Propagation of Endangered Species *Pinus armandii* var. *amamiana* by Tissue Culture©

Katsuaki Ishii, Yoshihisa Hosoi, Emilio Maruyama, Seiichi Kanetani
Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan.

For propagation via organ culture, mature embryos were excised from the seeds of *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima, an endangered species only inhabiting the south west islands of Japan. They were cultured in vitro under different tissue culture conditions. Adventitious buds were induced on the surface of the embryo on ½ DCR (Gupta and Durzan 1985) medium containing BAP and they grew to shoots after subculturing to medium containing activated charcoal or a low concentration of thidiazuron. From the elongated shoots, root primordia and roots were induced in medium containing IBA as an auxin. We found that a low concentration of zeatin or BAP added to the medium was beneficial for plant regeneration of mature embryos of this species. There were many abnormal chlorophyll germinants from seeds collected in an isolated tree.

For propagation via somatic embryos, embryogenic cell suspensions were induced from mature and immature seeds of *P. armandii* Franch. var. *amamiana* on MS liquid medium supplemented with 1 μM 2,4-D and 3 μM BAP. The suspensions were incubated in the dark at 25 ºC. Induced suspension cells were transferred to ammonium-free MS liquid medium supplemented with 1 μM 2,4-D, 3 μM BAP, and 30 mM L-glutamine and subcultured every 2 weeks. In the other set of experiments, the induction rate of somatic embryogenesis was high with ammonium-free half-strength MS medium (Table 1, 2). In order to develop somatic embryos, the suspension cells were transferred to ammonium-free MS medium supplemented with 10 μM ABA, 0.2% activated charcoal, 10% PEG (MW6000), 30 mM L-glutamine and 6% maltose. The cultures were incubated under a 16-h light/8-h dark photoperiod. After 1–2 months of culture, differentiation of embryos progressed and cotyledonary embryos were obtained. These embryos were transferred on ammonium-free MS solid medium under 16-h photoperiod. After 2–3 weeks plantlets with roots and green cotyledons were obtained. Plantlets were transplanted to vermiculite containing modified MS liquid medium in 200 ml culture flasks, then out-planted after habituation procedure.

LITERATURE CITED

Gupta, P.K. and D.J. Durzan. 1985. Shoot multiplication from mature trees of Douglas-fir (*Pseudotsuga menziesii*) and sugar pine (*Pinus lambertiana*). Plant cell reports 4:177-179.

Table 1. Modified MS media for somatic embryogenesis of *Pinus armandii* var. *amamiana*

Nutrients [mg·L^{-1}]	Basic media			
	A	B	C	D
NH_4NO_3	1650			
KNO_3	1900	1900	950	950
$MgSO_4 \cdot 7H_2O$	370	370	185	185
$CaCl_2 \cdot 2H_2O$	440	440	220	220
KH_2PO_4	170	170	85	85
$FeSO_4 \cdot 7H_2O$	27.8	27.8	13.9	13.9
Na_2EDTA	37.3	37.3	18.7	18.7
$MnSO_4 \cdot 4H_2O$	22.3	22.3	22.3	22.3
$ZnSO_4 \cdot 7H_2O$	8.6	8.6	8.6	8.6
H_3BO_3	6.2	6.2	6.2	6.2
KI	0.83	0.83	0.83	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.03	0.03	0.03	0.03
$CoCl_2 \cdot 6H_2O$	0.03	0.03	0.03	0.03
myo-Inositol	100	100	100	100
Nicotinic acid	0.5	0.5	0.5	0.5
Pyridoxine HCl	0.5	0.5	0.5	0.5
Thiamine HCl	0.1	0.1	0.1	0.1
Glycine	2	2	2	2
Casein, acid hydrolysate		500		
Sucrose (g·L^{-1})	30	30	30	30

Table 2. Effects of combination of 2,4-D and BAP on induction rate of embryogenesis of *Pinus armandii* var. *amamiana*

Basic medium A

	2,4-D μM			
BAP μM	0.3	1	3	10
0	5	4.8	9.5	15
1	5	4.8	0	0
3	0	5	9.5	10.5

Basic medium B

	2,4-D μM			
BAP μM	0.3	1	3	10
0	0	0	0	0
1	0	0	10	5.9
3	0	0	5	0

Basic medium C

	2,4-D μM			
BAP μM	0.3	1	3	10
0	0	11.1	11.1	14.3
1	11.1	10.5	10.5	7.7
3	17.6	10.5	21.1	8.3

Basic medium D

	2,4-D μM			
BAP μM	0.3	1	3	10
0	10	17.4	9.1	5.3
1	15	8.7	4.5	0
3	0	0	10	9.5