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In-vivo propagation of *Aristolochia tagala* – A rare medicinal plant species of Assam, India

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Abstract
In-vivo propagation was developed for *Aristolochia tagala*, a rare medicinal plant, using softwood cuttings in different rooting media and different concentrations of Indole-3- butyric acid (IBA). Softwood cuttings were taken from the soft, succulent new spring growth of the plants. Cuttings were disinfected by soaking in 1% w/v solution of bavistin and treated with four different rooting media i.e. the river sand, 1:1 ratio by volume river sand and peat moss, 1:1 ratio by volume river sand and garden soil, and 1:1:1 ratio by volume river sand, garden soil and peat moss and four different concentrations of rooting hormone IBA (500 ppm, 1000 ppm, 3000 ppm, and 5000 ppm). Data recorded were effective IBA concentrations in cuttings, the effect of the soil media, percentage of survival, mean number, and length of adventitious roots per cutting. The result showed remarkable growth differences in the various rooting media and hormone concentrations for the variables evaluated. Cuttings treated in rooting media containing river sand, garden soil, and peat moss (1:1:1 ratio by volume) with 3000 ppm IBA concentration had the best result of all the parameters observed and therefore recommended for invivo propagation of *A. tagala*.

Keywords: *Aristolochia tagala*, softwood cutting, rooting media, Indole butyric acid

Introduction

The genetic diversity of medicinal plants in the world is becoming endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for the production of medicines. Further, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization, etc., are also some important factors [1, 2]. Hence, there is a strong need for proactive understanding in the conservation, cultivation, and sustainable usage of important medicinal plant species for future use. Northeast India including Assam is represented by about 130 different tribes out of a total 427 of India having their traditional practices. Many herbal remedies individually or in combination have been recommended for the cure of different diseases in traditional medicinal practices by the ethnic communities of Northeast India [2].

Aristolochia tagala Chamisso, a climbing shrub belonging to the family aritolochiaceae is a rare medicinal plant distributed in India, Sri Lanka, China, Malaysia, Burma, Java, and Australia [3]. The chromosome number of *A. tagala* is 2n=12 [4]. Various parts of *A. tagala* are extensively used in traditional medicine. The juice from the leaves is used as a specific antidote for Cobra poison [5]. The roots are strongly aromatic and are used to treat snake bites, bone fractures, malaria, indigestion, rheumatism, toothaches, and various dermatological conditions by the Kani tribe of Thiruvananthapuram and Tirunelveli hills, India [3, 6]. Leaves are used to treat colic fits and bowel complaints. Due to indiscriminate harvesting of roots for local medicine and trade, the species has become rare in its natural habitat [3, 6].

Aristolochia species are sources of several physiologically active compounds of different classes. Aristolochic acid derivatives with various carbon skeletons, aporphines, benzyliso- quinolines, isoquinolines, protoberberines protopines, amides, chlorophylls, mono-, sesqui-, and diterpenoids, lignans, diphenyl ethers, flavonoids, tetralones, benzenoids, and steroids have been identified from different Aristolochia species [7]. The plant has been studied for several biological activities and was reported to have antiproliferative, anti-inflammatory, and antioxidant properties [8, 9]. Hadem *et al*., (2014) [10] reported the potential anticancer activity of aqueous-methanol extract of *A. tagala* in diethylnitrosamine-induced hepatocellular carcinoma (HCC) in BALB/c mice. The anticancer/antitumor activity has also been reported by Anilkumar *et al*., (2014) [11], Garg *et al*., (2007) [12], and Angeles *et al*., (1970) [13] against different cancer cell lines.

A. tagala has traditional uses as an anticancer, antifungal, antibacterial, and anti-infective agent [8]. Antimicrobial activity of the ethanolic extract of A.tagala roots has been reported in strains of Staphylococcus aureus [14]. The antibacterial activity of methanolic extract of *Aristolochia tagala* against resistant strains of clinical isolates of *Enterococcus faecalis* and *Staphylococcus aureus* has been reported by Lyngdoh *et al*. 2020 [15].

The compounds identified in the LC-HRMS analysis of crude aqueous-methanol extract of *A. tagala* by Hynniewta Hadem and Sen, 2018 [16] are *Aristolochia tagala* has also ecological importance. The caterpillars of the common birdwing (*Troides helena cerberus*) and common rose (*Pachliopta aristolochiae*) butterflies feed on the leaves of this plant [17].

For commercial cultivation and conservation, it is important to standardize the propagation technique of *A. tagala*. But in *A. tagala*, seed germination is low due to the presence of insufficient endosperm, which leads to low seed viability [18].

Hence, the objective of this study was to determine the possibility of using softwood cuttings for propagating *A. tagala* and to determine the effects of different rooting media, levels of IBA, and their interaction on rooting and survival of macropropagated *A. tagala*.

Table 1

Name of Compounds	Chemical formula
Aristolactame C IIIa	$C_{18}H_{15}NO_4$
Dehydrooxoperezinone	$C_{15}H_{14}O_4$
Pyriferine A	$C_{16}H_{22}N_2O_6$
Isocorydine	$C_{20}H_{23}NO_4$
Lagesianine A	$C_{20}H_{23}NO_5$
Kaempferol	$C_{15}H_{10}O_6$
3,5-Di-O-caffeoylquinic acid	$C_{29}H_{28}O_{15}$
Aristolactam IIIa; N-b-D-Glucopyranosyl isomer	$C_{22}H_{19}NO_9$
Aristolactam IIIa; O-β-D-Glucopyranoside isomer	$C_{22}H_{19}NO_9$
Aristolochic acid I	$C_{17}H_{11}NO_7$
4-O-beta-D-glucosyl-4-coumaric acid	$C_{15}H_{18}O_8$
Laptantine	$C_{15}H_{27}NO_6$
Medolin W/K/A/R	$C_{15}H_{22}O_2$
Perillyl acetate	$C_{12}H_{18}O_2$
Beta sistosterol	$C_{29}H_{50}O$
Δ-13, 14-2-Oxokolavenic acid	$C_{20}H_{30}O_3$
Medolin L/M/S	$C_{16}H_{24}O_2$
3-Oxoishwarane	$C_{15}H_{22}O$
Aristolactone	$C_{15}H_{20}$
Stigmasterol	$C_{29}H_{48}O$
Stigmastane-3,6-diol; (3b, 5a, 24R)-form, Diketone	$C_{29}H_{48}O_2$

Materials and method

Plant material

The experimental plant, *Aristolochia tagala* was collected from Lakhimpur district (27°27′16.8″ N, 94°12′11.58″ E) of Assam, India. The plant is quite glabrous, shrubby, twining leaves large cordate upper often narrow subsagittately lanceolate lower or all ovate or broadly ovate-oblong pedately 5-7 nerved, upper with the 2 principal nerves produced far beyond the middle, lower with all the nerves spreading, flowers in racemose puberulous cymes, lip of parianth villous. Quite glabrous, shrubby, twining, leaves large cordate upper often narrow subsagittately lanceolate lower or all ovate or broadly ovate-oblong pedately 5-7 nerved, upper with the 2 principal nerves produced far beyond the middle, lower with all the nerves spreading, flowers in racemose puberulous cymes, lip of parianth villous [19].

Phenology: Flowering: April- June; Fruiting: November-January.

Softwood cuttings

Softwood cuttings of *Aristolochia tagala* were taken from soft, succulent new spring growth of the plants. Cuttings were taken early in the morning when the plants are turgid and the cuttings were grouped based on their physiological ages. Cuttings were kept moist by wrapping with moist Sphagnum moss. The cuttings were separated into two-node with a leaf from the apex. The base of each cutting was cut with a slant. The leaves were cut into half of their original size to reduce transpiration. Cuttings were disinfected by soaking in 1% w/v solution of Bavistin. The cuttings were bundled into 24 pieces representing the number of assessment units per treatment. There were three replications with 8 cuttings per replication and 24 cuttings per treatment [20].

Rooting media

For softwood cuttings, four different rooting media i.e. River Sand (A1), 1:1 ratio by volume River sand and Peat moss (A2), 1:1 ratio by volume River sand and Garden soil (A3), and 1:1:1 ratio by volume River sand, Garden soil and Peat moss (A4) and four different ppm concentrations of rooting hormone IBA (500 ppm, 1000 ppm, 3000 ppm, and 5000 ppm) were used. Each rooting medium was sterilized by direct exposure to sunlight for five days and then treated with the fungicide bavistin. The medium was used to fill black polyethylene bags measuring 30 cm x 20 cm. The base of the bags was perforated to create room for drainage.

Experimental design

The experimental design was a 3 × 3 factorial experiment in a completely randomized design (CRD) under 65% shading. Treatment A represents the rooting media [River Sand (A1), 1:1 ratio by volume River sand and Peat moss (A2), 1:1 ratio by volume River sand and Garden soil (A3) and 1:1:1 ratio by volume River sand, Garden soil, and Peat moss (A4)], while treatment B represents IBA concentrations [distilled water (0 ppm), 500 ppm, 1000 ppm, 3000 ppm, and 5000 ppm]. Data were collected 60 days after planting of cuttings. Data recorded were effective IBA concentrations in cuttings, the effect of the soil media, percentage of survival (percentage of cuttings with roots and shoots divided by the total number of cuttings planted), mean number of roots per cutting (total number of roots divided by total number of cuttings with roots), mean length of adventitious roots per cutting (total length of all roots divided by total number of roots) [20]. These parameters were statistically analyzed.

Results and Discussion

In this experiment, we observed the response of cuttings in different rooting media and different concentrations of IBA alone and their cumulative effect (Tables1, 2, and 3). The rooting media A4 i.e. the mixture of river sand, garden soil, and peat moss (1:1:1 ratio by volume) showed the best result than the other three rooting media when it was treated alone. Percent survival, mean number of roots per cutting, mean length of the adventitious roots per cutting recorded in this rooting media were 83.3 ± 2.8 %, 2.8 ± 0.03 and 4.14 ± 0.09 cm respectively (Table: 1). Rooting media containing only river sand gave a poor result in all the parameters observed in this experiment. Further, the 3000 ppm IBM solution showed the best result than the other three IBA concentrations and the control solution when treated alone. Here the percent survival, mean number of roots per cutting, mean length of the adventitious roots per cutting recorded in 3000 ppm concentration of IBA were 88.6 ± 2.8 %, 3.14 ± 0.09 and 5.12 ± 0.06 cm respectively (Table: 2). The cumulative effect of rooting media and different concentrations of IBA was also observed. The highest percent survival (96.6± 2.8%), mean number of roots per cutting (3.6± 0.07), mean length of the adventitious roots per cutting (5.16 ± 0.04 cm) was observed in combination of River sand, Garden soil and Peat moss (1:1:1 ratio by volume) with 3000 ppm IBA concentration (A4B4) (Table 3). Rooting media containing only river sand with 500 ppm IBA concentration gave a poor result in all the parameters observed in this experiment (Table 3).

Table 1: Percent survival, mean number of roots per cutting and mean length of adventitious roots of *Aristolochia tagala* as affected by rooting media

Rooting media	% survival	Mean number of roots/cutting	Mean length of adventitious roots (cm)/cutting
River Sand (A1)	44.3± 2.8	1.2± 0.06	2.2± 0.07
River sand and Peat moss (A2)	63.3± 2.8	2.14± 0.07	3.14± 0.08
River sand and Garden soil (A3)	65.3± 2.8	2.08± 0.08	3.12± 0.07
River sand, Garden soil and Peat moss (A4)	83.3± 2.8	2.8 ± 0.03	4.14 ± 0.09

(Data mean of 3 replicates ± S.D.)

Table 2: Percent survival, mean number of roots per cutting and mean length of adventitious roots per cutting of *Aristolochia tagala* as affected by levels of IBA

IBA level (ppm)	% survival	Mean number of roots/cutting	Mean length of adventitious roots (cm)/cutting
Control (B1)	0	0	0
500 (B2)	61.6± 2.8	2.3± 0.07	3.3± 0.03
1000 (B3)	71.3± 2.8	2.8± 0.07	3.9± 0.04
3000 (B4)	88.6± 2.8	3.14 ± 0.09	5.12± 0.06
5000 (B5)	76.6± 2.8	2.8± 0.04	4.2± 0.08

(Data mean of 3 replicates ± S.D.)

Table 3: Percent survival, mean number of roots per cutting and mean length of adventitious roots per cutting of *Aristolochia tagala* cuttings as affected by interaction effects of rooting media and levels of IBA

Rooting media X Levels of IBA	% survival	Mean number of roots/cutting	Mean length of adventitious roots (cm)/cutting
A1B1	44.3± 2.8	1.2± 0.06	2.2± 0.07
A1B2	61.6± 2.8	1.5± 0.06	2.5± 0.04
A1B3	70.2± 2.8	1.8± 0.08	2.8± 0.04
A1B4	90.3± 2.8	2.1± 0.04	3.1± 0.06
A1B5	75.6± 2.8	1.8± 0.04	2.8± 0.06
A2B1	63.3 ± 2.8	2.14± 0.07	3.14± 0.08
A2B2	70.6± 2.8	2.2± 0.08	3.16± 0.09
A2B3	75.3± 2.8	2.21± 0.05	3.16± 0.09
A2B4	83.3± 2.8	2.6± 0.06	4.08± 0.05
A2B5	80.6± 2.8	2.3± 0.08	4.01± 0.05
A3B1	65.3 ± 2.8	2.08± 0.08	3.12± 0.07
A3B2	78.3± 2.8	2.1± 0.06	3.17± 0.09
A3B3	80.6± 2.8	2.3± 0.05	4.1± 0.04
A3B4	88.6± 2.8	2.8± 0.05	4.16± 0.06
A3B5	81.6± 2.8	2.3± 0.05	4.1± 0.08
A4B1	83.3 ± 2.8	2.8 ± 0.03	4.14 ± 0.09
A4B2	83.6± 2.8	3.1± 0.08	4.18± 0.05
A4B3	85.6± 2.8	3.3± 0.07	5.01± 0.05
A4B4	96.6± 2.8	3.6± 0.07	5.16 ± 0.04
A4B5	91.3± 2.8	3.3± 0.05	5.08± 0.06

(Data mean of 3 replicates ± S.D.)

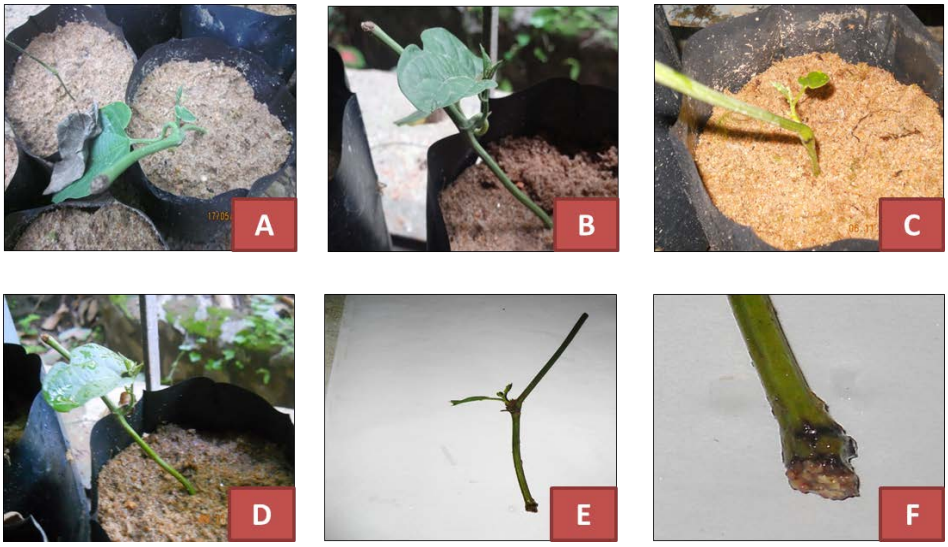

Fig 1: Photograph showing different stages of development of softwood cuttings of *Aristolochia tagala* tried in mixture of rooting media containing river sand, garden soil, and peat moss (1:1:1 ratio by volume) with 3000 ppm IBA.

The number of adventitious roots and their length were affected by the concentration of IBA and rooting media. Percent survival of *A.tagala* in different rooting media after 60 days showed significant differences between treatments (Table 1, 2, and 3). The combination of river sand, garden soil, and peat moss (A4) had the highest mean survival (83.3 ± 2.8%), while river sand (A1) gave the lowest survival (44.3 ± 2.8%). There was an increase of 39% survival using mixture of river sand, garden soil, and peat moss (1:1:1 ratio by volume) over that of using river sand alone. Again using different rooting media improves the production of adventitious roots (number and length) of *A. tagala* cuttings (Table 1). Application of IBA increased percentage of survival by 96.6 ± 2.8% for cuttings treated with 3000 ppm IBA over untreated cuttings (Table 3). Cuttings treated with 500 and 1000 ppm IBA (B2 and B3) had shorter roots, while those stem cuttings that received 3000 ppm IBA (B4) had tough and long adventitious roots with several second-order roots or root hairs. In *Milicia excelsa*, 0.2% IBA treatment increased the final rooting percentage by 9% above that of the control [21]. Likewise, hormone application in *Eucalyptus camaldulensis* increased the root number and root vigor but not the rooting percentage [22]. Cuttings treated with 3000 ppm IBA (B4) gave the highest number of adventitious roots, over those treated with 500 ppm (B2), 1000 (B3), and 5000 ppm IBA (B5) (Table 2). The number of adventitious roots was improved in 3000 ppm IBA (3.14 ± 0.09) as compared with the untreated cuttings (Table 2). The same finding was noted in *E. camaldulensis*, which gave a higher root number through hormone application [22]. Cuttings treated with 3000 ppm IBA (B4) produced the longest root (5.12 ± 0.06 cm) as compared with those treated with 500, 1000, and 5000 ppm IBA (B1, B2, B3, B5) with a mean of 3.3 ± 0.03, 3.9 ± 0.04 and 4.2 ± 0.08 cm respectively (Table 2). Shorter roots were developed on those treated with 500 and1000 ppm IBA (B2, B3) (Table 2). Interaction of rooting media and levels of IBA in the study revealed significant differences in percent survival and length of adventitious roots (Table 3, Figure 1). The survival rate ranged from 44.3 ± 2.8% to 96.6 ± 2.8%. The highest survival (96.6 ± 2.8%) was observed on cuttings planted in a mixture of river sand, garden soil, and peat moss (1:1:1 ratio by volume) treated with 3000 ppm IBA (A4B4). The mixture of river sand, garden soil and peat moss (1:1:1 ratio by volume) treated with 3000 ppm IBA (A4B4) produced the longest root with a mean of 5.16 ± 0.04 cm. This was significantly longer as compared with the rest of the treatments. Stem cuttings that were rooted in pure river sand and without IBA (A1B1) with a mean of 2.2 ± 0.07 cm produced the shortest root. During the harvesting of *A. tagala* stem cuttings, profuse rooting was noted in all cuttings treated with IBA as compared with the control. The elongated roots exhibited by cuttings rooted in a mixture of river sand, garden soil, and peat moss (1:1:1 ratio by volume) treated with 3000 ppm IBA (A4B4) can be an advantage in the growth and development of *A.cathcartii*.

Conclusions

We present invivo propagation technique of *Aristolochia tagala*, a rare medicinal plant of Assam, India. We tried softwood cuttings of *A. tagala* in different rooting media and different concentrations of Indole-3-butyric acid. Cuttings treated in a mixture of rooting media containing river sand, garden soil, and peat moss (1:1:1 ratio by volume) with 3000 ppm IBA concentration had the best result of all the parameters observed. As a result, the findings would be extremely useful in the conservation and commercial cultivation of *A. tagala.*

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The authors received no specific funding for this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

List of abbreviation

IBA: Indole-3-Butyric Acid
PPM: Parts Per Million

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