韓國林學會誌 80(4) : 416~419. 1991.
Jour. Korean For. Soc. 80(4) : 416~419. 1991.

In Vitro Propagation Using Stool Shoots of Mature *Betula platyphylla* var. *japonica*[1]

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자작나무 성숙목의 근주맹아를 이용한 기내증식[1]

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ABSTRACT

Effective micropropagation was achieved by axillary bud culture from stool shoots of 15-year-old *Betula platyphylla* var. *japonica*. Shoot development and proliferation from the explants were successful on WPM supplemented with 0.5 or 1.0mg/1 BAP. All the regenerated shoots rooted when transfered to GD medium containing 0.2mg/1 IBA. After transplaning to soil more than 95% of the plantlets survived and showed normal growth. The results demonstrate that masspropagation of selected mature trees is feasible using tissue culture technique.

Key words : *Axillary bud culture*, Betula platyphylla *var.* japonica, *micropropagation*.

적 요

15년생 자작나무의 근주 맹아지 액아를 이식절편체로하여 기내배양시켜 효과적으로 증식시킬 수 있었다. 줄기증식에는 WPM에 BAP 0.5와 1.0mg/1 첨가한 배지가 효과적이었다. 기내발근에는 GD배지를 이용하였고 0.2mg/1 IBA 첨가시 100% 발근되었다. 이렇게 증식된 식물체는 토양에 이식하여 95% 이상 활착되었으며 활착후 정상적인 생장을 보였다. 이상의 결과는 자작나무 성숙목의 기내 대량 증식 가능성을 시사한다.

Introduction

Betula species have been planted frequently for ornamental or timber trees in Korea. Most of the plantations have been established with seedlings. Although *Betula* species could be propagated by cuttings, it is still difficult to establish a large scale plantations using cuttings because of the poor rooting efficiency[6,7]. Now attempts are being made to select plus trees for mass production of superior phenotypes in this Institute. In order to maintain high genetic gain, it is sometimes necessary to propagate the selected trees by clonal propagations. If selected trees could be propagated through tissue culture techniques, the improvement would be great. Recent works indicated that *in vitro* regeneration would not only prove a quick method for vegetative propagation but also maintain genetic characteristics of any species[1,14]. However, mature trees are

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generally recalcitrant to vegetative propagation due to their age[4,5,16]. Many workers have reported successful *in vitro* propagation and good morphogenetic response in *Betula* species using various juvenile tissues[2,3,10,13]. Although there have been some reports on *in vitro* propagation with adult tissue explants, the data of transplanting to soil and survival afterwards are very rare[8,9,11,15].

In this paper we report regeneration of Japanese white birch (*Betula platyphylla* var. *japonica*) plantlets from axillary bud explants, transplantation to soil, and survival of the plantlets in the field.

Materials and Methods

Sprouts of 15 year old *Betula platyphylla* var. *japonica*, growing at the arboretum of the Institute of Forest Genetics, Suwon, Korea, were used as material. Shoots were collected from basal part of a single tree in mid-june, 1990. Shoot were cut into 3-5cm long pieces, surface sterilized with 70% ethanol for 30 sec followed by 3 min soaking in 2% NaClO, and rinsed three times with sterile distilled water. They were then submerged in sterile distilled water for 30 min, cut into 1.5 to 2.0cm piece with one axillary bud, and implanted into 25×150mm test tubes containing 8ml of culture medium. WPM supplemented with 0.5mg/1 BAP, 3%(w/v) sucrose, 100mg/1 inositol, and 0.75%(w/v) agar was used. The pH was adjusted to 5.8 before autoclaving at 121℃, 15 psi for 15 min. Cultures were maintained under cool white fluorescent light (2,000-3,000 Lux, a 16hr photoperiod) at 23±3℃.

After one week, shoots bursted from axillary buds of the explants were cut and subcultured to fresh medium for shoot proliferation. After 3 to 4 times of subculture for 12-16 weeks, the induced shoots were subcultured onto WPM or MS medium containing various combinations of BAP and NAA to find the optimal culture condition (Table 1). After 4 weeks in culture, the regenerated shoots in different media were counted. For mass propagation, new shoots, 2 to 4cm long, were excised from the original cultures and subcultured to fresh WPM containing 1.0mg/1 BAP at 4 week interval. *In vitro* rooting was tested in two kinds of media, GD and half-strength GD media supplemented with 0.2mg/1 IBA. After 3 weeks in culture, adventitious primary roots were counted. Rooted plantlets were transplanted to pots containing a 1 : 1 mixture of peatmoss and perlite, and nurtured on automatic environmental control room (25±2c, 80±2% relative humidity with 16 hr photoperiod (3,000 Lux) for 3 weeks. Survived plantlets were moved out-door and transplanted to soil in the field.

Results and Discussion

All the buds from the explants bursted within a week in culture and developed into shoots. After 4 week in culture, the shoots were elongated to 2-3cm long. The basal end of all explants formed greenish callus. Small callus was observed on the leaf part in contact with the medium.

Rapid shoot elongation and proliferation were obtained on WPM supplemented 0.5 or 1.0mg/1 BAP in combination with 0.01 mg/1 NAA (Table 1). Whereas 1.0mg/1 BAP was more effective than 0.5

Table 1. Effect of media and growth regulators on shoot proliferation of *B. platyphylla* var. *japonica*. Axillary bud explants from 15 year old tree were cultured for 4 weeks.

Media	Growth regulators (mg/1)		No. of explants cultured	No. of shoots obtained	Mean no. of shoots induced
	BA	NAA			
M S	Control		52	52	1.0
	0.2		30	30	1.0
	0.5		30	30	1.0
	0.5	0.05	14	30	2.1
	1.0		30	30	1.0
	1.0	0.05	30	30	1.0
WPM	0.5		30	120	4.0
	1.0	0.01	28	146	5.2

mg/1 BAP in shoot proliferation, 0.5mg/1 BAP gave better results in shoot elogation. Multiple shoots were regenerated mainly from axillary bud explants. Only a single shoot was developed from the explants derived from apical bud. WPM seemed to be the better medium for shoot proliferation and growth in this species. Shoots developed on MS media either failed to further elongate or were stunted after 3-4 weeks in culture. Shoot-tip necrosis and browning was frequently observed when MS medium was used. Sha *et al.*[12] reported that shoot-tip necrosis is associated with a Ca deficiency in a actively -growing shoot culture. They also conjectured that aeration in the culture vessel might be responsible for the browning. However, it is not true in this species since shoot-tip necrosis was not problem when WPM was used. The highest rate of proliferation(5.2 shoots per explant) was obtained on WPM with 1.0mg/1 BAP and 0.01 mg/1 NAA(Table 1).

About 20% of the shoots rooted spontaneously on WPM supplemented with 1.0mg/1 BAP after 3 to 4 weeks in culture. The spontaneous rooting in the presence of BAP seems to be due to auxin produced from the shoots. It may also be possible that BAP supplied to the medium could be used up in 3-4 weeks of culture. However, these shoots rooted very well on GD medium containing 0.2mg/1 IBA. We tested half strength and full strength media in both rooting rate and the number of roots obtained. There seemed to be no difference between the two concentration. Rooted plantlets were transplanted to pots containing a 1 : 1 mixture of peatmoss and perlite, and incubated in a culture room for 3 weeks before transplanting outside. About 95% of the plantlets survived and grew normally(Fig. 1).

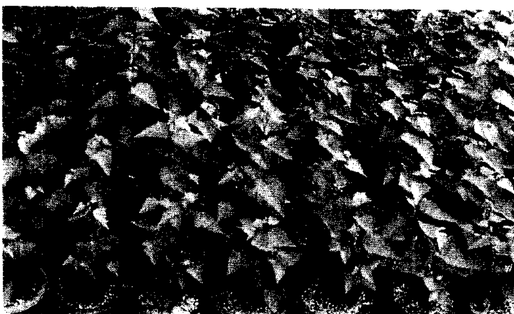

Fig. 1. Acclimatized plantlets after transplanting to soil.

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