Multiplication of *Bharangi*—*Clerodendrum serratum (L.*) Moon: An Ayurvedic Important Plant

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**ABSTRACT**

**Aim:** To develop effective *in vitro* multiplication protocol for rare and endangered medicinal plants of *Clerodendrum serratum* (L.) Moon.

**Materials and methods:** Trials were carried out using nodal segments as explants, which were inoculated on Murashige and Skoog medium (MS) plain medium and different concentrations of phytohormones viz., benzylaminopurine (BAP), kinetin (Kn), indol-3-butyric acid (IBA), indol-3-acetic acid (IAA), and naphthaleneacetic acid (NAA), and incubated for 8 hours of photoperiod using cool-white fluorescent tubes with a light intensity of 3,000 lux at 22°C ± 2°C.

**Results:** The maximum (7.0 ± 0.045) numbers of shoots were developed from the nodal segment inoculated on MS fortified with BAP (4 mg/L) ± 0.1 % polyvinylpyrrolidone (PVP) with shoots of 1.72 ± 0.018 cm height. Auxin BAP at 4 mg/L proved to be effective for bud proliferation and production of multiple shoots. The best and early root induction was achieved on MS + IBA (1–4 mg/L).

**Conclusion:** The developed *in vitro* protocol would be beneficial for the fast multiplication of *bharangi* plants and help to minimize the burden on supply and demand, so that the huge demand for the drug is fulfilled and also helpful to protect the plant.

**Keywords:** Conservation, Growth regulators, *In vitro* propagation, Micro-propagation, Node.

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**INTRODUCTION**

*Bharangi*—*Clerodendrum serratum* (L.) Moon belongs to the Verbenaceae family. It is one of the important medicinal plants used in the treatment of respiratory diseases in the Indian System of Medicines. It is a perennial shrub found throughout India at up to 1,500 m altitude, growing in deciduous forests, peninsular India and Western and Eastern Himalayas.¹ ¹ ² *Bharangi* is pungent and astringent in taste, having pungent postdigestive effect and hot potency.³ Roots are mainly used as drugs in various Ayurvedic and herbal preparations viz., *Bharangi* guda, *Bharangyadi kwatha*, *Bharagimoola pralepa*, *Kanakasava*, *Bharagyadi leha*, *Pippalyadi kwatha*, *Kaphhaladi kwatha*, *Maha Panchagavya Ghrita*.⁴ *Bharangi* root has anti-inflammatory, carminative, anthelmintic, expectorant, antispasmodic, sudorific, and digestive actions. Roots are used in the treatment of allergic rhinitis, asthma, fever, inflammation, hiccup, cough, bronchitis, flatulence, skin diseases, etc. Leaves are useful for external application in cephalalgia and ophthalmia. Seeds are used in dropsy.⁵

Saponins, stigmasterol, queretaric acid, D-mannitol, terpenoids, serratagenic acid, oleanolic acid, ursolic acid, scutellarein, β-sitosterol, lupeol, arabinose, baicalein, ferulic acid, caffeic acid two iridoid glycosides and minerals such as Mg, Al, Ca, etc. are present in the roots. Luteoline, spinossterol, catechin, diterpin–clerodin, and cruteuarein are reported in leaves.⁶

*Bharangi* roots are mostly collected from wild habitat. Habitat destruction, overexploitation, and poor seed germination lead to a decrease in natural resources of this plant species and *bharangi* plant is becoming rare and endangered in Gujarat,⁶ Chhattisgarh, and Madhya Pradesh regions.⁷ Day by day the demand for the drug is increasing in pharmacies due to its valuable medicinal property. Hence, it is necessary to develop an alternative propagation technique for *bharangi* to meet the demand of the industry.

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**Conflict of interest:** None

The paper deals with the mass multiplication of *bharangi* plants through *in vitro* propagation using the nodal segment as explants. This developed protocol will also be useful for the conservation of the plant.

**MATERIALS AND METHODS**

**Plant Material and Source of Explants**

Plantlets of *bharangi* were collected from the Mulashi area of Pune district, Maharashtra state, India. Plantlets were planted in the medicinal plant garden at the Regional Ayurveda Institute for Fundamental Research, Pune. Nodal segments were collected from the healthy plants growing in the garden and used as explants.

**Identification and Authentication of the Plant**

The collected plant was identified by botanists of the Institute and authenticated with the help of floras.¹ ¹ ¹ ² Herbarium specimens were

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Preparation

Explant Preparation

In vitro propagation trials were carried out using nodal segments as explants. Explants were inoculated on Murashige and Skoog (MS) plain medium and MS medium was fortified with different concentrations of phytohormones viz., benzylaminopurine (BAP), kinetin (Kn), indol-3-butric acid (IBA), indol-3-acetic acid (IAA), and naphthaleneacetic acid (NAA). Explants were treated with 5% Tween 20 solution (Hi media) for half-hour, followed by thorough washing using running tap water for 30 minutes. Further, these explants were surface sterilized with 0.1% mercury chloride solution (Hi media) for 1 minute and rinsed thrice with sterile distilled water under aseptic conditions.

Media Preparation and Inoculation

The MS9 was used as the basal medium which was supplemented with 3% sucrose (w/v) (Hi media, India) and 0.8% (w/v) Agar (Hi media, India). The pH of the medium was adjusted between 5.7 and 5.8 with 0.1 N NaOH or HCl before the addition of agar. After digestion of the medium, 20 mL was poured in each test tube and plugged with nonabsorbent cotton covered with a double-layered gauze cloth. Then the media was autoclaved for 20 minutes at 121°C at 15 lbs/inch² pressure. The MS salt medium fortified with different concentrations of growth hormones, BAP (1–5 mg/L) and Kn (1–4 mg/L) supplemented with 0.1% PVP to induce shoots.

Results

Shoot Regeneration from Nodal Segments

Nodal segments inoculated on to the MS plain medium and MS medium augmented with different concentrations of growth hormones, BAP (1–5 mg/L) and Kn (1–4 mg/L) supplemented with 0.1% PVP showed bud sprouting from the axillary bud of the nodal segment at all concentrations within 15 days. The maximum average of 7.0 ± 0.045 number of shoots was recorded from BAP (4 mg/L) with shoots of 1.72 ± 0.018 cm. Whereas the minimum mean number of shoots of 1.29 ± 0.032 with height of 4.21 ± 0.038 cm was observed on MS + BAP (1 mg/L) + 0.1% PVP. Direct organogenesis and multiple shoot formation were achieved with BAP 3–5 mg/L. Details of results obtained are displayed in Table 1; Figures 1, 2 and 5.

Nodal segments inoculated on MS medium fortified with different concentrations of Kn (1 to 4 mg/L), with the addition of 0.1% PVP, exhibited induction of callus at the cut ends and also bud sprouting which produced multiple shoots at all concentrations. Among these, the highest (12.5 ± 0.059) number of shoots achieved with MS + Kn (3 mg/L) + 0.1% PVP and lowest (3.64 ± 0.031) average number of shoots was achieved with MS + Kn (1 mg/L) + 0.1% PVP. Longest (3.55 cm) shoots were recorded with Kn (2 mg/L) + 0.1% PVP. Details of shoot formation are provided in Table 1; Figures 1, 2 and 5.

Root Formation

In vitro grown, 6-week-old shoots were transferred on to the rooting media, namely, ½ MS plain, MS plain, and MS supplemented with IBA, NAA, and IAA in varying concentrations. In vitro developed shoots implanted on ½ MS and MS plain medium for induction of root did not produce any root for up to 4 weeks’ period.

Table 1: Effect of different concentrations of benzylaminopurine on nodal segment of Clerodendrum serratum (L.) Moon

| Concentrations of BAP + 0.1% PVP | Average number of shoot with ± SE | Average height of shoot in cm with ± SE |
|---------------------------------|----------------------------------|---------------------------------------|
| MS                             | – –                             | 0.0                                   | 0.0                                    |
| 1 mg/L                         | + –                             | 1.29 ± 0.032                          | 4.21 ± 0.038                           |
| 2 mg/L                         | + +                             | 1.57 ± 0.039                          | 2.86 ± 0.024                           |
| 3 mg/L                         | + +                             | 2.5 ± 0.041                           | 2.23 ± 0.022                           |
| 4 mg/L                         | + +                             | 7.0 ± 0.045                           | 1.72 ± 0.018                           |
| 5 mg/L                         | + +                             | 4.18 ± 0.047                          | 2.37 ± 0.025                           |

Fig. 1: Effect of different concentrations of benzylaminopurine and kinetin on the number of shoot Clerodendrum serratum (L.) Moon
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The MS medium supplemented with IBA (1–4 mg/L), IAA (1–4 mg/L), and NAA (1–4 mg/L) induced root primordia and produced roots in all concentrations. The best and early root induction was achieved with MS + IBA (1–4 mg/L) as compared with IAA and NAA (1–4 mg/L).

Shoots transferred on MS + IBA (2 mg/L) produced maximum (7.52 ± 0.051) number of roots of length 1.44 ± 0.091 cm. Details of results obtained are exhibited in Table 3; Figures 3 to 5.

Whereas MS + IAA (3 mg/L) induced maximum average number of roots (2.9 ± 0.019) with length of 2.01 ± 0.017 cm. However, NAA (2 mg/L) produced a maximum average number of roots of 3.18 ± 0.028 with length of 1.93 ± 0.089 cm. Details of observations are shown in Tables 4 and 5; Figures 3 to 5.

Hardening

The in vitro grown plants were kept under hardening conditions and then transferred to nursery wherein 66.66% of plants survived in the field.

**Discussion**

The response of explants may vary based on age, size, and season of collection and also the combination of cytokinins and auxins. In our experiments, direct organogenesis was recorded with BAP at all concentrations. Maximum multiple shoots (7.0 ± 0.045) was achieved with BAP (4 mg/L). In previous work, shoot proliferation has also been established on different concentrations of BAP. Maximum (8.01) numbers of shoots were reported from BAP 1.5 mg/L combined with NAA (0.3 mg/L) with Lloyd and Mc Crown (LM) medium. It is reported that the MS medium supplemented with 0.2 mg/L of BAP produces an average number of shoots (1.4 ± 0.84), with a mean shoot length of 3.5 ± 2.01 cm. The BAP at a lower concentration of 0.5 mg/L showed best shoot bud induction and multiplication. Bud sprouting and shoot formation

**Table 2: Effect of different concentrations of kinetin on nodal segment of Clerodendrum serratum (L.) Moon**

| Concentrations of Kn + 0.1% PVP | Shooting Callusing | Average number of shoot | Average height of shoot in cm |
|---------------------------------|-------------------|-------------------------|------------------------------|
| MS + Kn | ++ | -- | 0.0 | 0.0 |
| 1 mg/L Kn | ++ | ++ | 3.64 ± 0.031 | 3.87 ± 0.028 |
| 2 mg/L Kn | ++ | ++ | 5.85 ± 0.042 | 3.55 ± 0.031 |
| 3 mg/L Kn | ++ | ++ | 12.5 ± 0.059 | 1.75 ± 0.0091 |
| 4 mg/L Kn | ++ | ++ | 5.21 ± 0.048 | 3.47 ± 0.027 |

**Table 3: Effect of different concentrations of indol-3-butyric acid on in vitro grown shoots of Clerodendrum serratum (L.) Moon observation after 4 weeks**

| Concentrations of IBA + 0.1% PVP | Rooting | Average number of root | Average length of root in cm |
|----------------------------------|---------|------------------------|-----------------------------|
| ½ MS + IBA | -- | -- | -- |
| MS + IBA | -- | -- | -- |
| 1 mg/L IBA | ++ | 4.56 ± 0.038 | 2.88 ± 0.021 |
| 2 mg/L IBA | ++ | 7.25 ± 0.051 | 1.44 ± 0.091 |
| 3 mg/L IBA | ++ | 3.75 ± 0.024 | 2.58 ± 0.021 |
| 4 mg/L IBA | ++ | 2.24 ± 0.019 | 3.52 ± 0.027 |
Fig. 5: *In vitro* grown *Clerodendrum serratum* (L.) Moon sprouting of nodes, multiple shooting, and hardening of the plant.

Table 4: Effect of different concentrations of indol-3-acetic acid on *in vitro* grown shoots of *Clerodendrum serratum* (L.) Moon observation after 4 weeks

| Concentrations of IAA + 0.1% PVP | Rooting | Average number of root | Average length of root in cm |
|---------------------------------|---------|------------------------|------------------------------|
| ½ MS                            | − − −   | − − −                  | − − −                        |
| MS                              | − − −   | − − −                  | − − −                        |
| 1 mg/L                          | ++      | 1.36 ± 0.091           | 3.09 ± 0.027                 |
| 2 mg/L                          | ++      | 1.71 ± 0.087           | 2.78 ± 0.021                 |
| 3 mg/L                          | + +     | 2.90 ± 0.019           | 1.93 ± 0.089                 |
| 4 mg/L                          | + +     | 2.09 ± 0.084           | 2.01 ± 0.014                 |

Different concentrations of growth hormones, BAP (1–5 mg/L) and Kn (1–4 mg/L) per explant developed from callus by indirect organogenesis and maximum average number of shoots (7.0 ± 0.045) per explant were recorded by direct organogenesis. Thus, the media used for the propagation trial is highly economical and easily reproducible as compared to the media and hormonal combinations published by other workers.

**Conclusion**

*In vitro* propagation trials were conducted by using nodal segments as explants. Trials were conducted on MS medium fortified with different concentrations of growth hormones, BAP (1–5 mg/L) and Kn (1–4 mg/L). Among the BAP, the number of shoots (12.5 ± 0.059) per explant developed from callus by indirect organogenesis and maximum average number of shoots (7.0 ± 0.045) per explant were recorded by direct organogenesis. Thus, the media used for the propagation trial is highly economical and easily reproducible as compared to the media and hormonal combinations published by other workers.

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हिंदी सारांश

एक महत्वपूर्ण आयुर्विदिक पादप: भारंगी—क्लेरोंड्रम सेराटम (एल.) मूल का बहुलीकरण

उद्देश्य: क्लेरोंड्रम सेराटम (एल.) मूल के दुर्लभ और लुप्तप्राय औषधीय पादप हेतु एक प्रभावी इन विध्रों बहुलीकरण प्रोटोकॉल विकसित करना।

सामग्री और विधियाँ: एक्सप्लांट्स का नोडल सेंगमेंट्स के रूप में प्रयोग कर परीक्षण किए गए, जिन्हें मुराशिम और स्कूग माध्यम (एमएस) प्लेन माध्यम और पादप श्रंचि रस्ता बेंजीलेमिनोपूरिन (बीएपी), काइनेटिन (कैपन), इंडोल 3-बूट्रिक अम्ल (आईबीए), इंडोल 3-एसिटिक अम्ल (आईएए) और नेपथिलिन एसिटिक अम्ल (एनएए) की विषम्बर संदर्भों पर किया गया और 22 दिमाग सेल्सियस + 2 दिमाग सेल्सियस पर 3,000 lux की हल्की तीव्रता से कूल-क्लाइंट प्लास्ट प्रयोग कर फोटोपोरियड हेतु 8 घंटे के लिए इंक्यूबेट किया गया।

परिणाम: नोडल सेंगमेंट से अधिकतम (7.0 ± 0.045) संख्या के शूटस एमएस फोटोआइड के साथ बीएपी (4 मिलीग्राम/लीटर)±0.1%, 1.72 ± 0.018 सेंटीमीटर उंचाई के शूटस के साथ पॉलीविनाइलपाराइलेडेन (पीवीपी) विकसित किए गए। कंत्री प्रजनन और बहुल शूटस के उत्पादन हेतु 4 मिलीग्राम/लीटर पर ऑक्सिजन बीएपी लाभकारी सिद्ध हुआ। एमएस+ आईबीए (1-4 मिलीग्राम/लीटर) पर उत्तम और शीघ्र स्तंभ इंक्यूबेशन प्राप्त किया गया।

निष्कर्ष: विकसित इन विध्रों प्रोटोकॉल भारंगी पादप के तेज़ बहुलीकरण के लिए लाभकारी होगा और यह मांग एवं आपूर्ति के बीड़ को भी कम करने में सहायक होगा, ताकि आयुर्विदिक की भारी मांग को पूरा किया जा सके और यह पादप को सुरक्षित रखने में सहायता भी करेगा।

मुख्य शब्द: संरक्षण, बीड़ नियामक, इन विध्रो प्रसार, माइक्रो-प्रसार, नोड।