Plant Biotechnology 24, 425–427 (2007)

Tissue Culture Note

Plant regeneration from leaf explants of auricula cultivars (*Primula*×*pubescens* Jacq.)

Michiaki Takihira[1], Motoyasu Otani[1,*], Shoji Tsuchiya[2], Takiko Shimada[1,a]

[1] Ishikawa Prefectural University, Ishikawa 921-8836, Japan; [2] Meijyo University, Aichi 486-0804, Japan
* E-mail: otani@ishikawa-pu.ac.jp Tel: +81-76-227-7504 Fax: +81-76-227-7557

Received April 23, 2007; accepted June 14, 2007 (Edited by M. Nakano)

Abstract Adventitious shoots were regenerated from leaf explants of 4 cultivars of auricula (*Primula*×*pubescens* Jacq.) on media supplemented with various cytokinins combined with NAA. One to four adventitious shoots per leaf explant were regenerated. The highest frequency of adventitious shoot formation was 46.6% for 'Borders Mixed' on the medium containing 0.2 mg l^{-1} TDZ and 2 mg l^{-1} NAA , 10% for 'Border Stripes' on the medium containing 0.2 mg l^{-1} TDZ and 2 mg l^{-1} NAA, 20% for 'Alpines Mixed' on the medium containing 0.02 mg l^{-1} TDZ and 2 mg l^{-1} NAA, 13.3% for 'Field House Mixed' on the medium containing 0.02 mg l^{-1} TDZ and 2 mg l^{-1} NAA. A higher concentration of NAA (2 mg l^{-1}) combined with TDZ in the medium may stimulate adventitious shoot formation from leaf explants in auricula cultivars. Zeatin was less effective for adventitious shoot regeneration from leaf explants. Regenerated shoot easily rooted on plant growth regulator-free LS medium. After acclimatization in a growth chamber, some of the regenerated plants flowered.

Key words: Auricula, plant regeneration, thidiazuron.

Cultivars of auricula (*Primula*×*pubescens* Jacq.) are descendants of a natural cross between *P. auricula* and *P. hirsuta*, which is a large family of plants comprising many thousands of hybrids. They have become popular as pot flowers in not only England but also other countries. All cultivars of auricula are hybrids and vegetative propagated by taking offsets from donor plants. Establishment of system for plant regeneration from cultured cells and tissues is necessary for micropropagating new cultivars and conserving the valuable old cultivars in danger of extinction.

There have been a few reports on plant regeneration from cultured cells and tissues of *Primula* species. Coumans et al. (1979) reported that young floral buds of *P. obconica* regenerated adventitious shoots when cultured on media with BA and NAA. Shimada et al. (1997) reported that regeneration of adventitious shoots and somatic embryos from leaf explants of *P. cuneifolia* var. *hakusanensis* was stimulated by TDZ or zeatin. Yamamoto et al. (1999) obtained regenerated plants from young expanding leaves of *P. sieboldii* on media with BA and NAA. Mizuhiro et al. (2001a) succeeded in plant regeneration from cell-suspension-derived protoplasts of *P. malacoides* and *P. obconica*, and produced somatic hybrids between them (Mizuhiro et al. 2001b). Plant regeneration from leaf calli of *P. vulgaris* and *P. elatior* was stimulated by a high level of TDZ (Schween and Schwenkel 2002, Schween and Schwenkel 2003). In the present study, we investigated the effect of cytokinins on adventitious shoot regeneration from leaf explants of auricula cultivars.

Four auricula cultivars, 'Borders Mixed', 'Border Stripes', 'Alpines Mixed' and 'Field House Mixed', were used in the present study. Seeds of each cultivar were kept in a refrigerator (4°C) until use. Seeds were surface-sterilized with a NaOCl solution containing 3% (v/v) active chlorine for 15 min followed by rinsing three times with sterilized distilled water, and then sown on plant growth regulator-free LS (Linsmaier and Skoog 1965) medium supplemented with 30 g l^{-1} sucrose and 2.5 g l^{-1} of gellan gum. After three-months of culture, seedlings grew into plantlets with 4 to 5 expanded leaves. Leaves were harvested from these plantlets, cut into 15×15 mm pieces and placed on LS media supplemented with 30 g l^{-1} sucrose, 2.5 g l^{-1} gellan gum and various concentrations (0–2 mg l^{-1}) and combinations of cytokinins (BA, Zeatin or TDZ) and NAA. For each medium, 30 leaf explants were cultured. Plant growth regulators were added to the medium and the medium was adjusted to pH 5.9 prior to autoclaving. All cultures were incubated at 20°C under a 14-h photoperiod with fluorescent lighting (about 3,000 lux).

[a] Present address: Department of Plant Sciences, University of Colombo, P.O. BOX 1490, Colombo 00300, Sri Lanka
Abbreviations: NAA, 1-naphtaleneacetic acid; TDZ, thidiazuron; LS, Linsmaier and Skoog; BA, 6-benzyladenine
This article canbe found at http//www.jspcmb.jp/

Table 1. Effect of plant growth regulators on adventitious shoot regeneration from leaf explants of auricula cultivars

Plant growth regulators (mg l^{-1})				No. of leaf explants with regenerated shoots (%)*1			
TDZ	zeatin	BA	NAA	'Borders Mixed'	'Border Stripes'	'Alpine Mixed'	'Field House Mixed'
0	0	0	0	0	0	0	0
0.02			0.2	0	1 (3.3)	0	0
0.02			2	3 (10)	0	6 (20)	4 (13.3)
0.2			0.2	10 (33.3)	1 (3.3)	1 (3.3)	0
0.2			2	14 (46.6)	3 (10)	1 (3.3)	0
	0.2		0.2	0	0	0	0
	0.2		2	0	0	0	1 (3.3)
	2		0.2	0	1 (3.3)	1 (3.3)	0
	2		2	0	0	2 (6.6)	2 (6.6)
	0.2	0.2	0.2	0	0	1 (3.3)	1 (3.3)
		0.2	2	0	0	0	0
		2	0.2	2 (6.6)	0	0	0
		2	2	2 (6.6)	1 (3.3)	4 (13.3)	2 (6.6)

*1 thirty leaf explants were used for each medium.

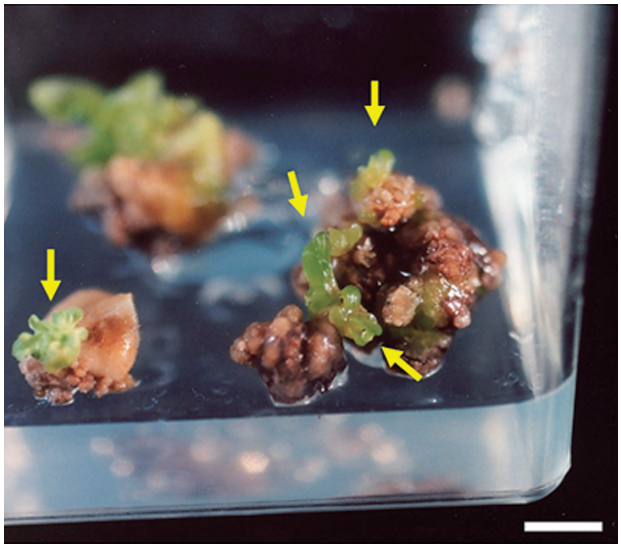

Figure 1. Adventitious shoot regeneration from a leaf explant of auricula 'Borders Mixed' on a medium containing 0.2 mg l^{-1} TDZ and 2 mg l^{-1} NAA after 60 days of culture. Arrows indicate adventitious shoots. Bar = 1 cm.

Figure 2. Regenerated plants of auricula 'Borders Mixed' growing in pots after 180 to 360 days of transplantation to a greenhouse. Bar = 4 cm.

After three-months of culture, leaf explants showed various responses to the kind of plant growth regulators as shown in Table 1. Adventitious shoots were regenerated directly from leaf explants in all cultivars (Figure 1). TDZ was clearly more effective for adventitious shoot formation than BA and zeatin. However, the optimum concentration of TDZ for adventitious shoot formation varied among the cultivars. The highest frequency of adventitious shoot formation was 46.6% for 'Borders Mixed' on the medium containing 0.2 mg l^{-1} TDZ and 2 mg l^{-1} NAA , 10% for 'Border Stripes' on the medium containing 0.2 mg l^{-1} TDZ and 2 mg l^{-1} NAA, 20% for 'Alpines Mixed' on the medium containing 0.02 mg l^{-1} TDZ and 2 mg l^{-1} NAA, 13.3% for 'Field House Mixed' on the medium containing 0.02 mg l^{-1} TDZ and 2 mg l^{-1} NAA (Table 1). A higher concentration of NAA (2 mg l^{-1}) combined with TDZ in the medium may stimulate adventitious shoot formation from leaf explants in auricula cultivars. One to four adventitious shoots per leaf explants were regenerated (Figure 1), and there were no large differences in the number of shoot per explants among the cultivars and plant growth regulators (data not shown).

The effect of TDZ on adventitious shoot regeneration from cultured cells and tissues have already reported in some *Primula* species, *P. cuneifolia* var. *hakusanensis* (Shimada et al. 1997), *P. vulgaris* and *P. elatior* (Schween and Schwenkel 2002, Schween and Schwenkel 2003). We also observed a promotive effect of TDZ on adventitious shoot regeneration in auricula cultivars. TDZ might be advantageous to plant regeneration from cultured cells and tissues in subgenus *Auriculastrum* of the genus *Primula* and European primrose. However, no somatic embryos were produced in the all cultivars of auricula used in the present study, although somatic embryogenesis in cultured leaf explants of *P. cuneifolia* var. *hakusanensis* was stimulated by TDZ. In addition,

the frequencies of adventitious shoot regeneration of auricula cultivars were still lower than those of the other *Primula* species reported. It is necessary to improve the frequency of adventitious shoot regeneration in auricula cultivars.

Regenerated shoots were transferred to plant growth regulator-free LS medium supplemented with 30 g l^{-1} sucrose and 2.5 g l^{-1} gellan gum, and cultured under the same conditions. Then, rooted plantlets were transplanted to pots containing a vermiculite : perlite (3 : 1) mixture and acclimatized under high humidity conditions at 20°C under a 14-h photoperiod with fluorescent lighting (about 5,000 lux) in a growth chamber. Almost all the regenerated plants were successfully acclimatized, and some of them flowered 180 to 360 days after transplantation to a greenhouse (Figure 2). There were no phenotypic differences among the regenerated plants.

We developed system for adventitious shoot regeneration from leaf explants of auricula cultivars. Regenerated plants could be acclimatized and showed normal phenotype in pots. The system developed in the present study may be useful for micropropagation of auricula cultivars.

Acknowledgments

We thank Ms. Akemi Yoko for useful advice.

References

Coumans M, Coumans-Gillès M-F, Delhez J, Gaspar Th (1979) Mass propagation of *Primula* obconica. *Acta Hort* 91: 287–294

Linsmaier EM, Skoog F (1965) Organic growth factor requirement of tobacco tissue culture. *Physiol Plant* 18: 100–127

Mizuhiro M, Yamada K, Ito K, Kadowaki S, Ohashi H, Mii M (2001a) Plant regeneration from cell suspension-derived protoplasts of *Primula* malacoides and *Primula* obconica. *Plant Sci* 160: 1221–1228

Mizuhiro M, Ito K, Mii M (2001b) Production and characterization of interspecific somatic hybrids between *Primula malacoides* and *P. obconica*. *Plant Sci* 161: 489–496

Schween G, Schwenkel HG (2002) In vitro regeneration in *Primula* ssp. via organogenesis. *Plant Cell Rep* 20: 1006–1010

Schween G, Schwenkel HG (2003) Effect of genotypes on callus induction, shoot regeneration, and phenotypic stability of regenerated plants in the greenhouse of *Primula* ssp. *Plant Cell Tissue Organ Cult* 72: 53–61

Shimada T, Matsushita T, Otani M (1997) Plant regeneration from leaf explants of *Primula cuneifolia* var. *hakusanensis*, "Hakusan-kozakura". *Plant Biotechnol* 14: 47–50

Yamamoto T, Magaya Y, Maruyama Y (1999) Mass propagation of *Primula sieborldii* E. Morr. Through leaf segment culture. *Bull Minami-Kyusyu Univ* 29 (A): 9–14