In Vitro PROPAGATION OF *Anthurium plowmanii* cv. WAVE OF LOVE AND PLANTLET ACCLIMATIZATION

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ABSTRACT

An efficient in vitro plant regeneration for *Anthurium plowmanii* cv. Wave of Love was established. Seed derived plants growing *in vitro* were used as sources of explants. One node cuttings of *Anthurium plowmanii* cv. Wave of Love were subjected to MS (Murashige & Skoog, 1962) medium supplemented with different concentrations of benzyladenine (BA), i.e. 0; 0,5; 1,0; 1,5; 2,0; 2,5; 3,0 and 5,0 mg/l and incubated under continuous fluoresent light of approximately 2000 lux. Shoots obtained in each treatment was observed after 8 weeks, 12 weeks and 16 weeks of cultures. Results showed that addition of BA to the medium from 0.5-5 mg/l significantly promoted shoot proliferation. In addition, increasing BA concentrations from 0.5 to 1.5 mg/l increased the shoot numbers as well as shoot fresh weight, while futher increase of BA concentrations (from 2.0 to 5 mg/l) in the medium resulted in levelling off the number of shoots per explant and shoot fresh weight. Maximum number of shoots and shoot fresh weights were obtained on medium supplemented with 1.5 mg/l BA. Shoot length tended to decrease with addition of and increasing concentration of BA in the medium. When one node cuttings from BA containing medium were subjected to MS medium free of growth regulators, with or without 2 g/l activated charcoal for 6 weeks, rooted plantlets were obtained. Subsequently, when these plantlets were acclimatized using mixes of pot plant media, 100% of the shoots were successfully acclimatized in the green house.

Keywords: *Anthurium plowmanii*, BA, *in vitro*, shoot proliferation, acclimatization..

INTRODUCTION

Anthurium is a genus consisting of about 1500 species of important ornamental plants. Common propagation method of *Anthurium* is by seeds, and the use of vegetative propagation methods has met with low success. Tissue culture techniques appear as a more efficient method to increase the number of propagules of *Anthurium* in a shorter time (George, 1996).

In vitro propagation of *Anthurium* has been reported through several modes of regeneration. Shoot organogenesis from callus of *Anthurium andreanum* was reported by Pierik et al. (1974), whereas direct shoot formation from lamina segments as explants was reported by Martin *et al*. (2003). In vitro regeneration of *Anthurium andreanum* through the formation of callus was established by Vargas *et al*. (2004). In this experiment, we describe efficient in vitro propagation of *Anthurium plowmanii* cv. Wave of Love through axillary branching from one node cuttings.

MATERIALS AND METHODS

Seed-derived plants of *Anthurium plowmanii* cv Wave of Love growing *in vitro* were used as sources of explants. Sterile one node cuttings were excised from the stock plants and used as explants for shoot proliferation experiment. Four explants were cultured in each bottle containing macro and micro salts of Murashige & Skoog (MS) (1962) supplemented with 3% sucrose, MS vitamines, 100 mg/l mio inositol, 0.7% agar and one of the treatment assigned, i.e., 0; 0,5; 1,0; 1,5; 2,0; 2,5; 3,0 and 5,0 mg/l benzyladenine (BA). The pH of medium was adjusted to 5.8 before autoclaving at 1.1 kg/cm^2 (121^{0}C). All cultures were incubated under continuous fluorescent light of

approximately 2000 lux intensity at 25 ^{0} ± 2^{0}C. This experiment was conducted in completely randomized design with 6 replicates. Each experimental unit consists of 5 culture bottles each of which contains 4 explants. Number of shoots per explants obtained in each treatment were observed after 8 weeks, 12 weeks and 16 weeks of cultures, whereas average of shoot length and shoot cluster-fresh weight were measured at 16 weeks after explanting.The analysis of variance of data was performed, followed by mean separation procedures using Tukey's Honest Significant Different (HSD) values.

In the second experiment, one node cuttings from BA-containing medium were subjected to MS medium free of growth regulators, with or without 2 g/l activated charcoal and incubated under the same condition described above for 2 weeks, then hardenned on benches with diffuse sun light for 3 weeks. Shoot length, number of roots were counted after 5 weeks. The rooted plantlets obtained from the second experiment were washed under tap water, soaked in fungicide solution (2 g/l Dithane-M45) and blotted on tissue paper, then planted in 8 cm Ø pot containing one of the mixed media, which consists of rice hulk charcoa l: sand (1:1), or sand : cocopeat (1:1), or rice hulk charcoal : sand : cocopeat (1:1:1) for acclimatization. Survival rate of plantlets during acclimatization was counted at 10 weeks after being transferred to ex vitro condition.

RESULTS AND DISCUSSION

Results

Effects of BA concentrations on number of shoots per explant.

Table 1. Number of shoots and shoot fresh weight as affected by benzyladenine (BA) concentrations in in vitro culture of *Anthurium plowmanii* cv. Wave of Love

BA concentrations (mg/l)	Number of shoots per explant			Shoot fresh weight per explant *)
	2 months after explanting *)	3 months after explanting *)	4 months after explanting *)	
0	1.52 d	1.52 d	1.59 f	0.60 f
0.5	5.87 c	10.48 c	12.51 d	3.89 cd
1.0	8.51 b	14.87 b	17.01 bc	4.73 ab
1.5	13.14 a	23.30 a	28.02 a	5.46 a
2.0	9.16 b	16.12 b	18.57 b	4.35 bc
2.5	8.06 b	14.34 b	16.65 c	4.26 bc
3.0	5.08 c	8.54 c	10.31 e	3.17 de
5.0	1.41 d	1.93 d	3.05 f	2.59 e

*) Numbers followed by same letters in column were not significantly different based on LSD (P<0.05)

Results of this experiments showed that addition of benzyladenin (BA) to the medium from 0.5 mg/l to 3 mg/l significantly increased number of shoots of *Anthurium* per explant, either at 2 months, 3 months or 4 months of cultures (Table 1). The trends of shoot multiplication upon BA effects were similar on the three observation times. At 2 months of cultures, the control teratment (without BA) resulting in 1.52 shoots per explant, and until 4 months of cultures, the figure was only 1.59 shoots per explant. Addition of BA starting at 0.5 mg/l significantly increased number of shoots per explant, and increasing culture period increased number of shoots per explant. Number of shoots per explant obtained at 2 months, 3 months and 4 months of cultures with addition of 0.5 mg/l BA were 5.87, 10.48 and 12.51 shoots per explant, respectively. Increasing BA concentrations to the medium from 0.5 to 1.5 mg/l increased the shoot numbers, while futher increase of BA concentrations (from 2.0 to 5 mg/l) in the medium resulted in levelling off the number of shoots per

explant, and the figures were similar in trends at 2, 3 and 4 months of culture. Maximum number of shoots at 2 months, 3 months and 4 months of cultures (ie., 13.14, 23.3 and 28.08 shoots per explant, respectively) were obtained on medium supplemented with 1.5 mg/l BA, while the lowest number of shoots were found on medium added with 5 mg/l BA.

Effects of BA concentrations on shoot fresh weight.

Consistent with results on shoot numbers, addition of BA to the medium also significantly increased fresh weight of shoot clusters per explant at 4 months of cultutres (Table 1). Average fresh weight of shoot clusters obtained in the control (without BA) treatment was only 0.6 g. Addition of 0.5 mg /l BA to the medium increased shoot cluster fresh weight by more than 6 times (3.89 g), and increasing BA concentration to 1.5 mg/l in the medium resulting in further increase of shoot cluster fresh weight to 5.46 g. However, futher increase of BA concentrations (2 - 5 mg/l) in the medium resulted in leveling off the shoot cluster fresh weight, with the lowest (2.59 g) found at 5 mg/l BA. Maximum value of shoot cluster fresh weight (5.46 g) was obtained at 1.5 mg/l BA.

Effects of BA concentration on shoot length.

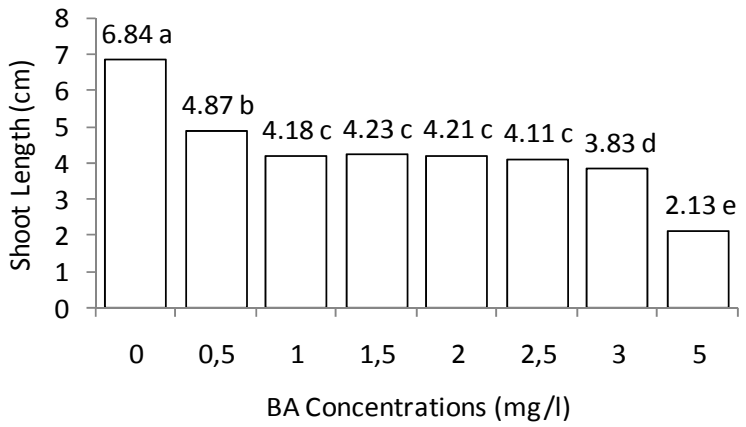

Figure 1. Effects of benzyladenine (BA) concentrations on shoot length of *Anthurium plowmanii* cv. Wave of Love cultured in vitro for 4 months. Numbers followed by the same letters were not different based on LSD (P<0.05).

Figure 1 showed effects of various BA concentrations on shoot length. Addition of BA to the medium decreased shooth length, and increasing concentraton of BA from 0.5 mg/l to 1.0 mg/l or more resulted in even decreased shoot length. The highest shoot length was obtained on medium without BA, whereas lowest was found at mg/l BA.

Culture appearance.

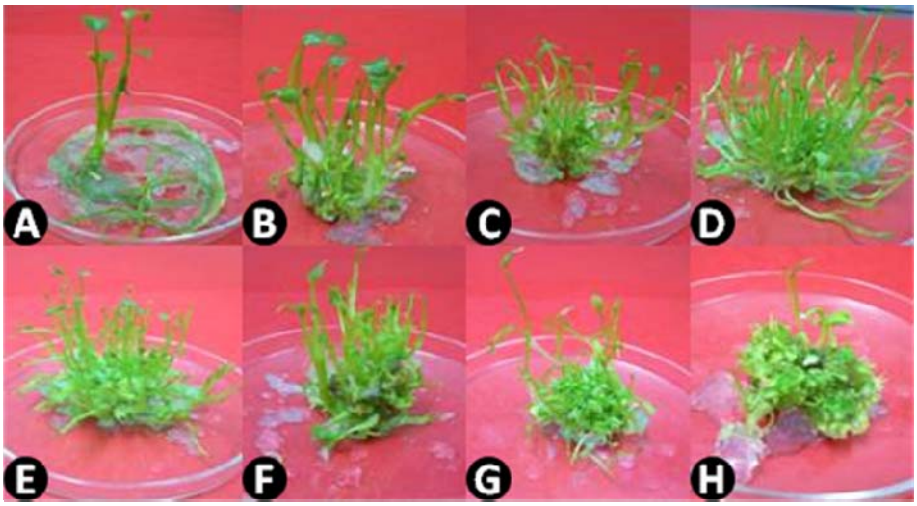

Figure 2. Multiple shoots of *Anthurium plowmanii* cv. Wave of Love cultured in vitro for 4 months as affected by benzyladenine (BA) concentrations. A = 0 mg/l BA, B = 0.5 mg/l BA, C = 1 mg/l BA, D = 1.5 mg/l BA, E = 2 mg/l BA, F= 2.5 mg/l BA, G =3 mg/l BA, and H = 5 mg/l BA.

Figure 2A-H showed shoot clusters formed at various concentration of BA. Culture of the control treatment (Figure 2A) showed only 2 shoots per explant, but the shoots had several roots and the highest in length. Cultures at BA- containing medium showed increased number of shoots, starting at 0.5 mg/l BA (Figure 2B) to 1.5 mg/l BA, which gave the maximum number of shoots per explant (Figure 2D). Then, upon further increase of BA from 2 to 5 mg/l in the medium, the shoot numbers and shoot lengths tended to decrease (Figure 2E-H). When one node cuttings from BA-containing medium were subjected to MS medium free of growth regulators with or without 2 g/l activated charcoal for 5 weeks, rooted plantlets were obtained (figures and data not shown). Most planlets were 3.5 - 4 cm in length, have 3 - 4 leaves and 4-5 roots.

Figure 3. Acclimatized *Anthurium plowmanii* cv. Wave of Love grown on potting media for 10 weeks

Plantlet acclimatization. Acclimatization was done by carefully planting well rinsed plantlets in 8 cm pot containing one mix of pot plant media consisting rice hulk charcoal: sand (1:1), or sand :

cocopeat (1:1), or rice hulk charcoal : sand : cocopeat (1:1:1). After 10 weeks, all plantlets were successfully acclimatized with 100 % survival rate (Figure 3).

RESULTS AND DISCUSSION

Plant propagation through in vitro techniques have been used widely for producing a large number of plants in a relatively short time. The procedure generally consists of several consecutive stages, each of which needs specific requirements, i.e., stage 0: selection of the source plants; stage 1: culture establishment; stage 2: shoot multiplication; stage 3: shoot elongation and rooting, and stage 4: hardening and acclimatization of plantlets (George, 1996). In this research, shoot multiplication, shoot elongation and rooting as well as acclimatization of *Anthurium plowmanii* cv. Wave of Love were studied.

Results obtained from this study clearly showed that addition of BA to the medium was essential for significant shoot proliferation. Without BA, almost no shoot proliferation was obtained, then by addition of low level of BA (0.5 mg/l) the shoot drastically increased by more than six fold. Effectiveness of BA for multiple shoot induction has also been reported by researchers in other plants such as banana (Anegra, 2008; Murad, 2008), pineapple (Hapsoro *et al*.,2006), and rose (Razavizadeh and Ehsanpour, 2008). Those plants have been successfully propagated through axillary branching that was also demonstrated in this present study.

Benzyladenine was also used to induce shoot multiplication of *Anthurium andreanum* cv Rubrun (Vargas *et al*., 2004). Vargas *et al.* (2004) reported that micro cuttings from 4 week-old *Anthurium* seedlings were inoculated on MS medium supplemented with 4.4 µM BA and 0.05 µM NAA to generate shoots for the source of explants. After 8 weeks of cultures, callus was formed at the stem based, and when callus segments were transferred to MS medium supplemented with 8.9 M BA + 2.7 M NAA, average of 6.6 small plantlets per 1 x 1 cm callus fragment were produced in 6 weeks.

Unlike report by Vargas *et al.* (2004) in which shoot multiplication occurred through callus formation, in this present study shoots underwent multiplication via axillary branching. This path of regeneration should be more suitable to produce planting materials with more trueness-to-type compared to that undergoing callus formation. This is because regeneration via axillary branching would result in plants with higher genetic fidelity. It should be also noted that efficient propagation has been obtained in this study because not only high shoot multiplication rate (28 shoots per explants) was achieved but also many nodal explants were produced for next cycle of multiplication because each shoot had at least 2 nodes.

To obtain plantlets which are ready to be acclimatized, one node cuttings from BA-containing medium must be transferred to the same basal medium without plant growth regulator, either with or without 2 g/l activated charcoal. In 6 weeks, most planlets were 3.5 - 4 cm in length, have 3 - 4 leaves and 4-5 roots. Activated charcoal has been widely used in plant tissue culture media for several advantages, namely to increase rooting, to absorb compounds exuded by cultured tissues or present in agar that would otherwise inhibit growth, and to promote embryogenesis (George, 1996). In this study, addition of activated charcoal to the medium resulted in increased root number of plantlets. However, the increase of root number in plantlets did not seem to affect survival rate of plantlets during acclimatization. When acclimatization was done by carefully planting well rinsed plantlets in 8 cm pot containing one mix of pot plant media consisting rice hulk charcoal: sand (1:1), or sand : cocopeat (1:1), or rice hulk charcoal : sand : cocopeat (1:1:1), all plantlets were successfully acclimatized with 100 % survival rate.

CONCLUSION

Successful in vitro propagation of *Anthurium plowmanii* cv. Wave of Love has been achieved in this study. Best shoot multiplication (28 shoots/explant) was attained using MS medium supplemented with 3% sucrose, MS vitamines, 100 mg/l mio inositol, 0.7% agar and 1.5 mg/l benzyladenine (BA). Regeneration of rooted shoots was obtained by culturing one node cuttings on MS basal medium without growth regulator for 6 weeks. Plantlets were successfully acclimatized with 100 % of survival on potting medium containing one mix of : rice hulk charcoal: sand (1:1), or sand : cocopeat (1:1), or rice hulk charcoal : sand : cocopeat (1:1:1).

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