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Establishment of Tissue Culture System of Chinese Poplars: *Populus tomentosa* and *Populus alba* cv. Pyramidalis×*Populus tomentosa*

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Introduction

Poplars have been planted widely in temperate zone because of their fast growth and utilization as pulpwood (FAO, 1979). Many improvements of poplars have been made by cloning and crossing (FAO, 1979) and recently biotechnology-based improvements such as micropropagation, protoplast culture, cell fusion and gene transfer system have been established in some species of poplars (FILLATTI *et al.*, 1987; DOUGLAS, 1989). Now reforestation of destroyed infertile land is required for environmental conservation, and poplars are expected to serve as materials for reforestation. But the improvements of poplars have been restricted to some species, so the ranges of resistance to environmental stresses are narrow in the improved varieties.

We have embarked on research attempting to develop improved varieties of poplars which are resistant to environmental stresses in destroyed infertile land. The start of this project is a collection of species and varieties of poplars. The collected species and varieties will be kept by tissue culture system for convenience to biotechnology-based improvement.

The seeds of *Populus tomentosa* and its hybrid, *Populus alba* cv. Pyramidalis×*Populus tomentosa* are obtained from China. These variations are planted in adverse conditions and considered to have drought tolerance. *P. tomentosa* is the species of natural crossing of *P. alba* and *P. davidiana*, and *P. alba* cv. Pyramidalis is the species of natural crossing of *P. alba* ×*P. nigra* (WATANABE, 1991). This report describes the establishment of tissue culture system in these two Chinese poplar species. Consequently, the two Chinese poplars will be provided stably as the materials for genetic improvements.

Materials and Methods

Plant Materials

Seeds of the two poplars, *Populus tomentosa* and *Populus alba* cv. Pyramidalis×*Populus tomentosa* were received from China. Seeds of poplars cannot maintain viability for a long period, so we sowed them in a pot with sand and soil as soon as possible. Some seeds germinated in 5 days, but most of them failed to germinate.

Induction of Adventitious Shoots

Hypocotyls with cotyledons were cut off from 5-day-old seedlings and were sterilized for 5 min in 0.3% $HgCl_2$. Explants were washed 3 times with sterile distilled water and were inoculated in culture tube (20×150 mm) containing 10 m*l* of 1/2 MS medium i. e., the medium wherein major non-organic components of MS medium (MURASHIGE and SKOOG, 1962) have been reduced to half concentrations (Table 1), supplemented with 2% sucrose, 0.8% agar and various concentrations of BAP (6-benzylaminopurine; 0, 0.4, 0.8 and 1.2 mg/*l*). The media were adjusted to pH 5.8 and autoclaved at 120℃ for 20 min. After inoculation

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Table 1. Components of the basic medium (1/2 MS medium) (mg/*l*)

NH_4NO_3	825	$CoCl_2 \cdot 6H_2O$	0.025
KNO_3	950	$CuSO_4 \cdot 5H_2O$	0.025
$CaCl_2 \cdot 2H_2O$	220	$Na_2MoO_4 \cdot 2H_2O$	0.25
$MgSO_2 \cdot 7H_2O$	185	KI	0.83
KH_2PO_4	85	H_3BO_3	6.2
$FeSO_4 \cdot 7H_2O$	27.8	Nicotinic acid	0.5
Na_2-EDTA	37.3	Pyridoxine acid	0.5
$MnSO_4 \cdot 4H_2O$	22.3	Thiamine HCl	0.1
$ZnSO_4$	8.6	myo-Inositol	100
		Glycine	2

Table 2. Formation of adventitious multiple shoots on hypocotyls with cotyledons excised from 5-day-old seedlings of *P. alba* cv. Pyramidalis×*P. tomentosa* treated with various concentration of BAP to 1/2 MS medium

BAP (mg/*l*)	Number of tested explants	Number of explants which developed multiple shoots	Number of explants which formed callus
0.0	16	0 (0%)	0 (0%)
0.4	16	0 (0%)	0 (0%)
0.8	16	1 (6%)	0 (0%)
1.2	16	0 (0%)	0 (0%)

Table 3. Formation of adventitious multiple shoots on hypocotyls with cotyledons excised from 5-day-old seedlings of *P. tomentosa* treated with various concentration of BAP to 1/2 MS medium

BAP (mg/*l*)	Number of tested explants	Number of explants which developed multiple shoots	Number of explants which formed callus
0.0	8	0 (0%)	0 (0%)
0.4	7	2 (29%)	0 (0%)
0.8	10	2 (20%)	4 (40%)
1.2	9	0 (0%)	5 (56%)

of explants the culture tubes were placed in a growth chamber maintained at 25℃ under fluorescent illumination (150 μmol/m^2/s at 400–700 nm) for a 16-hour photoperiod.

Induction of Roots from Adventitious Shoots

Elongated shoots were transferred to culture tubes containing 10 m*l* of 1/2 MS medium supplemented with 2% sucrose and 0.8% agar. And the effect of NAA (α-naphthylacetic acid) for root induction was tested at various concentrations (0, 0.002, 0.02, 0.1 and 1.0 mg/*l*).

Acclimatization of Plantlets

Plantlets were transferred to a mixture of peat moss and vermiculite (1 : 1) in pots. The pots were maintained in high relative humidity (85–95%) at 25℃ under fluorescent

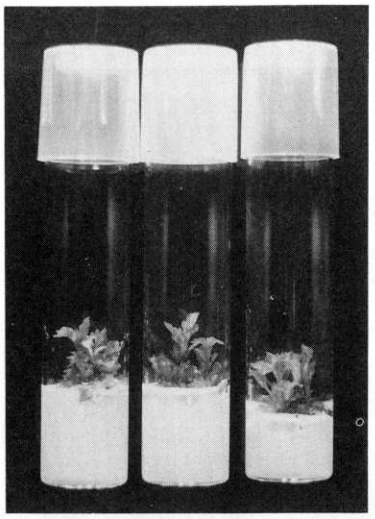

Fig. 1. Adventitious multiple shoots of *P. alba* cv. Pyamidalis×*P. tomentosa* subcultured on the 1/2 MS medium containing 0.8 mg/*l* of BAP.

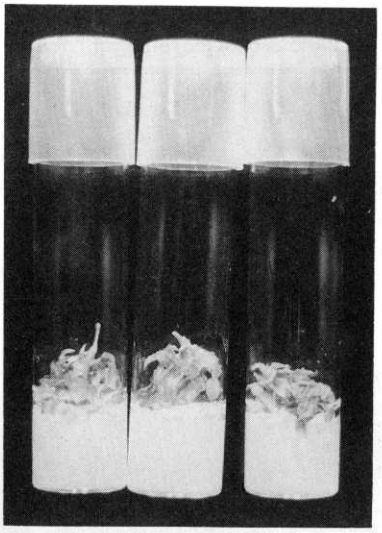

Fig. 2. Adventitious multiple shoots of *P. tomentosa* subcultured on the 1/2 MS medium containing 0.4 mg/*l* of BAP.

illumination (220 μmol/m^2/s at 400–700 nm) for a 16-hour photoperiod in a growth chamber. One week later, relative humidity was gradually reduced for 2 weeks.

Results and Discussion

Adventitious Shoots Formation

Explants of 5-day-old seedlings were tested on their ability to produce multiple shoots by using various concentrations of BAP (Table 2, 3). In the case of *P. alba* cv. Pyramidalis ×*P. tomentosa*, adventitious multiple shoots were induced on the 1/2 MS medium containing 0.8 mg/*l* BAP after 4 weeks of culture (Table 2). The adventitious shoots regenerated well by the subsequent culture on the same medium (Fig. 1).

In the case of *P. tomentosa*, adventitious multiple shoots were induced on the 1/2 MS medium containing 0.4 mg/*l* or 0.8 mg/*l* BAP after 4 weeks (Table 3). But the explants on the 0.8 mg/*l* BAP-containing medium formed calluses at the base of the shoots, and died after 6 weeks of culture. The explants on the 0.4 mg/*l* BAP-containing medium formed no calluses, and showed good growth. The adventitious shoots formed on the 0.4 mg/*l* BAP-containing medium were successfully subcultured on the same medium (Fig. 2).

In both of the poplars, the explants without shoot development turned white by bleaching, because sterilization of the explants was too strong. Consequently, only a small number of the explants produced multiple shoots, so more experiments are needed to determine the optimal concentration of BAP for shoot induction. But almost all the multiple shoots were successfully subcultured on the 0.4 or 0.8 mg/*l* BAP-containing medium.

In *in vitro* propagation of *P. tremula*, MS medium supplemented with a low concentration of BAP (0.2 mg/*l*) and with auxin (IBA 0.1 mg/*l*) stimulated bud formation and shoot elongation. While the shoot number increased with increasing concentration of BAP (0.2 to 1.0 mg/*l*), the high concentration of BAP (2.0 mg/*l*) resulted in the formation of numerous

Table 4. Root formation of adventitious shoots on *P. alba* cv. Pyramidalis×*P. tomentosa* in various concentration of NAA to 1/2 MS

NAA (mg/*l*)	Number of tasted shoots	Number of shoots which formed roots	Number of shoots which formed callus
0	28	26 (93%)	0 (0%)
0.002	10	6 (60%)	10 (100%)
0.02	9	6 (67%)	9 (100%)
0.1	20	16 (80%)	18 (90%)
1.0	20	17 (85%)	20 (100%)

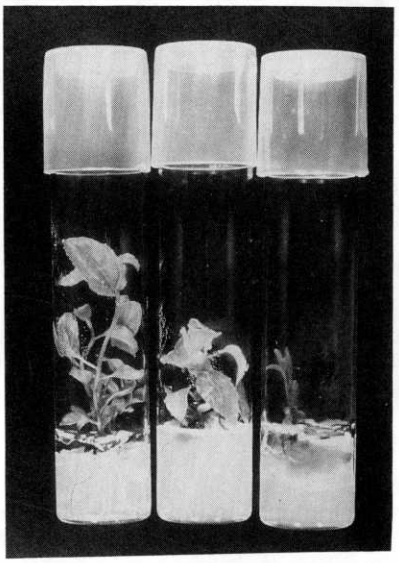

Fig. 3. Root formation of the shoots of *P. alba* cv. Pyramidalis×*P. tomentosa* on the 1/2 MS medium containing 0, 0.1 and 1.0 mg/*l* of NAA (left to right).

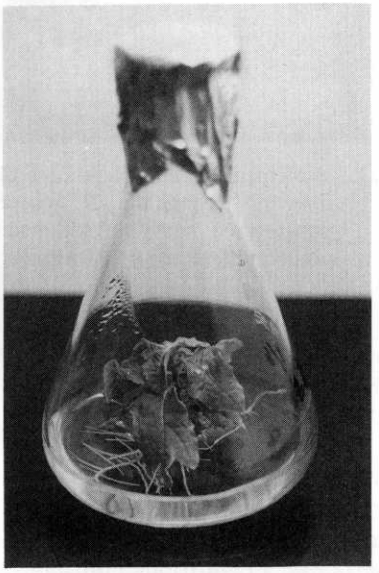

Fig. 4. Root formation of the shoots of *P. tomentosa* on the 1/2 MS hormone-free medium.

short shoots (CHALUPA, 1985). This is almost the same BAP levels which induced multiple shoots in our experiments.

Rooting of Adventitious Shoots

Elongated shoots of *P. alba* cv. Pyramidalis×*P. tomentosa* were tested to reveal their ability of root formation by using various concentrations of NAA (Table 4). Rooting of *P. alba* cv. Pyramidalis×*P. tomentosa* was observed regardless of NAA concentrations. But calluses were formed at the base of the shoots after 10 to 12 days of culture on the NAA-containing medium, and the shoots were vitrified (Fig. 3). In that medium, nascent roots were observed in 2 weeks after transplantation to the medium, but the growth of roots was suppressed (Fig. 3). On the contrary, the roots continued to develop and elongated for over 8 weeks on the hormone-free medium (Fig. 3). In this experiment, it is concluded that rooting of the shoots of *P. alba* cv. Pyramidalis×*P. tomentosa* could expected

on the hormone-free 1/2 MS medium.

We also confirmed that the shoots of *P. tomentosa* induced root formation in the hormone-free 1/2 MS medium (Fig. 4).

Soaking of cut-end of adventitious shoot in a solution with IBA (ZHOU and Gui, 1983) or transferring to NAA-containing medium (SAITO, 1989) are effective for root formation of *Populus* species. But in our experiment, roots were successfully formed on hormone-free medium and NAA was not suitable.

Acclimatization of Plantlets

The rooted shoots (plantlets) were subsequently transferred to nonsterile soil mixture consisting of peat moss and vermiculite (1 : 1). The plantlets were maintained under high relative humidity conditions for 1 week, and then brought to ambient moisture levels for an additional 2 weeks for hardening, and nearly 100% survival rates could be obtained (Fig. 5).

In the micropropagation of *P. alba*×*P. grandulosa*, the plantlets were kept in the greenhouse with high humidity (80–90%) for 4 to 6 weeks, and over 90% survival rates could be obtained consequently (KIM *et al.*, 1981). Programmed control of relative humidity has not been applied to acclimatization of *in vitro* cultured *Populus* yet. We could obtain a high survival rate of plantlets. Therefore, it is concluded that this successful procedures was suitable for acclimatization of these poplars.

This experiment proved that organogenesis and plantlet formation from explants of young seedling tissue of *P. tomentosa* and *P. alba* cv. Pyramidalis×*P. tomentosa* can be successfully induced. And also these two poplars can be successfully maintained by tissue culture system.

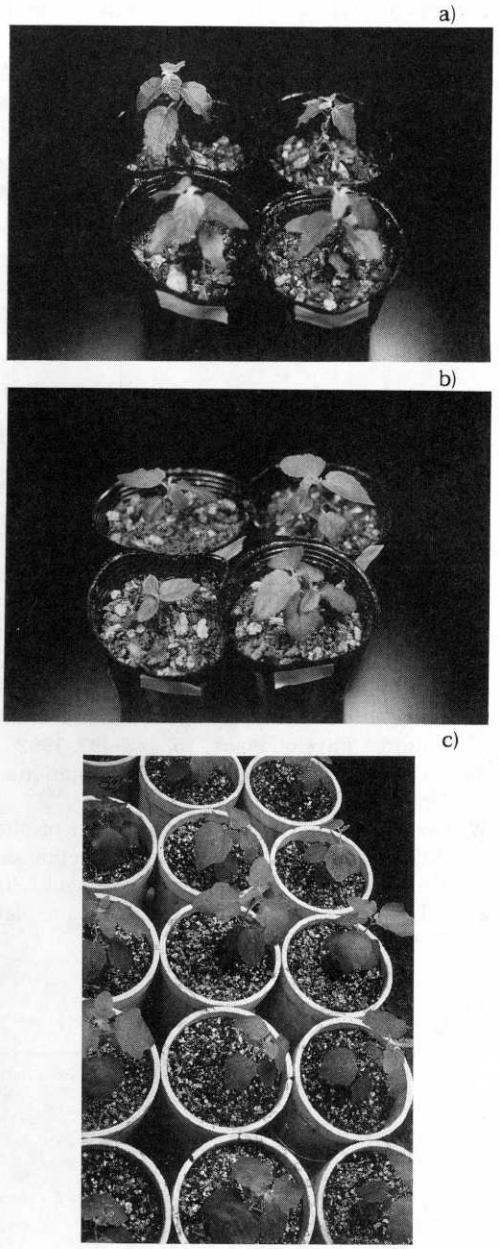

Fig. 5. Plantlet hardened-off to greenhouse environment.
a) *P. alba* cv. Pyramidalis×*P. tomentosa*, b) *P. tomentosa*, c) Successful acclimatization of adventitious plantlets.

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Summary

For the improvement of poplars which are resistant to environmental stresses, and for

conservation of genotypes, we established the protocols for inducing organogenesis *in vitro*. And plantlets were regenerated from explants obtained from young seedlings of *Populus tomentosa* and *P. alba* cv. Pyramidalis×*P. tomentosa*.

Hypocotyls with cotyledon were excised from 5-day-old seedlings and were surface sterilized. Explants formed multiple shoots on 1/2 MS medium containing BAP (0.4, 0.8 mg/*l*). Elongated shoots were transferred to hormone-free 1/2 MS medium and roots were induced on it. Regenerated plantlets were acclimatized in a mixture of peat moss and vermiculite under controlled relative humidity during 3 weeks. Subculture of multiple shoot was possible by plantation of shoots on the same medium of the first culture.

Key words: Chinese poplar, Seedling, Tissue culture, Regeneration of plantlet, Conservation of genotypes

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中国産ポプラ (*Populus tomentosa, P. alba* cv. Pyramidalis ×*P. tomentosa*) の組織培養系の確立

康　才明・小島克己・井出雄二

要　　旨

中国産ポプラの毛白楊 (*Populus tomentosa*) と毛新楊 (*P. alba* cv. Pyramidalis×*P. tomentosa*) の芽生えを外植体とした組織培養系を確立した。発芽後の子葉と胚軸を切り出し，BAP (0.4, 0.8 mg/*l*) を添加した 1/2 MS 培地上でマルチプルシュートを得た。伸長したシュートをホルモンフリーの 1/2 MS 培地に移植し，発根を誘導し幼植物体を得た。これをピートモスとバーミキュライトを用土として順化させた。またシュートは初代培養と同じ培地を用いて継代培養することができた。

キーワード:　中国産ポプラ，芽生え，組織培養，幼植物体再生，系統保存