SEED WEIGHT AND IN VITRO BUD INDUCTION POTENTIAL IN PSEUDOTSUGA MENZIESII COTYLEDONS CULTURED IN VITRO

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(Received for publication 28 November 1985; revision 2 April 1986)

ABSTRACT

Differences in bud induction on cotyledons from seedlings of six full-sib families and a wild seedlot of **Pseudotsuga menziesii** (Mirb.) Franco were observed after culturing on a half-strength Murashige & Skoog basal medium containing 5.0 μM benzyladenine (BA) and different auxin supplements. Naphthaleneacetic acid (NAA) at a concentration of 0.05 μM or a mixture of indoleacetic acid at 2.8 to 5.7 μM plus indolebutyric acid (IBA) at 2.5 to 4.9 μM induced high percentages of explants to differentiate buds. Cotyledons from full-sib families gave substantially more bud induction than cotyledons from wild stock. No significant interaction was observed between seed parents and treatments for bud induction. Seeds of full-sib families weighed significantly (at $p = 0.05$) more than seeds from the wild seedlot used in this study. A strongly positive correlation between seed weight and **in vitro** bud induction was observed.

Keywords: tissue culture; heterogeneity; seed weight; **Pseudotsuga menziesii**.

INTRODUCTION

Pseudotsuga menziesii (Douglas fir) is predominantly wind-pollinated, and wild seedlots are genetically heterogeneous (Hermann & Lavender 1968; Rehfeldt 1974). Consequently, individuals within full-sib families produced by crosses between genetically heterozygous parents should show considerable genetic variation as a result of gene recombinations. Significant differences in *P. menziesii* seedling growth rates and other characteristics were found among full-sib families (Campbell & Rediske 1966; Campbell 1972). *Pinus taeda* L. seed families, which are also genetically variable, responded differently in frequency and intensity of bud induction in embryo cultures. These differences can be reduced by adjusting the growth regulator concentrations (Mott *et al.* 1977). Similar genotypic variations were observed in *Pinus* spp. embryo cultures from wild seedlots or from half-sib families (Brown & Sommer 1977).

Pseudotsuga menziesii cotyledons from wild stock seedlings have produced adventitious shoots when cultured *in vitro*. Some of these have rooted to form plantlets (Cheng & Voqui 1977). In preliminary experiments, the number of bud primordia produced *in vitro* ranged from 21 to 265 per single seedling from a wild population (AboEl-Nil &

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Wochok 1977). The objective of this research was to quantify the bud induction potential of *P. menziesii* cotyledons from various seed sources, cultured *in vitro,* and to identify the most suitable auxin and auxin concentration for each source, and seed weight effect.

MATERIALS AND METHODS

Pseudotsuga menziesii seeds of full-sib families and wild stock from Coos Bay, Oregon, collected in 1978, were used. Seeds were germinated and their cotyledons were sterilised as described by Cheng & Voqui (1977). Decontaminated cotyledon explants were cultured on half-strength Murashige & Skoog (1962) medium (MS) solidified with 0.4% DIFCO agar for 1 week to identify residual contamination.

Subsequently, sterile cotyledons were cut into 2- to 4-mm explants and placed on the surface of a polyester fabric saturated with half-strength liquid MS medium containing 5.0 μM BA and supplemented with auxins at various concentrations (Table 1). Cultures were incubated at 21° ± 2°C under an 18-h photoperiod (c. 60 $\mu E/m^2/sec$) by Gro-Lux fluorescent lamps. Cotyledons from six full-sib families and a wild seedlot were tested on five auxin treatments in 12 to 33 replicate seedlings per treatment.

Cultures were observed periodically, and the numbers of explants that differentiated buds were recorded after 38 to 42 days of culture. The number of explants exhibiting bud differentiation as a function of total explants cultured per seedlings was expressed in percentage terms, and analysed with analysis of variance, Duncan's multiple range, and the least significant difference (LSD) tests. The arcsin transformation was used on percentage data before analysis.

To determine the seed weight distribution within each full-sib family and wild population seedlot, the following procedure was used. Random samples of 100 filled seeds from each full-sib family and the wild seed stock from the same seedlot used in tissue culture experiments were separated by X-ray radiography and individually weighed. Data were analysed by analysis of variance, Duncan's multiple range, and the least significant difference (LSD) tests (Freese 1967; Steel & Torrie 1960).

RESULTS AND DISCUSSION

The percentage of explants differentiating buds in response to the five auxin treatments illustrated wide differences in the bud induction ability between seedlings within families (Table 1). The impressively wide range (0–100%) of response among and within the families tested indicated considerable variability in each family population, which is expected in the progeny of heterozygous parents. A comparison of the means of percentages for bud induction of full-sib families and wild stock (Table 1) indicated that cotyledons from wild stock were significantly less responsive than cotyledons from full-sib families. The progeny of the reciprocal crosses of Parents 28 and 88 were significantly different, which may have been the result of maternal effects. Responses to treatments with 0.05 μM NAA, 5.7 μm IAA + 4.9 μM IBA, and 2.8 μM IAA + 2.5 μM IBA did not differ significantly, but bud induction was significantly higher than in the treatment with 0.0005 μM NAA (Table 1).

TABLE 1—Means (and ranges) of percentage of explants per family with differentiated buds after 38–42 days of incubation on five different auxin treatments. Sample size varied from 25 to 50 explants per replicate per treatment

Seed parentage*	Auxin concentration (μM)					Family mean†
	NAA 0.05	NAA 0.005	NAA 0.0005	IAA 5.7 + IBA 4.9	IAA 2.8 + IBA 2.5	
28 × 88 [12]	83.7 ± 3.7 (65–100)	73.9 ± 6.4 (14–96)	63.7 ± 7.6 (20–100)	66.1 ± 5.4 (25–90)	74.7 ± 6.3 (20–95)	72.4 ± 2.6 a
57 × 65 [24]	50.2 ± 5.8 (0–100)	50.9 ± 5.6 (0–90)	43.1 ± 5.6 (0–85)	57.1 ± 4.2 (11–85)	56.9 ± 4.2 (19–90)	51.6 ± 2.3 b
53 × 71 [16]	48.1 ± 7.7 (3–96)	37.4 ± 8.6 (0–90)	27.7 ± 9.4 (0–93)	66.9 ± 6.8 (3–100)	65.1 ± 7.1 (7–98)	49.0 ± 3.5 b
3 × 76 [33]	52.0 ± 4.7 (0–100)	49.9 ± 4.9 (0–93)	39.6 ± 4.8 (0–100)	42.4 ± 3.3 (15–77)	47.6 ± 3.3 (5–92)	46.3 ± 1.9 b
88 × 28 [17]	45.7 ± 8.5 (0–100)	32.8 ± 9.3 (0–100)	28.7 ± 8.3 (0–86)	59.1 ± 7.0 (12–100)	57.8 ± 7.0 (19–100)	44.8 ± 3.5 b
53 × 35 [19]	52.3 ± 6.8 (12–96)	33.1 ± 7.4 (0–97)	29.1 ± 7.0 (0–96)	44.2 ± 4.3 (11–83)	44.3 ± 6.2 (5–100)	40.6 ± 2.8 b
Wild [21]	34.6 ± 5.3 (0–81)	21.7 ± 5.9 (0–97)	12.8 ± 4.8 (0–66)	31.3 ± 4.9 (0–81)	38.2 ± 5.6 (0–97)	27.7 ± 2.3 c
Treatment mean	52.4 ± 2.3	42.8 ± 2.5	34.9 ± 2.4	52.4 ± 1.8	54.9 ± 2.0	47.5 ± 1.0

* Number of replicates in brackets.

† Indices followed by the same letter are not significantly different ($p = 0.05$) by Duncan's multiple range and least significant difference tests.

Analysis of variance for bud induction (Table 2) showed both family and treatment effects were highly significant (at the 1% level), but revealed no statistically significant interaction between families and treatments (at the 5% level).

TABLE 2—Analysis of variance of arcsin percentage of bud induction after 38–42 days

Source of variation	DF	F-values	Significance of F ratio
Treatment (T)	4	11.008	**
Parentage (P)	6	18.682	**
T × P	24	1.35	NS
Error	675		

** Significant at the 1% level.

Differences in seed weight means among full-sib families and the wild seedlot were highly significant (Table 3). The wild seedlot had the lightest seeds (10.8 ± 0.2 mg), while the full-sib family 28 × 88 had the heaviest seeds (15.9 ± 0.1 mg). There was a significant difference in seed weight of reciprocal-cross progeny with means of 15.9 ± 0.1 mg and 12.9 ± 0.1 mg for families 28 × 88 and 88 × 28, respectively. Ranking of tested seed parentages, according to bud induction *in vitro* and seed weight (Table 3), showed impressive similarities in five situations. Family 28 × 88 ranked first, family 57 × 65 ranked second, wild stock ranked last, family 88 × 28 ranked significantly lower than 28 × 88, and there was no significant difference between 53 × 71 and 53 × 35. The rank correlation coefficient was 0.82 and simple correlation coefficient of the means was 0.90 ($p < 0.01$). It appears that there is a positive correlation between seed weight and the development of buds from *P. menziesii* cotyledons cultured *in vitro*.

TABLE 3—Mean percentage of explants differentiating buds and mean single seed weights of the six full-sib families and wild seedlot. Simple correlation coefficient between means of bud differentiation percentage and means of seed weights was 0.90 ($p < 0.01$)

Parentage	Mean percentage bud differentiation	Mean* seed weight (mg) ± s.e.
28 × 88†	72.4	15.9 ± 0.1 a
57 × 65	51.6	15.0 ± 0.2 b
53 × 71‡	49.0	13.3 ± 0.2 d
3 × 76	46.3	13.8 ± 0.2 c
88 × 28†	44.8	12.9 ± 0.1 e
53 × 35‡	40.6	13.6 ± 0.1 cd
Wild	27.7	10.8 ± 0.2 f

* Means followed by same letter are not significantly different ($p = 0.05$) by Duncan's multiple range and least significant difference tests.
† Reciprocal crosses.
‡ Same female parent.

In a study utilising 27 explants per treatment of wild stock of an unknown number of seedlings, Cheng (1977) reported that NAA at a concentration of 0.0005 μM induced 96% of cultured explants to form buds while 0.005 μM and 50 μM produced 93 and 85% respectively. She concluded that the maximum expression of morphogenesis (bud induction) occurred at a BA concentration of 5 μM plus NAA at either 0.0005 or 0.005 μM. In contrast, the present study showed that NAA treatment at 0.05 μM induced significantly higher bud formation than either 0.005 μM or 0.0005 μM treatments (Table 1) for the crosses and wild seedlot tested in this study. This difference may be the result of differences in seed origin, considerable heterogeneity of wild stock, and the small sample size used by Cheng (1977).

Seed weight was found to be positively correlated with germination, survival, and early plant size in *Pinus strobus* L. (eastern white pine) (Spurr 1944) and with seedling height and weight in *Pinus radiata* D. Don (radiata pine) (Griffin 1972). Differences have been observed in seed weight and height growth between wind- and control-pollinated *Pseudotsuga menziesii* progenies (Sorensen 1973), with the latter significantly more vigorous. The differences, which lasted for at least 3 years, were attributed to the effect on the seeds by the bag micro-environment during control-pollination, inbreeding of the wind-pollinated seed, or other unknown factors (Sorensen 1973). Possible causes for significantly higher bud induction in cotyledons of the full-sib families are gene combinations favourable to bud induction and bag micro-environmental effects in the pollination bag which tended to favour larger seed.

This is the first report to show that considerable heterogeneity for *in vitro* bud induction among *P. menziesii* progenies of different parentage was correlated with seed weight. It, therefore, suggests that bud induction may be maternally influenced.

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