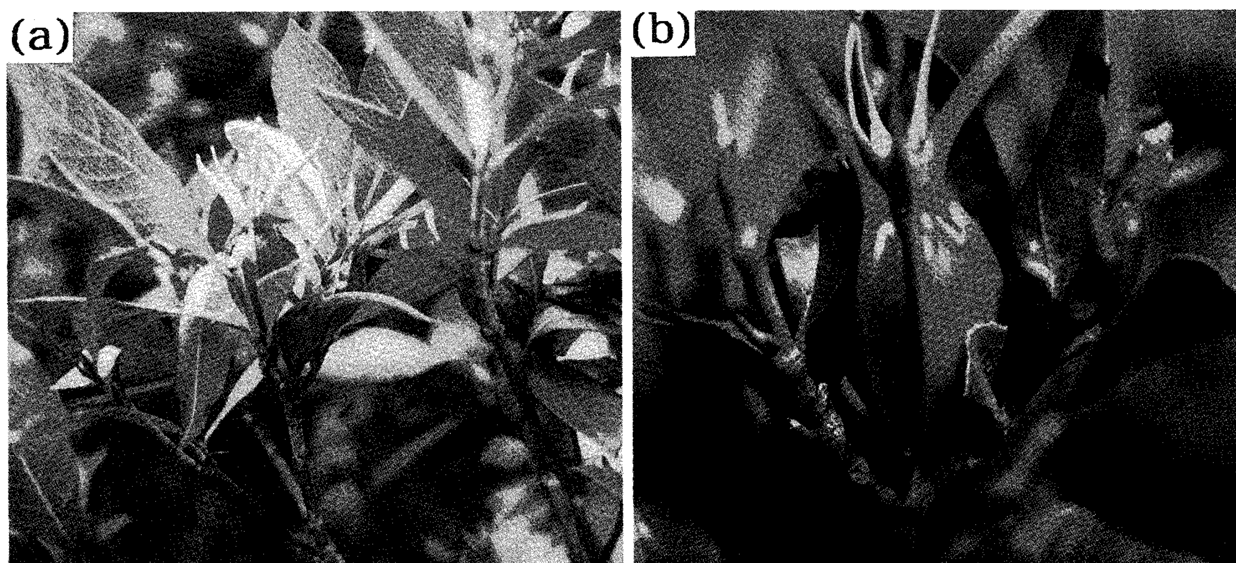

Fig. 1. *Gardenia jasminoides*((a), (b)).

Two types of calli were formed: one was transparent and friable(Fig.3.(a))and the other was opaque green and compact(Fig.3.(b)).

Fig.4. shows the frequency of embryogenic callus formation from fruit after 44 days culture. In particular, the better results were obtained with the medium containing 0.01μM of IAA and 0.01μM of kinetin(Fig.4.(a)). On the other hand, the better results were obtained with the medium containing more than 0.1μM of 2,4-D(Fig.4.(b)).

Plant regeneration from somatic embryo

Since somatic embryos developed slowly and occasionally ceased to grow on MS growth regulator-free medium, they had to be transferred onto the plant regeneration medium to develop tubers from the embryos(Fig.5.). When tubers with buds and roods were transferred to the growth medium, they further developed shoots and roods(Fig.6.(a)). Approximately 90% of them developed into mature plants in a growth chamber, after plantlets of 10 flesh were transferred to pots.

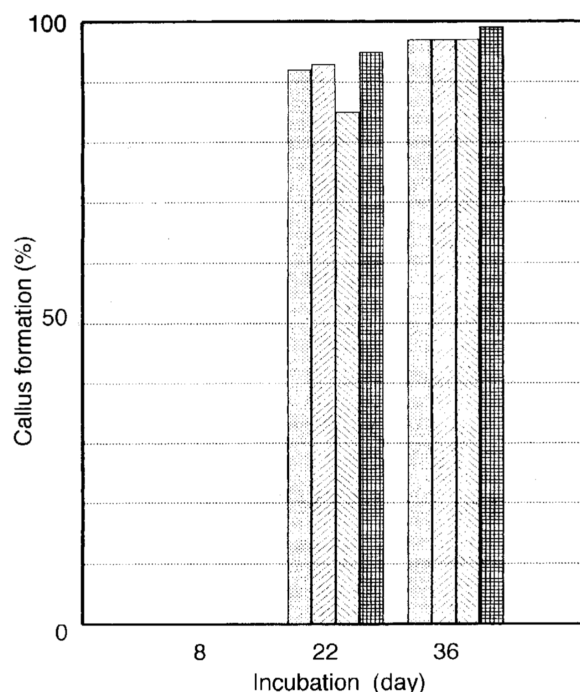

Fig. 2. Effects of NAA, IAA, BA and kinetin on callus formation in *G. jasminoides* flesh.
[dotted], NAA and kinetin; [hatched /], NAA and BA; [hatched \], IAA and kinetin; [grid], IAA and BA.

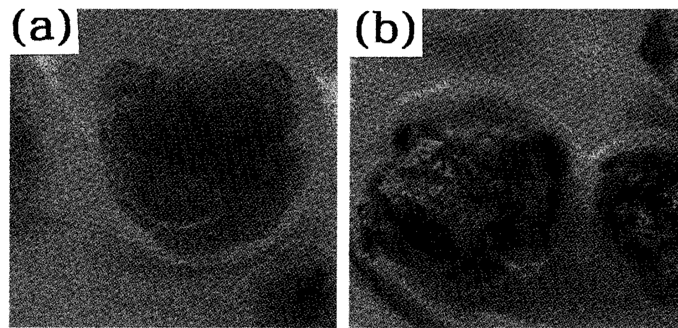

Fig. 3. Callus formation from a peeled flesh of *G. jasminoides*.
(a) Callus of transparent and friable type.
(b) Callus of opaque green and compact type.

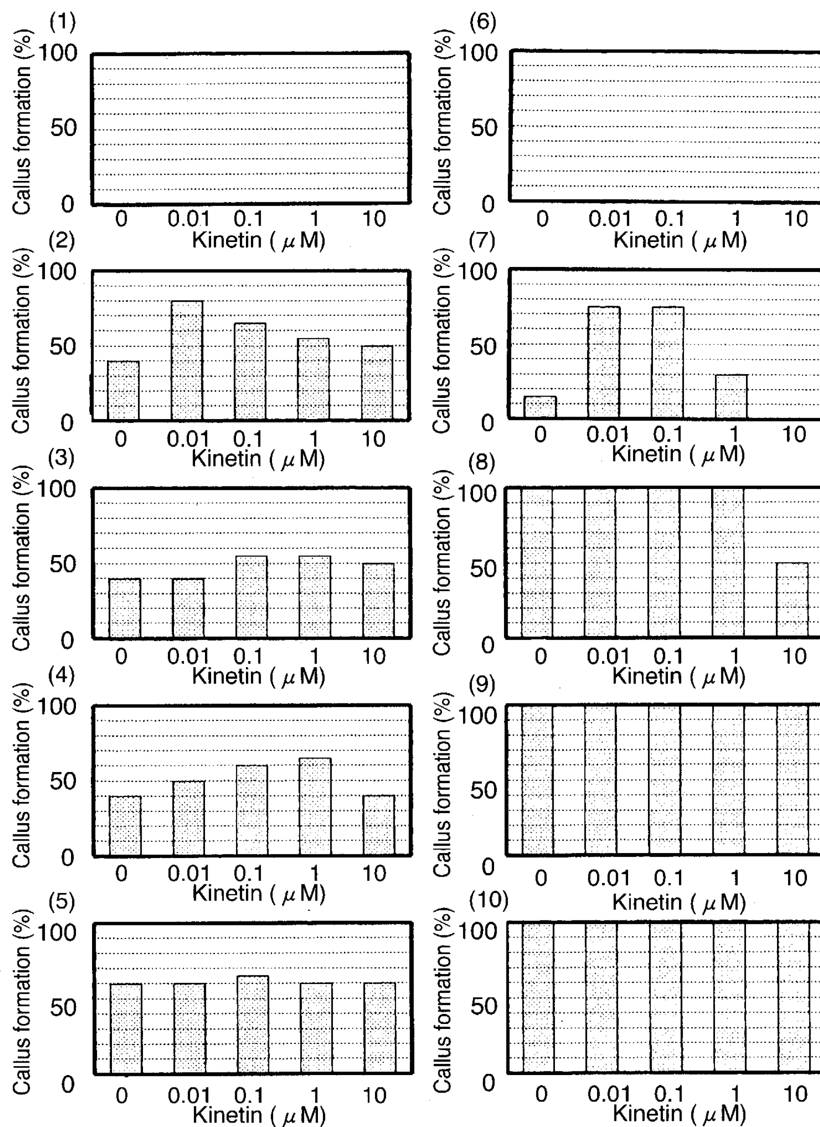

Fig. 4. Effects of concentration of IAA, 2,4-D and kinetin on callus formation in *G. jasminoides* flesh.
(1), 0; (2), 0.01; (3), 0.1; (4), 1; (5), 10μM concentration of IAA and (6), 0; (7), 0.01; (8), 0.1; (9), 1; (10), 10μM concentration of 2,4-D was added to the medium, respectively. Callus was observed after 44 days culture.

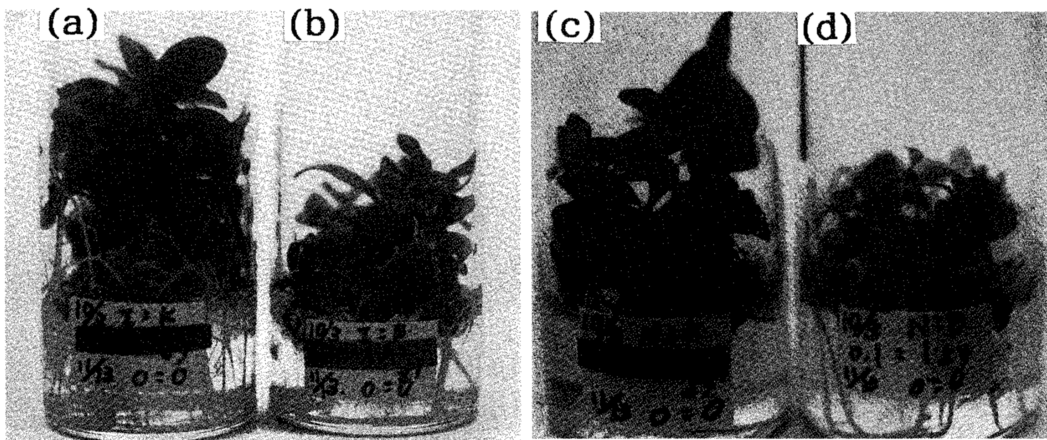

Fig. 5. Plantlet growing on plant growth medium.
Plantlet was derived from callus on the medium containing IAA(0.1μM) and kinetin(1μM)(a), IAA(0.1μM) and BA(1μM)(b), NAA(10μM) and kinetin(1μM)(c) and NAA(10μM) and BA(1μM)(d).

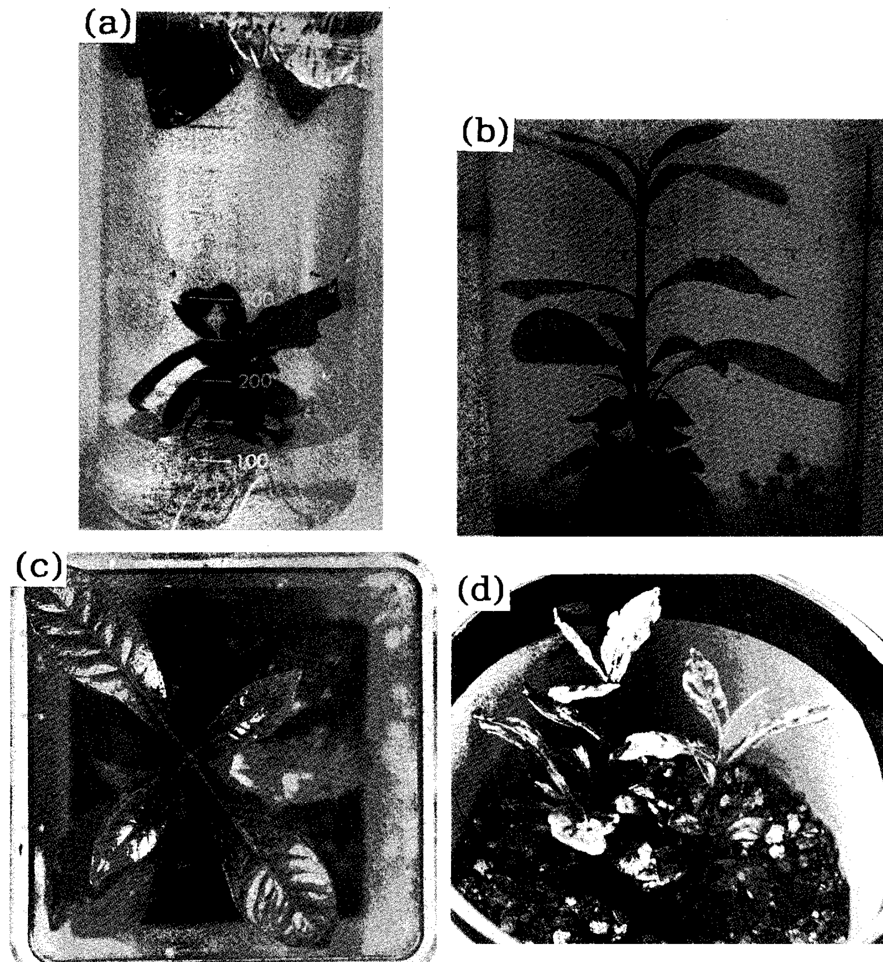

Fig. 6. *G. jasminoides* via callus.
(a) A plantlet growing on plant growth medium
(b) Plantlets transferred to pots (side).
(c) Plantlets transferred to pots (upside).
(d) Plants that grown in a greenhouse.

Regenerated plants were transferred to pots containing a 2 : 1, vermiculite and perlite mixture (Fig.6.(b), (c)). These potted plants were maintained at 25℃ under a 16 hour–photoperiod (6000 lux)in a growth chamber. And then, 3 plants have grown in a greenhouse(Fig.6.(d)), and have exhibited no morphological variation.

Acknowledgments

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References

1) Endo, T. and Taguchi, H., *Chem. Pharm. Bull.*, **21**, 2684–2688(1973)
2) Murashige, T. and Skoog, F., *Physiol. Plant.*, **15**, 473–497(1962)