BMC Plant Biology

RESEARCH Open Access

Unlocking the biotechnological potential of *Decalepis arayalpathra*: exploring synthetic seed production, metabolic profiling, genetic stability, and the impact of photosynthetic photon flux density on acclimatization

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Abstract

Decalepis arayapathra is an important medicinal plant known for several medicinal values, however, due to overharvesting, habitat destruction, and its limited geographical distribution, *D. arayapathra* faces severe threats of extinction. A synthetic seed protocol was developed for this plant, representing a novel approach in its propagation and conservation. Nodal segments (NS) were encapsulated in a sodium alginate (SA) matrix. 3% SA with 100 mM $CaCl_2$ solutions was best to obtain ideal beads with fine texture. Murashige and Skoog (MS) medium consisting of BA 5.0 µM + NAA 0.5 µM + ADS 20.0 µM resulted in a maximum regrowth frequency of 71.26% with 3.13 shoots per bead and a shoot length of 4.10 cm after six weeks of culture. Rooting in the microshoots was better observed with half- strength MS + 2.5 µM NAA, resulting in 3.1 roots per microshoot and a root length of 3.0 cm after four weeks of culture, followed by successful acclimatization. The study investigated the effect of photosynthetic photon flux density (PPFD) levels of 50 and 300 PPFD on various physiological and biochemical parameters during the acclimatization of in vitro-derived plants. Results showed an increase in photosynthetic pigments, including chlorophyll and carotenoids, as well as an enhanced net photosynthetic rate (P_N) and stomatal conductance (gs) with prolonged acclimatization, with higher PPFD being more effective. Antioxidant enzyme activities, including SOD, CAT, APX, and GR, increased over time, except for SOD, which began to decline after 21 days under both light conditions. Stress markers such as malondialdehyde (MDA) and electrolyte leakage decreased over time, indicating successful acclimatization. Genetic fidelity was confirmed through clear and monomorphic banding patterns obtained using RAPD and ISSR markers. Quantification of 2H4MB (2-hydroxy-4-methoxy benzaldehyde) in synseed-derived roots using HPLC revealed a concentration of 16.27 µg/ml. Metabolic profiling of the synseed-derived root tuber using GC-MS identified several major and minor metabolites. This study offers a breakthrough in the conservation of *D. arayapathra* through synthetic seed technology, enabling sustainable

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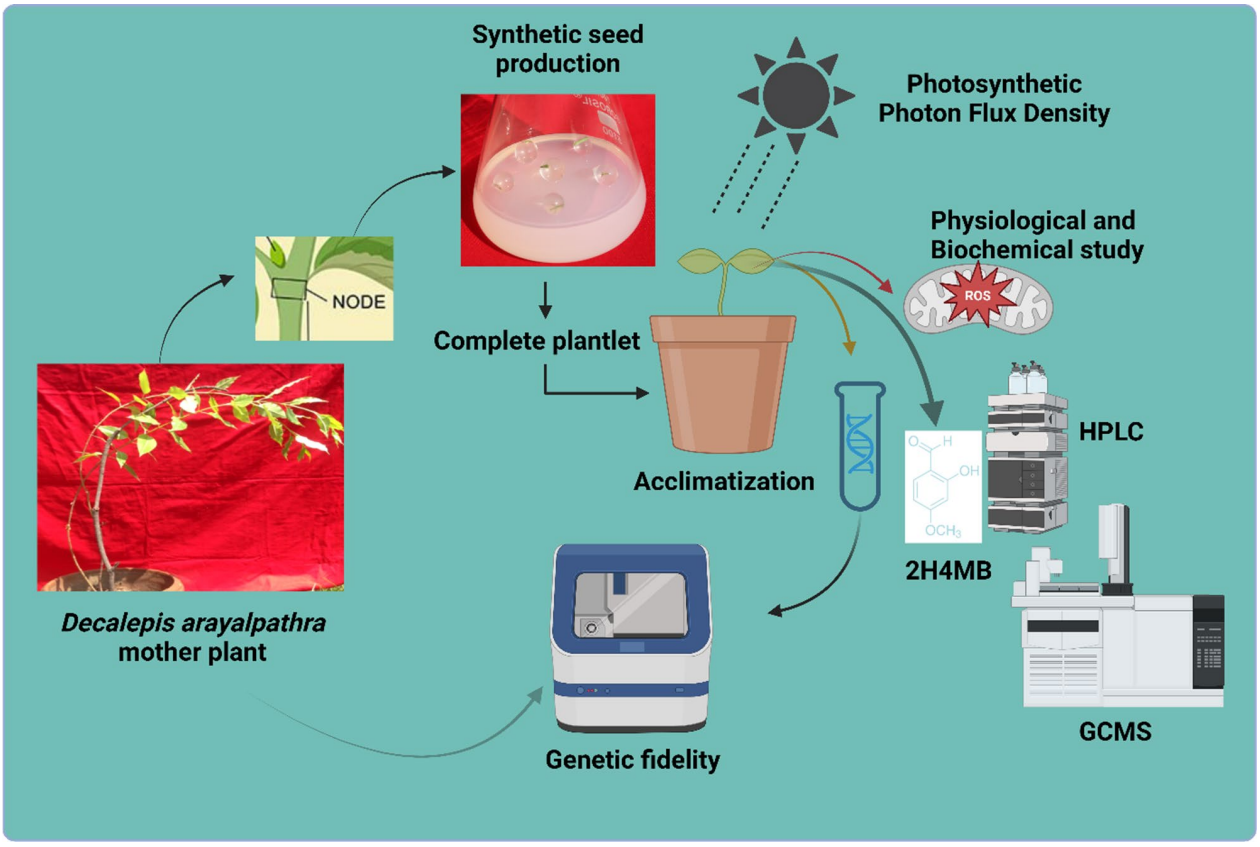
propagation while preserving genetic stability. It ensures a consistent supply of the bioactive compound 2H4MB, promoting medicinal research and commercial applications.

Graphical Abstract



Keywords Antioxidant enzyme, Chromatography, Clonal integrity, Ex situ conservation, Micropropagation, Stress markers.

Introduction

Encapsulated somatic embryos, shoot buds, or other plant cells that have the potential to develop into entire plants are called synthetic seeds, sometimes referred to as artificial seeds [1]. The gel-like material used to create these encapsulations typically sodium alginate (SA) protects the plant tissue and allows for a regulated release of nutrients. With the benefit of being created in a regulated in vitro setting, synthetic seeds are made to resemble natural seeds [2]. Regardless of the season, synthetic seeds may be made and sown at any time of the year. Meeting consumer needs and maintaining continuous production cycles both benefit from this flexibility [3]. Plant genetic resources can be effectively conserved through the use of synthetic seeds. Their ability to be stored for long periods of time without losing their viability is crucial for the protection of rare and endangered species [2]. One such plant is *Decalepis arayalpathra* (Joseph & Chandras) Venter belongs to Apocynaceae and known for several medicinal applications (http://www.theplantlist.org/tpl1.1/record/kew-2758601). The most important part of the plant is its tuberous root, which is rich in phenolic, alkaloids, and most importantly, 2-hydroxy-4-methoxybenzaldehyde (2H4MB), an important secondary metabolite and an isomer of vanillin that makes up 96.8% of the root [4, 5]. It functions as an antifungal, antivirulant, antioxidant, and antibacterial [6, 7] and it also found to effective against cellular apoptosis [8].

The species is unique to certain forest regions in the Southern Western Ghats of India, and its population has declined due to the region's increasing deforestation [9]. The natural regeneration and species distribution of *D. arayalpathra* have been hampered simultaneously by its unique niche, disease assault, poor fruit setting, low rate of seed germination, and resilience to root from stem cutting [10]. The species' restricted geographic area and dispersed populations make it severely endangered, and the National Biodiversity Authority of India (NBA) has

designated it as a species of high conservation concern [11]. This necessitates urgent conservation and propagation efforts to preserve its genetic diversity, prevent extinction, and ensure the sustainable utilization of its important secondary metabolites in various industries.

Although in vitro propagation of this plant has been explored to some extent [12, 13], this study focuses on developing a synthetic seed technique, which provides an alternative propagation method to address specific conservation and propagation challenges. To confirm the genetic uniformity of plants derived from synthetic seeds, we used two molecular markers in our study: RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeat). High Performance Liquid Chromatography (HPLC) was used for the evaluation of 2H4MB and Gas chromatography and mass spectrometry (GCMS) was used for the metabolic profiling of the root. In addition, we have investigated various physiological and biochemical parameters that provide information on the stress tolerance and acclimatization of the plants and optimize the protocols for successful establishment in the field. Investigating the effects of photosynthetic photon flux density (PPFD) on acclimatization of in vitro grown plants is crucial for optimizing light conditions that promote efficient photosynthesis, increase growth and improve survival rates when transitioning to an ex vitro environment. It helps to understand the light intensity requirements for better physiological adaptation. This ensures the successful establishment of plants outside of controlled laboratory conditions.

The aim of developing a synthetic seed process for *D. arayalpathra* is to facilitate the effective replication and preservation of this threatened species, ensuring its existence and accessibility for future applications. As a critically endangered plant with significant medicinal and ecological value, it requires innovative propagation techniques to overcome limitations in conventional methods. Synthetic seeds offer unique advantages, such as ease of storage, transport, and large-scale propagation, ensuring accessibility for future applications while preserving genetic integrity. To ensure the quality and consistency of propagated plants, genetic homogeneity is verified using RAPD and ISSR markers. Additionally, the evaluation of physiological and biochemical parameters provides valuable insights into the plants' ability to withstand transplant shock and adapt to environmental changes, optimizing protocols for successful field establishment. This research will support the sustainable use of *D. arayalpathra* in various sectors by safeguarding its genetic diversity and ecological value for future generations. This study fills a research gap by developing a synthetic seed propagation technique for *Decalepis arayalpathra*, an endangered species that has not been successfully propagated in vitro with synthetic seeds. It ensures the conservation and sustainable use of this species by preserving its genetic diversity and enabling large-scale propagation for future medical and industrial applications.

Methodology

Plant materials, media preparation and culture condition

Nodal segment (NS) (0.3–1.0 cm) were collected from a 4-year-old *Decalepis arayalpathra* mother plant growing in the Department of Botany, Aligarh Muslim University, Aligarh, India. The plant material was identified by an expert and the specimen has been added to the department herbarium with accession number 34,173. The shoots collected were thoroughly rinsed under running tap water for 30 min to eliminate any dust or debris. Subsequently, they were treated with a 1% (m/v) Bavistin fungicide solution for 30 min, followed by a 15-minute wash using a 5% (v/v) Labolene liquid detergent solution. The shoots were then rinsed 6–7 times with sterilized double-distilled water. Surface sterilization and further procedures were performed as described by Ahmad et al. [12]. Murashige and Skoog [14] containing 3% sucrose (w/v) sucrose as a carbon source and 0.8% agar were used for solidification. The pH was adjusted to 5.8 prior to adding agar, using either 1 N NaOH or 1 N HCl as needed. Approximately 45–50 cm^3 of the medium was dispensed into each wide-mouth flask with a capacity of 100 mL. The medium and instruments were sterilized by autoclaving at 121 °C and 1.06 kg/cm^2 ψ for 15 min. The cultures were maintained under controlled conditions at a temperature of 25 ± 2 °C with a 16-hour photoperiod, illuminated at a light intensity of 50 μmol m^{-2} s^{-1} using 40 W fluorescent tubes. The relative humidity in the culture room was maintained at 55 ± 5%.

Encapsulation of nodal segment

Encapsulation matrix, formation of beads and germination

For encapsulation, NS with axillary buds were utilized. For polymerization, varying concentration of SA (1–4%) prepared in liquid MS media and $CaCl_2$ concentrations (25–150 mM) prepared in DDW were used. The pH of both the solutions was set to 5.8 before autoclaving at 121 °C and 1.06 kg/cm^2 ψ for 15 min. The beads were prepared by dropping the SA matrix containing sterile NS into $CaCl_2$ solution with an aqua jet pipette and the beads were left in the $CaCl_2$ for 30 min till it becomes fully polymerized. Then after, the beads were washed with DDW to eliminate the residues of $CaCl_2$. It will be followed by inoculation on various nutrient media consisting of MS basal medium augmented with different concentration of cytokinin like BA (6-benzyladenine) (1.0–7.0μM), Auxin like NAA (α-naphthalene acetic acid) (0.1-1.0μM) and growth additives like Ads (Adenine sulphate) (10–30 μM). The nutrient medium MS lacking

PGR was served as control. The flask was kept in controlled condition as stated in Sect. 2.1. The optimized medium, consisting of MS supplemented with optimal concentrations of BA, NAA, and Ads, was utilized for matrix encapsulation.

Rooting and acclimatization

After being removed, healthy microshoots were moved to full- or half-strength MS media supplemented with auxin, namely NAA (1.0–5.0 µM), IAA (Indole-3-acetic acid) (1.0–5.0 µM), and IBA (Indole-3-butyric acid) (1.0–5.0 µM), at varying concentrations for obtaining roots. After 4 weeks, the plants with healthy shoots and roots were taken out from the culture and carefully cleaned and placed in thermocol cups containing autoclaved soilrite™ (Irish peat moss, 75% + expanded perlite, 25%). Sixty plants in all were split into two groups at random and put in a growth environment that was kept at 25 ± 2 °C and 60–65% relative humidity. Plants in both groups were grown with a 16-hour photoperiod, however their PPFDs varied from plant to plant. For one set of plants, low-light conditions were maintained (LL) intensity, 50 lmol $m^{-2}s^{-1}$; in contrast, plants in a different group were kept in high-light (HL) intensity, 300 lmol $m^{-2}s^{-1}$. Leaf samples were taken at various intervals, namely 0, 7, 14, 21, and 28 days after being moved to a growth room to allow for acclimatization. These samples were then frozen in liquid nitrogen until required.

Low temperature storage of synthetic seed

To study the low temperature (4 °C) storage and regenerability of synseeds, a comparative analysis was conducted using three different encapsulation matrices; (1) MS + 5.0 µM BA + 0.5 NAA µM + 20 µM Ads; (2) MS basal medium; (3) encapsulation matrix made with distilled water; and (4) non-encapsulated nodal segments were included in the analysis. These were evaluated across storage duration of 0, 1, 2, 4, 6, and 8 weeks. The beads from each treatment were carefully moistened with DDW, placed in a sterilized beaker, sealed securely with Parafilm®, and stored at 4 °C in a laboratory refrigerator. The beads were planted onto the optimal medium (MS + 5.0 µM BA + 0.5 µM NAA + 20 µM Ads for shoot regrowth. Shoot regrowth was analyzed after 6 weeks of transplantation followed by their transfer to rooting media in the same manner as described before.

Physiological analysis

Pigment analysis, photosynthesis and related attributes

Ten micropropagated seedlings were selected at random. On day 0 (control) and after 7, 14, 21 and 28 days of acclimatization, the fully developed leaves were removed. Extraction with 80% (v/v) acetone was then performed and the mixture was filtered using Whatman filter paper No. 1. An ultraviolet-visible (UV-Vis) spectrophotometer was used to measure the absorbance for chlorophyll and carotenoids at 663, 645 and 466 nm, respectively. A mobile photosynthesis system (Li-COR 6400, Lincoln, NE, USA) was used to measure net photosynthetic rate (P_N) and stomatal conductance (gs), with the in Ahmad et al. [12].

Membrane permeability was evaluated by electrolyte leakage (EL), as explained by Lutts et al. [15]. The samples were washed three times with double distilled water (DDW) to remove impurities from the surface. After cutting the young leaves with a hole punch, they were placed in a closed vessel containing 10 milliliters of DDW and incubated on a rotary shaker for 24 h. The electrical conductivity (EC1) of the solution was then determined. The samples were autoclaved at 120 °C for 20 min. After the solution cooled to room temperature, its electrical conductivity (EC2) was measured and EL was calculated as EL (%) = EC1/EC2 × 100.

The concentration of malondialdehyde (MDA) was measured using an altered version of the Cakmak and Host [16] technique. Ten milliliters of 0.1% (w/v) trichloroacetic acid were used to grind 0.5 g of fresh tissue, which was then centrifuged for five minutes at 15,000 rpm. A new test tube was filled with one milliliter of the supernatant and four milliliters of 0.5% (w/v) TBA prepared in 20% (w/v) TCA. Further measurement and calculation was carried out as mentioned in [17].

Biochemical analysis

Approximately 0.5 g of fresh, healthy leaves and 2.0 milliliters of an extraction buffer were combined in a pre-chilled mortar and pestle to investigate the antioxidant enzyme activity. 0.11 gs of ethylene diamine tetra acetic acid (EDTA), 1% Triton X-100, and 1% polyvinylpyrrolidone (PVP) made up the extraction buffer. After that, the mixture was centrifuged for 20 min at 15,000 rpm after being filtered through Whatman No. 1 filter paper. The antioxidant enzyme activity was determined using the isolated supernatant. This procedure was carried out in the dark and at a temperature of 4 ∘C. The activities of SOD, CAT, GR and APX were expressed as enzyme units (EU) mg-1 of protein according to the methods of Dhindsa et al. [18], Aebi [19], Rao [20], Nakano and Asada [21].

Genetic fidelity

Genetic fidelity was assessed using two molecular marker systems: five ISSR primers and six RAPD primers. DNA extraction was done from nine *D. arayapathra* plantlets that were produced in vitro, as well as the mother plant, using the CTAB method was carried out [22]. The reaction was carried out using a thermocycler with ISSR and RAPD primer, as previously mentioned by Ahmad et al.

Table 1 Effect of different sodium alginate concentration with 100 mM calcium chloride on the encapsulation of NS explant and regrowth frequency in *D. Arayalpathra* after six weeks of culture on MS medium (means ± SE)

Sodium Alginate (% w/v)	Regrowth frequency (%)	Remarks
1.0	0.0 ± 0.0^{e}	NA
2.0	0.0 ± 0.0^{e}	NA
2.5	28.66 ± 1.76^{c}	Fragile beads with soft texture
3.0	57.7 ± 1.15^{a}	Beads with soft texture
3.5	40.0 ± 1.15^{b}	Hard beads
4.0	17.33 ± 1.4^{d}	Hard beads

*Tukey's test indicates that there is no significant difference between mean values followed by the same letter with a 5% probability

Table 2 Effect of different $CaCl_2 \cdot 2H_2O$ with 3% sodium alginate on the encapsulation of NS explants and regrowth frequency in *D. Arayalpathra* after six weeks of culture on MS medium (means ± SE)

Calcium chloride (mM)	Regrowth frequency (%)	Remarks
25	0.0 ± 0.0^{e}	NA
50	0.0 ± 0.0^{e}	NA
75	29.66 ± 1.76^{c}	Fragile beads with soft texture
100	57.7 ± 1.15^{a}	Beads with soft texture
125	38.68 ± 0.88^{b}	Hard beads
150	18.33 ± 1.45^{d}	Hard beads

*Tukey's test indicates that there is no significant difference between mean values followed by the same letter with a 5% probability

[23], the reaction mixture was prepared and the amplification cycle was set up. Electrophoresis (0.8%, w/v agarose gels + ethidium bromide 4 µl in TAE buffer, running time 2 h at 50 V) was used to separate the amplified products. Using a UV trans-illuminator, the gel was visualized.

2H4MB (2-Hydroxy-4-Methoxybenzaldehyde) quantification and metabolic profiling of roots

To make the root powder, the root was removed from the in vitro-derived plant, cleaned under running water, dried, and then crushed in a mortar and pestle with Liquid N_2. The extraction process was carried out using the Soxhlet method with methanol acting as a solvent. A 0.22 µm filter syringe was utilized to purify the material prior to use. Using methanol, 2H4MB (98% purity, standard, Sigma Aldrich, New Delhi, India) was used to prepare a stock solution and several gradient solutions. he procedure followed the earlier description provided by Ahmad et al. [12] for the chromatographic condition and identification of the substance. The GCMS profiling was done by following the procedure of Ahmad et al. [5].

Statistical analysis

Experimentation was carried out with 10 replicates and three repetitions. One-way ANOVA was used to statistically evaluate the data. The significance of variances among means was accredited using Tukey's test at the 5% level of significance and data denoted as mean ± standard error (SE).

Results

Encapsulation and regrowth

The form and texture of the beads (Ca-alginate) were dependent on both Na-alginate and $CaCl_2$ after NS was employed for encapsulation (Tables 1 and 2). After 6 weeks of culture on MS basal medium without any PGR, we recorded a conversion response of 57.7 ± 1.15% on Na-alginate (3%) + $CaCl_2$ (100 mM), which produced an excellent bead in terms of its morphology (Fig. 1a). At this concentration, a clear, solid, and isodiametric synthetic seed was formed. Lower concentrations of $CaCl_2$ and sodium alginate resulted in the formation of fragile and brittle beads, which were difficult to handle and prone to breakage. Conversely, higher concentrations of these compounds produced excessively hard beads, which, while more robust for handling, were less conducive to germination and showed a reduced conversion frequency.

The synseed containing NS regrew 52.83 ± 0.67% when inoculated on MS medium without PGR (Table 3). However, PGR-enriched media significantly improved regrowth frequency and the combination of optimal cytokinin with auxin NAA at 0.5 µM resulted in a regrowth frequency of 64.40 ± 1.15% with a mean number of shoots of 1.80 ± 0.044 and a shoot length of 2.22 ± 0.058 cm. However, addition of growth additives provides an optimum growth with regrowth frequency of 71.26 ± 1.12% with mean number of shoots of 3.13 ± 0.096 and shoot length of 4.10 ± 0.044 cm on BA (5.0 µM) + NAA (0.5 µM) + 20 µM Ads (Fig. 1c).

In vitro rooting and acclimatization

Following a period of incubation of four weeks, a maximum of 3.1 ± 0.1 roots per microshoot, measuring 3.0 ± 0.1 cm in length, were seen in 82.1% of the cultures. The optimal ½ MS combined with 2.5 µM NAA was used to root these microshoots (Table 4; Fig. 1d).

Higher doses of auxins slowed the rate of root development, but the control rooting media without auxins showed no induction. The second-best hormone in this case was 2.5 µM IAA, which resulted in an average number of 2.2 ± 0.1 roots per shoot with a root length of 2.8 ± 0.1 cm. The synthetic seeds developed into healthy plantlets that were properly hardened according to the materials and methods. When the plant acclimatized in

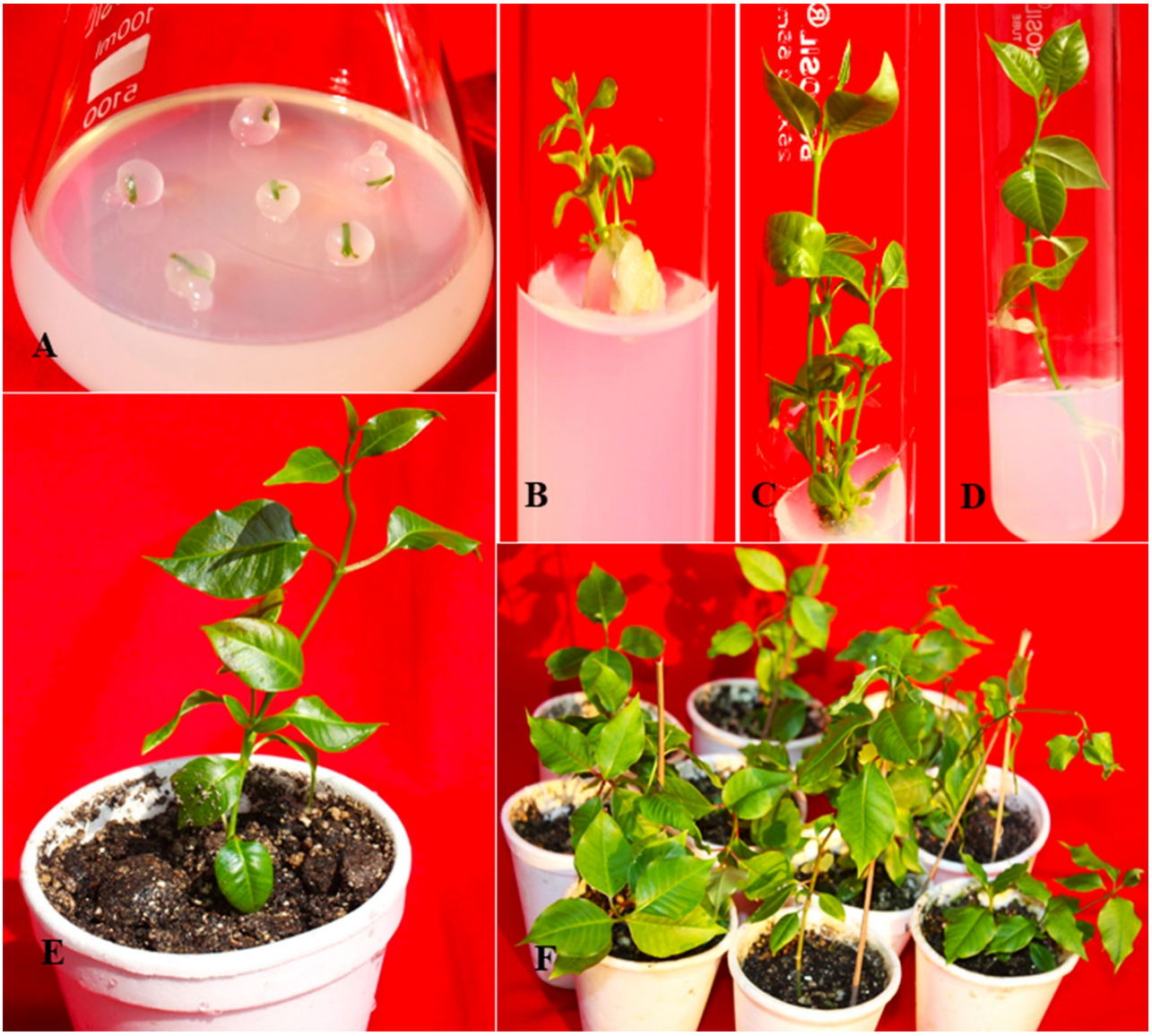


Fig. 1 Synthetic seed formation and regrowth in *D. araylpathra*. (**A**) Encapsulated in vivo derived NS on MS basal medium augmented with optimal PGRs, 1-day-old culture. (**B**) Initiation of shoots from nutrient encapsulated NS with MS + 5.0 µM BA + 0.5 µM NAA + 20 µM Ads after 1week culture. (**C**) Proliferating synthetic seed derived culture maintained on same culture media and condition after 6 weeks of culture. (D) Development of fibrous roots on ½ MS + 2.5 µM NAA after 4 weeks of culture. **E**&**F**) Successfully acclimatized in vitro rooted plantlets in soilrite

Soilrite™ was transferred to the greenhouse, it showed good growth and a survival rate of 89.3% (Fig. 1e&f).

Impact of short-term storage and germplasm preservation on regeneration capacity

After 0 weeks of storage, the frequency of non-encapsulated NS regrowth peaked, then decreased linearly, and after 6 weeks, there was no regeneration seen (Table 5). In terms of regrowth frequency, it was discovered that the encapsulation matrix made in MS basal medium + 5.0 µM BA + 0.5 NAA µM + 20 µM Ads was more optimal. After 0 weeks of storage, the maximum regrowth frequency of 78.13 ± 1.27% was noted, which was gradually followed by a progressive fall and a maximum regrowth frequency of 44.10 ± 1.24% after 8 weeks of storage.

Evaluation of physiological and biochemical parameters during acclimatization

Pigments, photosynthesis and stomatal conductance

Seedlings exposed to both 50 (LL) and 300 PPFD (HL) after acclimatization showed an increase in pigment content. There was a significant increase in total chlorophyll, carotenoids and chlorophyll a and b compared to the plantlets at day 0. However, after one week of acclimatization, a decrease in these pigments was observed. A significant increase in chlorophyll and carotenoids was

Table 3 Effect of various PGR treatments on *D. Arayalpathra* encapsulated NS regrowth following a 6-week culture period

PGRs (µM)	Regrowth frequency (%)	No. of shoots/bead	Mean shoot length (cm)
0.00	52.83 ± 0.67^{d}	1.07 ± 0.034^{g}	1.10 ± 0.019^{d}
BA (1.0)	58.14 ± 1.20^{cd}	1.16 ± 0.006^{g}	1.20 ± 0.074^{e}
BA (2.5)	60.77 ± 1.86^{bc}	1.3 ± 0.045^{e}	1.90 ± 0.044^{d}
BA (5.0)	64.97 ± 1.86^{a}	1.6 ± 0.037^{f}	2.10 ± 0.044^{e}
BA (7.0)	61.17 ± 1.27^{b}	1.47 ± 0.041^{e}	1.9 ± 0.080^{d}
BA (5.0) + NAA (0.1)	63.90 ± 1.08^{abc}	1.67 ± 0.044^{c}	2.02 ± 0.037^{b}
BA (5.0) + NAA (0.5)	64.40 ± 1.15^{abc}	1.80 ± 0.044^{e}	2.22 ± 0.058^{c}
BA (5.0) + NAA (1.0)	62.53 ± 0.97^{abc}	2.32 ± 0.091^{d}	2.68 ± 0.037^{b}
BA (5.0) + NAA (0.5) + 10 Ads	70.83 ± 1.80^{ab}	2.93 ± 0.096^{b}	3.80 ± 0.044^{b}
BA (5.0) + NAA (0.5) + 20 Ads	71.26 ± 1.12^{a}	3.13 ± 0.096^{a}	4.10 ± 0.044^{a}
BA (5.0) + NAA (0.5) + 30 Ads	68.30 ± 1.12^{ab}	2.27 ± 0.034^{g}	2.90 ± 0.019^{d}

*Mean values followed by the same letter are not significantly different according to Tukey's test at 5% probability

Table 4 Effect of various auxins on *D. Arayalpathra* microshoots' in vitro root induction on agar gelled media following four weeks of culture

Auxin (µM)	% Response	Mean no. of roots/ shoot	Mean root length (cm)
MS (Control)	00.0 ± 0.0^{g}	0.0 ± 0.0^{e}	0.0 ± 0.0^{g}
½ MS	40.3 ± 1.1^{f}	0.3 ± 0.0^{d}	2.4 ± 0.2^{e}
½ MS + NAA (1.0)	68.0 ± 0.3^{b}	1.1 ± 0.1^{c}	3.4 ± 0.1^{a}
½ + NAA (2.5)	82.1 ± 0.3^{a}	3.1 ± 0.1^{a}	3.0 ± 0.1^{b}
½ + NAA (5.0)	58.8 ± 0.3^{c}	1.0 ± 0.2^{d}	2.9 ± 0.2^{c}
½ + IAA (1.0)	69.2 ± 0.3^{b}	0.8 ± 0.1^{d}	2.5 ± 0.1^{d}
½ + IAA (2.5)	57.3 ± 0.3^{d}	2.2 ± 0.1^{b}	2.8 ± 0.1^{b}
½ + IAA (5.0)	49.1 ± 0.2^{e}	0.9 ± 0.1^{d}	2.0 ± 0.1^{e}
½ + IBA (1.0)	0.0 ± 0.00^{g}	0.0 ± 0.0^{e}	0.0 ± 0.0^{g}
½ + IBA (2.5)	38.0 ± 0.0^{f}	0.6 ± 0.1^{d}	1.0 ± 0.0^{f}
½ + IBA (5.0)	0.00 ± 0.0^{g}	0.0 ± 0.0^{e}	0.00 ± 0.0^{g}

Data represent mean ± SE of 20 replicates per treatment in 3 repeated experiments. Mean values followed by the same letter are not significantly different according to Tukey's test at 5% probability

observed in HL during acclimatization (Fig. 2A, B, C & E). The maximum content of chlorophyll a, b, total and carotenoids was 1.3 mg g^{-1} FW, 0.7 mg g^{-1} FW, 2.0 mg g^{-1} FW, and 0.58 mg g^{-1} FW, respectively at HL after 28 days of acclimatization. Similarly, P_N also showed similar trend and maximum value was 3.8 µmole CO_2 $m^{-2}s^{-1}$ on HL after 28 days of acclimatization (Fig. 1F). The value of stomatal conductance was found to be increase as the days of acclimatization increases on both HL and LL in comparison to the control. The maximum value was 0.28 mol $m^{-2}s^{-1}$ at HL in comparison to the days 0 (Fig. 2D).

Electrolyte leakage and lipid peroxidation

When the plantlets were exposed to light stress, their MDA and electrolyte levels were much higher than those of the plantlets on day 0. The MDA content and electrolyte loss were higher in the plantlets treated with HL than in those treated with LL. The maximum accumulation of MDA was 3.8 & 4.0 µmole g^{-1} FW at LL and HL respectively and similarly EL was 55 and 59% at LL and HL during first week of acclimatization (Fig. 3E&F). However, the values decrease as the days of acclimatization increases on both type of lights.

Enzymatic activities

During the first week of acclimatization, changes in SOD activity were observed (Fig. 3A). Differences in SOD activity were observed in both LL and HL intensity. On the other hand, 21 days after acclimatization, a higher level of catalase activity was detected at both light intensities, followed by a decrease in SOD activity (Fig. 3A&B). The maximum value of SOD and CAT was 2.8 Unit mg^{-1} protein min^{-1} at 21 days of acclimatization and 278 µmole min^{-1} mg^{-1} protein after 28 days of acclimatization, respectively at HL. However, the variation in light has not much effect on the APX activity throughout the acclimatization period (Fig. 3C). But significant difference was recorded as compared to the days 0. On the other hand, the activity of GR was affected by the variation of the light and maximum content of GR was 2.8 µmole min^{-1} mg^{-1} protein after 28 days of acclimatization on HL (Fig. 3D).

Genetic fidelity

A total of 11 primers (six RAPD and five ISSR) were used to amplify genomic DNA, producing clear and reproducible banding patterns (Table 6; Fig. 4). Six RAPD primers produced a total of 20 amplified bands, with sizes ranging from 200 to 2000 bp. Primer OPU-08 generated the highest number of bands (6), with a size range of 500–2000 bp. The least number of bands (1) was observed with primer OPL-01, with a size range of 200–400 bp.

Five ISSR primers produced a total of 20 amplified bands, with sizes ranging from 300 to 3500 bp. Primer UBC-812 exhibited the highest polymorphism with 6 bands, covering a size range of 300–2500 bp. Primer UBC-810 showed the least number of bands (2), with a size range of 500–1200 bp.

2H4MB quantification and metabolic profiling of the roots

When the extract's chromatogram and the standard 2H4MB peak were evaluated, both showed comparable retention times (RT). The similarity between the RT of 2H4MB (3.718 min) and the RT of the extract (3.717 min) under the same chromatographic conditions is clearly

Table 5 Effect of different storage duration on regeneration ability (%) of encapsulated and non-encapsulated NS of *D. Arayalapthra* on MS medium

Storage duration (weeks)	Regrowth frequency of NS segment that has been encapsulated (%) (encapsulation matrix made in MS basal medium plus 5.0 µM BA, 0.5 NAA µM, and 20 µM Ads)	Regrowth frequency of NS segments that have been encapsulated (%) (encapsulation matrix made in MS basal media)	Frequency of NS regrowth when encapsulated (encapsulation matrix made with DDW water)	Non-encapsulated NS regrowth frequency (%)
0	78.13 ± 1.27^{a}	70.10 ± 1.15^{a}	61.13 ± 1.16^{a}	90.20 ± 1.15^{a}
1	72.13 ± 1.10^{b}	60.13 ± 1.16^{b}	48.26 ± 1.21^{b}	60.23 ± 1.24^{b}
2	70.10 ± 1.24^{b}	53.00 ± 1.73^{c}	39.56 ± 0.95^{c}	40.23 ± 1.03^{c}
4	60.80 ± 1.56^{c}	30.20 ± 1.10^{d}	20.50 ± 1.02^{d}	18.16 ± 1.21^{d}
6	50.06 ± 1.09^{d}	18.40 ± 1.90^{e}	0.00 ± 0.00^{e}	08.86 ± 0.87^{e}
8	44.10 ± 1.24^{e}	09.93 ± 0.79^{f}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}

Beads prepared with 3.0% (w/v) sodium alginate and 100mM $CaCl_2.2H_2O$

Data represents Mean ± SE of 20 replicates per treatments in 3 repeated experiments

Mean values followed by the same letter are not significantly different according to Tukey's test at 5% probability

shown in Fig. 5A&B. The evaluated content of 2H4MB was 16.27 µg/ml.

The root tuber from mother plant and in vitro established plant were collected and extracted through methanol and further subjected for the GC-MS analysis. The source of the leaf and the preparation of the extract have already been described in the Material and Method section. The identified bioactive compounds along with their retention time (RT), area and percentage area obtained in in vitro sources of *D. arayalpathra* are documented in Table 7. The obtained GC-MS chromatogram has been shown in Fig. 6. Different phytocompounds such as 2-hydroxy-4-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 3-methoxyphenol, Eicosane, Isopropyl Myristate, n-Hexadecanoic acid, Methyl commate, Octadecanoic acid, n-Hexadecanoic acid, 3-Dodecanol, Diethyl Phthalate, 3-Heptadecanol, Pentacosane, Cyclopropane, Tetrapentacontane etc. The most important compound in the root system of the plant is 2-hydroxy-4-methoxybenzaldehyde, which is responsible for the aromatic properties of the root and is present in the plant cultivated in vitro. The names of the identified compounds with their respective retention time are listed in Table 7.

Discussion

Encapsulation, regrowth, germination, rooting, acclimatization and effect of short-term storage on regeneration efficiency

Synthetic seeds are created by encapsulating somatic embryos or shoot buds in an alginate matrix, enabling storage, transport, and germination without relying on conventional seeds [24]. It is crucial for the preservation of threatened species, making it possible to widely reproduce elite genotypes, and promoting the transfer of germplasm between geographical areas. In our study, nodal segments (NS) of *D. arayalpathra* were used for encapsulation and 3% SA with 100 mM $CaCl_2.2H_2O$ was found to be optimal to obtain normal and well-structured beads and maximum regrowth frequency. A similar concentration was also used in alginate encapsulation of micro-cutting in *Satureja Khuzistanica* [25], NS encapsulation in *Decalepis salicifolia* [26]. Additionally, it was found that the best solution for promoting ion exchange between Na^{+} and Ca^{2+} and creating compact, translucent, isodiametric beads was a 3% solution of SA and 100 mM $CaCl_2$. Lower SA concentrations (1% and 2%), however, produced abnormally shaped and extremely brittle capsules. In contrast, greater concentrations (4% and 5%) produced extremely firm capsules that impeded the conversion of synthetic seeds and prevented the development of propagules. However, 2.5% SA and 100mM $CaCl_2.2H_2O$ or 2.5% SA and 75mM $CaCl_2.2H_2O$ were also reported as optimal combination for ideal beads as seen in *Tinospora cordifolia* [27] and *Celastrus paniculatus* [28].

It was observed that the frequency of regrowth of MS medium was lowest in the absence of PGRs and increased in the presence of PGRs, either alone or in combination. The combination of BA and NAA was found to be optimal for regrowth of the encapsulated matrix in a similar set of results previously described in *H. indicus* and *D. salicifolia* [26, 29]. Furthermore, it was discovered that adding growth additives, such as Ads, to the combination treatment improved organogenesis in *D. arayalpathra* [12]. Better germination outcomes were also observed in *Sachharum officinarum* [30] and *Crinum malabaricum* [31] when auxin and cytokinin were combined. The combination of auxin and cytokinin is essential for promoting germination of encapsulated nodal segments because they together regulate root and shoot development, balance cell division and elongation, prevent callus formation, and help overcome dormancy [32, 33]. This combination creates an ideal environment for the nodal segment to regenerate into a healthy plantlet during synthetic seed germination.

Non-embryonic synseeds, also known as synthetic seeds, are unipolar because they only contain a portion

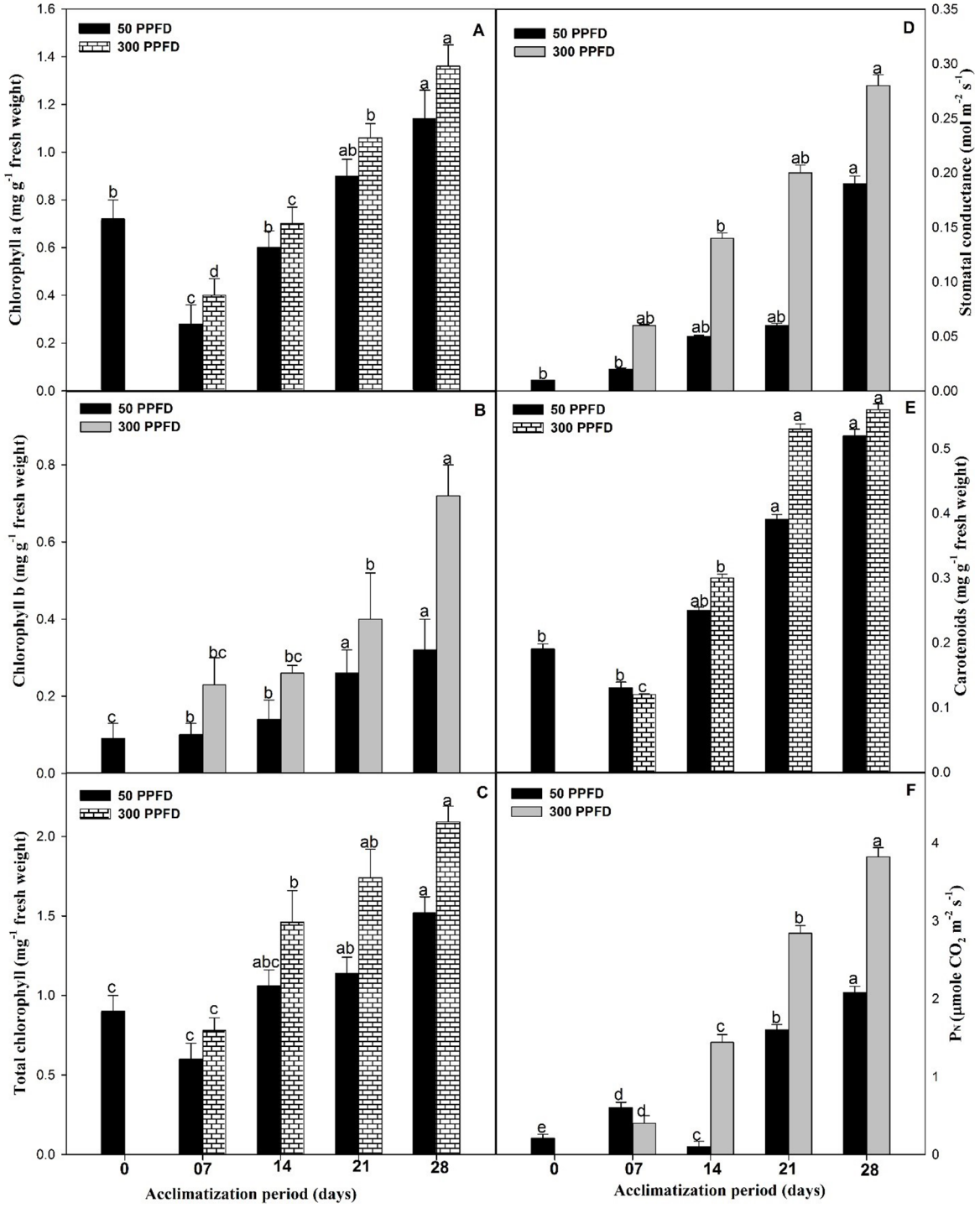


Fig. 2 Changes in chlorophyll content (**A**, **B**, **C**), stomatal conductance (**D**), carotenoids (**E**), Net photosynthetic rate (**F**) in synthetic seed derived plants of *D. arayalpathra* during different stages of acclimatization

of plant tissue, such as a nodal segment, shoot tip, or other vegetative parts [2]. Non-embryonic synthetic seeds, unlike somatic embryos, may have limited regeneration potential; however, they offer a practical and scalable approach for conserving and propagating plant material, particularly for species where somatic embryogenesis is not feasible or well-established [29, 30]. This lack of inherent root development necessitates the use of a separate rooting medium with appropriate auxin concentrations to stimulate root formation. In our study, microshoots rooted well on a semi-solid growth medium enriched with NAA. Similar kind of results were also

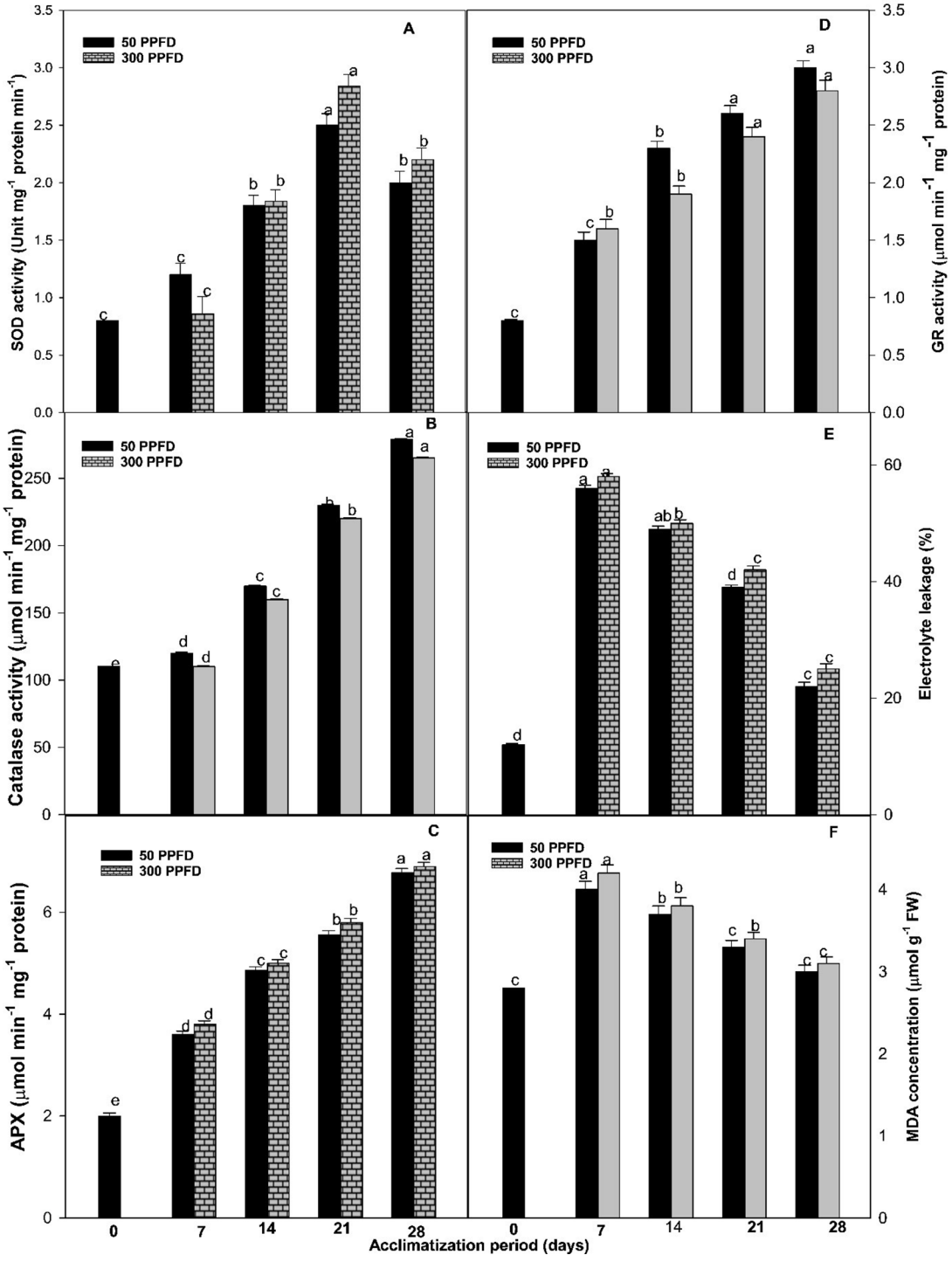


Fig. 3 Changes in SOD (**A**), CAT (**B**), APX (**C**), GR (**F**), Electrolyte leakage (**E**), MDA content (**F**) and in synthetic seed derived plants of *D. arayalpathra* during different stages of acclimatization

observed in *Aloe elegans* [34] and *Sesuvium portulacastrum* [35]. However, it was shown that half strength with IAA was ideal for rooting in microshoots of *Basilicum polystachyon* [36] and *Prunus dulcis* [37]. Auxins promote the differentiation of undifferentiated cells in the explant into root tissues by enhancing the expression of root-specific genes and proteins [38, 39]. This leads to the successful formation of root primordia, which is critical for the establishment of microshoots. They activate root primordia and initiate cell division in the pericycle cells, which leads to root development [40]. The effectiveness of auxins like IBA and NAA in promoting root formation

Table 6 List of RAPD and ISSR primers and their sequences along with scorable bands and size range

Molecular marker	Primer code	Primer sequences (5′-3′)	Amplified band	Size range (bp)
RAPD	OPL-01	5′GGCATGACCT3′	1	200–400
	OPL-04	5′GACTGCACAC3	4	300–700
	OPL-06	5′GAGGGAAGAG3′	2	400–1000
	OPU-05	5′TAGGAGGTGG3′	4	600–1500
	OPU-08	5′TGCGAGAGAA3′	6	500–2000
	OPU-10	5′TGACAGAGTT3′	3	300–500
ISSR	UBC-810	(GA)8T	2	500–1200
	UBC-812	(GA)8 A	6	300–2500
	UBC-830	(AG)8YT	4	1000–3500
	UBC-841	(GAA)8	5	200–1500
	UBC-870	(GGGGT)3T	3	1200–2800

in in vitro microshoots may vary depending on the species or variety, as they play a role in inducing root primordia, regulating cell differentiation, and supporting polar transport mechanisms crucial for root development [38]. For the in vitro propagation approach to be successful, roots acclimatization and field survival of regenerated plants are the most important steps. Soilrite is often considered the best medium for acclimatizing in vitro plants for several reasons: optimal drainage and aeration, nutrient availability, sterile and disease-free environment, and consistent pH. The combination of these factors makes Soilrite an excellent choice for the acclimatization of in vitro microsprouts and plantlets in an ex vitro environment and promotes successful establishment and growth [41, 42].

Encapsulated and non-encapsulated NSs were tested for their ability to regenerate after being stored at 4 °C for 1, 2, 4, 6, or 8 weeks. The primary goal of synseed production is to increase the stability of propogules. At lower temperatures for example 4 °C, metabolic processes within the encapsulated embryos or microshoots are significantly slowed down [2]. This reduces the consumption of stored energy reserves, which can prolong the viability of the synthetic seed and prevent premature germination during storage [3]. Storing synthetic seeds at higher temperatures can lead to cellular damage or stress responses that may affect viability. At 4 °C, enzymatic activities, respiration rates, and oxidative processes are minimized, which helps in preserving the integrity of the cells and tissues within the seed [3]. There are numerous sources that state that 4 °C is the ideal temperature for storage, like in Blackberry [43], *Taraxacum pieninicum* [44], and *Rauvolfia serpentina* [45]. Among the various comparisons, the encapsulated NS showed a higher frequency of regeneration compared to the non-encapsulated NS. However, the regeneration frequency of the non-encapsulated NS was affected by the storage time, and the regeneration frequency started to decrease with increasing storage time. In addition, the encapsulation matrix also has an effect on the regrowth of the encapsulated NS, and the encapsulation matrix prepared with the optimal PGR concentration showed a better effect. Similar results were also observed in *D. salicifolia* [26], *Casuarina equisetifolia* [41] and *T. pieninicum* [44]. The regrowth of synthetic seeds is significantly influenced by both the storage period and the type of encapsulation

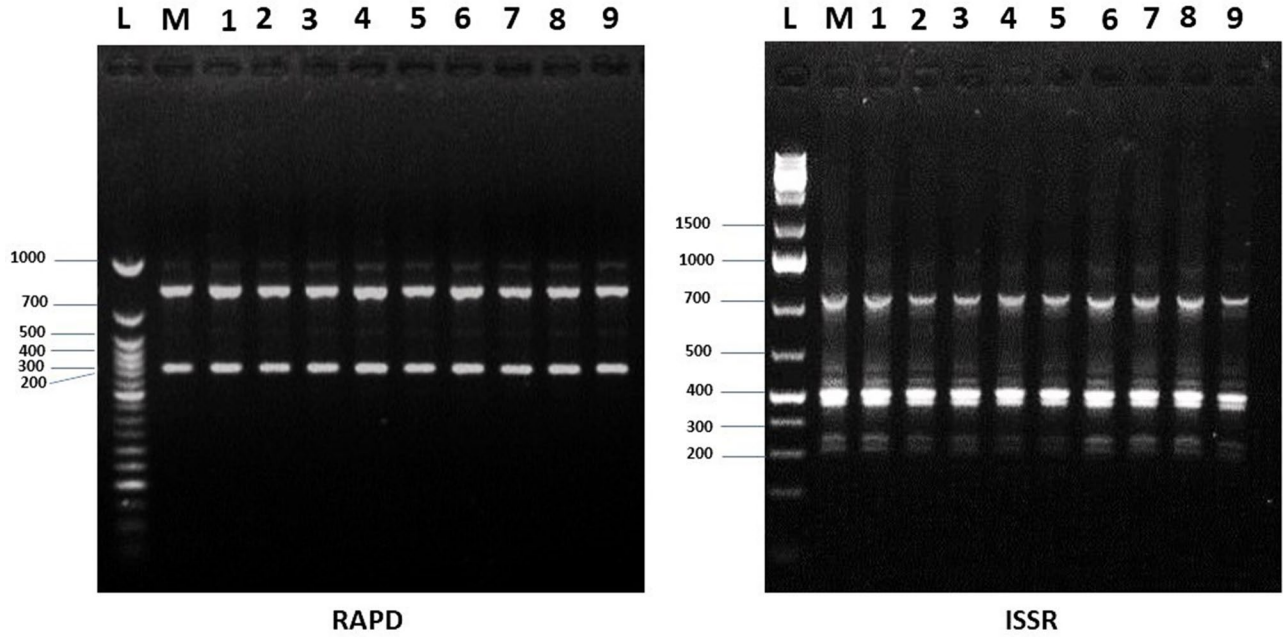


Fig. 4 DNA profiles of the mother (Lane M), micropropagated plants (Lanes 1–9), and DNA ladder (Lane L). (**A**) RAPD (OPU-05). (**B**) ISSR (UBC-812)

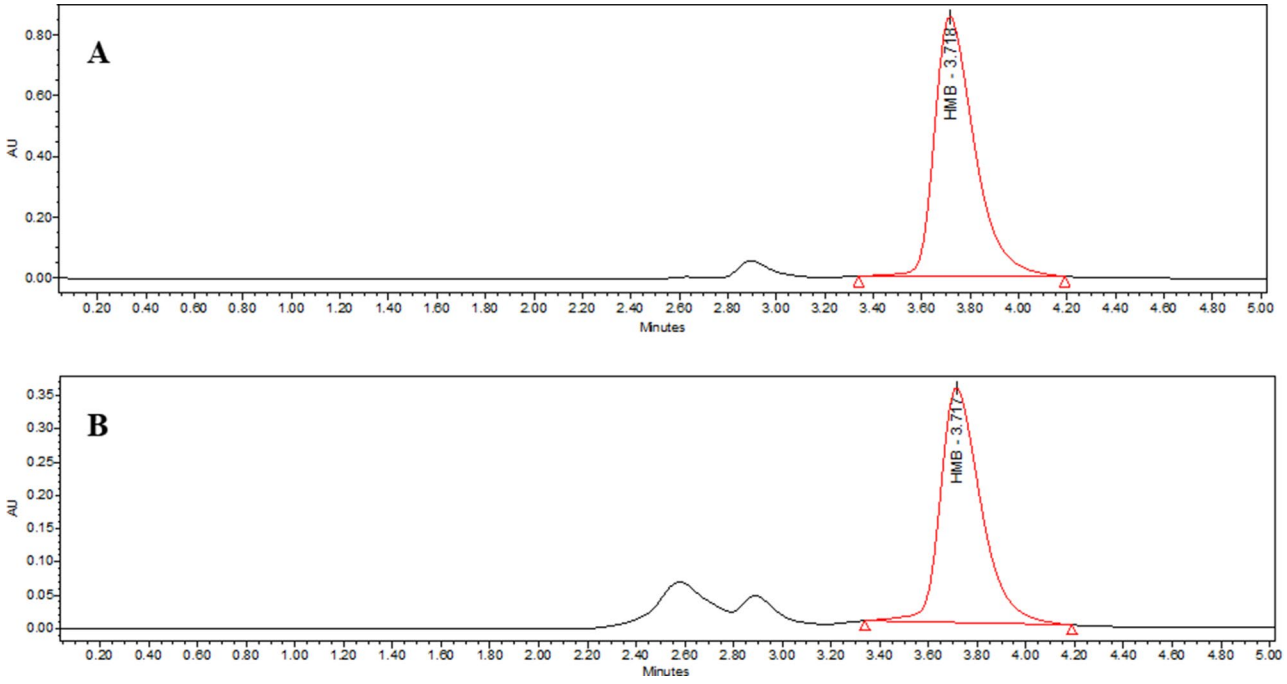


Fig. 5 HPLC chromatograms of the reference chemical, 2-hydroxy-4-methoxy benzaldehyde (2H4MB), at 280 nm (**A**) and the root extract at 280 nm following a 10-week acclimatization period (**B**)

matrix used. These factors affect the viability and ability of synthetic seeds to regenerate into whole plants after storage. Prolonged storage can have a negative impact on regrowth due to various factors such as moisture loss, depletion of internal energy reserves or changes in the encapsulation matrix over time. For example, prolonged storage can lead to dehydration or crystallization of the matrix, which impairs seed viability. The growth rate generally decreases after long-term storage, as the synthetic seed components are less vigorous [3].

Evaluation of physiological and biochemical parameters during acclimatization

Under high light conditions, plants often respond by upregulating enzymes involved in chlorophyll biosynthesis, such as glutamyl-tRNA reductase, which is crucial for the production of 5-aminolevulinic acid (ALA), a precursor of chlorophyll [46]. This increase in chlorophyll levels, particularly chlorophyll a and b, supports enhanced light-harvesting capacity, which allows the plants to capture more light for photosynthesis [47]. Chlorophyll a and b are primarily associated with the light-harvesting complexes (LHC) of Photosystem II (PSII). The increase in these pigments ensures efficient energy capture and transfer within the photosystems. Despite the exposure to high PPFD (photosynthetic photon flux density), which can often lead to photoinhibition, the plantlets managed to maintain their core PSII complex integrity [47]. The results of our investigation showed that both types of light had a significant effect on total chlorophyll, P_N, and chlorophyll a and b, as well as carotenoid levels, which increased with the number of days of acclimatization. Similar kind of finding was also observed in *Rauvolfia tetraphylla* [17] and *Azalea* [48]. However, a decrease in chlorophyll and carotenoids was observed in the first week of acclimatization compared to the first 0 days, which is usually due to a sudden change in environmental conditions. These plants, cultivated under controlled in vitro conditions with high humidity, low light intensity and constant nutrient supply, are exposed to various stress factors such as light and water stress, nutrient availability and ROS accumulation [49–51]. After one week of acclimatization, the increase in chlorophyll and carotenoid content in in vitro-raised plants can be attributed to the gradual adaptation of the plants to the new environmental conditions. As plant adjust several factors such as stomatal function and water regulation, acclimatization to light intensity, activation of defense mechanism and nutrient uptake [50, 52]. In addition, the role of light for example 50 and 300 PPFD plays a significant role in affecting these parameters and possible reason is at lower light intensities plants tend to produce a moderate amount of chlorophyll and carotenoids because light is one of the main drivers of photosynthesis. At higher light intensities, such as 300 PPFD, plants have access to more photons, which can stimulate higher rates of photosynthesis [53, 54]. This higher light availability leads to increased synthesis of chlorophyll and carotenoids to support a higher P_N. Carotenoids, in particular, play a protective role in quenching excess light energy and

Table 7 Metabolic profiling of methanolic root tuber extract of in vitro raised *D. Arayalpathra*

S.no.	*R*.Time	Area	Area%	Name
1	10.993	5,516,804	10.69	BENZALDEHYDE, 2-HYDROXY-4-METHOXY-
2	12.607	69,481	0.13	1,6,10-DODECATRIENE, 7,11-DIMETHYL-3-METHYLE
3	12.714	713,569	1.38	GUANOSINE
4	12.987	249,882	0.48	Cycloheptasiloxane, tetradecamethyl-
5	14.404	216,215	0.42	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER
6	14.861	398,704	0.77	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXY
7	14.998	746,932	1.45	.BETA.-D-GLUCOPYRANOSE, 1,6-ANHYDRO-
8	15.775	3,625,358	7.03	MOME INOSITOL
9	16.253	381,255	0.74	.alpha.-D-Galactopyranoside, methyl
10	17.012	523,858	1.02	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[
11	17.465	118,977	0.23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	18.264	2,219,114	4.30	HEXADECANOIC ACID
13	18.609	2,141,727	4.15	Trehalose
14	19.435	288,653	0.56	HEXADECANOIC ACID
15	19.572	384,005	0.74	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE #
16	19.976	4,111,542	7.97	cis-Vaccenic acid
17	23.825	82,810	0.16	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, [1R-(1.alpha.,3.b
18	24.626	731,217	1.42	Kaur-16-en-18-oic acid, 13-hydroxy-, methyl ester, (4.alpha.
19	25.337	251,905	0.49	1,2-BENZENEDICARBOXYLIC ACID
20	28.133	214,295	0.42	d-Glucosamine
21	28.518	268,952	0.52	Squalene
22	31.542	182,749	0.35	Stigmasta-7,22-dien-3-ol, acetate, (3.beta.,5.alpha.,22E)-
23	31.784	123,406	0.24	Vitamin E
25	33.774	368,543	0.71	Stigmasterol
26	34.711	1,723,856	3.34	STIGMAST-5-EN-3-OL, (3.BETA.)-
27	35.477	199,905	0.39	.alpha.-Amyrin

protecting the chloroplasts from photooxidative damage [55]. The gradual increase in stomatal gas exchange during acclimatization without an initial decrease can be attributed to the plant's adaptation to external environmental conditions such as light intensity, humidity and CO_2 availability. During the acclimatization phase, plants grown in vitro under controlled conditions with typically high humidity must first adapt to the lower humidity under ex vitro conditions, leading to a gradual improvement in stomatal function. Under high light conditions, the CO_2 demand increases, leading to a greater opening of the stomata for improved gas exchange. The higher photon flux stimulates photosynthesis, which requires increased CO_2 uptake and leads to a greater increase in stomatal conductance during the acclimatization phase. A similar observation was also made in tomato plants and other species, which showed higher stomatal conductance and gas exchange rates when exposed to higher PPFD, resulting in better carbon assimilation and acclimatization of the chloroplasts [56, 57].

During the acclimatization of in vitro-derived plants, reactive oxygen species (ROS) such as superoxide (O_2•−), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) are produced due to environmental stress factors like fluctuating light intensity, temperature, and oxygen levels [58, 59]. Plants use both enzymatic and non-enzymatic antioxidant systems to scavenge ROS and protect cells from oxidative damage. The non-enzymatic components (e.g., ascorbate, glutathione, flavonoids, carotenoids, tocopherols) and enzymatic antioxidants (e.g., SOD, CAT, APX, GR) play crucial roles in this defense mechanism [58, 60]. In addition, EL is a marker of membrane integrity and stress and high ROS levels can cause lipid peroxidation, leading to membrane damage. Similarly, MDA content is an indicator of lipid peroxidation and oxidative stress, commonly used measure the extent of membrane damage caused by ROS [60, 61]. In our study, a gradual increase in SOD, CAT, GR and APX was observed with increasing duration of acclimatization, while SOD decreased after 21 days of acclimatization. On the other hand, a sharp increase in EL and MDA content was observed in the first week of acclimatization, followed by a decrease in the content with increasing duration of acclimatization. The reason for the high EL and MDA levels is the high ROS production during the adaptation of the plants to new environmental conditions, especially at high light intensity. The sudden increase in photosynthetic activity can initially cause oxidative stress to the plants, resulting in increased MDA levels due to lipid peroxidation of the membrane. At this stage, the antioxidant enzyme systems such as SOD, CAT and APX may not yet be fully upregulated to counteract the ROS increase, leading to an accumulation of lipid peroxidation products such as MDA [62]. The high light (300 PPFD) tends to induce a higher initial MDA increase due to greater ROS production, while lower light (50 PPFD) results in a more moderate change. However, as plants acclimatize and their antioxidant systems become more efficient, MDA levels will gradually decrease as ROS are neutralized and membrane damage is minimized. Similar changes in these enzymes and stress indicators can also be observed in other plants [48, 63–65].

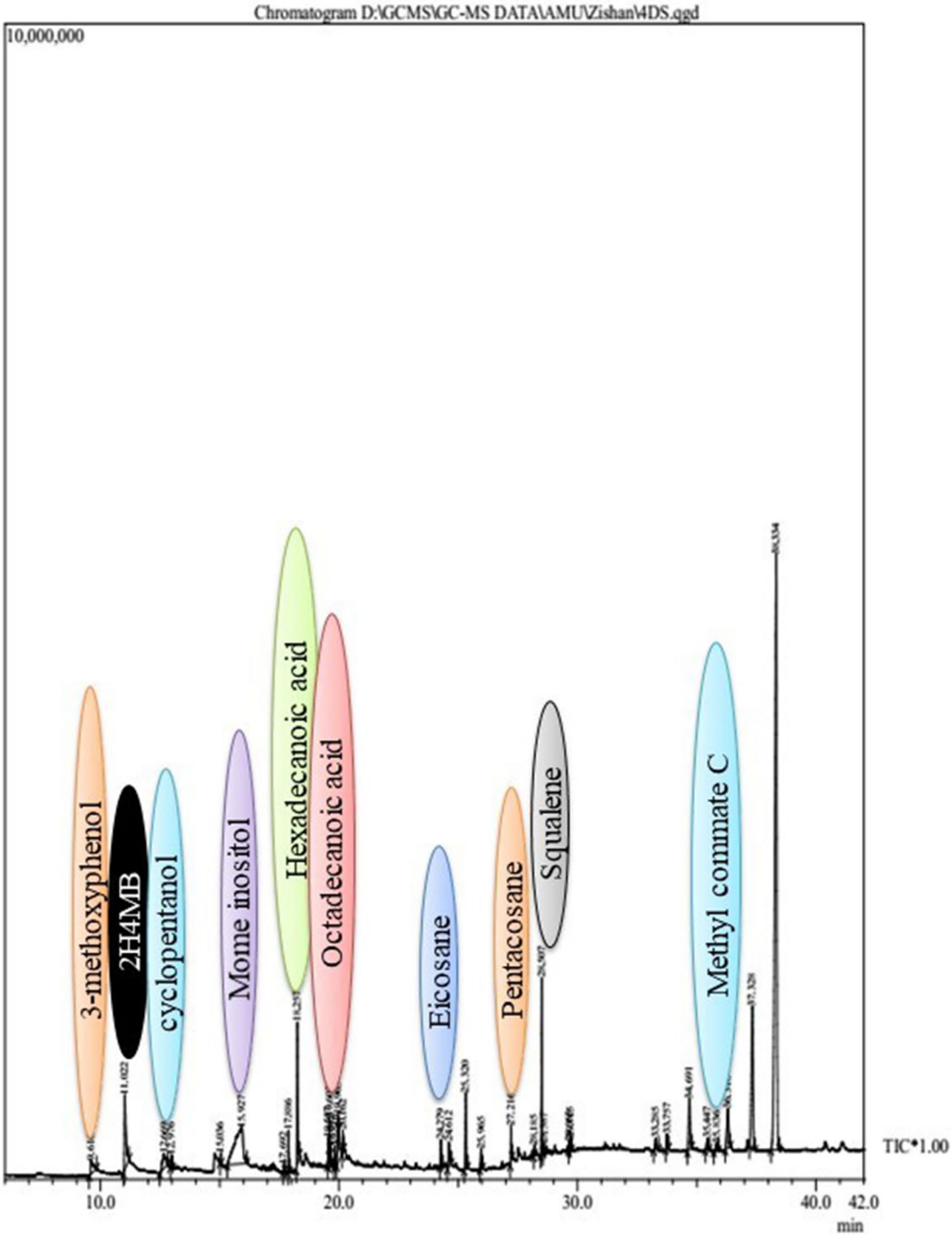


Fig. 6 GC-MS chromatogram for methanolic root tuber extract of in vitro derives *D. arayalpathra*

Genetic fidelity

RAPD and ISSR markers are commonly used for genetic fidelity testing of in vitro derived plants due to several key advantages such as polymorphic detection, simplicity and speed, no prior sequence information required, cost effectiveness, applicability to different species and high sensitivity. Both the marker widely utilized for the genetic fidelity testing in plants [66–69]. Maintaining genetic fidelity between the mother plant and the plants grown in vitro is crucial for several reasons, especially in agriculture, horticulture, conservation and research. In our study, a monomorphic banding pattern was observed in the in vitro grown plants compared to the mother plant D. arayalpathra, and no polymorphism was detected. The

results of this investigation support the idea that direct regenerated plants retain their genetic a similarity to their mother plants throughout the in vitro procedure. The RAPD and ISSR marker have been recently employed in *Piper longum* [69], *Camelina sativa* [70], *Cymbidium aloifolium* [71] and *Strychnos potatorum* [68].

2H4MB quantification and metabolic profiling of the roots

The evaluation and quantification of 2H4MB in in vitro-derived roots of *D. arayapathra* are important for several key reasons. 2H4MB is a major bioactive compound found in the roots of *D. arayalpathra* and responsible for many plants' medicinal properties as mentioned in introduction. 2H4MB can serve as a marker compound to standardize the quality of in vitro-derived *D. arayapathra* roots, ensuring that they meet the therapeutic and pharmacological standards of naturally sourced roots. *Decalepis arayapathra* is an endangered plant species, and its overharvesting from the wild has raised concerns about conservation. In vitro propagation offers a sustainable alternative for the production of bioactive substances without depleting natural populations. The quantification of 2H4MB in tissue culture-derived roots ensures that these plants can be used as a viable substitute for wild harvested roots in pharmaceuticals, thus supporting conservation efforts. In our study, we used the HPLC method for the analysis as it is very sensitive and accurate. The RT values for the plant extract obtained in vitro and the mother of *D. arayalpathra* show approximately similar values, indicating the persistence of 2H4MB in the extract. Similar kind of results was also observed in *D. salicifolia* [23] and *H. indicus* [29].

Comprehensive metabolic profiling is made possible by the excellent sensitivity and specificity of GC-MS for the detection of volatile chemicals. It is perfect for metabolite identification as it is able to separate complicated combinations, which increases accuracy. It also provides structural details that are essential for understanding metabolic pathways. GC-MS is particularly helpful for screening metabolites of in vitro derived plants of *D. arayalpathra* by providing a detailed analysis of volatile and semi-volatile compounds. It allows for the identification and quantification of key metabolites, which can reveal potential bioactive compounds and their variations in different growth conditions. This information is crucial for understanding the biochemical profile of the plant and can guide further research in the fields of pharmacology, ecology and biotechnology. Moreover, it aids in optimizing cultivation methods to enhance desired metabolites. GCMS has been widely utilized for the screening of several medicinal plants such as in *Aporosa cardiosperma* [72], *Gaillardia pulchella* [73] and *Boehmeria nivea* [74].

Conclusion

The development of a synthetic seed protocol for *Decalepis arayapathra* marks a significant step in conserving and propagating this endangered medicinal plant. By optimizing sodium alginate encapsulation and Murashige and Skoog medium conditions, regrowth, shoot and root development, and acclimatization were successfully enhanced. Genetic fidelity was maintained, and consistent production of the bioactive compound 2H4MB was ensured. Metabolic profiling through GC-MS enabled precise identification and quantification of metabolites in root samples, offering insights into metabolic pathways and plant responses to stress. This knowledge is critical for drug development and improving the efficacy of natural products, addressing challenges like drug resistance. The approach contributes to alleviating pressure on wild populations and supports biodiversity conservation. Future research should focus on scaling up synthetic seed technology for commercial propagation, optimizing growth conditions for higher bioactive compound yield, and exploring cryopreservation for long-term storage. Further studies on the ecological interactions of *D. arayapathra* will aid in successful reintroduction into natural habitats. Additionally, investigating the pharmacological properties of 2H4MB will help unlock the full medicinal potential of this plant.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06174-w.

Supplementary Material 1

Acknowledgements

The graphical abstract has been created using https://www.biorender.com/. We express our gratitude to Prof. M. Badruzzaman Siddiqui, a taxonomist from the Department of Botany at Aligarh Muslim University, for identifying the plant material.

Author contributions

ZA, AS: Conceptualization and experiment design. ZA, VY, MR: Writing– original draft, Visualization. ZA, OB, SV, VY, and MR: Writing– review & editing and data analysis; OB and SV: Funding acquisition.

Funding

The research methodology was developed with the financial support of the Russian Science Foundation (Agreement No. 22-16-00044).

Data availability

The datasets generated and/or analyzed during the current study do not include any protein sequences, DNA/RNA sequences, or other data types that require deposition in public repositories. Our study is focused on tissue culture experimentation, and all relevant experimental protocols and results are included in the manuscript. The authors confirm that all experimental research on Decalepis arayalpathra, including the collection of plant material, was conducted in accordance with the guidelines set by the University department.

Declarations

Ethics approval and consent to participate
We declare that the manuscript reporting studies do not involve any human participants, human data, or human tissue, and thus, it is not applicable.

Consent for publication
Not applicable.

Clinical trial number
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 12 October 2024 / Accepted: 29 January 2025
Published online: 13 February 2025

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