REGULATING ASYNCHRONY IN *DENDROBIUM* CV. SONIA *IN VITRO* CULTURES

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ABSTRACT

Dendrobium is one of the most popular orchid types cultivated in the floriculture industry. In vitro cultures of Dendrobium cv. Sonia have been successfully initiated and maintained in the plant tissue culture laboratory at Nilai University. However, the cultures grew asynchronously, producing plantlets at different stages of growth. Asynchrony is considered disadvantageous as it hampers seedling maintenance and transplanting. The present study was carried out to evaluate the role of explant type and NAA in regulating synchronous shoot development to obtain rapid production of plantlets for acclimatisation. The findings of this study have significant implications for the field of plant tissue culture and orchid cultivation, as they provide insights into how to minimise asynchrony in vitro cultures, thereby enhancing the efficiency and productivity of orchid propagation. The explants (protocorm-like-bodies or PLBs and shoots) were cultured in ½ MS medium supplemented with banana homogenate and NAA (1.0, 3.9, and 5.0 mg/L). The findings revealed that PLBs would multiply in vitro and produce cultures at different stages of growth. PLBs that were in contact with the culture medium continued to produce PLBs, while PLBs that were not in contact with the culture medium germinated. It appeared that asynchrony would be minimised in vitro culture if the shoots of at least 0.5 cm were segregated and cultured into fresh medium. These shoots would elongate, producing well-developed shoots and roots. Moreover, larger explants developed further, producing rooted shoots rather than PLBs and young shoots. ½ MS medium containing 5.0 mg/L NAA resulted in the highest shoot and root length.

Keywords: *Dendrobium, in vitro* culture, asynchrony, explant segregation

INTRODUCTION

Dendrobium is one of the most popular orchid types cultivated in the floriculture industry. *In vitro* cultures of *Dendrobium* cv. Sonia have been successfully initiated and maintained in the plant tissue culture laboratory at Nilai University. However, the cultures grew asynchronously, producing plantlets at different growth stages. Though asynchrony is generally a function of the plants' biology, physiology, biochemistry and adaptation to various environments, it is considered disadvantageous as it hampers seedling maintenance and transplanting. This nonuniformity of plant growth and development also reduces the researchers' or the commercial facility's ability to obtain consistent results.

With its unique ability to offer precise control over the physical and chemical environment, tissue culture presents opportunities for cell synchronisation in plant tissues. Our research is part of a more considerable effort to enhance the efficiency of *in vitro* propagation for various orchid species, particularly *Dendrobium* cv. Sonia. The present study evaluated the role of explant type and NAA in regulating synchronous shoot development to obtain rapid production of plantlets for acclimatisation.

MATERIALS AND METHODS

Plant Materials

The shoot cultures of *Dendrobium* cv. Sonia, meticulously initiated and maintained in the laboratory, were used as a source of explants for the study. Three types of explants, protocorm-like bodies, 0.5-1.0cm, and 1.5-2.0cm young shoots, were chosen for the study.

Preparation of Culture Media and Culture Conditions

½ MS medium (Murashige & Skoog, 1962) was used in the experiments. It was supplemented with 30g/L sucrose, 2.0g/L activated charcoal, 10% (v/v) banana homogenate, NAA (1-5 mg/L) and 0.7% agar (Sigma Aldrich, USA).

Fresh ripe bananas were bought on the day of media preparation from local stores to prepare the banana homogenate. The skin-peeled bananas were sliced into small pieces and completely crushed using a pestle and mortar before adding to the medium. The pH of the culture medium was adjusted to 5.8 with 0.1N NaOH or HCl before adding agar into the medium. The medium was autoclaved at 121℃ for 15 minutes under 1kg cm^{-2} pressure. Autoclaved medium (50mL) was poured into GA7 containers (Magenta Corp., Chicago, USA).

The explants were cultured in 50mL of the culture medium. Six shoots and 2.0g of PLB explants were cultured per GA7 container. The explants were placed upright in the medium, with the lower half dipped inside the agar. The GA7 containers were sealed with two layers of cling wrap. All cultures were kept under cool, fluorescent light (12h photoperiod) at 25 ± 2ºC under 12/12 (light/dark cycle) in the culture room for 16 weeks.

Assessment of Growth and Statistical Analysis

The present study investigated 3 explant types (PLBs, 0.5-1.0 cm shoots, and 1.5-2.0 cm shoots). Each explant type had 5 cultures and was independently repeated 5 times under the same conditions. The cultures were observed weekly, and growth parameters were gathered after 16 weeks of *in vitro* culture. The parameters recorded included the number and length of shoots per culture and the length of roots.

RESULTS AND DISCUSSION

Effect of Explant Types

The protocorm-like-bodies (PLBs) and young shoots of *Dendrobium cv.* Sonia were cultured in ½ MS medium supplemented with 30g/L sucrose, 10% banana homogenate, different concentrations of NAA (1.0mg/L, 3.0mg/L, and 5.0mg/L) along with 2g/L activated charcoal and 7g/L agar. During the 16 weeks, the 3 types of explants multiplied and developed into a culture composed of a mixture of shoots at different stages of growth (Figure 1). Most explants produced PLBs, shoots without roots, and shoots with roots at varying amounts (Table 1).

Table 1. Percentage of cultures with PLBs and shoots after 16 weeks.

Explant type	Percentage of cultures with PLBs	Percentage of cultures with shoots without roots	Percentage of cultures with rooted shoots
PLBs	100	100	78
0.5-1.0 cm shoots	71	100	100
1.5-2.0 cm shoots	10	70	100

When PLBs were used as the original explant, 100% of the cultures produced PLBs after 16 weeks in culture. In addition, 78% and 100% of the PLB-derived cultures produced rooted shoots and shoots without roots, respectively (Table 1). The cultures that made the least PLBs and shoots without roots were derived from 1.5-2.0 cm shoot explants. The larger explants developed further to produce rooted shoots than PLBs and young shoots. These findings suggest that the size and type of the explant play a significant role in the development of the culture, which can be crucial for enhancing the efficiency and productivity of orchid propagation.

The conversion of PLBs usually occurs 7 – 15 days after culture and can be determined by the first leaf primordium's colour change from light green to dark green. The conversion of PLBs into plantlets takes place at different timings, and there are various stages of growth in a culture. The PLBs in contact with the culture medium tend to divide and continuously produce PLBs, whereas the PLBs away from the culture medium are developed into shoots.

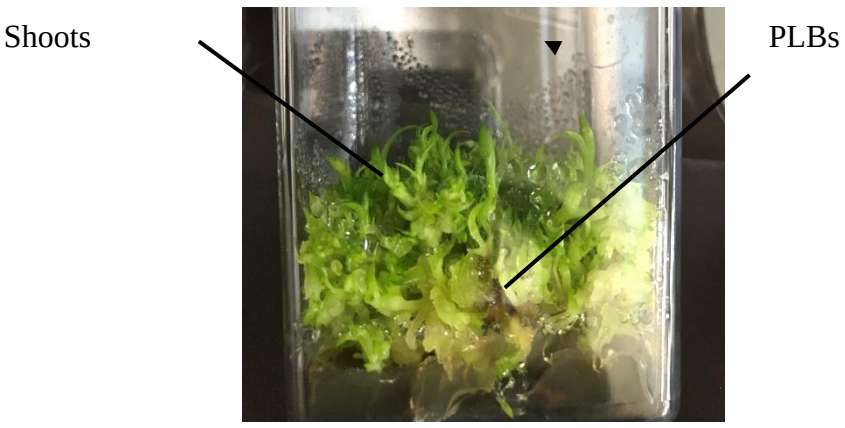

Figure 1. Mixed culture consisting of PLBs and shoots

Synchrony of Growth

Table 2 and Figure 2 show that besides the explant type, the concentration of NAA in the culture medium also affected synchronous growth in *Dendrobium* cultures. The addition of NAA in the culture medium influenced different stages of micropropagation in plant tissue culture, which enhanced micropropagation. Shoot development can be completed without NAA or by adding a higher concentration (5.0 mg/L) of NAA to the culture medium. Culture media supplemented with a low concentration of NAA promoted shoot formation, while a high concentration of NAA initiated roots. When the concentration of NAA is increased to 5 mg/L, the explants grow taller, with longer roots ranging from 0.2 to 3.6 cm.

Table 2. Height of shoots and length of roots of *Dendrobium* cv. Sonia, after 16 weeks in culture.

Explant type	Treatment #	NAA concentration (mg/L)	Mean no. of shoots per culture	Height of shoots (cm) range	Length of roots (cm) range
PLBs	T0	0	51.6	0.5 - 1.8	0.1 - 2.0
	T1	1.0	9.40	0.5 - 1.5	0.4 - 1.0
	T2	3.0	2.80	0.5 -1.7	0.5 - 1.1
	T3	5.0	12.25	0.5 - 1.2	0.1 - 2.4
0.5-1.0 cm shoots	T4	0	14.00	0.5 - 1.5	0.1 - 1.5
	T5	1.0	16.75	0.5 - 2.2	0.2 - 2.4
	T6	3.0	13.20	0.5 - 1.5	0.4 - 1.7
	T7	5.0	17.80	0.5 - 2.0	0.2 - 3.0
	T8	0	21.25	0.5 - 2.4	0.2 - 2.6
1.5-2.0 cm shoots	T9	1.0	15.00	0.5 - 2.3	0.3 - 2.5
	T10	3.0	10.20	0.5 - 2.5	0.4 - 2.6
	T11	5.0	9.20	0.6 – 3.6	0.2 - 3.6

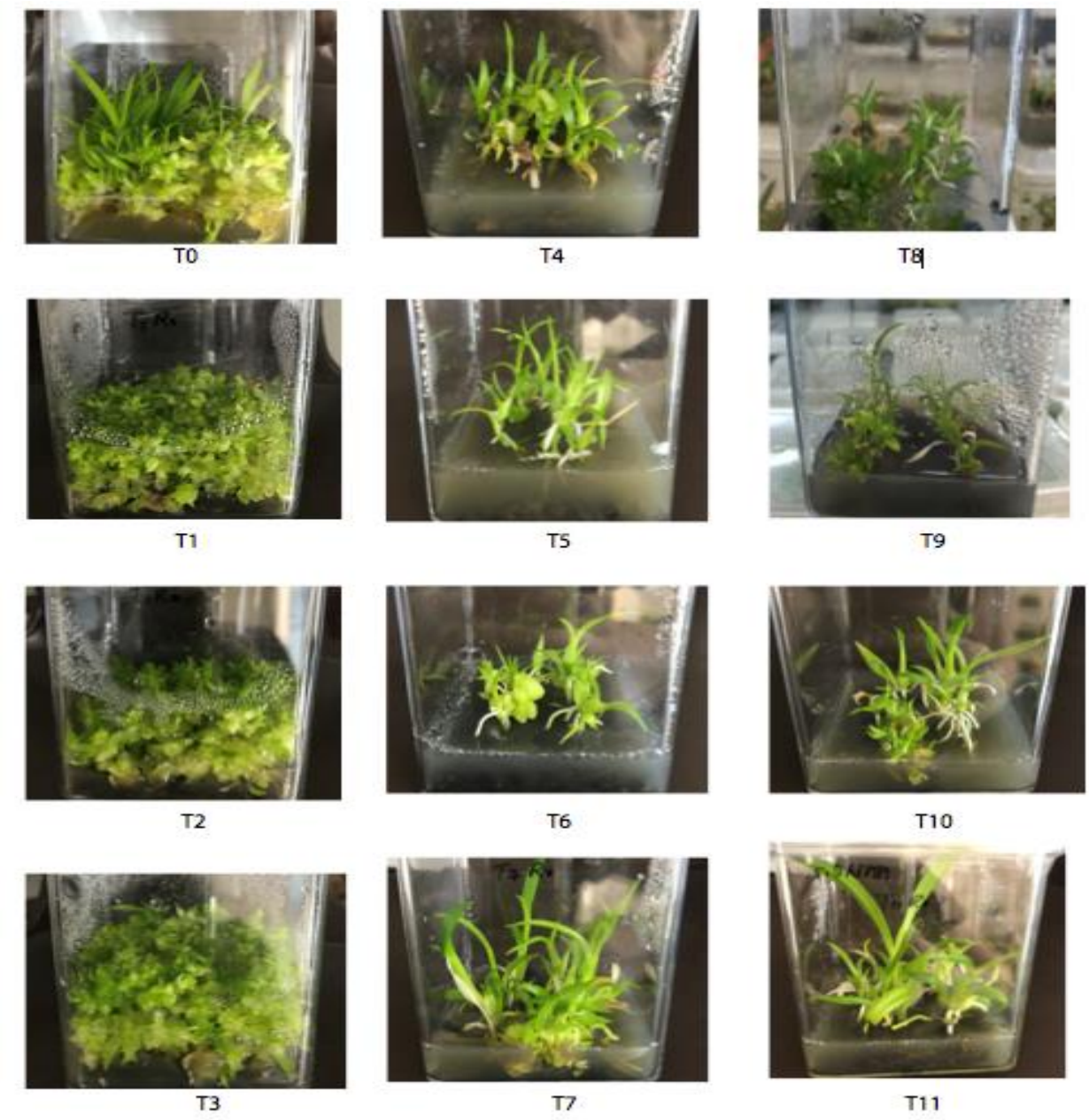

Figure 2: The growth of *Dendrobium* cv. Sonia, after 16 weeks in culture

Because *asynchronous* growth does occur in most *plant tissue culture* systems, synchronising tissue growth has been obtained by controlling the chemical microenvironment and manipulating the cell cycle stage at the beginning of the culture period. According to King (1980), repeated synchronous division may be induced by adding auxin to quiescent cells or starving and regrowing plant cell cultures. Cell division synchrony is associated with discontinuous biosynthetic events in several such cases.

For example, Nishida et al. (1992) established a system of synchronous cell division to suspension cultures of cells of *Catharanthus roseus* L. cv. Little Pinky by auxin starvation, followed by its readdition. When cells in the stationary phase were transferred to a fresh medium free of 2,4-dichlorophenoxyacetic acid (2,4-D), cells were arrested preferentially at the G_1 phase. After 2 days in the 2,4-D free medium, the readdition of 2,4-D induced the synchronous division of cells.

In cotton, friable clumps of cells selectively collected over filter mesh 40 and subjected to one cycle of Myo-inositol starvation have induced highly synchronised embryogenesis in the culture. The protocol gave 100% of embryos at the globular stage, out of which more than 80% developed into bipolar torpedo-stage embryos (Kumar &Tuli, 2004). Similarly, in another cotton cultivar, Jing-Lin Cao et al. (2008) reported combining suspension culture and solid culture (with filter paper) to improve somatic embryogenesis frequency and synchronous development of mass somatic embryos. Sieving embryonic calli a few times before placing them on the solid medium containing 2.46 μmol L^{-1} indole-3-butyric acid (IBA) and 0.70 μmol L^{-1} kinetin resulted in a

more synchronised development of embryos. About 70.2% for globular, 52.3% for torpedo-shaped, and 73.0% for cotyledonary embryos were obtained during the culture.

In our study with *Dendrobium*, asynchrony would be minimised *in vitro* culture if the shoots of at least 0.5 cm were selected, segregated and cultured into a fresh medium containing 5 mg/L of NAA.

CONCLUSION

Explant type and NAA improved synchronous growth of *Dendrobium cv.* Sonia shoots. Culturing PLBs and young shoots on half-strength MS, with or without the addition of NAA, results in the successful formation and proliferation of PLBs and shoot development. Moreover, larger explants developed further, producing rooted shoots rather than PLBs and young shoots. ½ MS medium containing 5.0 mg/L NAA resulted in the highest shoot and root length. Asynchrony would be minimised *in vitro* culture if the shoots of at least 0.5 cm were selected, segregated and cultured into a fresh medium containing 5 mg/L of NAA.

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