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Survival and Early Growth of *Populus alba* × *P. grandidentata In Vitro* Culture Plantlets in Soil[1]

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Populus alba × *P. grandidentata* 組織培養苗의 土壤에서의 活着과 生長[1]

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ABSTRACT

This study was undertaken to find out the effects of three kinds of potting media and two sources of explants on the survival and early growth of new plantlets of *Populus alba* x *P. grandidentata* in the greenhouse. The results obtained can be summarized as follows; 1) Among three potting media, Terralite was best for early growth and survival of plantlets. 2) Like humidifier, an intermittent misting system can be effective in keeping relative humidity high for the plantlets. 3) Survival rates over 80% could be obtained if humidity was kept high during the hardening period. 4) During hardening period, the plantlets showed the juvenile characteristics such as smaller leaves, thinner stems, and shorter internodes. 5) There were no differences on morphological characteristics between the plantlets originating from axillary buds and the plantlets originating from multiple shoots while they were growing at the greenhouse. 6) The plantlets originating from bud culture grew normally comparing to regular cuttings.

Key words: Populus alba × P. grandidentata; *potting medium; terralite; bud culture.*

要 約

無菌狀態와 最適의 環境條件에서 培養된 *Populus alba* × *P. grandidentata* 組織培養苗를 一般 溫室의 環境條件에 硬化시키고, 높은 活着率과 빠른 早期生長을 위해서 3가지 培養土에 移植하여 3週間 misting bench에서 硬化시킨 後 生育狀態를 比較 관찰하였다. Terralite, Jiffy mix, Jiffy-7-pellet의 3가지 培養土中 Terralite가 組織培養苗의 活着과 早期生長에 가장 좋았으며, 溫室移植 3週後의 活着率은 각각 平均 90%, 86.7%, 86.7%였다. 腋芽에서 起源한 組織培養苗와 multiple shoot에서 起源한 組織培養苗에 對한 外部形態의 比較에는 差異가 없었으나 生長에 對한 比較에는 有意性이 인정되었다. 溫室內에서 硬化期間동안 작고 좁으며 가는 잎과 줄기, 짧은 internode와 같은 幼性이 組織培養苗에 나타났으나, 移植 5～6週後에는 正常的인 植物體의 形態로 生長이 진작되었다.

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INTRODUCTION

For clonal propagation through *in vitro* culture of forest tree species, most of work has been concentrated on shoot and root induction (John, 1983; Dodds, 1983). Relatively few researchers have investigated the transfer of plantlets from *in vitro* conditions to the greenhouse (Leach, 1979; Mckeand and Wisniewski, 1982).

Several environmental conditions are essential in the initial hardening period while new plantlets which are produced through *in vitro* are transplanted to the greenhouse. One is maintenance of high relative humidity for two to three weeks to protect the plantlet from desiccation and enable it to initiate new roots and new shoots. The second requirement is a loose, aerated, welldrained rooting medium, which allows new roots to develop quickly. Desiccation and wilting are major causes of low survival when plantlets are transplanted. In aseptically cultured plum (Fuchigami et al., 1981) and carnation plantlets (Sutter and Langhans, 1979), lack of epicuticular wax was believed responsible for excessive transpiration. Brainerd et al. (1981) have also shown that aseptically cultured plum plantlets have smaller palisade cells, larger intercellular spaces, and low stomatal frequency. They were more easily injured by water stress than transferred plants which were acclimatized to the greenhouse.

To prevent the desiccation and wilting, plantlets were always covered with a thin layer of water during the early hardening period. There are two major methods to keep high relative humidity for the plantlets when they are transplanted from test tubes to the greenhouse. One is to supply the high humidity by intermittent mist (Cheng, 1978) and another is by humidifier (Kim et al., 1981). Kim et al. (1981, 1982) reported that over 90% survival could be obtained while the humidity of the greenhouse was kept high by a humidifier during the hardening period in hybrid poplar. Several person have shown that 70% to 100% of relative humidity was essential to harden new plantlets in the growth chambers or the greenhouse (Chalupa, 1974; Cheng, 1978; Reilly and Washer, 1977; Leach, 1979; Werner and Boe, 1980).

To promote the autotropic growth of new plantlets, new roots have to develop quickly when plantlets are transplanted to a normal soil environment. Many materials could be used as a potting medium such as sand, peat, perlite, sphagnum moss, vermiculite, pumice and loam, but for functional and economic reasons, peat-vermiculte mixtures predominate (Chalupa, 1974; Cheng, 1978; Whitehead and Giles, 1977; Reilly and Washer, 1977; Christie, 1978; Leach, 1979; Werner and Boe, 1980).

This study was undertaken to find out the effects of three kinds of potting media and two sources of explants on the survival and early growth of new plantlets of *Populus alba* × *P. grandidentata* when they were transferred to the greenhouse. Other problems associated with hardening off of new plantlets were also discussed.

MATERIALS AND METHODS

Three clones of *Populus alba* × *P. grandidentata* at the Iowa State University Forestry Department greenhouse were collected for use in this study. These three clones were Crandon, Hansen and Sherrill.

To culture the axillary buds of these three clones, axillary buds were removed from the upper one third of actively growing shoots of each clone. Buds were prepared for sterilization by trimming off leaves while retaining a small piece of petiole attached to the stem. The buds were dipped in 95% ethanol for ten seconds prior to a five minutes surface sterilization in a solution of 5% sodium hypochlorite and 0.04% Tween 20 in a laminar flow hood. This was followed with three sterile distilled water rinses.

Excised buds, 5 to 8 mm long, were cultured on Gresshoff and Doy (1972) medium.

For multiplication of these induced explants on Gresshoff and Doy (GD) medium, the axillary shoots of three clones were cut into five to six

segments, each with one axillary bud and one or two leaves. Then, to induce axillary shoots and multiple shoots, these axillary buds were subcultured on GD medium containing 0.2 mg/l Indole-3-butylic acid (IBA) and on Murashige and Skoog (1962) medium containing 0.2 mg/l 6-Benzylaminopurine (BAP).

When the desired number of axillary shoots and multiple shoots were produced for this study on both media, these two sourses of explants were subcultured to induce the roots on GD medium with 0.2 mg/l IBA for 20 days.

Culture room conditions were as follows: the day time temperature was controlled at 25-28°C with a 16 hour photoperiod and photosynthetically active radiation level of 50-60 $m\mu$ E/m^2/s from cool white fluorescent tubes. The night time temperature was controlled at 20-23°C with 8 hours darkness.

Three commercial potting media, Terralite, Jiffy mix and Jiffy-7-pellets, were tested to find the effect of potting media on transplanting of new plantlets.

The plantlets of each clone were randomly selected to be transplanted to the potting media and to be inserted in expanded Jiffy-7-pellets. The styrofoam round cups 8cm x 10cm in length were used as pots.

Transplants were then hardened in the greenhouse by intermittent misting under 30% shade for a period of three weeks. The misting system operated for 30 seconds on each five minute period. Three weeks after transplanting these plantlets were transplanted again into Terralite medium in the 4 inch round standard pots and the screen was removed.

Begining in the fourth week after transplanting, 20 ml of Peters water soluble fertilizer (20-20-20) was supplied twice a week to these plantlets.

The plantlets transplanted to the potting media and Jiffiy-7-pellets were arranged in a completely randomized design. The design included 18 treatments consisting of three clones, two sources of explants and three potting media. Fifteen replications of each treatment were tested. The survival rate at planting and early growth rate were measured every week for eight weeks.

RESULTS AND DISCUSSION

Most of the mortality occurred within the first three weeks after transplanting, but some additional plants died after transplanting from the small pots to the large ones. Twelve percents of the plantlets died within three weeks (Table 1). Eight weeks after transplanting, 18% of the plantlets had died. These good results show that intermittent misting can be successful in keeping the relative humidity high, similar to what Kim et al. (1981) achieved with a humidifier. Analysis of variance for the percent of survival of plantlets indicated that clone, potting medium and source of explant were not significant.

Table 1. Percent of survival three weeks and eight weeks after transplanting to the potting media

clone		Crandon		Hansen		Sherrill	
source		axillary bud	multiple shoot	axillary bud	multiple shoot	axillary bud	multiple shoot
Time (wks)	potting medium						
3	Terralite	86.7	86.7	93.3	93.3	100	80.0
	Jiffy mix	86.7	86.7	93.3	80.0	86.7	86.7
	Jiffy-7-pellet	86.7	93.3	80.0	80.0	86.7	93.3
8	Terralite	80.0	73.3	86.7	86.7	86.7	80.0
	Jiffy mix	80.0	80.0	93.3	73.3	86.7	86.7
	Jiffy-7-pellet	86.7	73.3	80.0	73.3	80.0	86.7

Surviving plantlets began shoot growth within 2 weeks after transplanting. The plantlets with large initial height tended to have greater initial shoot growth, but this relationship was not related to the

mortality. On the contrary, Leach (1979) reported that mortality and shoot growth of the plantlet of *Pinus taeda* appeared related to height at time of transplanting.

Five weeks after transplanting, shoot growth began accelerating though the plantlets still showed the juvenile conditions such as smaller leaves, thinner stems, and shorter internodes. The regular leaf traits and longer internode started to show about five to six weeks after transplanting.

Reversion from mature to juvenile characteristics, evidenced by hybrid poplar bud culture in this study, may be a general phenomenon in plant tissue culture (Scorza and Janick, 1980; Bonga, 1982). The cause of juvenile reversion in hybrid poplar bud culture is unknown. Sometimes, leaves with fine root system on petiole were found in direct cuttings of multiple shoots. Lyrene (1981), for example, found that shoots from tissue culture of blue berry cultivars rooted faster than shoots from field grown plants of the same cultivars. Callus from flowering cultures of *Nicotiana tabacum* L. stopped flowering after 4 subcultures, with the number of flowers decreasing with each subculture (Wardell and Skoog, 1973). This rapid loss of flowering potential may have signalled a return to the juvenile state.

Mean height of the plantlets at each weeks measurement is shown in Figure 1. The mean increases in shoot growth for three weeks and eight weeks after transplanting from axillary buds with the shoots originating from multiple shoots, the shoots originating from multiple shoots, the mean increase in shoot growth of the former was better

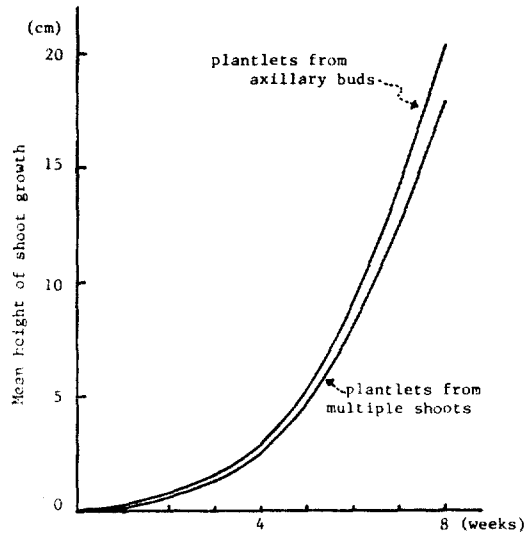

Fig. 1. Mean increase in shoot growth of the plantlets for eight weeks

than the latter. This result was probably because multiple shoots were conditioned in a high cytokinin medium to favor shoot proliferation over shoot elongation during multiplication stage (Hartmann and Kester, 1983). However, there were no differences on morphological characteristics between two explants sources. The Crandon clone was showed the best mean increase of shoot growth among the three weeks. But at eight weeks after transplanting, Sherrill clone was showing the best mean shoot growth. For potting soil, Terralite was best for early growth and survival of new plantlets during the hardening period of the first three weeks. Analysis of variance showed the difference in mean increase of shoot growth for the first three weeks to

Table 2. The mean increase in shoot growth for three and eight weeks after transplanting (mm)

clone		Crandon		Hansen		Sherrill	
source		axillary bud	multiple shoot	axillary bud	multiple shoot	axillary bud	multiple shoot
Time (wks)	potting medium						
3	Terralite	16.6	13.6	18.7	17.9	13.8	10.5
	Jiffy mix	19.9	14.0	17.9	12.7	11.5	12.6
	Jiffy-7-pellet	17.3	11.3	13.8	14.0	10.9	10.8
8	Terralite	208.9	173.0	185.8	175.6	215.3	208.2
	Jiffy mix	202.8	185.0	209.6	166.6	225.5	200.1
	Jiffy-7-pellet	183.6	165.2	186.6	167.3	225.5	181.0

be highly significant (0.0115, 0.0001 and 0.0001 level) for clone, source, and potting medium, respectively. However, the potting medium was not significantly different in the increase of shoot growth at eight weeks after transplanting. Terralite potting medium was best for early growth and survival of plantlets among the three potting media. This was probably because of the larger vermiculite mixed in Terralite than that in Jiffy mix. The air space in the more coarse vermiculite improves aeration and creates natural passageways for drainage.

Root development was not quantified, but the plantlets had developed fine, fibrous, new secondary root systems by two weeks after transplanting. However, Mckeand and Wisniewski (1982) have shown that loblolly pine tissue culture plantlets tended to have unbalanced and less fibrous root system comparing with seedlings. They also found that unlike seedling, plantlets of loblolly pine do not develop numerous secondary roots near the soil surface.

When these plantlets, with their first potting media, were retransplanted to the larger pots containing Terralite potting medium, there was no significant difference in shoot growth at eight weeks after transplanting. This result is a further indication that the potting medium was an important factor for the early growth of new plantlets, which were transplanted to the greenhouse.

These hardened plantlets originating from bud culture grew normally compare to regular cuttings at the nursery beds. This result was in accord with Lawrence's opinion (1981) that axillary bud multiplication is the most genetically conservative of the preferred system alternatives. Kim et al. (1981), for example, reported that there were no differences between the plantlets produced by bud culture and cuttings of *Populus alba* × *P. glandulosa* during one growing season. In contrast, callus cultures have a tendency to be genetically unstable (Thrope, 1983). The main reasons of genetically unstable are that apparently pieces of, or whole chromosomes are lost or duplicated during long term callus culture (Faltonson et al. 1984). Lester and Berbee (1977) reported that black poplar, derived from callus culture, showed a wide range of variation for height, number of branches, and leaf traits after one growing season.

For effective hardening off of the new plantlets, problems associated with environmental conditions other than humidity and potting media must be carefully considered. One is protection from various pathogens until some resistance has developed. Another is exposure of new plantlets to high light intensity after transplanting from culture vessels where plantlets were conditioned to low light intensity. When multiple shoots were taken directly to the greenhouse for rooting, there was a significant difference in rooting between shaded and non-shaded treatments(Chun, 1984). The probable reason for this result was that because the condition of multiple shoots was heterotrophic. Even though the leaves of multiple shoots were green, they may not actually be capable of photosynthesis (Hartmann and Kester, 1983).

It is clear from the data presented in this report that the kind of potting media influences the early growth and survival of plantlets in the greenhouse.

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