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Response of Explant Segment and Culture Medium on Callogenesis of Dragon Fruit (*Hylocereus undatus* L.)

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Abstract- This study was aimed to select the best explant for callus initiation with morphogenic response among basal, middle and top portions of the immature stem segments and axillary bud explants of dragon fruit (*Hylocereus undatus*). The sterilized explants were placed on Murashige and Skoog (MS) medium with 3.0 mg/l thidiazuron (TDZ) and 0.5 mg/l 1-naphthaleneacetic acid (NAA) for initial culture establishment. The results revealed that the highest responded explant (46.1% callus initiation) was basal portion of the immature stem segment than the other explants. Further, the experiment was done to select the suitable medium for callogenesis from the basal part of the immature stem explant cultured on the MS medium containing 3.0 mg/l TDZ alone and also in combination with 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4 D) or 0.5 mg/l NAA. Friable callus was formed in the cultured explants (31.4%) on medium supplemented with TDZ and 2,4 D within 3 weeks of culture. The basal portion of immature stem segments had high potential to produce callus, and MS medium with TDZ and 2,4 D showed stronger effect on friable callus production. Moreover, early and late immature stem segments were cultured on MS medium with TDZ and 2,4 D for 4 weeks and then callus were transferred to 3.0 mg/l BAP alone or in combination with 0.01 mg/l NAA or 0.01 mg/l gibberellic acid (GA3) to evaluate the effect of BAP on somatic embryogenesis. It was found that the early immature stem segments performed well, and high percentage (58.3%) of embryogenic callus was noted in the culture medium supplemented with BAP and NAA.

Keywords: 6-benzylaminopurine, dragton fruit, explant, friable callus, thidiazuron

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1. Introduction

Dragon fruit is grown in tropical countries including Sri Lanka and it belongs to the family Cactaceae. The Fruits are mostly consumed directly due to their flavour and delicious taste. The high amounts of vitamin C and water-soluble fiber are available in dragon fruits (Ruzainah et al., 2009). The dragon plant is also used as an ornamental plant in some countries. The seeds rarely propagate plants because of low seed viability. Dragon seeds are coated with mucilage in the pulp of the fruit and also occur only in specific fruiting seasons (Gunasena et al., 2007). Hence, stem cuttings from healthy mother plants are widely used for plant propagation. A large number of quality plant materials are required for commercial cultivation. Thus, *in vitro* culture techniques can be used for mass plant propagation in a short time. For large-scale production, suitable explants and optimal concentrations of correct plant growth regulators are required for plant regeneration directly or indirectly to produce healthy planting materials.

The advanges of tissue culture technique is the potential method for large numbers of clonal plant propagation with identical selected genetic traits (Maximova et al., 2002). Normally immature stem segments are used as explants for direct or indirect organogenesis of dragon fruit plants. Stem explants exhibited much higher shoot regeneration ability than leaf explants cultured on Murashige and Skoog (MS) medium supplemented with benzyladenine (BA) and 1-naphthaleneacetic acid (NAA) (Dahanayake and Ranawake, 2011). Bud explants in MS medium containing thidiazuron (TDZ) and NAA showed the highest *in vitro* response (Thinesh and Seran, 2015). The *in vitro* culture technique is a very profitable, faster and efficient method of plantlet production to meet the market demand for planting materials of many species (Choffe et al., 2000). Hence present study aimed to find out the effect of explants and plant growth regulars on the morphogenic response of dragon fruit for shoot organogenesis under *in vitro* conditions.

2. Materials and Methods

Present study was conducted in 2019, at the Tissue Culture Laboratory, Eastern University, Sri Lanka. The stem cuttings were collected from healthy dragon fruit mother plants grown in a net house. Immature axillary buds and stem segments (0.5 cm long) were excised from the cuttings and dipped in 70% ethyl alcohol for 30 sec. Subsequently, they were surface sterilized with 20% Clorox^{TM} (5.25% sodium hypochlorite) for 20 min and then they rinsed thoroughly by using sterilized distilled water four times before inoculation.

A. Explant For Callus Induction

In this study, explants, including axillary buds and stem segments from various positions, were excised from immature green cuttings. Then placed on MS medium with the supplement of 3.0 mg/l TDZ and 0.5 mg/l NAA for the callus induction.

B. Callogenesis From Stem Explant

The well performed and quick responded explant from the experiment 1 was used to inoculation on the MS media containing 3.0 mg/l TDZ alone or in combination with 0.5 mg/l NAA or 0.5 mg/l 2,4- dichloro phenoxy acetic acid (2,4 D).

C. Morphogenic Response of Callus

Late immature stem segments (green colour) and early immature stem explants (light green colour) in size of 0.5 cm were excised from the sterilized stem cuttings and cultured on MS media containing 3.0 mg/l TDZ and 0.5 mg/l 2,4 D. After 4 weeks of culture, they were transferred to MS medium supplemented with 3.0 mg/l 6-benzylaminopurine (BAP) alone or in combination with 0.01 mg/l NAA or 0.01 mg/l Gibberellic acid (GA3) for the production of somatic embryoids. A total of 36 explants were cultured for each treatmentt.

All cultures were incubated at 25 ± 2ºC temperature under white inflorescent light in photoperiod of 16 hrs light and 8 hrs dark conditions. The cultures were observed daily and percentages of survival, callus formation and morphogenic response were recored at regular inverval. The experiments were designed in a Completely Randomized Design with three replicates. The data obtained were analyzed by using Sstatistical Analysis Software. The mean comparisons between treatments were done by using Tukey's test at 5 % significant level.

3. Results and Discussion

A. Explants for callus induction

The results showed that axillary bud explants had a lower survival percentage when compared with stem explants (Figure 1). Survival percentage of both explants gradually decreased with over time in the culture medium. A possible reason for this is that most of the axillary bud explants were contaminated due to their thorny nature. But, survival explants remained greenish colour for 4-6 weeks and exhibited a sign of forming callus such as swollen edges of explants, slightly colour changes from green to white, increase the size of the explants due to cell division and enlargement.

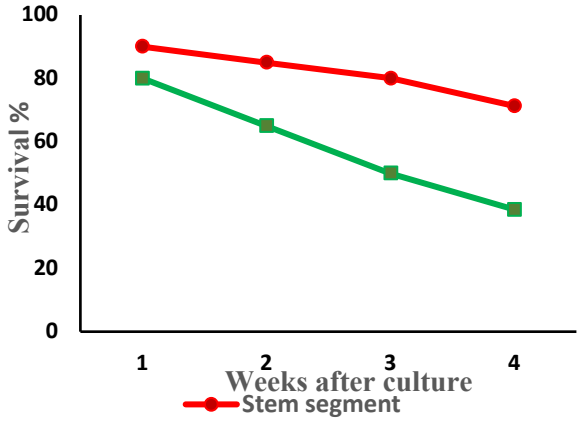

Figure 1. *Survival percentage of two different explants cultured in 3.0 mg/l TDZ and 0.5 mg/l NAA*

Callus induction percentage was gradually increased over time. Callus with different colours was formed on the survivng explants. However, when the explants reached their maximum potential for callus formation, utilizing basic nutrients and other requirements, no further callus formation was observed. The optimum callus size was observed at 4 weeks after culturing. Immature stem segments responded more quickly than axillary bud explants within two weeks of culture.

Callus derived from stem explants were whitish yellow or green in colour. Nodule like structures were observed in axillary bud explants and the callus was greenish yellow in colour. From the immature stem part, basal portion explants quickly induced friable callus (46.1%) more effectively than the other two types (Table 1). This is likely because the basal part of the immature stem segment is closer to the main stem of the plant and it may contain more nutrients than the top portions. Based on the *in vitro* response and callus induction percentage, the most suitable explant was basal portion of the immature stem segment. Dahanayake et al. (2010) and Rumiyati et al. (2017) stated that the type of explants in dragon fruit greatly influenced the regeneration ability of shoot buds in culture medium.

Table 1
In vitro response of the different explants of dragon fruit cultured on basic MS medium with 3.0 mg/l TDZ + 0.5 mg/l NAA after 4 weeks of culture.

Explants	Callus induction* [%]	Remarks
E1	33.5±4.7[b]	Whitish yellow, friable colour callus Callus formed in swollen edges of explants
E2	36.1±5.6[ab]	Whitish yellow, friable callus Callus formed in swollen explants
E3	46.1±7.3[a]	Whitish green colour callus Callus formed in swollen explants
E4	9.5±3.2[c]	Greenish yellow colour, compact callus Nodules like structures formed
F test	p<0.01	-

E1: Top portion of the immature stem explant
E2: Middle portion of immature stem explant
E3: Basal (Bottom) portion of the immature stem explant
E4: axillary bud explant
*Value represents mean ± standard error of three replicates. Means with the same letter are not significantly different using Tukey's HSD Test at 5% significant level.

B. Callogenesis from stem explants

The suitable explant (basal portion of the immature stem segment) from the previous experiment was used to study the effect of TDZ with two different auxins on callogenesis of dragon fruit. Callus formation occurred first on the explants cultured in MS medium supplemented with of 3.0 mg/l TDZ and 0.5 mg/l 2-4-D where yellowish white, friable callus was formed on the outer surface of explants (Figure 2). MS medium with 3.0 mg/l TDZ and 0.5 mg/l NAA was the second quickest medium to induce callus formation after 4 weeks, and the callus was light green in colour and relatively less friable. Moreover, callus formation was lowest (15.6%) in MS medium with only 3.0 mg/l TDZ (Table 2), and the callus size is lesser than that in the other two media.

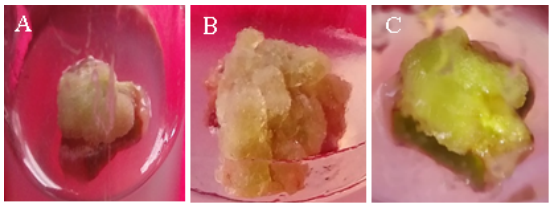

Figure 2. *Callus formation in the cultured immature stem explants on MS medium with plant growth regulators after 4 weeks of culture (explants cultured on MS + 3.0 mg/l TDZ (A), MS + 3.0 mg/l TDZ + 0.5 mg/l 2-4-D (B) and MS + 3.0 mg/l TDZ + 0.5 mg/l NAA (C)*

Table 2

Effect of TDZ with two different auxin for callogenesis of dragon fruit stem explants after 4 weeks of culture

Medium	Callus forrmation* [%]	Remarks
M1	15.6±4.8[b]	Yellowish white. Colour friable callus Swollen edges of explants
M2	31.4±7.4[a]	Whitish yellow colour friable callus More swollen explants
M3	25.3±1.8[b]	Light green colour less friable callus Slightly thickened explants
F test	P<0.01	

M1: MS + 3.0 mg/l TDZ
M2: MS + 3.0 mg/l TDZ + 0.5 mg/l 2,4 D
M3: MS + 3.0 mg/l TDZ + 0.5 mg/l NAA
** Values represent mean ± standard error of 3 replicates. Means with the same letter are not significantly different using Tukey's HSD test at 5% significant level.

It can be suggested that the callus was formed from culture medium that contained NAA may be more suitable for organogenesis, while MS medium contained 2,4-D may be better for somatic embryogenesis. Generally, 2,4-D is used for rapid callus induction during *in vitro* practices because it is a strong auxin and stimulates dedifferentiation to induce callus. This is in agreement with Rumiyati et al. (2017), who mentioned that friable callus with dark orange brown colour was fromed on the basal MS medium with 2,4-D at 1 μL^{-1} concentration. Thinesh and Seran (2015) stated that callus formation from the cultured stem segments was relatively high in MS medium with TDZ and NAA compared to TDZ alone. In addition, 2,4-D acts as auxin and promotes callus formation in plant tissues (Medeiros et al., 2005; Bejarano and López, 2011).

C. Morphogenic Response On Callus

The effect of culture media containing BAP with NAA or GA3 on calli derived from the cultured early and late immature stem explants on MS medium with 3.0 mg/l TDZ and 0.5 mg/l 2,4 D was observed. MS medium with the supplement of 3.0 mg/l BAP and 0.01 mg/l NAA showed the highest survival rate for both types of explants. BAP in combination with NAA is much better for *in vitro* morphogenic response than BAP with GA_3 (Table 03). MS medium with BAP alone exhibited the least performance. Among three media, MS medium with 3.0 mg/l BAP and 0.01 mg/l NAA was the best for the production of somatic emryoids like structures. Meanwhile MS medium containing 3.0 mg/l BAP and 0.01 mg/l GA3 also gave quick response by forming embryogenic callus within 4 weeks after transferring to this medium.

Table 3

Effect of BAP with NAA and GA_3 on embryogenic callus formation in the cultured stem explants of dragon fruit after 4 weeks of culture.

Treatments		**Embryogenic callus formation*[%]**
Explants	**Culture media**	
Late immature stem	MS + 3 BAP	12.00±0.50[b]
	MS + 3BAP + 0.01 NAA	33.33±4.70[a]
	MS + 3BAP + 0.01 GA3	19.22±2.89[b]
Early immature stem	MS + 3 BAP	29.17±4.17[a]
	MS + 3BAP + 0.01 NAA	58.33±4.17[b]
	MS + 3BAP + 0.01 GA3	41.67±4.17[c]
F test		
Explant		P<0.001
Medium		P<0.001
Explant*Medium		P<0.01

*Value represents mean ± standard error of 3 replicates. Means with the same letter are not significantly different using Tukey's HSD Test at 5% significant level.

Based on the results, MS medium containing NAA and GA_3 responded better than the medium containing only BAP. NAA and GA_3 both responded similarly, but GA_3 responded quickly than NAA. On the other hand, based on callus formation and its morphological features, NAA produced well developed embryogenic callus compared to GA_3.

Among two types of explants, early immature (tender) stem explants, which are 0.5 cm in size, quickly exhibited for somatic embryoids like structures than late immature stem explants selected from the previous experiment. The early immature stem segments had a high potential for embryogenic callus. This is in agreement with Molphe et al. (1998) who found that benzyladenine (BA) treatments including NAA produced greater callus than those with BA alone. Furthermore, Upendri and Seran (2020) confirmed that 0.5 cm sized aerial stem explants of turmeric plants produced somatic embryos with the supplementation of 2 mg/l BAP.

4. Conclusion

Present study concluded that among the four different explants excised from immature stem segments and axillary bud segments, the basal portion of the immature stem segment was the most responsive explant for the callus induction in dragon fruit. In addition, MS medium supplemented with 3.0 mg/l TDZ and 0.5 mg/l 2,4 D was the most effective culture medium for rapid friable callus production from the basal portion of immature stem explants. Callus derived from early immature stem segments on MS medium with 3.0 mg/l TDZ and 0.5 mg/l 2, 4-D had a higher potential for forming somatic embryoids-like structures compared to the explants from late immature stem segments cultured on MS medium containing 3.0 mg/l BAP and 0.01 mg/l NAA.

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