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BRIEF COMMUNICATION

The influence of thidiazuron on shoot regeneration from leaf explants of fifteen cultivars of *Rhododendron*

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Abstract

The influence of cytokinin thidiazuron (TDZ) and auxin indole-3-acetic acid (IAA) on *in vitro* shoot organogenesis of fifteen *Rhododendron* genotypes was investigated and a protocol for high frequency adventitious shoot regeneration from leaf explants was developed. High genotypic variation was observed and regeneration frequencies ranged from 0 to 100 %. Genotype Ovation had the highest number of shoots (26.4 per explant) after 12 weeks on medium with 0.57 μM IAA and 1.20 μM TDZ, but only 65 % of explants regenerated. Catawbiense Grandiflorum had 17.7 shoots per explant and 75 % regeneration on medium with 5.70 μM IAA and 0.45 μM TDZ and Van Werden Poelman had 14.3 shoots per explant and 100 % regeneration on medium with 0 57 μM IAA and 0.45 μM TDZ.

Additional key words: auxin, cytokinin, plant growth regulators, tissue culture.

The techniques of genetic engineering opened the way to the generation of novel colours and forms in various ornamental plants including *Rhododendron* species (Ueno *et al.* 1996, Pavingerová *et al.* 1997, Hsia and Korban 1998a, Knapp *et al.* 2001). The most common method of genetic engineering is *Agrobacterium*-mediated transformation of leaf discs followed by direct plant regeneration with minimum callus formation on the cut edge of explants. However, in *Rhododendron* spp., the frequency of plant regeneration from leaf explants is very different and the efficiency of growth regulators is cultivar specific (*e.g.* Preece and Imel 1991, Mertens *et al.* 1996, Hsia and Korban 1998b, Iapichino *et al.* 1991, Tomsone and Gertnere 2003). We previously showed that transformation of explants with meristematic tissues produced many problems such as the appearance of chimeric plants with gradual loss of transgenes during vegetative propagation (Pavingerová *et al.* 1997, Pavingerová and Šedivá 1999). Therefore, it is useful to optimise the regeneration system from somatic tissue explants. The aim of this study was to investigate the effect of thidiazuron (TDZ) in combination with indole-3-acetic acid (IAA) on adventitious shoot regeneration from leaves of *Rhododendron*. We investigated fifteen cultivars of *Rhododendron* with a view to find an acceptable method and cultivars for genetic transformation.

In vitro shoot cultures of 15 rhododendron cultivars (America, Azurro, Bohumil Kavka, Catawbiense grandiflorum, Catharine van Toll, Dr. H.C. Dresselhuys, Erato, Eva, Mars, Nova Zembla, Ortrud, Ovation, Purple Splendour, Rebe and Van Werden Poelman) were obtained from the Silva Tarouca Research Institute of Landscape and Ornamental Gardening, Průhonice, Czech Republic. For testing of influence of growth regulators the halves of young leaves were placed horizontally (ten per vessel) with the abaxial surface in contact with the medium and were cultivated under 16-h photoperiod with irradiance of 90 μmol m^{-2} s^{-1} and temperature of 23 °C. The basal medium consisting of Anderson's salts (Anderson 1984), 80 mg dm^{-3} adenine hemisulphate, 100 mg dm^{-3} myo-inositol, 0.4 mg dm^{-3} thiamine HCl, 30 g dm^{-3}sucrose and 2 g dm^{-3} polyvinylpyrrolidone (PVP) K30, was solidified with 8 g dm^{-3} agar. The medium was adjusted to pH 5.2 before autoclaving. The growth regulators were added to the medium after autoclaving by filter sterilisation. TDZ (0.45, 1.20, 2.30, 4.54 and 22.70 μM) in combination with IAA (0.57, 5.70, 11.40 and 22.80 μM) was used.

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Abbreviations: IAA - indole-3-acetic acid, PVP - polyvinylpyrrolidone; TDZ - thidiazuron.
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Table 1. The most effective concentrations of TDZ and IAA on explant survival and adventitious shoot formation on rhododendron leaf explants after 12 weeks of incubation. Means ± SE, $n = 20$.

Cultivar	TDZ [μM]	IAA [μM]	Explants survived [%]	Explants with shoots [%]	Shoot number [explant^{-1}]
Ovation	1.20	0.57	85	65	26.4 ± 3.8
	1.20	5.70	100	85	6.5 ± 1.3
Mars	2.30	0.57	65	60	13.7 ± 2.1
	0.45	5.70	75	75	10.7 ± 1.6
Nova Zembla	0.45	0.57	100	10	8.0 ± 1.9
	1.20	0.57	60	20	6.5 ± 1.4
Van Werden Poelman	0.45	0.57	100	100	8.0 ± 0.9
	1.20	0.57	100	100	6.7 ± 1.6
Rebe	4.54	5.70	70	65	7.1 ± 1.2
	0.45	11.40	80	30	6.3 ± 1.4
Dr. H.C.Dresselhuys	1.20	5.70	100	85	5.3 ± 1.2
	0.45	5.70	80	75	4.1 ± 0.5
Azuro	0.45	11.40	100	100	5.4 ± 1.1
	0.45	0.57	100	100	4.6 ± 0.8
Erato	4.54	5.70	70	40	4.1 ± 0.6
	22.70	22.80	100	30	4.1 ± 0.7

Each experiment had two replicates, each consisting of a Petri dish containing 10 explants. Shoot regeneration was expressed as the percentage of leaf segments that regenerated shoots (> 2 mm in length) and as the number of shoots per regenerating explant. Data were recorded after 12 weeks of culture.

Significant genotype effects were obtained during the growth of callus and regeneration of adventitious shoots on media with various concentrations of TDZ and IAA (Table 1).

The most frequent regeneration was observed in cv. Catawbiense Grandiflorum (Table 2) and also in cvs. Dr. H.C. Dresselhuys, Ovation and Rebe. In these cultivars we observed a high percentage of surviving explants, extensive growth of callus and a sufficient number (when we consider the potential use in genetic transformation) of regenerated shoots per explant.

Somatic embryogenesis was observed in cv. Dr. H.C. Dresselhuys during the first three weeks of cultivation on medium with 0.57 μM IAA and higher concentrations of TDZ (4.54 and/or 22.70 μM). Globular embryos and heart-stages were evident, and the embryos formed directly from the leaf epidermis without the initiation of primary callus. However, the torpedo-stages were not observed and organogenesis gradually predominated.

A large number of shoot buds was observed in the cvs. Catharine van Toll, Mars, Nova Zembla and Van Werden Poelman but these were short, compact, and difficult to count.

The cvs. Azurro, Erato, Ortrud and Purple Splendour formed the big callus on the surface of leaf discs in the majority of surviving explants, but regeneration of shoots was sporadic.

We observed almost 100 % survival of regenerants from leaf discs of cvs. Ortrud and Purple Splendour on all media tested. In cv. Ortrud, about 20 % of leaf discs produced adventitious shoots with a frequency from 1.0 to 3.4 shoots per explant in all combinations of

Table 2. Effect of TDZ and IAA on explant survival and adventitious shoot formation on leaf explants of *Rhododendron* cv. Catawbiense Grandiflorum. Means ± SE, $n = 20$.

TDZ [μM]	IAA [μM]	Explants survived [%]	Explants with shoots [%]	Shoot number [explant^{-1}]
0.45	0.57	80	65	14.0 ± 2.2
0.45	5.70	75	85	17.7 ± 2.5
0.45	11.40	100	100	8.8 ± 1.8
0.45	22.80	65	65	13.0 ± 2.3
1.20	0.57	60	45	15.3 ± 2.3
1.20	5.70	80	80	16.5 ± 1.4
1.20	11.40	90	85	8.7 ± 0.8
1.20	22.80	90	50	8.1 ± 0.8
2.30	0.57	75	65	11.3 ± 1.6
2.30	5.70	95	95	13.5 ± 3.7
2.30	11.40	100	100	9.9 ± 1.9
2.30	22.80	75	75	5.0 ± 0.6
4.54	0.57	90	85	15.2 ± 3.0
4.54	5.70	95	95	11.6 ± 2.9
4.54	11.40	80	80	7.5 ± 0.9
4.54	22.80	70	65	6.3 ± 1.2
22.70	0.57	85	80	14.5 ± 2.3
22.70	5.70	100	50	14.9 ± 3.5
22.70	11.40	50	50	6.9 ± 1.2
22.70	22.80	85	85	5.4 ± 0.7

growth regulators.

Explant survival of cv. Erato was higher at higher concentrations of IAA in the medium. With higher concentrations of TDZ in the medium leaves primarily formed callus and no adventitious shoots. However, the number of regenerated shoots was very low and rather sporadic.

The combination of TDZ and IAA was entirely unsuitable for regeneration of the cvs. America, Bohumil Kavka and Eva.

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