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Research Article
Effect of Growth Regulators on *Begonia* sp. Julau Propagation

[1]Penkhae Rungrueng, [2]Petcharat Chuntaratin and [3]Kiriya Sungthongwises

[1]Kasertsart Program, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok, Thailand
[2]Department of Agricultural Technology, Faculty of Science and Technology, Bangkok Thonburi University, Bangkok, Thailand
[3]Agronomy Section, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

Abstract

Background and Objective: *Begonia* sp. Julau is an ornamental plant that has attractive leaves and flowers. Importing the Julau begonias effects the survival of plant and difficult for transplanting in natural condition. Therefore, this research aimed to increase the number of *Begonia* sp. Julau by the plant tissue culture technique. **Materials and Method:** Research on asexual propagation of *Begonia* sp. Julau started by searching for a suitable part to sterilize. Murashige and Skoog (MS) medium was used to investigate shoot and root induction with the different concentrations of Benzyl Adenine (BA) and Indole-3-Butyric Acid (IBA). **Results:** The results showed that MS medium supplemented with 0.4 mg L^{-1} of BA induced a maximum number of shoots (41 shoots) within 4 months. MS medium with 0.5 mg L^{-1} of IBA induced longer roots than MS medium without plant growth regulator within 2 months. **Conclusion:** The leaves are a suitable part of *Begonia* sp. Julau for plant tissue culture. The survival rates were 100% after transplanting with sphagnum moss in plastic box conditions.

Key words: *Begonia*, auxin, cytokinin, growth, propagation, tissue culture

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Correspon ding Author: K. Sungthongwises, Agronomy Section, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand Tel: 00 66 090-3241635

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Thailand, the *Begonia* sp. is called Som koong, which is estimated to have about 60 species[1]. Begonias are popular flowering and ornamental plants to grow and export, due to the beautiful, colourful leaves and flowers[2] (Fig. 1). Generally, *Begonia* species are propagated by stem and leaf cuttings. Their vegetative propagation can be improved by application of micropropagation techniques to produce more number of adventitious shoots[3]. Currently, *Begonia* sp. Julau has been imported at an expensive price. After importing, *Begonia* sp. Julau often encounters problems of weakness, dying easily, making them difficult to grow in natural conditions. Generally, the propagation of begonias could be done with a seedling, shoot and leaf cuttings and plant tissue culturing, which is a technical method to produce a large number of plants in a short time. Micronutrients of Heller medium and macronutrients of Murashige and Skoog medium (MS) supplemented with BA induced shoots in *B. erythrophylla*, whilst supplementing with IBA, or without any growth regulator supplement, induced roots[4]. Tissue culturing of *B. tuberhybrida* leaf parts can promote 90% of shoots with a maximum number of 132 shoots per piece, in MS medium with Naphthaleneacetic Acid (NAA) and Thidiazuron (TDZ). The petiole provided 82% of shoots, with a maximum number of 33 shoots per piece in MS medium with NAA and TDZ. For root induction, MS medium with NAA can induce roots within 3 months[5]. The maximum number of shoots (44.33 shoots per piece) of *B. rex* Putz. was induced by MS medium with BA and NAA[6]. Also, MS medium with Benzyl Amino Purine (BAP) and NAA induced 20.8 shoots in *B. fallax*, with the highest average shoot height of 4.0 cm. For root induction, it was found that MS with NAA produced root growth[3]. In Thailand, pods have been found to be the most suitable parts for sterilization and propagation of *B. darthvaderiana*, in MS medium with BA. The shoots can be induced to root and can be transplanted in conditions of high humidity systems with 100% of the organisms surviving. To produce *Begonia* sp. Julau as a commercial ornamental plant in the future, tissue culture of *Begonia* sp. Julau need to investigate the suitable concentration of growth hormones for shoots and roots induction. The research objective was to investigate the effect of BA and IBA at different concentrations for asexual propagation of *Begonia* sp. Julau, by the plant tissue culture technology.

Fig. 1: *Begonia* sp. "Julau"

MATERIALS AND METHODS

Study area: The experiment was conducted at the Tissue Culture Laboratory, Agriculture and Agricultural Technology Section, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Thailand from January, 2019-March, 2020.

Disinfecting *Begonia* sp. "Julau": The study was laid out in a Completely Randomised Design (CRD), with four replications. Different parts of *Begonia* sp. Julau, such as leaves petiole leaves, shoot tips, lateral buds and nodes were washed by allowing water to flow through the plant parts for 30 min and then were soaked with 70% alcohol for 1 min. They were transferred to 5 and 10% sodium hypochlorite mixed with 2 drops of Tween-20® for 5 and 10 min, respectively, to sterilize; then the plant parts were immersed in 0.1% $HgCl_2$(w v^{-1}) for 2 min and then washed with sterile water four times, before moving to culture on MS medium[7] without growth regulators. They were cultured at 25±2°C, under 30 m mol m^{-2} s^{-1} of photon flux from a cool white fluorescent lamp for 16 h day^{-1}. The survival percentage of the *Begonia* sp. Julau parts was recorded.

Shoot propagation of *Begonia* sp. "Julau": The MS medium containing BA 0 (control), 0.1, 0.2, 0.3, 0.4 and 0.5 mg L^{-1} with 30 g L^{-1} of sucrose and adjusted to pH 5.7 were used for shoots propagation of the *Begonia* sp. Julau. They were cultured at 25±2°C, under 30 m mol m^{-2} s^{-1} photon flux from a cool white fluorescent lamp for 16 h day[1]. They were sub cultured to a new medium every month, with the growth characteristics and numbers of shoots being recorded within 4 months.

Root induction of *Begonia* sp. "Julau": For root induction, the shoots from experiment 1 were cultured on MS medium, both without growth regulator and with 0.5 mg L^{-1} of IBA culture added, otherwise under the same conditions as experiment 1, with four replications within 2 months. The percentage of

shoots forming roots was recorded and analysis of variance was conducted on data obtained for each parameter in each treatment. All analyses were calculated at a significance level of 0.05 to test for significant differences among treatments. The plants with shoots and roots were transplanted in natural conditions in sphagnum moss and the percentage of survival after transplanting was recorded.

Statistical analysis: An analysis of variance was conducted on data obtained for each parameter in each treatment. All analyses were carried out using Statistics software, version 8.0. Duncan's Multiple Range Tests (DMRTs) were calculated at a significance level of 0.05 to test for significant differences among treatments.

RESULTS

Suitable parts of *Begonia* sp. "Julau" for tissue culture: After sterilizing the different parts of *Begonia* sp. Julau and transferring them onto MS medium without growth regulator, the results showed that 90% of *Begonia* sp. Julau nodes were not infected by microorganisms, followed by 85.40 and 77% of petiole and leaves, respectively, whilst the lateral buds exhibited a significantly lower percentage of the explants free from microbes (Table 1). When these tissues were cultured on MS medium with 0.2 mg L^{-1} of BA, it was found that the leaf (Fig. 2a), shoot tips (Fig. 2b) and lateral buds (Fig. 2c) were able to grow and create new shoots, whilst other parts could not create new shoots; those tissues became brown and died. The leaf part was selected to do the shoot propagation, according to show the highest percentage of free from microbes.

Shoot propagation of *Begonia* sp. "Julau": Leaf part was able to regenerate multiple shoots. Therefore, formula to increase the number of shoots induced by MS medium containing different concentrations of BA was studied. The results showed that the multiple shoots grow and develop as normal *Begonia* sp. Julau plants. The MS medium with 0.1, 0.2, 0.3, 0.4 and 0.5 mg L^{-1} of BA could be used to increase the number of shoots (Fig. 3b-f) when compared with the control (Fig.3a) and there was a significant difference; the use of BA at 0.3, 0.4 and 0.5 mg L^{-1} had the highest significant difference from the numbers of shoots produced by the other concentrations of BA (41, 41 and 40 shoots, respectively) (Table 2) (Fig. 3d-f). However, MS medium with 0.5 mg L^{-1} BA produced a little shoots and leaves which were different from the normal *Begonia* sp. Julau pattern (Fig. 3f).

Table 1: Percentage of explants free from microbes; whether new shoots were generated after disinfection

Explant	% Explant free from microbes[1]	Shoots generated
Leaf	77.00[b]	no
Petiole	85.40[a]	no
Shoot tip	62.50[b]	yes
Lateral bud	45.80[c]	yes
Node	90.00[a]	no
CV (%)	31.60	
F-test	**	

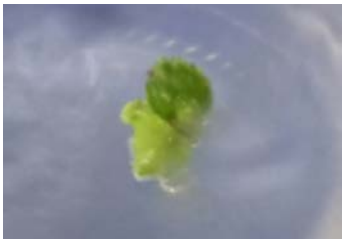
[1]Means with the same letter within a column are not significantly different by the DMRT at P £ 0.05, ** = Significant difference at p£0.01

Table 2: Number of shoots of *Begonia* sp. "Julau" after culturing explants on MS medium with different concentrations of BA for 4 months

Concentration of BA (mg L^{-1})	Number of shoots[1]
0 (control)	10.50[c]
0.1	29.80[b]
0.2	30.80[b]
0.3	41.30[a]
0.4	41.40[a]
0.5	40.80[a]
CV (%)	34.70
F-test	0

[1]Means with the same letter within a column are not significantly different by the DMRT at p £ 0.05, * = significant difference at p£0.05, BA: Benzyl Adenine

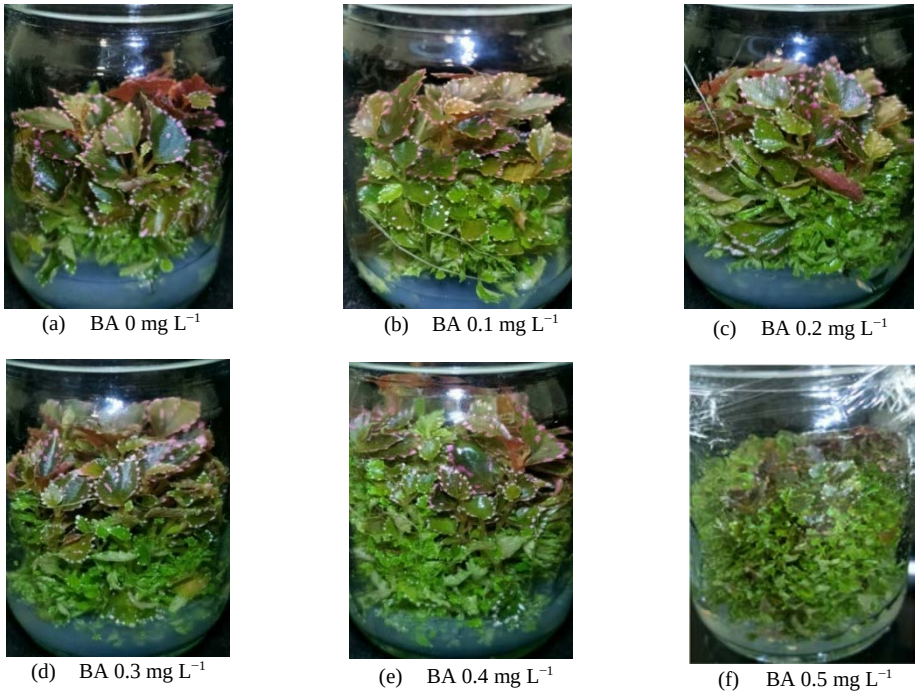
(a) Leaf



(b) Shoot tip

(c) Lateral bud

Fig. 2(a-c): Explants of *Begonia* sp. "Julau" inducing new shoots, regenerated after culturing on MS medium with 0.2 mg L^{-1} of benzyl adenine



(a) BA 0 mg L^{-1} (b) BA 0.1 mg L^{-1} (c) BA 0.2 mg L^{-1}

(d) BA 0.3 mg L^{-1} (e) BA 0.4 mg L^{-1} (f) BA 0.5 mg L^{-1}

Fig. 3(a-f): Shoot characteristics of *Begonia* sp. "Julau" after culturing on MS medium with different concentration of BA for 4 months
BA: Benzyl Adenine

(a) IBA 0 mg L^{-1} (b) IBA 0.5mg L^{-1}

Fig. 4(a-b): Root formation of *Begonia* sp. "Julau" on MS medium with 0 and 0.5 mg L^{-1} of IBA within 2 months
IBA: Indole-3-Butyric acid

Root induction of *Begonia* sp. "Julau": When shoots were cultured on MS medium with 0 and 0.5 mg L^{-1} of IBA for 2 months, 100% of the shoots had roots induced (Fig. 4a-b). However, MS medium with 0.5 mg L^{-1} of IBA provided a root length longer than MS medium without growth regulator (Fig. 4b). When the shoots were transferred to a plastic box with sphagnum moss and moisture, 100% of them survived (Fig. 5).

Fig. 5: *Begonia* sp. "Julau" characteristics after transplanting in plastic box condition

DISCUSSION

The process of plant sterilizing is important for imported *Begonia* sp. propagated by tissue culture since most parent plants are removed from the forest, which may contain many microbes. Also, transportation that uses inappropriate packaging may affect the plants' health and presents a chance of easy infection by pathogens. The selection of appropriate plant parts for the aseptic technique, free from microbes, depends on the plants' health characteristics, freedom from pathogens and the use of appropriate sterilization methods.

Most previous tissue culture research on *Begonia* sp. used the leaves or petiole leaves[8,9,10]. The *B. darthvaderiana* pod is the most suitable plant part to increase multiple shoots by tissue culture, according to the seeds inside. However, with imported begonias, there is little chance of their having a pod. From the current experiment, the leaves, shoot tips and lateral buds were suitable parts, when coupled with the use of appropriate growth regulators, for the rapid propagation of *Begonia* sp. Julau. In the experiment, BA is a plant growth regulator in the cytokinin group which is inexpensive compared to other hormones, especially for commercial propagation. The use of BA at 0.3 and 0.4 mg L^{-1} tend to produce the highest numbers of *Begonia* sp. Julau shoots (41 shoots). Although the MS medium with 0 and 0.5 mg L^{-1} of IBA could be able to induce *Begonia* sp. Julau root, means the producer can save cause of using IBA by MS medium without growth regulator. However, MS medium with 0.5 mg L^{-1} of IBA stimulates roots growth to grow to a length adequate for planting in a shorter time than 2 months as provided a root length longer than MS medium with 0 mg L^{-1} of IBA. To transfer *Begonia* sp. Julau from a tissue culture bottle to grow in a natural environment, it should first be grown in a container with a high humidity for 7–14 days, as is the case with *B. darthvaderiana*, in order to improve the strength of *Begonia* sp. Julau. Different to the *in vitro* plant regeneration from leaf and petiole explants of *B. rubro venta* var. meisneri C.B. Clarek, a maximum 65 shoots/petiole were formed on MS medium supplemented with 0.1 mg L^{-1} TDZ. The *in vitro* raised shoots formed roots in MS medium containing 0.1 mg L^{-1} indole 3-acetic acid (IAA), with a maximum of 13.80 roots/shoot. About 73.33% of the 500 plantlets transferred acclimatized successfully within 4 weeks in a glasshouse. On being transferred to the field, all the acclimatized plantlets survived after 8 weeks[11]. On complex regeneration variant V1 – mineral basic medium culture MB - MS supplemented with 1 mg L^{-1} BA, the rooting process was absent, but according to this carried out research, plantlets were obtained by rooting the elongated shoots on MS basic mineral media containing 1 mg L^{-1} IBA, respectively a basic mineral medium culture without growth regulators, producing a much better organogenesis, where the phenomenon was greater in rooting process[4]. However, *Begonia* sp. Julau is still difficult to transfer under natural conditions, therefore a very high relative humidity. In addition keeping color level of the dark pink leaves must be raised in a condition with a temperature cooler than 25 °C.

CONCLUSION

For the propagation of *Begonia* sp. Julau by tissue culture, shoot tips and lateral buds are suitable parts for success in disinfecting and generation shoots. MS medium with BA at 0.4 mg L^{-1} is the most suitable formula for producing the highest number of shoots (41 shoots). MS medium with IBA 0.5 mg L^{-1} provides longer roots than MS medium without growth regulators.

SIGNIFICANCE STATEMENT

This study discovered the suitable parts of *Begonia* sp. "Julau" and growth hormones that can be beneficial for asexual propagation of *Begonia* sp. Julau by tissue culture technique. This study will help the researchers to uncover the critical areas of *Begonia* sp. "Julau" shoot and root induction for commercial ornamental plants that many researchers were not able to explore. Thus a new theory on an effect of temperature to increase colour level of the leaves may be arrived at"

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