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The Effect of Plant Growth Regulators on Micropropagation of *Anthurinum andreanum* Linden

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ABSTRACT

Anthurium andreanum Linden plant species which belong to Araceae family has high commercial value. In this research, two varieties of A.andreanum have been micropropagated from calli by tissue culture techniques instead of classic production to increase the production. Plant regeneration of A.andraeanum varieties "Red Love" and "Pink Champion" have been achieved through adventitious shoot formation from calli. Leaf and petiol explants were harvested from mature certificated plants which were grown in the pots. The best combination for calli biomass production was obtained with combination of 0.1mg/L 2,4-D and 1.5mg/L BAP. Best shoot regeneration capacity was calculated as 86% per explant in the half strength MS medium which was supplemented with 0.5 mg/L BAP for 'Red Love' variety. As a result of our research, in vitro optimization of "Red Love" and "Pink Champion" varieties have been realized in tissue culture conditions. Furthermore, micropropagation of Red Love variety was successfully achieved during our experiment.

Keywords: *Anthurium andreanum*, Red Love, Pink Champion, Micropropagation, tissue culture.

INTRODUCTION

Ornamental plants have increased market share in recent years. Some of ornamental plants can easily produce in their natural environment without any reproduction barrier, while some of them does not produce cause gathering of the people and environmental factors. Tissue culture technics were used widely for micropropagation of plants. In the global market Anthurium species have second rank after orchids **[1,2].** Anthurium species constitute 27% of the Araceae family **[3].** It belongs to *Anthurieae*, the largest one of the six orders of subfamily *Pothoideae*. This subfamily is characterized by thin reticular vein structures on the leaves, geniculate stems and perfect flowers **[4, 5, 6, 7, 8, 9].** This economically significant subfamily includes approximately 1500 tropical species **[10].** 1 mg/L BA, 0.08 mg/L 2,4-D were used in a research conducted on ten cultivars of *Anthurium* species. After one hundred days the greatest callus development was observed in *Pistache* cultivar **[11].** 0.5 mg/L BAP was used as shoot induction medium in another research where 1 mg/L BAP and 0.08 mg/L 2,4-D ile ½MS were used as an callus induction medium for leaf explants of *A. andreanum* plant **[12].** Shoots were cultivated from "*Arizona*" and "*Sumi*" leaf calli of *A. andraeanum* plant. Callus induction medium was comprised of 0.6 mg/L 2,4-D, 1 mg/L BA and ½ strength MS basal salt. Root medium was supplemented with 1mg/L IBA and 0.04% active carbon **[13].** In a study where MS medium added with 3 mg/L NAA was used for callus development of *A.andreanum* plant, it was found that maximum shoot development was achieved an MS medium comprised of a combination of 0.5 mg/L NAA and 2 mg/L 2 IP. The shoots were rooted in ½ strength MS medium supplemented with 2 mg/L IBA **[14].** A study with petiol explant was conducted in order to stimulate callus formation and measure organogenesis in *Casino* and *Antadra* varieties of *A. andreanum* plant. It is observed that the best result in callus stimulation was obtained with

0.5 mg/L NAA+3 mg/L BA in 65 days, and the best result for shoots was obtained with a medium of 0.01 mg/L NAA+1 mg/L BA **[15].**

In scope of our research, healthy calli has been developed from leaf and petiol explants of Red Love and Pink Champion varieties of *A. andreanum* species. Micropropagation has been performed by cultivation of shoots from calli and in MS medium supplemented with various combinations and concentrations of plant growth regulators. In the result of this research, *in vitro* optimization and abundant cultivation of *Red Love* variety have been achieved.

MATERIALS AND METHODS

Plant Material

Certified mature plants of *A. andreanum* were used as plant material. Red Love and Pink Champion varieties of have been provided from Buse Flowering Company.

Explant source and surface sterilization

A. andreanum leaf explants were excised from two years old mature plants. Surface sterilization were realized with 70% ethanol for 1 minute and 20% sodium hypochlorite (containing 5% active chlorine) for seven minutes then replaced to the 0.1% (v/v) tween-20 for 20 minutes, after surface sterilization process explants were rinsed three times with sterilized distilled water.

Preparation of Plant Growth Medium

MS nutrient mediums supplemented with various concentrations of 2-4D, NAA and BAP have been used as callus stimulation mediums to develop calli from leaf explants of *A.andreanum* plant.

In vitro Culture Conditions

Red Love and Pink Champion varieties leaf explants were excised from two years old mature plants of *A. andreanum* and were placed to the nine different testing series with tree replicates in petri plates. Explants were cultured in petri plates, containing MS medium, with 3% (w/v) sucrose and 0.8% (w/v) agar (pH:5.75). Petri plates were incubated at 27±2°C and 16/8 photoperiod with 72µmolm^{-2}s^{1}. MS medium supplemented with different concentrations of 2,4-D, NAA and BAP.

Calli Induction from Leaf Explants

Leaf explants (1x1 cm) and petiols (1 cm) were cultured on MS supplemented with different concentrations of 2,4-D (0.1, 0.2, 0.6, 1 mg/L), NAA (2, 4, 5 mg/L) and BAP (1, 1.5, 2, 2.5, 3 mg/L) and MS0 for callus induction. Six explants in per petri dishes were used for each trial with three replicates. Twenty petri was used for each three replication. Petri plates were incubated at 27±2°C during four weeks **[16, 17]** in the beginning of fifth week petri plates were incubated at 25±2°C and 16/8 photoperiod with 72µmolm^{-2}s^{1} in the plant growth chamber conditions. After five weeks, formed calli were subcultured in every four weeks on MS medium containing initial hormone levels for calli production. Subculturing have been realized in the VFSS 1206 Dan-Laf Laminar Flow. Development of calli were photographed with Olympus SP800-UZ and were observed with SZ51 stereo microscope.

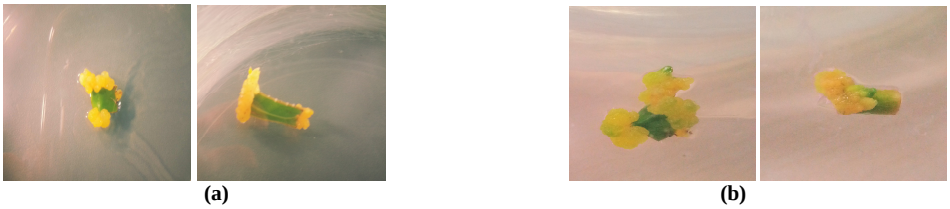
Shoot Proliferation from Calli

Best shoot regeneration capacity were calculated as 86% for per explant in the half-strength MS medium which supplemented with 0.5 mg/L BAP for 'Red Love' variety.

RESULTS AND DISCUSSION

Calli Induction Results

Best callus induction medium has been obtained in the MS medium which supplemented with 0.1mg/L 2,4-D and 1.5mg/L BAP. Pink Champion variety has been showed weak callus induction capacity eight weeks after incubation. Because of this reason calli induction experiments have been continued with Red Love variety (Figure 1).



(a) **(b)**

Figure 1. Calli induction in Red Love variety ten weeks (a) and 13 weeks (b) after transferring. MS medium supplemented with 0.1mg/L 2,4-D and 1.5mg/L BAP

Seedling Induction Results

For the shoot production experiments, thirteen weeks calli which obtained from Red Love variety has been transferred to the new medium. In this stage, passing from yellow to greenish in the calli were significant. Main organ was not absent in this stage. Only young shoots have been observed. In six weeks shoots have been shown in Figure 2.

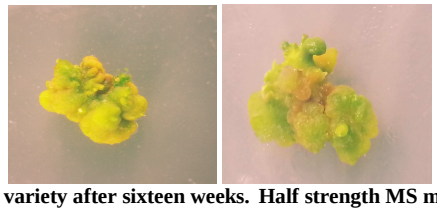


Figure2. Shoot primordium in Red Love variety after sixteen weeks. Half strength MS medium supplemented with 0.5 mg/L BAP

In scope of tissue culture studies, developing calli from each explant were compared in regard of their shoot forming potential. In these comparisons, calculation of incidence of shoot formation in per explant have been made based on 20 petri plates which is carrying five explants in each petri plates. For calculation, ten weeks old *Red Love* shoot primordium was developed in the medium which supplemented with 0.5 mg/L BAP ½ MS. And shoot primordium have been counted one by one. Shoots were showed excessive development in the eac\h petri plates. As a result of our calculations, the shoot percent for per callus have been found as 86%. In the tenth week the seedlings were develop from shoot primordia (Figure 3).

(a) **(b)** **(c)**

Figure 3. Seedlings in tenth (a), thirteenth (b) and eighteenth weeks (c) in Red Love variety. Half strength MS medium supplemented with 0.5 mg/L BAP

Acclimatization of In Vitro Seedlings

In vitro developed seedlings have been transferred to the magenta containers which is containing sterilized turf:perlite mix in 3:1 ratio. It has seen that the seedlings have healthily adapted to the in vivo conditions (Figure 4).

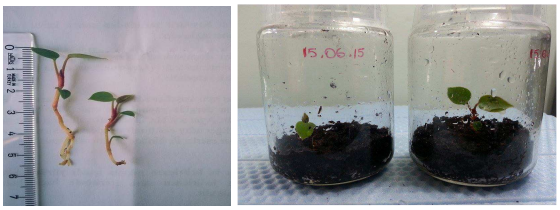


Figure 4. Acclimatization of twentieth weeks *Red Love* seedlings

DISCUSSION

In scope of our study, micropropagation of *Red Love* variety has been completed successfully. However, calli stimulation in *Pink Champion* variety has been found very weak despite of trying the series of various plant growth regulators. Because of that, in vitro developing process could not be forward to the shoot and micropropagation stages. At the previous studies on *A. andreanum* species it was found that different varieties of this species display different *in vitro* potentials. In this regard our study has similar results to previous studies.

In another study where callus development from leaf explants of *A. andreanum* plant a MS medium containing 0.05 mg/L NAA and 0.8 mg/L BA was used for shoot development. Furthermore, MS medium containing 0.1 mg/L NAA or IBA have provided successful results in root development **[18].** In one of the research has been performed on *Anthurium* cultivars the study was performed in a full MS medium containing 1 mg/L BA, 0.08 mg/L 2,4-D plant growth regulators. One hundred days after application the greatest calli development was only observed in one variety, while some of the varieties does not showed any calli development **[11].** In the another research, half strength MS medium which supported with 1 mg/L BAP and 0.08 mg/L 2,4-D was used as the medium for calli stimulation in the leaf explants of *A.andreanum* plant. As a result of this research best shoot stimulation was found in the MS medium supplemented with 0.5 mg/L BAP **[12].** In the other research, best results for callus development

has been found in the MS medium which supplemented with 1 mg/L BAP and 0.5 mg/L 2,4-D. The best shoot stimulation medium was found as MS medium which supplemented with 0.3 mg/L BAP **[20].** In conclusion of our research, successful shoot development have been achieved by use of half strength MS medium which supplemented with 0.5 mg/L BAP as the most suitable medium for shoot development from calli. These results are similar to results of the Bejoy study. Leaf explants of "*Arizona*" and "*Sumi*" varieties of *A.andraeanum* species were used to develop calli, and these were used to develop shoots. Callus stimulation medium was comprised of 0.6 mg/L 2,4-D, 1 mg/L BA and half strength MS basal medium. It was found that the number of shoots developed with *Arizona* variety were larger than the number of shoots developed with *Sumi* variety **[13].** In scope of our study, shoot development ratio has been found as 86% in the tenth week of shoot primordia in *Red Love* variety. This shoot development half strength MS medium were supplemented with 0.5 mg/L BAP.

CONCLUSION

Conclusions of our study are important in its focused on the micropropagation of economically important plants heavily preferred as decorative plants and its emphasis on development of seedlings of commercially significant decorative plants by further development of acclimatization methods. With this research it is shown that how to produce ornamental plant seedlings quickly with the *in vitro* micropropagation methods.

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