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Induction of Somatic Embryogenesis in *Torenia fournieri* Lind.

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ABSTRACT

Somatic embryogenesis is one biotechnological method that can be used to produce genetically identical plants for use in plant breeding. In this research, we attempted to induce the formation of embryogenic callus from young leaves excised from *in vitro Torenia* spp. by culturing in the dark for 1, 2, 3 and 4 weeks on Murashige and Skoog (MS) medium with Picloram solution added at the concentrations of 0.5, 1, 1.5 and 2 mg^{-l} and 2,4-D solution added at the concentrations of 0.5, 1, 1.5 and 2 mg^{-l}. The leaf explants from all the 2,4-D treatments turned brown and died, but the leaf explants cultured on media with Picloram added at every concentration tested formed soft, loosely aggregated callus tissue. Callus tissue was induced from leaves of diploid *Torenia* to the greatest degree (95% callus formation at 3 weeks) when cultured on MS medium containing Picloram at 1.5 mg^{-l} in the dark, and callus tissue tended to turn brown with increasing Picloram concentration and increasing incubation time. For polyploid *Torenia*, the highest percentage of callus formation was observed on leaves cultured on MS medium containing Picloram, and callus tissue also tended to turn brown with increasing Picloram concentration and increasing incubation time. Following transfer of the embryogenic callus tissue to hormone-free MS medium under light conditions (16-h photoperiod), the greatest rate of somatic embryo formation was observed, in case of both diploid and polyploid Torenia accessions.

1. Introduction

Wishbone flower, or *Torenia* spp., used to be classed in the family Scrophulariaceae (Jala,

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2011) but has recently been moved to the family Linderniaceae. Most Torenia species are perennial herbs that can grow in various kinds of soils in moist areas with partial sunlight. The plant is trailing to semi-recumbant and grows to height of about 15 cm. Most varieties have purplish blue flowers with a yellow throat. True to its name, Torenia displays two short wishbone-shaped curved stamens. Commercially, Torenia has been exploited as an ornamental plant that is popular in beds, pots and hanging baskets. Most often it is propagated by seed, but this method is relatively slow, requiring 10-15 days for germination. It can also be quickly propagated by stem cuttings, and experiments have been done using leaf cuttings. When leaf cuttings with intact petiole bases are planted upright in soil, they can root and sprout new shoots, and can be used with appropriate methods to induce mutations and chromosome doubling (Sawangmee, 2011).

Conventional plant breeding by hybridization via hand fertilization can result in undesirable characteristics being passed down along with desired characteristics, so biotechnological techniques of plant breeding have been developed to overcome these difficulties. Random mutations can be induced using chemicals or radiation, and in many cases advanced gene transfer techniques can be used to obtain very specific objectives. Somatic embryogenesis via plant tissue culture is one biotechnological procedure that can help make plant breeding more efficient (Galom, 2012).

The objective of this research was to investigate the most appropriate type and concentration of plant growth regulator, and the time period required, for inducing embryogenic callus from young in vitro leaves of both diploid and polyploid accessions of *Torenia*, as well as the steps required to induce the development of somatic embryos in diploid and polyploid accessions of *Torenia*.

2. Materials and Methods

2.1 Plant material

Both diploid and polyploid (from colchicine tablet induction) lines of purple-flowered native Thai *Torenia* (*Torenia fournieri* Lind.) with a semi-recumbent, semi-erect habit were the subject for this study.

The plants were maintained in a greenhouse at a day temperature of 33–35 °C and 60–65% relative humidity and a night temperature of 29–33 °C and 65–70% relative humidity.

2.2 Culture Establishment and Growth

Torenia plants were grown in the greenhouse. Axillary bud explants were surface sterilized by soaking with 70% alcohol for 1 min, followed by 10% (v/v) Clorox™ (1.4 % Sodium hypochlorite) containing 10 drops/l Tween 20 for 10 min, 5% (v/v) Clorox™ for 15 min and washed with sterilized distilled water 3 times for 5 min each, to remove the Clorox™. The ends of the explants were cut off on both sides and they were cultured on MS (Murashige and Skoog, 1962) medium. After 2 weeks, clean cultures were transferred to new MS medium. The cultures were maintained at 25 ± 2° C under a 16-hour photoperiod with illumination provided by cool fluorescent lamps at an intensity of 60 $\mu molm^{-2}sec^{-1}$ (TLD 36 w/853350 lm Phillips, Thailand).

2.3 Callus Induction

Leaves from *in vitro* Torenia plantlets were cultured on MS medium supplemented with 0, 0.5, 1.0, 1.5 and 2.0 mg/l of Picloram or 0, 0.5, 1.0, 1.5, 2.0 mg/l of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 3% sucrose and 0.25% phytagel. The cultures were maintained at 25 ± 2° C under dark conditions. After 0, 1, 2, 3 and 4 weeks of culture, callus was transferred to hormone-free MS medium and cultured under light conditions (16-hour photoperiod with illumination provided by cool fluorescent lamps at an intensity of 60 $\mu molm^{-2}sec^{-1}$) to observe somatic embryogenesis and subsequent shoot formation.

2.4 Statistical Analysis

Statistical differences were tested using Duncan's new multiple range test at the $P<0.01$ level.

3. Results and Discussion

The results showed that Torenia young leaf explants that were cultured in media containing no exogenous plant growth regulators in the dark grew and developed into somatic embryos without passing through an intermediate step of embryogenic callus development. Young leaf explants that were cultured on semi-solid MS medium containing 2,4-D at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg/l turned brown and stopped growing at a greater rate than the control group. Embryogenic callus tissue developed the most on young leaf explants that were cultured on MS medium containing Picloram at the rates of 0.5, 1.0, 1.5 or 2.0 mg/l. Callus was first detected after one week of culture. The callus consisted of white to yellowish loosely aggregated clumps of cells (Figure 1). After 3 weeks of culture in the dark, the highest percentage of callus formation (95%) from young leaves of diploid Torenia was observed in the treatment on MS supplemented with Picloram at the rate of 1.5 mg/l. When the cultures were left for longer than

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3 weeks, the percentage of tissue turning brown and dying increased with increasing time, especially with higher concentrations of Picloram. For the polyploid accession of *Torenia*, the treatments cultured in MS medium with Picloram added at the rates of 1.0 and 1.5 mg/l exhibited the highest percentage of callus formation (92.5%) after culture in the dark for 3 weeks. Again, after 3 weeks, the percentage of tissue browning increased with increasing time and with higher concentrations of Picloram (Table 1). These results are consistent with those in several previous reports in which callus was induced from leaf explants of other species, for instance *Saintpaulia* (Sunpai and Kanchanapoom, 2002), *Curcuma amada* (Prakash *et al*., 2004), *Curcuma aromatica* (Mohanty *et al*., 2008), and *Kaempferia galanga* (Rahman *et al*., 2004).

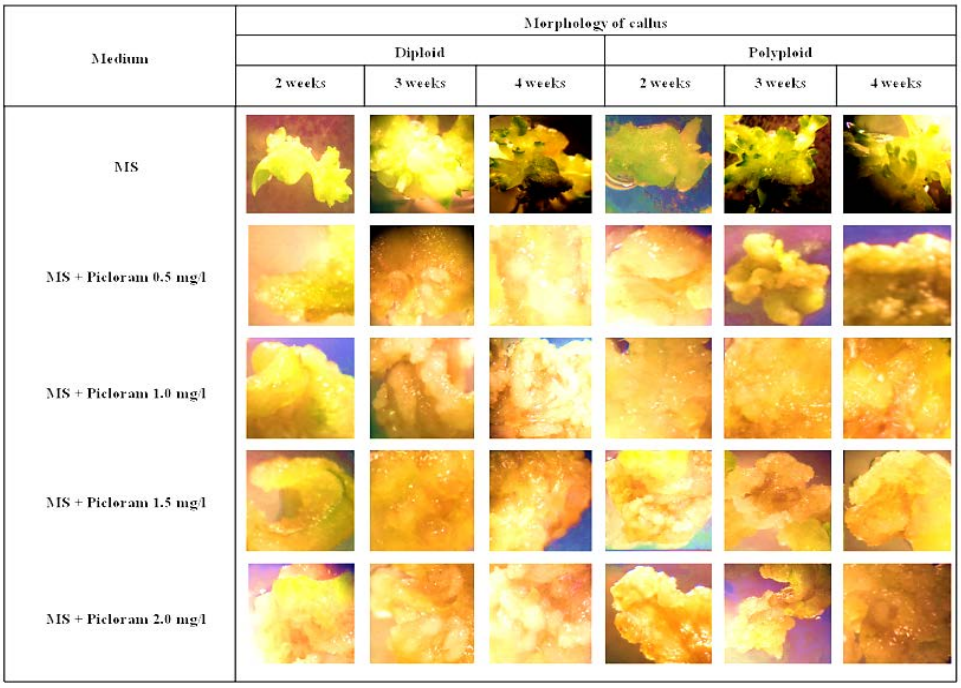

Figure 1: Morphology of callus tissue derived from *Torenia fournieri* Lind. leaf explants on MS medium and MS medium with different concentrations of Picloram after 2, 3 and 4 weeks in the dark.

When embryogenic callus from the callus induction step was transferred to hormone-free semi-solid MS medium and introduced to light conditions (16-hour photoperiod), somatic embryos and new shoots developed within 3 weeks (Figure 2). For both diploid and polyploid Torenia, the number of somatic embryos generated was the greatest in the callus that came from the Picloram 1.5 mg/l treatment (Table 2). As expected, the new shoots developed from polylpoid Torenia tissue tended to be larger than those from diploid Torenia (Figure 3) As previous researchers have pointed out, the correlation between the surface-to-volume ratios of the nucleus and the cell indicates that polyploid nuclei might be required for the formation of large plant cells. The physiological role of genetically programmed polyploidy is, however, elusive as it might contribute to or be a consequence of cell-differentiation programs. Multiplication of the genome has been proposed to increase metabolic activity, rRNA synthesis

and transcriptional activity (Nagl, 1976; Baluska and Kubica, 1992).

Table 1: Percentage of callus formed from *in vitro* leaf explants of *Torenia fournieri* Lind. cultured on MS medium supplemented with different concentrations of Picloram after 1, 2, 3 and 4 weeks.

Medium	Callus formation (%)							
	Diploid Torenia				Polyploid Torenia			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
MS + Picloram 0.5 mg/l	N	27.5 ± 0.96^{b}	80.0 ± 0.82^{b}	60.0 ± 0.82^{a}	N	55.0 ± 0.58^{bc}	75.0 ± 0.58^{a}	75.0 ± 0.58^{a}
MS + Picloram 1.0 mg/l	N	40.0 ± 0.81^{ab}	85.0 ± 0.58^{ab}	67.5 ± 1.26^{a}	N	65.0 ± 0.58^{ab}	92.5 ± 0.96^{a}	80.0 ± 0.82^{a}
MS + Picloram 1.5 mg/l	N	45.0 ± 0.58^{a}	95.0 ± 0.58^{a}	55.0 ± 1.29^{ab}	N	70.0 ± 0.82^{a}	92.5 ± 0.96^{a}	85.0 ± 0.58^{a}
MS + Picloram 2.0 mg/l	N	52.5 ± 0.50^{a}	55.0 ± 0.58^{c}	37.5 ± 0.50^{b}	N	50.0 ± 0.82^{c}	55.0 ± 1.29^{b}	42.5 ± 0.50^{b}
F-test	-	**	**	**	-	**	**	**
% CV	-	19.95	9.16	20.75	-	19.97	13.90	9.78

**Means within the same column followed by different superscripts are significantly different using DMRT, $p \leq 0.01$
N = no callus.

Figure 2: Plants regenerated from embryogenic callus on hormone-free MS medium after 4 weeks under light conditions: diploid (left) and polyploid (right).

Table 2: Number of shoots developed after 30 days in light conditions on hormone-free MS medium from *Torenia fournieri* Lind. callus tissue that was formed on MS medium containing different concentrations of Picloram in the dark.

Original callus induction medium	Number of shoots developed from callus after transfer to hormone-free MS medium							
	Diploid				Polyploid			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
MS + Picloram 0.5 mg/l	1.25 ± 0.50^{c}	1.00 ± 0.82^{b}	4.75 ± 0.50	4.50 ± 0.58	1.75 ± 0.96	3.50 ± 1.29^{a}	1.00 ± 0.82^{b}	4.25 ± 0.50^{a}
MS + Picloram 1.0 mg/l	1.75 ± 0.50^{bc}	1.00 ± 0.82^{b}	5.00 ± 0.00	4.25 ± 0.50	1.75 ± 0.96	1.50 ± 0.58^{ab}	1.00 ± 0.82^{b}	4.50 ± 0.58^{a}
MS + Picloram 1.5 mg/l	2.75 ± 0.50^{ab}	2.50 ± 0.58^{a}	5.50 ± 0.58	3.75 ± 0.96	1.50 ± 0.58	1.25 ± 0.96^{a}	5.25 ± 0.96^{a}	3.50 ± 0.58^{ab}
MS + Picloram 2.0 mg/l	3.75 ± 0.50^{a}	3.25 ± 0.50^{a}	4.75 ± 0.50	3.75 ± 0.96	1.56 ± 0.82	3.00 ± 0.82^{ab}	4.75 ± 0.50^{a}	2.50 ± 0.58^{b}
F-test	**	**	ns	ns	ns	**	**	**
% CV	21.05	35.72	9.12	19.13	48.09	40.92	26.35	15.15

**Means within the same column followed by different superscripts are significantly different using DMRT, $p \leq 0.01$
ns: non significant

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4. Conclusion

MS medium with 3% sucrose, 2.5 g^{-l} Phytogel and Picloram added at the rate of 0, 0.5, 1.0 and 2 mg^{-l} can induce the formation of friable embryogenic callus from young leaves of *in vitro* Torenia. Callus tissue was induced from leaves of diploid Torenia to the greatest degree (95% callus formation at 3 weeks) when cultured on MS medium containing Picloram at 1.5 mg^{-l} in the dark, and callus tissue tended to turn brown with increasing Picloram concentration and increasing incubation time. For polyploid Torenia, the highest percentage of callus formation (92.5%) was observed on leaves cultured on MS medium containing Picloram at the rates of 1.0 and 1.5 mg^{-l} for 3 weeks in the dark, and callus tissue also tended to turn brown with increasing Picloram concentration and increasing incubation time. Following transfer of the embryogenic callus tissue to hormone-free MS medium under light conditions (16-h photoperiod), the greatest rate of somatic embryo formation was observed in the callus derived from the 1.5 mg^{-l} Picloram treatment, in the case of both diploid and polyploid Torenia accessions.

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