DIFFERENTIATION OF ADVENTITIOUS BUDS ON DOUGLAS-FIR EMBRYOS IN VITRO

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Abstract: Conditions for obtaining adventitious buds on embryos of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) are given. These buds have been excised and rooted to produce plantlets.

Problems inherent in the genetic improvement of trees are linked to their long generation time, heterozygosity, and the difficulties of vegetatively propagating mature individuals. Possibly, the propagation of selected trees can be accomplished through tissue culture. For other plants, such as orchids, tissue culture has proved to be a practical means to the rapid multiplication of selected cultivars, but although the literature contains many references to the tissue culture of gymnosperms (1), there are few recorded instances of organogenesis. Recently it was reported that plantlets of *Pinus palustris* Mill. had been obtained by rooting adventitious buds differentiated on embryos grown in vitro (i.e., embryo cultures) (5). However, when the method described by Sommer, Brown and Kormanik (5) was tested using embryos of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), adventitious buds were obtained only sporadically. Subsequently, investigations were undertaken to improve the frequency of their development.

MATERIALS AND METHODS

In general, the culturing procedures have been described previously (5). The composition of the basal media is given in Table 1. NAA and 6-benzyl adenine were added to the basal media at the concentrations indicated and the cultures maintained at approximately 25°C. Illumination was approximatley 500 ft.-c. from Gro-Lux lamps, with a 15-hour photoperiod. Wild seed lots were used.

RESULTS

Initially the NAA and 6-benzyl adenine concentrations were varied widely to obtain a broad index of embryo response. In all, 35 combinations were tested with 10 embryos in each combination. As might be expected, differentiation of adventitious buds was favored by no or low auxin (Table 2). Results were more consistent, most rapid, and the buds better formed at lower cytokinin levels.

Using a different seed lot, the response to NAA and 6-benzyl adenine was investigated using lower concentrations. Twenty-

eight combinations were tested, with 25 embryos in each combination. The response for basal media I and II were also checked (Tables 3-4). Again, the lower concentrations of NAA favored the differentiation of adventitious buds with the lowest (0.01 ppm) producing slightly more buds per embryo under most conditions than no NAA. Basal medium I appeared superior to medium II with respect to both the percentage of embryos producing buds and also the number of adventitious buds produced at favorable hormone concentrations. Adventitious buds from these cultures were excised, placed on different media and, in some instances, rooted to form plantlets; shoot extension followed.

DISCUSSION

The method here described is not a means of mass-producing clones of planting stock from selected individuals but it does represent a significant step in that direction. It demonstrates that a high percentage of Douglas-fir embryos can form adventitious buds, and that plantlets can be obtained. In time, the technique may be modified and expanded as a means to producing plantlets from callus.

Table 1. Composition of basal media

Component	Medium I	Medium II
Major salts	Gamborg and Eveleigh (2)	Witham, Blaydes and Devlin (6)
Trace salts	Gamborg and Eveleigh (2)	Gamborg and Eveleigh (2)
Iron	Murashige and Skoog (4)	Murashige and Skoog (4)
Vitamins	Greshoff and Doy (3)	Greshoff and Doy (3)
Sucrose	20g/l	20g/l
Agar	7g/l	7g/l
pH	5.6-5.8	5.6-5.8

Table 2. Embryos producing adventitious buds after 1 month on medium II.

NAA Concentration	Percent of embryos with adventitious buds[1]						
	0.0ppm BA[2]	0.5ppm BA	1.0ppm BA	2.0ppm BA	4.0ppm BA	8.0ppm BA	16.0ppm BA
0.0 ppm	0	100	89	80	60	78	80
0.1	0	70	67	80	67	30	43
0.5	0	10	50	20	22	30	0
1.0	0	10	0	40	30	0	0
2.0	0	11	0	0	11	0	17

[1] 10 embryos per treatment at start
[2] 6-benzyl adenine

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Table 3. Adventitious bud differentitation on medium I.

NAA Concentration	Percent of embryos with adventitious buds[1]						
	0.0ppm BA[2]	0.1ppm BA	0.25ppm BA	0.5ppm BA	0.75ppm BA	1.0ppm BA	4.0ppm BA
0.0 ppm	0	92 (3.5)[3]	80 (3.7)	96 (3.8)	88 (4.5)	80 (3.7)	88 (4.6)
0.01	0	84 (4.6)	92 (3.9)	92 (3.4)	84 (4.6)	80 (4.2)	80 (4.0)
0.5	0	76 (2.7)	80 (2.7)	80 (3.3)	88 (3.7)	72 (3.6)	84 (3.7)
1.0	4(1)	44 (2.2)	84 (3.4)	56 (2.8)	64 (2.6)	80 (3.7)	88 (4.6)

[1] 25 embryos per treatment at the start
[2] 6-benzyl adenine
[3] numbers in parenthesis are average of adventitious buds/bud bearing embryo

Table 4. Adventitious bud differentiation on medium II.

NAA Concentration	Percent of embryos with adventitious buds[1]						
	0.0ppm BA[2]	0.1ppm BA	0.25ppm BA	0.5ppm BA	0.75ppm BA	1.0ppm BA	4.0ppm BA
0.0 ppm	0	80 (2.1)[3]	75 (2.8)	80 (3.1)	64 (3.3)	76 (3.4)	42 (2.0)
0.01	0	64 (4.7)	68 (4.4)	80 (3.6)	72 (2.7)	76 (4.0)	52 (3.2)
0.5	0	60 (2.5)	68 (3.4)	60 (2.6)	52 (3.5)	48 (2.4)	48 (2.8)
1.0	0	52 (1.9)	64 (2.0)	56 (2.1)	40 (2.7)	40 (2.8)	36 (1.6)

[1] 25 embryos per treatment at the start
[2] 6-benzyl adenine
[3] numbers in parenthesis are average number of adventitious buds/bud bearing embryo

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MODERATOR BATCHELLER: We have time now for a few questions for our last panel of speakers.

BILL BARR: I have heard reports of health problems with Benlate, of it building up in your body. Does anybody know anything about this? We aren't using Benlate now. We have stopped using Benlate because of this.

VOICE: Nothing specific on the buildup of it, but you want to be aware that a lot of people do have a severe allergic reaction to Benlate and it is not limited to dermatitis. I had some folks working for me using it in propagation and getting severe headaches which indiciates some kind of absorption into the body.

BRUCE BRIGGS: If you will check in the 1974 *Proceedings*, I believe you will find in the Eastern region meeting last winter that this problem occurred in Australia. They did show that it was very toxic in that area. There are more details in the *Proceedings*. There have been other people in the United States that have said there were adverse reactions but from that I cannot quote any data where there has been research done.

RALPH SHUGERT: Adding to this Jolly, Bill Flemer, of Princeton Nurseries, Past-President of the IPPS Eastern Region, had some employees become quite ill last winter. If anyone wants to explore it a little further, they could write to Bill personally.