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In vitro Plantlet Regeneration from Petiole Culture of Poplar Hybrids and Their Competence for Adventitious Bud Formation

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Introduction

Poplars have been used as rapid growing tree species for afforestation in the world (FAO, 1979). There are numerous hybrids developed for various usage (FAO, 1979). The Tokyo University Forests has been collected these hybrids from 1950's. We are now keeping over 300 clones of poplars in Tanashi Experimental Station and The University Forest in Hokkaido (The University Forests Council, 1989). These collections have been used for various kinds of silvicultural experiments. We have to maintain these precious gene resources for future researches.

For the preservation of gene resources, *in vitro* tissue culture has great potential. We can store a lot of clones in narrow room by tissue culture. Moreover, cryopreservation of tissues allows almost eternal storage of propagules. Tissue culture techniques of poplars are the most advancing among woody perennials (DOUGLAS, 1989, LUBRANO, 1992). Therefore we intend to apply the techniques for preservation of our popular hybrids.

We selected petioles as starting explants for *in vitro* culture because we can obtain a sufficient number of materials for tissue culture without giving any severe damage to the donor trees. While plant regeneration from protoplasts isolated from poplar petiole callus (LEE, 1987) and a possibility of direct adventitious bud induction from poplar petiole tissue (IDE, 1989) was reported, direct regeneration of plantlets from poplar petioles has not been completed yet.

In this paper we report adventitious buds induction from petioles of 37 poplar hybrids and plant regeneration from the buds, focusing on the competence for adventitious buds formation of hybrids.

Materials and Methods

Leaves with petioles were collected between mid-May and mid-June in 1992 and 1993 from 2–3 year-old trees of 37 poplar hybrids which were propagated by cuttage at nursery of Tanashi Experimental Station (Table 1). Petioles were cut from leaves and surface sterilized. They were soaked in 1% neutral detergent (Mama-lemon, Kao Co., Ltd.) for 3 min, washed by running tap water, and then soaked with agitation in 70% ethanol for 1 min, in sodium hypochlorite solution containing 1% effective chlorine for 8 min, and in 3% hydrogen peroxide for 5 min successively.

One centimeter long explants were cut from the petioles by removing both ends which were damaged by surface sterilization, and cultured on agar media containing different concentrations of BAP (N6-benzylaminopurine). The basic medium of these media was a half-strengthened MS medium where all inorganic components of MS medium (MURASHIGE and SKOOG, 1962) were reduced to a half concentrations and contained 20 g/l sucrose

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Table 1. List of tested poplar hybrids

No.	Hybrid	Scientific name*	Parental sections**	Tested year	
				1992	1993
1	alba×grandis	*P. alba*×*P. euramericana* cv. grandis	L×A	+	+
2	B-21	Unknown		+	+
3	C. B. D.	*P.*×*euramericana* cv. C. B. D.	A×A	+	+
4	FS-51	*P. maximowiczii*×*P. plantierensis*	T×A	+	+
5	FS-224	*P. deltoides* cv. virginiana×*P. nigra* cv. caudina	A×A	+	+
6	FS-228	*P. deltoides* cv. virginiana×*P. nigra* cv. caudina	A×A	+	+
7	I-45/51	*P.*×*euramericana* cv. I-45/51	A×A	+	+
8	I-72/51	*P. deltoides* cv. I-72/71	A	+	+
9	I-81/59	Unknown		+	+
10	I-476	*P.*×*euramericana* cv. I-476	A×A	+	+
11	Kamabuti	*P. nigra*×*P. maximowiczii*	A×T	+	+
12	Lo-5	Unknown		+	+
13	Lw-42	*P.*×*euramericana* cv. jacometii	A×A	+	+
14	Lw-43	*P.*×*euramericana* cv. serotina-erecta	A×A	+	+
15	OP-29	*P. charkowiensis*×*P. tricocarpa*	A×T	+	+
16	OP-226	*P. deltoides* cv. virginiana×*P. nigra* cv. caudina	A×A	+	+
17	Peace	*P. koreana*×*P. tricocarpa*	T×T	+	+
18	Robusta	*P.*×*euramericana* cv. robusta	A×A	+	+
19	W-7	*P.*×*euramericana* cv. robusta	A×A	+	+
20	W-18	*P.*×*euramericana* cv. robusta	A×A	+	+
21	W-22	Unknown		+	+
22	W-59	*P. candicans*	T	+	+
23	W-73	Unknown		+	+
24	W-77	Unknown		+	+
25	W-80	×*P. regenerata*×Unknown sp.	A×?	+	+
26	W-84	×*P. regenerata*×Unknown sp.	A×?	+	+
27	Wis-5	Unknown		+	+
28	Kiusiu	Unknown		+	−
29	W-76	Unknown		+	−
30	FS-41	*P. maximowiczii*×*P. tricocarpa*	T×T	−	+
31	FS-42	*P. maximowiczii*×*P. tricocarpa*	T×T	−	+
32	I-105/56	Unknown		−	+
33	L-230	*P.*×*euramericana* cv. robusta	A×A	−	+
34	OP-20	*P. charkowiensis*×*P. nigra* cv. caudina	A×A	−	+
35	OP-285	*P. nigra*×*P. tricocarpa*	A×T	−	+
36	W-2	Unknown		−	+
37	W-70	×*P. gelrica*	A×A	−	+

* Scientific names were referred to INOKUMA, 1958a, b and 1959.
** Classification by sections were referred to CHIBA, 1962.
L: Leuce, A: Aigeiros, T: Tacamahaca. +: Tested in this year, −: Not tested in this year.

(designated as 1/2MS medium). The media were solidified by 0.8% agar and their pHs were adjusted to 5.6 before autoclaving. Petioles were put on 10 m*l* slanted medium in a culture tube (25 mm in diameter, 120 mm in length) as the basal end facing downward.

Ten (1992) or fifteen (1993) petioles were prepared for each treatment of a hybrid petioles with a cirtain BAP concentration except for W-22 of which 20 explants were cultured in 1992.

Table 2. Proportion of explants on which adventitious buds formed

No.	Hybrid	BAP (mg/*l*)	0.4	0.4	0.8	0.8	1.2	1.6	Highest record*
		Tested year	1992	1993	1992	1993	1993	1993	
1	alba×grandis		90.0	60.0	70.0	13.3	7.1	0.0	90.0
2	B-21		0.0	14.3	N	6.7	—	0.0	14.3
3	C. B. D.		100.0	46.2	70.0	6.7	0.0	0.0	100.0
4	FS-51		40.0	35.7	22.2	26.7	0.0	0.0	40.0
5	FS-224		70.0	33.3	11.1	0.0	6.7	0.0	70.0
6	FS-228		0.0	0.0	10.0	0.0	—	0.0	10.0
7	I-45/51		60.0	20.0	14.3	0.0	0.0	0.0	60.0
8	I-72/51		50.0	28.6	20.0	8.3	0.0	0.0	50.0
9	I-81/59		100.0	69.2	77.8	36.4	30.8	38.5	100.0
10	I-476		90.0	75.0	80.0	18.2	7.7	6.7	90.0
11	Kamabuti		0.0	—	22.2	0.0	0.0	0.0	22.2
12	Lo-5		20.0	N	20.0	0.0	—	0.0	20.0
13	Lw-42		100.0	80.0	90.0	35.7	0.0	0.0	100.0
14	Lw-43		60.0	20.0	70.0	0.0	6.7	0.0	70.0
15	OP-29		0.0	73.3	0.0	26.7	6.7	0.0	73.3
16	OP-226		100.0	40.0	80.0	0.0	6.7	0.0	100.0
17	Peace		0.0	—	10.0	8.3	7.1	0.0	10.0
18	Robusta		50.0	15.4	0.0	22.2	0.0	0.0	50.0
19	W-7		30.0	0.0	0.0	18.2	8.3	0.0	30.0
20	W-18		10.0	0.0	11.1	0.0	0.0	0.0	11.1
21	W-22		36.8	0.0	40.0	7.1	0.0	0.0	40.0
22	W-59		—	0.0	N	0.0	0.0	0.0	0.0
23	W-73		90.0	0.0	25.0	0.0	0.0	7.1	90.0
24	W-77		71.4	53.3	55.6	46.7	35.7	0.0	71.4
25	W-80		A	13.3	16.7	0.0	0.0	0.0	16.7
26	W-84		37.5	—	0.0	—	—	—	37.5
27	Wis-5		N	0.0	—	0.0	0.0	0.0	0.0
28	Kiusiu		40.0	—	0.0	—	—	—	40.0
29	W-76		10.0	—	0.0	—	—	—	10.0
30	FS-41		—	40.0	—	0.0	0.0	0.0	40.0
31	FS-42		—	69.2	—	20.0	14.3	0.0	69.2
32	I-105/56		—	100.0	—	92.9	38.5	0.0	100.0
33	L-230		—	0.0	—	0.0	0.0	0.0	0.0
34	OP-20		—	50.0	—	0.0	0.0	0.0	50.0
35	OP-285		—	70.0	—	0.0	0.0	0.0	70.0
36	W-2		—	0.0	—	0.0	0.0	0.0	0.0
37	W-70		—	84.6	—	80.0	30.8	0.0	84.6

* Highest proportions of adventitious bud formation among tested BAP concentrations and years.
—: No explant was cultured or all explant was contaminated. A: Insufficient number (1–4) of explant cultured; Adventitious bud formation was observed. N: Insufficient number (1–4) of explant cultured; Adventitious bud formation was not observed.

Elongated adventitious shoots were transplanted to 1/2MS medium without any growth regulator which is known to induce rooting of adventitious shoots in other *Populus* species (KANG *et al.*, 1992) after a few month of culture.

The cultures were kept at 25℃, under 6,000 lux fluorescent illumination with a interval of 16 h light and 8 h dark throughout the experiment.

Table 3. Proportion of hybrids which developed adventitious bud on petioles after 40 days culture

Year	BAP (mg/*l*) (%)				Total
	0.4	0.8	1.2	1.6	
1992	78.6 (22/28)	71.4 (20/28)	— —	— —	89.3 (25/28)
1993	75.9 (23/31)	51.5 (17/33)	41.9 (31/31)	9.1 (3/33)	79.4 (27/34)

Number in parentheses indicates number of hybrids developed adventitious bud and number of hybrids tested.

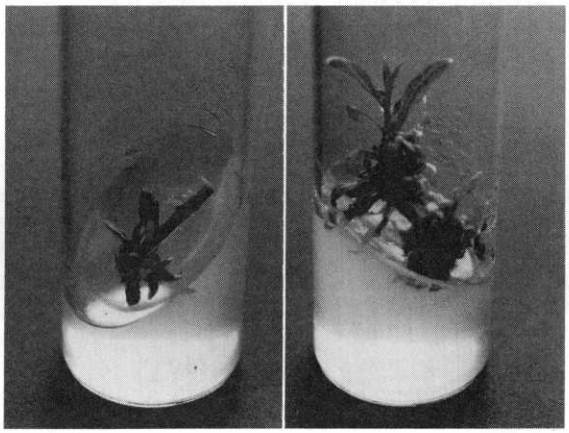

Fig. 1. Adventitious bud formation on cut ends of petioles.
Left hybrid was alba×grandis after 46 days culture. Right hybrid was FS-42 after 56 days culture. Medium was 1/2MS containing 0.4 mg/*l* BAP. Diameter of test tubes are 25 mm.

Results and Discussion

Adventitious bud formation and plant regeneration

The proportions of contaminated explants were 13.5% in 1992 and 19.3% in 1993. The hybrids of which the number of non-contaminated explants were less than five were eliminated from the discussion on the proportion of explants forming adventitious buds.

After one week culture, first adventitious bud was observed. Adventitious bud formations after 40 days culture were summarized in Table 2. About 76% of hybrids formed adventitious buds after 40 days culture (Table 3). Consequently 89% of tested hybrids (33 out of 37) formed adventitious buds in two years experiment. The proportion of hybrids which formed adventitious buds was highest on the medium containing 0.4 mg/*l* BAP in both years. The proportion was gradually decreased with the increment of BAP concentrations.

Adventitious buds were induced on the cut ends of petioles (Fig. 1). They were induced mainly from the basal ends of petioles irrespective of BAP concentrations (Table 4). The same phenomena was also observed when segments were cultured on flat agar medium (data not shown). There seems to be a general tendency that the adventitious buds form on proximal end of a poplar petiole segment.

Among 33 hybrids which formed adventitious buds, 26 hybrids (78.8%) showed the

Table 4. Proportion of adventitious bud formation from opposite ends of petioles after 40 days culture

Year	BAP (mg/*l*)	Distal end* (%)	Proximal end* (%)	Both ends* (%)	Total (%)
1992	0.4	11.8	45.2	9.1	48.3 (263)
	0.8	10.2	29.7	7.4	32.0 (256)
1993	0.4	13.0	31.3	11.7	33.9 (386)
	0.8	6.5	12.1	3.5	15.4 (431)
	1.2	2.3	5.9	1.0	7.2 (388)
	1.6	0.0	1.8	0.0	1.6 (442)

* Segments forming buds on both ends were also counted in these criteria.
Numbers in parentheses are total number of cultured explants.

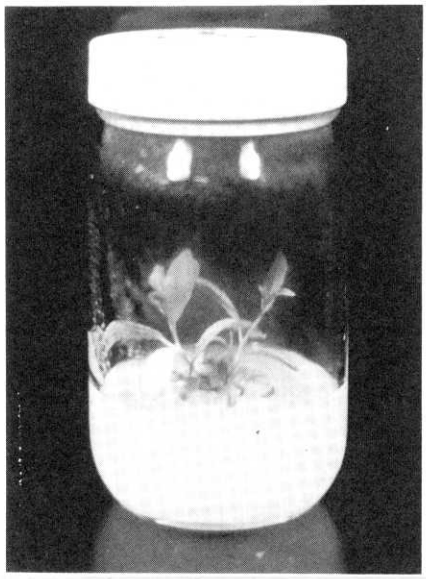

Fig. 2. Multiple shoot elongation from adventitious buds after one month subculture.
Hybrid was Peace. Medium was 1/2MS containing 0.8 mg/*l* BAP. Height of culture bottle was 90 mm.

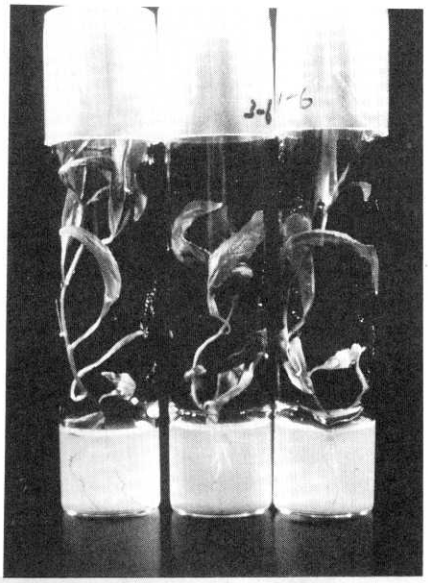

Fig. 3. Plant regeneration after one month culture on rooting medium.
Hybrid was W-73. Medium was 1/2MS without any plant growth regulator. Diameter of test tubes were 25 mm.

highest rate of adventitious bud formation on the medium containing 0.4 mg/*l* BAP. Only eight hybrids showed the best results on the medium with 0.8 mg/*l* BAP. Although most hybrids did not show high adventitious bud formation rate on the medium containing 1.2 and 1.6 mg/*l* BAP, some hybrids as I-81/59, W-77, I-105/56 and W-70 showed comparatively high rate (over 30%) even on the medium containing 1.2 or 1.6 mg/*l* BAP. This indicates that there are differences on the responses for BAP among hybrids.

From these results we could conclude that the optimum BAP concentration for

adventitious bud formation on petioles was around 0.4 mg/*l* for the poplar hybrids tested in this experiment. Higher concentrations of BAP was not suitable for adventitious bud formation. Adventitious buds are strongly induced on the medium containing 0.8 mg/*l* BAP in the culture of Japanese white birch petioles (SAITO and IDE, 1985). Poplars differentiated adventitious buds by lower BAP levels than Japanese white birch. Douglas (1989) commented that low concentration (0.05–0.3 mg/*l*) of cytokinin (BAP or zeatin) are required for shoot proliferation of poplars. We should survey lower BAP concentrations for efficient petiole culture.

Adventitious buds were subcultured in 1/2MS medium containing 0.4 mg/*l* of BAP. Adventitious buds of some hybrids elongated into shoots longer than 1 cm in primary culture (Fig. 2) and subculture (data wasn't shown). Rest of hybrids did not elongate adventitious buds into shoots and died. The long shoots were cut and transplanted to a rooting medium. After one month of culture on rooting medium, we obtained rooted plantlets of 11 hybrids, i.e., FS-51, Peace, W-73 and Robusta in 1992 and alba×grandis, FS-41, FS-42, FS-51, OP-20, OP-29, OP-285 and W-7 in 1993 (Fig. 3).

The regenerated plantlets were successfully acclimatized on a mixture of peat moss and vermiculite (1 : 1 (v : v)) in plastic pot (12cm in diameter, 10 cm in height) within 2–4 weeks (Fig. 4).

Competence for adventitious bud formation of tested hybrids

There were large differences between the proportions of adventitious bud formation in 1992 and in 1993 in some hybrids (Fig. 5). These differences may be caused by physiological differences in the starting materials and differences in culture technique such as strength of surface sterilization.

However, we could find significant correlation (coefficient (r=0.511) in 5% level) between the rates of adventitious bud formation on petioles of the other hybrids in both years. This suggests that the competence for adventitious bud formation differs from hybrid to hybrid.

Poplars are classified into five sections; i.e. Leuce (aspens and white poplars), Tacamahaca (balsam poplars), Aigeiros (black poplars), Leucoides and Turanga (International poplar commission, 1958). Twenty four tested hybrids could be identified the sections to

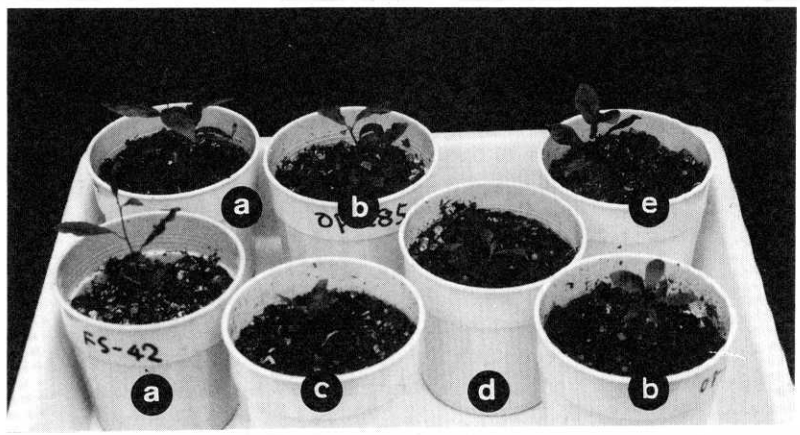

Fig. 4. Successfully acclimatized plants of poplar hybrids.
Hybrids were a: FS-42, b: OP-285, c: W-7, d: FS-51 and e: OP-29. Sixteen days after transplanting to the potting soils. Potting soil was peat moss and vermiculite (1 : 1/v : v).

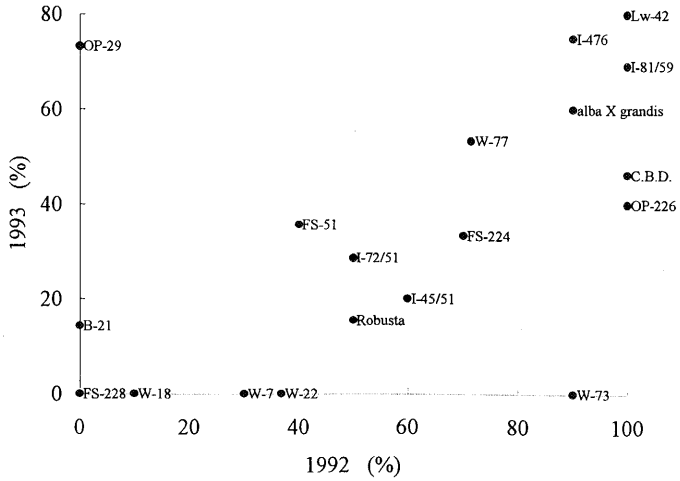

Fig. 5. Correlation of the proportions of adventitious bud formation on the medium containing 0.4 mg/*l* BAP between 1992 and 1993.
Each dot shows the data of different hybrid.

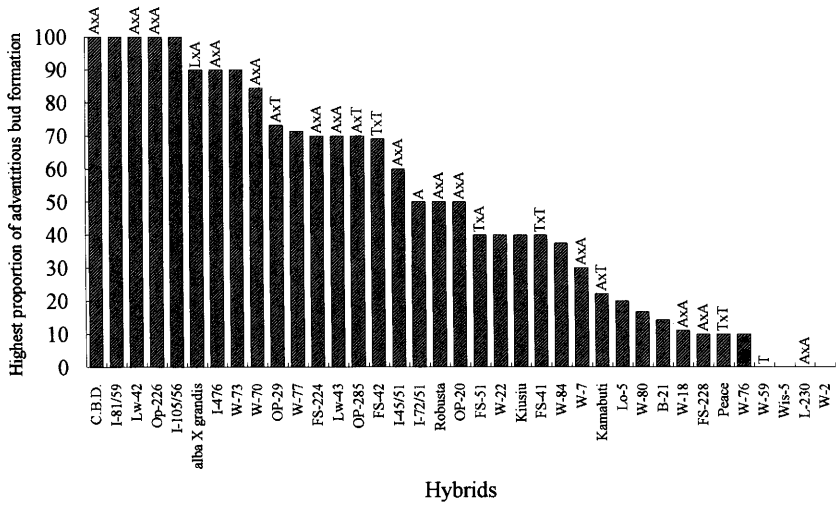

Fig. 6. Highest proportions of adventitious bud formation for each hybrids recorded during two years test.
Alphabet at the top of bars indicate the sections to which the hybrid's parent plants belonged.

which their parent plants belonged (Table 1) (INOKUMA 1958a, 1958b, 1959, CHIBA, 1962, CHIBA, 1993). Though we could not find significant relationship between the competence of adventitious bud formation of poplar hybrids and the sections to which the parent species of hybrids belonged (Fig. 6).

In this experiment we established the techniques of plant regeneration from petioles of poplar hybrids. Petiole segments of eleven hybrids among 37 tested hybrids regenerated plantlets. Those of the other 26 hybrids may have the potential of plant regeneration by

petiole culture. However there are differences in the competence of adventitious bud formation among hybrids, we would continue experiment to determine optimal condition for plant regeneration of these remaining hybrids. By the establishment of petiole culture, gene conservation of poplar hybrids by tissue culture would advance very much.

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Summary

A protocol for *in vitro* plant regeneration of poplar hybrids from petiole segments was established. When petioles of 37 poplar hybrids were cultured on a 1/2MS medium containing 0.4 mg/*l* N6-benzylaminopurine, adventitious buds were differentiated on the segments of 33 hybrids and developed to elongated shoots. The elongated shoots of 11 hybrids regenerated roots after being transferred onto a 1/2MS medium without any plant growth regulators. They were all successfully acclimatized and grown up to plantlets. While it was suggested that there exist differences in the competence of adventitious bud formation among hybrids.

Key words: Poplar hybrids, *Populus* spp., Petiole culture, Adventitious bud, Plant regeneration

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雑種ポプラの葉柄培養による植物体再生と不定芽形成能力の品種間差異

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要　　旨

雑種ポプラ (*Populus spp.*) の葉柄培養による植物体再生法を確立した。雑種ポプラ 37 品種の葉柄を 0.4 mg/*l* の N6-ベンジルアミノプリンを含む 1/2MS 培地上で培養したところ，33 品種で葉柄の切り口からの不定芽の形成およびシュートの伸長が認められた。また，伸長したシュートを切り取って，成長調節物質を含まない 1/2MS にさしつけたところ，そのうち 11 品種が発根して，幼植物体を再生した。再生した植物体はポットに移植し，順化させることが可能であった。これにより，ポプラの葉柄培養による植物体再生法が確立された。一方，不定芽の形成能力には，品種の違いに起因する差が存在することが指摘された。

キーワード：　ポプラ品種，*Populus* spp.，葉柄培養，不定芽，植物体再生