IN VITRO FLOWERING OF SELECTED ORNAMENTAL PLANTS

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ABSTRACT

In vitro flowering of three selected ornamental species from Malaysia was studied. *Oxalis triangularis*, *Celosia cristata* and *Begonia x hiemalis* were found to be able to flower on MS (Murashige and Skoog, 1962) medium supplemented with various hormones, such as Benzyl aminopurine (BAP), Naphthalene acetic acid (NAA), etc at different concentrations. In tissue culture, *in vitro* flowering is normally induced for many reasons. One of the most important ones being to shorten the life cycles of plants, other aims include to gather knowledge on *in vitro* flowered plantlets for the formation of fruits and seeds, to overcome problems associated with premature fruit drop or poor seed set. In the present work, we focused on the effect of different hormones and concentrations, on *in vitro* flowering of the three selected species. The effect of Adenine and sucrose at different concentrations were also tested for *Begonia x hiemalis*.

Keywords: Plant regeneration, *in vitro* flowering, adenine, sucrose concentration

INTRODUCTION

Flowering is a unique development event in plants which involves the transition of vegetative shoot apex to form either an inflorescence or a floral meristem followed by initiation and subsequent maturation of the floral organ (Sim et al.,2007). The reproductive stage or flowering process is one of the critically important stages in plant development and is vital for the completion of the life cycle and seed production (Ziv and Naor, 2006). The timing of the transition from vegetative growth to flowering is paramount important in agriculture, horticulture and plant breeding because flowering is the first step of sexual reproduction (Bernier et al.,1993). Under natural growth, flower formation usually commences when a plant attains maturity (Virupakshi et al.,2002). The application of tissue culture technique can be used for the potential of increasing the efficiency of *in vitro* flowering induction of selected plants. Takimoto (1960) found that the plant cultured on the sucrose medium did not require high intensity of light for flower initiation.

The present study reports on *in vitro* flowering of three selected ornamental species i.e *Oxalis triangularis*, *Celosia cristata* and *Begonia x hiemalis*. Begonias, known as versatile temperate plants comprise an enormous group of lovely and very spectacular flowering plants. Begonia are prized equally for dainty cluster of blossoms as well as their cheerful and interesting leaves. They are popularly known as Begonia Rose because of rose-like flower shaded-tolerant pot plant. The objectives of the present work are

to investigate whether it is possible to induce *in vitro* flowering in the three species during tissue culture and also to study the effects of sucrose and adenine on *in vitro* flowering.

MATERIALS AND METHODS

Explants used in this research were flower peduncles, petals, leaves, petioles, stem and inflorescence. The explants were sterilized using chlorox or sodium hypochlorite and 70% alcohol to remove dirt and avoid contamination during culture (Awal, 2009). The explants were then cultured onto solid MS (Murashige and Skoog, 1962) medium supplemented with different concentration of hormones, NAA and BAP. Screening process for suitable adenine and sucrose concentrations for *in vitro* flowering induction were also done. The cultures were maintained under control environments at 25 ±1°C with 16 hour photoperiod and 8 hr darkness. Twenty five explants were cultured in each case.

RESULTS AND DISCUSSION

Table 1 showed the effect of different concentrations of NAA and BAP on shoot and root formation in Begonia. It was found that MS supplemented with 1.0 mg/L BAP and 1.0 mg/L NAA was the best medium for shoot formation from the various explants. The most responsive explant in culture was petiole. However, further experiments showed that direct *in vitro* flowering or reproductive shoots were obtained only from inflorescence explants cultured on MS medium supplemented with 1.0 mg/L BAP, 1.0mg/L NAA, 4% sucrose and 40 mg/L adenine (Tables 2 and 3). The floral buds bloomed after 6-8 weeks of incubation period. Addition of both adenine and sucrose resulted in floral initiation and floral development after 4 weeks. The results also showed that 40 mg/L adenine would increase the *in vitro* flowering bud formation in Begonia. Among the different concentration of sucrose tested in this study, 4.0% sucrose induced the maximum numbers of flowers. In some cases abnormal red inflorescences devoid of reproductive organs were obtained which failed to develop into matured flowers even after transferred to MS media supplemented with other hormones.

CONCLUSIONS

In vitro flowering is possible in *Oxalis triangularis*, *Celosia cristata* and *Begonia x hiemalis*. The best explant source for *in vitro* flowering in Begonia was infloresence. For *Oxalis triangularis*, leaves was the best source of explant. Addition of sucrose and adenine could influence *in vitro* flowering in Begonia but not in the other species. Higher concentration of sucrose (5.0%) caused the flowers to abscise while lower concentration of sucrose (1.0-2.0%) reduced the number of flowers.

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Table 1: Shoot and root organogenesis from different explants of *Begonia* x *hiemalis* Fotsch. cv. *Schwabenland Red* cultured on MS medium supplemented with NAA and BAP. The cultures were maintained at 25 ± 1 °C with 16 hours light and 8 hours dark.

MS + hormone (mg/l) NAA	BAP	Explant	Observations	*Explants with shoots (%) (Mean ± SE)	*Explants with roots (%) (Mean ± SE)
0.1	0.1	Leaf	Normal shoots	$15.33 \pm 1.33_{h}$	$5.00 \pm 0.00_{f}$
		Peduncle	Multiple micro shoots	$11.83 \pm 3.06_{h}$	$5.00 \pm 0.00_{e}$
		Petiole	Normal shoots	$33.33 \pm 4.54_{de}$	$6.67 \pm 0.63_{d}$
		Stem	Multiple micro shoots	$27.67 \pm 3.48_{d}$	$6.00 \pm 0.53_{g}$
	0.5	Leaf	Multiple micro shoots	$29.33 \pm 4.13_{e}$	0
		Peduncle	Multiple micro shoots	$85.33 \pm 1.92_{a}$	$0.33 \pm 0.00_{g}$
		Petiole	Multiple micro shoots	$65.33 \pm 4.52_{a}$	$0.22 \pm 0.00_{f}$
		Stem	Multiple micro shoots	$65.00 \pm 2.39_{a}$	$0.87 \pm 0.48_{h}$
	1.0	Leaf	Multiple micro shoots	$40.00 \pm 4.14_{d}$	$3.67 \pm 0.50_{g}$
		Peduncle	Multiple micro shoots	$38.67 \pm 4.87_{de}$	$1.07 \pm 0.53_{ef}$
		Petiole	Multiple micro shoots	$68.67 \pm 1.33_{a}$	$4.40 \pm 0.81_{d}$
		Stem	Multiple micro shoots	$23.33 \pm 3.33_{de}$	$0.67 \pm 0.45_{h}$
	1.5	Leaf	Multiple micro shoots	$26.00 \pm 3.20_{ef}$	No response
		Peduncle	Multiple micro shoots	$24.00 \pm 2.35_{f}$	No response
		Petiole	Multiple micro shoots	$22.67 \pm 2.06_{f}$	No response
		Stem	Multiple micro shoots	$22.67 \pm 2.06_{e}$	No response
	2.0	Leaf	Multiple micro shoots	No response	No response
		Peduncle	Multiple micro shoots	No response	No response
		Petiole	Multiple micro shoots	No response	No response
		Stem	Multiple micro shoots	No response	No response
0.5	0.1	Leaf	Multiple micro shoots	$25.00 \pm 3.78_{ef}$	$12.33 \pm 2.12_{d}$
		Peduncle	Multiple micro shoots	$18.33 \pm 2.11_{fg}$	$7.00 \pm 0.65_{e}$
		Petiole	Multiple micro shoots	$30.00 \pm 2.67_{e}$	$9.00 \pm 1.77_{e}$
		Stem	Multiple micro shoots	$26.33 \pm 2.82_{de}$	$20.67 \pm 2.84_{d}$
	0.5	Leaf	Multiple micro shoots	$60.33 \pm 4.03_{ab}$	$5.00 \pm 0.00_{f}$
		Peduncle	Multiple micro shoots	$60.00 \pm 3.27_{bc}$	$5.00 \pm 0.00_{e}$
		Petiole	Multiple micro shoots	$25.00 \pm 0.00_{e}$	$5.00 \pm 0.00_{d}$
		Stem	Multiple micro shoots	$50.00 \pm 0.00_{b}$	$5.00 \pm 0.00_{g}$

Table 2.1: Continued

MS + hormone (mg/l) NAA	BAP	Explant	Observations	*Explants with shoots (%) (Mean ± SE)	*Explants with roots (%) (Mean ± SE)
		Leaf	Multiple micro shoots	$55.00 \pm 5.56_{c}$	$5.00 \pm 0.00_{f}$
0.5	1.0	Peduncle	Multiple micro shoots	$45.00 \pm 3.27_{c}$	$5.00 \pm 0.00_{e}$
		Petiole	Multiple micro shoots	$43.33 \pm 2.95_{c}$	$5.00 \pm 0.00_{d}$
		Stem	Multiple micro shoots	$31.67 \pm 5.27_{d}$	$5.00 \pm 0.00_{g}$
		Leaf	Multiple micro shoots	$34.33 \pm 3.12_{d}$	0
	1.5	Peduncle	Multiple micro shoots	$40.00 \pm 3.27_{de}$	$3.40 \pm 0.53_{g}$
		Petiole	Multiple micro shoots	$32.33 \pm 5.36_{d}$	$0.67 \pm 0.67_{e}$
		Stem	Multiple micro shoots	$36.33 \pm 3.73_{d}$	0
		Leaf	Multiple micro shoots	$60.33 \pm 5.3_{ab}$	$2.00 \pm 0.82_{g}$
	2.0	Peduncle	Multiple micro shoots	$60.33 \pm 5.33_{bc}$	$3.67 \pm 1.33_{ef}$
		Petiole	Multiple micro shoots	$30.33 \pm 5.27_{d}$	$1.67 \pm 0.79_{e}$
		Stem	Multiple micro shoots	$42.33 \pm 5.43_{c}$	$2.40 \pm 0.82_{h}$
		Leaf	Normal shoots/Rooting	$6.00 \pm 0.53_{i}$	$17.00 \pm 2.00_{c}$
1.0	0.1	Peduncle	Normal shoots/Rooting	$6.33 \pm 0.60_{h}$	$12.33 \pm 2.12_{d}$
		Petiole	Normal shoots/Rooting	$10.33 \pm 1.14_{f}$	$14.00 \pm 2.14_{c}$
		Stem	Normal shoots/Rooting	$9.00 \pm 1.00_{g}$	$29.00 \pm 4.34_{c}$
		Leaf	Normal shoots/Rooting	$51.67 \pm 6.67_{c}$	$12.00 \pm 2.17_{d}$
	0.5	Peduncle	Normal shoots/Rooting	$58.33 \pm 5.27_{bc}$	$7.33 \pm 0.67_{e}$
		Petiole	Normal shoots/Rooting	$25.00 \pm 0.00_{de}$	$11.33 \pm 2.26_{c}$
		Stem	Normal shoots/Rooting	$47.33 \pm 1.18_{b}$	$6.00 \pm 1.56_{g}$
		Leaf	Normal shoots/Rooting	$68.67 \pm 4.77_{a}$	$18.67 \pm 4.10_{c}$
	1.0	Peduncle	Normal shoots/Rooting	$66.00 \pm 5.23_{c}$	$15.00 \pm 1.89_{d}$
		Petiole	Normal shoots/Rooting	$51.00 \pm 2.40_{b}$	$6.67 \pm 1.26_{d}$
		Stem	Normal shoots/Rooting	$51.67 \pm 4.80_{b}$	$22.00 \pm 3.96_{d}$
		Leaf	Multiple micro shoots	$47.00 \pm 3.93_{c}$	$38.00 \pm 4.02_{a}$
	2.0	Peduncle	Multiple micro shoots	$47.00 \pm 3.90_{c}$	$7.67 \pm 1.37_{e}$
		Petiole	Multiple micro shoots	$41.67 \pm 3.98_{c}$	$7.00 \pm 0.65_{d}$
		Stem	Multiple micro shoots	$53.67 \pm 2.41_{b}$	$29.67 \pm 4.15_{c}$

* Mean values within a column followed by the same letters are not significantly difference at the 0.05 level according to LSD test between the same explant.

Table 2: The effect of different sucrose concentration on the *in vitro* flowering induction

Sucrose concentration % (w/v)	Observations		Explants with green shoots (vegetative) (%)a (Mean ± SE)		Explants with green shoots (reproductive)(%)b (Mean ± SE)	
	Inflorescence	Peduncle	Inflorescence	Peduncle	Inflorescence	Peduncle
0	Browning	Browning	n.r	n.r	n.r	n.r
1	Vegetative green shoots and roots	Vegetative green shoots and roots	11.50±6.09	26.00±5.03	n.r	n.r
2	Vegetative green shoots and roots	Vegetative green shoots and roots	31.00±6.94*	30.50±8.87*	n.r	n.r
3	Vegetative green, red shoots and roots	Vegetative green, red shoots and roots	40.05±9.98*	48.00±5.72*	8.50±2.35*	7.75±2.55*
4	Vegetative brownish shoots and roots	Vegetative brownish shoots and roots	36.50±11.25*	37.00±3.18*	5.55±2.22*	11.00±6.41*
5	Vegetative reddish shoots and roots	Vegetative brownish shoots and roots	31.75±8.15*	45.00±8.43*	5.55±2.22*	13.75±5.59*

*mean within a column are significantly different at the 0.05 level according to LSD test

Table 3:The effect of different adenine concentration on the *in vitro* flowering induction

ADENINE % (w/v)	Observations		Explants with green shoots (vegetative) (%)a (Mean ± SE)		Explants with green shoots(reproductive)(%)b (Mean ±SE)	
	Inflorescence	Peduncle	Inflorescence	Peduncle	Inflorescence	Peduncle
2	Multiple green shoots, flower buds and roots	Multiple green shoots, flower buds and roots	42.00±6.16	40.00±10.26	2.65±1.42	6.70±2.00
4	Multiple green shoots, flower buds and roots	Multiple green shoots, flower buds and roots	38.50±1.09	24.00±9.40	3.60±0.82	n.r
6	Multiple green shoots, flower buds and roots	Multiple shoots and roots	15.00±5.13*	8.40±3.79*	2.05±1.35	n.r
8	Multiple brownish shoots and roots	Multiple brownish shoots and roots	10.25±3.79*	5.00±0.00*	n.r	n.r
10	Multiple reddish shoots and roots	Multiple brownish shoots and roots	5.00±0.00*	n.r	n.r	n.r

*mean within a column are significantly different at the 0.05 level according to LSD test

Figures

Fig 1: *Oxalis triangularis* i) and ii) flower bud formation, iii) *in vitro* flowering in subculture media MS without hormone from explant cultured on MS with 0.5mg/1NAA and 2.0mg/1BAP after 12 weeks

Fig 2:*In vitro* flowering of *Celosia cristata* i) red in colour ii) yellow in colour in subculture media MS without hormone after 5 weeks incubation. iii) *in vitro* flowering of *Begonia x hiemalis* Fotsch