International Journal of Advanced Scientific Research
www.allscientificjournal.com
ISSN: 2456-0421
Received: 08-08-2026, Accepted: 07-09-2026, Published: 22-09-2026
Volume 11, Issue 3, 2026, Page No. 92-100

In *vitro* callus induction, multiple shoot regeneration and comparative phytochemical profiling of *Abelmoschus Moschatus* Medik. An important medicinal plant of Bangladesh

Joyantee Biswas, Sweety Majumder, Tapash Kumar Bhowmik*, Md. Mahbubur Rahman
Department of Botany, Faculty of Biological Sciences, Plant Tissue Culture and Biotechnology Laboratory, University of Chittagong, Chattogram, Bangladesh, India
DOI: https://doi.org/10.66856/ijasr.2026.11.3.11075

Abstract
Abelmoschus moschatus Medik. is a highly valuable medicinal and aromatic plant belonging to the Malvaceae family, widely recognized for its diverse pharmacological activities and secondary metabolites. However, natural populations are rapidly declining due to over exploitation and poor seed *via*bility. This study establishes a highly efficient, reproducible *in vitro* propagation protocol for *A. moschatus* through direct and indirect organogenesis using nodal, leaf and shoot apices segments, alongside a comparative qualitative phytochemical analysis of *in vitro* and naturally grown plants. Explants were cultured on Murashige and Skoog (MS) media supplemented with varying concentrations of auxins and cytokinins. For indirect organogenesis, maximum callus induction frequency was observed in nodal segments (95%) and leaf segments (93%) on MS media supplemented with 1.0 mg/l 2,4-D, requiring only 11-19 days. Calli derived from nodal segments exhibited maximum shoot proliferation (96%) with an average of 5.78 ± 0.27 shoots per vessel on MS supplemented with 1.0 mg/l BAP and 0.5 mg/l Kn. Direct organogenesis from shoot apices yielded optimal multiple shoot buds (MSBs) on MS with 1.0 mg/l BAP + 1.0 mg/l NAA (5.67 ± 0.45 shoots/explant). The highest shoot elongation (5.63 ± 0.13 cm) occurred on MS fortified with 1.0 mg/l BAP + 0.5 mg/l Kn. Optimal rooting (87%) with a maximum root number (5.81 ± 0.08) and length (4.73 ± 0.03 cm) was achieved on full strength MS media containing 1.0 mg/l BAP + 1.0 mg/l NAA. Seedlings were successfully acclimatized in a soil compost mixture with high survival rates. Comparative phytochemical profiling revealed the robust presence of alkaloids, flavonoids, steroids, glycosides and tannins in both sources. Interestingly, *in vitro* developed plantlets demonstrated a higher accumulation of phlobatannins, quinine and coumarin compared to their wild counterparts, suggesting that this optimized tissue culture protocol can be successfully utilized for both conservation and the commercial mass production of targeted secondary metabolites for the pharmaceutical industry.

Keywords: *Abelmoschus Moschatus*, callus induction, organogenesis, phytochemical screening

Introduction

Abelmoschus moschatus Medik., commonly known as Musk mallow, is a critically important aromatic and medicinal plant belonging to the Malvaceae family. Globally, medicinal plants serve as the primary source of curative agents, with an estimated 80% of rural populations in developing countries depending on botanical resources for primary healthcare (Rahman *et al.* 2013) [1]. In Bangladesh, the diverse agro-climatic conditions support a rich repository of medicinal flora, heavily utilized in both traditional Ayurvedic and modern therapeutic practices (Mia 1990, Ghani 1998) [2, 3]. A. moschatus is esteemed for its seeds, which yield an essential oil rich in ambrettolide and ambrettolic acid and its vegetative parts, which are traditionally utilized to treat gastrointestinal disorders, respiratory ailments and inflammatory conditions.

Despite its tremendous pharmaceutical potential, A. moschatus faces severe ecological threats. The unmonitored and destructive harvesting of its vegetative parts and seeds by local practitioners and pharmaceutical industries has drastically decimated its wild populations. Furthermore, the natural traditional propagation of A. moschatus is heavily impeded by prolonged seed dormancy, low germination viability and high susceptibility to environmental biotic and abiotic stressors. Consequently, it is imperative to develop alternative, sustainable propagation strategies that mitigate the pressure on wild habitats while ensuring a continuous supply of uniform, disease free plant material for commercial utilization (Fowler *et al*. 1993, Rout *et al*. 2000) [4, 5].

Plant tissue culture offers a robust biotechnological tool for the rapid, mass clonal multiplication of threatened medicinal plants using minimal initial explant material (Afolayan and Adebola 2004) [6]. In *vitro* techniques not only facilitate germplasm conservation but also enable the production of pathogen free propagules irrespective of seasonal constraints (Murch *et al.* 2000) [7]. While micropropagation protocols have been successfully established for several Malvaceae species, the morphogenetic responses of specific vegetative explants such as nodal segments, leaf segments and shoot apices in A. moschatus to complex interactions of plant growth regulators (PGRs) remain insufficiently explored. The precise balance of auxins such as 2,4-Dichlorophenoxyacetic acid (2,4-D) and α-Naphthaleneacetic acid (NAA) and cytokinins such as 6-Benzyl aminopurine (BAP) and Kinetin (Kn) must be optimized to harness the full totipotent potential of the specific explants.

Moreover, the medicinal efficacy of A. moschatus is unequivocally linked to its diverse secondary metabolites, including alkaloids, flavonoids, steroids and glycosides (Fraenkel and Gottfried 1959, Harborne 1973) [8, 9]. Secondary metabolites often play a pivotal role in plant defense against herbivores and microorganisms and serve as the biochemical foundation for modern drug synthesis (Oksman-Caldentey and Inze 2004) [10]. A critical research

gap exists in evaluating whether in *vitro* regenerated A. moschatus plants retain or exceed the biosynthetic capacity of their wild grown counterparts. Tissue culture environments, particularly the constant presence of exogenous PGRs, have been hypothesized to act as elicitors, occasionally hyper accumulating specific bioactive compounds in cultured tissues compared to naturally grown plants (Julsing *et al*. 2007, Kharde *et al*. 2018) [11, 12].

Therefore, the primary objectives of the present study are: (i) to establish a highly efficient, reproducible protocol for the direct and indirect micropropagation of A. moschatus using nodal, leaf and shoot apices segments; (ii) to evaluate the morphogenetic effects of various PGRs combinations on callus induction, shoot proliferation, elongation and rooting; and (iii) to conduct a comprehensive qualitative comparative phytochemical screening between in *vitro* developed plantlets and naturally grown wild plants to validate their pharmaceutical viability.

Materials and Methods

1. Plant material and explant source

Vigorous and disease free field grown plants of Abelmoschus moschatus Medik. were selected as the source of explants. Nodal, leaf and shoot apices segments were meticulously excised and washed thoroughly under running tap water to remove surface detritus. The excised tissues were treated with 1% savlon (ACI Pharma, Bangladesh) and liquid soap for 5 to 10 minutes with constant agitation, followed by 3-4 consecutive washes with distilled water to remove detergent residues. The materials were then transferred to a laminar airflow cabinet. Surface sterilization was achieved by briefly rinsing the explants with 70% ethanol for less than 60 seconds, followed by immersion in 0.1% Mercuric chloride ($HgCl_2$) for optimized durations (7-10 minutes depending on the explant tissue). Finally, the explants were rinsed 4-5 times with sterile distilled water to eliminate all traces of the sterilant prior to inoculation. The explants were cut into 0.5 - 1.0 cm pieces using a sterile surgical blade.

2. Media preparation and culture conditions

The basal nutrient medium consisted of Murashige and Skoog (MS) salts and vitamins (Murashige and Skoog 1962) [13], [13], supplemented with 3% (w/v) sucrose as the carbon source. All analytical grade chemicals, including macro and micronutrients, organic salts and plant growth regulators (PGRs), were procured from BDH (England), Merck (Germany) or Sigma (USA). The medium was solidified using 0.8% (w/v) agar (Himedia, India). The pH of the culture media was strictly adjusted to 5.7-5.8 using 0.1 N NaOH or 0.1 N HCl prior to the addition of agar. The media were dispensed into culture vessels and autoclaved at 1.06 kg/cm² pressure (121°C) for 20 minutes. All inoculated cultures were maintained in a highly controlled culture room at a temperature of 25 ± 2°C under a 14-hour continuous light and 10-hour continuous dark photoperiod provided by cool white fluorescent tubes (2000-3000 lux intensity).

3. Callus induction and multiple shoot regeneration protocols

To induce callogenesis, standardized nodal (NS) and leaf segments (LS) were cultured on MS basal media supplemented with diverse concentrations of 2,4-D (0.5 - 3.0 mg/l) and BAP (0.2 - 2.0 mg/l), as well as synergistic BAP and NAA combinations. Developing calli were continuously monitored for induction frequency, morphogenic texture, color and days required for initiation.

For indirect organogenesis, the derived calli were subcultured onto MS media containing various concentrations of BAP independently or in combinations with NAA (0.5 - 1.0 mg/l) and Kn (0.5 - 1.0 mg/l). For direct organogenesis, excised nodal segments and shoot apices were inoculated onto MS media fortified with varying levels of BAP (0.3 - 2.0 mg/l), either individually or combined with NAA or Kn. Proliferated multiple shoot buds (MSBs) were selectively rescued and subdivided into clusters of 2-3 shoots using a sterile scalpel for regular sub-culturing at 15-20 day intervals to encourage further multiplication.

4. Shoot elongation and rooting

Differentiated in *vitro* shoot buds were meticulously isolated and subcultured onto specialized elongation media comprising MS basal salts enriched with specific combinations of cytokinins (BAP, Kn) and auxins (NAA). The cultures were monitored for 30 days. Following sufficient elongation (2-4 cm in height), micro-shoots were transferred to rooting media. Root induction was evaluated using half and full strength MS media. For highly profuse rooting, MS media were fortified with differing regimes of IBA (0.5 - 2.5 mg/l), IAA, NAA and unique combinations such as MS + BAP + NAA.

5. Acclimatization

For ex *vitro* acclimatization, culture vessels containing well-rooted plantlets were transferred to ambient room temperature and exposed gradually over three to five days. The seedlings were carefully extracted and the roots were gently washed under running tap water to completely remove the adhering agar matrix, thereby preventing subsequent fungal contamination. Seedlings were then transferred to plastic pots containing a sterile soil compost mixture (1:1 ratio, treated with 0.1% agrosan fungicide) and maintained under high humidity for 3-5 days before eventual transfer to earthen pots in the natural outdoor environment.

6. Phytochemical screening protocol

For the comparative extraction, 25 g of dried, powdered samples from both in *vitro* developed plantlets and naturally grown wild plants were immersed in 50 ml of methanol in conical flasks. The mixtures were mechanically shaken for 30 minutes, incubated overnight, re-shaken and sonicated for 10 minutes prior to filtration through Whatman filter paper. This extraction process was repeated three times. The filtrates were concentrated via a rotary evaporator below 51°C to yield the crude extracts.

Qualitative assessment for the presence of secondary metabolites was executed utilizing established colorimetric methodologies (Harborne 1973, Sofowora 1993, Bandiola 2018) [9, 14, 15]. Alkaloids were screened utilizing five distinct reagents: Dragendorff's (D), Hager's (H), Mayer's (M), Wagner's (W) and Tannic acid (T). Ten additional secondary metabolites such as Phlobatannins, Flavonoids, Terpenoids, Steroids, Glycosides, Quinine, Coumarin, Anthraquinone, Saponin and Tannins were evaluated. Intensity of precipitation and color change was recorded sequentially: '+++' (highest quantity), '++' (moderately high), '+' (minimum) and '-' (absent).

7. Statistical analysis

The in *vitro* experiments were arranged in a completely randomized design (CRD). Data representing the mean ± Standard Error (SE) were collect for multiple parameters, including callus induction, days to shoot induction, number of MSBs per explant, shoot length, root number and root length. All quantitative data were collect from a minimum of five replicates.

Results

1. Callus induction from nodal and leaf segments

In order to induce callus, naturally grown nodal and leaf segments of A. moschatus were aseptically cultured on MS medium supplemented with 2,4-D and BAP, individually and in specific combinations (Table 1). The experimental outcomes demonstrated significant variations based on explant type, PGRs combinations and concentrations (Table 1). The data clearly highlight the superiority of 2,4-D for rapid callogenesis. The highest callus induction frequency for nodal explants (95%) was recorded on media containing 1.0 mg/l 2,4-D within 11-18 days, yielding a whitish brown friable callus (WBFC, Fig. 1). Similarly, leaf segments demonstrated maximum callogenesis (93%) on the same medium within 12-19 days, producing a distinct brownish friable callus (BFC, Fig. 2).

Conversely, the use of BAP alone was notably less effective. The minimal induction frequencies for nodal (65%) and leaf (75%) segments were observed in treatments utilizing 1.0 mg/l BAP, requiring highly extended durations (20-29 days) for callus initiation. When BAP was combined with NAA, the response improved significantly, but still fell short of the optimal 2,4-D treatments.

2. Multiple shoot buds (MSBs) induction via direct organogenesis

Direct shoot regeneration bypasses the callus phase, heavily decreasing somaclonal variation and shortening propagation time (Table 2). Culturing nodal segments directly on modified MS media triggered rapid MSBs emergence within 18-20 days (Fig. 3). The synergistic combination of 1.0 mg/l BAP and 0.5 mg/l Kn proved exceptional, inducing direct shoot proliferation in 96% of the nodal explants and yielding a maximum average of 5.78 ± 0.27 MSBs per explant. A combination of BAP and NAA (1.5 mg/l + 1.0 mg/l) also yielded excellent results (92%, 5.60 ± 0.45 MSBs, Fig. 4). Conversely, increasing the BAP concentration to 2.0 mg/l while keeping NAA low (0.5 mg/l) resulted in the weakest response (75%, 2.49 ± 0.46 MSBs).

Shoot apices demonstrated immense morphogenic plasticity and were highly responsive to direct MSBs induction (Table 2), showing proliferation ranges from 70% to 96% within an accelerated timeframe of 6-15 days. Unlike nodal segments that favored dual cytokinins, shoot apices thrived under a balanced cytokinin-auxin regime. A media composition of 1.0 mg/l BAP and 1.0 mg/l NAA induced the maximum response (96%) with 5.67 ± 0.45 MSBs generated per apex (Fig. 5-6). The lowest developmental rate (70%, 2.29 ± 0.24 MSBs) occurred on MS fortified with a slight hormonal imbalance of 1.5 mg/l BAP and 0.5 mg/l NAA.

3. Multiple shoot buds regeneration via indirect organogenesis

To stimulate morphological differentiation, established calli were transferred to MS media infused with varying cytokinins and auxins (Tables 3). Both WBFC and BFC successfully multiplied and initiated shoot bud organogenesis within 15-28 days. The data indicates that the source of the callus strongly influenced the optimal PGRs requirement for shoot induction.

For calli derived from nodal segments, the absolute maximum shoot regeneration frequency (95%) occurred on MS media supplemented with 1.5 mg/l BAP and 1.0 mg/l NAA (Fig. 7). This treatment produced the highest statistical mean of 5.18 ± 0.17 multiple shoot buds (MSBs) per vessel within 15-19 days (Fig. 8). Conversely, utilizing a combination of 1.5 mg/l BAP and 0.5 mg/l Kn severely restricted shoot formation (62%), yielding the lowest mean of only 1.20 ± 0.24 MSBs per vessel over an extended 19-28 day period.

For leaf segment derived calli, the morphological preference shifted. The peak morphogenic response (96%) was isolated in MS media fortified with dual cytokinins: 1.0 mg/l BAP and 0.5 mg/l Kn (Fig. 9-10), resulting in the rapid generation of 5.78 ± 0.18 MSBs per vessel. High concentrations of a single cytokinin proved detrimental; the lowest efficiency (68%) and shoot count (1.32 ± 0.12 MSBs) were recorded utilizing a lone 2.0 mg/l BAP treatment.

4. Elongation of induced MSBs of A. moschatus.

Newly differentiated micro-shoots, which often form dense, stunted clusters, required specialized media to drive internodal elongation (Table 4). Cytokinin-auxin combinations strongly dictated elongation rates over a 30-day culture period. Optimal vertical extension reaching 5.63 ± 0.13 cm was recorded on MS containing a dual cytokinin formulation of 1.0 mg/l BAP + 0.5 mg/l Kn (Fig. 11). Substituting Kn for NAA (1.5 mg/l BAP and 0.5 mg/l NAA) also yielded excellent elongation (5.29 ± 0.08 cm, Fig. 12). Conversely, media combining higher concentrations of BAP and NAA (2.0 mg/l BAP + 1.0 mg/l NAA) severely retarded shoot growth, restricting elongation to a mere 2.51 ± 0. 27 cm.

5. Root induction and acclimatization

Elongated shoots measuring 2-4 cm were excised and subjected to rhizogenesis utilizing half and full strength MS media. Root initials visually pierced the basal epidermis between 18-25 days of inoculation (Table 5). While half strength MS media supplemented with auxins like IBA or NAA traditionally promote rooting in many species, they yielded moderate success in A. moschatus (ranging from 65% to 75% rooting, generating only ~2.5 to 3.3 roots per shoot).

Remarkably, high efficiency rhizogenesis (87%) was induced on a full strength MS basal medium supplemented concurrently with a cytokinin and an auxin: 1.0 mg/l BAP and 1.0 mg/l NAA (Fig. 13-14). This synergistic interaction generated an impressive average of 5.81 ± 0.08 roots per shoot, alongside substantial root elongation (4.73 ± 0.03 cm), heavily outperforming pure half strength IBA/NAA formulations. Decreasing the NAA to 0.5 mg/l alongside 1.0 mg/l BAP severely depressed rooting efficiency to 60%, yielding only 2.44 ± 0.02 roots per shoot.

Ex *vitro* acclimatization involved systematically transferring robust in *vitro* plantlets into soil compost matrices. The hardened plants demonstrated robust morphological integrity and continuous vegetative development outside the laboratory conditions, proving the practical viability of this protocol (Fig. 15).

6. Qualitative phytochemical profiling

Extracted secondary metabolites act as crucial defensive markers and therapeutic agents. Alkaloids from in *vitro* generated and naturally wild grown plants were reacted against five reagents (Table 6). While Dragendorff's reagent yielded an intense (+++) positive for both variants, revealing a strong baseline of alkaloids, differences emerged under other tests. Hager's, Mayer's and Tannic acid reagents revealed a visibly higher concentration of alkaloids (++) in in *vitro* plants compared to their naturally grown (+) counterparts. Furthermore, Wagner's reagent confirmed an elevated alkaloid density (+++) in *vitro* compared to the wild type (++).

Furthermore, ten additional metabolites were screened (Table 7). Highly concentrated accumulations (+++) of Flavonoids, Steroids, Glycosides and Tannins were uniformly identified in both the wild and tissue cultured tissues, indicating that in *vitro* growth does not compromise these vital therapeutic compounds. Remarkably, in *vitro* specimens outperformed wild variants by biosynthesizing higher levels of specific defensive and medicinal metabolites: Phlobatannins (+++ vs. +), Quinine (+++ vs. ++) and Coumarin (+++ vs. ++). Conversely, naturally grown A. moschatus expressed slightly higher Terpenoid and Saponin densities (+++ vs. ++). Neither tissue type produced detectable Anthraquinones or Phenol.

Table 1: Effects of 2,4-D and BAP individually and BAP in combination with NAA on induction of callus from natural nodal and leaf explants of A. moschatus.

Plant growth regulators (mg/l)		Type of explants	% of explants produced callus	Time (d) required for callus induction	Colour and texture of induced callus
2,4-D	0.5	NS	80	11 – 16	WBFC
		LS	78	12 - 18	BFC
	1.0	NS	95	11 - 18	WBFC
		LS	93	12 - 19	BFC
	2.0	NS	77	11 – 15	WBFC
		LS	82	12 – 19	BFC
	3.0	NS	80	15 – 19	WBFC
		LS	87	16 – 22	BFC
BAP	0.2	NS	74	12 – 29	WBFC
		LS	82	15 – 19	BFC
	0.5	NS	81	16 – 18	WBFC
		LS	85	15 – 16	BFC
	1.0	NS	65	20 – 29	WBFC
		LS	75	20 – 25	BFC
	2.0	NS	83	13 – 19	WBFC
		LS	82	16 - 28	BFC
BAP + NAA	1.0 + 0.5	NS	75	16 – 19	WBFC
		LS	80	15 – 29	BFC
	1.0 + 1.0	NS	78	14 – 16	WBFC
		LS	82	15 – 19	BFC
	1.5 + 0.5	NS	79	16 – 19	WBFC
		LS	85	17 – 25	BFC
	1.5 + 1.0	NS	87	16 – 22	WBFC
		LS	88	17 - 29	BFC

NS= Nodal segment, LS= leaf segment; BFC= Brownish friable callus; WBFC= Whitish brown friable callus.

Table 2: Effects of BAP individually and in combination with NAA or Kn on induction of MSBs in natural nodal and shoot apices explants of A. moschatus.

PGRs	Conc. (mg/l)	% of explants giving response	Time (d) require for induction of MSBs	Average no. of MSBs/explant ($\bar{x}$ ± SE)	% of explants giving response	Time (d) require for induction of MSBs	Average no. of MSBs/explant ($\bar{x}$ ± SE)
		Nodal segment			Shoot apices segment		
BAP	0.2	80	22 – 25	3.44 ± 0.69	83	11 – 15	3.32 ± 0.37
	0.3	82	18 – 20	3.55 ± 0.67	82	10 – 12	3.22 ± 0.27
	1.0	78	18 – 28	2.89 ± 0.89	84	11 – 15	3.38 ± 0.46
	2.0	80	20– 22	3.47 ± 0.66	85	10 – 12	3.43 ± 0.51
BAP + NAA	1.0 + 0.5	88	19 – 22	4.66 ± 0.47	92	12 – 14	5.38 ± 0.40
	1.0 + 1.0	87	18 – 28	4.51 ± 0.45	96	07 – 12	5.67 ± 0.45
	1.5 + 0.5	76	19 – 28	2.54 ± 0.62	70	13 – 15	2.29 ± 0.24
	1.5 + 1.0	92	22 - 27	5.60 ± 0.45	90	08 – 15	5.07 ± 0.12
	2.0 + 0.5	75	19 – 25	2.49 ± 0.46	82	10 – 15	3.24 ± 0.17
	2.0 + 1.0	76	20 – 27	2.55 ± 0.26	83	13 – 15	3.30 ± 0.34
BAP + Kn	1.0 + 0.5	96	18 – 20	5.78 ± 0.27	89	10 – 12	4.13 ± 0.05
	1.0 + 1.0	77	20 – 22	2.64 ± 0.40	75	10 – 13	2.56 ± 0.29
	1.5 + 0.5	80	18 – 22	3.42 ± 0.77	84	12 – 15	3.36 ± 0.24
	1.5 + 1.0	82	18 – 20	3.56 ± 0.72	85	10 – 14	3.40 ± 0.34
	2.0 + 0.5	88	18 – 24	4.64 ± 0.38	76	09 – 15	2.57 ± 0.44
	2.0 + 1.0	87	26 – 28	4.57 ± 0.41	83	07 - 12	3.28 ± 0.32

d = days; MSBs = Multiple Shoot Buds; Values are the means of 5 replicates.

Table 3: Effects of BAP individually and in combination with NAA or Kn in MS medium of induced callus tissue culture from nodal and leaf segments of A. moschatus.

PGRs	Conc. (mg/l)	% of explants produced callus	Time (d) required for MSBs induction	No. of shoot buds/vessel ($\bar{x}$ ± SE)	% of explants produced callus	Time (d) required for MSBs induction	No. of shoot buds/vessel ($\bar{x}$ ± SE)
		Nodal segment			Leaf segment		
BAP	0.2	80	15 - 20	2.33 ± 0.33	82	18 - 25	4.23 ± 0.03
	0.5	75	17 – 25	1.26 ± 0.35	74	16 – 27	2.15 ± 0.15
	1.0	78	16 – 28	1.94 ± 0.36	76	15 – 22	2.35 ± 0.11
	2.0	72	15 – 28	2.26 ± 0.29	68	16 – 28	1.32 ± 0.12
BAP + Kn	1.0 + 0.5	85	15 – 19	4.18 ± 0.17	96	15 – 17	5.78 ± 0.18
	1.0 + 1.0	75	15 – 25	3.20 ± 0.21	76	17 – 28	2.65 ± 0.02
	1.5 + 0.5	62	19 – 28	1.20 ± 0.24	70	15 - 25	1.98 ± 0.23
	1.5 + 1.0	79	15 – 19	2.20 ± 0.17	78	18 – 24	2.95 ± 0.09
BAP + NAA	1.0 + 0.5	81	17 – 28	2.25 ± 0.23	85	19 – 22	4.59 ± 0.23
	1.0 + 1.0	78	15 – 25	2.18 ± 0.13	88	20 – 25	4.89 ± 0.05
	1.5 + 0.5	73	15 – 19	1.22 ± 0.27	76	25 – 28	2.36 ± 0.12
	1.5 + 1.0	95	15 – 19	5.18 ± 0.17	80	24 – 28	4.98 ± 0.17

d = days; MSBs = Multiple Shoot Buds; Values are the means of 5 replicates.

Table 4: Effects of different concentrations and combination of PGRs on the elongation of directly and indirectly produced multiple shoot buds of A. moschatus.

PGRs	Conc. (mg/l)	Initial length (cm) of individual shoot buds ($\bar{x}$ ± SE)	Length (cm) of individual shoot buds after 30d of culture ($\bar{x}$ ± SE)
BAP + NAA	1.0 + 0.5	2.18 ± 0.13	4.17 ± 0.11
	1.0 + 1.0	2.54 ± 0.12	4.25 ± 0.07
	1.5 + 0.5	1.51 ± 0.52	5.29 ± 0.08
	1.5 + 1.0	2.58 ± 0.14	3.24 ± 0.09
	2.0 + 0.5	1.23 ± 0.07	2.64 ± 0.04
	2.0 + 1.0	1.21 ± 0.11	2.51 ± 0.27
BAP + Kn	1.0 + 0.5	2.43 ± 0.26	5.63 ± 0.13
	1.5 + 1.0	1.15 ± 0.19	3.16 ± 0.68
	2.0 + 0.5	1.16 ± 0.17	3.17 ± 0.54
	2.0 + 1.0	1.19 ± 0.14	3.15 ± 0.29
	2.5 + 0.5	2.15 ± 0.17	4.15 ± 0.37
	2.5 + 1.0	2.18 ± 0.13	4.32 ± 0.98

d = days; Values are the means of 5 replicates.

Table 5: Effects of different concentrations and combinations of PGRs on the development of roots in elongated MSBs of A. moschatus on half and full strength MS medium.

PGRs	Conc. (mg/l)	Days to root induction	% of micro shoot produced roots	Average number of roots/ micro shoot ($\bar{x}$ ± SE)	Average length (cm) roots after 30d of culture ($\bar{x}$ ± SE)
½ MS0	0.0	-------	-------	-------	-------
½ MS + IBA	0.5	20 – 25	68	2.71 ± 0.13	2.63 ± 0.12
	1.5	18 – 22	67	2.56 ± 0.16	2.82 ± 0.10
	2.0	20 – 22	65	2.47 ± 0.09	2.35 ± 0.02
	2.5	20 – 25	66	2.49 ± 0.10	2.62 ± 0.23
½ MS + IAA	0.5	------	------	-------	--------
	1.0	-------	-------	-------	--------
½ MS + NAA	0.5	19 – 21	70	3.14 ± 0.05	2.33 ± 0.02
	1.0	18 – 22	72	3.26 ± 0.03	2.34 ± 0.04
	2.0	19 – 21	73	3.27 ± 0.03	2.43 ± 0.07
	3.0	20 – 22	75	3.37 ± 0.03	2.44 ± 0.03
MS + BAP + NAA	1.0 + 0.5	20 – 21	60	2.44 ± 0.02	1.24 ± 0.04
	1.0 + 1.0	18 – 22	87	5.81 ± 0.08	4.73 ± 0.03

d = days; --- = No response; Values are the mean of 5 replicates.

Table 6: Qualitative test for alkaloids of Abelmoschus moschatus Medik.

Plant sample used	Qualitative estimation of alkaloids by different reagents				
	D	H	M	W	T
In *vitro* developed plant	+++	++	++	+++	++
Naturally grown plant	+++	+	+	++	+

Notes: Name of the reagents, D-Dragendroff's reagent, H-Hager's reagent, M-Mayer's reagent, W-Wagner's reagent and T- Tannic acid reagent.

Table 7: Qualitative test for ten secondary metabolites of Abelmoschus moschatus Medik.

Plant sample Used	Secondary metabolites(% of coloration)										
	Phl.	**Flv.**	**Ter.**	**Str.**	**Gly.**	**Qui.**	**Cou.**	**Anthro.**	**Sap.**	**Tann.**	**Phe.**
In *vitro* developed plant	+++	+++	++	+++	+++	+++	+++	-	++	+++	-
Naturally grown plant	+	+++	+++	+++	+++	++	++	-	+++	+++	-

Notes: Phl.= Phlobatannins, Flv.= Flavonoids, Ter.= Terpenoids, Str.= Steroids, Gly.= Glycosides, Qui.= Quinine, Cou.= Coumarin, Anthro.=Anthroquinone, Sap.=Saponins, Tann.= Tannin, Phe = Phenol.

Fig 1: Induction of whitish brown friable callus in nodal segment on MS medium with 1.0 mg/l 2,4-D.

Fig 2: Induction of brownish friable callus in leaf segment on MS medium with 1.0 mg/l 2,4-D.

Fig 3: Induction of MSBs from nodal segment on MS medium with 1.0 mg/l BAP and 0.5 mg/l Kn.

Fig 4: Proliferation of MSBs from nodal segment on MS + 1.5 mg/l BAP + 1.0 mg/l NAA.

Fig 5: Induction of MSBs from shoot apices on MS + 1.0 mg/l BAP + 1.0 mg/l NAA.

Fig 6: Proliferation of MSBs from shoot apices on MS + 1.0 mg/l BAP + 1.0 mg/l NAA.

Fig 7: Initiation of MSBs from whitish brown callus in MS medium with 1.5 mg/l BAP and 1.0 mg/l NAA.

Fig 8: Proliferation of MSBs from whitish brown callus in MS medium with 1.5 mg/l BAP and 1.0 mg/l NAA.

Fig 9: Initiation of MSBs from brownish friable callus in MS medium with 1.0 mg/l BAP and 0.5 mg/l Kn.

Fig 10: Proliferation of MSBs from brownish friable callus in MS medium with 1.0 mg/l BAP and 0.5 mg/l Kn.

Fig 11: Elongation of MSBs on MS medium with 1.0 mg/l BAP and 0.5 mg/l Kn.

Fig 12: Maximum elongation of MSBs on MS medium with 1.5 mg/l BAP and 0.5 mg/l NAA.

Fig 13: Induction of root on full strength MS medium with 1.0 mg/l BAP and 1.0 mg/l NAA.

Fig 14: Maximum number of roots on full strength MS medium with 1.0 mg/l BAP and 1.0 mg/l NAA.

Fig 15: In vitro grown seedling in plastic pot at outside environment.

Discussion

The successful exploitation and conservation of medicinal plants like Abelmoschus moschatus requires highly optimized biotechnological interventions. Tissue culture bypasses the natural reproductive constraints of such species, offering a framework to understand their physiological and morphogenetic plasticity. This study documents substantial variability in direct and indirect organogenesis contingent upon the explant source and specific exogenous hormonal gradients.

The induction of callus, an unorganized mass of actively dividing dedifferentiated cells, is fundamentally governed by the delicate ratio of auxins to cytokinins (Krikorian and Berquam 1969) [16]. In our findings, 1.0 mg/l 2,4-D efficiently promoted high-frequency callogenesis (93-95%) in both nodal and leaf explants of A. moschatus. The superiority of 2,4-D over cytokinins (like BAP) for inducing calli in Malvaceae and other dicots is extensively documented; it profoundly triggers gene expressions leading to rapid cellular dedifferentiation and vascular meristematic activity, whereas BAP alone often results in delayed and inferior callus mass generation (Biswas *et al*. 2007) [17]. Following callus stabilization, the regeneration of multiple shoot buds (indirect organogenesis) was heavily reliant on the presence of cytokinins. The highest regeneration was noted in nodal calli subjected to 1.5 mg/l BAP + 1.0 mg/l NAA. This specific synergistic interaction between a potent cytokinin (BAP) acting to stimulate lateral meristem development and a supplementary auxin (NAA) facilitating vascular connection to the nascent shoot buds is a classic morphogenetic response mirrored in several preceding studies on related botanicals (Jawahar *et al*. 2008, Rout *et al*. 2009) [18, 19].

A critical success of this research is the establishment of direct organogenesis, eliminating the callus intermediary to ensure true-to-type, somaclonally stable plantlets. We determined that a synergistic combination of 1.0 mg/l BAP and 0.5 mg/l Kn induced the maximal emergence of MSBs directly from nodal segments. The deployment of dual cytokinins frequently breaks intense apical dominance more effectively than a solitary cytokinin, activating dormant axillary meristems through modified endogenous hormonal balancing, a trend also observed in the direct regeneration of other herbaceous medicinal plants (Lithy *et al*. 2011) [20]. Interestingly, shoot apices favored a BAP and NAA combination, highlighting that endogenous auxin-cytokinin levels vary significantly between tissue zones, thus dictating distinct exogenous PGRs requirements (Kumar and Nandi 2015) [21].

For the induction of in *vitro* rooting, which dictates the eventual ex *vitro* survival rate of micro-shoots, our study registered anomalous but highly favorable results using full strength MS media enriched with 1.0 mg/l BAP and 1.0 mg/l NAA. While standard rhizogenesis usually depends

heavily on auxins (IBA or NAA) executed on reduced strength media (to lower nitrogen concentration), the successful utilization of an auxin-cytokinin amalgamation in A. moschatus suggests a highly species-specific biochemical necessity for cytokinin-mediated root meristemoid organization, aligning with specific morphogenetic behaviors reported in isolated taxa (Teshome *et al*. 2016) [22].

In assessing the qualitative phytochemical matrix, in *vitro* generated plants proved biosynthetically robust. They accumulated higher concentrations of alkaloids, phlobatannins, quinine and coumarin than naturally grown populations. Plant in *vitro* cultures are essentially chemical factories (Ramachandra Rao and Ravishankar 2002) [23]. The enhanced metabolic flux observed can be scientifically attributed to the constant availability of exogenous plant growth regulators, particularly auxins and cytokinins, which routinely function as potent elicitors inside culture vessels. These PGRs actuate the transcription factors responsible for secondary metabolic pathways, effectively 'stressing' the tissue to hyper accumulate defensive phyto-compounds like alkaloids and phlobatannins, which are vital for antibacterial, antioxidant and anti-inflammatory drug formulations (Sheena and Jothi 2015, Kharde *et al*. 2018) [12, 24]. Therefore, in *vitro* propagated A. moschatus maintains uncompromised and in some biochemical aspects superior, pharmacological potential.

Conclusion

This study establishes a highly effective, robust and reproducible protocol for the mass micropropagation of the endangered medicinal species Abelmoschus moschatus Medik. through direct and indirect organogenesis. By optimizing explicit PGR combinations for distinct vegetative explants (favoring 2,4-D for callogenesis, BAP+Kn for nodal direct shoot regeneration and BAP+NAA for rapid shoot apex proliferation), rapid clonal multiplication, elongation and resilient rooting were successfully achieved. Crucially, comparative phytochemical investigations confirmed that in *vitro* regenerated plantlets retain and occasionally surpass, the biosynthetic capacity of their wild counterparts regarding highly valued secondary metabolites like alkaloids, phlobatannins, quinine and coumarin. This optimized protocol subsequently serves as a critical dual-action framework: it effectively mitigates the ecological pressures of wild harvesting for conservation and establishes a continuous, reliable and high-yielding biological pipeline for the global pharmaceutical industry.

Acknowledgement

The authors formally acknowledge the substantial infrastructural support and research facilities provided by the Plant Tissue Culture and Biotechnology Laboratory, Department of Botany, University of Chittagong, Chattogram-4331, Bangladesh.

References

1. Rahman MM, Akhi UR, Biswas A. Micropropagation of Mentha viridis L. - an aromatic medicinal plant. International Journal of Pharmacy and Life Sciences,2013:4(9):2926-2930.
2. Mia MMK. Traditional Medicine. In: Ghani A, editor. Jahangirnagar University, Savar, Dhaka, 1990, 73.
3. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd ed. Asiatic Society of Bangladesh, Dhaka, 1998, 373.
4. Fowler MR, Rayns FW, Hunter CF. The language and aims of plant cell and tissue culture. In *Vitro* Cultivation of Plant Cells. Oxford: Butterworth-Heinemann Ltd, 1993, 1-18.
5. Rout GR, Samantaray S, Das P. In *vitro* manipulation and propagation of medicinal plants. Biotechnology Advances,2000:18:91-120. DOI: 10.1016/S0734-9750(99)00027-9
6. Afolayan AJ, Adebola PO. In *vitro* propagation: A biotechnological tool capable of solving the problem of medicinal plants decimation in South Africa. African Journal of Biotechnology,2004:3:683-687.
7. Murch SJ, Choffe KL, Victor JMR, Slimmon K, Raj P, Saxena PK. Thiazuron induced plant regeneration from hypocotyl cultures of St. John's wort (Hypericum perforatum L. cv. Anthos). Plant Cell Reports,2000:19:576-581. DOI: 10.1007/s002990050816
8. Fraenkel GS, Gottfried S. The raison d'Etre of secondary plants substances. Science,1959:129(3361):1466-1470. DOI: 10.1126/science.129.3361.1466
9. Harborne JB. Phytochemical Methods. London: Chapman and Hall Ltd, 1973, 49-188.
10. Oksman-Caldentey KM, Inze D. Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. Trends in Plant Science,2004:9(9):433-440. DOI: 10.1016/j.tplants.2004.07.006
11. Julsing MK, Rijpkema M, Woerdenbag HJ, Quax WJ, Kayser O. The Engineering of Medicinal Plants: Prospects and Limitations of Medicinal Plant Biotechnology. In: Medicinal Plant Biotechnology,2007:978-3-527-314443-0.
12. Kharde AV, Kore SV, Khetmalos MB. Elicitation of Bacoside Content Using Plant Growth Regulators in Cell Suspension Culture of Bacopa monnieri (L.) Wettst. Bangladesh Association for Plant Tissue Culture and Biotechnology,2018:28(2):191-199. DOI: 10.3329/ptcb.v28i2.39680
13. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum,1962:15(3):473-497. DOI: 10.1111/j.1399-3054.1962.tb08052.x
14. Sofowora A. Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd, 1993, 289.
15. Bandiola TMB. Extraction and Quantitative Phytochemical Screening of Medicinal plants: A Brief Summary. International Journal of Pharmacy,2018:8(1):137-143.
16. Krikorian AD, Berquam DL. Plant cell and tissue cultures: The role of Haberlandt. The Botanical Review,1969:35:59-67. DOI: 10.1007/BF02858911
17. Biswas A, Bari MA, Roy M, Bhadra SK. In *vitro* plant regeneration of an endangered medicinal plant Abrus precatorius L. Plant Tissue Culture & Biotechnology,2007:17(2):201-209. DOI: 10.3329/ptcb.v17i2.3237
18. Jawahar M, Ravipaul S, Jeyaseelan M. In *vitro* regeneration of Vitex negundo a multipurpose woody

aromatic medicinal shrub. Bangladesh Association for Plant Tissue Culture and Biotechnology,2008:18(1):37-42. DOI: 10.3329/ptcb.v18i1.3262
19. Rout GR, Das P, Goel S. Tissue culture of woody ornamentals: a review. Biotechnology Advances,2009:18:91-120.
20. Lithy ME, Bahr MI, Amin MH, Hamada M. In *vitro* propagation and ex *vitro* acclimatization of Abelmoschus moschatus Medik. Journal of Applied Sciences Research,2011:7(12):1949-1956.
21. Kumar MS, Nandi SC. High frequency plant regeneration with histological analysis of organogenic callus from internode explants of Asteracantha longifolia Nees. Journal of Genetic Engineering and Biotechnology,2015:13(1):31-37. DOI: 10.1016/j.jgeb.2014.12.006
22. Teshome I, Teshome S, Soromessa T, Feyissa T. Development of an efficient in *vitro* propagation protocol for Satureja punctata - A rare aromatic and medicinal plant. Taiwania,2016:61(1):41-48.
23. Ramachandra Rao S, Ravishankar GA. Plant cell cultures: Chemical factories of secondary metabolites. Biotechnology Advances,2002:20(2):101-153. DOI: 10.1016/S0734-9750(02)00007-X
24. Sheena EV, Jothi G. Comparative phytochemical screening and antioxidant properties of in *vitro* and in *vivo* propagated Orthosiphon stamineus Benth (Lamiaceae). Asian Journal of Pharmaceutical and Clinical Research,2015:8(4):216-220.