

Impact of *Anthurium* spp. genotype on callus induction derived from leaf explants, and shoot and root regeneration capacity from callus

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Abstract

In this study, the most critical step in *Anthurium* micropropagation was the induction of primary calli from leaf segments. Genotype played an important role during *in vitro* multiplication of *Anthurium*. Callus induction from leaf segments was examined in ten *Anthurium* cultivars: 'Carnaval', 'Neon', 'Choco', 'Sonate', 'Midori', 'Pistache', 'Tropical', 'Safari', 'Arizona' and 'Cancan' on MS medium supplemented with 1 mg L⁻¹ BA, 0.08 mg L⁻¹ 2,4-D, 30 g L⁻¹ glucose, 8 g L⁻¹ agar and adjusted to pH 6.0. After 100 days, leaf segments of eight genotypes formed calli, among them, cultivar 'Pistache' had the highest callus induction ratio (65.1%) and two genotypes, 'Carnaval' and 'Cancan', showed no response. After multiplication, calli were subcultured on shoot regeneration medium, 1/2 MS with NH₄NO₃ level adjusted to 0.206 g L⁻¹, added with 20 g L⁻¹ glucose, 1 mg L⁻¹ BA, 8 g L⁻¹ agar and adjusted to pH 6.0. Shoots were obtained from all cultivars with different potential of shoot regeneration. The average number of shoots per explant in 'Tropical' (10.1) was higher than that of 'Choco' (4.3) and 'Pistache' (3.5), and shoots (at least 10 mm high) were excised and cultured on rooting medium, 30 g L⁻¹ glucose, 8 g L⁻¹ agar and 1 g L⁻¹ activated charcoal added to 1/4 MS medium. All shoots consistently formed roots after 30 days and plantlets developed well after being transferred to the nursery. The propagation process took 10 and a half months to complete.

Key words: *Anthurium andraeanum*, genotypes, leaf explants, callus induction, shoot regeneration, root regeneration.

Introduction

Anthurium sp., a perennial herbaceous plant, is an economically important genus of Araceae. It is native to the tropics of Central and South America, and is enjoyed for its colorful attractive, luxurious flowers and exotic foliage. It is cultivated commercially as cut flower and potted plant throughout the world. The necessity to find a rapid and effective propagation method is required to satisfy the increasing demand of *Anthurium*.

Different explants, leaves, immature petioles, inflorescence stalks, spathe, spadices and axillary nodes have been used for micropropagation. Hamidah *et al.* (1997) induced somatic embryogenesis from leaves, petioles and roots on medium to which various concentrations of sugar and several types of growth regulators were added. For somatic embryos, leaf pieces from micropropagated plants were found to be best on MS medium (Murashige and Skoog, 1962) with 0.1 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) and a high sucrose concentration (6%), but a low glucose concentration (3%) was better to induce callus. MS with 1 mg L⁻¹ 6-benzyladenine (BA) was the best medium for shoot regeneration and 1/2 MS plus 0.1 mg L⁻¹ α -naphthalene acetic acid (NAA) for shooting (Hamidah *et al.*, 1997). Pierik (1976) developed a method of *Anthurium* propagation using solid and liquid media alternatively. Geier (1986) used culture media derived from Nitsch's basal medium (1969), which contained minerals, vitamins, sucrose and agar. Of the various media tested, including NH₄NO₃ concentration and the presence or absence of different auxins and cytokinins, NH₄NO₃ level had the most significant effect on callus and

shoot formation, a low level being best for regeneration in all the genotypes investigated. Cytokinin was also necessary to induce callus from leaf explants, but if auxin and cytokinin were combined, shoot induction and development were better in subculturing. Kuehnle and Sugii (1991) examined the influence of growth regulators on callus induction derived from leaf segments. Callus was produced most successfully on a modified Pierik medium containing 2,4-D and BA, while sucrose was better than glucose as a carbon source.

Another factor that plays an important role during *Anthurium* *in vitro* propagation is the effect of genotype. Pierik (1976) examined 38 genotypes of *Anthurium andraeanum* and observed moderate to strong callus formation from leaf segments in 31 genotypes, very poor callus formation in four and no response in the remaining three genotypes. In another study, Geier (1986) found that out of 18 genotypes, three did not show any regeneration, five produced only callus and ten formed caulogenic callus when leaf explants were cultured on Nitsch medium. Not only callus induction but also shoot regeneration from callus was dependent on the genotype. Observing the average number of shoots per culture, Geier (1986) obtained <1 in five genotypes, 1-10 in three genotypes, and >10 in two genotypes. This result showed that different genotypes had different responses to the same medium and the identical procedure could not be applied for the *in vitro* propagation of all *Anthuriums*. Although many researches have already been conducted, the main objective of this study was to investigate the effect of genotype on callus induction derived from leaf explants and its influence on shoot and root regeneration capacity, which allows establishing a

suitable procedure suited to each *Anthurium*, which can be used for commercial production.

Materials and methods

Plant material: Leaf segments of ten valuable cultivars of *Anthurium andraeanum*: 'Carnaval', 'Neon', 'Choco', 'Sonate', 'Midori', 'Pistache', 'Tropical', 'Safari', 'Arizona' and 'Cancan' were used as material. Young leaves having approximately 1/2-2/3 of the final length were surface-sterilized with HgCl₂ 0.1%, and then sectioned to about 1 cm² pieces.

Callus induction and multiplication: To investigate the effect of genotype on callus induction derived from leaf explants, 100 leaf explants from each genotype were transplanted onto callus induction medium: 1/2 MS basal medium supplemented with 30 g L⁻¹ glucose, 1 mg L⁻¹ BA, 0.08 mg L⁻¹ 2,4-D and 8 g L⁻¹ agar. Two leaf segments were placed in each culture vessel, and callus was induced in darkness at 20 ± 2°C, 75-80% relative humidity. When callus had formed and achieved the required size (about 2.0 to 2.5 cm²), it was divided and three 0.5 cm² pieces were transferred to callus multiplication medium (1/2 MS medium with 20 g L⁻¹ glucose, 1 mg L⁻¹ BA and 8 g L⁻¹ agar) to get a large amount of calli.

Shoot regeneration: To investigate the effect of genotype on shoot regeneration from callus, calli obtained in four month subcultured 'Tropical', 'Choco' and 'Pistache' were transferred to shoot regeneration medium: 1/2 MS with NH₄NO₃ lowered to 0.206 g L⁻¹, 20 g L⁻¹ glucose, 1 mg L⁻¹ BA and 8 g L⁻¹ agar. Friable, etiolated calli were chosen to increase the frequency of shoot regeneration and each culture vessel contained three 0.7 cm² callus pieces.

Rooting: For more rapid and consistent rooting, 10 mm tall shoots of 'Tropical', 'Choco' and 'Pistache' were isolated and transplanted to rooting medium: 1/4 MS medium supplemented with 30 g L⁻¹ glucose, 8 g L⁻¹ agar and 1 g L⁻¹ activated charcoal.

Culture conditions (*in vitro* and *ex vitro*): pH was adjusted to 6.0 in all media except for the rooting medium (pH 6.2) and media were autoclaved for 30 minutes at 121°C and 1 atm. Callus multiplication, shoot regeneration and root formation were carried out under a 10-hour photoperiod, 45 µmol m⁻² s⁻¹ illumination, and at 25 ± 2°C and 70-75% relative humidity.

Plantlets of 'Tropical', 'Choco' and 'Pistache' with more than 2 well-developed roots and 2.5-3.0 cm in height were isolated and transferred to a 50-70% of daylight shaded nursery. Plantlets were cultivated on tree fern fiber substrate at 22 ± 2°C and 80-85% relative humidity, sprayed with water 2 times a day, Komix BFC as fertilizer 3 times a week and 5 g L⁻¹ fungicide solution mixture including Zodiac 80 WP and Manozep 80 WP 2 times a week.

Data was analyzed for significance by ANOVA with the mean separation by Duncan's multiple range test (Duncan, 1995).

Results and discussion

Effects of genotype on callus induction from leaf segments: Two 1 cm² leaf pieces were cultured in each callus inducing medium vessel. After 30 days of culture in darkness, callus formation was first observed in 'Midori' and 'Safari' with 18.2% and 12.5% explants inducing callus, respectively (Table 1). Leaf pieces of other cultivars were green but without traces of callus.

Table 1. Effect of genotype on callus induction of *Anthurium* after different periods

Cultivar	Callus induction rate (%)		
	30 days	60 days	100 days
'Carnaval'	0	0	0
'Neon'	0	2.0	2.0
'Choco'	0	12.5	12.5
'Sonate'	0	28.6	28.6
'Midori'	18.2	20.0	35.2
'Pistache'	0	61.8	65.1
'Tropical'	0	34.3	35.0
'Safari'	12.5	16.7	20.8
'Arizona'	0	9.8	10.7
'Cancan'	0	0	0

Explants were continuously left in the dark. After 60 days of culture, all cultivars induced callus except for 'Carnaval' and 'Cancan'. While the callus formation rate of cultivars 'Midori' and 'Safari' was slow (18.2 to 20.0% in 'Midori' and 12.5 to 16.7% in 'Safari'), some cultivars which did not form callus after 30 days of culture, had high rates of callus induction (28.6% in 'Sonate', 34.3% in 'Tropical' and 61.8% in 'Pistache'), even higher than those of genotypes inducing calli early. Explants of other genotypes also formed callus but at lower rates: 12.5% in 'Choco', 9.8% in 'Arizona' and 2% in 'Neon'. And after 100 days, the regeneration rate of the ten cultivars gradually increased with a corresponding rise in fresh weight of calli.

In 'Choco', 'Pistache' and 'Safari', leaf explants started to turn yellow, yet calli still developed. 'Safari' explants induced callus early but had a low induction rate (20.8%) while 'Pistache' had the highest callus induction rate (65.1%). The induction rate of 'Midori' and 'Tropical' were similar (about 35.2 and 35.0%, respectively), however the size of 'Tropical' calli were profusely larger. In addition, 28.6% of 'Sonate' leaf explants induced large calli, which developed quickly afterwards. Other cultivars showed little change: a low induction rate in 'Choco' (12.5%), 'Arizona' (10.7%) and 'Neon' (2.0%). 'Carnaval' and 'Cancan' did not induce callus.

These results indicate that ten *Anthurium* genotypes had different responses to the same callus induction medium and culture conditions. They could be clustered into four groups: group of 'Pistache' having high callus induction rate; group of 'Midori', 'Tropical', 'Sonate', 'Safari', 'Choco' and 'Arizona' having average callus induction rate; group of 'Neon' having low callus induction rate and group of 'Carnaval' and 'Cancan', which could not induce callus.

Effect of genotype on shoot regeneration from callus: Shoot formation was investigated in 'Choco', 'Pistache' and 'Tropical', which had different callus induction rates. After 30 days of culture, shoot regeneration was first observed in 'Tropical', while other cultivars did not form shoots.

Calli of three cultivars still survived after 100 days of culture. Among them, the highest number of regenerated shoots was obtained in 'Tropical' (10.1 shoots per explant), which were firm with small leaves; moreover, much caulogenic calli was induced. 'Choco' had lower number of regenerated shoots (4.3 per explant), which had large leaves and developed slowly, but its calli turned darker. The lowest number of shoots formed in 'Pistache' (3.5 per explant) (Table 2).

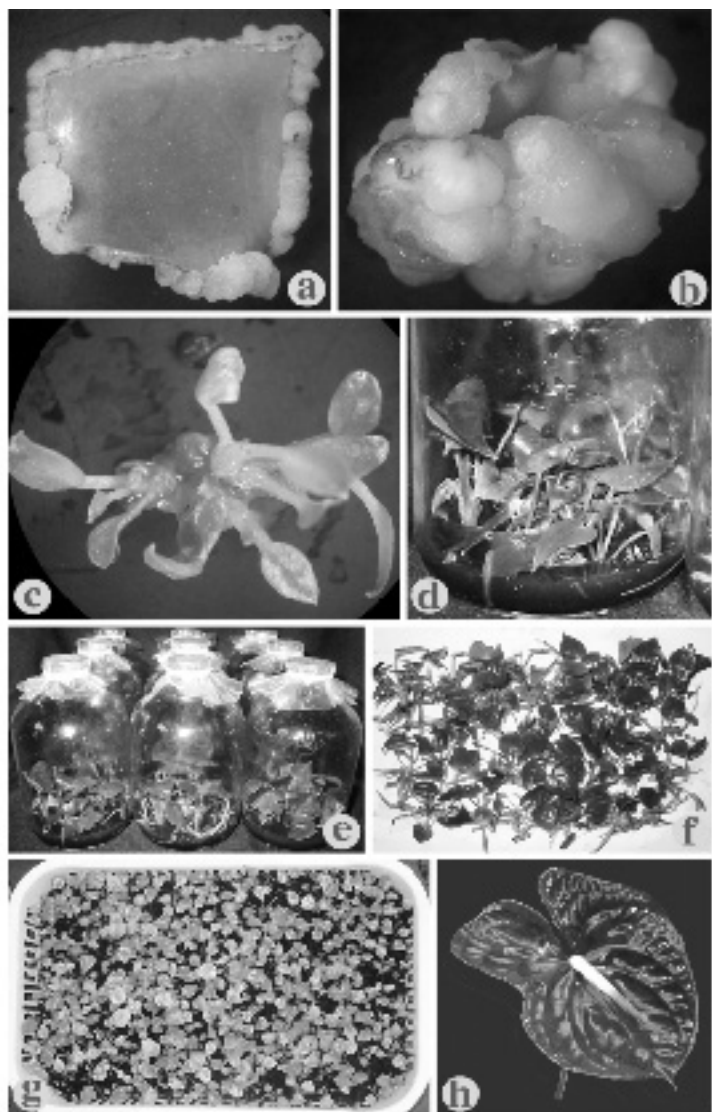


Fig. 1. Micropropagation by leaf explant culture of *Anthurium* spp.: (a), (b) callus induced from leaf segment; (c) shoots regenerated from callus; (d), (e) root formation; (f) plantlets; (g) *ex vitro* plantlets; (h) *Anthurium* 'Choco' flower.

Table 2. Effect of genotype on shoot regeneration from callus of *Anthurium*

Cultivars	Callus survival rate (%)	Number of shoots per explant
'Choco'	100	4.3b ^x
'Pistache'	100	3.5c ^s
'Tropical'	100	10.1a ^s

^x Different letters within a column indicate significant differences at $P \leq 0.05$ by Duncan's multiple range test.

Effect of genotype on root formation from shoots: Shoots of 'Choco', 'Pistache' and 'Tropical' were isolated and transferred to shoot formation medium without plant growth regulators (10 shoots per vessel), which is necessary for firm and rapid root formation. Although shoots and roots could regenerate naturally when cultured over a long period and under illumination on MS medium, root formation depended on the size and developmental stage of shoots, and culture media. Shoots formed roots when placed on media with low concentration of macroelements and without a cytokinin, BA. Results showed that all shoots of the three cultivars formed fully developed root systems after 30 days of culture with a comparable number of roots. However, based

on the characteristics of each cultivar, formed roots had different shapes and sizes. Shoots with small leaves of 'Tropical' formed long and thin roots, while shoots with larger leaves of 'Choco' and 'Pistache' formed short but thick roots.

Effect of genotype on *ex vitro* plantlet development: Five hundred homomorphous plantlets each of 'Choco', 'Pistache' and 'Tropical' were placed in the nursery. All of them survived and developed well after 30 days of culture. Some plantlets with very short roots or even without roots also grew and formed roots post planting, although it took more time.

Anthurium micropropagation by leaf explant culture was characterized by the use of different media at different stages of morphogenesis. The most difficult step in this propagation process was callus induction from leaf explant. This is due to the restricted dedifferentiation of mature tissue and depends strongly on genotype. *Anthurium* 'Pistache' could best induce calli on 1/2 MS medium supplemented with 30 g L⁻¹ glucose, 1 mg L⁻¹ BA, 0.08 mg L⁻¹ 2,4-D, 8 g L⁻¹ agar and adjusted to pH 6.0 (65.1% explants induced calli after 100 days of culture), whereas 'Carnaval' and 'Cancan' could not. Genotype affected shoot regeneration slightly but did not affect root formation. The critical factor in shoot regeneration stage was the effect of NH₄NO₃ concentration. 'Tropical' had the highest number of regenerated shoots per explant (10.1 shoots per explant after 100 days) when its calli were cultured on 1/2 MS with NH₄NO₃ lowered to 0.206 g L⁻¹, 20 g L⁻¹ glucose, 1 mg L⁻¹ BA, 8 g L⁻¹ agar (pH 6.0), and all genotypes formed roots normally (after 30 days) on 1/4 MS medium supplemented with 30 g L⁻¹ glucose, 8 g L⁻¹ agar, 1 g L⁻¹ activated charcoal (pH 6.2). All plantlets developed well when transferred to the nursery. According to the results of this study, the propagation process took ten and a half months to complete: four months for callus induction, two and a half months for mass multiplication, three months for regenerating shoots and one month for forming roots. This process was best for 'Pistache', appropriate for 'Tropical', but had to be adjusted to obtain more regenerated shoots of 'Pistache' and 'Tropical' calli. More researches are required to define the appropriate requirements for each of the other cultivars.

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References

- Duncan, D.B. 1995. Multiple range and multiple F test. *Biomet.*, 11: 1-42.
- Geier, T. 1986. Factors affecting plant regeneration from leaf segments of *Anthurium scherzerianum* Schott (Araceae) cultured *in vitro*. *Plant Cell Tiss. Org. Cult.*, 6: 115-125.
- Hamidah, M., A.G.A. Karim and P. Debergh, 1997. Somatic embryogenesis and plant regeneration in *Anthurium scherzerianum*. *Plant Cell Tiss. Org. Cult.*, 48: 189-193.
- Kuehnle, A.R. and N. Sugii, 1991. Callus induction and plantlet regeneration in tissue cultures of Hawaiian *Anthurium*. *HortSci.*, 26: 919-921.
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant Physiol.*, 15: 473-496.
- Nitsch, J.P. 1969. Experimental androgenesis in *Nicotiana*. *Phytomor.*, 19: 389-404.
- Pierik, R.L.M. 1976. *Anthurium andraeanum* plantlets produced from callus tissues cultivated *in vitro*. *Plant Physiol.*, 37: 80-82.