

ラクダキリ(Paulownia catalpifolia)組織培養育成シュートの葉片と葉柄からの植物体再生

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著者名	宋,碩林 須田,邦裕 石井,克明 齋藤,明 大庭,喜八郎
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短 報

Plantlet Regeneration from Leaf and Petiole Explants of
in vitro Cultured *Paulownia catalpifolia**¹Shou Lin SONG,*² Kunihiro SUDA,*³ Katsuaki ISHII,*⁴
Akira SAITO,*⁴ and Kihachiro OHBA*²

I. Introduction

Paulownia catalpifolia STEUD is a fast-growing deciduous tree which has wood of great commercial value and is cultivated widely in China (12). Most species of *Paulownia* are very easy to propagate with conventional root cuttings, but they suffer from severe damage from witches' broom, the disease spreads extensively through root cuttings (3, 4). However, no pharmaceutically effective cure for the disease has been found. Meristematic culture (8) and its combination with a heat treatment (10) seemed to be effective in producing witches' broom-free plantlets from *Paulownia* trees infected by the mycoplasma like organism (MLO), the pathogen of the *Paulownia* witches' broom.

Several reports of successful tissue culture of *Paulownia* spp. (7, 9, 11) have been published since 1978, but only one (6) is on plantlet regeneration from leaf fragments, that of *P. elongata* S.Y. Hu. In our previously reported experiment, plantlets were regenerated from two mature trees of *P. catalpifolia* by meristematic culture (11), but no reports have been published on the leaf or petiole explant culture of this species. If we can succeed in leaf or petiole explant culture, we can have more explants for successive cultures and more chances for genetic manipulation of *Paulownia* with leaf cultures like *Populus* (2). Therefore, it is important to develop an *in vitro* leaf or petiole explant culture technique to regenerate and propagate a large number of plantlets in a short time.

This paper describes successful plantlet regeneration from leaf and petiole explant cultures of *P. catalpifolia*.

II. Materials and methods

1. Explants

On June 17, 1989, meristems about 0.5 mm long taken from two mature trees of *P. catalpifolia*, were cultured on BTM (Broad-leaved Tree Medium) and 1/2 BTM both with 1.0 mg/l of BAP (6-benzylaminopurine), and shoots were induced from the survived meristems (11). These shoots had been subcultured seven times at 30 day intervals on BTM with 1.0 mg/l of BAP, until leaf and petiole explants were prepared from the shoots.

2. Shoot initiation from the leaf and petiole explants

BTM (1) and MS (MURASHIGE, and SKOOG Medium) (5) were used as culture media for shoot initiation from leaf and petiole explants because these two media were regarded as fitting for shoot initiation from the meristems of *Paulownia* spp. (9). Fifteen combination of five kinetin and three NAA (1-naphthaleneacetic acid) concentration including a control as zero, were applied for both BTM and MS.

Leaves with petioles were collected from the shoots cultured on BTM with 1.0 mg/l of BAP. Petiole explants, about 0.8 cm long, were cut off, and leaf-fragment explants, about 0.8 cm in length and 0.5 cm in width, were taken from the leaves. Both of these types of explants were cultured on the slope surface of BTM and MS with 1.0% agar placed in 18 mm × 180 mm test tubes. One explant was placed in each test tube, and 10 explants were cultured for each of the 15 combination treatments.

*¹ 宋 碩林・須田邦裕・石井克明・齋藤 明・大庭喜八郎：ラクダギリ (*Paulownia catalpifolia*) 組織培養育成シュートの葉片と葉柄からの植物体再生

*² Inst. of Agric. and for., Univ. of Tsukuba, Ibaraki 305 筑波大学農林学系

*³ Akita Pref. For. Center, Akita 010-12 秋田県林業センター

*⁴ For. and Forest Prod. Res. Inst., Ibaraki 305 森林総合研究所

All test tubes were incubated at $25 \pm 1^\circ\text{C}$ for a 16 h photoperiod with fluorescent lamps having a light intensity of about 4,000 lx for 30 days.

3. Rooting of shoots initiated from the leaf and petiole explants and their acclimatization

Sixteen shoots, longer than 1.0 cm, were chosen randomly from the shoots initiated from the leaf and petiole explants. These shoots were cut to a length of about 1.0 cm and were cultured on BTM in 18 mm $\times$ 180 mm test tubes supplemented with 1.0 mg/l of IBA (Indol-3-butyric acid) for root induction. One shoot was placed in each test tube. Ten of the regenerated plantlets were transplanted into pots filled with vermiculite. The plantlets were grown in an incubator at $25 \pm 1^\circ\text{C}$ for a 16 h photoperiod with fluorescent lamps having a light intensity of about 2,000~3,000 lx. All pots were kept in a plastic box covered with a thin transparent plastic sheet for one week, and then the sheet was opened slightly for another week. After gradually making opening larger by each week, it was removed completely.

III. Results and discussion

1. Shoot initiation

Many flat leaf fragments became bent, and swelling first was seen at the point of cutting of the leaf fragment nearest the petiole on MS and BTM with NAA after ten days of culture. Meanwhile many

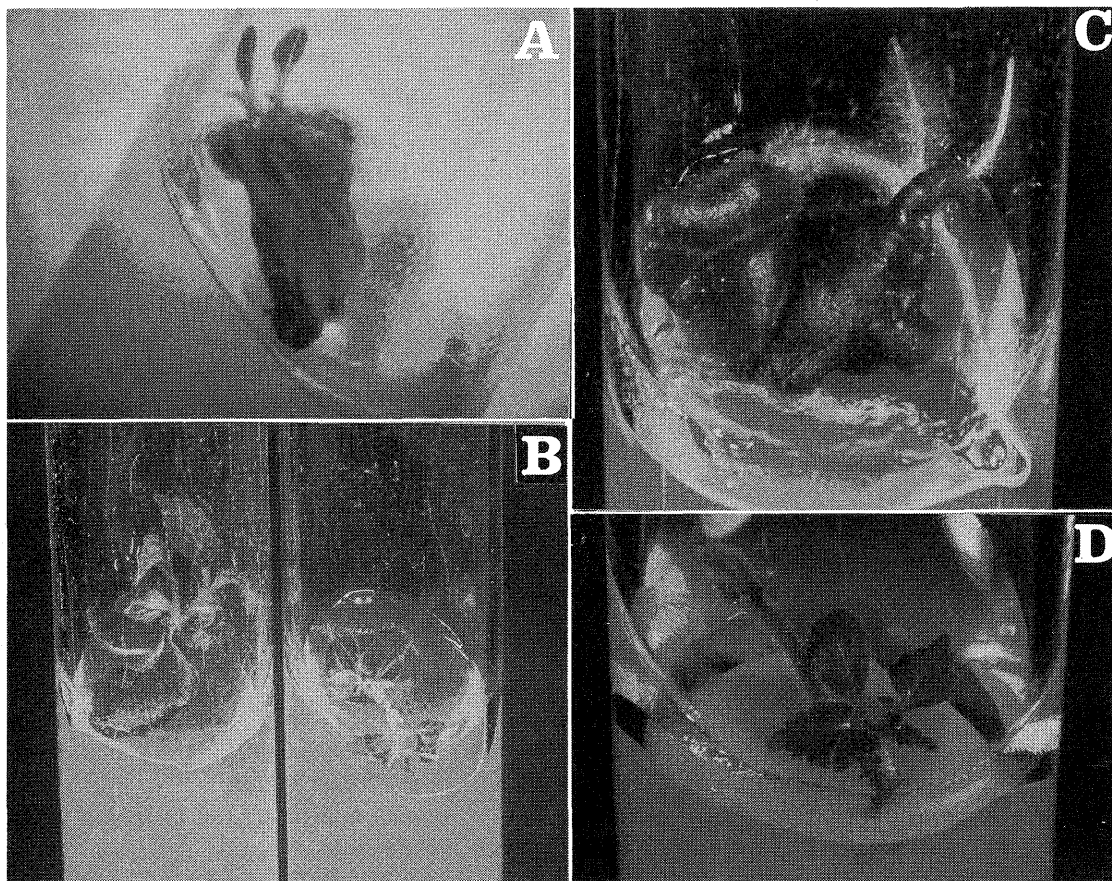


Fig. 1. Shoot and root formations from leaf fragment or petiole explants of *P. catalpifolia*

(A), Shoot formation from a leaf fragment explant 17 days after the beginning of culture ; (B), Shoot formation from leaf fragment explants 30 days after the beginning of culture ; (C), Root formation from a leaf fragment explant 30 days after the beginning of culture ; (D), Shoot formation from a petiole explant 30 days after the beginning of culture.

Note : Diameter of the test tubes is 18 mm.

Table 1. Shoot and root formations on the leaf and petiole explants^{*1}

Medium	Growth regulator (mg/l)		Shoot formation ^{*2}		Root formation ^{*3}	
	NAA	Kinetin	Leaf fragment	Petiole	Leaf fragment	Petiole
MS	0.0	0.0	— ^{**}	—	—	—
		0.5	—	—	—	—
		1.0	—	—	—	—
		2.0	—	—	—	—
		8.0	3/10	—	—	—
	0.5	0.0	—	—	—	3/10
		0.5	—	—	—	1/10
		1.0	1/10 ^{*5}	—	2/10	—
		2.0	—	2/10	—	—
		8.0	5/10	2/10	—	—
	1.0	0.0	—	—	4/10	4/10
		0.5	—	—	3/10	2/10
		1.0	1/10	1/10	3/10	2/10
		2.0	1/10	—	—	1/10
		8.0	5/10	4/10	—	2/10
BTM	0.0	0.0	—	—	—	—
		0.5	—	1/10	—	—
		1.0	1/10	2/10	—	—
		2.0	4/10	—	—	—
		8.0	4/10	—	—	—
	0.5	0.0	—	—	7/10	7/10
		0.5	—	—	1/10	—
		1.0	—	—	—	—
		2.0	—	—	—	—
		8.0	—	3/10	—	—
	1.0	0.0	—	—	6/10	9/10
		0.5	—	—	1/10	—
		1.0	—	—	1/10	—
		2.0	—	—	—	—
		8.0	—	—	—	—

^{*1} The data was taken 30 days after the beginning of culture. ^{*2} Numerator, No. of leaf or petiole explants with shoot formations; Denominator, No. of leaf or petiole explants cultured. ^{*3} Numerator, No. of leaf fragment or petiole explants with root formations; Denominator, No. of leaf fragment or petiole explants cultured.

^{**} No shoot or no root formations. ^{*5} Both shoot and root formations on the same leaf explant.

on BTM with 1.0 mg/l of NAA had root formations. Only one leaf explant, that one cultured on MS with 0.5 mg/l of NAA and 1.0 mg/l of kinetin, initiated both a shoot and a root (Table 1).

Although shoot initiations from leaf and petiole explants were small in number, this technique will be useful for genetic transformation studies of *Paulownia* species in the future.

2. Rooting of shoots initiated from leaf and petiole explants and their acclimatization

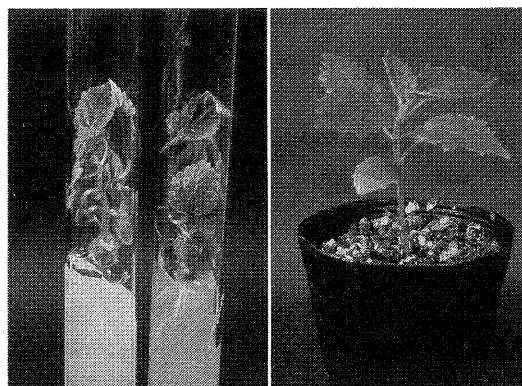
About six days after the beginning of culture, root formations were observed on the shoots, and one more week later nearly 80 % of the shoots being cultured had rooted. All of the shoots rooted after 30 days of culture (Fig. 2 left), and ten of them were transplanted into pots filled with vermiculite. All of these had survived 30 days after transplanting (Fig. 2 right).

explants of leaf fragments and petioles cultured on MS without NAA began to become necrotic. The first initiation of root was seen singly on petiole explants cultured on MS and BTM with NAA singly after eight days of culture, and then four days later the first shoot initiation was observed on a petiole explant cultured on BTM with 0.0 mg/l of NAA and 0.5 mg/l of kinetin. Then after a few days, shoot and root formations occurred frequently (Fig. 1). After 30 days of culture, shoot formation from the leaf explants was induced only on the media with greater kinetin concentrations of 1.0 mg/l and more. Fifty percent of the leaf explants developed shoots on MS with 8.0 mg/l of kinetin in combination with 0.5 and 1.0 mg/l of NAA, but there were none on the BTM with NAA (Table 1). Shoots were initiated from the petiole explants cultured on MS with NAA and the larger kinetin concentrations of 1.0 mg/l and more, whereas the shoots were formed on petiole explants cultured on BTM without NAA, but with concentrations of kinetin of 0.5 mg/l and 1.0 mg/l. No shoots were formed on the petiole explants with BTM and 1.0 mg/l of NAA. A leaf explant cultured on MS with 8.0 mg/l of kinetin and 0.5 mg/l of NAA formed seven shoots which was the maximum number in this experiment, and the formation of one to three shoots from one explant was very common (Table 2). No root formations were found on both MS and BTM without NAA, although roots were initiated on both MS and BTM with NAA. Greater numbers of the leaf and petiole explants cultured on both MS and BTM with 1.0 mg/l of NAA initiated roots than did the leaf and petiole explants cultured on both MS and BTM with 0.5 mg/l of NAA. Especially on MS with 1.0 mg/l of NAA, root formations were observed at every kinetin concentration level ranging from 0 to 8 mg/l, and 90 % of the petiole explants cultured

Table 2. Number of shoot formed on leaf and petiole explants*¹

Medium	Growth regulator(mg/l)		Leaf* ²	Petiole* ³
	NAA	Kinetin		
MS	0.0	8.0	6/3	—* ⁴
	0.5	1.0	1/1	—
		2.0	—	3/2
		8.0	12/5	8/2
	1.0	1.0	2/1	2/1
		2.0	1/1	—
		8.0	11/5	8/4
BTM	0.0	0.5	—	1/1
		1.0	2/1	2/2
		2.0	5/4	—
		8.0	8/4	—
	0.5	8.0	—	5/3

*¹ The data was taken 30 days after the beginning of culture. *² Numerator, Total number of shoots formed on the leaf explants; Denominator, No. of leaf explants with shoot formations. *³ Numerator, Total number of shoots formed on the petiole explants; Denominator, No. of petiole explants with shoot formations. *⁴ No shoot or no root formation.

Fig. 2. Plantlet regeneration and acclimatization of *P. catalpifolia*

Left, Rooting of the shoots formed on leaf or petiole explants; Right, Plantlet during acclimatization.

Note: Diameter of the test tubes is 18 mm.

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* The titles are tentative translation from the original Chinese titles by the authors of this paper.

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