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Full Length Research Paper

Micropropagation of *Anthurium andreanum* cv. Terra

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Anthurium anderanum **cv. Terra from family Araceae, is a plant with a high commercial value. Conventional propagation of this plant performed via suckers and seeds planting that nowadays missed its application have been abolished. This study aimed to establish producible protocol for indirect *in vitro* regeneration of the Terra genotype. For this purpose, the effects of 16 different plant growth regulators treatments on callus induction and regeneration of leaf explants were studied. Callus with the highest fresh and dry weight was produced on modified Murashige and Skoog medium containing 0.1 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) and 1.5 mg/L 6-benzylaminopurine (BAP). For regeneration stage, induced calli were transferred to modified MS medium without plant growth regulators, and after six weeks, shoots formed. For rooting, regenerated shoot were transferred to MS medium without any plant growth regulators. Regenerated plantlets potted on mixture of coco pit and perlite were succesfully acclimated.**

Key words: *Anthurium*, micropropagation, growth regulators, regeneration.

INTRODUCTION

The *Anthurium* genus comprises about 1500 tropical species with economically important genera in the family of Araceae. *Anthurium* species are produced for many proper goals, including cut-flower, flowering potted plants and landscape plants (Nowbuth et al., 2005). *Anthurium* are traditionally propagated by seeds (Dufour and Guerin, 2003), however, vegetative propagation methods applied to these plants have not shown good results and tissue culture techniques appears as an alternative to increase the production (Pierik et al., 1974; Chen et al., 1997). Propagation through seeds is not worthwhile because of cross-pollination and the progenies are heterozygous. Moreover, it is hampered by the poor germination rate and low viability of the seeds. (Martin et al., 2003).

Micropropagation of *Anthurium* has been achieved with various tissues. The tissue culture of *Anthurium* was first reported by Pierik et al. (1974). They achieved regeneration of *Anthurium andreanum* through adventitious shoots formation from callus (Pierik et al., 1974; Pierik and Steegmans, 1976) and also direct shoot regeneration from lamina explants were achieved (Martin et al., 2003). Vargas et al. (2004) established an alternative method for regeneration of *Anthurium* plants. They obtained *in vitro* plants from germinated seeds and plantlets from micro-cuttings culture. Joseph et al. (2003) reported the calli induction from leaf explants of *Anthurium* in modified Murashige and Skoog (MS) medium with 0.88 µM 6-benzylaminopurine (BAP) + 0.9 µM 2,4-dichlorophenoxy acetic acid (2,4-D) + 0.46 µM kinetin (Kin). Chen et al. (1997) also regenerated *Anthurium* plants from root explants. In addition, Yi-xun et al. (2009) induced calli from proto-corm-like of *A. andreanum* Hort. Micropropagation by callus induction from leaf explants is a difficult step for *in vitro* propagation Nevertheless, credible proliferation of callus and subsequent plant regeneration is important for enormous plant propagation. This article describes the establishment of rapid method for regeneration of

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Table 1. Growth regulator composition of culture media used in callus induction (mg/L).

Treatment	2,4-D	BAP
1	0.5	-
2	1	-
3	1.5	-
4	2	-
5	-	0.5
6	-	0.7
7	-	1
8	-	1.5
9	-	2
10	-	2.5
11	0.1	0.5
12	0.1	1
13	0.08	0.5
14	0.08	1.5
15	0.1	1.5
16	0.16	1.5

Table 2. The effects of BAP on callus induction.

BAP (mg/l)	Percentage of callus genesis
0.5	30^{e}
0.7	50^{d}
1	60^{c}
1.5	80^{b}
2	80^{b}
2.5	90^{a}

Means followed by the same case letters are not significantly different at the 5% probability level by Duncan's new multiple-range test.

Anthurium andreanum cv Terra from callus tissue through organogenesis.

MATERIALS AND METHODS

Commercial cultivar Terra from *A. andreanum* was selected. The leaf explants from this variety was used as plant material. The leaves were sterilized for 20 min in 3% hypochlorite sodium and then rinsed three times with sterile water. Sterile leaves were sectioned to about 1×1 cm^2.

Media

For the propagation by indirect organogenesis, MS medium (Murashige and Skoog, 1962), half strength MS and modified MS (half strength of MS medium except of $MgSO_4$ and $CaCl_2$) were used.

Callus induction

Leaf explants were transplanted into dishes supplemented with 30 g/L sucrose, and different treatment of 2,4-D and BAP. The pH was adjusted to 5.7 before autoclaving. The media differed in concentrations of plant growth regulators and light conditions of culture.

Shoot regeneration

For shoot regeneration, calli of leaf explants were transferred to modify MS with 0.1 mg/L 2, 4-D and 1 mg/L BAP, and kept at 16/8 light and darkness photoperiod.

Rooting

The regenerated shoots longer than 3 cm with a pair of leaves were transferred to the MS medium without plant growth regulators.

Statistical analysis

The results are presented as mean values ± standard errors. All experiments were repeated four times. The data on callus induction and wet and dry weight were subjected to analysis of SPSS ver19, with the means separation ($p<0.05$) by Duncan's multiple range test. The charts were drawn by Excel 2007 (Table 1).

RESULTS

Callus formation of *Anthurium* was observed on the leaf explants using modified MS medium. The stable of leaf explants in MS medium was on the average. Callus formation was performed 60 days after culture of explants. Table 1 shows the rate of growth regulators used for callus induction from leaves. 2,4-D alone had no effect on callus induction of *Anthurium*. The effects of BAP on the percentage of callus formation are given in Table 2. Maximum of callus genesis with BAP occurred at a concentration of 2.5 mg/L. The percentage of callus induction with BAP differed significantly ($P<0.05$).

Moreover, the best medium for callus induction from leaf explants was modified MS with 0.1 mg/L 2,4-D +1.5 mg/L BAP. Interplay of 2,4-D and BAP was significant ($P<0.05$). Figure 2 shows the rate of wet and dry weight in the different plant growth regulators treatments. Leaf explants cultured on half strength MS were ruined finally. Explants were transferred to 16/8 light and dark photoperiod to enhance organogenesis in calli. After four weeks, pink organs were formed which showed negative gravitropism, and after two weeks, leaf primordial were engendered. For rooting, the regenerated shoot from callus was transferred to MS medium without any plant growth regulators. Figure 1 shows the callus induction from leaf explants, organogenesis, regeneration and compatibility of plantlets.

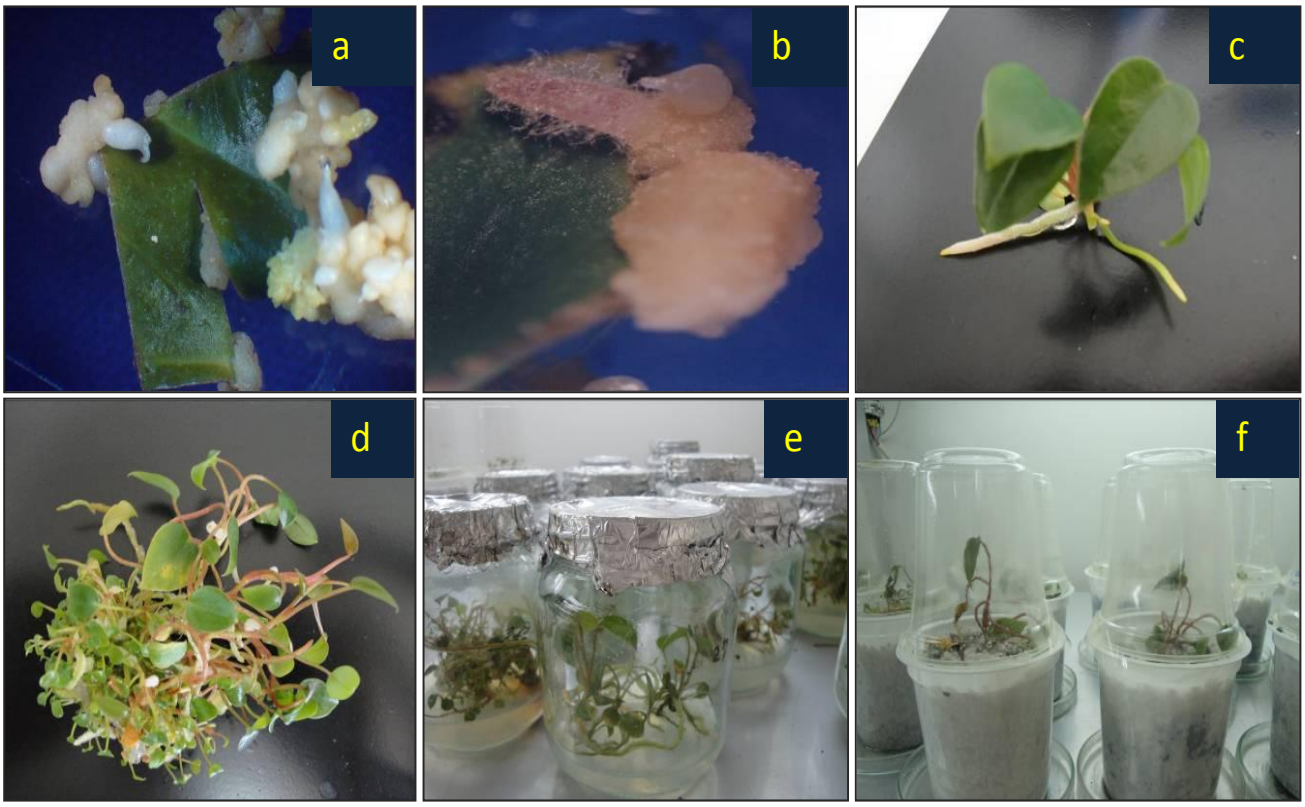

Figure 1. Shows the callus induction from leaf explants, organogenesis, regeneration and compatibility of plantlets.

DISCUSSION

Many investigators have reported that the vegetative propagation of many *Anthurium* species is a very difficult method (Hamidah et al., 1997; Pierik and Steegmans, 1976; Pierik et al., 1974). Micropropagation is an alternative method of propagation that is appropriate for selection of spices. In this research, we achieved a rapid regeneration system of *A. andreanum* from callus culture. Nutrition, genotypes and growth regulations are the most important cultural factors that a commercial *Anthuriums* grower can control. Silva et al. (2005) concluded that various physical and biological factors including media play important roles in propagation of *A. andreanum*. Geier (1986) analyzed the influence of NH_4NO_3 on callus and shoot formation from leaf tissues. Leaf explants in MS medium had average stability, whereas those in half strength of MS medium showed deficiency of nutrient material and were ruined finally. The rate of NH_4NO_3 in MS medium appeared high in callus induction of *Anthuriums* and must be lower (Joseph et al., 2003; Cimen and Ozge, 2009). With lower rate of NH_4NO_3, the calli induction occurred. Our investigation also showed of course that BAP can induce callus, but when used with 2,4-D, had more effect on callus induction.

The success of tissues culture is related to the correct choice of explants material (George et al., 2008). In this research, we used leaf explants. On the other hand, Techato et al. (2006) used leaves, node and internodes of three genotypes of *Anthurium*. They cultured explants on MS medium, modified MS, woody plant medium (WPM) and Nitsch and Nitsch medium (NN) and obtained the highest callus formation from leaf explants in MS and modified MS. Moreover, Nhut et al. (2006) studied the effect of ten different *Anthurium* genotypes and reported different responses from their genotype. Our research also show that the application of 2,4-D with BAP could be positive for the propagation of *A. andreanum* cv. Terra. Cimen and Ozge (2009) used 1 mg/L IBA and 0.04% active charcoals to initiate roots. In addition, Joseph et al. (2003) transferred shoots regenerated from *A. andreanum* Hort. onto half strength MS medium supplemented with 0.54 µM of naphthaleneacetic acid (NAA) for rooting. The rooting process occurred on medium without plant growth regulators. Plantlets potted on mixture of coco pit and perlit were succesfully acclimated.

This paper establishes a rapid method for micropropagation of *A. andreanum* cv. Terra. We concluded that in addition to mineral salt of mediums and plant growth regulators, age and genotype of explants are very important in the success of plant tissue culture

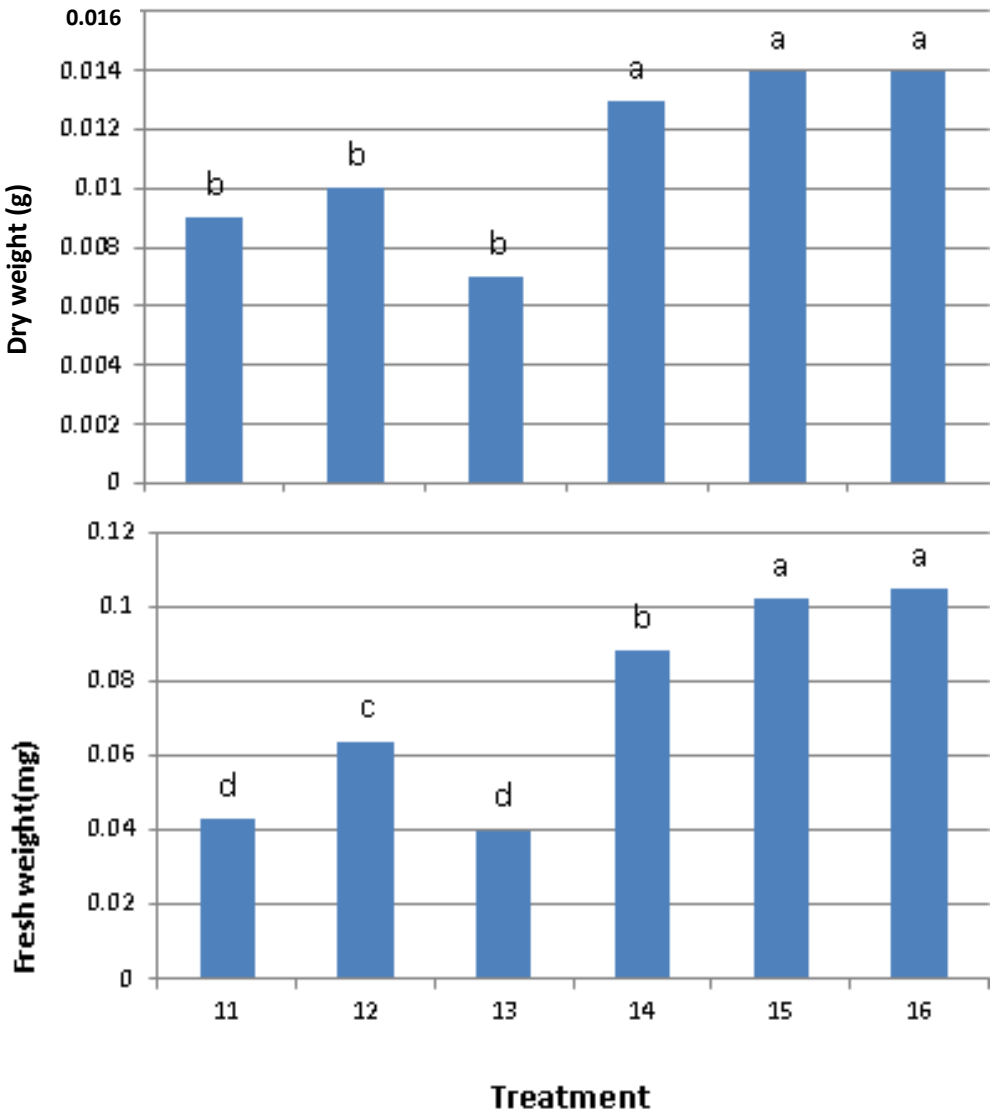

Figure 2. The effects of various hormonal compounds on fresh and dry weights of induced calli. Each bar represents the mean of 20 replications. Bars indicated by similar letters are not significantly different at P = 0.05 (Duncan's new multiple-range test).

experiment.

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