EFFECTS OF EXPLANT TYPE ON *IN VITRO* CULTURE OF *DENDROBIUM* CV. SONIA

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ABSTRACT

Tissue culture is considered an important technology for developing countries in the large-scale production of fairly uniform, disease-free, good quality planting materials. Accordingly, it plays a crucial role in the micropropagation of orchid plants that provide income for growers in Southeast Asia. However, in vitro orchid cultures usually develop asynchronously which may impede plantlet production and acclimatisation. Hence, a study was undertaken to evaluate the effects of explant type in the regeneration and development of Dendrobium cv. Sonia plantlets in vitro. Different explant types: protocorn-like-bodies (PLBs) and young shoots (0.5 – 1.0 cm and 1.5-2.0 cm long) were cultured on ½ strength MS medium with 30g/L sucrose, 2g/L activated charcoal and 10% (v/v) banana homogenate. The results showed that PLB explants would produce more PLBs and very few shoots with or without roots after 16 weeks in culture. Shoot explants 0.5-1.0 cm long would produce PLBs and new shoots in nearly equal amounts. On the other hand, larger explants (1.5 – 2.0 cm long) would continue to grow producing elongated leaves and well-developed roots and new shoots and very few PLBs. These observations indicate that selection and segregation of morphologically uniform shoots is an essential step in the micropropagation protocol for Dendrobium cv Sonia. Asynchrony in in vitro cultures of Dendrobium cv. Sonia may be minimised if shoots of at least 0.5 cm long are periodically segregated and cultured into fresh medium.

Keywords: Micropropagation, Dendrobium, explant type, asynchrony

INTRODUCTION

Biotechnology, especially plant tissue culture, contributes to food security by providing important tools for the improvement of agricultural productivity. Plant tissue culture permits the large-scale production of relatively uniform, disease-free, high-quality planting material of high value horticultural crops. In commercial applications, it also creates employment, particularly for women. Reddy (2007) reported that women constitute nearly 90% of the workforce in the production divisions of plant tissue culture companies in India.

Tissue culture is an important technology in the production of orchid plants that provide considerable source of foreign exchange and income for small growers in Southeast Asia. The export of Malaysian orchids increased to RM13.2 million (3 million USD) in 2017 from RM11.9 million (2.7 million USD) in 2015 indicating that orchids can bring gains to the country (The Sun Daily, 2017). Among the tropical orchids, *Dendrobium* is the most popular-cut-flower export commodity in Malaysia.

The genus *Dendrobium*, which is composed of about 1,500 species of epiphytic orchids are predominantly found in tropical and subtropical Asia, Pacific islands, and Australia (Encyclopedia Britannica).

Given the importance of *Dendrobium*, *in vitro* micropropagation protocols have been developed and established (reviewed in Teixeira *et al*., 2015). The review has identified the most commonly used parameters, in the tissue culture of *Dendrobium* species, hybrids and cultivars. The explants commonly used include nodal segments, *in vitro*-derived PLBs, shoot tips, transverse thin cell layers (tTCLs) from protocorms and young stems, leaves, pseudobulbs, *in vitro* seedlings, axillary buds, and callus. These explants are often cultured in MS basal medium supplemented with growth regulators BA and/or NAA, banana extract or coconut water as additives and agar as the gelling agent.

Tissue cultures of *Dendrobium* cv. Sonia have also been successfully initiated and maintained for at least a year in the Nilai University Biotechnology laboratory. However, majority of the orchid cultures developed asynchronously. Asynchrony in the present report refers to the non-uniformity in the growth of shoots and is entirely a phenotypic criterion. Generally, the cultures were composed of a mixture of protocorm-like bodies (PLBs) and shoots developed at different growth stages with varying growth rates. Such a scenario would impede seedling transplantation and acclimatisation processes in the greenhouse or field conditions due to the lack of sufficient number of morphologically uniform plantlets.

In *Dendrobium lituiflorum*, synchronous growth and development of seed-derived plantlets was obtained in KC medium supplemented with 20% banana extract after 30 days of fourth subculture (Vyas *et al*., 2011). The addition of 10% (v/v) banana homogenate in the culture medium for *Dendrobium* cv. Sonia in the present study unfortunately did not solve the issue of culture asynchrony. Hence, a study was undertaken to evaluate the effects of explant type in the regeneration and development of *Dendrobium* cv. Sonia shoots.

MATERIALS AND METHODS

Shoot cultures of *Dendrobium* hybrid cv. Sonia were used to generate the stock cultures as a source of explants. Different explant types: protocorn-like-bodies (PLBs) and young shoots (0.5 – 1.0 cm and 1.5-2.0 cm long) were segregated from the stock cultures. Approximately, 0.5 gram of each explant was cultured on autoclaved ½ strength MS medium (Murashige and Skoog, 1962) supplemented with 2.0 g/L activated charcoal, 10% (v/v) banana homogenate, 2% (w/v) lab grade agar and 3% (w/v) of sucrose (De Cruz *et al*., 2018). The pH of the culture media was adjusted to pH 5.7-5.8 with 1M NaOH or 1M HCl prior to autoclaving for 20 minutes under 1 kg cm^{-2} pressure.
All cultures were incubated at 25± 2 ºC under 12 hours dark/12 hours light cycle and irradiance provided by cool-white fluorescent tubes (Philips, Thailand).

The explant type-experiment was set up in a completely randomised design. Each explant type consisted of 10 cultures and trials were repeated once. Fresh weights of cultures were recorded after 16 weeks in culture. Other growth parameters that were studied were the number of shoots per culture, number of shoots with roots, number of shoots without roots, length of shoots, number of roots per shoot and length of roots. Data collected were subjected to analysis of variance (ANOVA) using SPSS software version 20 (SPSS Inc., Chicago) and means were compared using the most significant difference test, Tukey's test in which $p<0.05$.

RESULTS AND DISCUSSION

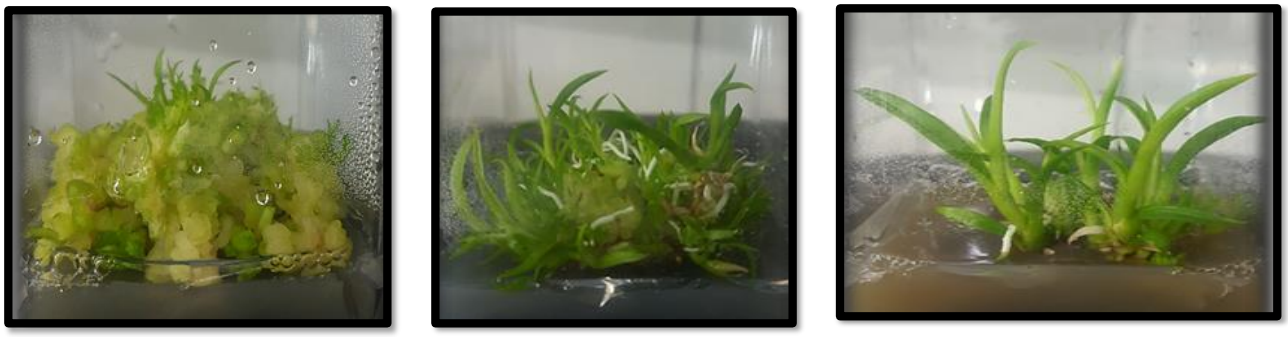
Subculturing or transferring of *in vitro* cultures into fresh culture medium is important in plant propagation application. It is carried out primarily either to maintain the cultures for an extended period or to multiply the number of cultured materials. However, the effect of subculturing on the proliferation rate of cultures has been shown to vary between plant species. A reduction in the multiplication rate was observed in various ornamental plants (Varjda & Varjda 2001), pineapple (Hamad & Taha, 2008) and cherry, plum and pear rootstocks (Vujović *et al.*, 2011) during extended growth and frequent subculturing of shoots on medium containing growth regulators. On the other hand, shoot multiplication increased with subculturing until the third subculture period in dwarf raspberry (Debnath, 2004). Similarly, the proliferation of cultures with extended time of culturing was reported in *Dendrobium* hybrids (Martin & Madassery, 2006) and *Anthurium* (Bejoy *et al*., 2008 & Atak and Çelik, 2009). Furthermore, multiplication of *Dendrobium* hybrids through PLB formation and subsequent shoot development was achieved when the culture medium was supplemented with growth regulators (Martin & Madassery, 2006). The addition of 6.97 mM Kinetin in ½ MS medium resulted to the conversion of more than 90% PLBs to shoots. The shoots developed also exhibited proliferation.

In the present study, the three types of *Dendrobium* explants clearly multiplied and developed into mixed culture in ½ MS medium supplemented with 2.0 g/L activated charcoal, 10% (v/v) banana homogenate and 3% (w/v) sucrose. All 3 types of explants produced PLBs and shoots with and without roots to varying amounts during the 16-week culture period (Table 1, Figure 1a-c). When PLBs were used as the original explant, 100% of the cultures produced PLBs. In addition, 90% and 60% of the cultures produced rooted shoots and shoots without roots respectively. The cultures that produced the least PLBs and shoots without roots were derived from 1.5-2.0 cm shoot explants. These larger shoot explants elongated and produced a well-formed root system.

Table 1: Percentage of cultures with PLBs and shoots after 16 weeks in ½ MS medium

Explant type	Number of cultures	Percentage of cultures with PLBs	Percentage of cultures with shoots without roots	Percentage of cultures with rooted shoots
PLBs	20	100	60	90
0.5 – 1.0 cm shoots	20	95	50	90
1.5 – 2.0 cm shoots	20	20	20	100

Figure 1. Dendrobium cv Sonia shoot cultures derived from different explants



(a) Derived from PLBs

(b) Derived from 0.5 – 1.0 cm shoot explants

(d) Derived from 1.5 – 2.0 cm shoot explants

Fresh Weight of Cultures

The PLBs and shoots from the cultures were carefully segregated and their fresh weights were determined. Table 2 shows that the fresh weight of PLBs derived from larger explants (1.5-2.0 cm shoots) was the lowest (0.16 g/culture) and accounted for only 8.65% of the fresh weight per culture. A larger portion of the fresh weight was observed in the shoots indicating that 1.5 – 2.0 cm explants continued to grow producing roots and new shoots during the 16-week culture period. The production of PLBs from this explant was minimal. On the other hand, the highest fresh weight was obtained with PLBs derived from very young explants (PLBs). The fresh weight of these PLBs accounted for 63.56% of the average fresh weight of the culture. This result suggests that PLBs produced more PLBs and very few shoots with or without roots.

Table 2: Mean fresh weight of *Dendrobium* cv Sonia cultures after 16 weeks in ½ MS medium

Explant type	Number of cultures	Mean fresh weight of culture (g±S.E.)	Mean fresh weight of PLBs (g±S.E.)	Mean fresh weight of shoots without roots (g±S.E.)	Mean fresh weight of rooted shoots (g±S.E.)
PLBs	20	6.12 ± 1.47 [a]	3.89 ± 1.01 [a] (63.56%)	1.43 ± 0.53 [a] (36.76%)	0.80 ± 0.23 [b] (13.07%)
0.5 – 1.0 cm shoots	20	6.57 ± 1.07 [a]	3.00 ± 0.74 [a] (45.66%)	0.49 ± 0.16 [ab] (16.33%)	3.08 ± 0.42 [a] (46.88%)
1.5 – 2.0 cm shoots	20	1.85 ± 0.21 [b]	0.16 ± 0.08 [b] (8.65%)	0.13 ± 0.1 [b] (81.25%)	1.56 ± 0.13 [b] (84.32%)

* Mean values of 20 replicates followed by the same letter are not significantly different at 5% level by Tukey's Test

The use of 0.5 – 1.0 cm shoots as explants produced cultures that were made up of PLBs and shoots in nearly equal amounts. The average fresh weight per culture was 6.57 g; 45.66% of which were from PLBs, 16.33% from shoots without roots and 36.88% from shoots with roots.

Number of Shoots

The mean number of shoots produced per culture in PLB explants was 21.35 g (Table 3). Of these shoots, 72.60% had roots and 27.40% did not have roots. The mean number of shoots per culture obtained for 0.5 – 1.0 cm shoot explant was 29.75; 72.61% of the shoots had roots and the remaining 27.39 produced shoots without roots. For larger explants (1.5 – 2.0 cm shoots), the mean number of shoots produced per culture was significantly lower (12.45) compared to the PLB explant type. Majority (93.98%) of the shoots had roots, only 6.02% of the shoots did not have roots. Consistent with the earlier observations, larger explants (1.5 – 2.0 cm shoots) continued to grow *in vitro* producing roots instead of PLBs and new shoots. Hence, the number of shoots obtained after 16 weeks was fewer than those from younger explants (PLBs and 0.5 – 1.0 cm shoots).

Table 3: Mean number of shoots per culture after 16 weeks in ½ MS medium

Explant type	Number of cultures	Mean number of shoots per culture (g±S.E.)	Percentage of shoots with roots	Percentage of shoots with without roots	Length of shoots (cm)
PLBs	20	21.35 ± 5.12 [a]	72.60	27.40	0.1 – 3.2
0.5 – 1.0 cm shoots	20	29.75 ± 4.79 [ab]	72.61	27.39	0.5 – 8.5
1.5 – 2.0 cm shoots	20	12.45 ± 0.9 [b]	93.98	6.02	0.1 – 5.5

* Mean values of 20 replicates followed by the same letter are not significantly different at 5% level by Tukey's Test

Number of Roots Per Shoot

When PLBs were used as the original explant, the mean number of roots of the *in vitro* shoots was the lowest (Table 4). On the other hand, shoots derived from larger explants (1.5 – 2.0 cm) were more and longer.

Table 4: Mean number of roots per shoot after 16 weeks in ½ MS medium

Explant type	Number of cultures	Mean number of roots per shoot (g±S.E.)	Mean length of roots (cm ±S.E)
PLBs	20	1.36 ± 0.23 [c]	0.47 ± 0.02 [b]
0.5 – 1.0 cm shoots	20	2.77 ± 0.33 [b]	0.66 ± 0.02 [a]
1.5 – 2.0 cm shoots	20	3.87 ± 0.27 [a]	0.68 ± 0.02 [a]

* Mean values of 20 replicates followed by the same letter are not significantly different at 5% level by Tukey's Test

The present study indicates that selection and segregation of shoots at different stages of growth is an essential step in the micropropagation protocol for *Dendrobium* cv. Sonia. A high degree of synchronous formation of shoots would be achieved if shoots of at least 0.5 cm in length from mixed cultures could be carefully segregated and subcultured into fresh medium for *in vitro* rooting. The present observations are consistent with the findings of Sinha and Roy (2004) who determined that shoots of same size need to be cultured in ½ MS medium without plant growth regulators in order to obtain morphologically uniform shoots of *Vanda teres*. The subculture of a clump or individual shoots to a rooting medium with or without growth regulators has been demonstrated in other orchids (Martin & Madassery, 2006; Rangsayatorn, 2009; Chookoh, 2019) This step is essential to avoid losing many of the smaller plantlets during the transplanting of rooted plants for hardening in the greenhouse.

CONCLUSION

The present study was undertaken to evaluate the effects of explant type in the regeneration and development of *Dendrobium* cv. Sonia shoots *in vitro* after they had been subcultured onto fresh culture medium. The results showed that very young explants (PLBs) would produce more PLBs and very few elongated shoots. Shoot explants (0.5 – 1.0 cm) would produce PLBs and shoots in nearly equal amounts. Both these explants would result in the production of cultures containing a mixture of PLBs and shoots at different growth stages and varying growth rates. On the other hand, larger explants (1.5 -2.0 cm) would continue to grow producing roots and new shoots and very few PLBs. These observations suggest that asynchrony in *in vitro* cultures would be minimised if shoots of at least 0.5 cm in length from mixed cultures could be carefully segregated and cultured into fresh medium for *in vitro* rooting. This step is particularly beneficial in commercial undertaking where mass scale synchronous plantlet formation is essential and desired.

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