MICROPROPAGATION OF CATAWBA HYBRID RHODODENDRONS, 'NOVA ZEMBLA', 'CYNTHIA', AND 'PINK PEARL'

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Abstract. The *in vitro* response of floret explants of three Catawba hybrid rhododendron cultivars—'Nova Zembla', 'Cynthia' and 'Pink Pearl' to varying indoleacetic acid (IAA) and (Δ^2 Insopentenyl)—adenine (2iP) levels is described.

Although the explants of the three cultivars under investigation differed in their exogenous growth regulator requirements, it was found that relatively high auxin (2.0 to 4.0 mg l^{-1}) and cytokinin (10.0 to 15.0 mg l^{-1}) concentrations were necessary to induce adventitious growth.

Caulogenesis of both 'Nova Zembla' and 'Cynthia' cultures was maximised only if IAA was omitted from the medium. Both cultivars had an absolute requirement for 2iP; the former within the range 5.0 to 15.0 mg l^{-1}: the latter, 5.0 mg l^{-1}. Maximum propagule development occurred at 1.6 mg l^{-1} for micropropagules derived from shoot tip explants used as the test material.

In vitro culture has proved to be an effective method for the rapid propagation of many plant genera, particularly herbaceous genotyes (12, 13). Within the past decade, considerable research efforts have been directed toward development of tissue culture systems for woody plant species (1, 17, 18). Rhododendron is one such species which responds to tissue culture (2, 3, 15). However, some factors require further elucidation and refinement. For example, the concentration of exogenously supplied auxin and cytokinin compounds to the culture medium at any specific stage.

There are conflicting reports regarding the recommended concentration of both IAA and 2iP which should be used in rhododendron tissue culture media. Currently, this is arbitrary, non-specific and often depends upon the investigator. Concentrations used have ranged between zero and 6.0 mg l^{-1} for IAA Anderson (4) and 1.6 and 15.0 mg l^{-1} for 2iP (4, 5, 6, 7, 9, 14).

This research was initiated to examine the effects of varying IAA and 2iP concentrations on floret explant material excised from three rhododendron cultivars.

MATERIALS AND METHODS

Propagation material of the three cultivars was harvested from 3 to 5 year old mother plants kindly supplied by Bord Na Mona shrub nursery, Lullymore, County Kildare. The florets were washed in 1% v/v detergent for 20 min. followed by immersion and agitation for 30 min. in 0.5% v/v sodium hypochlorite solution containing 0.1% v/v Tween 20. They were rinsed three times with sterile distilled water.

Papery coverings which surrounded the florets were aseptically removed using a forceps and scalpel; each was dissected leaving the maximum amount of intact pedicel tissue and placed within a 50 mm sterilin petri dish containing a nutrient agar medium. Where adventitious tissue clusters were used as the innoculum, they were simply sub-cultured onto a fresh medium. Where micropropagules were used as innoculum, four nodal shoots excised below the 1st and 5th internodes were singularly placed horizontally on the surface of the culture medium within 100 ml sterilin specimen containers. Ten replications were used in each treatment. The basal medium composition was that of Anderson's 1984 revised rhododendron one. The pH was adjusted to 5.0 ± 0.1 with 1 m KOH or HCl prior to the addition of agar and was autoclaved at 0.7 kg cm^{-2} and 110°C for 20 min. The medium was allowed to cool to 37°C, thereupon the thermolabile substances were filter sterilized using Miller-HA 0.45 μM filters attached to a 50 ml Millipore syringe. The medium was dispensed (20 ml) into the respective culture vessels using a 10 ml BD Cornwall automatic dispensing apparatus.

Experiments with a range of IAA and 2iP concentrations (0.0, 2.0, 4.0 mg l^{-1} and 5.0, 10.0, 15.0 and 20.0 mg l^{-1}), respectively, were carried out for all experiments except where nodal shoots of 'Pink Pearl' were used. In this instance 2iP was supplied at 0.0, 1.6, 2.5, 5.0, 10.0, 15.0, and 20.0 mg l^{-1}. The cultures were incubated in a growth chamber at a temperature of 25°C and a photon flux density of 30 ± 10 UE m^2 sec^{-1}. A 16 hour photoperiod was maintained using Phillips warm white florescent tubes.

The results were evaluated by measuring the size of the induced adventitious tissue growth after 8 weeks growth (Tables 1, 2, 3); by counting the total number, and number of usable shoots, at 12 and 16 weeks (Tables 4, 5, 6, 7, 8). The data was statistically analysed using the factorial analysis of variance and Duncan's Multiple Range Test

RESULTS

The addition of auxin and cytokinin compounds to the culture medium had a highly significant effect on adventitious growth and development of floret explants for the three cultivars investigated.

'Nova Zembla' explants established rapidly and produced adventitious growth clusters measuring 913 and 1021 mm^3, when auxin was added at 2.0 and 4.0 mg l^{-1} respectively (Table 1). These values were not significantly different. When auxin was omitted from the medium, adventitious tissue growth was significantly retarded (Table 1).

Table 1. Effect of auxin and cytokinin concentration on adventitious growth development (mm^3) from floret explants of *Rhododendron* 'Nova Zembla'

Auxin mg l^{-1}	Cytokinin mg l^{-1}				
	5	10	15	20	Weighted mean
0	100	545	392	—	432 b
2	1121	—	692	844	913 a
4	1063	1232	905	802	1021 a
Mean	1028 a	971 a	673 a	814 a	

Means followed by the same letter are not significantly different at the 5% level.

Comparing cytokinin concentrations for the above cultivar, there was a general decrease in adventitious tissue development with increasing cytokinin concentration (Table 1). Maximum growth occurred at 5.0 mg l^{-1}, whilst the minimum occurred at 15.0 mg l^{-1} 2iP. However, these differences were not significant.

Although no significant interaction occurred between the relative auxin and cytokinin concentration, development was maximised between 2.0 and 4.0 and 5.0 and 10.0 mg l^{-1} IAA and 2iP, respectively (Table 1).

'Cynthia' floret explants established and grew rapidly in culture. They grew slowly in the absence of auxin and rapidly when it was added to the culture medium at 2.0 mg l^{-1}. This difference was highly significant. (Table 2).

Explant growth rate was intermediate at 4.0 mg l^{-1} also being significantly better than when auxin was omitted (Table 2).

Increasing cytokinin concentrations tended to increase adventitious growth, and generated adventitious tissue clusters measuring between 449mm^3 and 752mm^2 at 5.0 and 50.0 mg l^{-1}, respectively (Table 2). However, there was no significant difference between any of the concentrations used.

Table 2. Effect of auxin and cytokinin concentration on adventitious growth development (mm^3) from floret explants of *Rhododendron* 'Cynthia'

Auxin mg l^{-1}	Cytokinin mg l^{-1}				
	5	10	15	20	Weighted mean
0	195	355	308	—	229 b
2	830	1385	747	915	918 a
4	—	339	1206	838	748 a
Mean	449 a	630 a	751 a	752 a	

Means followed by the same letter are not significantly different at the 5% level.

A significant interaction occurred between the two growth regulators for promotion of floret growth, at 2.0 and 10.0 mg l^{-1} IAA and 2iP, respectively (Table 2).

'Pink Pearl' explants grew best at 2.0 mg l^{-1} IAA; at 4.0 mg l^{-1}

IAA growth was significantly retarded whilst at 0.0 mg l^{-1} IAA, no growth occurred. (Table 3).

In common with 'Cynthia' floret explant growth, those of 'Pink Pearl' also grew in response to increased cytokinin concentrations up to 15.0 mg l^{-1} 2iP (Table 3). Although growth was greatest at this concentration, it was not significantly different from any of the others under test. No significant interaction occurred between auxin and cytokinin for the given parameter (Table 3). However, the results indicate an optimum IAA and 2iP combination of 2.0 and 10.0 to 15.0 mg l^{-1}, respectively, for adventitious tissue development of floret explants of this cultivar (Table 3).

Table 3. Effect of auxin and cytokinin on adventitious growth development (mm^3) from floret explants of *Rhododendron* 'Pink Pearl'.

	Cytokinin mg l^{-1}				
Auxin mg l^{-1}	5	10	15	20	Weighted mean
0	—	0	8	0	3 c
2	93	526	735	348	428 a
4	—	35	173	79	120 b
Mean	128 a	206 a	292 a	222 a	

Means followed by the same letter are not significantly different at the 5% level.

The auxin and cytokinin concentrations used in the shoot induction medium had a highly significant effect on caulogenesis of 'Nova Zembla' and 'Cynthia' cultures only. For instance, propagule induction in 'Nova Zembla' cultures was greatest when no auxin and least when 4.0 mg l^{-1} IAA was added to the medium. The greatest decrease (49%) occurred between 0.0 and 2.0 mg l^{-1} IAA (Table 4).

Table 4. Effect of auxin and cytokinin concentration on shoot production from *in vitro* derived masses of adventitious buds of *Rhododendron* 'Nova Zembla' after 16 weeks in culture.

	Cytokinin mg l^{-1}				
Auxin mg l^{-1}	5	10	15	20	Weighted mean
0	58	38	54	44	49 a
2	11	15	26	45	25 b
4	6	8	23	43	21 b
Mean	24 b	23 b	38 a	44 a	

Means followed by the same letter are not significantly different at the 5% level.

The level of cytokinin in the medium also significantly influenced caulogenesis of 'Nova Zembla' cultures. In direct contrast with adventitious tissue development, caulogenesis increased with increasing 2iP concentration, and was maximised at 20.0 mg l^{-1} (Table 4). However, at this level it was not significantly different

from that at 15.0 mg l^{-1}. At 5.0 and 10.0 mg l^{-1} caulogenesis was significantly inhibited (Table 4). A significant interaction between auxin and cytokinin also occurred. Caulogenic growth was greatest at 0.0 and 5.0 mg l^{-1} IAA and 2iP respectively (Table 4).

Further analysis showed that the number of usable 'Nova Zembla' propagules (>10mm) was also significantly influenced by the levels of the respective growth regulators in the medium. To this end the absence of auxin was critical (Table 5). In fact the number of usable shoots dropped 340% compared with production when IAA was added at 2.0 mg l^{-1} (Table 5). In contrast the cytokinin concentration was not critical, and no significant difference occurred irrespective of the concentration used (Table 5). However, high 2iP levels (15.0 mg l^{-1}) favoured the development of such propagules (Table 5).

Table 5. Effect of auxin and cytokinin concentration on the number of usable shoots produced from *in vitro* derived masses of adventitious buds of *Rhododendron* 'Nova Zembla' after 16 weeks in culture.

	Cytokinin mg l^{-1}				
Auxin mg l^{-1}	5	10	15	20	Weighted mean
0	19	18	23	9	17 a
2	4	0	2	5	5 b
4	1	—	2	7	4 b
Mean	8 a	8 a	12 a	9 a	

Means followed by the same letter are not significantly different at the 5% level.

Although an auxin/cytokinin interaction occurred, it was not highly significant. The maximum number of usable propagules occurred at 0.0 and 15.0 mg l^{-1} IAA and 2iP respectively.

In common with the cultivar, 'Nova Zembla', 'Cynthia' cultures also produced more propagules when auxin was omitted from the medium. Growth decreased significantly; 34% when IAA was increased from 0.0 to 2.0 mg l^{-1} and 65% when it was increased from 0.0 to 4.0 mg l^{-1}, respectively (Table 6). Similarly there was also a significant decrease when it was increased from 2.0 to 4.0 mg l^{-1}.

The concentration of cytokinin in the culture medium also significantly influenced caulogenesis of 'Cynthia' cultures. It was best at 15.0 and least at 20.0 mg l^{-1} 2iP, respectively (Table 6).

Although no significant interaction occurred between the two, 0.0 and 5.0–15.0 mg l^{-1} IAA and 2iP, respectively, induced the maximum number of propagules.

Further analysis disclosed that the numbers of usable propagules (>10mm) harvested from 'Cynthia' cultures varied in accordance with the relative auxin and cytokinin concentration used in the culture medium (Table 7).

Table 6. Effect of auxin and cytokinin concentration on shoot production from *in vitro* derived masses of adventitious buds of *Rhododendron* 'Cynthia' after 16 weeks in culture.

Auxin mg l^{-1}	Cytokinin mg l^{-1}				
	5	10	15	20	Weighted mean
0	52	46	51	42	47 a
2	19	24	46	22	31 b
4	14	25	17	7	16 c
Mean	26 b	35 a	39 a	25 b	

Means followed by the same letter are not significantly different at the 5% level.

Table 7. Effect of auxin and cytokinin concentration on the number of usable shoots produced from *in vitro* derived masses of adventitious buds of *Rhododendron* 'Cynthia' after 16 weeks in culture.

Auxin mg l^{-1}	Cytokinin mg l^{-1}				
	5	10	15	20	Weighted mean
0	19	11	16	7	12 a
2	9	8	18	7	12 a
4	2	3	1	2	2 b
Mean	9 ab	8 ab	13 a	6 b	

Means followed by the same letter are not significantly different at the 5% level.

At low auxin concentrations (0.0 and 2.0 mg l^{-1} IAA) no difference occurred in the number of such propagules generated. However, at 4.0 mg l^{-1} their number decreased six fold (Table 7).

There was no significant difference in the number obtained at 2iP concentrations between 5.0 and 15.0 mg l^{-1}, respectively. However, at the 20.0 mg l^{-1} there was a significant decrease (Table 7).

No significant interaction between auxin and cytokinin occurred for this parameter, neither was there little difference in the number of usable propagules produced at 0.0 and 5.0 mg l^{-1} and 2.0 and 15.0 mg l^{-1} IAA and 2iP respectively. Adventitious tissue clusters of 'Pink Pearl' remained recalcitrant and failed to differentiate sufficient numbers of usable propagules derived from florets. However, when micropropagules derived from shoot tip cultures of 'Pink Pearl' were tested under seven 2iP concentrations alone, its addition to the culture medium had a marked influence on their subsequent growth and caulogenic rates. The highest number of propagules occurred within the range 1.6 to 5.0 mg l^{-1}, but was greatest at the former (Table 8). The lowest rates occurred at concentrations in excess of 10.0 mg l^{-1} (Table 8).

Table 8. The effect of seven concentrations of 2iP on caulogenesis and shoot size of *Rhododendron* 'Pink Pearl' propagules.

Treatment	Mean shoot No.	Mean shoot No. >10mm	Mean shoot No. <10mm
2iP (mg l^{-1})			
0	0.43[a]	0.16[a]	0.27[a]
1.6	3.16[b]	1.90[b]	1.26[b]
2.5	2.26[bc]	1.02[cd]	1.60[b]
5.0	2.75[bc]	1.55[bc]	1.20[b]
10.0	2.23[cd]	0.85[de]	1.38[b]
15.0	1.73[d]	0.45[de]	1.28[b]
20.0	1.28[d]	0.30[e]	1.58[b]

Means followed by the same letter are not significantly different at the 5% level.

The maximum number of usable shoots (>10mm) occurred at 1.6 and 5.0 mg l^{-1} whilst the minimum number occurred at higher levels: 10.0 to 20.0 mg l^{-1}, respectively. These differences were highly significant (Table 8). In contrast there was no difference in the number of non-utilizable shoots (<10mm) produced at all levels, except when 2iP was omitted from the medium.

DISCUSSION

The results demonstrate the variability between floret explants of rhododendron cultivars in response to the inclusion of IAA and 2iP at varying ratios in the culture medium. In general 'Nova Zembla' was the most responsive irrespective of the growth regulator concentrations used; 'Pink Pearl' was the least and, in some instances, failed to regenerate adventitious tissue, especially when IAA was omitted from the medium.

In accord with the findings of Anderson (1980) these results demonstrate an absolute requirement for IAA in the explant medium to promote explant development and growth. Similarly, with respect to the 2iP concentration used, these results compare with those of Strode *et al* (16), Hannapel *et al* (9), Anderson (3), and Ettinger and Preece (7), insofar as high 2iP concentrations (10.0 to 15.0 mg l^{-1}) promoted moss-like adventitious growth, a necessary step in the evolution of micropropagules derived from floret explants.

In contrast with the findings of Economou (6), Meyer (11), and Anderson (4), additions of IAA to the sub-culture medium neither enhanced caulogenesis nor improved micropropagule quality. In fact, the cultures lost their requirement for exogenous auxin and grew better at all cytokinin concentrations in its absence. On the contrary, if it were added to the medium, a rapid decline in caulogenic rates occurred at all 2iP concentrations, thus masking any cytokinin activity. This was greatest at 4.0 mg l^{-1} IAA for both 'Nova Zembla' and 'Cynthia'. This phenomenon has not previously

been reported for rhododendron tissue cultures. It is probable that the cultures either accumulated sufficiently high endogenous IAA concentrations or became habituated and autonomously developed auxin biosynthetic pathways during the establishment phase, thus enabling them to grow and develop rapidly when sub-cultured.

The results suggest that, unlike IAA, the 2iP concentration in the sub-culture medium is not critical and may range between 5.0 and 15.0 mg l^{-1}, depending on the cultivar, for material derived from floret explants. However, when 'Pink Pearl' cultures derived from shoot tip explants were sub-cultured, the maximum number of propagules occurred at 1.6 mg l^{-1}. This compares with the findings of McCown and Lloyd (10). It contrasts with the above findings, and also with those of Strode *et al* (16) and Anderson (4) who recommended 1.0 and 5.0 mg l^{-1} IAA and 2iP, respectively. Similarly, it contrasts with those of Fordham *et al* (8), Douglas (5), and Ettinger and Preece (7) who recommended levels as high as 15.0 mg l^{-1}, irrespective of the attendant risk of adventitious tissue induction at such levels.

In the latter experiment IAA was omitted from the medium and its deletion may explain the efficacy of the lower 2iP concentration and conceivably enhance its effectiveness at such low levels.

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PROPAGATION OF *EMBOTHRIUM COCCINEUM*, *CARPENTERIA CALIFORNICA*, AND *FREMONTODENDRON* 'CALIFORNIA GLORY'

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There are often many different ways of propagating a particular plant which will achieve the same result. What is described in this paper are the methods used at Hewton Trees and Shrubs for the propagation of *Embothrium coccineum*, *Fremontodendron* 'California Glory', and *Carpenteria californica*, all of which are often considered to be difficult subjects. All three types of plant are propagated in a similar way so the majority of the paper is devoted to the description of *Embothrium coccineum*, reference being made to any differences in the method for the other plants.

BACKGROUND AND BOTANICAL INFORMATION

Embothrium coccineum, a native of Chile and bordering parts of Argentina from about 37° south to Tierra del Fuego, was first introduced to Great Britain by William Lobb in 1846. In the wild it grows from the coast up to the tree line and as a small bush up to an 8 metre tree; in cultivation it can be even taller.

Plants growing in the United Kingdom are either evergreen or semi-deciduous. There is confusion over nomenclature, but all are synonyms of *Embothrium coccineum*, and all produce a profusion