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Induction of Callus from Japanese Hackberry (*Celtis sinensis* Pers. var. *japonica* Nakai) and its Root Organogenesis

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エノキの組織培養と不定根の誘導

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Résumé

A green compact callus was induced from sterilized shoots of Japanese hackberry (*Celtis sinensis* Pers. var. *japonica* Nakai) on a Murashige-Skoog medium supplemented with 1.0mg/ℓ 1-naphthaleneacetic acid (NAA) and 0.1 mg/ℓ 6-benzylaminopurine (BAP). The callus grew fast on this medium in PLANTEX to attain 9 fold amount of dry weight at 9 weeks incubation at 25℃ with a continuous illumination of 2,500 lux. Roots were regenerated from the callus after incubation for 4 weeks under dark at hormonal combination of 0.5mg/ℓ NAA and 1.0 mg/ℓ BAP at a relatively high frequency of 67%.

和文要旨

エノキ (*Celtis sinensis* Pers. var. *japonica* Nakai) の成木の新枝よりナフタレン酢酸 (NAA) 1.0 mg/ℓ, 6-ベンジルアミノプリン (BAP) 0.1 mg/ℓを含むイオン強度を半分にした改変 Murashige-Skoog (MS) 培地を用い緑色カルスを誘導した。このカルスの生育は早く，プランテックスを用いて25℃で 2,500 lux の照光下培養した場合，9週間で絶乾重量が9倍になった。このカルスを0.5 mg/ℓのNAAと1.0 mg/ℓのBAPを含む改変MS培地を用いて暗黒下培養したところ，67%の率で根が誘導された。

1. INTRODUCTION

Japanese hackberry (*Celtis sinensis* Pers. var. *japonica* Nakai) is one of the several trees of the genus *Celtis* in the elm family (*Ulmaceae*) and distributed in temperate areas in Japan. It has bright green ovate-lanceolate leaves with singly toothed margins, a slightly curved tapering apex, and three prominent veins arising from the base of the blade. It has been often used as a milepole and planted as garden and park trees, attaining heights of about 20 m and producing edible purplish-black fruits in autumn. In addition, it has often become a host of evergreen mistletoe. While investigating host-parasite interactions between

mistletoe and its host, we previously induced mistletoe and beech calli and examined what happened when both calli were co-cultured[1]. However, since growth of the green beech callus was not fast enough to investigate further the host-parasite cell-cell interactions, it is desired to develop a new *in vitro* system which permits to carry out a rapid analysis. Induction of callus from *Ulmaceae* has been focused on elm[2-4] and zelkova[5] trees, but no work has so far been reported about *in vitro* cell cultures of *Celtis*.

The purpose of this research is to describe a procedure for the induction of rapid growing callus from Japanese hackberry which is able to regenerate root.

2. EXPERIMENT

2.1 Plant

Newly born shoots of Japanese hackberry (*Celtis sinensis* Pers. var. *japonica* Nakai) growing at the Experimental Forest Station on the Campus of Kyoto University, Sakyo-ku, Kyoto, was harvested in May and June, 1991.

2.2 Induction of calus

The shoots were cut into small pieces, which were sterilized and incubated on a modified Murashige-Skoog medium (MS)[1] having a half of the ionic strength of the original medium[6] containing 1% agar as shown in Table 1. Preliminary experiments indicated that kinetin was not a favorable cytokinin for induction of callus as was the case of beech[1]. Therefore, the induction of callus was operated under various combinations of 0.1-10.0 mg/ℓ of 1-naphthaleneacetic acid (NAA) and 0.01-5.0 mg/ℓ of 6-benzylaminopurine (BAP) at 25℃with a continuous illumination of about 2,500 lux (cool white fluorescent tubes). The induced green callus was subcultured in the same modified MS medium supplemented with 1.0 mg/ℓ BAP and 0.1 mg/ℓ NAA.

Table 1 Composition of the half - ionic strength Murashige - Skoog medium (pH 5.7)

Constituent	Final conc. (mg/ℓ)	Constituent	Final conc. (mg/ℓ)
NH_4NO_3	825	KI	0.415
KNO_3	950	$Na_2MoO_4 \cdot 2H_2O$	0.125
$CaCl_2 \cdot 2H_2O$	220	$CuSO_4 \cdot 5H_2O$	0.0125
$MgSO_4 \cdot 7H_2O$	185	$CoCl_2 \cdot 6H_2O$	0.0125
KH_2PO_4	85	*myo*-Inositol	100
$MnSO_4 \cdot 4 \sim 6H_2O$	11.15	Glycine	2
$ZnSO_4 \cdot 7H_2O$	4.3	Nicotinic acid	0.5
H_3BO_4	3.1	Pyridoxine-HCl	0.5
Na_2EDTA	18.63	Thiamine-HCl	0.1
$FeSO_4 \cdot 7H_20$	13.98	Sucrose	30000

2.3 Growth rate of callus

About 150 mg of callus in wet state was planted on a presterilized polyester fiber mat in a PLANTEX CCP-102 (TOYOBO Co., Ltd.)[7] containing 10 ml of the medium supplemented with 1.0 mg / ℓ NAA and 0.1 mg/ ℓ BAP, and incubated as described above. The growth rate was estimated as increase of dry weight of the callus relative to the original dry weight. The dry weight of the callus was measured after dryness in an oven at 79℃ and expressed as the average dry weight of 5 samples.

2.4 Regeneration of root from callus

Several calli subcultured for about 6 months after the commencement of callus formation were transplanted on the modified MS media (40 ml) containing 1% Gellan Gum (Scott Lab., Inc.) in 100 ml Erlenmeyer flasks and incubated for 2 months under dark at 25℃. The media was supplemented with various combinations of NAA (0.1－10.0 mg/ ℓ) and BAP (0.001-10.0 mg/ ℓ).

3. RESULTS AND DISCUSSION

3.1 Induction and growth of callus from Japanese hackberry

The induction of green hard callus tissue was successful at the cut surface of internodal pieces within one month culture. The induction and relative growth of calli varied with the concentration of hormones as shown in Table 2. The cultures on medium having low and high hormone concentrations did not induce a callus. The combination range of 0.5-2.0 mg/ ℓ NAA and 0.1-1.0 mg/ ℓ BAP gave green calli with the best quality of callus at combination of 1.0 mg/ ℓ NAA and 0.1 mg/ ℓ BAP as shown in Fig. 1. At this hormonal range, when NAA was at a concentration higher than 1.0 mg/ ℓ, the root regeneration proceeded prior to the induction of callus.

The induced callus was subcultured onto the same medium for 5 months at interval of about 3 weeks. The relative growth rate of the callus in PLANTEX is shown in Fig. 2. As

Table 2 Effects of hormones on induction of cullus from Japanese hackberry.

		NAA conc. (mg/ ℓ)					
		0.1	0.5	1.0	2.0	5.0	10.0
BAP conc. (mg/ℓ)	0.01	—	—	—	—	—	—
	0.05	—	—	—	+	—	—
	0.10	—	+	+++	++	+	—
	0.50	—	++	++	++	+	—
	1.00	—	+	+++	+++	+	—
	2.00	—	+	+	+	+	—
	5.00	—	—	—	—	—	—

Visual estimation: −, none ; +, poor; ++, good; +++, very good.

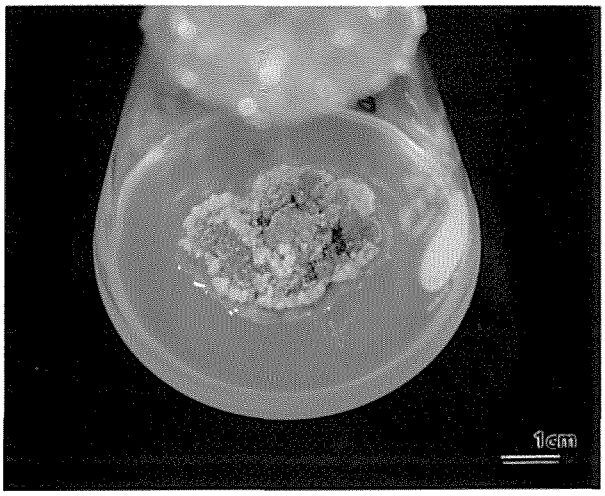

Fig. 1 Callus of Japanese hackberry. Green callus subcultured for 3 months on the half-ionic strength MS medium supplemented with 1.0 mg/ℓ NAA and 0.1 mg/ℓ BAP.

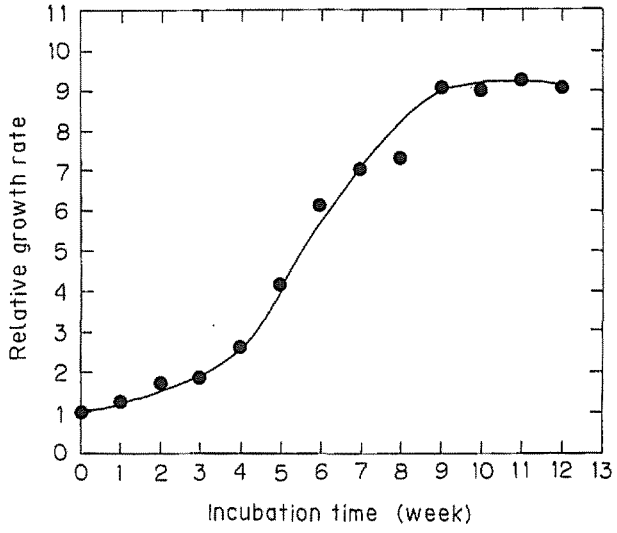

Fig. 2 Growth curve of callus cells of Japanese hackberry in PLANTEX. Green callus on the half-ionic strength MS medium supplemented with 1.0 mg/ℓ NAA and 0.1 mg/ℓ BAP.

already shown in the previous report[1], PLANTEX is suitable for this purpose because it is easy to pick up the callus from the white fiber mat inside the PLANTEX. The result indicated that the growth reached maximum after 9 weeks incubation giving about 9 times more amount of dry weight resulting in 4.5 times faster growth rate than that of green beech callus grown in the presence of 1.0 mg/ℓ NAA and 0.5 mg/ℓ BAP[1].

3.2 Regeneration of root from callus

The calli subcultured for more than one month were transferred onto the medium containing various combinations of NAA and BAP. The callus turned brown and ceased to grow on the hormone-free medium. After 5 weeks culture, 67% of the calli underwent reproducible root differentiation only at a combination of 0.1 mg/ℓ NAA and 1.0 mg/ℓ BAP as shown in Fig. 3. Some roots seem to lose geotropism as observed in the case of aspen[8].

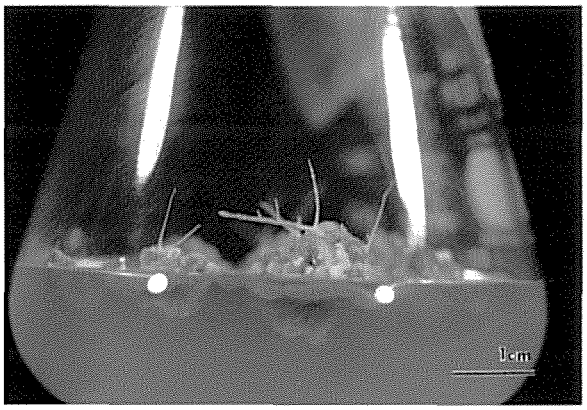

Fig. 3 Root regeneration from callus of Japanese hackberry. Regenerated roots after 9 weeks incubation on the half-ionic strength MS medium supplemented with 0.5 mg/ℓ NAA and 1.0 mg/ℓ BAP.

Another combination of hormones at 5.0 mg/ℓ NAA and 0.1 mg/ℓ BAP sometimes regenerated roots but the result was not reproducible due to an unfavorable callus growth. Shoot regeneration was difficult to achieve, as this species seems to be highly callus-producing and rhizogenic as in the case of *Populus*[9]. When BAP was replaced by zeatin (N^6-(*trans*-4-hydroxyisopentenyl)adenine)[10], an adventitious bud-like organ was produced only at the hormonal condition of 0.5 mg/ℓ of zeatin without NAA, but the regeneration of shoots from the calli was unsuccessful. Further efforts are needed to regenerate plants from the callus cells.

The present results show that a rapid growing callus could be developed from a newly grown shoot of Japanese hackberry which was the most favorable habitate of mistletoe in Japan. This system would be a potential candidate to be utilized in characterization of cell-cell interactions between mistletoe and its host.

4. ACKNOWLEDGEMENTS

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