Application of in vitro ovary culture for cottonwood (*Populus deltoides*) breeding

Hoduck Kang* and Min-Soon Lee[1)]
Forest Biotechnology Lab., Department of Forest Resources, Dongguk University, Seoul 100-715, Korea
[1)]Department of Environment and Forest Products, Joongbu University, Kumsan 312-800, Korea

ABSTRACT

Five different poplar hybrids were tested for rescuing embryo to elongate *in vitro* plantlets after hybridization. Ovaries and ovules were cultured on Woody Plant Medium (WPM) supplemented with cytokinins, 6-benzylamine (BA) and zeatin. Multiple shoots were initiated from half section of capsule with immature embryos after 21 days from pollination and tiny shoots were formed after the expansion of cotyledons in ovule cultures.

Germinating response was better in intraspecific hybrids (6.53 ± 1.66) than interspecific crosses (0.93 ± 0.54) from half section of capsules on WPM medium. In general, zeatin was better than BA in inducing multiple shoots from isolated ovules. The highest average number (19.40 ± 4.53) of shoots was produced from immature ovules of 21 days post-pollination of WPM medium supplemented with 5.0 mg/L zeatin. The highest percentage of germination was 93% from the half section of *in vitro* ovary cultures. Soil acclimation was successfully conducted in cell tray containing artificially mixed soil with 96% survival rate.

Key words **:** Embryo rescue, immature ovule and ovary culture, in vitro germination, multiple shoots, intra- and interspecific poplar hybrids

INTRODUCTION

Interest in poplars is increasing because they are rapid growing trees and has value for lumber and biomass production (Hall *et al.*, 1982; Ahuja, 1987). Poplars can be propagated by grafting, root suckers, and stem cuttings (Herrmann and Seuthe, 1982; Hall *et al.*, 1989, Kang and Hall, 1996a).

Poplar tissue culture has been intensively studied for plant regeneration from axillary buds (Coleman and Ernst, 1990), stem internodes (Douglas, 1984; Kang and Hall, 1996a), leaf discs and root segments (Kang and Hall, 1996a), callus (Son and Hall, 1990), and protoplasts (Russel and McCown, 1986).

In poplar breeding program, poplar hybridization is usually conducted in a greenhouse where floral branches can be forced to flower and then pollinated under controlled conditions (Savka *et al.*, 1987). After pollination, to be maximized shooting ability from immature ovules or embryos, poplar seeds are required a long maturation period of time which is optimized upto 20 days (Kang and Hall, 1996b). Due to this long period, many crosses fail to produce seeds. The major obstacles are the premature abscission of branches

*Corresponding author : **Hoduck Kang**, E-mail : HDK0225@dongguk.edu

bearing catkins and early dehiscence of the entire capsules (Raquin *et al.*, 1992). To overcome these problems, *in vitro* systems such as immature ovule, embryo and ovary culture have been applied to rescue these premature seeds and to obtain the complete plantlets in poplar breeding programs (Kang and Hall, 1996b; Kouider *et al.*, 1984; Noh *et al.*, 1986; Raquin *et al.*, 1993; Savka *et al.*, 1987).

The objectives of the present studies were to (1) rescue premature embryos through *in vitro* germination in poplar breeding program, (2) enhance the ability of the complete plantlets of hybrids, (3) compare the percentage of *in vitro* germination among the different crosses after pollination, and (4) apply large-scale propagation to produce the diverse germplasms.

MATERIALS AND METHODS

Plant materials

Poplar hybridizations were conducted with the cooperation of diverse research groups; Iowa State University, Ames, University of Washington, Seatle, and Boise Cascade Company in the United States. To hybridize the cottonwood species, male and female flowers were collected in the early January around mid west regions; Iowa, Illinois, Minnesota, and Missouri. Pollinations were conducted in Forestry greenhouse at Iowa State University. The branches cut from male trees were planted to pots supplied with water in a greenhouse until ready to be pollinated. Most pollen sources were harvested after flowering of male trees. The duration of post-pollination expanded to 21 days in greenhouse condition controlled with 23± 2℃.

In vitro germination from capsule culture and shoot regeneration of immature ovules

Among the 22 combinations of hybridized cottonwoods, three of *P. deltoides* hybrids and two of *P. deltoides* X *P. nigra* hybrids were selected for *in vitro* culture to germinate shoots from premature embryos. Twenty-one days matured ovaries were used as explants for *in vitro* germination and shoot regeneration. Poplar capsules were sterilized with 70% ethanol for 1 min., 20% Clorox for 20 min. and then rinsed four times with distilled water. After disinfested, the capsules were dissected longitudinally to two pieces under aseptic condition and inoculated onto WPM medium supplemented with sucrose 30 g/L, Sigma agar 7 g/L excluding any plant growth regulators.

Immature ovules were isolated aseptically from the capsules and inoculated on the surface of 20㎖ of WPM medium supplemented with various concentrations of BA and zeatin of cytokinins. After 2 weeks of culture, the explants of immature ovules started to proliferate on the medium. *In vitro* germination and regeneration were recorded as successful for any explants that produced at least one shoot in the media. The total number of shoots was observed after four weeks of initial culture.

Shoot elongation and transfer to soil

Multiple shoots were transferred to elongation medium containing 0.02 mg/L NAA and allowed to grow for 1 month in a growth chamber. The cultures were maintained in the same condition at 23± 1℃ mean dry temperature, 23± 2℃ mean night temperature. Photoperiod was programmed at 16 h with an irradiance of 50-60$\mu Em^{-2}S^{-1}$ provided by cool-white fluorescent tubes.

Elongated shoots in rooting media were transplanted to soil in a cell tray after agar was removed with tap water. The plantlets were acclimated under shaded, intermittent mist for two weeks, transferred to a regular greenhouse bench under shading condition and grown for one month for hardening them. Survival data were collected after one month period in the green house. Each treatment was composed of five replications and every replication contained five explants of ovary or immature ovules.

RESULTS AND DISCUSSION

In vitro germination in capsules

After 2-3 days of culture, embryos in the half section of capsules were starting to germinate on WPM medium. Shoot formation depended on embryo maturation after pollination. In preliminary experiment, 21 days post-pollination was appropriate to produce shoots in *in vitro* condition. Similar result was reported from embryo culture of *Populus deltoides* (Kang and Hall, 1996b). The aged embryos more than 28 days post-pollination produced single shoot in *Populus deloides* (Kouider *et al*, 1984). An normal capsule contains 15 to 20 ovules in a natural condition. In our experiment, half section of capsule was inoculated on WPM medium to germinate in *in vitro* condition. It means that the maximal number of shoots is 7 to 10 from half segment of capsule. Among 5 hybrids, PD 9311 X PD 9309 as an intraspecific cross, produced the highest number of shoots (6.53 ± 1.66) in the half section of capsules (Table 1, Fig. 1-A). In addition, the embryos of intraspecific hybrids elongated well in in vitro condition containing WPM salts (Fig. 1-C). The expanded shoots appeared dark green with healthy condition (Fig. 1). However, interspecific hybrids showed less then one shoot from the half section of capsules and newly formed shoots were not expanded well with reddish calllus (Fig. 1-B). The capacity of seedling production was ranged from 42% to 93% which is different from intra- and interspecific hybrids.

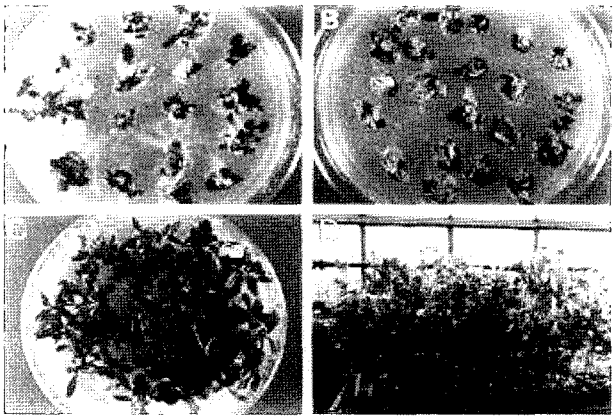

Fig. 1. *In vitro* germination from immature ovules of poplar breeding program. (A) Shoot germination from immature ovules of intraspecific hybrid (PD 9311 X PD 9309), (B) callus formation from *in vitro* culture of interspecific hybrid (PD 9301 X PN 21), (C) shoot elongation on sub-culture of intraspecific hybrid (PD 9311 X PD 9309), (D) acclimatization in greenhouse.

The capsules of intraspecific hybrids showed more fatty than interspecific one.

Multiple shoot formation from in vitro ovule culture

In preliminary experiment, poplar hybridization was conducted with the various sources of male and female plants (Fig. 2-A). After 2 weeks of culture, immature ovules on WPM medium supplemented with BA or zeatin as a cytokinin source started to produce multiple shoots. Shoot formation was significantly affected by the type of crosses and the kind of plant growth regulators (Fig. 2-4). Also, there were variations in the

Table 1. Plantlet formation from the in vitro ovary culture with half section of capsules in cottonwood hybrids

Hybridization (Crosses)	Post pollination	Capsules tested	Shoot morphology	Shoots / capsule	Seedling produced	
					No.	Percent
PD9311 X PD9309	21 days	60	Fast elongated	6.53 ± 1.66	56	93
PD9314 X PD9303	21 days	60	Yellowish	4.60 ± 1.58	45	75
PD9301 X PD9308	21 days	60	Yellowish	1.50 ± 0.70	38	63
PD9311 X PN24	21 days	60	Reddish	0.93 ± 0.54	25	42
PD9301 X PN21	21 days	60	Callus-like	0.53 ± 0.21	25	42

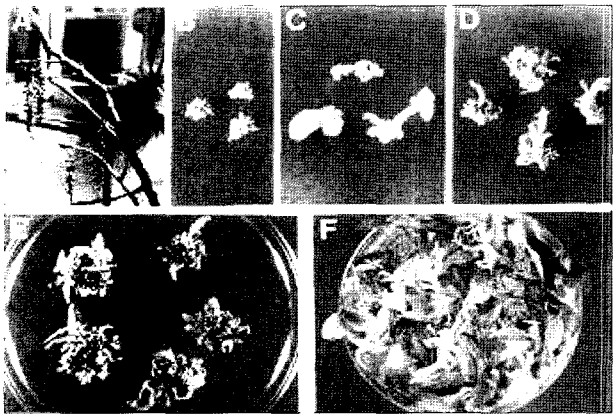

Fig. 2. Shoot formation from immature ovules of poplar breeding program. (A) Hybridization of *Populus* species in greenhouse, (B-D) embryo rescue from shoot multiplication of immature ovules and cotyledons in intraspecific hybrid (PD 9311 X PD 9309), (E) shoot formation from immature ovules of interspecific hybrid (PD 9301 X PN 21), (F) shoot elongation of multiple shoots of intraspecific hybrid (PD 9311 X PD 9309).

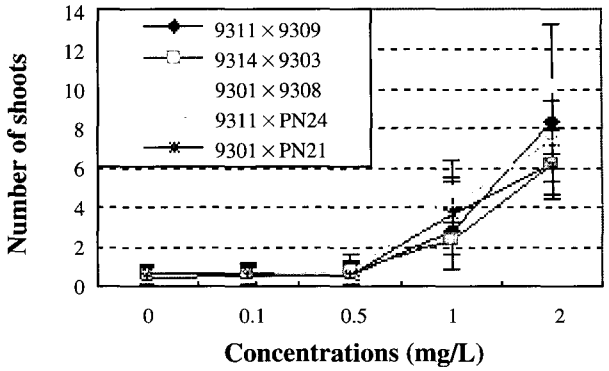

Fig. 3. The effect of BA on shoot proliferation from immature ovules of poplar hybrids after 21 days pollination.

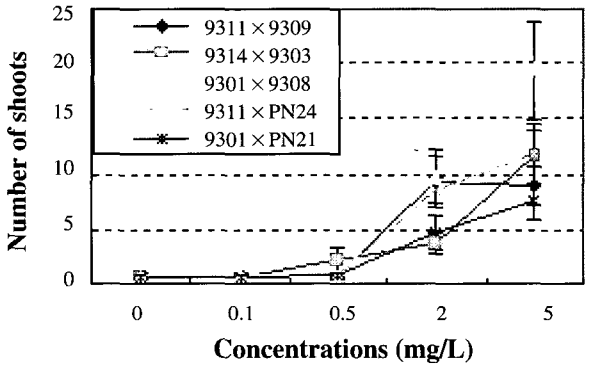

Fig. 4. The effect of zeatin on shoot proliferation from immature ovules of poplar hybrids after 21 days pollination.

position of shoot induction on proliferation medium. Most shoots were produced from immature embryos and some small shoots were initiated from the ends of expanding cotyledons. However, the regenerated shoots were hard to elongate in same medium (Fig. 2-C).

In BA treatment, the cross (PD 9301 X PD 9308) manipulated the highest number of shoots (10.20± 3.01) among five crosses. Even though the cross was poor in shooting capacity rather than other intraspecific hybrids, multiplication was the best performed in BA medium. However, interspecific hybrids produced less than one shoot in BA treatment.

In zeatin treatment, the highest number of shoots (19.40±4.53) was produced from intraspecific hybrid of PD 9301 X PD 9308 (Fig. 2-B, C and D, Fig. 4). In addition, intraspecific hybrids showed multiple shoots upto 11.84±2.59 in average. Shooting pattern was quite different between intra- and interspecific hybrids of *Populus* species. Intraspecific hybrids appeared to be elongated multiple shoots with main stems (Fig. 2-D). However, interspecific hybrids produced multiple shoots with small leaves. They were hard to elongate plantlets in *in vitro* condition.

In general, tiny shoots were formed on proliferation medium supplemented with higher concentrations of BA or zeatin. The age of the explants had a strong influence on the production of multiple shoots. Similar studies have been reported with *Populus deltoides* (Kouider *et al.*, 1984). They resulted that multiple shoots produced from immature embryos on MS medium supplemented with 0.5 mg/L. In-ovary culture was developed with *Populus* species in which half exised capsules were inoculated into MS medium. However, they germinated immature ovules to rescue

embryo desiccation through the half section of capsules and did not provide multiplication results in poplar breeding program (Raquin *et al.*, 1993).

Shoot elongation and acclimation to soil mix

Shoots regenerated from immature ovules were excised and transferred to elongation medium supplemented with half strength WPM salts and 0.02 mg/L NAA as an auxin source. After 1 month of subculture, the plantlets fully expanded in *in vitro* condition (Fig. 1-C and 2-F). When higher concentrations of auxin were added, the induced roots showed malformations such as thicker diameter and stunted length. After removing agar, the expanded plantlets were transferred to cell trays containing an artificial soil mixture (vermiculite : perlite : peat moss = 1 : 1 : 1 by volume) in a shaded mist bench under greenhouse conditions. After 2 weeks, these plantlets were moved to a regular bench (Fig. 1-D). The survival rate was upto 96% in greenhouse (data not shown).

In conclusion, the system of *in vitro* germination was developed and the capacity of shoot proliferation was increased by the zeatin treatment. Our results suggest that poplar hybridization can be applied for rescuing embryo desiccation to produce various crosses from intra- and interspecific hybrids.

ACKNOWLEDGEMENT

This work was supported by the research fund from the Korea Institute of Environmental Science and Technology, the Ministry of Environment, Korea.

LITERATURE CITED

Ahuja, M. R. 1987. *In vitro* propagation of poplar and aspen. *In*: Bonga J.M., Durzan D.J.(Eds) Cell and Tissue Culture in Forestry, pp. 207-223. Martinus Nijhoff Publishers, Dordrech /Boston/Lancaster.

Coleman, G. D. and S. G. Ernst. 1990. Axillary shoot proliferation and growth of *Populus deltoides* shoot cultures. Plant Cell Rep. 9: 165-167.

Douglas, G. C. 1984. Formation on adventitious buds in stem internodes of *Populus* spp. cultured *in vitro* on basal medium: influence of endogeneous properties of explants. Plant Physiol. 116: 313-321.

Hall, R. B., G. D. Hilton, and C. A. Maynard. 1982. Construction lumber from hybrid aspen plantations in the central states. J. For. 80: 291-294.

Hall, R. B., J. P. Colletti, R.C. Schultz, R. R. Faltonson, S. H. Kolison, Jr., R. D. Hanna, T. D. Hillson, and J. W. Morrison. 1989. Commercial-scale vegetative propagation of aspens. USDA Forest Service, General Technical Report NC-140, pp. 211-219.

Herrmann, S. and H. Seuthe. 1982. Vegetative Vermehrung von Hybrid-Aspen durch Okulation. Holzzucht 36:17-22.

Kang, H. and R. B. Hall. 1996a. Shoot proliferation from in vitro nodal cultures of cottonwood hybrid (*Populus deltoides* X *P. nigra*). Kor. J. Plant Tiss. Cult. 23: 39-44.

Kang, H. and R. B. Hall. 1996b. Light scanning electron microscope observation of shoot formation in developing ovules of cottonwood (*Populus deltoides*). Kor. J. Plant. Tiss. Cult 23: 83-88.

Kouider, M., R. M. Skirvin, K. P. Saladin, J. O. Dawson, and J. J. Jokela. 1984. A method to culture immature embryos of *Populus deltoides in vitro*. Can. J. For. Res. 14: 956-958.

Noh, E. R., Y. B. Koo, and S. K. Lee. 1986. Hybridization between incompatible poplar species through ovary and embryo culture. Res. Rep. Inst. For. Gen. 22: 9-14.

Raquin, C., L. Troussard, and M. Villar. 1993. In-ovary embryo culture as a tool for poplar hybridization. Can. J. Bot. 71: 1271-1275.

Russel, J. A. and B. H. McCown. 1986. Culture and regeneration of *Populus* leaf protoplasts isolated

from non-seedling tissue. Plant Sci. 46: 133-142.

Savka, M. A., J. O. Dawson, J. J. Jokela, and R. M. Skirvin. 1987. A liquid culture method for rescuing immature embryos of eastern cottonwood. Plant Cell Tiss. Org. Cult. 10: 221-226.

Son, S. H. and R. B. Hall. 1990. Plant regeneration capacity of callus derived from leaf, stem, and root segments of *Populus alba* L. X *P. grandidentata* Michx. Plant Cell Rep. 9: 344-347.

(Received Jun. 25, 2004)
(Accepted Aug. 6, 2004)