



# Effects of Plant Growth Regulators on *in-vitro* regeneration of a potential medicinal plant -*Scilla hyacinthina* (Roth.)J.F. Macbr

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## ABSTRACT

A suitable plant regeneration protocol has been developed from scale leaf and bulb scale explants of *Scilla hyacinthina*, a medicinally important plant species belonging to the family liliaceae. The maximum number of shoots (13.2), with average length 6.5 cm, was achieved with medium containing 1.5 mg/l TDZ. Rooting of shoots was achieved on half-strength MS medium supplemented with 1.5 mg/l IAA.

**Abbreviations:** MS – Murashige and Skoog medium, BAP – 6-benzylamino-purine; KIN-Kinetin; Thidiazuron (TDZ); Indole-3-acetic acid (IAA); Indole-3-butyric acid (IBA); GA<sub>3</sub>Gibbrellic acids; HgCl<sub>2</sub> . mercury chloride

## 1. INTRODUCTION

*Scilla hyacinthina* (Roth.)J.F. Macbr (Liliaceae) is an ephemeral grasses on the floor of scrub, located in India and some Asian countries [1]. The leaves are simple, oblong, succulent with purplish on the upper surface of leaves. The plant produce flush of white inflorescence and perennial tunicated bulb, bulb range from 10-14gm [2]. The plant bulb is used as anthelmintic, cardiac stimulant, digestive, diuretic, emmenagogue and expectoram [3]. It's also used in asthma, cough, bronchitis, paralytic attacks, ailments of the hearts, calculous affection, rheumatism and skin disease. The bulb paste to cure wounds and join the broken bone. The plant have major amount of carbohydrate and secondary metabolites such as alkaloids, flavonoids, saponins [4]. Plant tissue culture is a rapid and regeneration of novel hybrid and large numbers of identical individuals, disease or pest resistant varieties, thus indirectly increasing the crop yield. Plant species are poor seed germination, frequency of seedling death and environmental challenges such as habitat destruction and illegal and indiscriminate collection [5]. *Scilla*, a monocotyledonous species

belonging to the liliaceae, is one of the most important medicinal plants, mainly because of its pharmacological properties and attractive flowers. The continuous improvement of specific characters, such as flower colour morphology, longevity and size is essential for successful commercial application of any variety. Conventional breeding is the *Scilla* hampered by its heterozygous state and self- incomparability among the species of the different groups. The members of liliaceae have long been known as an amenable species, which adapt easily to most tissue culture techniques. Plant regeneration via organogenesis easily achieved from a vast array of explants ranging from flower organs to bulb scales [6,] [7]. Non-conventional breeding methods offer new promising strategies for the improvement of crop but these techniques require a reproducible in vitro regeneration protocol.

Meristems is considered to be one of the most promising ways for plant regeneration and it's true type of selected variety also. Such individuals are genetically similar showing the same agronomic characteristics [8]. However, a major difficulty in this plants, just grow up after the rain fall and seed not fertility. Due to above the reason for plant propagated by *in vitro* technique. In this technique, produce more plantlets from piece of parts and disease free plants. An *in vitro* method has been successfully propagated for example species from a leaf 3 (anther) [9]. In the present studies to investigate of *Scilla hyacinthina* using different explants.

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## 2. MATERIALS AND METHODS

The fresh bulb materials of *Scilla hyacinthina* (Fig. 1) was collected from Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The collected samples were authenticated by Dr. V. Nanadagopalan, Associate Professor, PG & Research Department of Botany, National College, Tiruchirappalli, Tamilnadu and a voucher specimen was deposited at Department of Botany, National College, and Tiruchirappalli.



Fig. 1: Natural Habitat.

The explants were washed with running tap water for 2h, followed by teepol solution for 10min and rinsed for 3 times in distilled water. Whereas, 0.1% HgCl<sub>2</sub> for 3 min and with 70% ethanol solution for 5 times. Then, these explants were rinsed for 5 min in sterile distilled water. MS medium supplemented with different hormones of BAP, TDZ, KIN, IBA&IAA.

All the *in vitro* cultures were placed in the culture room maintained at 25 ± 2 °C under continuous photoperiod with a photosynthetic photon flux density (PPFD) of 45 μmol m<sup>-2</sup> s<sup>-1</sup> provided by cool white fluorescent lamps. The pH of all the media was adjusted to 5.7 – 5.8 before autoclaving at 121 °C for 13 min under a pressure of 1.05 kg cm<sup>-2</sup>.

## 3. RESULTS

The fresh plant materials were collected from field grown and these plant materials were used for culture on MS medium supplemented with various concentrations of BAP, TDZ & KIN. During the study period, bacterial and fungal contaminations were observed on bulbs scale explants and these contaminants were eliminated by fungicide (Bavastin). For the initial time of culture, the explants were kept in dark condition for resting time.

The multiple shoot was observed in high concentration of 2.0mg/L<sup>-1</sup>BAP and TDZ at 45 day of culture (Table 1; Fig. 2a). Cytokinin concentration gradually induced multiple shoot regeneration from bulb scale and leaf scale explants.

Highest multiple shoot were observed at 2.0 mg/L<sup>-1</sup>at BAP and TDZ in 12.7±1.0, 13.2±1.9 and, 12.5±1.6, 13.4±1.7 from bulbs scale and scale leaf explants, respectively. The control plants (without PGR) were no shoot regeneration response from both the explants. KIN were also exhibited highest shoot regeneration from scale leaf explants.

The shoot were excised and inoculated on MS medium supplemented with various concentrations of IBA and IAA for induction of root regeneration.

**Table 1:** Effect of different concentration of BAP, TDZ and KIN on direct shoot formation from bulb scale and scale leaves explants of *Scilla hyacinthina*.

| S.No | Hormones<br>Concentration (in mg/L) | Number of shoot |                 |
|------|-------------------------------------|-----------------|-----------------|
|      |                                     | Bulbs scale     | Scale leaves    |
| 1    | Control                             | -               | -               |
| 2    | 0.5                                 | 4.5±1.0         | 1.9±0.8         |
| 3    | 1.0                                 | 6.3±1.5         | 2.8±0.7         |
| 4    | 1.5                                 | 7.5±1.0         | 4.8±0.9         |
| 5    | 2.0                                 | <b>12.7±1.8</b> | <b>13.4±1.7</b> |
| 6    | 2.5                                 | 5.5±1.0         | 6.6±1.0         |
| 7    | 0.5                                 | 3.1±0.8         | 1.0±1.0         |
| 8    | 1.0                                 | 5.7±1.0         | 3.5±0.8         |
| 9    | 1.5                                 | 7.7±1.5         | 7.1±1.1         |
| 10   | 2.0                                 | <b>13.2±1.9</b> | <b>12.5±1.6</b> |
| 11   | 2.5                                 | 6.7±1.1         | 7.8±1.0         |
| 12   | 0.5                                 | 2.3±1.1         | 1.1±0.7         |
| 13   | 1.0                                 | 3.4±1.0         | 3.9±1.1         |
| 14   | 1.5                                 | <b>6.9±1.1</b>  | <b>12.6±1.5</b> |
| 15   | 2.0                                 | 5.5±1.0         | 8.6±0.9         |
| 16   | 2.5                                 | 4.3±0.9         | 5.6±1.1         |

No roots were observed without hormones. The highest number of roots was observed at 1.5 mg/L<sup>-1</sup> of IAA (7.3±6.9) from bulb scale explants. IAA was more efficient root regeneration while compared with IBA (Table-2; Fig. 2b).

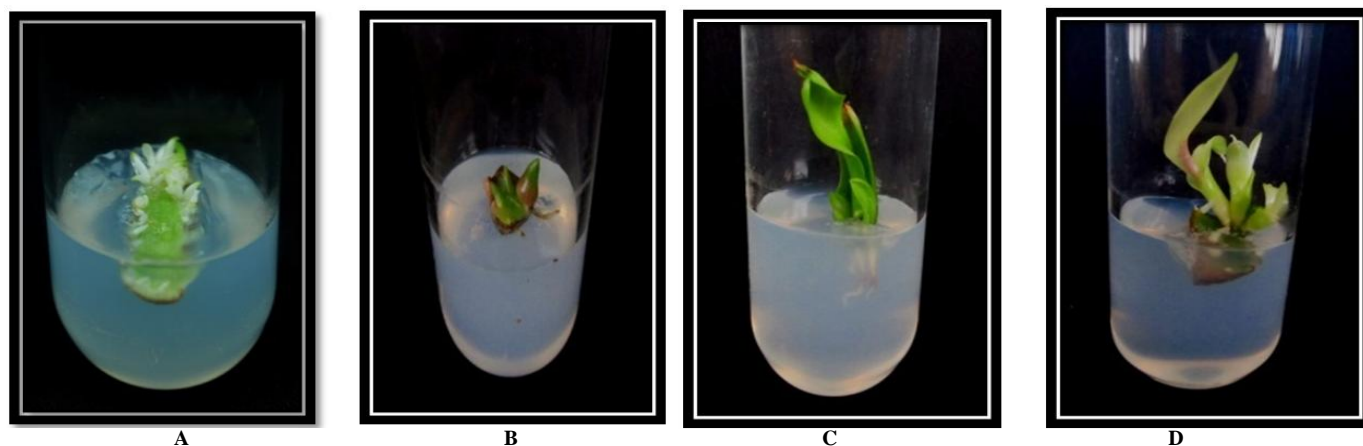
**Table 2:** Effect of different combinations of IBA and IAA on root formation of bulb scale and scale leaf explants of *Scilla hyacinthina*

| S.NO | Hormones<br>Concentration (in mg/L) | Number of roots |            |
|------|-------------------------------------|-----------------|------------|
|      |                                     | Bulb scale      | Scale leaf |
| 1    | Control                             | -               | -          |
| 2    | 0.5                                 | 1.0±0.4         | 0.6±0.5    |
| 3    | 1.0                                 | 4.7±0.7         | 1.8±0.7    |
| 4    | 1.5                                 | 7.3±0.9         | 1.8±0.7    |
| 5    | 0.5                                 | 2.6±0.2         | 2.1±0.6    |
| 6    | 1.0                                 | 4.6±0.3         | 3.7±1.1    |
| 7    | 1.5                                 | 7.6±1.8         | 6.5±0.2    |

**Table 3:** Different concentration of GA<sub>3</sub> for shoots elongation of *Scilla hyacinthina*

| S.NO | Hormones<br>Concentration (in mg/L) | Shoot length (in cm) |
|------|-------------------------------------|----------------------|
|      |                                     |                      |
| 1    | Control                             | -                    |
| 2    | 0.5                                 | 2.6±0.2              |
| 3    | 1.0                                 | 4.6±0.3              |
| 4    | 1.5                                 | 6.5±0.2              |
| 5    | 2.0                                 | 5.3±0.1              |
| 6    | 2.5                                 | 4.6±0.1              |

GA<sub>3</sub> were used for shoot elongation of plantlet, it's 1.5mg/L<sup>-1</sup> of GA<sub>3</sub> showed highest results (Table 3; Fig. 2c & d).



**Fig. 2:** Micropropagation studies on *Scilla hyacinthina* a. Initial stage of bulblet production from leaf scale explants, b. Root formation from bulblet on MS medium amended with IAA, c. Elongated shoots with many scale leaves, d. Formation of bulb as well as root.

#### 4. DISCUSSION

The natural reproduction of seeds was more unfertile on *Scilla hyacinthina*. The bacterial and fungal contamination was observed on the bulb scale explants during study period. The bulb explant is often associated with heavy bacterial and fungal contamination [10], [11]. It is more difficult to large scale multiplication of these plants. There is urgent need to develop alternate method for production of without microbial contamination free plants. Plant tissue culture techniques are recalcitrant crops, especially monocotyledonous species and grass species. Previous studies reported that darkness to stimulate more direct shoot production than light condition [12] [13].

The effect of light can be interpreted as acting on metabolism and sugar uptake [14]. Bulb scales were most commonly used for *in vitro* micropropagation of geophytes. In the present work, bulb scale and scale leaf explants of *S. hyacinthina* were cultured on MS medium supplemented with different concentration of BAP, KIN and TDZ for shoot induction of explant. The highest concentration of BAP and TDZ shows generally increased the bulblet production than KIN. Similarly, stem *Bergia fischerina* of TDZ were produced several shoot formation from bulb scale and immature embryo explants [15]. Recently, Uranbay, [16] observed that BAP, KIN and 2iP were favorable bulblet production on 2-4 bulb scale of *Muscari aucheri*. Previous reports that observed cut treatment of bulb scale base explant of *Hippeastrum* to stimulate bulblet production [17]. In the present study, IAA was more efficient in promoting root formation than IBA. It has been described that IAA improve the number and quality of root in several species because it stimulates the cellular division of radial primordium, promotes the synthesis of specific proteins, and increases the sugar release into phloem [18]. In conclusion, the micropropagation

protocol reported here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots were easily developed. This is highly advantageous for the production of uniform source of selected potential medicinal plant for a range of further biotechnological applications.

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