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In vitro callus induction and indirect organogenesis of *Brucea mollis* Wall. ex Kurz – A potential medicinal plant of Northeast India

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ABSTRACT

The present investigation was carried out to develop an efficient protocol for *in vitro* propagation of *Brucea mollis* Wall. ex Kurz, an important medicinal plant, confined to only Karbi Anglong district of Assam. The plant is extensively used to cure malaria. Due to several natural factors like inefficient pollination, seed dispersal mechanisms and anthropogenic activities, the plant is facing extinction. Through indirect organogenesis from leaf and internode explants on MS (Murashige and Skoog) media, callus induction was achieved at a maximum (100%). However, the minimum time (7–15 days) was observed for induction of callus on B5 as compared to MS media. The color and texture of the callus varied depending on the type of explants as well as the concentrations of growth regulators used in both the media. Best response for percentage of callus induction (100%) was obtained from the leaf explants on MS media, when the media was supplemented with the four combinations of growth regulators, *i.e.*, (i) BAP (8.88 μM) + NAA (1.61 μM), (ii)BAP (8.88 μM) + NAA (2.68 μM), (iii) BAP (8.88 μM) + 2,4-D (2.27 μM) and (iv) BAP (8.88 μM) + 2,4-D (4.54 μM). While in the case of internode explants, maximum (100%) percentage for callus induction was obtained with BAP (8.88 μM) + NAA (1.61 μM) and BAP (8.88 μM) + NAA (2.68 μM). Multiple shoot regeneration was achieved from the callus of all the three types of explants when transferred to shoot regeneration media (both MS and B5 media) supplemented with BAP, NAA, Kinetin and IBA at different concentrations and combinations. The maximum percentage of shoot regeneration (100%) from the calli of all explant types were obtained with the combination of BAP, NAA and IBA. However, the best response in terms of minimum number of days taken to regenerate shoots and maximum shoot length was obtained using the leaf explants at BAP (8.88 μM) + NAA (2.68 μM) + Kinetin (13.95 μM) on MS media. The regenerated shoots were cultured for rooting on half strength media supplemented with IBA and NAA singly, and the highest rooting percentage was achieved when MS media was supplemented with 14.76 μM IBA. About 60% of the plantlets survived during acclimatization and were successfully transferred to the field. The regeneration protocols standardized for *B. mollis* could be found most effective for mass propagation of the medicinally important plant as well as its conservation.

1. Introduction

Brucea mollis Wall. ex Kurz is an important medicinal shrub belonging to the family Simaroubaceae. It is reported to occur in China, Bhutan, Cambodia, Malaysia, Laos, Myanmar, Philippine, Nepal, Vietnam and Thailand (Bharati and Singh 2012; Das et al. 2017; Borthakur et al. 2018). In India, the plant is mainly confined to Northeastern regions *viz.,* Assam, Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Sikkim and West Bengal (Darjeeling) (Gupta et al. 2004).

Brucea mollis is locally known as '*Koinine*', which is confined to only Karbi Anglong district of Assam. It is a popular antimalarial plant used by several ethnic communities of Northeastern region. Its fruit and root decoction is used by the local people of Karbi Anglong District to cure malaria (Singh et al. 2003; Pullaiah 2006; Bhutani 2008).

There are reports of phytochemical compounds in *B. mollis* which display potential bioactive compounds such as antiplasmodial, cytotoxic, pesticide, antimalarial, anticancer, antitumor activities etc. (Ouyang et al. 1994a, 1994b; Ouyang et al. 1995; Dembitsky 2005; Liu et al. 2009; Bharati and Singh 2012; Prakash et al. 2013). Due to its importance in folk medicine, the species has drawn attention in pharmacological research.

Inspite of immense potentiality, the population size of *B. mollis* is drastically reduced due to over exploitation and different anthropogenic activities. The species has been recorded as endangered during the CAMP (Conservation assessment and Management Plan) survey in 2003 (Baruah et al. 2017; Kakati and Borthakur 2017; Borthakur et al. 2018). Therefore, development of its mass propagation technique is urgently needed for both conservation as well as availability of plant

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Table 1
Effect of BAP, NAA and 2,4- D on callus induction in different explants on MS media.

Plant growth regulators (μM) in MS media			Leaf explants		Node explant		Internode explant	
BAP	NAA	2,4 D	Percentage of callus induction	No. of days taken for callus induction	Percentage of callus induction	No. of days taken for callus induction	Percentage of callus induction	No. of days taken for callus induction
8.88	1.61	–	100.00 ± 0.00^{a}	15.53 ± 0.50^{b}	60.00 ± 20.00^{bc}	13.06 ± 0.23^{cd}	100.00 ± 0.00^{a}	12.93 ± 1.36^{c}
8.88	2.68	–	100.00 ± 0.00^{a}	15.33 ± 0.30^{b}	53.33 ± 11.54^{bcd}	11.46 ± 0.57^{a}	100.00 ± 0.00^{a}	11.06 ± 0.64^{ab}
8.88	5.37	–	80.00 ± 20.00^{b}	14.06 ± 2.15^{b}	53.33 ± 11.54^{bcd}	12.73 ± 0.70^{b}	80.00 ± 20.00^{ab}	12.06 ± 0.83^{abc}
8.88	10.74	–	73.33 ± 11.54^{bc}	15.20 ± 0.80^{b}	46.66 ± 11.54^{cd}	13.26 ± 0.94^{bc}	86.66 ± 11.54^{ab}	11.73 ± 0.75^{abc}
8.88	–	2.27	100.00 ± 0.00^{a}	10.80 ± 0.80^{a}	73.33 ± 11.54^{ab}	13.53 ± 0.92^{bcd}	93.33 ± 11.54^{ab}	10.66 ± 0.46^{a}
8.88	–	4.54	100.00 ± 0.00^{a}	12.00 ± 0.52^{a}	93.33 ± 11.54^{a}	13.06 ± 0.75^{bc}	73.33 ± 11.54^{bc}	11.13 ± 0.41^{ab}
8.88	–	9.08	86.66 ± 11.54^{ab}	15.00 ± 0.60^{b}	53.33 ± 11.54^{bcd}	14.80 ± 0.60^{d}	73.33 ± 11.54^{bc}	12.73 ± 0.46^{c}
8.88	–	13.63	86.66 ± 11.54^{ab}	15.53 ± 0.41^{b}	33.33 ± 11.54^{d}	14.26 ± 0.80^{cd}	53.33 ± 11.54^{c}	12.46 ± 0.50^{bc}

Values are Mean ± SD of five replicates from three repeated experiments. Means with common superscript within each column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT).

material for bioprospection. The conventional technique of *B. mollis* propagation is less efficient due to limited seed availability as well as low seed germination (Kakati and Borthakur 2017). As a result, the plant becomes rare in its natural habitats. Therefore, there is a need to develop an efficient micropropagation protocol. The present study deals with indirect organogenesis through callus mediated induction of leaf, node and internode explants of *B. mollis*. The protocol established will certainly benefit germplasm conservation of this endangered medicinal plant as well as expand pharmaceutical prospects.

2. Methods and materials

2.1. Explants, culture medium and culture establishment

Leaves, nodes and internodes of 8–9 months old plant of *B. mollis* were collected as explants from the Botanical Garden of Gauhati University, Assam. Young leaves (10–15 days old) as well as nodes and internodes (of about 8–9 months old plants) were surface sterilized by washing under tap water to remove the dust particles, followed by exposure to 0.1% $HgCl_2$ for 5 min then finally rinsed with sterile distilled water three times and dried under aseptic conditions (Sarma and Tanti 2017). Explants of 1 cm × 0.5 cm were used for inoculation. The explants were cultured on MS (Murashige and Skoog 1962) and Gamborg B5 media (Gamborg et al. 1968) supplemented with 3% w/v sucrose, 0.8% w/v agar and supplemented with plant growth regulators auxins (NAA, 2,4-D, IBA) and cytokinins (BAP, Kinetin). pH was maintained at 5.8 ± 0.2 before adding agar and autoclaved at 121 °C for 20 min at 15 lb. pressure. The cultures were maintained at 25 ± 2 °C and 60–70% relative humidity with 3 K light intensity for 16 h light and 8 h dark conditions. Each treatment was repeated thrice with five replicates per treatment at 1 month interval (Hore and Tanti 2018).

2.2. Callus induction

Calli were induced on MS and B5 media supplemented with BAP (8.88 μM), NAA (1.61 μM, 2.68 μM, 5.37 μM, 10.74 μM) and 2,4-D (2.27 μM, 4.54 μM, 9.08 μM, 13.63 μM) in two different sets of combinations (Table 1-2). The explants were cultured in test tubes (55 ml) and subcultured in 12–15 days interval on the culture medium containing the same concentrations of plant growth regulators. The data pertaining to callus induction percentage, time taken for callus induction, texture and color were recorded after six weeks of culture. The number of days taken for callus induction was calculated by mean ± standard deviation (Tables 1–2). After 3–4 repeated sub-culturing cycles, the calli were excised and transferred to different treatments, *i.e.*, (i) BAP + NAA + Kinetin and (ii) BAP + NAA + IBA in four different concentrations.

2.3. Indirect organogenesis

For shoot regeneration, the calli were cultured on both media containing BAP (8.88 μM), NAA (1.61 and 2.68 μM), Kinetin (2.32, 4.65, 9.30 and 13.95 μM) and IBA (2.46, 4.92, 9.84 and 14.76 μM) in different combinations. The parameters, *i.e.*, percentage of shoot regeneration, number of days taken for shoot regeneration, number of shoots regenerated, number of leaves and shoot length (cm) were taken after 30 days (Tables 3–4).

2.4. In vitro root initiation

In vitro derived microshoots were cultured on full and half strength MS and B5 media containing IBA (2.46, 4.92, 9.84, 14.76 and 19.68 μM) and NAA (2.68, 5.37, 10.74, 16.11 and 21.48 μM), respectively. The observed parameters were percentage of root initiation; number of days taken for root initiation, number of roots regenerated and root length (cm) (Figs. 1–2).

Table 2
Effect of BAP, NAA and 2,4- D on callus induction in different explants on B5 media.

Plant growth regulators(mg/L) in MS media			Leaf explant		Node explant		Internode explant	
BAP	NAA	2,4 D	Percentage of callus induction	No. of days taken for callus induction	Percentage of callus induction	No. of days taken for callus induction	Percentage of callus induction	No. of days taken for callus induction
8.88	1.61	–	86.66 ± 11.54^{ab}	10.80 ± 0.91^{c}	80.00 ± 20.00^{a}	11.80 ± 1.24^{d}	93.33 ± 11.54^{a}	12.00 ± 0.87^{d}
8.88	2.68	–	86.66 ± 11.54^{ab}	10.66 ± 1.81^{c}	86.66 ± 11.54^{a}	10.06 ± 1.00^{c}	86.66 ± 11.54^{ab}	10.66 ± 0.46^{bc}
8.88	5.37	–	83.33 ± 15.27^{ab}	8.53 ± 0.94^{ab}	80.00 ± 20.00^{a}	8.06 ± 0.30^{b}	73.33 ± 11.54^{ab}	10.06 ± 0.57^{b}
8.88	10.74	–	73.33 ± 11.54^{ab}	8.06 ± 0.41^{a}	80.00 ± 20.00^{a}	7.16 ± 0.57^{a}	73.33 ± 11.54^{ab}	8.33 ± 0.57^{a}
8.88	–	2.27	93.33 ± 11.54^{a}	10.13 ± 0.30^{bc}	86.66 ± 11.54^{a}	11.66 ± 0.83^{d}	80.00 ± 20.00^{ab}	11.93 ± 0.23^{d}
8.88	–	4.54	86.66 ± 11.54^{ab}	11.20 ± 0.34^{c}	80.00 ± 20.00^{a}	11.80 ± 1.00^{d}	93.33 ± 11.54^{a}	11.20 ± 0.34^{cd}
8.88	–	9.08	66.66 ± 11.54^{bc}	13.93 ± 0.46^{d}	66.66 ± 11.54^{ab}	8.73 ± 0.75^{bc}	66.66 ± 11.54^{bc}	10.26 ± 0.30^{bc}
8.88	–	13.63	66.66 ± 11.54^{bc}	14.73 ± 1.33^{d}	66.66 ± 11.54^{ab}	9.33 ± 0.94^{bc}	46.66 ± 11.54^{c}	8.4 ± 0.52^{a}

Values are Mean ± SD of five replicates from three repeated experiments. Means with common superscript within each column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT).