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Differential Effects of Sucrose and Plant Growth Regulator on Shoot Multiplication and Bulbil Formation in *Oxalis Versicolour* In Vitro

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ABSTRACT

Explants from young leaves and stem nodes of *Oxalis versicolour* were used and cultured on MS medium supplemented with different concentration of 2,4-D. The best result showed that cluster of callus were formed and proliferated around the base of explants on MS medium supplemented with 0.1 mg/l 2,4-D. Callus transferred to MS medium supplemented with various concentrations of NAA and BA. After nine weeks, callus regenerated to be new shoots. The highest average length of stolon was from MS medium supplemented with 0.1mg/l NAA and 0.1 mg/l BA and number of plantlets was from MS medium supplemented with 4.0 mg/l NAA and 5.0 mg/l BA. Plantlets were cultured on MS medium supplemented with different concentrations of sucrose for ten weeks. It was found that all parameters: number of plantlets, bulbil sized, length of stolon, and number of nodes were significant difference ($p \leq 0.05$). Number of flowers and sized of flowers found only in MS medium supplemented with 9–10 % of sucrose.

1. Introduction

Bicolored *Oxalis Versicolor* (Candy Cane Sorrel) is a unique bulb with really spectacular

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flowers. Oxalis is belonging to Oxalidaceae. Their origin is in Americas and South Africa. The toxic principle is soluble oxalate. This oxalis is very beautiful in full bloom. They are even more stunning when they have not quite opened up completely and display a striking red and white striped pattern. They can be planted in the garden or in a planter on the patio or window-box. The foliage is three very narrow petals on each leaf and is not much like the clover we are used to seeing. The bulbs are very tiny, about the size of a fingernail. The thin plants will spring up in no time at all and are to produce many dainty 1/2" flowers and watering once the soil has become dry, however, do not soak.

This plant propagated by growing bulb. Plant tissue culture is one choice that can micropropagated and increase about 10000 plants within 2 months. Direct micropropagation system through enhanced young leaves or axillary bud development and organogenesis has been reported for different species of gladiolus (Jala, 2013). Various results have also been reported for the role of cytokinins in plant regeneration from callus initiated from different organs of Gladiolus such as young leaves and cormel slices (Kamo 1994). Since the callus initiation and regeneration depend variously on cultivated varieties, explants and growth regulators used in culture media (Kamo 1994, 1995), the present investigation was undertaken to determine the proper concentrations of growth regulators for callus initiation and regeneration of a locally cultivated, taking young leaves as explant.

2. Materials and Methods

2.1 Culture Establishment and Growth

Oxalis plants were grown in green house. Young leaf and stem nodes were used as explants. Explants were surface sterilized by soaking with 70% alcohol for 10 sec, followed by 10%(v/v) Clorox (NaOCl) containing 15 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 in both sides and cultured on MS (Murashige and Skoog, 1962). After 2 weeks, cleaned cultures were transferred to MS medium supplemented with different combination of 2,4-D 2% sucrose, 0.25% gelrite at pH 5.7 and autoclaving at 121^{o} C for 20 min. 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 µmolm^{-2} sec^{-1} (TLD 36 w/853350 lm Phillips, Thailand). These cultures were maintained in a

proliferating state by subculturing every 3 weeks into the same medium 4 times.

2.2 Multiplication

Callus were cultured on MS medium supplemented with combination of (0.1, 1, 2, 3, 4 mg/l) NAA and (0.1, 1, 2, 3, 4, 5 mg/l) BA and various concentrations of (2,3,4,5,6,7,8,9,and 10 %) sucrose. These treatments were subcultured every 3 weeks for 4 times. Shoot proliferation, bulb formation and their growth are displayed in Table 2 and 3.

3. Statistical Analysis

Experiments were set up in Completely Randomized Design (CRD). Each treatment consisted of 20 replicates for the first second and third experiment. The test of statistical significance was done by applying DMRT at 1% and 5% confidence level using SAS statistical software.

Table 1: Effect of 2,4-D on sized and color of callus after cultured for 8 weeks.

2,4-D conc. (mg/l)	Sized of callus	Color of callus
0.1	4.60 ± 0.45a	Light green
1	1.64 ± 0.39b	Light green
2	1.29 ± 0.32b	Light green
3	1.76 ± 0.38b	cream
5	1.68 ± 0.23b	Cream
F-test	**	-
% C.V.	16.68	-

** Mean within the same column followed by the same alphabet were not significant difference using DMRT, $p \leq 0.05$.

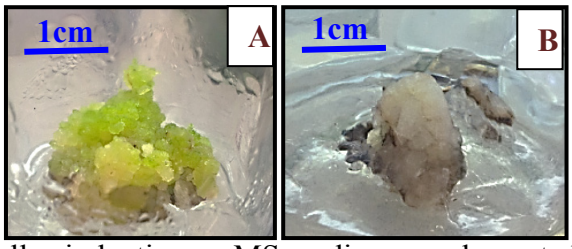

Figure 1: Callus induction on MS medium supplemented with 2,4–D
A - 0.1 mg/l, B - 5.0 mg/l.

4. Results

After 2 weeks, explants from the disinfestation process resulted in a survival rate about 75 %, These explants were cultured on MS medium supplemented with different concentration of 2,4-D. After cultured for 4 weeks, callus were formed and proliferated around the base of explants which attached to the medium. The sized of callus were

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significant differences ($p \leq 0.05$). Cluster of callus were formed with different sized and depended on concentration of 2,4-D, as showed on Table 1 and Figure 1.

4.1 Result of Multiplication

Callus transferred to MS medium supplemented with various concentrations of NAA and BA. After cultured for 4 weeks, it was found that callus regenerated to be new shoots. Average length of stolon and number of plantlets were depended on concentration of NAA and BA in the MS medium and were highly significant difference ($p \leq 0.01$) (Table 2). The result showed that MS medium supplemented with 0.4 mg/l NAA and 0.5 mg/l BA gave the highest number of plantlets (71.6 plantlets). MS medium supplements with 0.1 mg/l NAA and 0.1 mg/l BA gave the longest length of stolon (11.10cm).

Table 2: Effect of NAA and BA concentration on inducing length of stolon and number of plantlets after cultured for 9 weeks.

Plant growth regulator		length of stolon (cm)	Number of plantlets
NAA (mg/l)	BA(mg/l)		
0	0	13.30a ± 4.62	1.10a ± 0.31
0.1	0.1	11.10a ± 2.80	4.10b ± 2.55
0.5		7.40b ± 2.73	3.00b ± 2.10
	0.5	4.60c ± 1.12	18.20b ± 4.90
1.0		5.10c ± 1.77	20.40c ± 10.30
	1.0	4.15c ± 0.70	26.40c ± 13.80
2.0		1.49d ± 0.26	24.50c ± 12.34
	2.0	0.47d ± 0.13	46.20c ± 8.05
4.0		0.61d ± 0.08	44.50d ± 7.61
	4.0	0.50d ± 0.11	49.20d ± 9.64
	5.0	0.46d ± 0.11	71.60d ± 5.12
F-test		**	**
% C.V.		43.57	28.91

**Mean within the same column followed by the same alphabet were not significant difference using DMRT, $p \leq 0.01$.

Plantlets were cultured on MS medium supplemented with different concentrations of sucrose for 10 weeks. It was found that all parameters: number of plantlets, bulbil sized, length of stolon, number of nodes, number of flowers and sized of flowers were significant difference ($p \leq 0.05$) (Table 3). Two and Three percentage of Sucrose gave the best result that gave the highest number of plantlets (10.5 – 10.75 plantlets, respectively) (Figure 5), number of nodes (10.75 – 10.50 nodes per, respectively) and length of stolon (29.25-28.5 mm, respectively). The biggest sized of bulbil were formed at 9–10 percentage of sucrose (18.65–19.05 mm, respectively) (Figure 4). At the ninth week, plant were flowering in the MS medium only contained 9-10 percentage of sucrose. Ten percentage of sucrose gave the highest number of flowers and the biggest sized of flower, also (Figure 3).

Table 3: Effect of sucrose concentration on number of plantlets, bulbil size, length of stolon , number of nodes, number of flower, and size of flowers after culture for 10 weeks.

Sucrose (%)	No. of plantlets	Size of Bulbil (mm)	Length of stolon(mm)	Number of nodes	Number of flowers	Size of flower (cm)
2	10.75a ± 1.50	9.72e ± 0.92	29.25a ± 0.96	10.75a ± 0.96	-	-
3	10.50ab ± 1.73	9.92e ± 0.82	28.50a ± 0.58	10.50a ± 0.58	-	-
4	5.50d ± 0.58	11.22de ± 1.21	26.50a ± 1.29	8.25b ± 0.96	-	-
5	9.50abc ± 4.20	13.05cd ± 0.31	19.25b ± 0.96	7.75b ± 1.89	-	-
6	6.50cd ± 1.29	16.07b ± 0.70	19.50b ± 1.91	8.00b ± 0.81	-	-
7	7.75bcd ± 1.26	14.80bc ± 1.98	19.25b ± 2.22	7.50b ± 0.58	-	-
8	8.75bcd ± 0.65	15.32bc ± 0.52	20.25b ± 1.70	8.25b ± 0.50	-	-
9	9.25abcd ±0.96	18.65a ± 0.47	18.50b ± 1.00	8.00b ± 0.82	0.50b ± 0.57	2.80b ± 3.23
10	12.75a ± 0.65	19.05a ± 1.97	13.25b ± 2.36	7.75b ± 1.25	4.75a ± 0.95	6.07a ± 1.00
F-test	*	*	*	*	*	*
% C.V.	19.96	8.09	7.23	11.88	30.10	60.29

Figure 3: Characteristic of *Oxalis versicolour* flower (the end of arrow) which cultured in MS medium supplemented with 10 % sucrose.

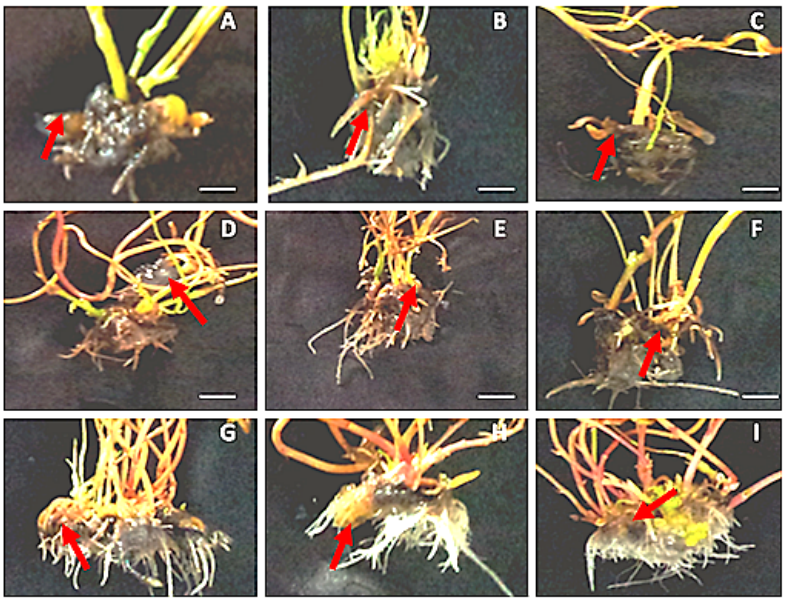

Figure 4: Characteristics of *Oxalis versicolour* bulbil cultured in MS medium supplemented with various concentrations of sucrose. A 2%sucrose, B. 3% sucrose, C.4%sucrose, D.5% sucrose , E. 6%sucrose, F.7%sucrose, G. 8%sucrose, H.9% sucrose, I.10% sucrose. (bar = 1cm)

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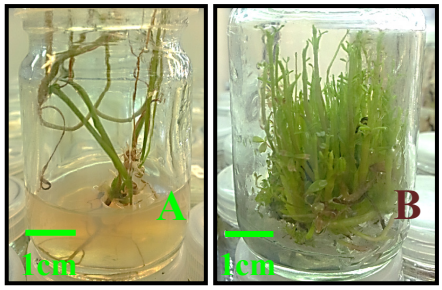

Figure 5: Various Characteristics of *Oxalis versicolour* after cultured on MS medium with various concentration of sucrose. A - plantlets which cultured in MS medium with free sucrose, B- Plantlet in MS medium with 10% sucrose.

5. Discussion

Since the callus initiation and regeneration was depend variously on cultivated varieties, explants and growth regulators used in culture media (Kamo 1994, 1995). The result showed that callus transferred to MS medium supplemented with various concentrations of NAA and BA formed different characters. After cultured for 4 weeks, callus regeneratedto be new shoots and the highest number of plantlets was in MS medium and supplemented with 0.4 mg/l NAA and 0.5mg/l BA, the longest Average length of stolon and number of plantlets were from MS medium supplemented with 0.1 mg/l NAA and 0.1 mg/l BA. This result was the same as Jala and Wassamon (2012) did in *Gynostemma pentaphyllum*, Gladiolus (Jala, 2013), and Globba sp. (Jala et al.,2013).

The result in this experiment showed that when cultured explants of *Oxalis versicolour* on MS medium supplemented with different concentration of (2, 3, 4, 5, 6, 7, 8, 9, 10 %) sucrose, the result showed that all parameters were significant difference ($p \leq 0.05$). The stimulation on bulbil sized, stolon length, number of node as Murashige and Skoog(1962) described their medium. Sucrose is the most common carbon source as well as anosmotic agent for plant tissue and organ culture. Sucrose also supports the maintenance of osmotic potential and the conservation of water in cells. However, high sucrose concentration in the media restricts the photosynthetic efficiency of cultured plants by reducing the levels of chlorophyll, key enzymes for photosynthesis and epicuticular waxes promoting the formation of structurally and physiologically abnormal stomata (Hazarika, 2006). On the other hand, earlier studies have shown that plantlets growing under tissue culture conditions do not fix enough CO_2 to sustain growth in the absence of sucrose, which is mainly due to limited CO_2 inside the vessel (Gautheret, 1955). Media with 3% sucrose have been the staple since Murashige and Skoog (1962) described their MS medium. Indeed, sucrose concentrations above 2.5 % repress proliferation of callus of various plants (Malamug et al., 1991). Low concentrations of sucrose favor the initiation of numerous shoots in tobacco callus and

depress the growth of callus (Barg et al., 1977). The effect of high concentration of sucrose are especially when conditions in vitro are in adequate for significant photosynthesis (Muller et al., 2011). The result showed that plantlets in the bottle were flowering by increasing concentrations of sucrose (Table 3). This result was the same as Aloni et al., (1996,1997) mentioned that light perceived by the plant increased the availability of sucrose to the flowers due to increase photosynthesis and translocation. Sucrose had taken up but the sink organ is metabolized, and Zrenner et al.,(1995) studied and indicated that sucrose synthase is a regulatory enzyme that controls sucrose cleavage and starch biosynthesis in sink tissue.

6. Conclusion

Young leaves and stem nodes of *Oxalis versicolour* were used as explants and cultured on MS medium supplemented with different concentration of 2,4-D. It was found that callus were proliferated at the base of explants and 0.1 mg/l 2,4-D gave the biggest sized of callus(4.6 mm) and their color was light green. Callus transferred to MS medium supplemented with various concentrations of NAA and BA. Callus regenerated to be new shoots and maximum shoots was formed in MS medium supplemented with 4.0 mg/l NAA and 5.0 mg/l BA. Plantlets were cultured on MS medium supplemented with different concentrations of sucrose (2, 3, 4, 5, 6, 7, 8, 9, 10 %) for 10 weeks. It was found that 2and 3% sucrose gave the best result in number of plantlets, bulbil sized, length of stolon, number of nodes. Only nine and ten percentages of sucrose gave flowers. Ten percentages of sucrose gave the highest number of flowers and the biggest sized of flowers.

7. References

Aloni B, L. Karni, Z. aidman, A.A. Schaffer. 1996. Changes of carbohydrates in pepper (*Capsicum annuum L.*) flowers in relation to their abscission under different shading regimes. Annals of Botany 78: 163-168.

Aloni, B., Z. Aloni, L. KarniI, Z., and A. A. Schaffer. 1997. Relationship between Sucrose Supply, Sucrose-cleaving Enzymes and Flower Abortion in Pepper. Annals of Botany 79: 601 – 605.

Altman, A., R. Goren. 1971. Promotion of callus formation by abscisic acid in citrus bud cultures. Plant Physiol 47: 844-846.

Arigita L, M.J. Cañal, T.R. Sánchez, A. González . 2010. CO_2-enriched microenvironment affects sucrose and macronutrients absorption and promotes autotrophy in the in vitro culture of kiwi (*Actinidia deliciosa* Chev. Liang and Ferguson). In Vitro Cell Dev-Pl 46: 312–322.

Barg, R. and N. Umiel. 1977. Effects of sugar concentrations on growth, greening and shoot formation in callus cultures from four genetic lines of tobacco. Z Pflanzenphysiol 81: 161-166.

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Dragan, V. and B.S. Vinterhalte. 1999. Hormone-Like Effects of Sucrose in Plant in vitro Cultures. Phyton (Austria) Special issue: Plant Physiology".v39: 57-60.

Hazarika BN (2006) Morpho-physiological disorders in *in vitro* culture of plants. Sci Hort 108: 105–120.

Jala, A. and Wassamon Patchpoonporn. 2012. Effect of BA and NAA and 2,4-D on Micropropagation of Jiaogulan(Gynostemma pentaphyllum Makino). International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies. 3(4):363-370.

Jala, A. 2013 The Effect of the 2,4-Dichlorophenoxy Acetic Acid, Benzyl Adenine and Paclobutrazol, on Vegetative Tissue-Derived Somatic Embryogenenesis in Turmeric (Curcuma var. Chatti). International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies. 4(2):105-111.

Jala, A., Nattapong Chanchula and Thunya Taychasinpitak. 2013. Multiplication New Shoots from Embryo Culture on Globba spp.). International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies. 4(3):207-214.

Kamo K. 1994. Effect of phytohormones on plant regeneration from callus of G*ladiolus* cultivar "Jenny Lee". In Vitro Cell. Dev. Biol. 30P : 26 - 31.

Kamo K. 1995. A cultivar comparison of plant regeneration from suspension cells, callus, and cormel slices of *Gladiolus.* In vitro Cell. Dev. Biol. 31P : 113 - 115.

Malamug J.J.F., H. Inden and T. Asahira. 1991. Plantlet regeneration and propagation from ginger callus. Scientia Hort. 48: 89–97.

Muller B, Pantin F, Génard M, Turc O, Freixes S, et al. (2011) Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. J . Bot. 62: 1715–1729.

Murashige, T . and F.A. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15 : 473 - 492..

Gautheret R.J.1955. The nutrition of plant tissue cultures. Annu Rev Plant Phys. 6: 433–484.

Zrenner R, Salanoubat M, Willmitzer L, Sonnewald U. 1995. Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants. *Plant Journal* 7: 97-108.

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