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Research Article

Effect of 2, 4-D on callus induction at nodal and internodal explants of *Brucea mollis* Wall. ex Kurz- an endangered plant of Northeast India

Prapty Das*, Bhaben Tanti and S. K. Borthakur

Department of Botany, Gauhati University, Assam, India, Pin-781014
*das.prapty@gmail.com

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Abstract

The present study deals with *in vitro* callus induction from the nodal and internodal explants of *Brucea mollis* Wall. ex Kurz, an endangered plant of Northeast India with 2,4-D at various concentrations (0.5. 1, 2, 3 mg/L) in MS media. The explants exhibited positive response at all the hormone concentrations. Both nodal and internodal explants showed 100% callus induction frequency at 2,4 -D (1 mg/L). The color of the calli varied from creamish to light brown and the texture was compact. The weight of the callus was highest at 2,4- D (1 mg/L) in both nodal and internodal explants. The callus showed shooting response at different combination of BAP and Kinetin. Both the explants showed 100% callus regeneration frequency with highest shoot number (7.2± 1.28) at BAP (4 mg/L) and Kinetin (0.5 mg/L)) in internodal explants. Among all the hormonal concentration BAP (4 mg/L) and Kinetin (0.5 mg/L) showed best shooting response in terms of shoot number, shoot length and number of leaves.

INTRODUCTION

Brucea mollis Wall. ex Kurz belonging to the family Simaroubaceae is an important medicinal plant confined to the Northeastern region of India. It is used traditionally for the treatment of malaria (Borthakur, 1976). In addition to having cardiovascular effect, the plant exhibits anticancer and diuretic activities (Dhawan *et al.*, 1977; Rastogi and Dhawan, 1990). The plant is also used to cure stomachache and used as anti tumor, insecticide, pesticide, plasmodicide, amebicide and anti plasmodial medicine (Bharati and Singh, 2012). It is reported from Bhutan, Cambodia, China, Laos, Malaysia, Myanmar, Nepal, Philippine, Thailand and Vietnam (Pullaiah, 2006). In India its distribution is restricted only to the Northeastern region (Gupta *et al.*, 2004). The species is considered to be endangered in Arunachal Pradesh and Assam during the CAMP workshop held in Guwahati (Anonymous, 2003). Further *B. mollis* has been listed as NT (near threatened) plant species of Meghalaya (Anonymous, 2005). The chemical compounds isolated from *B. mollis* has been reported along with their biological activities. (Bharati and Singh, 2012, Chen *et al.*, 2011, Liu *et al.*,2009, Ouyang *et al.*,1994a, Ouyang *et al.*,1994b, Ouyang *et al.*, 1995, Tung *et al.*, 2013). Recently, studies on indigenous knowledge and approach for conservation of *B. mollis* was reported and was suggested for conservation of this endangered medicinal plant (Kakoty and Borthakur, 2017). This is the first report on callus induction of *Brucea mollis* through tissue culture approach. Considering the above facts in mind the present study was taken

up to develop a protocol for callus induction using 2,4- D hormone in nodal and internodal explants of this endangered plant of Northeast India .

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MATERIALS AND METHODS

Plant material

Young stems consisting of nodes and internodes were taken as the explants from 6 months old plant of *B. mollis*. It was collected from the experimental garden of Department of Botany, Gauhati University, India

Surface sterilization

Young stems consisting of nodes and internodes were first washed under tap water for 3-4 min to remove the dust particles. The explants were then rinsed with autoclaved distilled water and surface sterilized with 0.1 % (w/v) mercuric chloride for 4-5 min under laminar flow chamber. After that the explants were again washed with autoclaved distilled water for 4-5 min in order to remove the traces of mercuric chloride. Finally the explants were soaked with sterilized filter paper.

Culture Medium

The nodal segments of about 0.5 cm and internodal segments of 1 cm were excised with sterilized scalpel and cultured in the test tubes containing MS (Murashige and Skoog, 1962) medium with 3 % sucrose and 0.8% agar supplemented with 2,4-D (2,4 dichlorophenoxyacetic acid) at 0.5, 1, 2 and 3 mg/L for inducing callus. The pH was maintained at 5.8 before adding agar and autoclaved at 121ºC for 20 min at 15 lb pressure.

Culture conditions

The cultures were maintained at 25±2ºC and 60-70 % relative humidity with 3 K light intensity for 16 hrs light and 8 hrs dark conditions. Each treatment was repeated thrice with 5 replicates per treatment. Sub culturing was done at 15 days interval and observations were recorded.

Data recording and analysis

The data pertaining to callus intensity, callus induction percentage, texture, color, initiation time were recorded. The callus weight was calculated as mean ± standard error.

$$\text{Callus induction \%} = \frac{\text{No. of explants callusing}}{\text{Total no. of explants in the culture}}$$

$$\text{Callus regeneration frequency \%} = \frac{\text{No. of callus regenerated in shoots}}{\text{Total no. of calli in the culture}}$$

RESULTS

Effect of 2,4 - D on callus induction in nodal explants

The nodal explants exhibited positive response at all the concentrations of 2,4- D. It took 7-16 days to initiate callus and all the calli were compact in texture. The callus initiated from the basal cut end portion of the explants and the color of callus varied from creamish to light brown. Highest percentage (100%) of callus induction frequency showed at 1 mg/L of 2,4- D in 30 days (Table 1, Fig 1). However, the percentage of callus induction decreases with increase in hormonal concentration. Weight of callus was taken at 30 and 50 days interval to record the growth potency. Highest weight of callus was observed at 2,4 D (1 mg/L) with 0.373±0.0048 g and 0.716±0.016 g on 30th day and 50th day respectively (Table 2).

Effect of 2,4 D on callus induction in internodal explants

Callus initiated from the cut end portion of the internode explants in 15-18 days. Color of the callus varies from cream to light brown and texture was compact. Best callogenic response was observed at 2,4- D (1 mg/L) with 100% frequency of callus induction in 30 days (Table 1, Fig 2). Frequency of callus induction however decreases with increase in concentrations. Highest weight of callus was observed at 2,4 D (1 mg/L) with 0.371±0.001 g and 0.618±0.015 g on 30th and 50th day respectively (Table 2).

Indirect shoot organogenesis from callus

The morphology of callus changed when they were subcultured for regeneration at a combination of BAP and Kinetin. The texture of the callus was hard and compact and the color changed into light green (Fig 3). Shoot initiation took place in both the explants when cultured at BAP (1, 2, 3, 4 mg/L) and Kinetin (0.5 mg/L).

Shoot initiation took place between 25-30 days in both the explants. Highest shoot length (4.4 ± 0.81) cm and (7.2 ± 0.604) cm was observed in BAP (4 mg/L) and Kinetin (0.5 mg/L) in nodal and internodal explants respectively. Best shooting response was found at BAP (4 mg/L) and Kinetin (0.5 mg/L) with highest number of shoots 5.0 ± 1.0 at nodal callus and 7.2 ± 1.28 at internodal callus. Callus regeneration frequency was found to be 100% at all the concentrations.

Table 1. Effect of callus induction at various concentration of 2,4- D in nodal and internodal explants

MS + 2,4-D	Source explant	No. of explants inoculated	Callus induction percentage	Intensity of callus formation	Initiation time in days	Colour	Texture
0.5 mg/L	Node	5	80%	+++	7	Light Brown	Compact
0.5 mg/L	Inter node	5	60%	++	15	Light Brown	Compact
1 mg /L	Node	5	100%	+++	10	Cream colour	Compact
1 mg/L	Inter node	5	100%	+++	11	Cream colour	Compact
2 mg/L	Node	5	60%	++	13	Cream colour	Compact
2 mg/L	Inter node	5	40%	++	12	Cream colour	Compact
3 mg/L	Node	5	40%	+	16	Cream colour	Compact
3 mg/L	Internode	5	40%	+	18	Cream colour	Compact

Observation: profuse callus +++, moderate callus ++, poor callus +

Table 2. Weight of the callus at different concentration of 2,4 D on 30th day and 50th day

MS + 2,4-D (mg/L)	Source Explants	Weight of callus on 30th day (g)	Weight of callus on 50th day (g)	Stage of the callus on 50th day
0.5	Node	0.362±0.006	0.529±0.034	Pre embryonic
	Internode	0.212±0.011	0.478±0.01	Pre embryonic
1.0	Node	0.373±0.0048	0.716±0.016	Pre embryonic
	Internode	0.371±0.001	0.618±0.015	Pre embryonic
2.0	Node	0.110±0.037	0.476±0.038	Pre embryonic
	Internode	0.152±0.028	0.188±0.022	Pre embryonic
3.0	Node	0.089±0.005	0.183±0.019	Pre embryonic
	Internode	0.083±0.003	0.098±0.002	Pre embryonic

Table 3. Effect of BAP and Kinetin on shoot regeneration from the nodal callus

Hormone	Hormonal concentration (mg/L)	Callus regeneration frequency (%)	Number of shoots	Number of leaves	Shoot length (cm)
BAP + Kinetin	1.0 + 0.5	100%	2.6±0.4	2.8±0.37	0.68±0.226
	2.0+0.5	100%	3.6 ± 0.509	3.0± 0.44	1.4 ± 0.367
	3.0+0.5	100%	4.4 ± 0.927	4.8 ± 0.86	2.94 ± 0.22
	4.0+0.5	100%	5.0 ± 1.0	4.1 ± 0.67	4.4 ± 0.81

Observation: Each treatment consists of 5 replicates and was recorded after 30 days from the day of subculture in regeneration media. The values are mean ± standard error.

Table 4. Effect of BAP and Kinetin on shoot regeneration from the internodal callus

Hormone	Hormonal concentration (mg/L)	Callus Regeneration frequency (%)	Number of shoots	Number of leaves	Shoot length (cm)
BAP + Kinetin	1+0.5	100%	3.8±0.734	4± 0.707	2.84 ± 1.12
	2+0.5	100%	5.4±0.927	5 ± 0.632	5.2 ± 0.969
	3+0.5	100%	5.8 ± 1.15	5 ± 0.707	5.4 ± 1.15
	4+ 0.5	100%	7.2 ± 1.28	7.4 ± 1.249	7.2 ± 0.604

Observation: Each treatment consists of 5 replicates and was recorded after 30 days from the day of subculture in regeneration media. The values are mean ± standard error.

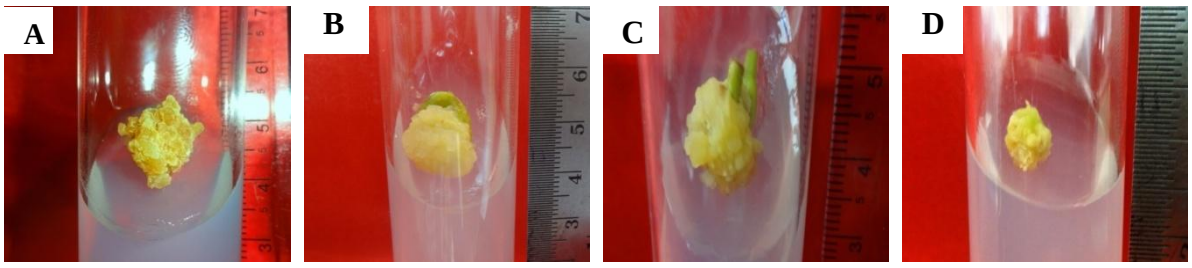

Fig. 1: Callus at different 2,4 -D concentrations of nodal explants, A. 0.5 mg/L, B. 1.0 mg/L, C. 2.0 mg/L and D. 3.0 mg/L

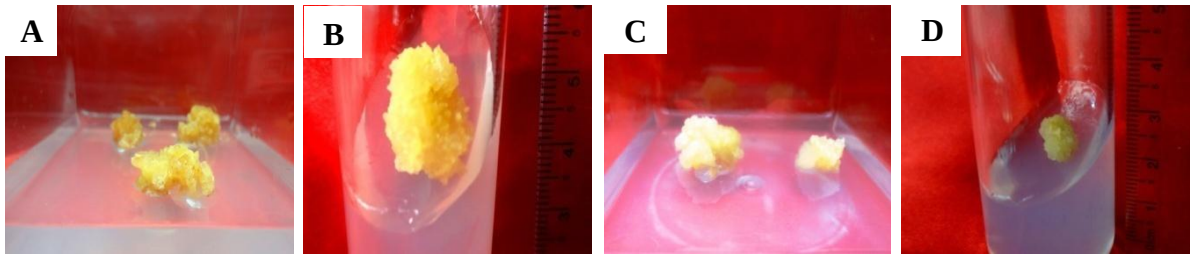

Fig. 2: Callus at different 2,4 -D concentrations of internodal explants, A. 0.5 mg/L, B. 1.0 mg/L, C. 2.0 mg/L and D. 3.0 mg/L

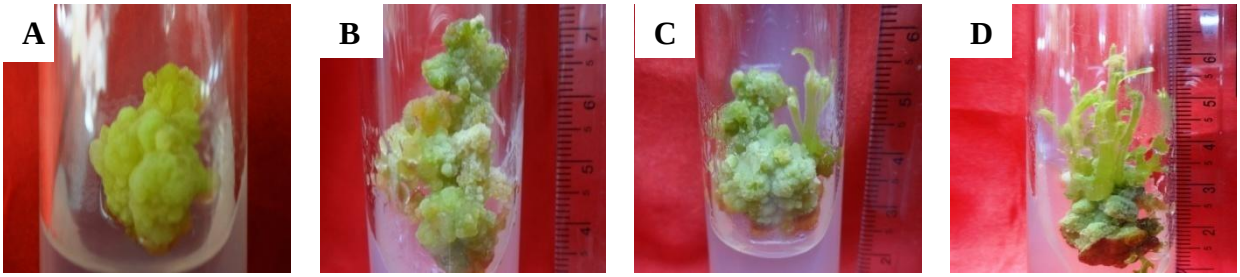

Fig. 3: Callus regeneration at BAP (4.0 mg/L) and Kinetin (0.5 mg/L). (A - B) Green and hard callus in 20 days, (C) shoot regeneration after 25th day, (D) Shoot elongation in one week

DISCUSSION

Effect of 2,4- D in callus induction has been studied in a number of medicinal plants like *Achyranthes aspera* (Sen *et al.*, 2014), *Ipomaea obscura* (Mungole *et al.*, 2009), *Vitex negundo* (Choudhury *et al.*, 2011), *Aquilaria agallocha* (Debnath, 2013), *Chlorophytum borivilianum* (Nakasha *et al.*, 2016), *Centella asiatica* (Biradar, 2017), etc. Though it is a synthetic hormone, its role in callus induction is widely acceptable. Color of callus varied from creamish white to light brown and the texture were compact in nature. Similar color and texture of callus were observed in *Simarouba glauca* DC. (Dudhare *et al.*, 2014). In the present study the best callogenic response exhibited at 2,4- D (1 mg/L) in both nodal and internodal explants of *Brucea mollis.* The percentage of callogenic response however decreases with the increase in concentrations of 2,4 D and this corroborate the findings on *Achyranthes aspera* (Sen *et al.*, 2014). The weight of the callus was recorded at intervals 30 and 50 days. With increasing number of days, the color of the calli changed to brown. All the callus showed pre embryonic stage when they were subcultured at the same initial concentrations. But when the callus were subcultured for regeneration at a combination of BAP and Kinetin the color and texture changes to green and became hard after 20 days from subculture. Shoot initiation took place after 25 days in both the explants with 100% callus regeneration frequency. The best shooting response was shown at BAP (4 mg/L) and Kinetin (0.5 mg/L) in both the explants with highest shoot number in internodal callus which exhibits similarity with the *Achyranthes aspera* (Sen *et al*., 2014). The effect of BAP and Kinetin in shoot regeneration was also previously studied by Biswas *et al.*, 2007, Muhammad *et al.*, 2007, Sen *et al.*, 2013. The present findings on shoot regeneration from callus at BAP and Kinetin showed good response which establishes a protocol for *in vitro* propagation of *B. mollis*.

Callus exhibit a morphogenic changes during *in vitro* culture at different level of hormone concentrations (Sudipta *et al.*, 2011). In *Brucea mollis* the morphology of callus is fully depend upon the age, type of the explants, media and hormonal concentration. Callus induction is important for large scale production of plant materials which play a pivotal role in producing secondary metabolites and bioactive compounds. Regeneration of *Brucea mollis* through callus can be made possible through nodal and internodal explants at different hormonal combination of BAP and Kinetin.

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