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ORIGINAL RESEARCH

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

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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 floras. Murashige and Skoog basal (MS) medium was prepared as per the standard protocol. Surface sterilization of explants was done with the help of disinfectant and 0.1% solution of $HgCl_2$. Media were autoclaved at 121°C under 15 lb/inch^2 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 (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.

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INTRODUCTION

Aristolochia bracteolata Lam. (syn. *Aristolochia bracteata* Retz.) belongs to family Aristolochiaceae known as *Dhumrapatra* in Ayurveda.[1] 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 repellant properties due to presence of aristolochic acid.[2-6]

In Indian system of medicine, *Dhumrapatra* is mainly used as a single drug.[7] The drug possesses purgative,[8,9] antipyretic,[8,9] anti-inflammatory,[6,8,9] anthelmintic, emmenagogue, alterative, and abortifacient properties.[3,10] Its root is used to treat syphilis, gonorrhea, expelling roundworms, and skin diseases.[3,8,9] Leaves are bitter and used to cure worm infestation, dysmenorrhea, joint pain, urticaria, skin disorders, intermittent fever, dry and weeping eczema, and ulcer.[3,7,8] In Northern Nigeria traditionally, it is reported in the treatment of gastric cancer, lung inflammation, dysentery, snake bite, and scorpion stings.[6]

The major chemical constituents isolated and reported from the plant are aristolochic acid, magnoflorine, N-acetylnornuciferine, aristolactam, β-sitosterol, and ceryl alcohol.[7] It also contain esters, aristolactams, aporphines, isoquinoline, benzylisoquinoline, amides, lignans, coumarin, terpenoids, benzoids, and many others.[8]

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.[5] Herbarium specimens were prepared[11] 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 supplanted 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

Medium	*Callus formation*	*Average number of shoot with ± SE*	*Root formation*
MS plain	–	–	–
MS + BAP (1 mg/L)	–	0.94 ± 0.72	–
MS + BAP (2 mg/L)	–	1. 81 ± 0.83	–
MS + BAP (3 mg/L)	–	2.23 ± 0.82	–
MS + BAP (4 mg/L)	–	1.27 ± 0.77	–

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

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

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

Medium	*Callus formation*	*Average number of shoot with ± SE*	*Root formation*
MS + TDZ (0.5 mg/L)	–	1.31 ± 0.62	–
MS + TDZ (1 mg/L)	–	1.27 ± 0.58	–
MS + TDZ (1.5 mg/L)	+	0.62 ± 0.42	–
MS + TDZ (2 mg/L)	+	0.51 ± 0.39	–

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

Medium	*Callus formation*	*Shoot formation*	*Root formation*	*Observations*
MS + BAP (1 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	–	+	–	Little sprouting of shoot
MS + BAP (2 mg/L) NAA (0.1 mg/L) + 0.1% PVP	–	+	–	Sprouting of single shoot observed
MS + BAP (3 mg/L) NAA (0.1 mg/L) + 0.1% PVP	–	+	–	Little sprouting of shoot observed
MS + BAP (4 mg/L) NAA (0.1 mg/L) + 0.1% PVP	–	+	–	Shoot sprouting observed

Table 5: Effect of IBA on *in vitro*-grown shoots of *A. bracteolata* Lam

Medium	*Callus formation*	*Average number of root with ± SE*
MS + IBA (1 mg/L) + 0.1% PVP	–	0.5 ± 0.35
MS + IBA (2 mg/L) + 0.1% PVP	–	2.34 ± 0.43
MS + IBA (3 mg/L) + 0.1% PVP	–	2.85 ± 0.59
MS + IBA (4 mg/L) + 0.1% PVP	+	0.49 ± 0.29

Root Formation

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.

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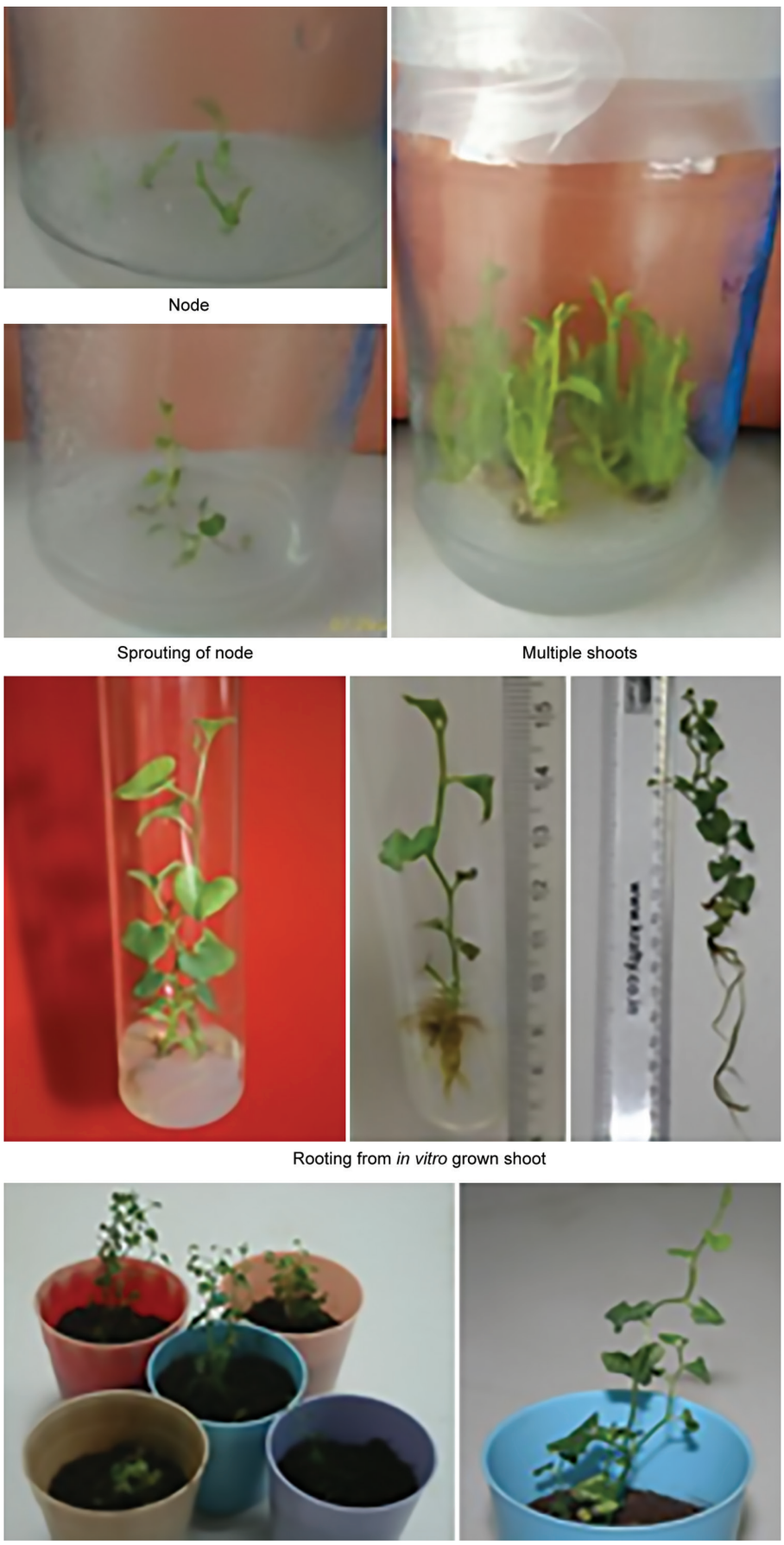

Fig. 1: *In vitro* grown shoots, roots, and hardening of the plant

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हिन्दी सारंश

धूम्रपत्र (*एरिस्टोलोकिआ ब्रैकटीओलेटा* लैम.) का सूक्ष्मप्रवर्धनः एक महत्वपूर्ण आयुर्वेदीय औषधीय पादप

उद्देश्यः भारतीय चिकित्सा पद्दति में प्रयुक्त *धूम्रपत्र,* एक महत्वपूर्ण औषधीय पादप है। अत्यधिक उपभोग तथा अव्यवस्थित संग्रहण के कारण भारत देश के कुछ हिस्सों में यह दुर्लभ तथा विलुप्तप्राय हो रहा है। प्रमाणित औषधी की आपूर्ति के लिए तथा बड़े पैमाने पर पौधे को उगाने के लिए, *धूम्रपत्र* की, इन विट्रो प्रोपेगेशन तकनीक को विकसित किया गया है।

सामग्री तथा विधियाँः फ्लोरा की मदद से संगृहीत पौधे की पहचान की गयी। मानक प्रोटोकॉल के अनुसार मुराषिगे तथा स्कूग बेसल माध्यम को तैयार किया गया। कीटाणुनाशक तथा 0.1% मरक्यूरिक क्लोराइड की मदद से एक्सप्लांटस का सतही रोगाणुनाशन किया गया। माध्यम को 121 डिग्री सेल्सियस तापमान तथा 15 एलबीएस/वर्ग इंच के दाब पर 15 मिनट्स के लिए ऑटोक्लेव किया गया। कल्चर्स को 22 प्लस माइनस 2 डिग्री सेल्सियस तापमान तथा तीन हजार लक्स की तीव्रता वाले प्रकाश युक्त फोटोपीरियड पर 8 घंटे इन्क्यूबेट किया गया। निरीक्षणों को चार सप्ताह पश्चात रिकॉर्ड किया गया।

परिणामः 0.5 मिग्रा प्रति लीटर केएन. के द्वारा संवर्धित एम्. एस. मिडिया पर सर्वश्रेष्ठ शूट (कुल संख्या – 20) को स्थापित किया गया। 3 मिग्रा प्रति लीटर आई.बी.ए. द्वारा एम्.एस. दृढ़ीकृत करने पर अधिकतम रूट्स (2–3 संख्या) प्राप्त हुए। एम्. एस. + केएन. (0.5 मिग्रा प्रति लीटर) + 0.1% पी.वी.पी. पर संचारित नोडल एक्सप्लांटस द्वारा अधिकतम शूट तथा रुट प्राप्त हुए।

निश्कर्षः *धूम्रपत्र* के लिए विकसित इन विट्रो प्रोपागेशन प्रोटोकॉल तीव्र गुणन तथा महत्वपूर्ण औषधीय पादप के संरक्षण हेतु लाभदायक सिद्ध होगा।

मुख्य शब्दः संरक्षण, कंजर्वेशन, हॉरमोन्स, रिजेनेरेशन, इन विट्रो