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IN VITRO SHOOT ORGANOGENESIS OF SNAKE PLANT (*Sansevieria trifasciata* L.) AS INFLUENCED BY EXPLANT ORIENTATION AND SUCROSE CONCENTRATION

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ABSTRACT

This experiment was carried out to study the effect of orientations of explants and concentrations of sucrose on shoot organogenesis of snake plant (*Sansevieria trifasciata* L.) under *in vitro* conditions. Explants were excised from healthy juvenile leaves and sterilized. Subsequently they were kept horizontal and vertical positions in MS medium containing 3% and 5% of sucrose concentrations. The results showed that sucrose at 3% was the most suitable concentration to produce the high number of shoots and sucrose at 5% was the most responsive concentration for the shoot elongation in the cultured leaf explants of snake plant. Further, the result exhibited that the most suitable explant orientation for the shoot induction and proliferation was horizontal placement of leaf explants.

Keywords: *In vitro* shoots**,** Leaf explants, Orientation**,** Sucrose, Snake plant

INTRODUCTION

Snake plant (*Sansevieria trifasciata* L.) is a perennial herb in tropical and subtropical parts of the world. *Sansevieria* species are among major foliage ornamentals because of the variegated and mottled leaves (Bos, 1998). The conventional propagation by leaf cuttings and division is mostly practiced to propagate *Sansevieria* plants however these plants are insufficient to meet the commercial demand (Hartman et al., 2002). Thus, *in vitro* technique can be used for mass propagation of snake plant for commercial scale plantings. Sarmast et al. (2009) reported that more than 100 *in vitro* plantlets can be obtained from 1 cm leaf segments. This technique is employed for rapid multiplication of disease-free clones and genetically improved plants (Bhojwani and Razdan, 1992).

Success of *in vitro* plant propagation mainly depends on explants and culture medium. Anis and Shahzad (2005) reported that the frequency of shoot regeneration was high in MS (Murashige and Skoog, 1962) medium containing 10 µM BAP (6-benzyl amino purine) and 0.1 µM NAA (Naphthalene acetic acid). Lavakumaran and Seran (2014) mentioned that number of microshoots per *in vitro* explant of aloe was high in MS medium containing 3 mg/l BAP and 0.5 mg/l NAA. BAP at 1.0 - 3.0 mg/l was the best concentration to form the shoots in *Aloe* micropropagation (Chukwujekue et al., 2003). Combination of BAP and NAA is generally better for plant regeneration through not only organogenesis but also somatic embryogenesis. This was supported by several researchers. Rapid and very efficient plant micropropagation can be obtained from vegetative meristems (Natali et al., 1990). Cytokinin and auxin concentrations play an important role in the frequency of shoot induction and proliferation under *in vitro* conditions. In addition to plant growth regulators, sucrose concentration in culture medium has effect on microshoot formation. Therefore, this

experiment was conducted to determine the suitable concentration of sucrose for the shoot formation and also to study the effect of orientation of leaf explants on shoot morphogenesis of snake plant.

MATERIALS AND METHOD

This study was done at the Tissue Culture Laboratory, Eastern University, Sri Lanka. Healthy juvenile leaves of snake plants were collected from the University Premises and used in this experiment. Middle portion of leaves were separated from each leaf.

Sterilization of explants

The collected leaf explants were washed thoroughly under running tap water for 30 min. and then dipped in 70% ethanol for 30 sec. After that, the leaf explants were disinfected by using 20% of sodium hypochlorite (5.25% active ingredients) with two drops of tween 20 for 10 min. Subsequently they were washed three times with sterile distilled water. After the sterilization of explants, they were taken inside the laminar hood to inoculate them on the culture medium for shoot induction.

Inoculation of explants

The basal medium used for the culture is MS medium (Murashige and Skoog, 1962) solidified with 0.8% w/v agar (Bacteriological grade, Himedia, India). Sucrose at 3% or 5% concentration (Analytical grade, Himedia, India) and plant growth hormones such as 2 mg/l BAP and 0.5 mg/l NAA were added to the culture medium. The pH of the medium was 5.8 and it was poured into the culture vessels. They were then autoclaved at 121^0C for 20 min at 15 psi and kept for four days before being inoculation of explants. Subsequently, leaf segments (1x1 cm^2) were excised from the sterilized explants and placed on the culture media in vertical and horizontal orientations. After inoculating the explants, the mouth of the vessels was tightly capped and sealed with parafilm. This experiment was repeated thrice.

Culture environment

The sterilized explants were inoculated on the MS media supplemented with two different concentrations (3% and 5%) of sucrose and growth hormones (2 mg/l BAP and 0.5 mg/l NAA). The culture vessels containing explants were then incubated at 25 ± 0.5^0C, under fluorescent tube light with 16 hours photoperiod. The light intensity of 2000-2500 lux and 70% relative humidity was maintained.

Shoot formation

After 8 weeks of culture, the explants from initial culture media were transferred into fresh MS medium containing sucrose and growth hormones as the same as their initial medium for shoot proliferation and further study. The cultures were observed regularly for any *in vitro* responses. After 16 weeks of culture, the elongated shoots were transferred into hormone free MS medium for the *in vitro* regeneration of plantlets.

Statistical analysis

Numbers of newly emerged shoots per explants and shoot length were recorded. The data obtained were analyzed by using SAS software. The mean comparisons between treatments were done by using LSD (Least Significant Difference) test at 5 % significant level.

RESULTS AND DISCUSSION

The objectives of the study were to perform the most suitable explant orientation and sucrose concentration for the establishment of *Sansevieria trifasciata* (L.) in MS (Murashige and Skoog, 1962) medium containing 2 mg/l BAP and 0.5 mg/l NAA under *in vitro* conditions for vegetative propagation. For this purpose, the excised explants were placed in vertical and

horizontal positions in MS medium containing two different sucrose concentrations (3% and 5%).

Survival of explants

Higher survival percentage of the cultures was observed in this experiment and contamination was less than 5%. It may be mainly due to the selection of explants excised from middle portion of the leaves and surface sterilization of explants in 20% sodium hypochlorite. Further, explants were excised into 1×1 cm^2 size where exposure of the explants' surface was low. This may also be one of the reasons for the less contamination. This was supported by Dey and Harborne (1997) and also Sathyagowri and Seran (2011) who stated that the risk of contamination is lesser as the size of explants is smaller.

Phenolic browning was less in *Sansevieria* leaf explants. It may be due to the antioxidant potential (Aliero et al., 2008). Sometimes, the kind of oxidative browning can be avoided by soaking or stirring the explants in antioxidant solutions as a pretreatment before transferring them on the media (Panaia et al., 2000; Wu and Toit, 2004). In this study, ascorbic acid was incorporated into culture media. As a result of the antioxidant property of ascorbic acid, the phenolic browning was at low level.

***In vitro* responses**

The cultured leaf explants showed no *in vitro* response during the first three weeks. Then callus formation was noted at the cut margins of the explants and subsequently it was observed over the entire leaf segment mainly explants placed in horizontal orientation. It may be due to the cut ends of leaf explants through which the nutrients and growth regulators can be absorbed efficiently from the medium (Reynoired et al., 1993). The result was agreed with Yadav et al. (2011) in woody plants.

In the present study, a few adventitious buds were later developed at the edge of cut surface from vertically placed explants in 5% of sucrose level followed by horizontally placed explants in 5% of sucrose, vertically placed explants in 3% of sucrose and horizontally placed explants in 3% of sucrose (Figure 1). Further, it was observed that the explants (leaf lamina) placed horizontally showed higher shoot bud initiation than the explants placed vertically which had less surface contact with the nutrient medium. It was in agreement with Mujib (2005) who reported that the high percentage of shoot bud formation was observed in the cultured leaf lamina which was kept horizontally on the medium but when leaf sections placed vertically, they did not show any response.

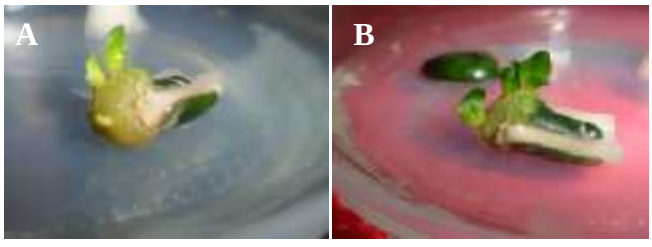

Figure 1: *In vitro* responses of leaf explants after 8 weeks of culture.

A. Horizontally placed explants in 3% of sucrose
B. Horizontally placed explants in 5% of sucrose

The result revealed that the number of leaves was high in the explants placed in 3% of sucrose than that in 5% of sucrose. This result was in agreement with several workers. Dahab et al. (2004) who stated that sucrose at 50 g/l in the medium significantly reduced the number

of leaves in *Ruscushypoglossum* when compared with 30 and 40 g/l sucrose concentrations but the number of leaves was considerably increased with increasing the sucrose concentration in the media from 10 to 30 g/l. Thus, the more number of leaves can be obtained by using MS medium supplemented with 30 gm/l sucrose. The finding was also reported by Pasqual et al. (1994) in *Nephrolepis exaltata*. Sucrose at 2%-3% concentration has been commonly used in plant regeneration under *in vitro* conditions.

Number of developed shoots

The higher number of shoots was formed from the horizontally placed explants in medium mainly containing 3% sucrose than the vertically placed explants (Table 1). This finding was supported by Dahab et al. (2004) stated that the MS (Murashige and Skoog, 1962) medium supplemented with sucrose at 30 g/l or 40 g /l gave higher number of shoots per explant of *Ruscushypoglossum.* L and sucrose at 50 g /l gave lower number of shoots per explants. It has been shown that horizontal placement of explants seems to be better than vertical placement of explants for shoot proliferation (Figure 2). Further, it was noted that the rate of shoot bud initiation from callus derived from leaf explants was favoured by the horizontal placement (Figure 2). Kumar and Reddy (2009) reported the regeneration efficiency and number of shoot buds were higher in horizontally placed explants than vertically placed explants. This has been also noted by Arockiasamy et al. (2002) in *Eryngium foetidum*. This may be due to the surface contact of the explants to the medium for nutrient absorption. In the vertical position, surface contact of the explant is relatively less compared to the horizontal position.

Table 1: Effect of explant orientations and sucrose concentrations on number developed shoots, length of shoots and *in vitro* responses of cultured explants after 16 weeks of culture.

Treatments		Number of developed shoots	shoot length	*In vitro* responses
Explant orientation	Sucrose %			
Vertical placement	3 %	2.00 ± 0.41ab	1.90 ± 0.09c	• Less friable callus mass • Shoots with about three leaves
	5 %	1.75 ± 0.25b	3.30 ± 0.09a	• Less callus mass • Shoots with about two leaves
Horizontal Placement	3%	3.00 ± 0.41a	1.75 ± 0.06c	• Compacted callus mass • Numerous shoot buds • Shoots with about two leaves
	5%	2.75 ± 0.25ab	2.77 ± 0.14b	• Very compacted callus mass • Numerous shoot buds • Shoots with about two leaves
F test		*	**	-

Values represent mean ± standard error of four replicates. F test* - P<0.05, ** - P<0.01.
Means with the same letters are not significantly different according to LSD test at 5% significant level in each column.

Longest shoot length

The longest shoot length was observed in 5% of sucrose where the explants placed vertically. Increasing the level of sucrose from 3% to 5% significantly increased the shoot length (Table 1). Dahab et al. (2004) reported that the longest shoots were obtained when the explants were cultured on MS medium containing 50 g/l sucrose and the shortest shoots were recorded on MS medium supplemented with 10 g/l sucrose. The promoting effect of the high level of sucrose on shoot length was also observed by Kozak (2000) on *Gloriosa rothschildiana* cv. Red Dark.

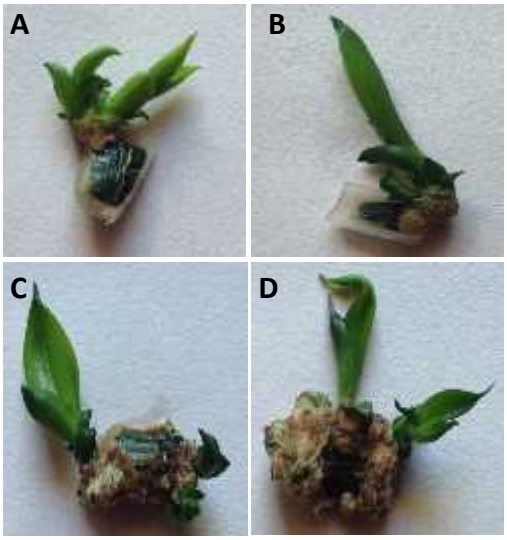

Figure 2: *In vitro* responses of leaf explants after 16 weeks of culture.

A. Vertically placed explants in 3% of sucrose.
B. Vertically placed explants in 5% of sucrose.
C. Horizontally placed explants in 3% of sucrose.
D. Horizontally placed explants in 5% of sucrose.

CONCLUSION

The results revealed that medium containing sucrose at 3% was the most responsive concentration to produce higher number of *in vitro* shoots from the cultured leaf explants of *Sansevieria trifasciata* (L.) among the two different concentrations of (3% and 5%) sucrose. Further, it was noted that sucrose at 5% was the most suitable concentration for the *in vitro* shoot elongation. The result also exhibited that the most appropriate orientation of leaf explants for the shoot proliferation was horizontal position on the culture medium.

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