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In Vitro Plant Multiplication from Axillary Buds of *Populus davidiana* Dode*

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사시나무(*Populus davidiana* Dode)의 腋芽를 이용한 器內大量增殖*

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ABSTRACT

An effective *in vitro* multiplication method was developed for clones of *Populus davidiana* Dode. Ten different media were tested for their effect on shoot multiplication. Both MS and LP medium with 0.2mg/l BAP appeared to be the best for the shoot multiplication with the rate of 9 shoots per explant. There were significant differences among the clones in both multiplication rate and shoot growth. While some clones did not require BAP to promote shoot formation, others did. More than 60% of *in vitro* shoots rooted on the half-strength GD medium containing 0.2mg/l IBA.

Key words : Populus davidiana Dode, *axillary bud, shoot regeneration, rooting, clonal difference*

要 約

*Populus davidiana*는 종자의 활력이 짧고(약 3주), 삽목시 발근율이 저조하여 전통적인 유성 · 무성번식법의 어려움이 있어서 효과적인 기내증식법을 연구하였다. 10가지의 기본 배지를 이용하여, 1-2개의 액아를 포함한 잎이 붙어 있는 줄기절편체를 0.2mg/l의 BAP가 첨가된 배지에서 배양한 결과 MS 배지와 LP배지에서 가장 많은 신초를 생산하였다. 두 배지에서 5주간 배양한 결과 절편체당 9개 이상씩 신초를 생산하였다. 신초생산과 신초의 생장에 있어 클론간의 상당한 차이를 보였다. 몇 클론은 생장조절물질을 첨가하지 않아도 신초를 생산하였다. 기내배양된 신초 중 60% 이상이 0.2mg/l의 IBA가 첨가된 1/2 GD배지에서 발근이 가능하였다.

INTRODUCTION

Poplars grow widely in the temperate regions of the northern hemisphere and are economically important due to their fast growth and short rotation time(Ahuja, 1986 ; Chun *et al.*, 1986 ; Hall *et al.*, 1982). To date, 32 poplar species have been known. The first large plantation was established in Europe where the economic value of poplar was recognized(Noh *et al.*, 1988). In Korea, domestic plantations supply about 60% of the annual poplar timber demand. Since the mid-1980's US $ 2 to 2.5 million have been spent per year for importation of the timber. To correct this imbalance, it is desirable to establish many more short-rotation plantations. Prior to 1981, Lombardy poplars(*Populus nigra var. italica*) were planted mainly in dry riverbeds and in mountain lowlands. Since 1981, however, the Ministry of Construction has strictly prohibited any riverbed plantation, fearing that the trees

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may cause the riversystem to overflow during the rainy season.

The selection of poplars suitable for growing in mountain regions is thus of paramount importance. For the rapid establishment of plantations, efficient propagation systems need to be developed.

Populus davidiana Dode is widely-distributed in Korea except in the southern parts of the penninsula, It grows faster at high altitudes and is more resistant to dry, poor and acidic soil than other *Populus* species that are endemic as well as introduced. However, as in other poplars, the seeds are too small to collect and handle. They reamin viable for only a short period of time(about 3 weeks). In addition, early seedling development is precarious. Traditional asexual propagation proved to be difficult because of their low rooting percentage(less than 20%) in cuttings(Noh *et al.*, 1989).

This paper reports the use of micropropagtion as a potential reliable method for the multiplication of *Populus davidiana*.

MATERIALS AND METHODS

1. Effect of media on shoot multiplication

In this study, 21 clones of *Populus davidiana* plus trees were used(Baekdam-BD ; Sogwang-SG ; Taehwa-TH ; Ohdae-OD ; Sukam-SA ; Hama-HM ; Chungnyang-CN ; Pukdae-PD and Hankye-HK). Nodal explants with 1-2 axillary buds were taken from *in vitro* shoot cultures that had been initiated from root sprouts of *Populus davidiana* plus trees and had been maintained *in vitro* for 2 years. Ten different media all containing 0.2mg/l 6-benzylaminopurine(BAP) were tested for their effect on shoot multiplication. They included mB5(Gamborg et al., 1988), DKW(Driver and Kuniyuki, 1984), GD(Gresshoff and Doy, 1972), H(Heller, 1965), LM(Litvay et al., 1981), LP(Quoirin et al., 1977), MS(Murashige and Skoog, 1962), SH(Schenk and Hildebrandt, 1972), W(White, 1963), and WPM(Lloyd and McCown, 1980). Ten explants were cultured on each medium for 5 weeks. The number of shoots formed was counted and their growth were measured.

To investigate the effect of explant position on shoot multiplication, 2~3cm long *in vitro* shoots were divided into two segments(upper and lower) containing 1-2 axillary buds with or without leaves. The upper segments contained terminal buds. Ten explants of each position were cultured on MS medium containing 0.2mg/l BAP.

Prior to adding of 0.2% gelrite(Sigma Co. w/v) all media were adjusted to pH 5.5-5.8. The gelrite was dissolved by microwaving. Eight ml of the media were then dispensed into 2×15cm glass test tubes. Capped test tubes were sterilized by autoclaving at 121℃ and 1.05kg/cm^2 for 15 minutes. All cultures were kept in a growth room at 25±2℃ under 70% humidity. Illumination of 60μmol m^{-2}s^{-1} was supplied by cool white fluorescent lamps with a sixteen-hour photoperiod. Between 5 and 20 replicates of each clone were used for each treatment. Data were analysed by Duncan multiple range test.

2. Clonal differences in shoot multiplication and rooting

MS medium containing 0.2mg/l BAP was used to investigate clonal differences in shoot multiplication. The media were prepared as discribed above. To compare the rooting ability among the clones, a half-strength GD medium containing 0.2mg/l indolebutyric acid(IBA) solidified with 0.7%(w/v) of agar(Gumagar, Sigma Co.) was used. Ten to twenty shoot explants derived from each of 21 clones were taken for the experiment. Incubation conditions were the same as those described above. Only the roots more than 0.5cm in size were counted.

RESULTS AND DISCUSSION

1. Effect of media on shoot multiplication and growth

Differences in shoot multiplication and growth were observed among the explants growing on different basal media(Table 1). LP and MS media gave the best results, although DKW supported shoot growth best. The following five media-mB5, GD, H, SH, and W were not recommendable for shoot proliferation of *Populus davidiana*.

Table 1. Effect of media on shoot multiplication of *P. davidiana*.. Culture period was 5 weeks.

Media	The number of shoots formed	Shoot length(cm)
mB_5	3.9 ± 1.9* a**	1.6 ± 0.7
DKW	7.0 ± 1.3 b	3.1 ± 1.4
GD	3.7 ± 1.3 a	1.5 ± 1.4
H	4.2 ± 1.9 a	1.5 ± 0.9
LM	6.9 ± 1.2 b	1.7 ± 0.7
LP	9.2 ± 2.4 c	2.3 ± 1.4
MS	9.2 ± 2.4 c	2.7 ± 1.4
SH	3.8 ± 1.0 a	1.4 ± 0.5
W	4.7 ± 1.8 a	1.3 ± 0.6
WPM	7.0 ± 1.6 b	1.9 ± 1.2

* : Mean ± standard deviation
** : The same letter is not different according to Duncan's multiple range test at p=0.05

Some differences in shoot and leaf morphology were observed among the explants grown on different media. For example, the explants cultured on WPM developed short shoots with broad leaves and formed big calli at the base of the explants. The basal parts of stems were swollen within one week in culture. Axillary buds also burst out. More multiple shoots were developed from the proximal axillary buds than from the distal buds. Our findings are constent with those of other worker(Christie, 1978). Christie(1978) reported that MS medium was preferable for poplar axillary bud culture. She suggested that 10^{10} plantlets could be produced per year from a single axillary bud if growth regulators were added at optimum concentrations(MS+BAP 0.2mg/l, NAA 0.02mg/l). In the present study, one explant could be practically multiplied to 2×10^6 within a year. While MS medium has also been used for the multiplication of poplars by other workers(e.g., Barocka *et al.*, 1985 ; Gebhardt, 1989 ; Rutledge and Douglas, 1988), WPM has successfully been used for mass propagation of *Populus tremula* and *P. tremuloides* with some minor modifications(Ahuja, 1984).

To test the effect of the explant positions on shoot proliferation, another experiment was carried out. The explants were cultured with leaves attached. These explants produced more shoots than did those without leaves. The presence of leaves might have influenced the growth of the shoots induced(Table 2). Longer shoots were produced from the explants taken from the upper part than from the lower part. It might have been caused by the terminal buds of the upper parts. The explants from the upper part elongated faster than those from the lower parts. Three weeks after the culture, roots were visible at the base of the explants. No rootings were observed from the explants derived from the upper parts. Geotropical accumulation of root-inducing substances might have caused these differences between the two types of the explants.

2. Clonal differences in shoot multiplication and growth

Much difference was found among the clones in the rate of shoot multiplication and growth (Table 3, Fig. 1). Some clones(SG 12, 13, and 14) did not seem to require BAP to produce shoots though most others needed it.

Douglas(1984) also observed multiple shoots when he cultured the axillary bud of *Populus detoides* on hormone-free MS medium. However, Christie(1978) reported that cytokinin was required to promote shoot formation in *Populus* spp.

In some clones(TH 8, TH 18, HM 4, and CN 3) the addition of BAP led to a high number of shoot formation. The clone TH 18 was the most responsive in shoot production and thus produced

Table 2. Effect of explant position on shoot mul-tiplication and growth

Position	Leaf	No. of shoots formed	Shoot length(cm)	Rooting rate(%)
Upper	with leaf	5.9 ± 1.7*	3.2 ± 1.5	0.0
	without leaf	4.4 ± 1.3	1.8 ± 0.9	0.0
Lower	with leaf	5.5 ± 2.2	3.0 ± 1.7	40.0
	without leaf	5.4 ± 2.4	1.6 ± 0.8	20.0

* : Mean ± standard deviation

Table 3. Clonal difference in shoot multiplication and growth

Clones		No. of shoots formed		Shoot length(cm)	
		BAP 0.0	BAP 0.2	BAP 0.0	BAP 0.2
BD	3	1.4 ± 4.2*	4.0 ± 4.2*	0.7 ± 0.1*	1.3 ± 0.8*
	7	1.0 ± 0.0	3.0 ± 1.0	1.0 ± 0.0	1.3 ± 0.5
	10	1.9 ± 1.3	3.8 ± 2.3	2.4 ± 1.3	1.3 ± 0.5
	13	1.0 ± 0.0	2.8 ± 1.5	1.0 ± 0.0	1.2 ± 0.3
	14	1.0 ± 0.0	3.2 ± 3.2	1.0 ± 0.0	1.4 ± 0.7
	15	1.5 ± 0.7	2.5 ± 0.7	3.4 ± 1.0	2.1 ± 1.1
	16	1.3 ± 0.8	3.3 ± 2.7	1.4 ± 0.0	1.5 ± 0.8
SG	12	5.4 ± 4.5	5.8 ± 2.7	1.7 ± 0.9	1.4 ± 0.6
	13	3.6 ± 2.2	3.5 ± 3.5	1.9 ± 1.0	0.9 ± 0.1
	14	5.1 ± 4.3	4.4 ± 2.4	2.3 ± 1.6	1.4 ± 0.6
TH	8	1.7 ± 1.2	6.8 ± 1.2	2.6 ± 1.3	2.5 ± 1.2
	18	2.2 ± 1.9	9.0 ± 1.0	2.3 ± 1.2	3.5 ± 1.1
OD	5	1.9 ± 1.2	2.0 ± 1.4	0.8 ± 1.2	1.3 ± 0.4
	11	1.0 ± 0.0	4.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.4
SA	2	1.4 ± 0.9	4.0 ± 2.6	2.0 ± 0.0	1.4 ± 0.6
	10	1.2 ± 0.4	4.0 ± 2.5	1.2 ± 0.0	1.9 ± 0.9
HM	4	1.7 ± 0.8	7.0 ± 2.8	0.8 ± 1.6	1.6 ± 0.6
	5	1.8 ± 1.6	4.0 ± 1.5	2.7 ± 1.1	1.5 ± 0.5
CN	3	1.7 ± 0.8	7.0 ± 2.8	0.8 ± 1.6	1.6 ± 0.6
PD	7	1.0 ± 0.0	5.0 ± 2.7	1.0 ± 0.0	1.7 ± 0.7
HK	11	2.7 ± 2.5	5.3 ± 2.2	2.7 ± 1.4	1.8 ± 1.1

* : Mean ± standard deviation

A B C

A. Clones of SG 12, 13, and 14 from left to right, developing many healthy, vigorous leaves.
B. Clones originated from BD 15, developing long nodes with several leaves.
C. Comparison of BD and SG clones. BD clones grew fast and showed long nodes with several leaves, while SG clones formed thick crown containing short nodes and many shoots. It needs further study to make use of these differences for early test.

Fig. 1. Clonal differences in shoot multiplication and growth

the longest shoots. The results observed in this work are similar to those obtained by others (Rutledge and Douglas, 1988 ; Coleman and Coleman, 1990). Rutledge and Douglas(1988) reported clonal differences in the number of shoots produced on the same culture medium among 12 commercially important poplar clones. Coleman and Ernst(1990) found differences in the multiplication rate among the clones of *Populus deltoides*. It is an interesting observation that each provenance manifested its specific characteristics during growth(Fig. 1). The BD clones produced shoots with a few leaves and did not form callus at the proximal end of explants. The HM clones,

Table 4. Clonal difference in rooting rates and root number

Clones	Rooting rate(%)	No. of roots formed	Clones	Rooting rate(%)	No. of roots formed
BD 3	70	1.3 ± 1.3* a**	TH 8	100	2.8 ± 2.2 ab
7	100	2.9 ± 1.8 ab	18	70	1.5 ± 1.4 a
10	100	2.8 ± 1.9 ab	OD 5	70	1.8 ± 1.6 ab
13	100	2.9 ± 1.2 ab	11	100	4.0 ± 2.7 bc
14	100	2.8 ± 1.8 ab	SA 2	80	1.7 ± 1.4 a
15	80	3.1 ± 2.1 ab	10	60	0.8 ± 0.8 a
16	100	2.3 ± 1.7 ab	HM 4	100	5.3 ± 1.8 cd
SG 12	90	9.3 ± 2.7 ef	5	100	5.6 ± 2.5 cd
13	100	7.4 ± 2.8 de	CN 3	100	2.6 ± 1.2 ab
14	100	11.4 ± 3.4 f	PD 7	100	2.6 ± 1.1 ab
			HK 11	100	5.8 ± 3.9 cd

* : Mean ± standard deviation

** : The same letter is not different according to Duncan's multiple range test p=0.05

however, produced shoots with many large leaves and formed a large callus at the base of the explants. The SG and the TH clones developed very green vigorous leaves, while the OD clones showed necrotic shoot-tips and leaves that fell down within 5-week culture period.

Conventional time-dependent tree breeding needs tools for prediction of the performance of trees at maturity based on juvenile traits. If *in vitro* characters shown in the test tubes are maintained in the field or are in some way linked to field performance, they could serve as a useful tool for early prediction. Therefore careful observation as well as maintenance of these plants should be made even after planting in the field.

3. Clonal differences in rooting

Populus dividiana has not been propagated by cuttings since it has poor rooting ability. However *in vitro* shoots showed very high rooting percentages(Table 4). The lowest rooting rate was 60% in the clone SA 10. Fourteen of the twenty-one clones showed 100% rooting. Differences in both secondary root formation and root color were also observed among the clones. There were remarkable differences in the number of roots formed among the clones. The SG clones produced 7 to 11 roots per explant, while SA 10 and BD 3 gave the least number of roots and low rooting ability.

Clonal differences in *in vitro* rooting have also been observed in other poplar species. Ahuja (1984) reported clonal differences in *in vitro* rooting of *Populus tremula*. Barocka *et al.*(1985) also reported differences among *Populus* clones in rooting and acclimatization. There was no difference among the explants of the same clone. This result is consistent with that of Wann and Einspahr(1986) who observed the same pattern among individuals of one clone in rooting ability in *P. tremuloides*.

The explants developing many roots should be transferred to the greenhouse for acclimatization since the roots started to twine if the explants stayed longer in the test tubes. Although the explants continued to grow even after rooting, new axillary bud break did not happen. Vitrification of the explants was not observed in any of the rooting treatments. Rooted plantlets are presently growing in the greenhouse.

The present study was carried out to establish the *in vitro* multiplication system for *Populus davidiana* Dode. For axillary bud culture, MS and LP media with 0.2mg/l BAP were recommendable. If the explants from the lower shoot segment are to be used, less than 0.2mg/l IBA may be added for vigorous rooting. Clonal differences, however, were signigicant in shoot multiplication and growth and in rooting capacity. Attention and careful set-up may be required in a large-scale mass-production program.

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