*Research & Reviews: Journal of Agricultural Science & Technology*
*Volume 1, Issue 1, April 2012, Pages* 12-17.

# Direct Regeneration from Leaf Explants of Petunia, Petunia Hybrida

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**ABSTRACT**

*Petunia is a very popular and high-value ornamental plant in the world. There is a high demand for petunia and hence plant nurseries are a great source of these plants. Owing to the fact that many high-value petunias are hybrids, production of plants through micro propagation is of great importance. As per the literature available, obtaining direct shoot proliferation from leaf explants has not been observed. In our study, direct regeneration was maximum in MS media supplemented with 2 mg/L BAP and 0.1 mg/L NAA and enhanced shoot number and length was observed in MS media supplemented with 2 mg/L Kn with 0.1 mg/L NAA. Micro shoots cultured on media with BAP alone produced the maximum number of shoots. Roots induced on half-strength MS medium with IBA at 1 mg/L, along with activated charcoal 2 g/L were long and of good quality with light green color. Plantlets were hardened with a success rate of 100% on agro peat substrate. The growth of these plants during hardening was extraordinary with hard stem and bunchy spread. Plants which were raised in the field showed extraordinary branching and gave a greater number of flowers than the seedling plants.*

***Keywords:*** *Direct regeneration, leaf explants, Petunia, ornamental plant, direct shoot proliferation*

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## 1. INTRODUCTION

Petunia of Solanaceae family is a widely cultivated genus of flowering plants of South American origin. Most of the varieties seen in gardens are hybrids (Petunia × hybrida). *Petunia hybrida* is an economically important ornamental plant species [5, 10, 13, 15].

Among the easiest of flowers to grow, petunias are a cheerful standby of cottage gardens everywhere. Now available in a dazzling array of colors, many are also fragrant. It is greatly diversified and available in a range of colors [3]. Petunias are annuals. They do demand full sun and adequate water, but are otherwise low-maintenance. The improvement of quality attributes such as flower color and longevity, plant shape, architecture, and creation of novel variation are important economic goals [2].

There is a high demand for petunia plants and plant nurseries are a great source of these plants. Further growth and success depends on the development of new technologies, such as tissue culture [8]. Different environmental factors were found to affect the *in vitro* organogenesis of petunia plant. Shoot organogenesis in *Petunia hybrida* leaf discs

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are affected by explant size, configuration and duration of exposure to benzyladenine (BA) [1]. Also, studies on anatomical changes [14], effect of light [12], sugar and $CO_2$, nitrogen and calcium [7], and ethylene [6] on shoot and root regeneration in the petunia plant, were done. Mutation, breeding and tissue culture technique are being utilized for the improvement of ornamental characteristics [9]. Several studies on somaclonal *in vitro Petunia hybrida* cultures were conducted. The evaluated characters were: leaf morphology, flower color, flower shape, and leaf variation [11]. Light and cytokinins were suggested to be associated with developmental processes in plants [4]. Owing to the fact that many high-value petunias are hybrids, production of plants through micropropagation is of great importance. Traditional vegetative propagation and seed propagation are not enough to meet the requirement of the demand. Hence the present study.

## 2. MATERIALS AND METHODS

The plant material for the experiments was collected from GKVK, University of Agricultural sciences, Bangalore, India. Healthy, mature and young leaves from shoots were used as the explants.

The explants were washed thoroughly under running tap water for 5 min. These were treated with 2% tween 80 (w/v) for 5 min, and again washed thoroughly in running tap water. After surface sterilizing with 0.05% $HgCl_2$ for 5 min, these were washed with sterile double distilled water inside the laminar airflow chamber to remove traces of $HgCl_2$.

Explants were cultured individually on MS medium containing different concentrations (0.1–4 mg/L) of BAP, Kinetin and NAA to induce shoots. The regeneration and rooting media contained 3% sucrose and gelled with 0.8% agar (Hi-Media, India). The pH was adjusted to 5.7 and autoclaved at 121 °C under 15 lb pressure for 15 min. All the cultures were maintained in a growth room with an 18 h photoperiod (cool, white fluorescent light – 2000 lux light intensity) and the temperature was maintained at 25 ± 2 ºC, with 50–70% relative humidity. After regeneration and sufficient elongation, the micro shoots were carefully excised and rooted on MS medium with 1 mg/L IBA and 2 g/L activated charcoal.

Rooted plantlets were removed from the culture bottles, roots were washed to get free of the adhering media and planted in polycups containing hardening media (sterile coco peat). The plantlets were immediately transferred to the mist house for primary hardening for one month. Then they were transferred to greenhouse for secondary hardening for one month and planted in the soil. Each treatment consisted of ten replicates and the experiment was repeated twice.

## 3. RESULTS AND DISCUSSION

Emergence of multiple shoot buds from the

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leaf explants on MS media supplemented with BAP, Kinetin and NAA was observed without an intervening callus phase. Shoots started proliferating after 30–40 days. The results obtained showed direct regeneration and high number of shoots in most of the combinations tested, although the frequency, number and length of multiple shoots varied with different concentrations and combinations of plant growth regulators. Maximum shooting frequency in the leaf explants (70–80) was observed in BAP (2 mg/L) in combination with NAA (0.1 mg/L) and Kinetin (2 mg/L) in combination with NAA (0.1 mg/L) (Table I and Figure 1).

***Table.I:*** *Effect of BAP, Kinetin and NAA on Shoot Regeneration from Leaf Explants of Petunia hybrida.*

| MS + Growth Hormones (mg/L) | | | % of Culture Response | No. of Multiple Shoots at 50 Days | Shoot Length (cm) | Kind of Response |
|---|---|---|---|---|---|---|
| **BAP** | **Kn** | **NAA** | | | | |
| 0.5 | - | 0.1 | 60 | 29.50 ± 0.34 | 3.25 ± 0.07 | Callus |
| 1.0 | - | 0.1 | 65 | 35.00 ± 0.37 | 3.41 ± 0.06 | Callus |
| 1.5 | - | 0.1 | 80 | 65.60 ± 0.48 | 4.24 ± 0.06 | Callus + Shoots |
| **2.0** | **-** | **0.1** | **90** | **82.40 ± 0.40** | **4.50 ± 0.07** | **Shoots** |
| 2.5 | - | 0.1 | 85 | 77.80 ± 0.44 | 4.47 ± 0.18 | Shoots |
| 3.0 | - | 0.1 | 80 | 65.10 ± 0.35 | 3.99 ± 0.06 | Shoots |
| 3.5 | - | 0.1 | 75 | 32.60 ± 0.45 | 3.73 ± 0.09 | Shoots + Embryogenic callus |
| 4.0 | - | 0.1 | 70 | 28.50 ± 0.40 | 3.22 ± 0.06 | Shoots + Embryogenic callus |
| - | 0.5 | 0.1 | 50 | 21.20 ± 0.42 | 2.99 ± 0.05 | Callus |
| - | 1.0 | 0.1 | 80 | 25.10 ± 0.57 | 3.07 ± 0.07 | Callus |
| - | 1.5 | 0.1 | 85 | 70.50 ± 0.48 | 4.06 ± 0.10 | Callus + Shoots |
| **-** | **2.0** | **0.1** | **90** | **80.30 ± 0.63** | **4.51 ± 0.06** | **Shoots** |
| - | 2.5 | 0.1 | 90 | 75.00 ± 0.56 | 4.32 ± 0.07 | Shoots |
| - | 3.0 | 0.1 | 80 | 60.00 ± 0.56 | 3.83 ± 0.06 | Callus + Shoots |
| - | 3.5 | 0.1 | 65 | 36.90 ± 0.53 | 3.56 ± 0.06 | Callus + Shoots |
| - | 4.0 | 0.1 | 60 | 24.90 ± 0.66 | 2.91 ± 0.12 | Shoots |

*Data were recorded at the 60 days. Values shown are means ± SE.*

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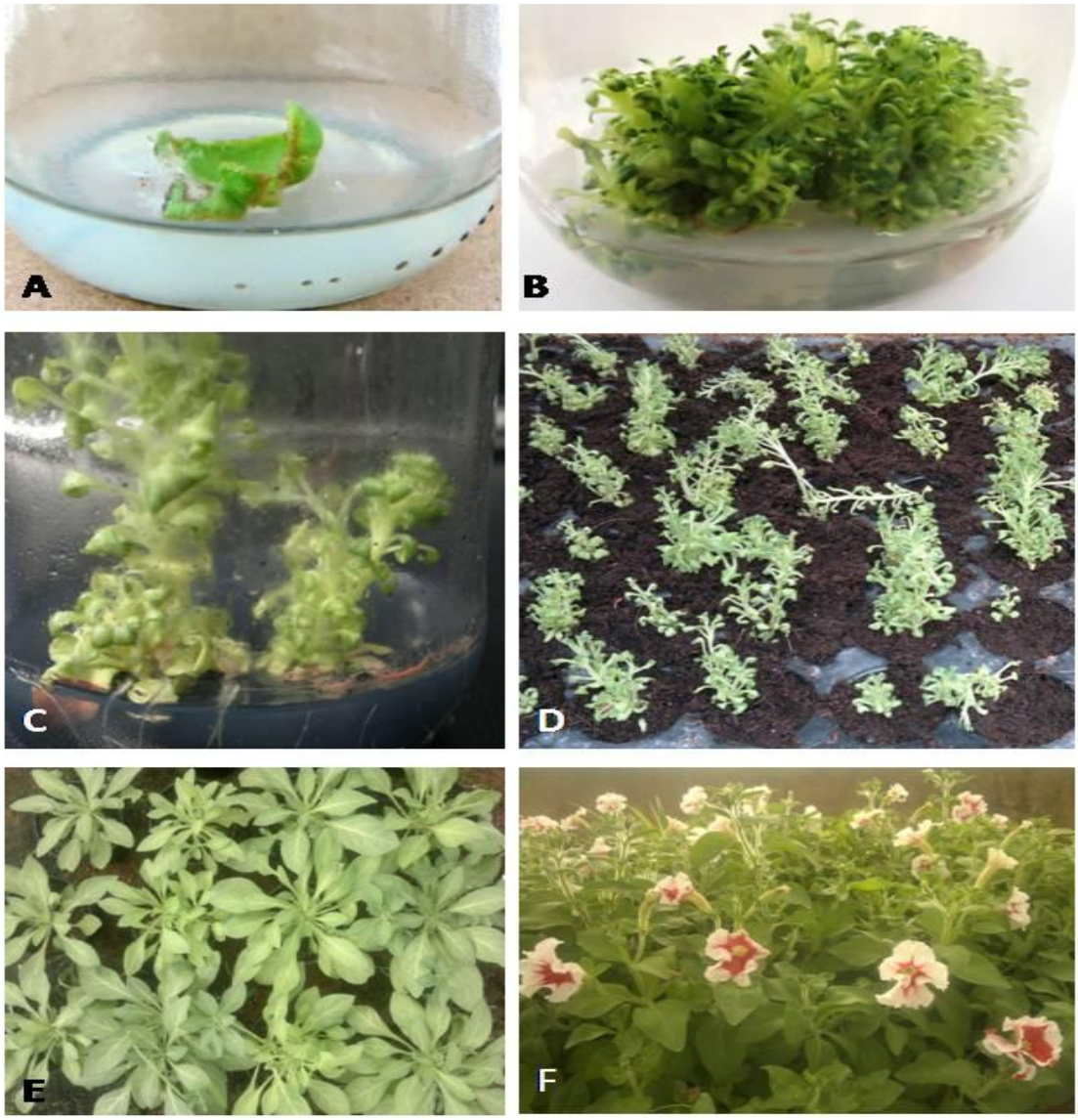


***Fig.1:*** *(A) Leaf Explants Initiated, (B) Multiple Shoots after 60 Days, (C) in vitro Hardening of Single Shoot, (D) Primary Hardened Plants in Polycups Containing Coco Peat, (E) Secondary Hardening in Pots, (F) Petunia Plant Generated through Tissue Culture Producing Flowers.*

Among the different combinations of media tried, exogenous supply of auxins favored the root formation. Addition of IBA to MS medium enhanced the rate of rhizogenesis (both frequency as well as number of roots). In all concentrations, root primordial appeared between 10–15 days of inoculation. According to our data, half strength MS salt fortified with

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activated charcoal (2 g/L) showed highest frequency and the maximum number of roots (7–8) was recorded with IBA (1 mg/L) (Table II).

***Table.II**: Effect of IBA and NAA on Root Induction of Petunia hybrida.*

| Growth Hormones | | | % of Roots Induction | No. of Roots per Explant at 30 Days. | Root Length (cm) |
|---|---|---|---|---|---|
| IBA (mg/L) | NAA (mg/L) | Activated Charcoal (g/L) | | | |
| 0.5 | - | 1 | 70 | 4.10 ± 0.30 | 4.41 ± 0.03 |
| 1.0 | - | 1 | 80 | 5.90 ± 0.25 | 4.48 ± 0.03 |
| 1.5 | - | 1 | 90 | 6.30 ± 0.26 | 4.81 ± 0.04 |
| 2.0 | - | 1 | 90 | 6.90 ± 0.53 | 4.99 ± 0.04 |
| 0.5 | - | 2 | 95 | 8.20 ± 0.28 | 5.19 ± 0.05 |
| **1.0** | **-** | **2** | **100** | **7.40 ± 0.32** | **5.46 ± 0.05** |
| 1.5 | - | 2 | 100 | 6.80 ± 0.34 | 4.80 ± 0.04 |
| 2.0 | - | 2 | 90 | 6.10 ± 0.37 | 4.42 ± 0.05 |
| - | 0.5 | 1 | 70 | 3.40 ± 0.39 | 3.05 ± 0.06 |
| - | 1.0 | 1 | 80 | 3.70 ± 0.24 | 3.14 ± 0.04 |
| - | 1.5 | 1 | 85 | 5.00 ± 0.21 | 3.56 ± 0.05 |
| - | 2.0 | 1 | 90 | 5.60 ± 0.34 | 3.82 ± 0.05 |
| - | 2.5 | 2 | 90 | 6.80 ± 0.33 | 4.21 ± 0.05 |
| - | 3.0 | 2 | 80 | 7.10 ± 0.30 | 4.00 ± 0.03 |
| - | 3.5 | 2 | 75 | 6.10 ± 0.37 | 3.79 ± 0.04 |
| - | 4.0 | 2 | 70 | 4.60 ± 0.46 | 3.99 ± 0.27 |

*Data were recorded at the 25 days. Values shown are means ± SE.*

Rooted plantlets were transferred to polycups containing coco peat. These plantlets were acclimatized well and transferred to green house and planted in the soil with 100% survivability.

Micro propagation of petunia provides an opportunity to harvest virus-free material and conserve important germplasm resources *in vitro*. It furnishes material for genetic transformation and for employing molecular techniques in breeding.

In conclusion, the findings of this work could be useful in further study. More investigation could be done to improve the regeneration of

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petunia from leaf explants. In addition, the molecular technique could be used for detection and conforming somaclonal variation.

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