eISSN: 2455-1716
Hore and Tanti, 2018
DOI:10.21276/ijlssr.2018.4.3.1

Research Article

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Regeneration of Plantlets from Rhizome Bud Explants of *Lasia spinosa* (Lour.) Thwaites- A Medicinal Plants of Assam

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ABASTRACT

In numerable medicinal plants are commercially propagated through tissue culture for large production of elite material (Rhizome buds of *Lasia spinosa* {Lour.}). Thwaites could be induced on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of kinetin (kin) and 6-benzyl amino purine (BAP) alone and in combination with Naphthaleneacetic acid (NAA). Lasia is one of the traditionally important plants of Assam, which is employed in the treatment of gastrointestinal diseases, respiratory diseases and skin infections. This plant is also a rich source of dietary fibers, reported containing polyphenols, ascorbic acid and hydrocyanic acid. The present study aimed to establish producible protocol for *in vitro* regeneration of *Lasia spinosa* using rhizome bud explants. For shoot proliferation, among the various concentrations, 3.0 mgL^{-1} BAP showed the highest shoot regeneration frequency of 88.2±2.8%. The highest number of shoot were recorded as 1.9 ± 0.45 in *L. spinosa,* but the highest shoot length (4.5±0.07 cm) was observed at reduced concentration of BAP (1.0 mgL^{-1}). Plantlets rooted in ½ strength MS medium augmented with 0.1–1.0 mgL^{-1} either NAA or IBA for *L. spinosa* for root formation. The highest percentage (79.5±2.6%), maximum number of rootlets/ shoot let (4.0±0.46) and mean length of rootlets (3.25±0.06 cm) were observed in *L. spinosa.* Our findings have paved a way for future investigation on another mode of regeneration like haploid production, anther culture etc and also for the commercial and rapid propagation of *L. spinosa.*

Key-words: *Lasia spinosa*, Rhizome bud, Plantlets, Kinetin, 6-benzylaminopurine

INTRODUCTION

Lasia spinosa (Lour.) Thwaites (Araceae) is an important plant in folklore medicine. Traditionally, the leaves of this herb are commonly employed in the treatment of gastrointestinal diseases, respiratory diseases and in skin infections. The plant parts of *Lasia spinosa* has a number of medicinal uses like leaves and corms are used to cure piles [1], tubers are used for the treatment of rheumatoid arthritis, constipation, and to purify blood in Rajshahi and Natore district of Bangladesh [2], rhizome possesses antioxidant capacity [3,4], antimicrobial property and cytotoxic activity [5,6]. In Northeast India, leaves and rhizomes are commonly used in traditional medicine for treating joint-pain and skin infections [7].

How to cite this article

Hore P, Tanti B. Regeneration of Plantlets from Rhizome Bud Explants of *Lasia spinosa* (Lour.) Thwaites- A Medicinal Plants of Assam. Int. J. Life Sci. Scienti. Res., 2018; 4(3): 1736-1743

Access this article online

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The genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines. As conventional propagation method through rhizome axillary buds is time consuming and provides a limited number of propagules, it is necessary to promote rapid production of *L. spinosa* through tissue culture techniques for its commercial availability and conservation.

MATERIALS AND METHODS

Explant sterilization- Excised micro-cutting of rhizome bud from the source plants were used as explants. The explants were coarsely trimmed to a size of 3 cm and washed thoroughly under running tap water for 10 min and then treated with liquid detergent [5% (v/v) Tween-20] for 15 min. Later these explants were washed with double-distilled water for 10 min. The explants were then sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min and washed several times with sterile H_2O to remove all traces of $HgCl_2$. After a final wash, the

eISSN: 2455-1716
Hore and Tanti, 2018
DOI:10.21276/ijlssr.2018.4.3.1

explants were spread on the pre-sterilized petri-dishes lined with sterile blotting paper inside a laminar airflow chamber. They were then trimmed finely to the appropriate size.

Inoculation and incubation- Excised micro-cuttings of rhizome bud (1 cm–2 cm) were dissected out and all the inoculation operations were carried out under strict aseptic condition inside a Laminar Air Flow chamber, which was made sterile by the incessant exposure of germicidal UV rays for half an hour before use. All operations were carried out using pre-sterilized instruments and glassware. Explants were then aseptically introduced into culture vessels. The culture tubes were then plugged tightly with non-absorbent cotton plugs and the culture bottles and petri-plates were sealed tight with sealing film. All cultures were incubated under irradiance of 70 µmol $m^{-2}s^{-1}$ for 16 hours photoperiod and temperature of 25±10°C and with a relative humidity of 55%–60%.

Regeneration of plantlets- Basal medium supplemented with different concentrations of kinetin (1.0, 2.0, 3.0, 4.0 mgL^{-1}) and Benzyl amino purine (BAP) (1.0, 2.0, 3.0, 4.0 mgL^{-1}) individually and in combinations with Naphthalene acetic acid (NAA) (0.5, 1.0 mgL^{-1}) were tested for the induction of callus and regeneration of shoot and root from micro-cutting of rhizomes bud explants [8]. Sub-culturing was done at 14-day intervals onto fresh medium for 6 weeks to induce *in vitro* regeneration of shoot. Shoot buds were further cultured for elongation in the same medium supplemented with low concentration of cytokinin. The responses of each explant with regard to the induction of shoots, the length of shoot and the percentage of response were recorded after 6 weeks in culture.

***In vitro* rooting-** *In vitro* regenerated shoots were rooted on half strength medium supplemented with different concentrations of auxin [9] (NAA and IBA) alone. The response of each explant with regard to the number of roots induced and root lengths per shoot after 2 weeks in culture were recorded.

Hardening and acclimatization- *In vitro* grown plantlets were gently removed from culture tubes and washed with slightly warm (37°C) sterile double distilled H_2O to remove all traces of nutrient medium [10]. After removing media, plants were dipped in 1% w/v solution of Bavistine to prevent any fungal infection to newly developed plants. After Bavistine treatment the plantlets were carefully planted in plastic pots containing soilrite. The plantlets were irrigated by sprinkling with 0.5 x MS inorganic salts for three to four times per day for seven days. Plantlets were acclimatized for two weeks in an aseptic culture room under (16 hrs photoperiod at 28±2°C; 8 h in dark at 25±2°C) conditions. Further, the plantlets were exposed gradually to sunlight for acclimatization and were maintained in a garden.

Statistical Analysis- Statistical data for the percentage of response per explants with different concentrations and combinations of cytokinin and auxin with basal MS medium (shoot regeneration, shoot lengths, number of roots and root lengths) were recorded. Thus obtained data were analyzed statistically using SPSS 16.0 software (IBM Corporation SPSS, North America) [11].

RESULTS

Regeneration potential of micro-cutting of rhizome bud was explored on MS medium supplemented with various plant growth regulators and results are summarized in Table 1. Micro-cutting of rhizome bud explant remained green and fresh but failed to develop multiple shoots in growth regulators free MS medium (control). All micro cuttings of rhizome bud cultured on MS medium supplemented with various concentrations of kinetin and BAP individually and in combination with NAA have developed healthy shoots. Micro-cuttings of rhizome bud cultured on MS medium fortified with cytokinin alone induced multiple shoots at a lesser frequency compared to the media supplemented with a combination of cytokinin and auxin (Fig. 1). All the concentrations of BAP and kinetin facilitated shoot bud differentiation but BAP being more efficient than kinetin in terms of percent regeneration, number of shoots and shoot length. Among the various concentrations of BAP and kinetin tested, 3.0 mg/L BAP showed the highest shoot regeneration frequency of 88.2±2.8%, the highest number of shoot were recorded as 1.9±0.45 in *L. spinosa,* but the highest shoot length (4.5±0.07cm) was observed at reduced concentration of BAP (1.0 mg/L).

The synergistic influences of auxin with cytokinin was evident when combination of optimal concentration of

eISSN: 2455-1716
Hore and Tanti, 2018
DOI:10.21276/ijlssr.2018.4.3.1

each cytokinin with different concentrations of NAA (0.5 and 1.0 mg/L) were tested (Table 1). Addition of NAA markedly enhanced the percent regeneration and number of shoots for the *Lasia spinosa* used for *in vitro* propagation. Among all the cytokinin and auxin combinations, the maximum percent regeneration was found as 90.6 ± 2.8 and number of shoots (3.6±0.55) per explants were obtained at 3.0 mg/L BAP+1.0 mg/L NAA. But the highest shoot length (4.21±0.06cm) was recorded at the combination of 1.0 mg/L BAP + 0.5 mg/L NAA.

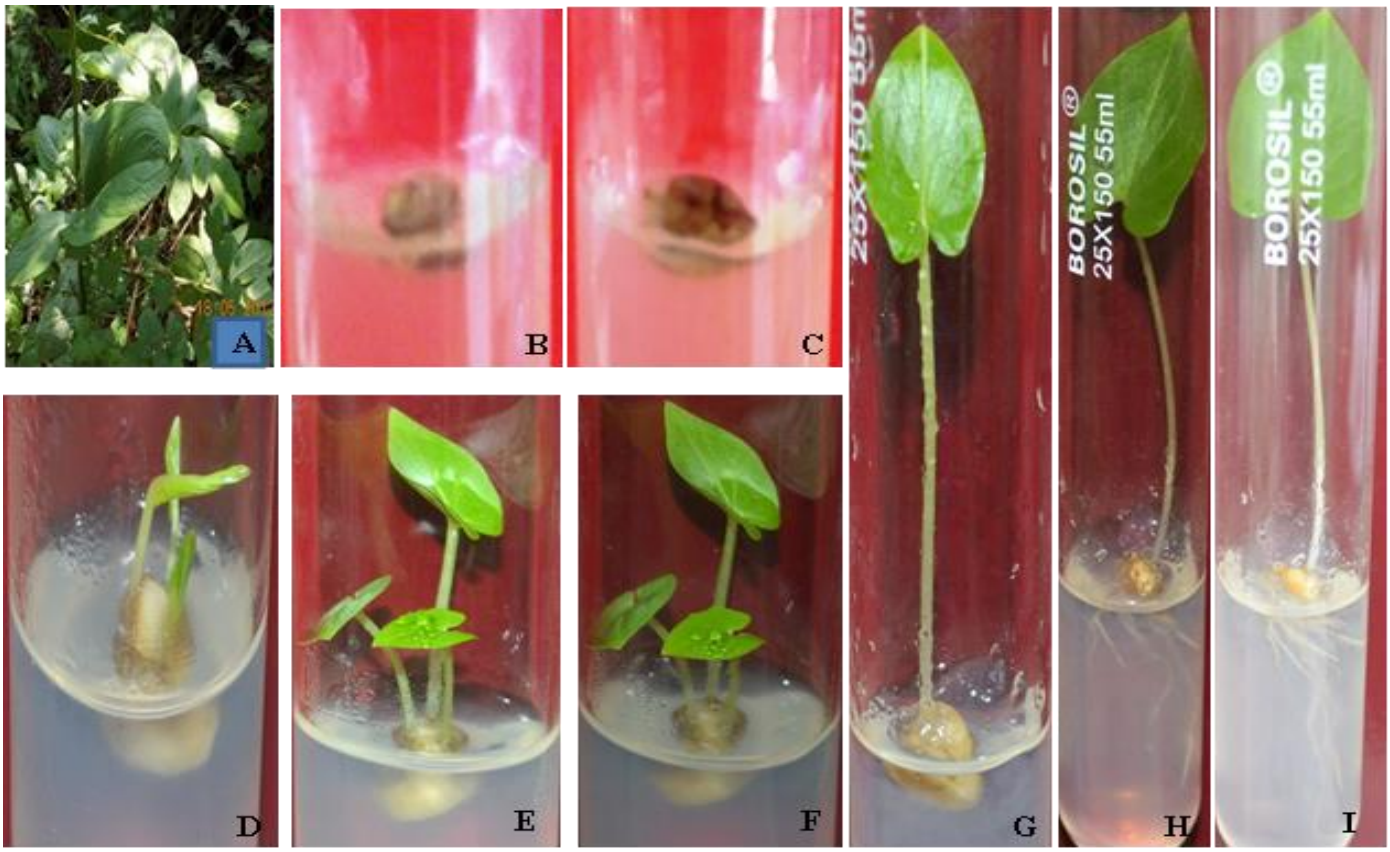

Fig. 1: Different stages of *in-vitro* regeneration of *Lasia spinosa* from micro-cutting of rhizome bud explant A= Plant in wild condition, B= Inoculation of micro cutting of rhizome bud in MS medium, C= Initial days after inoculation in MS medium, D= Direct organogenesis from explant in 3:1 mgL^{-1} of BAP and NAA in MS medium, E-F= multiple shoot regeneration in 3:1 mgL^{-1} of BAP and NAA, G= Shoot elongation in 1 mgL^{-1} BAP, H-I= Stages of rooting in 0.5 mg/L IBA in ½ MS medium

Table 1: Effect of cytokinin and auxin individually and in combinations for organogenesis from micro cuttings of rhizome bud of *L. spinosa* (6 weeks)

Concentration of plant growth regulators (mgL^{-1})			Response of micro cutting of rhizome bud (%)	Number of shoots/explant (Mean ± SD)	Shoot length/explant (cm) (Mean ± SD)
Kinetin	BAP	NAA			
Control (PGR free)			0	0	0
1			0	0	0
2			69.6 ± 2.8	1.6 ± 0.42	3.60 ± 0.06
3			72.6 ± 2.8	1.2 ± 0.46	3.01 ± 0.02

crossref DOI:10.21276/ijlssr.2018.4.3.1

4			67.2 ± 2.8	1.0 ± 0.43	2.5 ± 0.02
1		0.5	0	0	0
2		1	72.0	1.8 ± 0.44	3.06 ± 0.12
3		1	82.3 ± 2.8	2.1 ± 0.32	2.77 ± 0.12
4		0.5	70.6 ± 2.8	1.2 ± 0.24	2.04 ± 0.05
	1		83.6	1.8 ± 0.34	4.5 ± 0.07
	2		76.3 ± 2.8	1.2 ± 0.56	3.84 ± 0.05
	3		88.2 ± 2.8	1.9 ± 0.45	3.28 ± 0.06
	4		78.0	1.4 ± 0.42	2.94 ± 0.06
	1	0.5	82.6 ± 2.8	2.1 ± 0.42	4.21 ± 0.06
	2	1	84.6 ± 2.8	2.8 ± 0.56	3.58 ± 0.05
	3	1	90.6 ± 2.8	3.6 ± 0.55	3.42 ± 0.10
	4	0.5	77.6 ± 2.8	2.6 ± 0.42	2.52 ± 0.05

Root induction- The *in vitro* raised shootlets were sub-cultured on ½ strength MS medium augmented with 0.1 -1.0 mgL^{-1} either NAA or IBA for *L. spinosa* for root formation. At 14th day, the *in vitro* raised shootlets were produced *in vitro* rootlets without any callus proliferation. Medium containing 0.5 mgL^{-1} of IBA was proved to be the most effective for rooting of micro shoots than that containing any other concentrations of NAA in case of both the plants evaluated (Table 2). Here, NAA did not significantly improve the parameters evaluated. The highest percentage (79.5±2.6%), maximum number of rootlets/ shootlet (4.0±0.46) and mean length of rootlets (3.25±0.06cm) were observed in *L. spinosa.* There were several reports regarding the effectiveness of IBA in rooting of *in vitro* induced shoots of medicinal plants [12,13].

Table 2: Effect of auxins for root induction of *Lasia spinosa*

Auxin concentration (mgL^{-1})		Response (%)	Numbers of roots/shoot	Root length/culture
NAA	**IBA**			
0.1		37.6 ± 2.6	1.7 ± 0.54	2.66 ± 0.12
0.5		54.2 ± 2.6	2.2 ± 0.54	2.22 ± 0.12
0.8		48.4 ± 2.6	2.8 ± 0.56	2.69 ± 0.14
1		44.2 ± 2.6	2.7 ± 0.46	2.46 ± 0.15
	0.1	66.3 ± 2.6	2.6 ± 0.45	2.76 ± 0.05

0.5	79.5 ± 2.6	4.0 ± 0.46	3.25 ± 0.06
0.8	63.2 ± 2.6	1.3 ± 0.56	3.15 ± 0.07
1	60.4 ± 2.6	3.1 ± 0.42	3.0 ± 0.07

Acclimatization and hardening- The rooted plantlet was successfully hardened off inside the growth room in sterile soilrite for 2 weeks and eventually established in natural soil. There was no detectable variation among the potted plants with respect to morphological and growth characteristics (Fig. 2).

After 15 days, *in vitro* raised plantlets were hardened in poly cups with soilrite, irrigated with 0.5× MS liquid medium. The plants were kept in a culture room for 14 days. Approximately, 75% of plants were successfully established in polycups. After 15 days the polycups hardened plants, these were transferred to pots placed in and kept in the poly house. Seventy percentages of plantlets were well established for *Lasia spinosa* in the poly house condition. After one month, regenerated plants were successfully transferred to the field.

The protocol optimized here for *in-vitro* propagation of *Lasia spinosa* using micro-cuttings of rhizomes bud through direct organogenesis was found to be efficient, reproducible and provide a rapid technique for mass propagation and multiplication of this potential medicinal plant and also could further be used in its improvement programme.

Fig. 2 (A, B): Acclimatization *in-vitro* grown plantlets of *Lasia spinosa* in Poly-house condition

DISCUSSION

In this study, an *in-vitro* propagation protocol has been developed for *L. spinosa* using micro-cutting of rhizome bud. The plants showed direct organogenesis from the micro-cutting of rhizome bud which was found to be more suitable for regeneration when cultured on MS medium using various concentrations of BAP (1.0–4.0) and kinetin (1.0–4.0) separately or in combination with low concentration (0.5 and 1.0 mgL^{-1}) of auxin (NAA). Intact rhizome buds of native ginger in Borneo, *Etlingera coccinea* were cultured on Murashige and Skoog medium supplemented with 0.1 to 2.0 µM Thidiazuron (TZD), 6-BAP and kinetin. Regenerated shoots developed well in the MS medium supplemented with 5.0 µM BAP by promoting an average of 5.5±0.25 proliferated shoots per explants, 3.0±0.061 cm shoot length, and 6.5±0.35 leaves per explants. It was observed that BAP in combination with NAA was more effective for shoot induction [8,14]. The best conditions for propagating *Homalomena pineodora* was found to be on MS medium supplemented with 3% sucrose and 0.5 mgL^{-1} BA (6- benzyladenine) under 24 hrs of cool fluorescent light which produced an average of 3.8 shoot per explants [15]. Treatments of BAP and NAA, 3.0 mgL^{-1} BAP + 1.0 mgL^{-1} NAA was found to be suitable and showed better response in *L. spinosa*. In this concentration, 90.6% explants induced to develop shoots. The number of shoot as well as length of shoot per explant was recorded as 3.6±0.55 and 3.42±0.10 cm respectively. BAP is considered one of the most suitable cytokinin for the

multiplication of axillary buds reported by many authors [16,19-21]. In the present investigation, combination of BAP with NAA was found more suitable than BAP and kinetin alone. But, highest shoot length was observed in low concentration of BAP *i.e.,* 4.21±0.06 cm. *In vitro* formed shoots were excised and rooted on a separate root inducing half strength basal MS medium. Regenerated shoots thus formed were carefully excised and then rooted on basal MS medium. Rooting on proliferated shoots of *Anthurium andreanum* were successfully obtained on addition of PGRs *viz.,* IBA (0.0, 0.5, 1.0 and 2.0 mg/L), NAA (0.0, 0.05, 0.1 and 0.25 mgL^{-1}) and KIN (0.0 and 0.2 mgL^{-1}) [17,22] .The *in vitro* raised shootlets were sub-cultured on ½ strength MS medium augmented with 0.1 -1.0 mgL^{-1} either NAA or IBA for *L. spinosa* for root formation. At 14th day, the *in vitro* raised shootlets were produced *in vitro* rootlets without any callus proliferation. The researcher performed rooting of plantlets of *Aglaonema* on MS medium containing 3 mgL^{-1} indole-3-butyric acid (IBA) [10,23]. Rooting is usually induced by auxin, and IBA is more effective for rooting compared with other auxins as reported for *Anthuriums* [9,24-26]. Medium containing 0.5 mgL^{-1} of IBA was proved to be the most effective for rooting of micro-shoots than that containing any other concentrations of NAA in case of both the plants evaluated. Here, NAA did not significantly improve the parameters evaluated. The highest percentage (79.5±2.6%), maximum number of rootlets/ shootlet (4.0±0.46) and mean length of rootlets (3.25±0.06cm) were observed in *L. spinosa*. The rooted plantlet were successfully hardened off, approximately 75% of plants were successfully established. After 15 days, hardened plants were transferred to pots, where seventy percentages of plantlets were found in well established manner.

CONCLUSIONS

Numerous elite material can easily be generate from callus as it has high potency. As well the genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines. There is a strong need for proactive understanding in the conservation, cultivation and sustainable usage of important medicinal plant species for future use**.** Hence tissue culture may lay to overcome many of the difficulties in the present day world. Our findings have paved a way for future investigation on another mode of regeneration like haploid production, anther culture, protoplast culture etc and also for the commercial and rapid propagation of *L. spinosa*. Above all regeneration of plants through micropropagation is an alluring alternative for mass multiplication of outstanding cultivars at faster rates than conventional methods.

ACKNOWLEDGMENTS

The financial assistance received as BSR-Junior Research Fellow under UGC-SAP (DRS-I), Govt. of India was highly acknowledged.

CONTRIBUTION OF AUTHORS

Research concept and design was framed by Bhaben Tanti and practical implementation, data collection, and analysis was carried out by Puspita Hore. Final review of work was carried out by Puspita Hore and Bhaben Tanti.

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eISSN: 2455-1716
Hore and Tanti, 2018
DOI:10.21276/ijlssr.2018.4.3.1

Received: 08 Feb 2018/ Revised: 23 Mar 2018/ Accepted: 21 Apr 2018