

## The influence of thidiazuron on shoot regeneration and proliferation of rhododendrons *in vitro*

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### Abstract

To improve shoot regeneration and proliferation methods for *Rhododendron L.*, the present study was initiated to test the effect of thidiazuron (TDZ) on (i) adventitious shoot regeneration from flower explants (each consisting of an ovary with pedicel) and (ii) shoot proliferation. For regeneration, flower explants of evergreen rhododendron cv. 'Irina' were placed on Anderson's medium supplemented with various combinations of TDZ (0 - 2 mg l<sup>-1</sup>), N<sup>6</sup>-Δ<sup>2</sup>-isopentenyl adenine (15 mg l<sup>-1</sup>) and indole-3-butyric acid (3 mg l<sup>-1</sup>). For proliferation two-node microshoot cuttings were placed on Anderson's medium supplemented with various concentrations of TDZ (0 - 2 mg l<sup>-1</sup>). Application of TDZ induced direct adventitious shoot regeneration from flower explants. Concentrations of 0.05 to 1 mg l<sup>-1</sup> TDZ were found to be efficient. A higher TDZ concentration irreversibly suppressed shoot elongation. As TDZ treatment inhibited further development of axillary buds, it could not be used for promotion of rhododendrons shoot proliferation *in vitro*.

**Key words:** proliferation, rhododendron, shoot regeneration, thidiazuron.

### Introduction

The improvement of the adventitious shoot regeneration system using tissue culture methods of *Rhododendron L.* genus plants is still important due to the diverse morphogenic potential of different genotypes. A good shoot regeneration system is a prerequisite for the development of a successful micropropagation protocol and the evolution of genetic transformation techniques. The shoot regeneration of rhododendrons has been stimulated *in vitro* from different types of explants by using various combinations of auxins and cytokinins (for summary see Tomsone, Gertnere 2003). Explants isolated from rhododendron flowers have several advantages, compared to other explants, the main being easier control of contamination and a long time interval for explant harvest (Mayer 1982; Dai et al. 1987). Therefore, attention has been focused on the improvement of direct shoot regeneration from flower explants. There are many reports showing that the application of thidiazuron (TDZ; N-phenyl-N<sup>1</sup>-1,2,3-thiadiazol-5-ylurea) results in a better shoot regeneration capacity in comparison with other cytokinins (Babaoglu, Yorgancilar 2000; Srikandarajah et al. 2001; Zhang et al. 2001). Concerning TDZ application to rhododendrons, some attempts have been made using leaf explants (Preece, Immel 1991) and stamens (Shevade, Preece 1993). The results of our previous investigation indicated that adding TDZ together with N<sup>6</sup>-Δ<sup>2</sup>-isopentenyl adenine (2iP) and indole-3-butyric acid (IBA) to the medium improved adventitious shoot regeneration from flower explants and

resulted in a higher explants survival rate (Tomsone, Gertnere 2003).

The objective of the present study was to determine the optimal concentration of TDZ for adventitious shoot regeneration. In addition to that, the effect of TDZ on the development of axillary buds on microshoots was investigated.

## Materials and methods

For shoot regeneration tests, evergreen rhododendron (*Rhododendron* L.) cv. 'Irina' flower buds were sampled in October from open fields of the Botanical Garden of the University of Latvia. Raceme buds were washed with an antibacterial soap and rinsed in running tap water. Subsequently, buds were surface disinfected for 15 min in 17 % of a commercial bleach (Domestos, Hungary) solution containing 5 % sodium hypochlorite and rinsed with sterile distilled water. The outer bud scales were removed, florets excised, ovary with pedicel isolated (named "flower explants") and then explants, one per tube (0.15 × 15 cm), were placed on the surface of the medium. Anderson's medium (1984) containing 20 g l<sup>-1</sup> sucrose, 10 g l<sup>-1</sup> glucose, 6.1 g l<sup>-1</sup> agar, supplemented with 15 mg l<sup>-1</sup> 2iP, 3 mg l<sup>-1</sup> IBA and 0 to 2 mg l<sup>-1</sup> TDZ was used for shoot regeneration for 12 weeks. Subsequent explants were transferred for shoot elongation to Anderson's medium with 3 mg l<sup>-1</sup> 2iP and cultivated for 8 weeks.

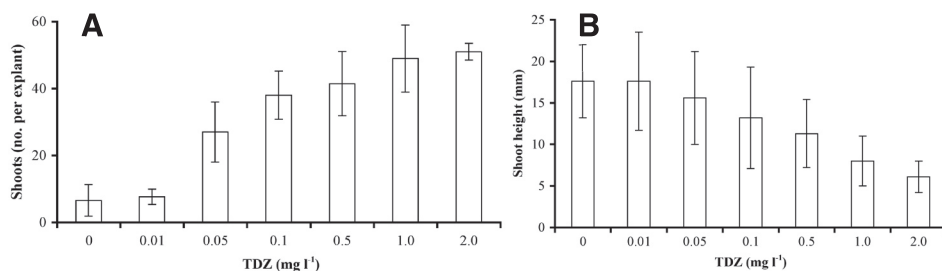
For proliferation tests, two node microshoot cuttings of cv. 'Irina', one per tube (0.15 × 15 cm), were placed on Anderson's medium supplemented with TDZ (0 to 2 mg l<sup>-1</sup>) and cultivated for 12 weeks.

The pH of the medium was adjusted to 5.5 prior to autoclaving. The cultures were incubated under cool white fluorescent light (35 - 50 mmol m<sup>-2</sup> s<sup>-1</sup>) with a 16-h photoperiod at 25 ± 2 °C. For each treatment 20 explants were used.

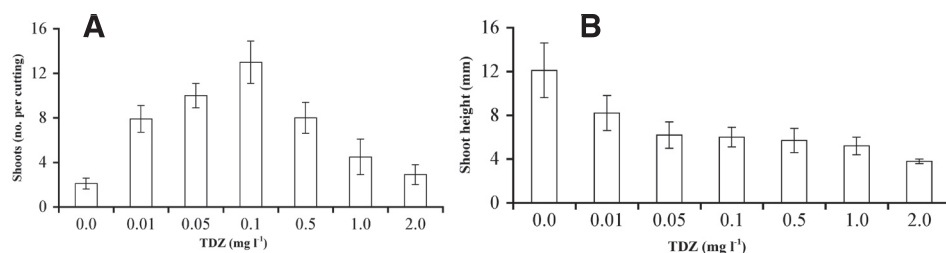
## Results and discussion

Without TDZ, the shoot regeneration capacity from rhododendron flower explants was extremely low. TDZ promoted production of granular masses of tissues and numerous shoot primordia directly on the surface flower explants. During a 12-week period, the highest shoot tips reached 3 to 5 mm in height and then stunted. As the minimal height of shoots suitable for further subcultivation was 5 mm, the number of shoots reaching at least 5 mm in height within 8 weeks of cultivation on shoot elongation medium was used as the criterion of evaluation of the effect of TDZ on regeneration. The results showed that TDZ stimulated shoot regeneration in a concentration-dependent manner (Fig. 1A). However, higher TDZ concentrations significantly reduced the height of newly formed shoots (Fig. 1B). Considering the positive effect of TDZ on shoot induction and the negative effect on shoot height, the optimal TDZ concentration for shoot regeneration from flower explants was in a range between 0.05 to 0.5 mg l<sup>-1</sup>. The obtained results confirm that TDZ stimulated adventitious organogenesis similarly as shown for stamen explants of rhododendrons (Shevade 1993) and leaf explants (Preece 1991). The addition of TDZ to the medium significantly improved shoot regeneration from flower explants, compared to traditionally used 2iP together with indole-3-acetic acid (Mayer 1982; Dai et al. 1987; Gertnere, Tomsone 1996) or 2iP together with IBA (Fig. 1).

When used on two-node microshoot cuttings, TDZ treatment resulted in the



**Fig. 1.** The effect of thidiazuron (TDZ) on shoot regeneration from flower explants of rhododendron cv. 'Irina'. A, shoot number; B, shoot height. Measurements were made after 12 weeks on regeneration medium with TDZ and a subsequent 8 weeks of cultivation on elongation medium. Data presented are means  $\pm$  SD from 20 explants. Only shoots at least 5 mm in length were measured.



**Fig. 2.** The effect of thidiazuron (TDZ) on shoot proliferation from two-node microshoot cuttings of rhododendron cv. 'Irina'. A, shoot number; B, shoot height. Measurements were made after 12 weeks of cultivation.

development of a number of short, deformed shoots (Fig. 2). The shoots developed on the surfaces of swollen axillary buds. It is obvious that TDZ efficiently stimulated direct adventitious shoot regeneration from flower explants of rhododendrons, but inhibited shoot elongation. As TDZ suppressed development of axillary shoots, it could not be used for the promotion of microshoot proliferation.

## Acknowledgements

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