Embryo Culture of *Pinus merkusii*

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Summary : The conditions of adventitious bud differentiation, rooting, and acclimation in embryo culture of *Pinus merkusii* were examined. Fifty embryos were inoculated on modified Gresshoff and Doy medium supplemented with 10 or 25 μM of 6-benzylaminopurine (BAP). In primary culture, two-thirds of the embryos died due to contamination. As adventitious buds were differentiated from the surviving embryos, they were subcultured. After 7 - 16 months from the biginning of culture, the shoots over 2 cm in length were cut from the cultures and were transplanted to the rooting medium. In 10 μM BAP, 7 - 442 shoots per embryo were obtained, and in 25 μM BAP, 12 - 698 shoots per embryo were obtained. There was a great difference among the embryos in shoot formation. Rooting percentage was 2.4 - 57.1 % on modified Risser and White medium supplemented with 10 μM of 3-indolebutyric acid. Although there was little difference in the rooting percentages among various IBA concentrations, there was a great deal of difference among the embryos. In acclimation, a maximum of 93 plantlets were acclimated from one embryo. Among rooting media, perlite was the most suitable for rooting.

1 Introduction

In the Forest Tree Breeding Center we are carrying out research concerning the tissue culture of tropical and subtropical tree species to make possible overseas cooperation. *Pinus merkusii* grows in Myanmar, Thailand, Indonesia and so on. As the wood of *P. merkusii* is water-resistant, it is frequently used for the construction of houses. *P. merkusii* is one of the most important afforestation tree species in these tropical countries[5].

In this study we examined the conditions of adventitious bud differentiation, rooting, and acclimation in embryo culture of *P. merkusii*.

2 Materials and Methods

The seeds of *P. merkusii* were provided by the Forest Tree Improvement Project of Indonesia conducted by the Japan International Cooperation Agency. They were bulk seeds collected in Sumatra in 1993.

The seeds were sterilized following the procedure of the authors[3]. The embryos were taken from the seeds aseptically and were placed on modified Gresshoff and Doy medium[4] supplemented with 10 or 25μM of 6-benzylaminopurine (BAP). Although 50 embryos were inoculated in each BAP concentration group, some embryos died due to contamination. The cultures in 9 cm petri dishes were incubated at 25 ℃ under white fluorescent illumination at 3,000 lux with a

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16-hr daylength. After 7 weeks the cultures were transplanted to the next BAP free medium, which was made up of the same components as the primary culture medium, and were subcultured every 4 weeks. After 7 - 16 months from the biginning of culture, the shoots over 2 cm in length were cut from the cultures and were transplanted to the rooting medium. As the shoots didn't grow simultaneously, transfer to the rooting medium was carried out several times. This medium was modified Risser and White medium[4] supplemented with 10 μM of 3-indolebutyric acid (IBA). A survey of the rooting was conducted after 3 months. In another rooting experiment, IBA concentration was examined at 1, 5, 10, and 25 μM. Ten shoots were taken from each culture, and a total of 50 shoots from 5 cultures were used in each IBA concentration. The rooted plantlets were taken out of the culture bottles, and the agar attached to the roots was removed. They were then transplanted to other culture bottles containing non-aseptic perlite and acclimated. A survey of the acclimation was conducted after 2 months. In another acclimation experiment, the kinds of media, Kanuma-soil, perlite, and vermiculite were examined. Ten shoots were taken from each culture, and a total of 50 shoots from 5 cultures were used in each medium. This experiment was conducted at the same time as the rooting experiment concerning IBA concentrations.

3 Results and Discussion

One week after inoculation, 16 embryos in the 10 μM BAP group and 17 embryos in the 25 μM BAP group survived. Approximately two-thirds of the embryos died due to contamination. In the seed used in this experiment there was more space between seed coat and endosperm than in *Pinus thunbergii* or in *P. densiflora* and this space appeared to make it more difficult to maintain sterile conditions.

The buds then differentiated in the surviving embryos. In the 10 μM BAP group, the number of shoots that separated from each embryo varied from 442 shoots in the No. 10-2 embryo to 7 shoots in the No. 10-1 embryo, with an average of 171 shoots

Table 1. Shoot formation, rooting and acclimation

10 μM BAP group							25 μM BAP group						
		Rooting and acclimation							Rooting and acclimation				
Embryo No.	No.of shoots obtained	No.of shoots examined	No.of shoots rooted	Rooting percentage (%)	No.of acclimated plantlets	Acclimation percentage (%)	Embryo No.	No.of shoots obtained	No.of shoots examined	No.of shoots rooted	Rooting percentage (%)	No.of acclimated plantlets	Acclimation percentage (%)
10- 1	7	7	4	57.1	2	50.0	25- 1	12	12	2	16.7	2	100.0
10- 2	442	302	102	33.8	93	91.2	25- 2	30	30	17	56.7	13	76.5
10- 3	17	17	6	35.3	6	100.0	25- 3	240	180	55	30.6	28	50.9
10- 4	331	271	133	49.1	70	52.6	25- 4	42	42	1	2.4	0	0.0
10- 5	147	87	40	46.0	32	80.0	25- 5	428	368	45	12.2	39	86.7
10- 6	147	147	36	24.5	20	55.6	25- 6	128	128	23	18.0	13	56.5
10- 7	46	46	14	30.4	7	50.0	25- 7	23	23	10	43.5	7	70.0
10- 8	25	25	9	36.0	4	44.4	25- 8	43	43	22	51.2	8	36.4
10- 9	236	176	36	20.5	31	86.1	25- 9	168	168	55	32.7	22	40.0
10-10	197	137	51	37.2	28	54.9	25-10	29	29	8	27.6	6	75.0
10-11	18	18	3	16.7	0	0.0	25-11	33	33	11	33.3	8	72.7
10-12	37	37	1	2.7	1	100.0	25-12	698	558	125	22.4	65	52.0
10-13	98	98	42	42.9	14	33.3	25-13	69	69	16	23.2	8	50.0
10-14	303	163	77	47.2	46	59.7	25-14	36	36	11	30.6	7	63.6
10-15	246	166	42	25.3	24	57.1	25-15	55	55	9	16.4	7	77.8
10-16	433	293	84	28.7	52	61.9	25-16	46	46	6	13.0	1	16.7
							25-17	23	23	8	34.8	4	50.0
Total(Mean)	(171)	1990	680	(34.2)	430	(63.2)	Total(Mean)	(124)	1843	424	(23.0)	238	(56.1)

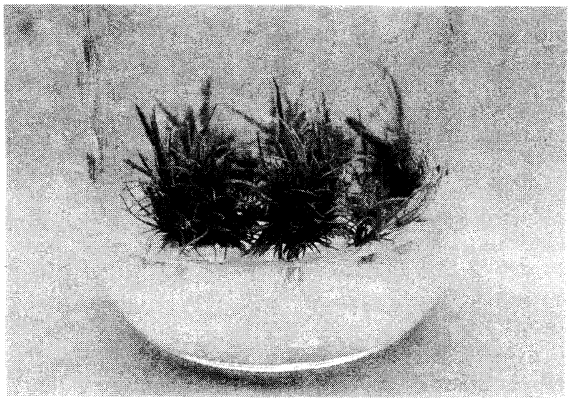

Photo. 1. Masses of shoots at 5 months after inoculation (No.25-5 embryo)

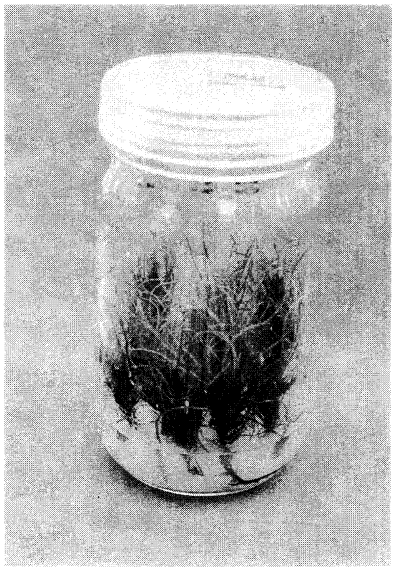

Photo. 2. Shoots just rooted (No.10-2 embryo)

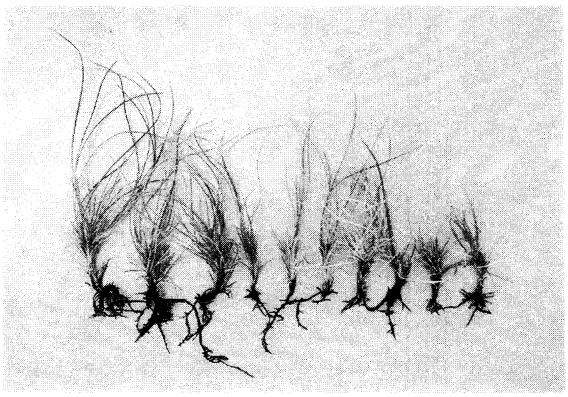

Photo. 3. Regenerated plantlets (No.10-4 embryo)

(Table 1). In the 25 μM BAP group, the number of shoots varied from 698 shoots in the No. 25-12 embryo to 12 shoots in the No. 25-1 embryo, with an average of 124 shoots (Table 1, Photo. 1). More shoots were obtained than in the cases of *P. thunbergii* or *P. densiflora* [1,2]. In the number of shoots, there was a greater difference among the embryos than among the BAP concentrations. Rooting from the shoots was successful (Photos 2, 3). The rooting percentages for the shoots varied from 57.1 % in the No. 10-1 embryo to 2.4 % in the No. 25-4 embryo, with averages of 34.2 % in the 10 μM BAP group and 23.0 % in the 25 μM BAP group (Table 1). There was also a great difference among the cultures. The acclimation percentages varied from 91.2 % in the No. 10-2 embryo to 33.3 % in the No. 10-13 embryo, with averages of 63.2 % in the 10 μM BAP group and 56.1 % in the 25 μM BAP group. There was little difference between the two groups.

Although the rooting percentage in the IBA-free medium was 14 %, those in the IBA-containing media were 26~30 % (Table 2). There was no significant difference among the IBA-containing media in ANOVA.

Among the supporting structure of rooting media, the maximum rooting percentage was 48 % in perlite,

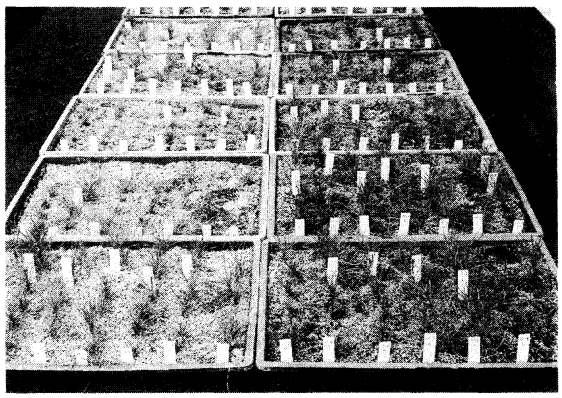

Photo. 4. Acclimation of regenerated plantlets

Table 2. Effect of IBA concentration on rooting

Embryo No.	IBA concentrations(μM)				
	0	1	5	10	25
10- 2	0	4	3	3	2
10-14	1	6	3	4	3
10-15	0	2	2	2	2
10-16	3	1	3	1	1
25-12	3	0	4	3	5
Total	7	13	15	13	13
Rooting percentage	14	26	30	26	26

Ten shoots were examined in each embryo and in each IBA concentration

Table 3. Effect of supporting structure on rooting

Embryo No.	Kanuma-soil	Perlite	Vermiculite
10- 2	0	5	3
10-14	1	6	3
10-15	0	4	0
10-16	0	4	0
25-12	1	5	0
Total	2	24	6
Rooting percentages	4	48	12

Ten shoots were examined in each embryo and in each medium

and there was a significant difference among the media at the 1 % level in ANOVA (Table 3). In another rooting experiment concerning IBA concentration conducted at the same time, agar was used as a medium, and the average rooting percentage was 26 % in 10 μM IBA. It may be reasonable to expect a higher rooting percentage when using perlite instead of agar as a medium.

Although the conditions for adventitious bud differentiation, rooting, and acclimation in the embryo culture of *P. merkusii* were clarified to some extent by the above results, there is still a great difference among embryos or the cultures in shoot formation, rooting, and acclimation. It is therefore necessary to examine the differences among families using half-sib families.

Literature cited

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*: This English title is a tentative translation from the original Japanese by the authors of this paper.