BIOLOGIA PLANTARUM 46 (3): 463-465, 2003

BRIEF COMMUNICATION

In vitro shoot regeneration from flower and leaf explants in *Rhododendron*

S. TOMSONE and D. GERTNERE

Plant Tissue Culture Laboratory, University of Latvia, Kandavas 2, Riga, LV-1083, Latvia

Abstract

Rhododendron shoot regeneration was accomplished using either flower explants (each consisting of ovary with pedicel) of *Rhododendron* cvs. Nova Zembla and Irina or leaves isolated from *in vitro* grown *Rhododendron catawbiense* Michx. Multiple shoot tip clumps were obtained on Anderson's medium containing 0.5 to 1.5 mg dm^{-3} thidiazuron (TDZ) in combination with 12 to 15 mg dm^{-3} N^6-[2-isopentenyl]adenine (2iP) and 1 to 3 mg dm^{-3} indole-3-butyric acid (IBA). After 16 weeks on the regeneration media, explants with shoot tip clumps were transferred for shoot elongation to Anderson's medium with 3 mg dm^{-3} 2iP. Two months later, the shoots have reached 5 to 40 mm in length and were fit for subcultivation.

Additional key words: indole-3-butyric acid, micropropagation, N^6-[2-isopentenyl]adenine, thidiazuron.

The development of efficient regeneration systems from somatic tissues will contribute to the improvement of *Rhododendron* micropropagation. In previous studies, direct and indirect shoot regeneration from different types of rhododendron explants has been stimulated by various combinations of auxins and cytokinins. Regeneration of leaf explants has been induced by indole-3-butyric acid (IBA) and thidiazuron (TDZ) (Preece and Imel 1991), and by IBA and N^6-[2-isopentenyl]adenine (2iP) (Iapichino *et al.* 1992), regeneration of pedicels and ovary bases - by indole-3-acetic acid (IAA) and 2iP (Meyer 1982), regeneration of ovaries - by IAA and 2iP (Dai *et al.* 1987), regeneration of stamens - by IAA, TDZ and 2iP (Shevade and Preece 1993), and regeneration of shoot tip calluses - by zeatin (Harbage and Stimart 1987). Meyer (1982) has already proved the advantages of isolating the explants from rhododendron flowers in facilitating establishment of sterile *in vitro* culture. Findings of our previous investigations have demonstrated that the best explant source is ovary with pedicel. However, regeneration from 19 tested rhododendron genotypes, proved insufficient when 2iP and IAA were used as growth regulators (Gertnere and Tomsone 1996). The objective of this study was to improve rhododendron adventitious shoot regeneration from flower explants and isolated *in vitro* leaves using TDZ in combination with 2iP and IBA.

Rhododendron spp. cvs. Nova Zembla and Irina (seedling derived from *Rhododendron catawbiense* Michx., registered 2000) flower buds were sampled in February from open fields of the Botanical Garden of the University of Latvia. Raceme buds were washed with antibacterial soap (*Safeguard, Procter & Gamble*, Amiens, France), subsequently surface-sterilised in 0.03 % ethanolmercuric chloride with 0.07 % N-cetyl-piridinum chloride for 15 min, and rinsed three times in sterile distilled water. The outer bud scales were removed, florets excised, ovary with pedicel isolated from each floret, and used as an explant (named "flower explant"). Flower explants were placed horizontally on the surface of the medium in culture tubes, one explant per tube. *Rhododendron catawbiense* Michx. explants were whole leaves isolated from proliferating *in vitro* shoot culture. Ten leaves per vessel were placed on the medium.

For shoot regeneration all explants were placed on Anderson's medium (Anderson 1984) containing 20 g dm^{-3} sucrose, 10 g dm^{-3} glucose, 7 g dm^{-3} agar (*Kräuter-Mix*

Received 17 December 2001, *accepted* 2 July 2002.
Abbreviations: IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; 2iP - N^6-[2-isopentenyl]adenine; TDZ - thidiazuron.
Acknowledgements: This study was supported by the Science Council of Latvia, Grant No. 01.0260.
Fax: (+371) 7450852, e-mail: signet@lanet.lv

GmbH, Abstwind, Germany), and supplemented with various combinations of 2iP, TDZ and IBA. After 16 weeks on the treatment media, explants were transferred for shoot elongation to Anderson's medium with 3 mg dm^{-3} 2iP and maintained there for 2 months. Medium pH was adjusted to 5.5 prior to autoclaving. The cultures were incubated under cool white fluorescent lamps (35 - 50 µmol m^{-2} s^{-1}) with a 16-h photoperiod at 25 ± 2 °C. For each treatment, 20 flower explants and 30 *in vitro* leaves were used.

During the first 10 weeks, shoot primordia – spherical structures and/or shoot tips – arose directly from the surface all around the explants. Some explants developed callus, unrelated to any specific medium or genotype. During the next four weeks, shoots of *Rhododendron* cv. Irina elongated to 5 mm in height, while simultaneously, new spherical structures and shoot tips continued to appear. The longest shoots of cv. Nova Zembla reached only 2 mm in height under the same conditions.

Table 1. Effects of TDZ, 2iP and IBA on shoot formation of *Rhododendron* cvs. Irina and Nova Zembla from flower explants and *Rhododendron catawbiense* Michx. from isolated *in vitro* leaves. Results were calculated after 16 weeks cultivation on regeneration medium and a subsequent 2 months cultivation on elongation medium. Mean ± SE, cv. Irina - 17 initial explants, cv. Nova Zembla - 20 initial explants, *R. catawbiense* - 30 initial explants per treatment. The shoot number and shoot length means were calculated including shoots 5 mm in length and longer.

Cultivar	TDZ [mg dm^{-3}]	2iP [mg dm^{-3}]	IBA [mg dm^{-3}]	Explants survived [%]	Explants with shoots [%]	Number of shoots [$explant^{-1}$]	Shoot length [mm]
Irina	0	15	3	12	100	3.3 ± 1.3	7.7 ± 0.9
	0.5	15	3	88	100	33.5 ± 2.0	9.5 ± 0.4
	0.7	15	3	88	100	25.2 ± 2.0	10.2 ± 0.5
	1.0	15	3	76	85	31.9 ± 1.9	9.9 ± 0.3
	1.5	15	3	65	100	28.0 ± 1.6	8.7 ± 0.3
	0.7	14	3	82	79	39.6 ± 3.6	11.5 ± 0.4
	1.0	13	3	94	100	33.8 ± 3.6	10.7 ± 0.3
	1.5	12	3	76	100	32.3 ± 1.5	9.1 ± 0.4
	0.5	15	0	47	100	16.9 ± 2.4	9.8 ± 0.4
	0.5	15	1	82	100	29.3 ± 2.6	10.2 ± 0.5
	0.5	15	2	65	100	30.5 ± 2.7	9.7 ± 0.4
	0.5	15	4	53	100	19.1 ± 2.8	9.2 ± 0.3
Nova Zembla	0	15	3	35	100	4.4 ± 1.1	6.2 ± 0.6
	0.5	15	3	50	100	15.9 ± 1.2	6.2 ± 0.2
	0.7	15	3	65	92	26.0 ± 2.4	8.8 ± 0.5
	1.0	15	3	65	100	18.9 ± 2.2	7.3 ± 0.2
	1.5	15	3	70	79	12.3 ± 1.4	7.3 ± 0.3
	0.7	14	3	70	85	15.3 ± 2.0	7.3 ± 0.3
	1.0	13	3	55	100	16.5 ± 1.8	6.8 ± 0.3
	1.5	12	3	70	71	11.4 ± 1.9	8.1 ± 0.3
	0.5	15	0	45	100	1.3 ± 0.3	5.2 ± 0.2
	0.5	15	1	90	94	4.9 ± 0.9	5.9 ± 0.1
	0.5	15	2	90	100	17.8 ± 1.8	7.5 ± 0.6
	0.5	15	4	40	100	3.4 ± 0.9	5.3 ± 0.1
R. catawbiense	0	15	3	43	100	7.0 ± 0.9	6.6 ± 0.6
	0.5	15	3	33	100	10.5 ± 1.0	5.6 ± 0.5
	0.7	15	3	63	100	3.5 ± 0.8	6.6 ± 0.2
	1.0	15	3	73	100	3.6 ± 0.8	5.8 ± 0.3
	1.5	15	3	50	100	0.6 ± 0.2	7.1 ± 1.0
	0.7	14	3	40	100	39.6 ± 3.7	7.3 ± 0.2
	1.0	13	3	47	100	21.0 ± 2.9	6.4 ± 0.2
	1.5	12	3	67	100	43.6 ± 3.0	7.1 ± 0.2
	0.5	15	0	0	-	-	-
	0.5	15	1	57	100	2.7 ± 0.6	5.8 ± 0.4
	0.5	15	2	47	100	22.8 ± 2.3	7.0 ± 0.2
	0.5	15	4	53	100	1.9 ± 0.5	5.8 ± 0.4

After 16 weeks, the explants were transferred for shoot elongation to Anderson's medium supplemented with 3 mg dm^{-3} 2iP. Three flower explants of cv. Irina from each treatment were left on regeneration medium. After 2 months, no increase in length was observed for these samples (means of shoot length were 3.0 to 5.6 mm depending on the treatment). In comparison, the shoots from the transferred explants continued to grow. The mean length of the shoots depended more on the genotype and the explant type than on the tested regeneration medium (Table 1). Minimal length of shoots suitable for subcultivation was 5 mm. Therefore, the criterium of evaluation of the regeneration medium influence was the number of shoots that have reached 5 mm in length and higher after 2 months cultivation on elongation medium. The lack of TDZ or IBA in the medium resulted in a lower explant survival rate (Table 1). Shoot regeneration of cv. Irina flower explants was stimulated by 0.5 to 1.5 mg dm^{-3} TDZ in combination with 12 to 15 mg dm^{-3} 2iP and 1 to 3 mg dm^{-3} IBA (Table 1). Shoot regeneration of cv. Nova Zembla flower explants was promoted by 0.5 to 1.5 mg dm^{-3} TDZ in combination with 12 to 15 mg dm^{-3} 2iP and 2 to 3 mg dm^{-3} IBA (Table 1). Shoot formation of *R. catawbiense* leaf explants was stimulated by 0.5 to 1.5 mg dm^{-3} TDZ in combination with 12 to 15 mg dm^{-3} 2iP and 2 to 3 mg dm^{-3} IBA (Table 1).

Overall, we observed direct shoot regeneration with a few explants developing an organogenic callus. Chalupa (1988) observed appearance of organogenic callus in *Quercus robur* L. exposed to TDZ, but Preece (1991) reported that callusogenesis of leaf explants of *Rhododendrom* hybrids occurred depending on IBA concentration. When TDZ was used together with IBA and 2iP, the callus formation rate was low and direct shoot regeneration occurred, which is a precondition for qualitative plant material production. Obtained results indicated that the inclusion of TDZ in the medium improved the explant survival rate as well as shoot regeneration. But, as it was expected, TDZ prevented shoot elongation. The optimal concentrations of TDZ, 2iP and IBA depended on the rhododendron genotype and the explant type. For shoot initiation we used 15 mg dm^{-3} 2iP, which is the treatment concentration level that Meyer (1982) and Dai (1987) have recommended for flower explants. Nevertheless, it would be useful to extend concentration ranges of 2iP and TDZ to determine the optimal treatment concentrations for shoot regeneration.

Addition of 1 to 3 mg dm^{-3} IBA to the regeneration medium did not affect shoot length, but resulted in a better shoot regeneration in general as well as in a higher explant survival rate. Preece (1991) reported that *in vitro* leaf explants survival rate and shoot regeneration improved as a result of the explants preculturing on the medium with IBA + 2iP and a subsequent transferring to the medium with IBA + TDZ. To reduce regeneration costs, we tried combining these treatments in one. The leaf explants survival rate was approximately 53 % (Table 1). We believe it could be improved by varying the growth regulators concentrations.

Meyer (1982), Dai (1987) and Shevade (1993) added IAA to the shoot regeneration medium. With respect to the shoot formation rate, IBA was more appropriate for the shoot regeneration as compared to IAA in our previous experiments (Gertnere and Tomsone 1996). Besides, the data also showed better shoot elongation when regeneration occurred on the medium supplemented with IBA as compared to the medium supplemented with IAA.

Due to relatively extended period of successful flower explant isolation, easy obtainable sterile culture, and high TDZ potency in shoot regeneration stimulation, the use of flower explants and isolated *in vitro* leaves under TDZ, 2iP and IBA treatments have a great potential for improvement of rhododendron shoot regeneration *in vitro*.

References

Anderson, W.C.: A revised tissue culture medium for shoot multiplication of rhododendron. - J. amer. Soc. hort. Sci. **109**: 343-347, 1984.

Chalupa, V.: Large scale micropropagation of *Quercus robur* L. using adenine-type cytokinins and thidiazuron to stimulate shoot proliferation. - Biol. Plant. **30**: 414-421, 1988.

Dai, C., Lambeth, V.N., Taven, R.: Micropropagation of *Rhododendron prinophyllum* by ovary culture. - HortScience **22**: 491-493, 1987.

Gertnere, D., Tomsone, S.: Induction of morphogenesis of various *Rhododendron* L. taxa flower buds *in vitro*. - Proc. Latvian Acad. Sci., Sect. B **50**: 315-319, 1996.

Harbage, J.F., Stimart, D.P.: Adventitious shoot regeneration from *in vitro* subcultured callus of *Rhododendron* Exbury hybrids. - HortScience **22**: 1324-1325, 1987.

Iapichino, G., McCulloch, S., Chen, T.H.H.: Adventitious shoot formation from leaf explants of *Rhododendron*. - Plant Cell Tissue Organ Cult. **30**: 237-241, 1992.

Meyer, M.M., Jr.: *In vitro* propagation of *Rhododendron catawbiense* from flower buds. - HortScience **17**: 891-892, 1982.

Preece, J.E., Imel, M.R.: Plant regeneration from leaf explants of *Rhododendron* 'P.J.M. Hybrids'. - Sci. Hort. **48**: 159-170, 1991.

Shevade, A., Preece, J.E.: *In vitro* shoot and floral organogenesis from stamen explants from a *Rhododendron* PJM group clone. - Sci. Hort. **56**: 163-170, 1993.