ORIGINAL ARTICLE

Cultivar, explant type and culture medium influencing embryogenesis and organogenegenesis in *Anthurium* spp.

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Abstract

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The effect of cultivars, culture media, explant type and wounding on culture response of anthurium were studied. Valantino gave the highest callus formation (83.73%) significantly different from Sonat (78.73%) and Plew Thien Phuket (45.66%). Valantino and Plew Thien Phuket gave meristematic nodular callus (MNC) whereas Sonat produced embryogenic-like callus (ELC). Modified Murashige and Skoog (MMS) medium gave the highest callus formation from both leaf (86.6%) and node (100%). Callus obtained in Nitsch and Nitsch (NN) and MMS was MNC while woody plant (WPM) medium provided ELC. For explant types, internode gave the highest callus formation (72.63%). Nodal and internodal explant gave ELC while the leaf explant yielded MNC. Wounding leaf blades tended to promote more MNC.

Key words : Anthurium, embryogenesis, organogenesis, meristematic nodular callus

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บทคัดย่อ

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อิทธิพลของพันธุ์ ชิ้นส่วน และอาหารเลี้ยงต่อกระบวนการเอ็มบริโอเจนนิซิส และออกาโนเจนนิซิส ในหน้าวัว

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ทำการศึกษาผลของอาหารเพาะเลี้ยง พันธุ์ ชนิดของชิ้นส่วนที่เพาะเลี้ยงและการสร้างแผลต่อการตอบสนองการเพาะเลี้ยงหน้าวัว ผลจากการศึกษา พบว่า พันธุ์วาเลนติโนให้การสร้างแคลลัสสูงสุด 83.73% ต่างจากพันธุ์โซเนต (78.73%) และพันธุ์เปลวเทียนภูเก็ต (45.66%) พันธุ์วาเลนติโนและเปลวเทียนภูเก็ตให้แคลลัสแบบปม (meristematic nodular callus: MNC) ในขณะที่พันธุ์โซเนตให้แคลลัสที่มีโครงสร้างคล้ายต้นอ่อน (embryogenic-like callus: ELC) สูตรอาหารดัดแปลงมูราชิกและสกุด (MMS) ให้การสร้างแคลลัสสูงสุดจากใบ (86.6%) และข้อ (100%) สูตรอาหาร MMS และนิชและนิช (NN) ให้ MNC ส่วนอาหารสูตร woody plant medium (WPM) ให้ ELC สำหรับชิ้นส่วนข้อและปล้องให้ ELC ในขณะที่ชิ้นส่วนใบให้ MNC การสร้างแผลให้กับแผ่นใบมีแนวโน้มส่งเสริมการสร้าง MNC

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Anthurium is one of the most popular and economically important genera in the family Araceae. Plew Thien Phuket, Valantino and Sonat cultivars are mainly grown for cut flower and pot plant in the tropics. Traditional propagation of this plant species is by top cuttings and sucker division. The establishment and improvement of micropropagation procedures by *in vitro* culture is more desirable.

Recently, micropropagation of anthurium is commercially used. However, bud culture faces either relatively slow or unreliable multiplication rate with occasional somaclonal variation (callus culture). Currently, over one thousand plant species have been regenerated in vitro via organogenesis and somatic embryogenesis. Somatic embryogenesis and organogenesis are among the most striking processes in plant micropropagation. Both processes are markedly affected by physical, physiological and genetical factors (Bhansali and Singh, 2000). Somatic embryogenesis is an alternative micropropagation method which provides the high multiplication rate. It has several distinct advantages over organogenesis. Recently, an embryogenic-like callus of *A. andraeanum* cultured on Murashige and Skoog (MS) medium containing 2, 4-D and BA was described (Kuehnle and Sugii, 1991) on modified half-strength MS medium supplemented with 2, 4-D and kinetin (Kuehnle *et al*., 1992).

So far, there are no reports on factors affecting somatic embryogenesis in anthurium. Here we report genetical, physical and chemical factors affecting culture response of the plant

Materials and Methods

Plant material

Three cultivars of anthurium namely, Plew Thien Phuket, Valantino and Sonat were used in this study. All genotypes were raised on *in vitro* plants and two-week-old shoots used as explant types (node, stem and leaf) for callus induction.

Effect of cultivars and explant type on culture response

Three explant types; leaves, node and internode of three genotypes; Plew Thien Phuket, Valantino and Sonat were excised and cultured on agar-solidified modified Murashige and Skoog (MMS) medium supplemented with 3% sucrose, 0.5 mg/l BA and 0.5 mg/l TDZ. After 8 weeks of culture in the dark callus formation from each explant in each genotype was recorded and statistically analysed.

Effect of culture media and explant type on culture response

Four kinds of culture media; MMS, Murashige and Skoog (MS), woody plant medium (WPM) and Nitsch and Nitsch (NN) were used for culturing two types of leaf explant, leaves and nodes of Sonat. All media were supplemented with 3% sucrose, 0.5 mg/l TDZ and 0.5 mg/l BA. These media were also solidified with 0.75% agar and adjusted pH to 5.7 before autoclaving at 1.05 $kg.cm^2$, 121ºC for 15 min. After 8 weeks of culture in the dark, the percentage of callus formation from the explant of each culture medium was recorded.

Effect of wounding and genotype on culture response

Wounding was applied to leaf explant of three cultivars of anthurium, Plew Thien Phuket, Valantino and Sonat. Wounding and non-wounding explants were cultured on MMS supplemented with 3% sucrose, 0.5 mg/l BA and 0.5 mg/l TDZ. After 8 weeks of culture in the dark, callus formation from wounding and non-wounding leaves of each cultivar was recorded and statistically analysed.

Results and Discussion

Effect of cultivars and explant type on culture response

Among the cultivars, Valantino gave the highest callus induction of 83.73%, which was significantly different from that of Sonat (78.67%) and Plew Thien Phuket (45.6%) (Table 1). The difference might be due to intra-metabolism of plant which affected cell division and differentiation. Te-chato *et al*. (2002) also reported that genotype affects callus formation in anthurium. Type of callus obtained from each cultivar was also different in this present study. The callus from Sonat was soft yellow in color having somatic embryo-like structure (ELC) whreas Valantino and Plew Thien produced meristematic nodular callus (MNC) (Figure 1).

Effect of culture media and explant type on culture response

MS and MMS medium gave the highest callus formation of 86.6% from leaf explant, followed by WPM (66.67%) and NN medium (40%). In the case of nodal explant, MMS medium

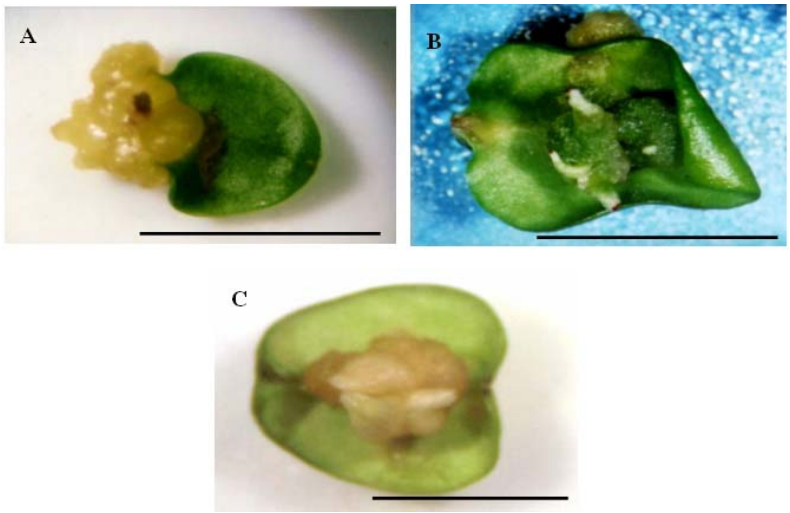

Figure 1. Callus from leaf explant of each genotype after 8 weeks of culture on MMS medium supplemented with 0.5 mg/l TDZ and 0.5 mg/l BA. (A) Sonat (B) Valantino (C) Plew Thien Phuket (bar = 1 cm).

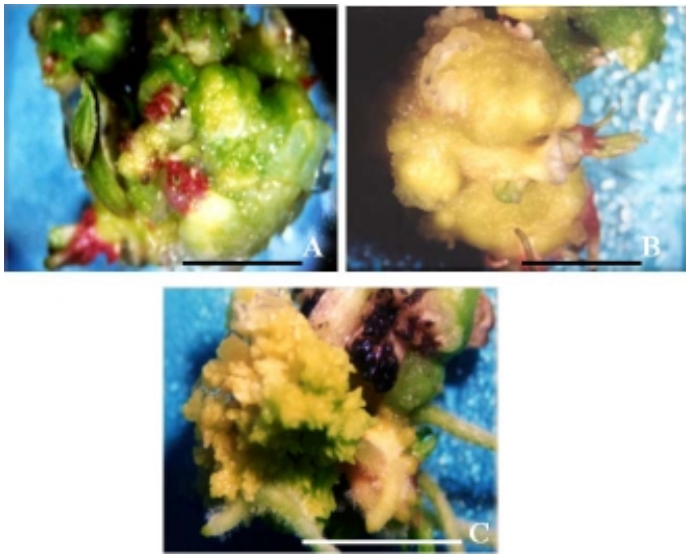

Figure 2. Callus from node of Sonat induced on MMS medium (A, B) and WPM medium (C) supplemented with 0.5 mg/l TDZ and 0.5 mg/l BA after 6 weeks of culture (bar = 0.5 cm).

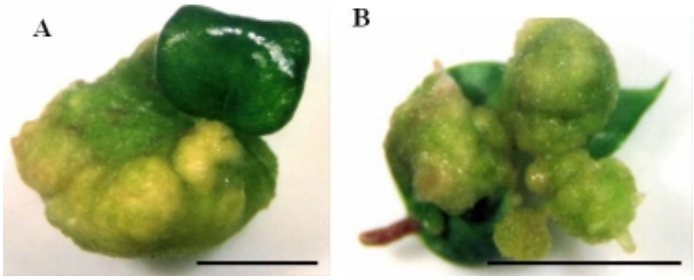

Figure 3. Position of callus formed on wounded leaf (A) and intact (B) of anthurium cv. Valentino at 6-7 weeks after culturing (bar = 1 cm).

gave the highest callus of 100% (Table 2). The callus on NN and MMS medium was found to be MNC whereas MS and WPM promoted ELC. Pierik *et al*. (1974) and Te-chato *et al*. (2002) reported MNC induction from zygotic embryo and leaf, respectively on MMS medium, which decreased macro and microelement to half of the original concentration except chelate iron. This situation induced meristemoid cells which underwent MNC, especially the addition of adenine sulphate. A high concentration of NH_4NO_3 in WPM and MS medium favor ELC (Figure 2). Gamborg *et al*. (1968) demonstrated that embryogenic callus could be induced on medium with high concentration of macroelements of MS and WPM. Bhansali and Singh (2000) described ammonium nitrate as a suitable form for the induction of somatic embryonic callus in tropical plant tissue culture. Reinert *et al*. (1967) and Furguson *et al*. (1958) suggested that potassium ion and Fe-EDTA have also been shown to be essential for somatic embryogenesis.

Wounding leaf blade tended to promote callus formation (58.86%) whereas non-wounding leaf gave 44.03%. However, significant difference

Table 1. Effect of explant type and genotype on callus formation on MMS supplemented with 0.5 mg/l TDZ and 0.5 mg/l BA after 8 weeks of culture.

Cultivars	Callus formation (%)			Average (cultivar)	F-Test (cultivar)	C.V. (%) (cultivar)	Characterisation of callus
	leaf	internode	node				
Plew Thien Phuket	45.3	50.3	41.2	45.6B	**	4.31	Yellow
Sonat	77.6	83.6	74.8	78.7A		Soft yellow	
Valantino	82.8	84.0	84.4	83.7A		Dark yellow	
Average (explants)	68.6	72.6	66.8				
F-test (explants)	ns						
C.V. (%)(explants)	30.01						

C.V. (%)(genotypes x explants) = 14.94
**** = Significant difference at P0.01**
ns = Nonsignificant difference

Table 2. Effect of culture medium on callus formation from leaf and nodal explant of anthurium. All media were supplemented with 3% sucrose, 0.5 mg/l TDZ and 0.5 mg/l BA and cultured for 8 weeks.

Medium	Number of cultured explants		Callus formation (%)		Type of callus
	Leaves	Node	Leaves	Node	
MMS	15	10	86.6	100	Yellow nodular callus
MS	15	10	86.6	70	Embryogenic-like structures
WPM	15	10	66.67	60	Embryogenic-like structures
NN	15	10	40	20	Red yellow nodular callus

Table 3. Effect of wounded leaf blades versus intact leaf blades on MMS medium with same concentration of TDZ and BA at 0.5 mg/l after 8 weeks of culture.

Cultuvars	Callus formation (%)		Average (cultivar)	F-Test (cultivar)	C.V. (%) (cultivar)	Remarks
	Wounded	Intact				
Plew Thien Phuket	78.6a	36.8c	57.70	ns	38.08	Non-browning
Sonat	50.8b	37.6c	44.2			Browning
Valantino	47.2b	37.6c	42.4			Browning
Average (explants)	58.86	44.03				
F-test (explants)	ns					
C.V. (%)(explants)	25.67					

C.V. (%) (species X explants) = 8.83
ns = Nonsignificant difference

was not obtained (Table 3). Callus developed mainly from wounds and from cut end of petiole only in non-wounding leaf (Figure 3).

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