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# *In vitro* Regeneration of Wild *Begonia palmata* D. Don from the Sikkim Himalayas

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## ABSTRACT

This study attempted to establish an *in vitro* protocol for *Begonia palmata* a wild *Begonia* species native to the Sikkim Himalayas, by employing direct organogenesis with leaf and petiole explants. Shoot buds were directly induced from the cut end of the explants and 80-90 % explants were observed devoid of fungal contamination by using surface disinfectants viz. 15 % NaOCl, 70 % ethanol, and 0.1 % $HgCl_2$ for 30 sec. Shoot initiation was observed after 25.80 ± 1.20 days of culture, explants exhibited adventitious shoots and progressed into the early stage of shoot development. The mean number of shoots per explant and number of plantlets per explant was found higher in MS + 2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA from leaves explant. The maximum shoot length (6.44 ± 0.28 cm) was recorded in MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA from leaves explant. Whereas, MS + 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA in petiole explant recorded better root initiation and length results. The protocols described here will be valuable for disease-free propagation, commercial ornamental cultivation, *ex situ* conservation, and genetic transformation of *B. palmata.*

**Keywords** Begonia, Conservation, Micropropagation, Ornamental plant.

## INTRODUCTION

*Begonia palmata* D Don. a member of Begoniaceae family is a monoecious, wild species found in various regions of the North Eastern states of India in general, Sikkim in particular. The species is recognized for its ornamental value due to its phenotypically different variants within the species level and its medicinal properties (Bhattarai and Rana 2020). Its attractive foliage with pinkish-white flowers makes it an excellent choice for ornamental plants. To conserve the wild and economically important species of Sikkim Himalaya an attempt has been made to develop *in vitro* micropropagation protocols for conserving the wild floras and potential introduction in floriculture markets.

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Ornamental plants are in high demand in both domestic and foreign markets and have significantly increased in recent decades. In developed countries, *in vitro* multiplication played a great role in propagating various ornamental plants such as *Ficus, Begonia, Saintpaulia, Chrysanthemum, Rosa, Anthurium,* and *Spathiphyllum* were micro-propagated on a large scale. Likewise, most of the indoor ornamental species were mass propagated through tissue culture techniques to eliminate the diseases and production of true-to-type planting materials (Aswathy and Murugan 2019). These propagation protocols are indeed applicable and beneficial to species that are difficult to propagate, and also economically important even for those species that are propagated easily (Awal *et al.* 2013).

*In vitro* regeneration and micropropagation of *Begonia via* organogenesis, meristem culture, somatic embryogenesis, and thin cell layer technology have been investigated by various workers (Mendi *et al.* 2009, Awal *et al.* 2013, Lai *et al.* 2018, Aswathy and Murugan 2019, Nabieva *et al.* 2019). However, not much reported on *B. palmata* despite its commercial importance. Plant tissue culture techniques are always required to meet the demand for potted/indoor plants in floriculture industries and are widely applied in both the research and development of improved crops (Maliro and Lameck 2004). Tissue culture techniques are used to propagate over 156 ornamental genera worldwide (Rout and Jain 2020).

This study aimed to develop a simple, efficient, and rapid *in vitro* plant regeneration system using leaf and petiole explants for conservation, *in vitro* gene banking, and commercial propagation. We report a highly efficient protocol for *B. palmata* regeneration via direct shoot bud formation (without a callus phase), achieving complete plantlet regeneration within 3-4 months.

## MATERIALS AND METHODS

### Plant materials and explant preparation

*B. palmata* D. Don plants were collected from their natural habitat in the Sikkim Himalayas. During the experimental period, these plants were maintained in the department polyhouse. Disease-free and healthy explants were selected for tissue culture. Generally, petioles and leaf discs were used to determine the optimal explant for *in vitro* regeneration of Begonia. The medium was sterilized by autoclaving at 121°C (15 psi) for 15 minutes. Sterilized media were stored in the culture room and used 3-4 days after preparation. The culture room was maintained at 23 ± 2°C with air conditioning, a 16-hour light/8-hour dark photoperiod, and racks fitted with white fluorescent tubes providing 1000 lux. A laminar airflow cabinet was located in a separate, enclosed room.

The explants were initially surface sterilized for 30 minutes under running tap water. Continuous stirring with distilled water containing two to three drops of Tween 20 for 15-20 minutes. Explants were immersed with 15% sodium hypochlorite mixed with two drops of Tween 20 for 20 minutes and then rinsed five times with sterile distilled water. Finally, the explants were rinsed with 70 % ethanol and followed by rinsing five times in sterile distilled water. After that explants were treated with 0.1% mercuric chloride solution for 30 seconds and again rinsed the explants five times with sterile distilled water. Each rinsed time lasted approximately one minute.

### Preparation of PGR's stock solutions

The stock solutions were prepared by dissolving auxin 100 mg (NAA) in a few drops of absolute alcohol and then volumes were made to 100 ml. Likewise, the stock solutions were prepared by cytokinin (BAP) 100 mg by dissolving in a few drops of 1N NaOH and final volumes were made to 100 ml with distilled water. Both hormones contained 1.0 mg $ml^{-1}$. Stock solutions of PGR's were stored in a refrigerator at 4º C in the liquid phase.

### Direct organogenesis

MS media supplemented with different combinations and concentrations of NAA and BAP were used for shoot and root formation. By using combinations of NAA and BAP, the explants from intact plants were cultured in the selected media. All media contained 3.0% (w/v) sucrose and 0.8% (w/v) agar. The pH was adjusted to 5.8 before autoclaving. Leaf discs

and petiole segments were cultured in the media supplemented with combinations of NAA and BAP. There were 24 treatments in this experiment, with combinations of three levels of NAA (0.1, 0.5, and 1.0 mg $l^{-1}$) and five levels of BAP (0.1, 0.5, 1.0, 1.5, and 2.0 mg $l^{-1}$) including one control with five replications per treatment. Cut leaf discs (1cm × 1cm) were cultured in the medium and petioles of 1cm were cultured in sterilized plankton, petridish, and test tubes containing MS media with combinations of NAA and BAP. Cultures were sub-cultured every two weeks and parameters were recorded.

### Statistical analysis

The experiments were set up in Completely Randomized Design. For data analysis, each treatment consisted of five replications. Statistical analysis was done with the use of ANOVA and Duncan's Multiple Range Test (determination of the significance of results with the use of LSD). R software 4.1.2 was used for statistical calculations.

## RESULTS AND DISCUSSION

During the investigation, it was observed that the explants treated with 15% sodium hypochlorite followed by 70% ethanol and devoid of mercuric chloride treatments resulted in profuse fungal contamination (90-100%). Adding mercuric chloride (0.1% for 30 sec) controlled moderate fungal contamination up to 80-90%. The effectiveness of different disinfectants has been reported by several authors on other plant species (Mahmoud and Al-Ani 2016, Kumari *et al.* 2017). The 84% survival rate of leaf disc explant was found maximum in MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA (Table 1). After a healthy explant's survival rate, explants were sub-cultured every week to avoid necrosis in the early stage of development. Similar reports on healthy survival rates have been reported by several authors on other plant species (Lai *et al.* 2018, Aswathy and Murugan 2019, Nabieva *et*

**Table 1.** Survival rate (%), days required for shoot initiation, and number of shoots per explant of *in vitro* micropropagation of *Begonia palmata* in MS media supplemented with different combinations of BAP and NAA (Mean values ± SE).

| Parameters/ Treatments | Survival rate % | | Days required for shoot initiation |
|---|---|---|---|
| | Leaf disc | Petiole | Leaf disc |
| MS + 0.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 8.00±4.90 h | 12.00±4.90 f | 89.60±2.93 a |
| MS + 0.1 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 12.00±8.00 gh | 12.00±8.00 f | 78.40±2.64 b |
| MS + 0.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 24.00±4.00 efgh | 24.00±4.00 ef | 71.40±3.17 bc |
| MS + 1.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 20.00±6.32 fgh | 28.00±4.90 def | 56.80±2.13 efg |
| MS + 1.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 28.00±4.90 efgh | 32.00±8.00 def | 58.20±2.91 def |
| MS + 2.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 32.00±8.00 efg | 36.00±7.48 de | 49.00±1.30 hi |
| MS + 0.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 32.00±4.90 efg | 36.00±4.00 de | 65.20±3.17 cd |
| MS + 0.1 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 44.00±11.66 cde | 48.00±14.97 bcd | 57.20±2.11 efg |
| MS + 0.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 40.00±10.95 def | 36.00±7.48 de | 52.40±4.28 fgh |
| MS + 1.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 64.00±9.80 abc | 64.00±7.48 abc | 49.40±2.11 hi |
| MS + 1.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 56.00±7.48 bcd | 64.00±7.48 abc | 48.40±2.11 hi |
| MS + 2.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 78.00±4.90 a | 80.00±4.47 a | 33.80±0.86 j |
| MS + 0.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 36.00±7.48 def | 44.00±7.48 cde | 62.80±2.85 de |
| MS + 0.1 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 36.00±4.00 def | 48.00±4.90 bcd | 52.60±2.94 fgh |
| MS + 0.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 24.00±4.00 efgh | 32.00±4.90 def | 49.20±3.20 hi |
| MS + 1.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 80.00±6.32 a | 76.00±7.48 a | 25.80±1.20 k |
| MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 84.00±7.48 a | 84.00±7.48 a | 29.80±1.74 jk |
| MS + 2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 80.00±6.32 a | 84.00±7.48 a | 32.40±0.24 jk |
| MS + 0.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 44.00±7.48 cde | 48.00±10.20 bcd | 63.40±3.33 de |
| MS + 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 64.00±7.48 abc | 68.00±8.00 ab | 50.60±2.68 ghi |
| MS + 0.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 68.00±8.00 ab | 64.00±7.48 abc | 49.60±2.71 hi |
| MS + 1.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 36.00±7.48 def | 32.00±4.90 def | 44.20±0.86 i |
| MS + 1.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 28.00±.90 efgh | 48.00±10.20 bcd | 34.40±2.98 j |
| MS + 2.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 80.00±8.94 h | 76.00±7.48 a | 33.40±0.81 j |
| CD 5% | **20.24** | **21.10** | **7.02** |
| SEM | **7.21** | **7.52** | **2.50** |

**Table 1.** Continued.

| Parameters/ Treatments | Days required for shoot initiation | No. of shoots per explant | |
|---|---|---|---|
| | Petiole | Leaf disc | Petiole |
| MS + 0.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 119.40±10.16 a | 8.60±0.40 i | 5.80±0.80 m |
| MS + 0.1 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 93.80±7.60 b | 15.40±0.75 hi | 11.60±1.12 jklm |
| MS + 0.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 76.20±5.27 c | 17.60±1.86 ghi | 10.60±1.21 lm |
| MS + 1.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 53.40±2.14 def | 26.40±2.73 fgh | 19.80±2.27 ghijk |
| MS + 1.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 54.60±3.23 def | 24.60±2.42 gh | 21.20±3.01 fghi |
| MS + 2.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 48.80±1.59 ef | 29.20±2.44 fg | 22.80±1.43 efg |
| MS + 0.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 71.80±2.27 c | 15.20±1.56 hi | 10.80±1.53 klm |
| MS + 0.1 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 57.60±3.94 de | 22.40±1.17 gh | 12.60±1.86 ijklm |
| MS + 0.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 54.60±2.77 def | 26.20±2.22 fgh | 22.40±1.33 efgh |
| MS + 1.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 50.40±1.69 ef | 38.80±5.27 ef | 30.60±2.04 cde |
| MS + 1.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 49.40±2.50 ef | 38.60±2.14 ef | 29.20±1.83 def |
| MS + 2.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 34.20±0.97 h | 113.20±13.94 b | 72.20±3.75 a |
| MS + 0.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 57.20±2.63 de | 21.00±1.48 ghi | 17.60±2.44 ghijkl |
| MS + 0.1 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 52.40±2.58 def | 24.60±2.11 gh | 20.20±1.32 fghij |
| MS + 0.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 49.60±2.42 ef | 26.40±2.11 fgh | 23.20±2.42 efg |
| MS + 1.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 30.20±1.43 h | 62.20±3.51 d | 38.80±2.69 c |
| MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 30.60±1.36 h | 64.80±12.48 d | 53.60±5.26 b |
| MS + 2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 32.80±0.97 h | 132.40±3.50 a | 50.40±8.97 b |
| MS + 0.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 61.20±3.89 d | 21.40±1.03 ghi | 13.60±1.57 hijklm |
| MS + 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 51.60±2.32 def | 26.80±2.71 fgh | 18.00±2.02 ghijkl |
| MS + 0.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 46.40±2.52 fg | 29.80±2.35 fg | 23.60±1.60 efg |
| MS + 1.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 46.80±2.52 fg | 45.40±2.82 e | 29.20±2.35 def |
| MS + 1.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 37.40±3.04 gh | 65.20±6.52 d | 33.40±3.85 cd |
| MS + 2.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 34.60±0.75 h | 80.60±5.10 c | 68.80±7.04 a |
| CD 5% | **10.12** | **13.32** | **9.18** |
| SEM | **3.61** | **4.75** | **3.27** |

*al.* 2019, Nabieva and Fershalova 2023). Research indicated that combining auxin and cytokinin in the regeneration medium improved regeneration efficiency, with the optimal combination identified for Begonia (Nabieva and Fershalova 2023).

As shown in Table 1, after 25.80 ± 1.20 days in culture, the explants developed adventitious shoots and entered the early stage of shoot development. Adventitious shoots emerged directly from the entire explant surface. The auxin-to-cytokinin ratio plays a crucial role in early shoot initiation. Rosilah *et al.* (2014) suggested that a low auxin concentration and a higher cytokinin concentration induce shoot organogenesis in the *in vitro* regeneration of *B. pavonina.* The best results were obtained with a combination of BAP and NAA. Plant growth regulator (PGR) application is a key factor in controlling the transition between vegetative and reproductive phases in plants.

The maximum average number of shoots per explant (132.40±3.50) was found in MS+2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA from leaf disc explant. The correct ratio of auxins and cytokinin plays a crucial factor in early shoot initiation. Statistically significant differences between the treatments were recorded (Table 1). Similar findings were also reported by Kaviani *et al*. (2015), Kumari *et al.* (2017), and Shobi and Viswanathan (2017). Kabirnataj *et al.* (2012) reported the highest adventitious shoot regeneration with an average of 41.6 from leaf explant after 5 weeks of culture on MS media fortified with 1 mg $l^{-1}$ BAP and 0.5 mg $l^{-1}$ IBA. In the leaf disc explant, significantly the maximum shoot length (6.44 ± 0.28 cm) was recorded in MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA. It has been observed that shoot length increases as the rate of BAP increases with the increasing rate of NAA and such findings are in close agreement with the other authors (Kumari *et al.* 2017, Shobi and Viswanathan 2017, Lai *et al.* 2018) also reported a significant increase in shoot length on the application of combined BAP and NAA.

**Table 2.**. Shoot length (cm) and number of plantlets per explant of *in vitro* micropropagation *Begonia palmata* in MS media supplemented with different combination of BAP and NAA (Mean values ± SE).

| Parameters/ Treatments | Shoot length (cm) | | No. of plantlets per explant | |
|---|---|---|---|---|
| | Leaf disc | Petiole | Leaf disc | Petiole |
| MS + 0.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 3.04±0.23 e | 3.12±0.12 f | 5.20±0.37 k | 3.40±0.51 n |
| MS + 0.1 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 3.10±0.21 e | 3.16±0.11 ef | 12.60±2.48 ghij | 7.80±0.58 klm |
| MS + 0.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 3.16±0.23 e | 3.22±0.29 def | 12.40±1.21 hij | 7.60±1.08 klmn |
| MS + 1.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 3.20±0.19 e | 3.24±0.35 def | 21.20±1.69 def | 12.80±1.71 fghij |
| MS + 1.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 3.28±0.33 de | 3.26±0.28 def | 19.60±1.89 defg | 12.40±0.51 ghij |
| MS + 2.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 3.38±0.22 de | 3.34±0.23 def | 17.80±2.15 efghi | 11.20±0.66 ijkl |
| MS + 0.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 3.26±0.13 de | 3.28±0.22 def | 8.80±0.66 jk | 5.60±0.81 mn |
| MS + 0.1 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 3.18±0.21 e | 3.22±0.26 def | 11.60±0.81 ijk | 7.20±1.20 lmn |
| MS + 0.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 3.48±0.12 de | 3.58±0.17 def | 18.20±1.85 efghi | 12.20±1.20 ghij |
| MS + 1.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 3.68±0.15 de | 3.74±0.22 def | 21.20±2.48 def | 18.20±1.62 e |
| MS + 1.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 5.22±0.35 bc | 5.04±0.24 bc | 23.40±3.25 de | 13.80±0.58 fghij |
| MS + 2.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 5.48±0.30 b | 4.78±0.15 bc | 38.40±2.73 c | 26.60±2.62 d |
| MS + 0.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 3.40±0.27 de | 3.42±0.36 def | 14.40±1.29 fghij | 10.40±0.68 jkl |
| MS + 0.1 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 3.66±0.49 de | 3.78±0.15 de | 18.20±1.02 efghi | 14.80±1.07 efghi |
| MS + 0.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 3.60±0.31 de | 3.82±0.14 d | 19.20±2.03 defgh | 16.80±1.80 ef |
| MS + 1.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 4.66±0.24 c | 4.58±0.12 c | 52.40±6.07 b | 29.20±1.66 cd |
| MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 6.44±0.28 a | 4.82±0.25 bc | 55.20±2.33 b | 31.60±2.46 bc |
| MS + 2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 5.22±0.20 bc | 5.82±0.35 a | 69.40±5.04 a | 34.00±1.41 b |
| MS + 0.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 3.48±0.18 de | 3.56±0.20 def | 16.60±1.75 efghi | 11.80±1.24 hijk |
| MS + 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 3.54±0.18 de | 3.58±0.30 def | 19.80±1.59 def | 15.20±1.36 efghi |
| MS + 0.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 3.60±0.19 de | 3.66±0.20 def | 20.60±1.33 def | 16.40±2.01 efg |
| MS + 1.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 3.64±0.25 de | 3.76±0.21 def | 22.60±1.03 de | 13.40±0.75 fghij |
| MS + 1.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 3.92±0.25 d | 4.74±0.16 c | 26.00±3.16 d | 15.60±1.29 efgh |
| MS + 2.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 5.76±0.14 ab | 5.40±0.20 ab | 55.60±3.20 b | 43.20±3.15 a |
| CD 5% | **0.70** | **0.65** | **5.20** | **3.40** |
| SEM | **0.25** | **0.23** | **12.60** | **7.80** |

**Table 3.** Days required for root initiation and root length (cm) of *in-vitro* micropropagation of *Begonia palmata* in MS media supplemented with different combination of BAP and NAA (Mean values ± SE).

| Parameters/ Treatments | Days required for root initiation | | Root length (cm) | |
|---|---|---|---|---|
| | Leaf disc | Petiole | Leaf disc | Petiole |
| MS + 0.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 120.60±8.39 a | 113.00±5.30 a | 0.72 ±0.12 j | 0.84±0.09 i |
| MS + 0.1 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 93.20±5.29 b | 96.20±5.89 b | 1.22±0.09 ij | 1.12±0.09 i |
| MS + 0.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 89.60±2.98 b | 97.60±1.36 b | 1.34±0.07 ij | 1.22±0.34 hi |
| MS + 1.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 85.80±2.29 bc | 92.80±2.08 b | 1.38±0.11 hij | 1.36±0.09 hi |
| MS + 1.5 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 78.60±2.89 cd | 85.40±2.04 c | 1.42±0.20 hij | 1.46±0.45 hi |
| MS + 2.0 mg $l^{-1}$ BAP + 0.0 mg $l^{-1}$ NAA | 73.80±2.73 d | 74.80±2.60 d | 1.44±0.10 hij | 1.52±0.15 hi |
| MS + 0.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 52.80±1.16 efg | 50.20±1.93 f | 1.58±0.21 hi | 1.70±0.53 hi |
| MS + 0.1 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 53.80±1.50 ef | 50.40±2.16 f | 2.20±0.51 gh | 2.04±0.23 h |
| MS + 0.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 36.40±2.50 k | 37.80±1.66 ijk | 3.32±0.23 def | 3.26±0.27 efg |
| MS + 1.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 72.20±3.93 d | 75.80±0.73 d | 3.96±0.57 bcd | 4.08±0.56 bcde |
| MS + 1.5 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 45.20±0.66 ghi | 44.40±1.33 fghi | 3.22±0.17 def | 3.52±0.27 efg |
| MS + 2.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA | 43.60±1.66 hijk | 42.80±2.94 ghij | 4.28±0.37 abc | 4.70±0.28 abcd |
| MS + 0.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 44.20±3.22 hijk | 42.20±2.24 ghij | 4.02±0.14 bcd | 3.86±0.21 def |
| MS + 0.1 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 59.20±1.50 e | 57.80±1.46 e | 3.68±0.47 cdef | 3.32±0.25 efg |
| MS + 0.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 50.20±2.80 fgh | 49.20±1.83 fg | 3.06±0.43 ef | 3.08±0.41 fg |
| MS + 1.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 44.80±0.92 ghij | 42.40±3.34 ghij | 3.18±0.33 def | 2.94±0.27 g |
| MS + 1.5 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 45.20±1.02 ghi | 44.00±1.30 fghi | 3.26±0.09 def | 3.66±0.28 efg |
| MS + 2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA | 42.40±0.87 hijk | 39.80±1.62 hijk | 3.48±0.32 cdef | 3.98±0.36 cde |
| MS + 0.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 39.80±2.22 ijk | 46.20±2.71 fgh | 2.92±0.47 fg | 3.88±0.24 def |
| MS + 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 36.80±1.36 jk | 34.80±3.20 k | 5.04±0.36 a | 5.44±0.42 a |
| MS + 0.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 39.60±2.58 ijk | 36.00±1.87 jk | 4.58±0.20 ab | 4.90±0.15 ab |

**Table 3.** Continued.

| Parameters/ Treatments | Days required for root initiation | | Root length (cm) | |
|---|---|---|---|---|
| | Leaf disc | Petiole | Leaf disc | Petiole |
| MS + 1.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 49.20±1.88 fgh | 47.20±1.66 fg | 3.84±0.14 bcde | 4.12±0.30 bcde |
| MS + 1.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 48.40±1.89 fgh | 45.40±0.81 fgh | 3.98±0.30 bcd | 4.86±0.49 abc |
| MS + 2.0 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA | 48.80±3.18 fgh | 47.80±2.13 fg | 4.28±0.33 abc | 4.88±0.22 ab |
| CD 5% | 8.31 | 7.18 | 0.85 | 0.89 |
| SEM | 2.96 | 2.56 | 0.30 | 0.32 |

The combination of BAP and NAA, at varying concentrations, significantly influenced the number of plantlets per explant. The data presented in Table 2 indicates a significant variation in the number of plantlets per explant in *B. palmata.* The average number of plantlets per explant developed from leaf disc explant (69.40 ± 5.04) was observed highest in MS +2.0 mg $l^{-1}$ BAP + 0.5 mg $l^{-1}$ NAA). The average minimum days required for root initiation (34.80 ± 3.20 days) was recorded in MS + 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA (Table 3). In this study, it has been noted that the petiole explant of *B. palmata* showed a higher rooting percentage as compared to the leaf disc, the best combination for root initiation in this study was obtained from MS media supplemented with 0.5 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA. These findings are more or less similar to earlier reports where 100% root induction was reported in the same medium (Kabirnataj *et al*. 2012, Yesmin *et al*. 2016). The present experiment indicates that the time required for root initiation decreased with the increased concentration of BAP. Bai *et al.* (2018) reported direct root induction in *Trichosanthes cucumerina* var. *cucumerina* from MS medium fortified with 0.5 mg $l^{-1}$ BAP, 0.5 mg $l^{-1}$ 2,4-D, and 1.0 mg $l^{-1}$ NAA. The highest value of root length may be due to enhanced growth of tissue on the application of plant growth hormones because the interaction between endogenous and exogenous levels of the hormone might have played an important role in plant growth. In the present study, a maximum root length of 5.44 ± 0.42 cm was recorded in MS media supplemented with 0.1 mg $l^{-1}$ BAP + 1.0 mg $l^{-1}$ NAA in petiole explant of *B. palmata.*

## CONCLUSION

This study reports the first investigation into the *in vitro* plant regeneration of *B. palmata* via direct organogenesis, utilizing leaf and petiole explants sourced from the Sikkim Himalayas. The synergistic interaction of 6-Benzylaminopurine (BAP) and α-Naphthaleneacetic acid (NAA) demonstrated high efficacy in inducing the formation of numerous micro shoots and robust *in vitro* plantlets, thereby facilitating large-scale propagation. The methodology detailed herein is anticipated to substantially contribute to the production of disease-free planting material suitable for the commercial ornamental cultivation of *B. palmata*. This approach offers a means of mitigating pressure on natural populations and provides a foundation for future investigations into the genetic transformation of *B. palmata.*

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