Micropropagation of *Dhumrapatra* (*Aristolochia bracteolata* Lam.): An Important Medicinal Plant of Ayurveda

**ABSTRACT**

**Aim:** *Dhumrapatra* is an important medicinal plant used in Indian system of medicine. Due to overexploitation and unsystematic collection, it is becoming rare and endangered in some parts of India. To meet the demand of the authentic drug and propagate the plant on a large scale, an *in vitro* propagation technique has been developed for *Dhumrapatra*.

**Materials and methods:** The collected plant was identified with the help of florists. Murashige and Skoog basal (MS) medium was prepared as per the standard protocol. Surface sterilization of explants was done with the help of disinfectant. Media were autoclaved at 121°C under 15 lb/inch² pressure for 15 minutes. Cultures were incubated at 22°C ± 2°C and 8 hours photoperiod with light intensity of 3000 lux. Observations were recorded after a 4-week period.

**Results:** Best establishment of shoots (20 nos.) was found in MS media augmented with 0.5 mg/L kinetin (Kn). Maximum roots (2–3 nos) produced in MS were fortified with 3 mg/L indolebutyric acid (IBA). Maximum number of shoots and roots were achieved on nodal explants inoculated on MS + Kn media fortified with 0.5 mg/L + 0.1% polyvinylpyrrolidone (PVP).

**Conclusion:** The *in vitro* propagation protocol developed for *Dhumrapatra* would be beneficial for rapid multiplication and conservation of important medicinal plant.

**Keywords:** Conservation, *Dhumrapatra*, Hormones, *In vitro*, Regeneration.


**Source of support:** Nil

**Conflict of interest:** None

**INTRODUCTION**

*Aristolochia bracteolata* Lam. (syn. *Aristolochia bracteata* Retz.) belongs to family Aristolochiaceae known as *Dhumrapatra* in Ayurveda. It is a perennial herb found in plains of northern India from Haryana and UP, southward to peninsular India up to Maharashtra, Andhra Pradesh, and as a weed in black soil. Locally, it is called as “Keetmari,” as it exhibits insect repellent properties due to presence of aristolochic acid.

In Indian system of medicine, *Dhumrapatra* is mainly used as a single drug. The drug possesses purgative, anti-inflammatory, anthelminitic, emmenagogue, alterative, and abortifacient properties. Its root is used to treat syphilis, gonorrhea, expelling roundworms, and skin diseases. Leaves are bitter and used to cure worm infestation, dysmenorrhea, joint pain, urticaria, skin disorders, intermittent fever, dry and weeping eczema, and ulcer. In Northern Nigeria traditionally, it is reported in the treatment of gastric cancer, lung inflammation, dysentery, snake bite, and scorpion stings.

The major chemical constituents isolated and reported from the plant are aristolochic acid, magnoflorine, N-acetylnornuciferine, aristolactam, β-sitosterol, and ceryl alcohol. It also contain esters, aristolactams, aporphines, isoquinoline, benzylisoquinoline, amides, lignans, coumarin, terpenoids, benzoids, and many others.

As there are no systematic protocols of *ex situ* and *in situ* conservation of this medicinally important plant, an efficient *in vitro* protocol has been developed and presented in this study.

**MATERIALS AND METHODS**

**Plant Material and Source of Explants**

Plantlets were collected from Ranjangaon area, District, Pune, Maharashtra state of India, and planted in the garden at Regional Ayurveda Institute for Fundamental Research, Pune.

**Identification and Authentication of Plant**

The plant was identified by the botanist of the institute and authenticated with help of flora of Maharashtra. Herbarium specimens were prepared and deposited in...
the herbarium section of the institute with the voucher specimen number 3456.

Explants Source and Preparation of Explants

In vitro regeneration studies were initiated using nodal segment as explants collected from the plants grown in the garden. The explants were thoroughly washed under running tap water for 30 minutes and then soaked in 5% Bavistin (v/v) solution for 1 hour and thoroughly washed under running tap water. These explants were again soaked in 5% Tween 20 solution (HiMedia) for half an hour and thereafter washed under running tap water for further 30 minutes. Thus, the washed explants were sterilized with 0.1% mercury chloride solution (HiMedia) for 1 minute and rinsed three times with sterile distilled water under aseptic conditions.

Media Preparation and Inoculation

The MS salt medium supplemented with 3% sucrose (w/v) (HiMedia, India) was used in all experiments. The pH of the medium was adjusted to 5.8 to 5.9 with 0.1 N NaOH or HCl prior to adding agar 0.8% (w/v) (HiMedia, India); 20 mL of this molten medium was dispensed into culture tubes, which were plugged with non-absorbent cotton wrapped in double-layered gauze cloth. The media was steam sterilized for 20 minute at 121°C at 15 lbs/inch 2 pressure. The MS basal medium was supplemented with different concentrations of benzylaminopurine (BAP), thidiazuron (TDZ), Kn, naphthalene acetic acid (NAA), and indol-3-butyric acid (IBA) singly or in combinations with other hormones.12

Surface-sterilized nodal segments were trimmed to about 2 cm long, possessing at least one axillary bud, and inoculated on to MS basal alone or MS fortified with different concentrations of plant growth regulators viz., BAP, TDZ, KN, NAA, and IBA singly or in combinations.

Culture Conditions

All cultures were incubated at 8-hour photoperiod under illumination of cool, white fluorescent tubes with light intensity of 3000 lux at 22°C ± 2°C. Cultures were examined after 2 to 3 weeks. All the experiments were repeated thrice with 21 replicates.

RESULTS

Direct Shoot Regeneration from Nodal Sector

Nodal segments obtained from in vivo-grown plants were inoculated on MS basal, MS fortified with different concentrations of phytohormones viz., BAP (1–4 mg/L), TDZ (0.5–2 mg/L), Kn (0.5–2 mg/L), and NAA (0.1 mg/L) singly as well as a combination of MS + BAP (1–4 mg/L) + NAA (0.1 mg/L), and 0.1% PVP was added to check the leaching in the medium. Out of these media, shoot formation was observed in BAP (1–4 mg/L), TDZ (0.5–2 mg/L), Kn (0.5–2 mg/L) and NAA (0.1 mg/L); however, leaching of phenols in the medium was observed. Addition of 0.1% PVP enhanced the shoot growth and reduced amount of the leaching. Multiple shoots were observed on Kn (0.5–2 mg/L). Maximum 5 to 20 numbers of shoots developed in Kn (0.5 mg/L) and lowest 2 to 10 numbers of shoots in Kn (2 mg/L). Details of results are displayed in Tables 1 to 4 and Figure 1.

Table 1: Effect of BAP on nodal sectors of A. bracteolata Lam

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus formation</th>
<th>Average number of shoot with ± SE</th>
<th>Root formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS plain</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MS + BAP (1 mg/L)</td>
<td>–</td>
<td>0.94 ± 0.72</td>
<td>–</td>
</tr>
<tr>
<td>MS + BAP (2 mg/L)</td>
<td>–</td>
<td>1.81 ± 0.83</td>
<td>–</td>
</tr>
<tr>
<td>MS + BAP (3 mg/L)</td>
<td>–</td>
<td>2.23 ± 0.82</td>
<td>–</td>
</tr>
<tr>
<td>MS + BAP (4 mg/L)</td>
<td>–</td>
<td>1.27 ± 0.77</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2: Effect of Kn on nodal sectors of A. bracteolata Lam

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus formation</th>
<th>Average number of shoot with ± SE</th>
<th>Average number of root with ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS plain</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MS + Kn (0.5 mg/L)</td>
<td>–</td>
<td>17.92 ± 0.94</td>
<td>3.85 ± 0.62</td>
</tr>
<tr>
<td>MS + Kn (1 mg/L)</td>
<td>–</td>
<td>13.78 ± 0.92</td>
<td>3.37 ± 0.58</td>
</tr>
<tr>
<td>MS + Kn (1.5 mg/L)</td>
<td>–</td>
<td>11.25 ± 0.88</td>
<td>2.83 ± 0.44</td>
</tr>
<tr>
<td>MS + Kn (2 mg/L)</td>
<td>–</td>
<td>7.93 ± 0.85</td>
<td>2.79 ± 4.43</td>
</tr>
</tbody>
</table>

Table 3: Effect of TDZ on nodal sectors of A. bracteolata Lam

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus formation</th>
<th>Average number of shoot with ± SE</th>
<th>Root formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + TDZ (0.5 mg/L)</td>
<td>–</td>
<td>1.31 ± 0.62</td>
<td>–</td>
</tr>
<tr>
<td>MS + TDZ (1 mg/L)</td>
<td>–</td>
<td>1.27 ± 0.58</td>
<td>–</td>
</tr>
<tr>
<td>MS + TDZ (1.5 mg/L)</td>
<td>+</td>
<td>0.62 ± 0.42</td>
<td>–</td>
</tr>
<tr>
<td>MS + TDZ (2 mg/L)</td>
<td>+</td>
<td>0.51 ± 0.39</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4: Effect of BAP and NAA on nodal sectors of A. bracteolata Lam

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus formation</th>
<th>Shoot formation</th>
<th>Root formation</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + BAP (1 mg/L) + NAA (0.1 mg/L) + 0.1% PVP</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Little sprouting of shoot</td>
</tr>
<tr>
<td>MS + BAP (2 mg/L) NAA (0.1 mg/L) + 0.1% PVP</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Sprouting of single shoot observed</td>
</tr>
<tr>
<td>MS + BAP (3 mg/L) NAA (0.1 mg/L) + 0.1% PVP</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Little sprouting of shoot observed</td>
</tr>
<tr>
<td>MS + BAP (4 mg/L) NAA (0.1 mg/L) + 0.1% PVP</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Shoot sprouting observed</td>
</tr>
</tbody>
</table>
Micropropagation of Dhumrapatra—(Aristolochia bracteolata Lam.)

In vitro-grown shoots were transferred to MS medium supplemented with IBA (1–4 mg/L) + 0.1% PVP. Root induction was observed in all concentrations of IBA, and maximum 2 to 3 roots per shoot were observed within three weeks in IBA (3 mg/L) + 0.1% PVP. Shoots inoculated on kinetin 1 to 4 mg/L also produced 2 to 3 roots per shoot simultaneously. Details are given in Tables 2, 5 and Figure 1.

ACCLIMATIZATION AND HARDENING

Acclimatization and hardening trials were conducted on cocopeat, garden red, black cotton soil, and mixture of black soil and sand (1:1); 70% of plantlets survived following transfer to the field.

DISCUSSION

In the present investigation, response of nodal segment on MS medium augmented with BAP, Kn, TDZ, NAA was studied. Maximum average 17.92 ± 0.94 number of shoot formation was observed on 0.5 mg/L Kn than the shoot formation on BAP (1–4 mg/L) and TDZ (0.5–2 mg/L). Similarly, Abu-Romman et al.13 observed the maximum shoot induction of nodal explants of Cucumber on MS fortified with Kn than other cytokinins viz., BAP and TDZ.

Earlier studies reported that BAP (4 mg/L) + NAA (0.5 mg/L) produced multiple shoots (6.5), while Kn combined with NAA did not induce shoots,14 and BAP (1 mg/L) + NAA (1 mg/L) regenerated 2.6 shoots from nodal segment.15 In the present study, it was observed that Kn alone at various concentrations 0.5 to 2 mg/L + 0.1% PVP produced profuse multiple shoots (20 nos.) within a 3-week period with 3 to 4 roots per explants. Response of nodal segment that was recorded using 1 to 4 mg/L BAP showed swelling of node and sprouting of shoots (1–3) after 4 weeks, and after the addition of 0.1% PVP, growth rate was enhanced and control over the leaching was achieved. In many plants, leaching of phenol content may change the chemical nature of medium and ultimately hamper the growth of explants. Medium containing TDZ (0.5–2 mg/L) also responded better but callus formation was observed; hence, TDZ was not found suitable for stimulating shoot induction as compared with the other hormones tried.

In contrast to our result, BAP was reported as the most effective plant growth hormone for shoot induction and multiplication in other species of Aristolochia. Chandra Prabha et al.16 reported that shoot tips and nodal segments of A. indica L. inoculated on MS medium fortified with BAP (1 mg/L) produced an average of 9.07 ± 0.37 and 13.71 ± 0.25; Kn (1.5 mg/L) showed 8.13 ± 0.39 and 10.33 ± 0.79; and TDZ (0.5 mg/L) produced 9.00 ± 0.48 number of shoots with 6.72 cm ± 0.25 average height per explants respectively. Similarly, in Aristolochia saccata Wall. and Aristolochia cathcartii Hook.f., the effect of BAP singly and in combination with NAA showed better response toward shoot formation. BAP (3 mg/L) + NAA (1.00 mg/L) produced maximum of 3.4 ± 0.55 number of shoots in A. saccata, whereas in A. cathcartii, BAP (4 mg/L) combined with 0.5 mg/L NAA produced 6.2 ± 0.44 number of shoots per explants.17 Also, combination of BAP (2 mg/L) + NAA (0.5 mg/L) was found to be beneficial in shoot production from nodal explants of Aristolochia tagala Champ. 18

Induction of roots from in vitro-grown shoot was easily achieved on IBA (1–4 mg/L) with 2 to 3 roots per shoots within 3 weeks in the present study. Similarly, the highest number of rootlets/in vitro-grown shoot 3.8 ± 0.26 was observed on ½ MS supplemented with 1.0 mg/L IBA.15,19 However, Sebastinraj and Sidique observed the maximum 11.1 ± 0.6 root induction with 10.2 ± 0.7 on 0.3 mg/L IBA.

CONCLUSION

The developed in vitro propagation protocol may be helpful for the mass production of plantlets which could be cultivated, transplanted in the barren land, or for commercial plantation. It would also be useful for conservation and multiplication of this important medicinal plant of Ayurveda.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1: *In vitro* grown shoots, roots, and hardening of the plant
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हिन्दी सारंश

शुक्रपद्म (एस्ट्रोलॉजिका ब्रैकटोइटोलेस लैम्ब) का सूक्ष्मप्रभाव: एक महत्वपूर्ण आयुर्वेदिय औषधीय पादप

वर्णन: भारतीय विज्ञान छात्रों में शुक्रपद्म एक महत्वपूर्ण औषधीय पादप है। अर्थशास्त्र उपयोग तथा अवश्यकता संबंध में कारण भारत देश के कुछ हिस्सों में यह दुर्लभ तथा विविधताधारी हो रहा है। प्रभावित औषधीय की आहुति के लिए तथा बड़े पैमाने पर पीढ़ियों के उपयोग के लिए, शुक्रपद्म की, इस विधिपरीक्षण तकनीक की विस्तारित किया गया है।

साभारी तथा विविधता: गैलरी की गणना से मायूसियत जीवों को पापात की गयी। मानक प्रोटोकॉल के अनुसार ग्ल्यूकोज़ तथा ल्यूटीन वेल्स गणना को करने का क्रियान्वयन किया गया। क्रियान्वयन के लिए 0.1% मर्यादा लेनसराइड की गणना से एक्स्प्रेस का साधारण लेनसराइड किया गया। गणना को 121 हिस्सों से जुड़ा तालमान तथा 15 एलकेएस/वर्ग इंच के साथ पर 15 मिनटों के लिए ऑपरेटर द्वारा किया गया। क्लास को 22 जंसाइ प्रजा 2 हिस्सों से जुड़ा तालमान तथा तीन हजार लक्ष्य की तीनता तथा प्रशासन एक्स्ट्रेम मेयरिनिंग पर 8 घंटों स्पष्ट किया गया। निदेशकों को चार प्रतिव हालत रिकॉर्ड किया गया।

परिणाम: 0.5 मिली ग्राम प्रति लीटर कोप्पी, के द्वारा संक्षिप्त एम. एल. मिडिया पर साफ़ शुद्ध (मूल संख्या - 20) को स्वाभाविक किया गया। 3 मिली ग्राम प्रति लीटर आई.वी.ए. द्वारा एबह्युलु पूर्वाकार करने पर आकर्षण बनाया कहर (2-3 साल) प्राप्त हुआ। एम. एल. + कोप्पी। (0.5 मिली ग्राम प्रति लीटर) + 0.1% पी-सी-आई. पर संचारित मोडल एक्स्ट्रेम प्रयोगक प्रयोग में शुद्ध तथा रेट प्राप्त हुए।

निश्चय: शुक्रपद्म के लिए विस्तारित इस विधि परीक्षण प्रोटोकॉल तीव्र गुणन तथा महत्वपूर्ण औषधीय पादप के संकेत हैं तथा लागभगह प्रजिया होगा।

मुख्य साधन: संतुलन, कांदेरियां, हार्मोन, जियोरिक्स, इन विद्युतो