フユボダイジュ成木の腋芽からの試験管内植物体再成

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短　報

In Vitro Plantlet Regeneration from Axillary Buds of Mature Trees of *Tilia cordata**

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I. Introduction

The clonal propagation of forest trees, which provides valuable methods for tree improvement programs, has been achieved using tissue culture techniques. A large number of selected genotypes can be produced in a short period of time.

Tilia species are valuable broadleaved forest trees. There are very few reports on tissue culture with *Tilia* species, whose seeds germinate poorly and irregularly because of their hard seed-coats and immature embryos. The first reports of tissue culture of *Tilia* species were made by BARKER (*1*), who induced calli from various source tissues of *Tilia americana* L., and CHALUPA (*3*) first established multiple shoots from axillary buds of juvenile seedlings of *Tilia cordata* MILL.

This report describes *in vitro* plantlet regeneration from axillary buds of 15-year-old trees of *T. cordata.*

II. Materials and Methods

Two 15-year-old *Tilia cordata* MILL. trees, growing in the arboretum of the Forestry and Forest Products Research Institute at Tsukuba, Japan, were used as the source of test materials. Axillary buds were taken in mid-May, 1987, from leafed branches which had elongated in the previous year. The buds were surface-sterilized by soaking in 70% ethanol for three minutes, and then three percent hydrogen peroxide for 15 min. Then they were dried on sterilized filter-paper on a clean bench and cut into about 10 to 15 mm lengths. These buds were cultured in autoclaved 18×180 mm test tubes containing 20 ml of agar-nutrient media.

The compositions of three basal media are shown in Table 1; a modified IDE and SAITO medium (*6*) (IS), a modified broadleaved tree medium (*2*) (BTM), and a modified woody plant medium (*5*) (WPM). For propagating shoots, all media contained 20 g/*l* of sucrose and 1% agar, and were supplemented with various concentrations of BAP (6-benzylaminopurine). The media were adjusted to pH 5.8 and autoclaved for 20 min at 120°C. For rooting, a half strength of each basal media containing 10 g/*l* of sucrose, three different amounts of IBA (indole-3-butyric acid), and 1.0 mg/*l* of NAA (*α*-naphthaleneacetic acid) was used.

Cultures were maintained with a 16 h light/8 h dark cycle under white fluorescent-light at an intensity of about 5,000 lx and a temperature of 25°C.

III. Results and Discussion

Bud burst occurred within two or three weeks on all media and at all concentrations of BAP, and the shoots developed from axillary buds about 3 to 4 cm of length after four weeks of culture (Fig. 1). Great differences of shoot development among media and concentrations of BAP were not observed (Table 2). All treatments resulted in large shoot-development rates of 60% or more excluding IS supplemented with 0.2 mg/*l* of BAP. The best results were obtained from WPM supplemented with 1.0, 2.0, or 5.0 mg/*l* of BAP. These results differ from those of juvenile stages where small dosages of BAP (0.2-1.0 mg/*l*) stimulated the best shoot growth from axillary buds (*3*), but they are similar to those reported by LEE and others (*4*) in *Quercus* who obtained large shoot-development rates even with large amounts of BAP. This may have resulted from differences of physiological conditions between juvenile and mature stages.

For further proliferation, shoots developed were subcultured on basal media containing 0.2 mg/*l* of BAP

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Table 1. Compositions of three basal media

(mg/l)

Component	IS	BTM	WPM
KNO_3	170	190	—
NH_4NO_3	680	165	400
$CaCl_2 \cdot 2H_2O$	—	44	96
$Ca(NO_3)_2 \cdot 4H_2O$	710	640	556
$(NH_4)_2SO_4$	—	240	—
K_2SO_4	—	860	990
KCl	140	—	—
$MgSO_4 \cdot 7H_2O$	370	370	370
KH_2PO_4	80	170	170
H_3BO_3	3.2	6.2	6.2
$MnSO_4 \cdot H_2O$	8.0	22.3	22.3
$ZnSO_4 \cdot 7H_2O$	9.0	8.6	8.6
KI	0.8	0.15	—
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.25	0.25	0.25
$CoCl_2 \cdot 6H_2O$	—	0.02	—
Fe-EDTA*	5.6	5.6	5.6
Sym-Diphenylurea	3.0	—	—
Urea	10.0	—	—
Thiamine HCl	0.1	1.0	1.0
Nicotinic acid	0.8	0.5	0.5
Pyridoxine HCl	0.1	0.5	0.5
Fumaric acid	1.0	—	—
Ascorbic acid	1.0	—	—
Glycine	—	2.0	2.0
Glutamine	—	2.0	2.0
Lysine	100.0	—	—
L-Tyrosine	10.0	—	—
myo-Inositol	100.0	100.0	100.0

* Ferric ethylenediaminetetraacetic acid

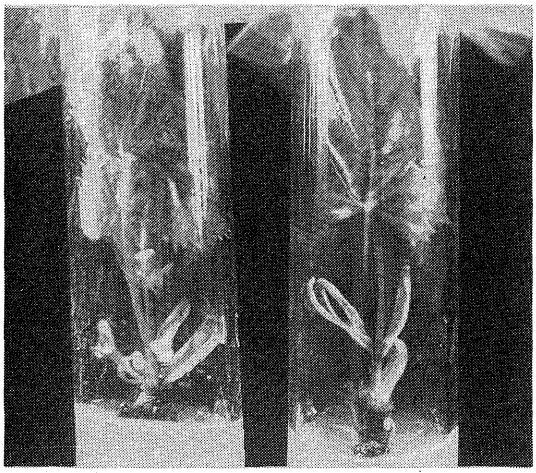

Fig. 1. Shoots developed from axillary buds on WPM medium with 1.0 mg/l of BAP

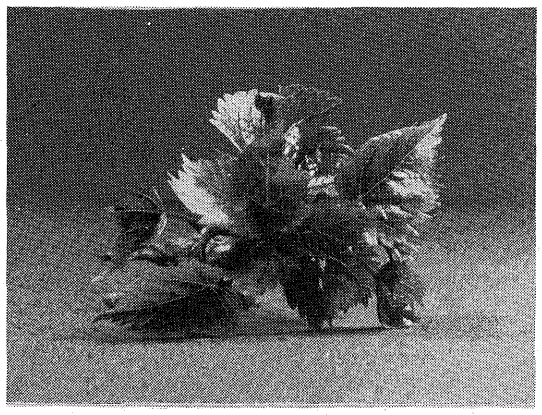

Fig. 2. Multiple shoots formed on WPM medium with 0.2 mg/l of BAP

Table 2. The effect of media and BAP on shoot development from axillary buds of 15-year-old *T. cordata**

Media	BAP mg/l					
	0.2	0.5	1.0	2.0	5.0	% **
IS	42.1	61.9	86.4	77.3	81.8	
BTM	80.9	71.4	61.9	59.1	73.9	
WPM	77.8	65.2	89.5	89.5	91.3	

* Data were taken after 4 weeks of culture.

** Percentages were calculated from 19~23 cultured buds for each combination of media and BAP.

Table 3. The effect of media and IBA on rooting from shoots developed (NAA supplied at 0.1 mg/l)*

Media	IBA mg/l			
	0.03	0.3	3.0	% **
1/2 IS	4.5	0.0	63.6	
1/2 BTM	4.7	0.0	25.0	
1/2 WPM	0.0	0.0	48.1	

* Data were taken after 4 weeks of culture.

** Percentages were calculated from 21~33 cultured shoots for each combination of media and IBA.

after 4 weeks of culture. An original shoot produced an average of 5.2 new shoots or internodal stems (Fig. 2), which were isolated and again subcultured on WPM containing 0.2 mg/l of BAP. This means that multiple shoots can be produced about five times within four weeks.

For rooting, shoots, 3 cm or more in lengh, were transferred to rooting media which were at half-strength for each basal media containing 10 mg/l sucrose, three levels (0.03, 0.3, and 3.0 mg/l) of IBA, and 0.1 mg/l

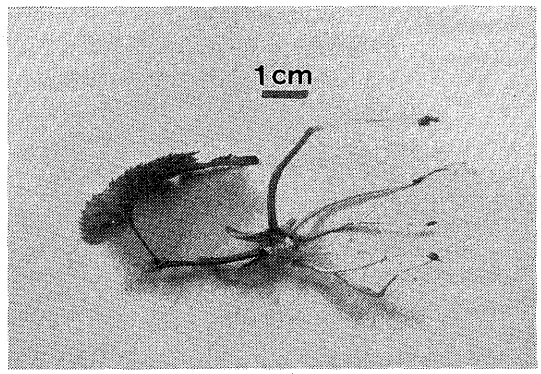

Fig. 3. Rooting on 1/2 IS medium with 3.0 mg/*l* of IBA and 0.1 mg/*l* of NAA

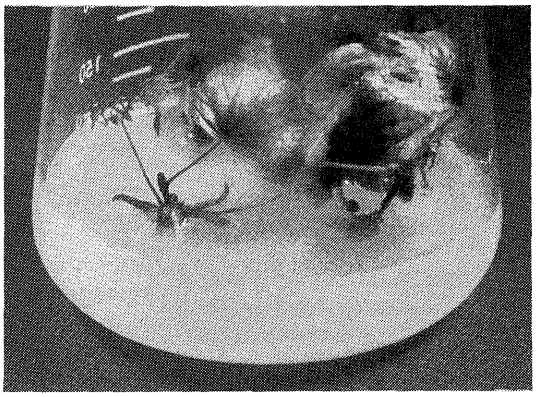

Fig. 4. Formation of plantlets from mature trees of *T. cordata* by axillary bud culture

of NAA. White calli were formed first at the bases of the shoots, and roots were formed through calli within three or four weeks (Fig.3). The best results were obtained from 1/2 IS supplemented with 3.0 mg/*l* of IBA and 0.1 mg/*l* of NAA. Root formation was better on 3.0 mg/*l* than on 0.03 or 0.3 mg/*l* of IBA for all media. The root-formation rates on 1/2 IS, 1/2 BTM, and 1/2 WPM with 3.0 mg/*l* of IBA and 0.1 mg/*l* of NAA were 63.6, 25.0, and 48.1%, respectively (Table 3). These results differ from those reported by CHAULPA (*3*) who observed that 80～100% of the shoots formed roots on WPM with 0.3 mg/*l* of IBA and 0.1 mg/*l* of NAA. This may have resulted from the differences of the amounts of endogenous auxins because the shoots and buds were cultured with the exclusion of auxins in this experiment.

After root development, the plantlets were transplanted into pots containing equal volumes of peat and vermiculite.

In this experiment, the possibility of rapid propagation from mature trees of *T. cordata* was proven by the culturing of axillary buds (Fig. 4). Propagation from mature stages generally is more practical and useful than from juvenile stage, that is, embryos or young seedlings, because it is possible to determine if these mature trees have superior genetic performance on important traits with greater certainty.

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