In vitro strategies for conservation of Wild Banana Germplasm: A Multifaceted Resource of Non Woody Forest Products

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Research Article

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Abstract

Ensete superbum (Roxb.) Cheesman (Wild banana; Musaceae) is an endemic plant species from Western Ghats. It has been used extensively in ayurvedic medicinal practices for relieving various stomach, kidney related disorders and on leucorrhoea. The overexploitation of wild banana, together with low natural regeneration, has resulted in a decrease in natural stands. There is an urgent need for the conservation of this through *in vitro* propagation protocol of *E. superbum* using zygotic embryo culture. Excise embryos were culture on MS growth medium with different concentration of growth regulators individual and in-combinations. Among the tested treatments embryos cultured on MS supplemented with 0.75 mg/l BA + 200 mg/l PVP showed most favourable response up to 95.77% germination within two to three weeks which grows up to 16.9 cm in length. The 20–25 days old hydroponically grown plants saplings were transferred on growth substratum 50% soil: 25% compost: 25% sand. *In-vitro* germinated seedlings were acclimatized in a hydroponic culture medium improve survival rate. These plants were transferred to the field and were found to be phenotypically normal, healthy and similar to donor plants. This is the first report for mass scale production of healthy plantlets through *in vitro* zygotic embryo culture and standardized nursery techniques. The study utilized hydroponic techniques in *in vitro* cultivated plantlets for initial development, with early acclimatization and hardening being observed. This technique could be an alternative solution for restocking and pave the way for rapid restoration of nearly threatened medicinally important *E. superbum*.

Key message

This is the first report on reliable and reproducible embryo rescue technique with intervention of hydroponics to early recovery of 100% plantlets. This protocol would be effective for satisfying the year-round demand of food, fodder, fibre and medicine.

Introduction

Western Ghats has significant status in terms of unique climatic conditions and harbours many endangered plant species (Sreekumar et al., 2020). Tribal communities in this area utilized local plant resources for food, feed and medicinal purposes. Overpopulation, fast rate urbanization and significantly increased tourism are directly effecting on native plant species (Patwardhan et al., 2021; Sundararajan et al., 2025). Wild banana (genus *Ensete*) is an indigenous plant species and a most promising seasonal source of food, fodder, fibre and medicine (Bezuneh and Feleke 1966; Mali and Bhadane 2008; Arinathan et al. 2007; Mohan et al., 2010; Kar and Bortharkur 2013; Kumar et al. 2013; Ponni and Nair 2019a). The plants are morphologically similar to bananas and are hence known as false or wild banana. The genus *Ensete* has seven species: *E. Superbum, E. glaucum, E. ventricosum, E. gilletti, E. homblei, E. perrieri* and *E. lecongkietii* (Simond., 1960,1962; Väre and Häkkinen 2011; Joe et al., 2016; Borrell et al. 2019).

Among these species, *Ensete superbum* (Roxb.) Cheesman (Wild banana; Musaceae) is an endemic plant species from Western Ghats. The whole plant has been extensively employed in traditional

medicinal practices for the treatment of various ailments, primarily to alleviate common health disorders such as diabetes, kidney stones, leucorrhoea. It contains active compounds mainly β-carboline, a non-steroidal phytosterol-4-hydroxyl-3-methyl-hex-5-enyl and used as a food additive. This multipurpose plant species is directly utilized as food, fodder and medicines (Kachroo et al., 2009; Kumar et al., 2010, 2013; Vasudharan et al., 2013; Sethiya et al., 2016). Tribal communities in the region traditionally consume the immature inflorescences, fruits, stems, and underground parts as components of their daily diet. Every part of the *Ensete* plant can be used to prepare items of high value and almost all can be transformed into value-added, commercial products. While the leaves are widely used as food plates/packaging of food, the shoots or entire plants are used for aesthetic ornamental purposes. Leaves and the pseudo stem are also used for animal feed (Patil and Bhaskar. 2006; Yashodharan and Sujana 2007; Khyade et al., 2009; Padal et al., 2010; Prabha et al., 2010; Bhise et al. 2015; Vasundharan et al., 2015; Kauthale et al., 2017; Borrell et al. 2019; Yemata 2020). Fruits are used as food, feed and also for ethno-medicine. These features indicate that *Ensete* provides a good genetic pool to be exploited for breeding and improving cultivars (Bhosale et al., 2009; Jadhav et al., 2015; Joga et al. 2021). However, the overexploitation of wild banana species, coupled with their limited natural regeneration capacity, has led to a marked decline in natural populations. Consequently, these species have been classified as nearly threatened in the Indian Red Data List. (IUCN., 2011; 2018; Naikawadi et al., 2022). Looking into the status of endangered, rare and threatened species particularly with reference to economic plants is important and wild bananas can be considered a potential crop to meet the challenges of climate resilience and sustainability (Raza et al., 2019; Matheka et al. 2019; Ponni and Nair 2019a, 2019b). Planting of *Ensete* will help improve the fragile marginal land ecosystem and socio- economic situation of ethnic communities (Jagtap et al., 2008; Bhandary and Chandrashekhar. 2014).

This species exhibits phenotypic similarity to cultivated bananas and its leaves are widely utilized as natural plates or for food packaging, while the shoots and entire plants are often employed for ornamental purposes. These diverse applications have contributed to large-scale, unregulated harvesting (Kumar et al., 2011; Bhise et al., 2015). On the other hand, seed germination remains poor (approximately 33%) and is further constrained by the lack of favourable environmental conditions, collectively impairing population sustainability (Pradheep et al., 2021; Ponni et al., 2019a). As a result, this endemic species of the Western Ghats has been categorized as rare and threatened. Notably, wild bananas possess remarkable drought tolerance, enabling survival under severe water stress (Naikawadi et al., 2022; Thingnam et al., 2023). To mitigate these challenges, both *in vivo* and *in vitro* mass propagation strategies, coupled with targeted plantation in selected localities, offer promising approaches for conservation and population recovery (Ponni et al., 2019b; Rohini 2020; Ahilawat et al., 2024; Manohar et al., 2024).

Within forest ecosystems, animals feed on a wide variety of plant species, among which wild banana represents a particularly important resource. In fact, wild banana serves as a major source of fodder for large mammals such as elephants. Several explanations have been proposed for elephant forays into new areas, including local overabundance of populations, habitat loss, scarcity of food and water resources and natural exploratory instincts. These factors often result in elephants invading agricultural

fields, causing damage to crops, property and occasionally human life, thereby intensifying human–wildlife conflict. In this context, the application of plant tissue culture techniques and biotechnological approaches to *E. superbum* is urgently needed. To date, few reports exist on the tissue culture studies of *E. superbum* (Mathew et al., 1996; 2000; 2003; Kulkarni et al., 2002; Ponni et al., 2019a, b), and none have demonstrated reliable successful establishment of germplasm or their subsequent elite clones plantation for conservation purposes.

Despite its considerable potential, wild banana from Western Ghats region remains at high risk due to habitat loss, intensive anthropogenic activities and overexploitation. Conventional propagation methods are time-consuming and challenging, largely because of low seed viability and prolonged dormancy. Substantial research efforts are therefore required to optimize *in vitro* embryo germination for the rapid production of plantlets, which is essential for biodiversity conservation and for realizing the bio-prospecting value of wild banana. Although *in vitro* propagation through organogenesis has been reported in certain medicinal species the process is typically lengthy, cost-intensive, and associated with high mortality rates of plantlets during acclimatization and field transfer. Zygotic embryo culture plays an important role in *in vitro* propagation of endangered forest species to overcome physical biotic interference (Raghavan, 2003; Rambabu et al., 2006; Ghorpade et al., 2010). This study first time report on the viability of embryos, establishes a protocol for zygotic embryo culture and mass scale plantlets development, incorporating hydroponic interventions for the year-round production and conservation of elite germ-plasm in *E. superbum.*

Material and methods

Source of plant material, authentication, source seeds and seed viability

Plant material collected from Chandmal Tarachand Bora College Botanical Garden Shirur (Lat: 18.8208; Long: 74.3738), Pune, Maharashtra, an adjoining region of Western Ghats, India. The healthy plant material mainly fruits of *E. superbum* were randomly selected. The fruits were separated from inflorescence and dried at room temperature (30–35 ^{0}C) for 5 days. The dried pulps were separated and seed were removed. Washed these seeds under tap water for five times. The seeds lot were separated according to its floating characteristics (non-viable seeds). To ensure that the seeds used for the experiment were viable and of high quality, the sample lots were subjected to viability test using the tetrazolium technique. Three replicates (20 seeds each replicate) were subjected to 2, 3, 5, triphenyl tetrazolium chloride (TTC) test 90, 120, 150, 180, 210 and 240 days of storage at root temperature (4°C). In this method, seeds were longitudinally sectioned and the sections were immersed in a 0.5% aqueous solution of TTC (pH 6.5) for 24 hrs at room temperature (25°C) under controlled dark conditions. The TTC solution was drained and sections were rinsed 3 times with tap water. The topographical staining pattern of the embryos and cotyledons were studied under a dissection microscope.

Surface sterilization of seed and aseptic seedling development

About 150 seeds of *E. superbum* were placed in a 500 ml sterilized distilled water for the 5 min. These seeds were sterilized with 0.1% freshly prepared sodium hypo chlorite solution for 7 min. These surface sterilized seeds were washed 10 times with distilled water. The surface sterilized dissected embryo from sterilised seeds were transferred aseptically for germination on (1%) sucrose - agar medium in culture tubes and kept in dark for 48 hours.

Zygotic embryo culture and exudation control

Zygotic embryos were excised and cultured on MS (Murashige and Skoog 1962) medium individual application of cytokinins (BA, Kin and TDZ 0.1 to 2.0 mg/l) and auxins (IAA, IBA, NAA and 2, 4- D 0.1 to 2.0 mg/l) and PVP (50, 100, 150, 200, 250 to 300 mg/l). All the operations were performed in a laminar air flow chamber. All the cultures were maintained under 8 hours light (2750 lux) and 16 hours dark period at 25 ± 2 ºC and relative humidity 50 to 60%. As per the requirement the cultures were maintained by sub culturing on fresh medium at interval 28 days.

From these preliminary experiments, the nutrient media consisting cytokinins and auxins were selected for maintenance of embryo culture. The selection was made on the basis of embryo germination as well as induction of callus mediated shoots. Sub-cultured shoots growth and development of plantlets was judge visually. Small shoots were subculture to fresh parental medium over a period of 18 months. Subculturing was done by transferring approximately one to two shoots at every four weeks intervals. The test tubes and culture bottles consisting shoot culture were incubated at 25 ± 2°C temperature, 8 hours light/16 hours dark photoperiod from cool white fluorescent tube lights with light intensity of 30 µmol m^{-2} s^{-1}, and about 70% humidity.

Elongation and rooting of shoots

Individual shoots were subculture on MS medium supplemented with 0.75 mg/l BAP with 3% sucrose with 20 days of interval. Elongated shoots of 3–4 cm in length were excised after 28 days of culture and transferred individually to full and half strength MS medium containing IAA (0.75 mg/l). The pH of the rooting media was adjusted to 5.8 and 0.25% w/v cleri gel was added before autoclaving at 120 ^{o} C and 104 kPa for 20 min. The cultures were incubated at 25 ± 2° C and about 80% relative humidity under an 8 h photo-period with light intensity of 30 µmol m^{-2} s^{-1} photon flux density provided by cool-white fluorescent tube. Each rooting treatment consisted of minimum of 20 replicates. At an interval of 28 days the data for frequency of root development and number of roots per shoot were recorded.

Acclimatization of plantlets

The *in vitro* rooted plantlets were removed from the culture tubes and washed thoroughly with water to make it free from agar and nutrients. *in vitro* rooted plantlets were transfer on hydroponic condition. Non-absorbent cotton was used as a seal for each rooted plantlet. This 500 ml plastic bottles were placed under shade condition upto 15 to 20 days, later rooted shoots were transfer on nursery bags (8×4 cm) with 50% soil: 25% compost: 25% sand mixture were used. The plants were irrigated with about 50 to 100 ml of water twice in a day for the first two weeks. For initial two weeks, the plantlets were placed in the shade with low light intensity (near 40 $\mu molm^{-2} s^{-1}$), high humidity 90 ± 5% and temperature 26 ± 4°C. After eighteen days the plants were exposed to higher light intensity (near 70 $\mu molm^{-2} s^{-1}$), lower humidity (70 ± 5%) for a week. Later, the plants were exposed to natural condition with maximum light intensity (maximum about 340 $\mu molm^{-2} s^{-1}$).

Maintenance of germplasm

Well acclimatised hydroponically grown two to three feet tall with four to five leaflets containing well developed plantlets was established at Chandmal Tarachand Bora College Botanical Garden Shirur (Lat: 18.8208; Long: 74.3738), Pune, Maharashtra. This site has come under a dry tropical climate. In next season, fruits were harvested for further experiments.

Statistical Analysis

The experiments were set up in a completely randomized design. Experiments were with minimum of 15 replicates per treatments and conducted at least thrice. Observations on the number of embryos responding were recorded at the intervals of seven days and wherever necessary, daily observations were also recorded. Data were analysed by analysis of variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Duncan's (1995) Multiple Range Test (DMRT) by using SPSS 16 software at the 5% probability level. Variability in data has been expressed otherwise as mean ± SE.

Results

3.1 Source of plant material

In natural habitat *E. superbum* plants are found at high elevation steep slope (Fig. 1A). Single plant produced single spadix in the month of September to October. It is showing monocarpic condition. This spadix, spirally arrange finger like banana fruits. In single circular rows approximately 14 to 16 banana fruits with diameter and length up to 2-4cm and 9-11cm respectively. Under whole spadix inflorescence contain 110 to 130 fruits, immature fruit contain white seeds show prominent embryo and transparent endosperm (Fig. 2). Single fruit contain approximately 60 to 220 black seed with diameter of 6.5-7.5mm at maturity (April to May) (Fig. 1F). In Fully matured seeds, the embryo is surrounded by powdery white endosperm.

Embryo extraction, seed viability and embryo culture

The mature seeds were first cut precisely into half and the embryo is visible at micropilar end. Embryo is T shaped or sometimes fully matured seed contain button mushroom liked structure. (TTC) 2,3,5-triphenyl tetrazolium chloride solution testing on freshly harvested seeds revealed a percentage viability of up to 95%, which continues to drop as the storage duration increases to 93.73, 84.11, 72.26, 59.57, 36.37, and 13.68% after 90, 120, 150, 180, 210, and 240 days (Table. 1). After surface sterilization embryo was isolated using sterile needle in laminar air flow, the isolated embryos were cultured on Murashige and Skoog (MS) medium. Only the embryos enlargement with late radicle appearance and the medium's blackening due to phenolic exudation are visible in full strength MS medium devoid of growth regulators and antioxidants (PVP). Progressive darkening of the culture medium occurred due to the release of phenolic compounds from the explants. Mild phenolic exudation appeared during the second to third week of culture, which subsequently intensified, resulting in complete blackening of the medium by the third week. Growth medium without anti-oxidant PVP leads to the death culture was observed. Hence, all further experiments PVP were used. Single leaf was expanded up to three to five cm. Embryo germination was initiated approximately 20–25 days after inoculation. By the end of the fourth week, the radicle elongated and penetrated into the culture medium, indicating successful establishment of the seedlings. Comparatively, faster and more efficient germination responses were recorded in MS medium supplemented with different concentrations of plant growth regulators applied either individually or in combination, suggesting a positive influence of hormonal supplementation on embryo development and improved germination efficiency. There are no any sign of multiple shoots or embryoids from inoculated explants. Swelling of embryo and induction of adventitious shoots were not seen on tested mediums. Single leaf was expanded up to three to five cm. within 21 days of incubation. Percentage of *In vitro* embryo germination were decline by application of agar with high concentration of sucrose (4–5%) as well as PVP concentration (250 to 300 mg/l).

Effect of cytokinins on embryo germination

In further experiment, embryos were cultured on various concentration of MS supplemented with various concentrations of 6-benzyladenine (BA), kinetin (Kin) and Thidiazuron (TDZ) individually and 200 mg PVP. Significant variation in embryo response in terms of shoot growth, and induction of roots and its development was observed depending on the type and concentration of cytokinins applied. Among the tested treatments embryos cultured on MS supplemented with 0.75 mg/l BA + 200 mg/l PVP showed most favourable response up to 95.77% germination with two to three weeks which grows upto 16.9 cm in length (Table. 2). This treatment resulted in the highest percentage of embryo germination, maximum shoot elongation, increased number of roots, and greater root length compared to all other treatments. The embryos cultured under this condition showed healthy differentiation and normal seedling development. Lower concentrations of BA (0.1–0.5 mg/l) supported moderate embryo germination and growth; however, the developmental response was comparatively reduced. Increasing BA concentration beyond the optimum level (1.0–2.0 mg/l) adversely affected morphogenesis. At higher concentrations,

cultures exhibited abnormal growth patterns, reduced differentiation, and poor organ development, indicating inhibitory effects of excess cytokinin.

A similar trend was observed with kinetin supplementation. Lower concentrations of kinetin promoted moderate germination and seedling formation, showing lower responses as compare to BA treatments. The best response among kinetin treatments was recorded at 0.5 mg/l concentration which is 88.27% embryo germination, shoot length upto 14.5cm however; it remained inferior to the optimal BA treatment (Table.2). Slightly higher kinetin levels induced tissue swelling and formation of a white, friable, snow-like callus within three weeks of culture initiation. The induced callus, when maintained on the same kinetin-containing media, failed to develop embryogenic structures or organized plantlets, suggesting its non-embryogenic nature.

The application of TDZ resulted in the lowest overall morphogenic response among the cytokinins evaluated. Embryo development under TDZ treatment exhibited a characteristic declined-shaped response pattern declining from lower to higher concentrations. The optimum response was recorded at 0.1 mg/l TDZ, where cultures showed moderate embryo germination (Table.2). Slightly higher TDZ concentrations (0.25–0.75 mg/l) significantly reduced morphogenic performance, with 0.1 mg/l producing the weakest response. Likewise, increasing TDZ concentration to 2.0 mg/l markedly inhibited embryo differentiation and subsequent seedling growth. Although both TDZ and kinetin supported embryo development to some extent, benzyladenine (BA) was distinctly superior in promoting efficient embryo germination and normal seedling formation in *E. superbum*. Higher cytokinin levels, irrespective of type, consistently suppressed organized morphogenesis, frequently resulting in reduced elongation and abnormal or stunted growth.

Effect of auxins on embryo germination

The response of *E. superbum* embryos cultured on MS medium supplemented with different auxins (IAA, IBA, NAA and 2, 4-D) varied significantly with respect to germination percentage, shoot growth, and root development (Table.3). Embryos cultured on hormone-free MS medium produced only one to two roots after approximately four weeks of culture and root elongation remained slow, reaching only 2–3 cm in length. Addition of auxins markedly improved rooting response and overall seedling development.

Among all treatments, indole-3-acetic acid (IAA) proved to be the most effective auxin. MS medium supplemented with 0.5 mg/l IAA recorded the highest embryo response (97.73%), maximum shoot height (15.82 cm), greatest number of roots (19.43), and longest root length (20.41 cm). Early root initiation was observed within the second week of culture at lower IAA concentrations (0.25 mg/l), producing three to four hairy roots with secondary branching without any callus formation. Moderate concentrations of IAA (0.5–1.0 mg/l) supported healthy seedling development; however, further increase to 2.0 mg/l reduced germination efficiency and promoted abnormal growth.

Indole-3-butyric acid (IBA) also enhanced embryo germination and rooting, although the response was comparatively lower than IAA. The optimal response under IBA treatment was observed at 0.75 mg/l, which produced high germination frequency (96.4%) along with increased shoot and root growth. Higher concentrations resulted in reduced growth performance and initiation of callusing. In contrast, α-naphthalene acetic acid (NAA) showed comparatively weaker effects on embryo conversion into complete seedlings. Response is only by means of swelling of explants and pseudo organogenesis was observed. Although very less germination occurred at intermediate concentrations, increasing NAA levels caused a decline in seedling development. Embryos frequently showed partial germination characterized by expansion of a single leaf blade above the medium, while complete plantlet formation was inhibited. Higher concentrations induced whitish, snowy callus and watery callus formation showed suppressed root primordial development.

The synthetic auxin 2, 4-D primarily promoted callus induction rather than organized growth. Lower concentrations produced limited germination and reduced shoot and root elongation, whereas higher concentrations resulted in profuse whitish, snowy lethal callus formation within two weeks of culture. No visible root initials were observed on the callus surface, indicating its non-organogenic nature Table.3.

Combined effect of cytokinins and auxins on embryo germination

When embryos were cultured on MS medium containing combinations of cytokinins and auxins, enhanced morphogenic responses were observed. Efficient embryo germination along with callus-mediated shoot formation and development of two to three shoots per explant occurred under specific hormonal combinations. Some treatments induced callus-mediated single shoot regeneration; however, continued maintenance of callus on the parental medium failed to produce embryogenic structures. After four weeks of incubation, slightly yellowish-green calli with limited morphogenic potential were observed. Repeated sub culturing on the same medium did not promote differentiation, and the calli gradually became necrotic by the fourth week. Overall germination frequency reached nearly 94.43%, with approximately 17 cm shoot length and with 22.57 roots per embryos with root length upto 19.29 cm on combination of 0.75 mg/l BA + 0.50 mg/l IAA. Followed by this 0.50 mg/l Kin + 0.75 mg/l IBA achieves 87.8% embryo germination surprisingly the shoot length, No of roots was more on 0.50 mg/l Kin + 0.75 mg/l NAA which is up to 15.63cm. The combination of auxins and cytokinins could lead to give healthy plantlets as compare to individual treatments (Table.4).

The types and concentration of growth medium and plant growth regulator significantly affected the frequency of callus formation. In order to determine suitable condition for embryonic callus induction the calli were cultured on a half strength MS medium containing a range of BAP combine with 2, 4, D. Prevention of blackening of zygotic embryo at developing embryonic calli was achieved in presence of PVP. In order to induced high rate of calli induction and proliferation full strength MS medium supplemented with high concentration of BAP was useful. The calli appear to be friable watery and turn

brown to blackish at the end of fourth week of initiation of culture. To form calli were classify into two types embryonic nodular compact calli and non-embryonic spongy loose calli. Applying 200 mg/l PVP is beneficial for reducing the browning of culture and induction of calli. Presence of higher concentration of growth regulator and sucrose lead to abnormal swelling and callusing embryo. Regular sub-culturing calli did not show any significant proliferation and shoot root differentiation after four weeks of culture calli become yellowish to brown in colour.

Acclimatization of plantlets

In this study, when healthy regenerated shoots where transfer MS basal medium supplemented with different concentrations of auxins (IAA, IBA and NAA) (Fig. 1). Significant percentage of rooting was observed in terms of number and length of root. In control medium half strength MS medium without growth regulator did not shows any root formation. Root immergence was observed at the end of embryo after two and half week of culture, after six to eight weeks later well-developed root system was observed. IAA was more effective than IBA and NAA, best response was recorded for rooting was on MS medium supplemented with 0.5 mg/l IAA. Further increase in concentration of IAA a resulted decreased in percentage of shoot producing roots and number of roots per shoot. The root forms in medium supplemented with IAA were thick long while those form on media supplemented with IBA and NAA were slender and week.

Well rooted (7 to 8 cm) plantlets with 2 to 3 leaves were used for further experiment. These plantlets were transferred to hydroponic media. MS Half strength hydroponics media without organic supplements shows significant results in terms of height of shoots, no. of roots, and length of roots. The application of liquid medium (hydroponics) is beneficial intermediate steps for the survival of in *in vitro* grown plantlets in terms of growth parameters. The composition of nutrient elements, show noticeable variation in further growth. 60 days old *in vitro* grown plantlets were treated with IAA of different strength.

Effective growth was noticed on half strength MS + 0.75 mg/l IAA, the average height of shoots 31.5cm, 16.14 average no. of roots and average length of roots 21.5 cm. Based on the hydroponically grown plantlets and simultaneously *in vitro* raised embryo cultured plantlets were grown on solid substratum gives negligible rate of survival (Table. 5). The 20–25 days old hydroponically grown plants saplings were transferred on growth substratum 50% soil: 25% compost: 25% sand. The parameters of vegetative growth mainly size of plantlets and length of leaves show significant growth various by application of different combinations of substratum. Plantlets of seedling show significant growth variation. This method of hardening gives a better way for surest recovery of healthy plantlets in short period of time.

Maintenance of germ-plasm

More than 250 plant saplings have been maintained at Chandmal Tarachand Bora College Shirur, Maharashtra (India). Vegetative growth, inflorescence development and dormant condition are similar to natural habitat plantlets. The pollination mechanism in this species through bats, hence forth regular visit of bats was seen during night condition. The fruits and seeds development, number of fruits per inflorescence and number of seed per fruits are similar to natural growing plants. The goal is to be reserve genetic diversity in sure resources for future research and breeding for maintained population for climatic adaptation and restoration.

Discussion

Source of plant material and seed viability

Under natural conditions, forest plant species have a multi-stage fruit and seed development process that is controlled by environmental, hormonal, and genetic variables to ensure that seeds maturity and spreading to appropriate habitats. This mechanism usually for species mobility, reducing mortality and ensuring colonisation of suitable sites (Oboho, & Nwaihu, 2016; Bareke, 2018). In case of *E. superbum* species influences matured in post monsoon season and seeds dried and disperse before next monsoon season. This process allows to avoid high seedling mortality near parent and reach safe sites for establishment (Naikawadi et al. 2022). Continuous checking of these phenology could help for separating seeds from fruits followed by physical cleaning and processing to ensure high quality, viable seeds used in *in-vitro* experiments. Still, *in vitro* maintenance of this species is visible but not extensively practiced.

Seasonal dependence, physiological dormancy, and slow germination rates are some of the major limiting factors in conventional seed propagation (Naikawadi et al. 2012). The significance of the seedling in plant population ecology has long been recognized. Naturally protein component present in the seed coat become inactive after seed maturation. Under favourable condition seed coat imbibed and activate protein component. (Baskin & Baskin 1998). A continuous decrease in seed viability was observed of different rhizomatous herbs of Himalayan region with storage period (Sharma et al. 2014) similar observations was recorded in present investigation. The variation in seed dormancy and the subsequent patterns of seedling emergence are controlled by environmental conditions. Important factors controlling the variation seed dormancy within species include the environment of the mother plant during the time of seed maturation and environmental conditions after the seeds have been released (Bewley 1997; Oboho, & Nwaihu, 2016; Bareke, 2018). Certain environmental conditions may be required to break dormancy, and other conditions are often required to permit germination after dormancy is broken (Bewley & Black 1994). Seeds of many species require days, weeks, or months at low temperatures to break dormancy (Bradbeer 1992; Penfield, 2017), whereas others require warm temperatures for after-ripening to germinate when permissive conditions arrive (Chauhan & Johnson 2008). Liu et al., 2004 stated *Leymus chinensis*, *In vitro* culture techniques show the importance in shortening the breeding cycles in high level of seed dormant plant species. Intact seed coat and phenolic compounds can inhibit germination, impair seedling formation and maintain seed dormancy for

particular period (Inácio et al., 2013; Klupczyńska and Pawłowski 2021; Lee et al., 2021; Capaci et al., 2026). The similar conditions were observed in case of *E. superbum* hard seed coat and phenolic compounds limit the seed germination as well as seedling growth.

In-vitro germination of zygotic embryo

The age of embryo is a critical factor in embryo rescue studies (Uma et. al., 2011). MS medium played a significant role along with the strength and type of the medium also plays significant role in embryo germination and it was found that in monocotyledonous species half strength medium showed significant vegetative development (Murthi et al., 2014). Appropriate concentration of source of carbohydrate (sucrose 3% w/v) proved superior in promotion of germination along with subsequent conversion to healthy seedling. In present investigation use of embryo as an explant is the ease to get *in vitro* shoots in short period of time. Juvenile embryo shows more vitality and responsive explants for various studies (Elhiti & Stasolla, 2010; Aygun & Dumanoglu, 2015; Ojosnegros et al., 2021). *E.superbum* contain high level of phenolic compounds in all type of explants causes media blackening. Addition of moderate concentration of PVP resulted to control the phenolic exudation. PVP is essential component with growth medium to avoid the *in vitro* culture early browning (Purohit et al., 1995; Vrundha,et al., 2023) similarly to overcome the limitation caused by the phenolic compounds the PVP was utilized. Example of regeneration studies of immature embryo with BAP alone and in combination of PGR involved *A. indica* (Dandapani et al. 2021), Peach (Grigolo et al. 2020), Citrus (Kurt et al. 2022) coinciding with these studies immergence of single root and single shoot from the embryo observed on application of low concentration of BAP. Increasing concentration of BAP the growth was also monitored shoot proliferation. BAP is an adenine type of cytokinin that has been widely used for shoot induction and proliferation of *in vitro* and also some embryo rescue studies. Survival and development of viable embryo under *in vitro* condition depend on several factors such as age of embryo, concentration of medium and growth regulator and culture condition (Nikam et al., 2010; Ghorpade et al., 2010; Naikawadi et al., 2025) Development and stabilisation of *in vitro* raised biomass achieved by maintaining culture conditions and regular sub-culturing. The uniform biomass development for specific time period is a crucial step in *in vitro* techniques (Zottini et al., 2006; Vescovi et al., 2012; Thacker et al., 2018; Di Bonaventura et al., 2024).

Acclimatisation of plantlets through hydroponics

Under controlled condition, rooting of shoots derived from zygotic embryos typically involves a multistage process where shoots organogenesis is followed by an induction phase using specific growth hormones (Elhiti & Stasolla, 2010). The rooting of *in vitro* raised shoots is a critical phase of micropropagation where physiological, biochemical, and anatomical changes are manipulated to induce adventitious roots (Abou Dahab et al., 2000; Liu et al., 2002; Nishesh et al., 2023; Boudadi et al., 2025). Successful rooting often depends on controlling the auxin/cytokinin balance, with high auxin-to-cytokinin ratios promoting root formation. Many workers reported that, Half MS medium stimulate better root induction (Chavez and Litz.1999; Choi et al., 2003; Rinaldi et al., 2019), and concentration of indole-3-butyric acids (Rosas et al., 2002; Tippani et al., 2017). Rooting of *in vitro* shoots often requires specific

hormonal, media, and environmental conditions, with auxins like IAA, IBA and NAA being crucial. In the present investigation best rooting of shoots was achieved on IAA 0.5 mg/l concentration rather than other auxins such as IBA and NAA.

Acclimatization involves transitioning plants from sterile, high-humidity, low-light, and sucrose-rich *in vitro* conditions to *ex vitro* environments, requiring physiological adjustments such as functional cuticle development and stomatal control as well as to initiate complete autotrophic growth in a greenhouse to improve survival rates. It involves overcoming anatomical abnormalities like absent epicuticular wax and dysfunctional stomata, which cause severe transplant desiccation (Chandra et al., 2010). Transitioning tissue-cultured plantlets to half-strength without organic supplements Murashige and Skoog (½ MS) hydroponics medium is an effective method for improving survival rates during acclimatization. This technique involves gradual environmental changes, including modifying the *in vitro* medium, transferring to a hydroponic system with ½ strength MS inorganic salts and managing humidity to promote robust root and leaf development (Mohapatra et al., 2021; Bansal et al., 2023). Regular routine procedure of mass scale *in vitro* production the main limitation of this technology is the period of acclimation of the fragile *in vitro* plants after they have been multiplied or regenerated. Most losses of *in vitro* plants occur when the plantlets are moved directly from the test tubes to the *ex vitro* conditions (Castañeda-Méndez et al., 2017). The best acclimatization program was floating culture in Hoagland nutrient salts solution in stipulated time (Hassan, 2017). Purohit et al., (2021) proves in commercially important nutritive plant species *Actinidia deliciosa* early development of shoots, roots, and leaves through hydroponics was advantageous in establishment of micro-propagated plants in a greenhouse. More than 95% plant survival was found by following proper acclimatization using hydroponic method. Castañeda-Méndez et al., (2017) established and maintain *in vitro* cassava *(Manihot esculenta)* shoots under hydroponic culture. Designed a simple, rapid, low-maintenance hydroponic system to improve survival rate of transplanting to the *ex vitro* conditions through the rapid acclimation process of *in vitro* plants. Similar results were noted *Typha latifolia* (Dethier et al., 1998); *Caesalpinia ferrea*, (Chaturvedi et al., 2004); *Solanum tuberosum* (Duong et al., 2006); *Azadirachta indica* A. Juss (Rambabu et al., 2006); *Trichosanthes kirilowii* (Daniel et al., 2018), *Givotia rottleriformis* (Duan et al., 2020), *Nerium oleander* (Nimoriya et al. 2021), *Ipomoea batatas* (Hu et al., 2022) and *C. arenaria* (Banaszczyk et al., 2025). In this study, we have developed a simple hydroponic system to accelerate the *Ensete* acclimation and multiplication process. Efficient intermediate protocol developed which increased survival. This system considerably increased the survival percentage of *in vitro* and/or transgenic lines and reduces the time requirement for multiplication by hydroponic acclimation.

Maintenance of germplasm

In-vitro raised *E. superbum* plantlets were well acclimatised, vegetative growth, inflorescence development and dormant condition are similar to natural habitat plantlets. Tissue culture techniques serve as an effective tool for conserving rare, endangered, threatened, and endemic elite seasonal plant species (Rajshekhar and Sahijram., 2015; Bettoni et al., 2024). Similar results were noted in other medicinal plants, *P. miniata* and *P. speciosa* (Ferreira et al., 2015); L. *mackliniae* (Sahoo et al., 2018); H.

biflora (Ravathi et al., 2019); *P. santalinus* (Chakraborty et al., 2023); R. *illyricus* (Kocot et al., 2024); B. *griffithii* (Targu and Kumaria et al., 2025); *R. Malabarica* (Kiran et al., 2025)

Conclusion

As an overall because of uncertain raining, change in climate, anthropogenic activities and over burden of human population, the conflict between human and wild animals is frequently recorded in the Western Ghats. This is mainly happening due to non-availability food and water. Therefore, the proposed work would be beneficial for propagation, characterization and plantation in forest for food, feed and pharmaceuticals and may partly help to overcome the situation. There is need of application of plant tissue culture method and biotechnological approaches to the wild banana *E. Superbum*. Therefore, the attempts in the proposed investigation will help in the establishment *in vitro* propagation protocol and large scale plantlets production and actual plantation at the required site of forest in the Western Ghats region. The developed protocol and attempt will be the model for beneficial development and sustainable availability of food and medicine in the area where the proposed plant species is endemic and on the verge of extinction.

Declarations

Ethical approval and consent to participate:

Not applicable

Consent for Publication:

Not applicable

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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Author Contribution

Author contributions VBN and TDN designed the study. ANG, SDD & HAS performed laboratory experimental work and data analysis. VBN, SDD and TDN wrote the manuscript. VBN, HAS and TDN edited and revised the manuscript and approved the final version of the manuscript.

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Tables

Table 1
Seed storage and embryo viability of E.superbum

Storage Duration (Days)	Seed viability (%)
90	88.27 ± 0.74^{a}
120	84.11 ± 0.21^{b}
150	72.27 ± 0.49^{c}
180	59.58 ± 0.14^{d}
210	36.38 ± 0.27^{e}
240	13.69 ± 0.14^{f}

Table 2

Effect of cytokinins on in vitro embryo culture of E.superbum

Growth Regulators	No. of embryo response (%)	Height of shoots (cm)	No. of Roots	Length of Roots
Control	**30.66 ± 0.66i**	**2.7 ± 0.02h**	**2.93 ± 0.42^{h}**	**3.06 ± 0.12^{j}**
BA (mg/l)				
0.1	73.69 ± 0.22^{g}	9.16 ± 0.02^{e}	4.36 ± 0.04^{j}	7.8 ± 0.02^{j}
0.25	82.63 ± 0.27^{d}	11.71 ± 0.03^{d}	7.4 ± 0.08^{h}	10.6 ± 0.03^{g}
0.5	84.29 ± 0.14^{c}	14.77 ± 0.28^{b}	12.37 ± 0.02^{e}	13.62 ± 0.11^{d}
0.75	95.77 ± 0.17^{a}	16.91 ± 0.01^{a}	17.32 ± 0.09^{a}	18.49 ± 0.12^{a}
1.0	73.37 ± 0.1^{g}	13.73 ± 0.04^{c}	15.15 ± 0.1^{c*}	15.18 ± 0.05^{c}
2.0	82.07 ± 0.15^{d}	11.61 ± 0.05^{d} EC	13.8 ± 0.06^{d} EC	9.19 ± 0.02^{h}
Kinetin (mg/l) (µM) (µM)				
0.1	64.73 ± 0.39^{i}	7.9 ± 0.01^{g}	3.44 ± 0.03^{k}	6.28 ± 0.08^{k}
0.25	75.67 ± 0.12^{f}	9.26 ± 0.02^{e}	6.32 ± 0.11^{i}	8.47 ± 0.02^{i}
0.5	88.27 ± 0.12^{b}	14.56 ± 0.03^{b}	15.44 ± 0.07^{b}	16.5 ± 0.05^{b}
0.75	82.57 ± 0.29^{d}	12.59 ± 0.09^{c}	11.71 ± 0.11^{f}	11.48 ± 0.25^{f}
1.0	80.6 ± 0.29^{e}	11.7 ± 0.03^{d*}	13.68 ± 0.05^{d*}	12.52 ± 0.08^{e}
2.0	68.73 ± 0.27^{h}	8.59 ± 0.08^{g*}EC	9.17 ± 0.09^{g}EC	8.59 ± 0.25^{i}
TDZ (mg/l)				
0.1	70.11 ± 0.14^{e}	10.03 ± 0.03^{d}	8.33 ± 0.08^{h}	9.62 ± 0.07^{h}
0.25	68.54 ± 0.16^{f}	9.12 ± 0.05^{e}	7.41 ± 0.05^{h}	8.84 ± 0.03^{i}

The values represent the mean ± SE calculated from three independent experiments, each based on a minimum 15 replicates. Values followed by same superscript letter were not significantly different at 5% level (DMRT). C explant produced callus, *callus interspersed root formation, EC Explant produced extensive callus.

Growth Regulators	No. of embryo response (%)	Height of shoots (cm)	No. of Roots	Length of Roots
Control	**30.66 ± 0.66i**	**2.7 ± 0.02h**	**2.93 ± 0.42^{h}**	**3.06 ± 0.12^{j}**
BA (mg/l)				
0.5	65.88 ± 0.25^{f}	8.92 ± 0.02^{e}	6.14 ± 0.07^{i}	7.95 ± 0.04^{i}
0.75	60.47 ± 0.18^{g}	7.34 ± 0.04^{f*}	5.20 ± 0.04^{j*}	6.40 ± 0.08^{j}
1.0	55.32 ± 0.20^{h}	7.01 ± 0.06^{f*}C	4.22 ± 0.06^{j*}C	6.18 ± 0.05^{j}
2.0	58.26 ± 0.22^{g}	6.21 ± 0.03^{g*}EC	3.10 ± 0.05^{k}EC	5.42 ± 0.06^{k}

The values represent the mean ± SE calculated from three independent experiments, each based on a minimum 15 replicates. Values followed by same superscript letter were not significantly different at 5% level (DMRT). C explant produced callus, *callus interspersed root formation, EC Explant produced extensive callus.

Table 3

Effect of auxins on in vitro embryo culture of E. superbum

Growth Regulators	No. of embryo response (%)	Height of shoots (cm)	No. of Roots	Length of Roots
IAA (mg/l)				
0.1	**91.53 ± 0.23^{l}**	**8.56 ± 0.06^{g}**	**9.26 ± 0.07^{n}**	**9.28 ± 0.00^{n}**
0.25	93.57 ± 0.07^{j}	11.91 ± 0.02^{de}	11.27 ± 0.05^{i}	14.43 ± 0.06^{i}
0.5	97.73 ± 0.27^{a}	15.82 ± 0.04^{a}	19.43 ± 0.07^{a}	20.41 ± 0.17^{a}
0.75	95.61 ± 0.07^{e}	13.55 ± 0.15^{bc}	15.08 ± 0.04^{c}	18.51 ± 0.25^{c}
1.0	93.1 ± 0.06^{c}	12.52 ± 0.07^{def}	17.31 ± 0.03^{d}	17.59 ± 0.11^{d}
2.0	87.42 ± 0.04^{f}	11.38 ± 0.08^{i}*	14.25 ± 0.04^{f}*	16.38 ± 0.04^{f}
IBA (mg/l)				
0.1	88.4 ± 0.06^{l}	7.68 ± 0.05^{i}	9.08 ± 0.08^{l}	11.61 ± 0.06^{l}
0.25	92.47 ± 0.09^{k}	10.63 ± 0.24^{efg}	10.26 ± 0.02^{j}	13.38 ± 0.09^{j}
0.5	94.37 ± 0.12^{f}	13.52 ± 0.09^{cd}	14.3 ± 0.06^{de}	17.36 ± 0.02^{de}
0.75	96.4 ± 0.06^{b}	14.68 ± 0.09^{b}	18.22 ± 0.06^{b}	19.4 ± 0.04^{b}
1.0	91.63 ± 0.12^{d}	11.5 ± 0.08^{g}	16.11 ± 0.11^{e}*	17.22 ± 0.12^{e}
2.0	83.47 ± 0.03^{g}	10.5 ± 0.02^{j}*	13.46 ± 0.18^{g}*	15.73 ± 0.04^{g}
NAA (mg/l)				
0.1	84.4 ± 0.21^{k}	7.55 ± 0.03^{j}	9.13 ± 0.07^{l}	11.59 ± 0.06^{l}
0.25	84.73 ± 0.09^{k}	9.99 ± 0.03^{j}	10.18 ± 0.04^{k}	12.47 ± 0.01^{k}
0.5	89.67 ± 0.26^{h}	12.78 ± 0.03^{h}	12.1 ± 0.1^{h}	15.44 ± 0.03^{h}

The values represent the mean ± SE calculated from three independent experiments, each based on a minimum 15 replicates. Values followedby same superscript letter were not significantly different at 5% level (DMRT). C explant produced callus, *callus interspersed root formation, EC Explant produced extensive callus.

Growth Regulators	No. of embryo response (%)	Height of shoots (cm)	No. of Roots	Length of Roots
IAA (mg/l)				
0.1	**91.53 ± 0.23^{l}**	**8.56 ± 0.06^{g}**	**9.26 ± 0.07^{n}**	**9.28 ± 0.00^{n}**
0.75	92.77 ± 0.37^{d}	14.48 ± 0.02^{efg}	16.19 ± 0.11^{d}*	17.53 ± 0.02^{d}
1.0	87.87 ± 0.15^{e}	10.78 ± 0.14^{i}*	15.19 ± 0.1^{f}*	16.43 ± 0.04^{f}
2.0	80.4 ± 0.31^{h}	9.76 ± 0.32^{k}**	12.13 ± 0.03^{i}**	14.49 ± 0.07^{i}
2,4 D (mg/l)				
0.1	76.6 ± 0.15^{m}	5.4 ± 0.06^{l}	9.17 ± 0.08^{l}	10.53 ± 0.1^{m}
0.25	83.53 ± 0.09^{l}	7.65 ± 0.03^{j}	7.11 ± 0.06^{m}*	11.57 ± 0.06^{l}
0.5	87.6 ± 0.15^{i}	9.87 ± 0.05^{i}*	*	*
0.75	91.77 ± 0.09^{f}	11.71 ± 0.03^{fg}*	**	**
1.0	87.87 ± 0.15^{h}	9.56 ± 0.18^{i}*	**	**
2.0	81.53 ± 0.03^{l}	6.35 ± 0.03^{k}**	***	***

The values represent the mean ± SE calculated from three independent experiments, each based on a minimum 15 replicates. Values followedby same superscript letter were not significantly different at 5% level (DMRT). C explant produced callus, *callus interspersed root formation, EC Explant produced extensive callus.

Table 4

Effect of cytokinin and auxins on in vitro embryo culture of E. superbum

Growth Regulators		No. of embryo response (%)	Height of shoots (cm)	No. of Roots	Length of Roots
0.75 mg/l BAP	0.50 mg/l IAA	94.43 ± 2.94^{a}	17 ± 0.35^{a}	22.57 ± 0.33^{a}	19.29 ± 0.25^{a}
	0.75 mg/l IBA	86.67 ± 1.93^{ab}	15 ± 0.12^{b}	20.18 ± 1^{bc}	15.11 ± 0.23^{d}
	075 mg/l NAA	83.33 ± 1.93^{bc}	16.5 ± 0.26^{a}	21.59 ± 0.36^{ab}	17.18 ± 0.18^{b}
	0.75mg/l 2,4-D	72.23 ± 2.94^{de}	1.57 ± 0.03^{h}	0 ± 0g	0 ± 0^{g}EC
0.5 mg/l Kin	0.50 mg/l IAA	81.13 ± 2.94^{bcd}	9.13 ± 0.23^{d}	19.59 ± 1.6^{d}	17.51 ± 0.08^{b}
	0.75 mg/l IBA	87.8 ± 1.1^{ab}	10.53 ± 0.38^{c}	19.55 ± 0.16^{d}	14.82 ± 0.04^{d}
	075 mg/l NAA	80 ± 1.91^{bcd}	15.63 ± 0.12^{b}	20.26 ± 0.5^{bc}	16.24 ± 0.23^{c}
	0.75 mg/l 2,4-D	62.2 ± 4.85^{f}	1.03 ± 0.03^{h}	0 ± 0^{g}	0 ± 0^{g} EC
0.75 mg/l TDZ	0.50 mg/l IAA	68.9 ± 1.1^{ef}	6.04 ± 0.16^{e}	7.76 ± 0.32^{e}	8.93 ± 0.39^{f}
	0.75 mg/l IBA	74.43 ± 1.13^{cde}	4.63 ± 0.39^{f}	6.02 ± 0.4^{ef}	13.02 ± 0.24^{e}
	075 mg/l NAA	72.23 ± 2.94^{de}	3.7 ± 0.1^{g}	4.61 ± 0.18^{f}	15.89 ± 0.12^{c*}
	0.75 mg/l 2,4-D	36.67 ± 5.07^{g}	0 ± 0^{i}	0 ± 0^{g}	0 ± 0^{g}EC

The values represent the mean ± SE calculated from three independent experiments, each based on a minimum 15 replicates. Values followedby same superscript letter were not significantly different at 5% level (DMRT). C explant produced callus, *callus interspersed root formation, EC Explant produced extensive callus.

Table 5

Effect of hydroponic condition on E. superbum samplings

Growth Regulators	Strength of media	Height of shoots	No. of Roots	Length of Roots
IAA (mg/l)	½ strength			
0.1		9.88 ± 0.03^{j}	7.11 ± 0.06^{i}	11.57 ± 0.06^{h}
0.25		19.53 ± 0.03^{d}	11.59 ± 0.12^{f}	13.29 ± 0.04^{f}
0.5		24.55 ± 0.02^{b}	13.93 ± 0.03^{c}	15.42 ± 0.1^{d}
0.75		31.55 ± 0.03^{a}	16.14 ± 0.03^{a}	21.5 ± 0.02^{a}
1.0		21.65 ± 0.12^{c}	12.13 ± 0.02^{e}	16.45 ± 0.01^{c}
2.0		12.47 ± 0.01^{g}	8.75 ± 0.13^{h}	11.56 ± 0.07^{h}
IAA (mg/l)	Full strength			
0.1		7.11 ± 0.06^{l}	6.13 ± 0.03^{j}	10.26 ± 0.06^{i}
0.25		11.59 ± 0.12^{i}	11.08 ± 0.19^{g}	12.7 ± 0.27^{g}
0.5		13.93 ± 0.03^{f}	12.99 ± 0.11^{cd}	14.63 ± 0.04^{e}
0.75		16.14 ± 0.03^{e}	15.31 ± 0.16^{b}	19.56 ± 0.03^{b}
1.0		12.13 ± 0.02^{h}	11.55 ± 0.26^{f}	15.24 ± 0.05^{d}
2.0		8.75 ± 0.13^{k}	8.55 ± 0.16^{h}	10.29 ± 0.08^{i}

The values represent the mean ± SE calculated from three independent experiments, each based on a minimum 15 replicates. Values followed by same superscript letter were not significantly different at 5% level (DMRT).

Figures

Figure 1

A) Habit and habitat of *E. superbum,* B) Fully developed Fruiting, C) Matured fruits, D) T.S fruits, E) Fully dried stalk, F) Matured Seeds.

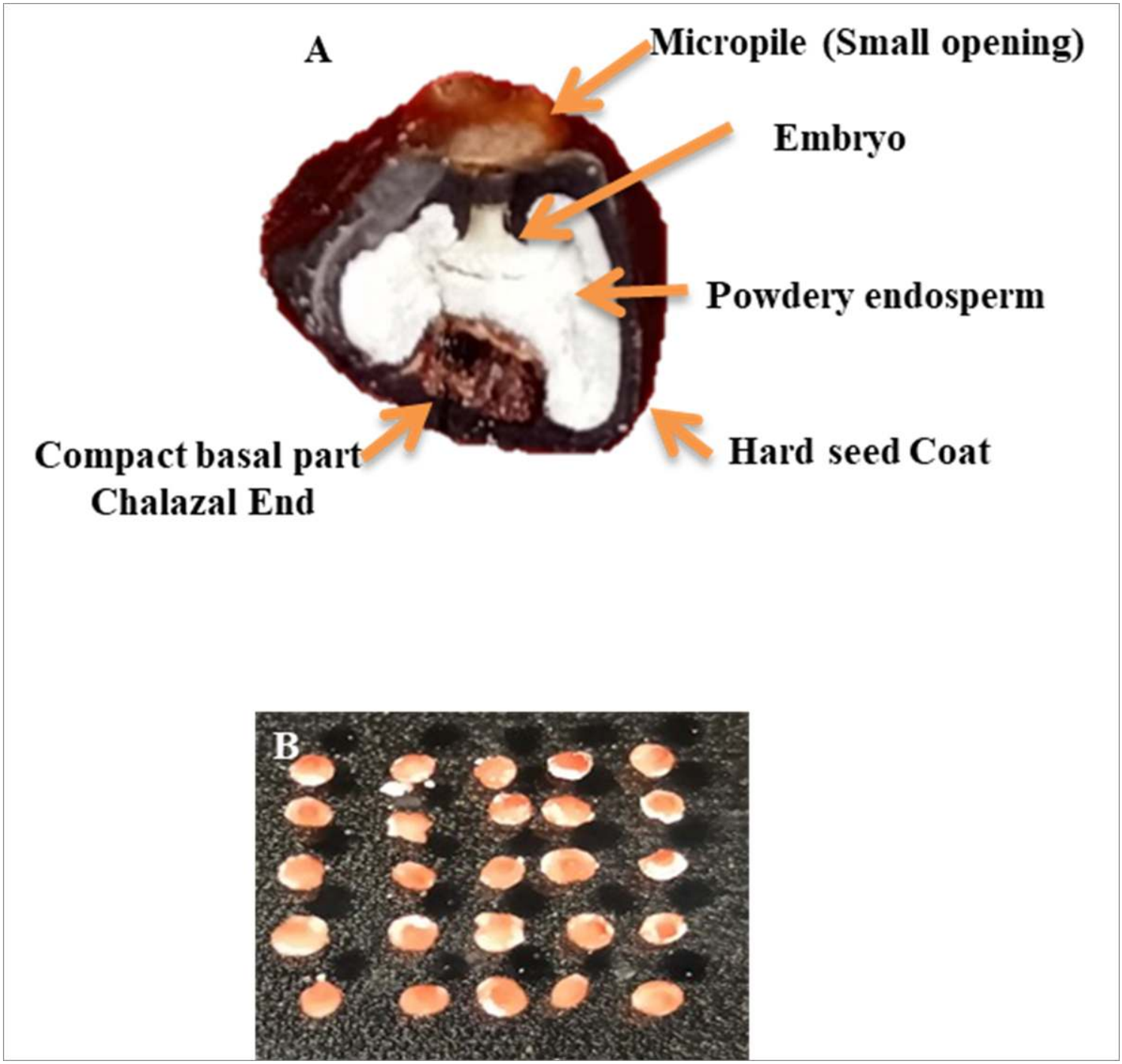

Figure 2

A) T.S of matured seeds B) shape of embryo, C) Embryo viability test Solution

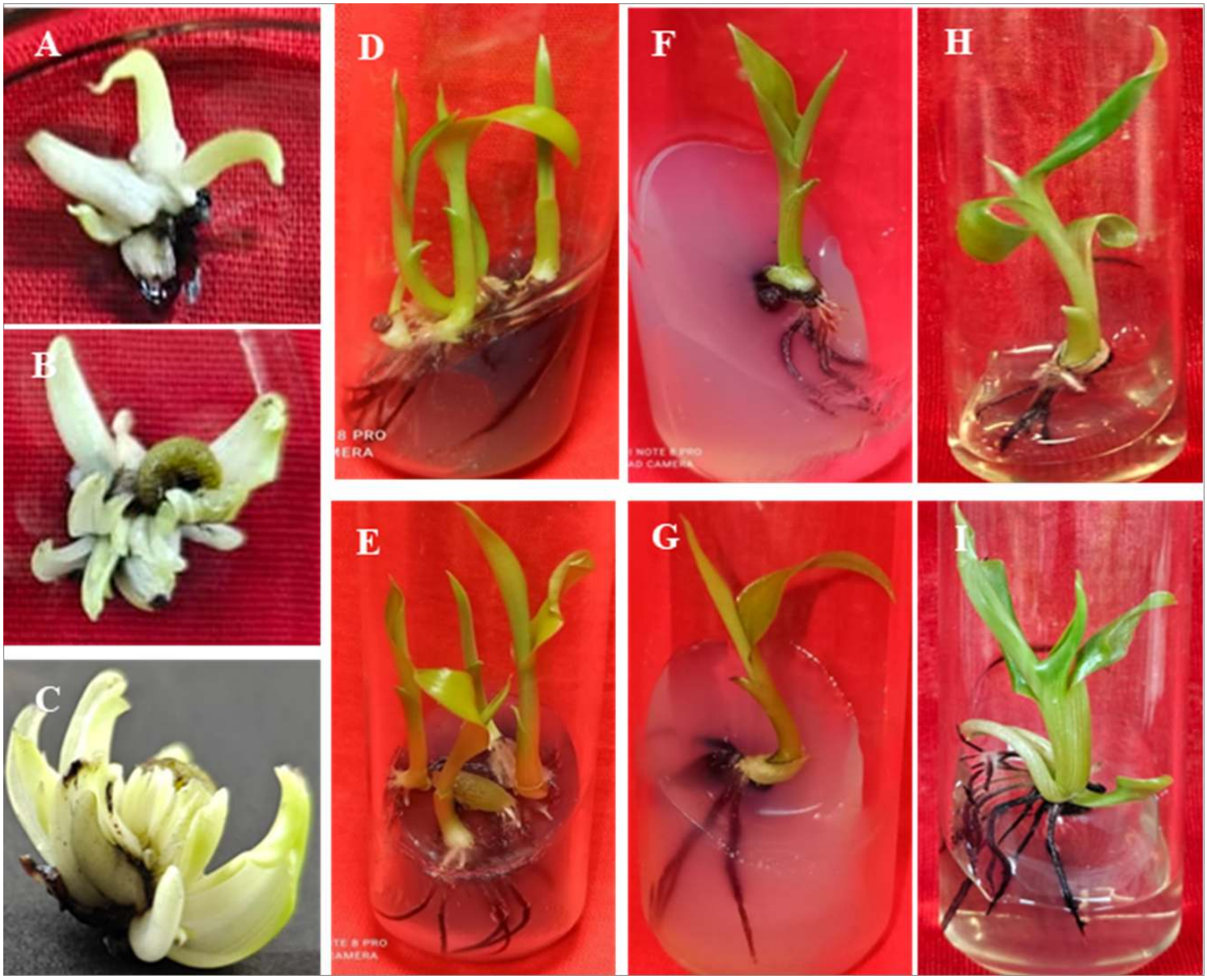

Figure 3

Effect of growth regulators on embryo germination of *E.superbum,* Three week old *in vitro* embryo germination on MS medium A) MS+ 0.25 mg/L BA, B) MS+ 0.5 mg/L BA, C) MS+ 0.75 mg/L BA, D) Shoots maintain on E), F) MS+ 0.75 mg/L BA, First sub-culture single shoot maintain on F), G) MS+ 0.5 mg/L BA, *in vitro*raised single on rooting medium H) MS + 0.25 mg/L IAA, I) MS + 0.5 mg/L IAA.

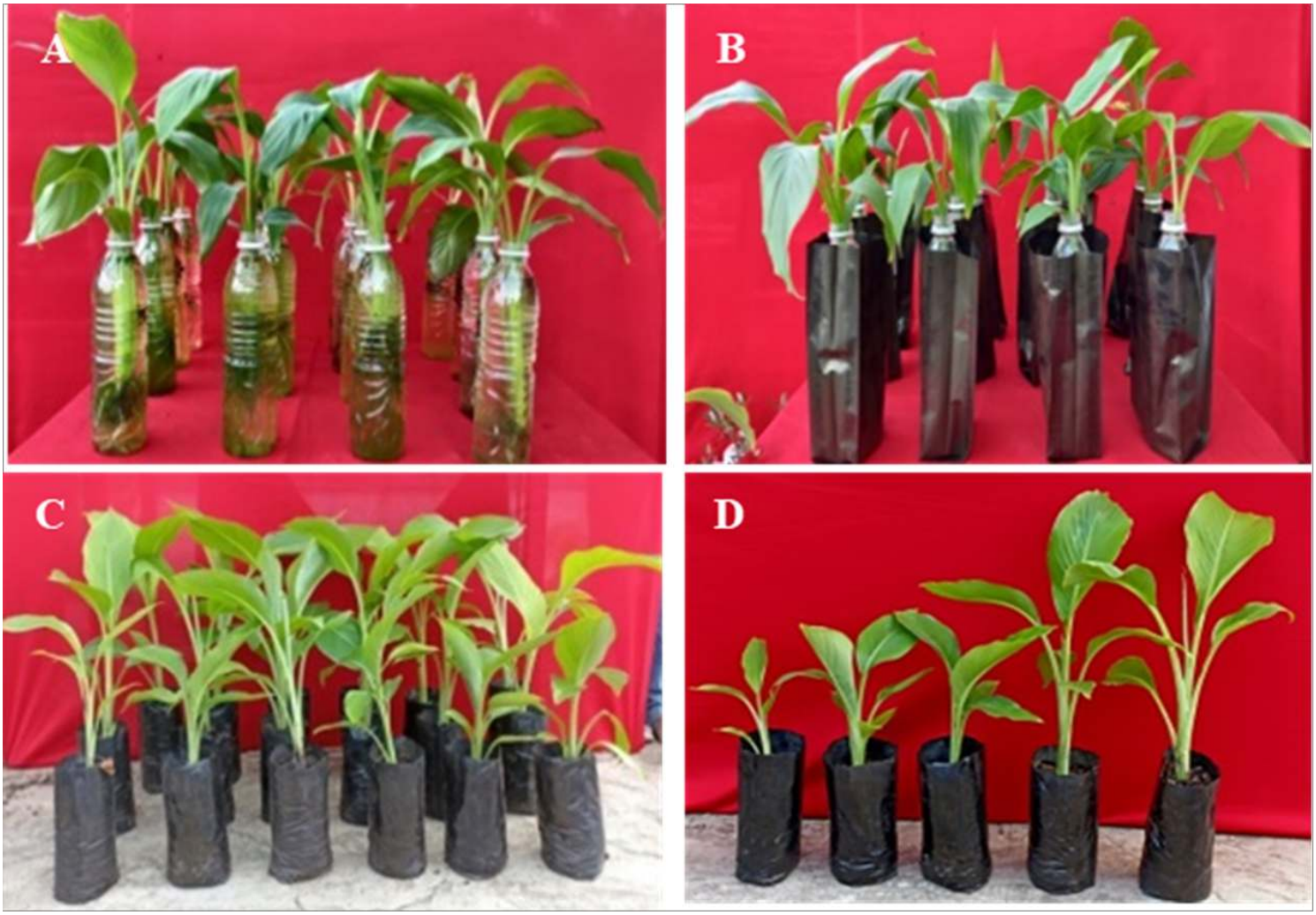

Figure 4

Primary acclimatization and hardening of *in vitro* grown *E.superbum,* Plantlets maintain with half strength MS liquid medium, A) under light condition, B) under dark condition, C) Well acclimatized plantlets, D) Fully developmental *Ensete*samplings.

Figure 5

A) Germplasms conservation program: Fully developed plantlets maintained at Botanical Garden B) 6 months old plants at flowering stage.