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REVIEW ARTICLE

Advancements and future prospects in micropropagation techniques for major palm species: Date palm, oil palm, arecanut, and coconut

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Abstract

Palms are widely distributed across warm temperate, tropical, and subtropical regions, showcasing their ecological significance and adaptability to diverse environments. Classification schemes play a crucial role in organizing the vast diversity of palms and provide valuable insights into their evolutionary adaptations. These frameworks enhance our understanding of key species such as date palms, oil palms, and coconuts. Micropropagated palm seedlings are economically essential for global palm industries of food, biofuel, and cosmetics, because they ensure consistent growth, increased yield, and disease resistance. Clonal propagation of superior cultivars is made possible by this technique, maximizing land utilization and increasing yield per hectare. Micropropagated seedlings support large-scale commercial plantations and promote sustainable agriculture in response to the growing demand for palm-derived products worldwide. Furthermore, governments and research- driven initiatives are fostering the adoption of tissue culture methods to meet market needs. To facilitate large-scale propagation, micropropagation techniques have been developed for palms, such as date palms, oil palms, coconuts, and arecanuts. These techniques produce consistent, disease-free planting materials through tissue culture. However, despite their potential, tissue culture techniques face challenges, including low effectiveness, high rates of contamination, and scaling limitations, particularly due to the recalcitrant nature of palm tissues to *in vitro* conditions. Improvement in tissue culture methods can support sustainable agriculture, drive economic growth, and conserve biodiversity. By enhancing propagation techniques, tissue culture has the potential to address global issues such as environmental sustainability and food security. To fully realize the potential of palms and ensure their continued use in industry and agriculture, further research and development in palm micropropagation are essential.

Keywords

arecanut; coconut; date palm; micropropagation; oil palm; organogenesis; palms; somatic embryogenesis

Introduction

Palm species such as date palm (*Phoenix dactylifera*), oil palm (*Elaeis guineensis*), arecanut (*Areca catechu*), and coconut (*Cocos nucifera*) play a

critical role in the economy and ecology of tropical and subtropical regions. Each species serves as a fundamental component of agricultural systems and provides numerous products essential to local and global markets. For example, the date palm, a staple in Middle Eastern diets, contributes significantly to the economies of arid regions through its high-value fruit (1). The oil palm, primarily cultivated in Southeast Asia, is a major source of vegetable oil and biofuels, meeting about 40% of the global demand for edible oils (2). Similarly, arecanut is widely used in traditional rituals and as a stimulant in South Asia, where it holds cultural and economic importance (3). Coconut, referred to as the "tree of life," provides food, fuel, fiber, and raw materials for various industries, particularly in tropical island economies (4).

The cultivation of these palm species faces several challenges, such as long growth cycles, susceptibility to diseases, and difficulties in large-scale propagation due to seed-based limitations. Traditional propagation methods, such as seed germination or offshoot transplantation, often fail to meet the demands of commercial production and conservation needs (5). This is particularly problematic for species like the date palm and coconut, where the seedling phase is lengthy and vegetative propagation is often constrained by low availability of offshoots (6). Consequently, micropropagation has emerged as a valuable technique for enhancing the sustainable cultivation and conservation of these palms, allowing for the mass production of high-quality, disease-free plants with desired traits (7).

Micropropagation, or the *in vitro* propagation of plants, offers a controlled environment for rapid, large-scale production of plantlets, bypassing the limitations associated with traditional methods. The use of techniques such as somatic embryogenesis and organogenesis has proven particularly effective for palm species, allowing the production of genetically stable and uniform plants (8). This technique is critical not only for commercial cultivation but also for the conservation of endangered palm species threatened by habitat loss and genetic erosion (9).

This review aims to provide a comprehensive overview of recent advancements in micropropagation techniques for major palm species, focusing on innovations that address specific challenges in the propagation of date palm, oil palm, arecanut and coconut. Additionally, the review highlights the future prospects in palm micropropagation, emphasizing technologies that can support sustainable agriculture and conservation efforts.

Overview of micropropagation techniques

Micropropagation is a specialized technique in plant tissue culture that enables the rapid production of large numbers of genetically identical plants within a controlled and sterile environment. This process has become a keystone of plant biotechnology, supporting commercial agriculture, conservation efforts, and genetic improvement by offering a reliable method to propagate high-value or endangered species (10). Unlike traditional propagation methods, which often rely on seeds or vegetative cuttings, micropropagation enables the efficient multiplication of plants through *in vitro* culture techniques, helping to meet demand for high-quality plants with uniform traits (11).

The choice of micropropagation techniques typically depends on the plant species, their growth characteristics, reproductive biology, and economic significance. For example, herbaceous plants like ornamentals and vegetables, which grow rapidly and are relatively easy to regenerate, often benefit from shoot tip cultures or direct organogenesis. Conversely, woody perennials such as forest trees and fruit crops, which have slower growth rates and more complex structural characteristics, require approaches like somatic embryogenesis or callus culture. Monocotyledons, including grasses, cereals, and palms, respond well to somatic embryogenesis and suspension cultures, which are particularly suited to their structural and physiological traits. Meanwhile, dicotyledons, such as many crops and ornamentals, are frequently propagated from nodal segments or axillary buds due to their potential for producing multiple shoots. Medicinal and aromatic plants, on the other hand, demand specialized conditions like hairy root cultures or organogenesis to preserve their bioactive compounds. By aligning specific techniques with the unique traits of various plant groups, micropropagation provides a practical *in vitro* propagation pathway for propagating a wide range of species (12).

Organogenesis involves the regeneration of plant organs like shoots or roots from explants. This approach is often preferred for its genetic stability and the relative simplicity of producing high-quality plantlets. On the other hand, somatic embryogenesis involves inducing non-reproductive (somatic) cells to form embryos that develop into complete plants (Fig. 1). This technique is especially valuable for large-scale production, as it can generate thousands of plantlets from a single explant (13, 14).

While micropropagation offers significant advantages, it also presents notable challenges, particularly for palm species. Palms are generally slow-growing and their extended juvenile phases make traditional propagation through seeds or offshoots difficult, often rendering them impractical for commercial-scale production (15). Additionally, palms are highly susceptible to contamination due to the large surface area of their tissues and the extended culture times required for growth. Ensuring sterile conditions is therefore critical but challenging, as even minor contamination can severely impact the success of a culture (16). Organogenesis and somatic embryogenesis in palms require specific culture media and environmental conditions. These processes often involve complex adjustments to balance growth regulators and nutrient needs, which can increase costs and limit scalability (15). These challenges underscore the need for ongoing research and optimization of micropropagation protocols tailored to each palm species. Innovations in bioreactor systems, molecular markers for

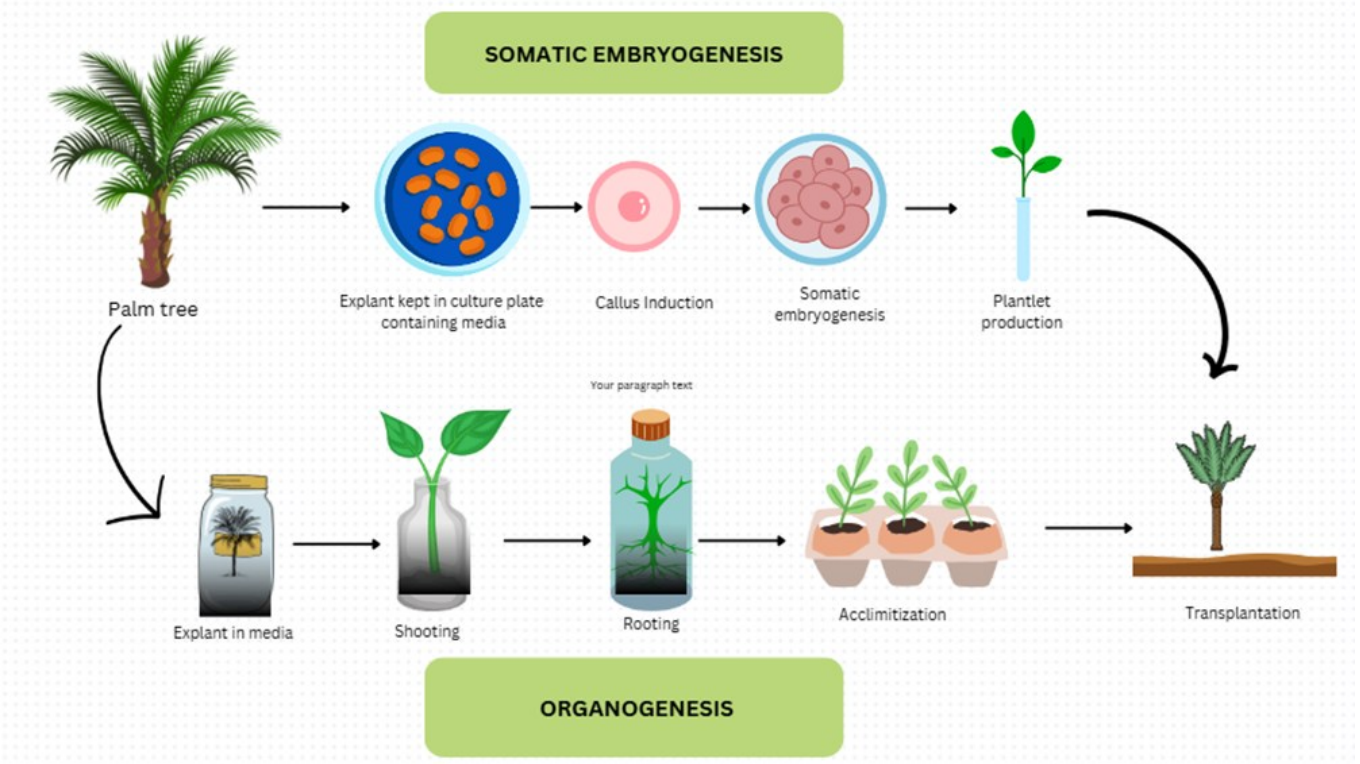

Fig. 1. Overview of micropropagation techniques.

genetic fidelity, and cryopreservation methods are essential to enhance both the efficiency and sustainability of palm micropropagation.

Species-specific advances in micropropagation techniques

Date palm (*Phoenix dactylifera*)

Traditional methods for propagating date palms present significant challenges, as seed propagation results in high genetic variability, leading to inconsistency in fruit quality and yield (17). Vegetative propagation through offshoots is often constrained by the slow growth rate and the limited availability of offshoots, especially in older palms (18). Recent advances in *in vitro* propagation have focused on techniques such as somatic embryogenesis, shoot tip culture, and liquid culture systems, which allow for the large-scale production of genetically uniform plants. Somatic embryogenesis, in particular, has enabled the efficient production of high-quality plantlets from callus tissue, thereby improving consistency in crop characteristics. The importance of environmental conditions in date palm propagation is emphasized. Date palms have thermoregulatory requirements during growth and fruit ripening (40°C or above). Temperature during flowering (12°C or below) must be avoided to prevent inhibition. Although they are potentially drought-tolerant in principle, they always need a proper water supply at certain developmental points, ideally *via* carefully designed irrigation plans. Well-drained, low-salinity soils and amendments are preferred, and salt-tolerant rootstocks are used to drastically reduce the effects of salinity. For instance, reduced humidity decreases the incidence of fungal infection but may also negatively affect pollen flight and ventilation. Maximum light and windbreak should always be provided for normal, vigorous growth and vegetative control. In order to stimulate root growth, such plants need to be routinely fertilized because they grow in nutrient-deficient arid soils. Climate change may influence the growth cycle, but these plants have the ability to survive in extreme conditions. All of these factors, when properly managed, make the cultivation of date palm in dry climates possible (19). Advanced tissue culture techniques such as immature inflorescence culture are also effective in enhancing growth rates in tissue culture (20).

To maintain genetic fidelity, genetic markers were utilized to assess and ensure clonal uniformity across propagated plants. Additionally, cryopreservation techniques are increasingly applied to store somatic embryos, preserving genetic material for long-term conservation and minimizing the risks associated with genetic drift (16).

Shoot tip culture

The direct organogenesis approach successfully regenerated and multiplied date palm plants by cultivating shoot tip explants without inducing callus formation. This method, which uses Murashige and Skoog (MS) media supplemented with specific growth regulators, minimizes the likelihood of somaclonal variation, ensuring the production of genetically stable plants. The medium was fortified with 1 mg/L naphthalene acetic acid (NAA), 1 mg/L naphthoxy acetic acid (NOA), 2.5 mg/L benzyl adenine (BA), and 2.5 mg/L isopentenyladenine (2iP). Under these conditions, adventitious buds proliferated in darkness from shoot tip explants, progressing through six subcultures spaced four weeks apart. The organogenesis process was observed in three distinct stages: the

initiation of bud formation, the differentiation into vegetative buds, and the multiplication of shoot buds (21). This systematic development enabled efficient multiplication, with the maximum frequency of organogenesis achieved at 5 mg/L of 2iP. For root induction, 1 mg/L of NAA proved most effective, while 5 mg/L of silver nitrate significantly enhanced shoot bud multiplication. These optimized conditions facilitated the large-scale production of superior planting material with minimal genetic heterogeneity (8, 22). Beyond propagation, shoot tip culture techniques have also been employed for short-term *in vitro* preservation of date palm germplasm. Sucrose served as an osmotic agent, while abscisic acid (ABA) was utilized as a growth retardant to maintain tissue viability. Importantly, molecular analysis confirmed the genetic fidelity of regenerated plants, demonstrating that the micropropagation protocol effectively preserved the original traits of the mother plant (22). The success in generating genetically stable, high-quality plantlets establishes the potential of direct organogenesis for commercial-scale date palm cultivation and conservation efforts.

Somatic embryogenesis

The process of somatic embryogenesis begins with the induction of an embryogenic callus from somatic tissues, followed by the differentiation of somatic embryos. This process is influenced by various factors, including hormonal balance. Notably, successful induction of somatic embryogenesis has been achieved using shoot tips cultivated on MS medium supplemented with auxins and cytokinins (17). High doses of 2, 4-dichlorophenoxyacetic acid (2, 4-D) were best for inducing callus, while lower quantities promoted the differentiation of somatic embryos (8). To regenerate date palms without endangering the mother tree, immature inflorescence explants were removed, surface sterilized, and then cultured. Both somatic embryos and embryogenic callus occur at 27 ± 2°C and throughout a 16-hour photoperiod. Bipolar-shaped embryos were formed from globular embryos and these were further cultured for additional development. Adding activated charcoal significantly enhanced embryo maturation in date palm cultures (23). Alternating maltose as a carbon source instead of sucrose improved the quality of somatic embryos (24). This method achieves high regeneration efficiency and provides a sustainable solution for propagating date palms. By using inflorescence explants and optimizing culture conditions with selective growth regulators, this approach enables the production of a large number of genetically uniform and disease-free plants within a short timeframe. This not only addresses the limitations of traditional propagation methods but also supports the conservation of genetic resources while meeting the growing demand for date palm cultivation (20).

Date palm somatic embryogenesis has been the subject of numerous investigations. Somatic embryo growth and development were greatly enhanced by adding antioxidants such as glutathione (GSH) at a concentration of 50 mg/L to maturation media (25). Temporary immersion Bioreactors (TIBs) have demonstrated potential for large-scale propagation by using meristematic tissues from *cv. Zambli* offshoots. This system operates with periodic immersions of 30 minutes every six hours, which have been shown to enhance germination and plantlet production. Longer immersion times (10 or 30 minutes) lead to greater biomass and plantlet production compared to shorter immersion times. The TIB system provides a controlled environment, maintaining temperatures at 25 ± 2°C and a 12-hour photoperiod using LED tube lights. This setup minimizes shear stress and promotes nutrient absorption during immersion, thereby supporting the development of embryogenic calli and somatic embryos. Plant growth parameters, such as shoot production and fresh/dry weight, indicate that TIBs significantly improve plant vigor and rooting, making this system ideal for *in vitro* propagation of date palms (26). Bayoud-resistant genotypes exhibited high embryogenesis frequencies in a medium supplemented with 225 µM 2, 4-D (27). In Mejhoul cultivars, somatic embryogenesis was optimized by 45 µM picloram and 5 µM 2iP, while NAA and BA promoted germination and PEG aided embryo development. Under greenhouse settings, the survival percentage of regenerated plantlets was 76%, indicating a strong potential for large scale multiplication (27).

Immature inflorescence culture

Immature inflorescences have emerged as one of the most promising explant sources for date palm tissue culture due to their high embryogenic potential and responsiveness to *in vitro* settings, as they include meristematic cells that can be reprogrammed to generate calluses and somatic embryos. In contrast to other explants like leaf or root tips, Bekheet showed that immature inflorescences had higher embryogenic potential. They are also suitable for large-scale propagation due to their high frequency of callus induction (8). The importance of developmental stage in callus and embryo creation was highlighted that immature inflorescences harvested at an early developmental stage (about 10-15 cm) produced more somatic embryos and higher quality callus than those harvested later (28). The successful induction of callus from immature inflorescences was achieved using a combination of 2,4-D (12 mg/L) and BAP (0.5 mg/L) in MS medium, underscoring the importance of optimizing plant growth regulators for this process (19). The addition of activated charcoal to culture medium enhances somatic embryo maturation by lowering oxidative stress and mitigating phenolic browning (28). However, extended subculturing and tissue manipulation can lead to somaclonal variation, causing genetic instability in regenerated plants (29). Furthermore, different date palm cultivars can respond differently to immature inflorescence culture; some cultivars show lower rates of callus induction and embryo production, suggesting that additional protocol adjustment may be required to optimize outcomes for more resistant or less responsive varieties (30).

Laboratories work on date palm micropropagation

Research on date palm micropropagation has greatly benefited from the contributions of several esteemed universities. With an emphasis on media refinement and genetic fidelity through the use of molecular markers, the National Research Center in Egypt has refined somatic embryogenesis and organogenesis techniques, optimizing media for genetic stability (31). Researchers at King Faisal University in Saudi Arabia focus strongly on avoiding somaclonal variation while cultivating premium varieties, such as Medjool and Khalas. To guarantee genetic stability, the Biotechnology Unit of Tunisia uses RAPD analysis and polyamines. The Desert Research Center in Egypt focuses on employing direct organogenesis to reduce somaclonal variance. King Abdulaziz University in Saudi Arabia has developed effective cryopreservation and liquid culture methods. The Centre for Advanced research in plant tissue culture at Anand Agricultural University, Anand, Gujarat, has optimized plant growth regulators and micropropagation media to produce true-to-type date palm plantlets on a large scale with a 100% field establishment rate. Cultivation techniques are significantly enhanced by tissue-cultured plants' early flowering, increased offshoot production, and elite genotype propagation. The success of micropropagation is increased by axenic cultures, which are produced from elite tree offshoots and provide steady, disease-free growth (32).

Oil palm (*Elaeis guineensis*)

Oil Palm propagation faces significant challenges due to high genetic variability, particularly with seed-based methods that often produce heterogeneity in plant traits. Genetic variability is recognized as a critical factor influencing traits such as oil yield and disease resistance. In terms of oil yield, genetic uniformity ensures consistent production, as variability can lead to a mix of high and low-yielding plants, reducing overall efficiency and profitability. Clonal propagation helps minimize this variability, producing plants with consistently high yields. Conversely, genetic variability can be advantageous for disease resistance, as a diverse population may include individuals resistant to specific pathogens, reducing the risk of widespread crop loss. However, in clonal propagation with minimized variability, uniform susceptibility to diseases poses a significant risk, as a pathogen infecting one plant can rapidly spread. To balance these needs, controlled genetic variability allows for the propagation of resistant genotypes, combining uniformity in yields with enhanced resilience (33). Callus induction and somatic embryogenesis have been instrumental in producing uniform plantlets, particularly when coupled with controlled bioreactor systems for scaling up production (34). These bioreactors offer an efficient and cost-effective solution for producing large numbers of clonal plants in a controlled environment, reducing the risk of contamination and ensuring genetic consistency. To address genetic stability, quality control measures such as molecular marker analysis and regular monitoring are employed, helping to identify and eliminate any off-types. Various types of markers such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), AFLP (Amplified Fragment Length Polymorphism) and SNP (Single Nucleotide Polymorphism), play major roles. These markers contribute significantly by detecting somaclonal variations, ensuring genetic consistency in regenerated plants, maintaining uniformity in key traits like oil yield and disease resistance. They are integral to quality control, identifying genetic drift or contamination in large-scale propagation and verifying the genetic identity of parent lines and conserved germplasm. This ensures that valuable traits are preserved during regeneration. By employing molecular markers, researchers can reliably monitor genetic integrity, making them critical for successful oil palm propagation and consistent commercial performance. This ensures that mass propagation does not compromise the genetic integrity of oil palm cultivars, which is critical for maintaining desirable traits such as high oil yield and disease resistance (35).

Plant growth regulators

Plant growth regulators (PGRs) play an important role in oil palm micropropagation, affecting processes such as callus formation, shoot initiation, and root growth. Auxins, especially 2,4-D (2,4-dichlorophenoxyacetic acid), are necessary to encourage the development of callus on explants, including embryos and shoot tips. The concentration of 2,4-D between 1 and 2 mg/L was ideal for inducing callus (36), whereas it emphasized the significance of lowering 2,4-D concentrations in order to encourage somatic embryo differentiation at later stages (23). To promote shoot regeneration from callus, cytokinins such as BAP (6-Benzylaminopurine) and Kin (Kinetin) have been employed and shown that a combination of 2,4-D and BAP promotes shoot development. To balance callus growth and shoot differentiation, the auxin-to-cytokinin ratio is essential (37). Gibberellic acid (GA_3) has also been shown to increase shoot vigor and elongation, particularly during early shoot regeneration, demonstrating that GA_3 supplementation can increase shoot length and overall growth (38).

Shoot tip culture

Shoot tip explants are appropriate for clonal replication and produce high-quality plantlets (5). Direct-shoot organogenesis is a successful technique for oil palm clonal replication that circumvents the problems associated with somaclonal variation that arise in somatic embryogenesis. With a 4-week incubation time and ½MS medium with 9.8 µM 2-iP, shoot induction achieved 54.2%, and true-to-type, genetically stable plants were successfully developed in a greenhouse. In this context, true to type simply means that plants generated through this process remain genetically identical to the parent plant, maintaining traits such as growth, high yield and quality. Using molecular markers or cytological analysis, researchers confirmed genetic stability, which has been an issue in regenerated plants

produced through somatic embryogenesis. In short, the process is perfect for producing uniform, high- quality clones that can be used for commercial purposes. For the propagation of exceptional oil palm clones, this technique provides a dependable substitute (39). By increasing the number of primary and secondary roots, the study showed that immersing unrooted oil palm plantlets in a 2 mM NAA solution during *ex vitro* acclimation greatly increased rooting frequency from 15% to 80% and improved root quality. *Ex vitro* rooting streamlined the procedure and decreased the cost of producing seedlings, but root length remained unchanged (40). The study demonstrated effectiveness of shoot tip culture for oil palm clonal propagation by using solid and liquid OPM media enriched with 0.01 mg/L IAA and 0.46 mg/L BAP (41). Shoot tip culture of oil palm and growth regulators for oil palm

Table 1. Growth regulators used in palm micro propagation techniques

Growth regulator	Concentration	Effect	Species	References
Thidiazuron (TDZ)	5 mg/L	Induction of high embryonic callus (93%)	*Phoenix dactylifera*	(81)
Kin (Kinetin)	0.5–1.0 mg/L	Shoot proliferation	*P. dactylifera*	(82)
Zeatin	2 mg/L	Maximum shoot generation (16 shoots/plant)	*P. dactylifera*	(83)
GA3	0.5 mg/L	Enhanced shoot length	*P. dactylifera*	(83)
NAA	0.2 mg/L	Optimal rooting (81%)	*P. dactylifera*	(83)
IBA (Auxin)	1.0–2.0 mg/L	Root development and elongation	*P. dactylifera*	(84)
BAP (Cytokinin)	0.5–1.5 mg/L	Shoot initiation and proliferation	*P. dactylifera cv. Aziza Bouzid*	(85)
ABA	0.5–1.0 mg/L	Embryo maturation	*P. dactylifera*	(23)
Activated Charcoal	1–3 g/L	Reduces phenolic browning, improves callus health	*P. dactylifera*	(84)
NAA	1.0 mg/L	Enhanced callus formation	*Elaeis guineensis*	(86)
2,4-D (Auxin)	10–50 mg/L	Induction of embryogenic callus	*E. guineensis*	(87)
BAP	2.0 mg/L	Increase in number of leaves (19.35) and Leaf length (39.75cm)	*E. guineensis*	(88)
NAA + BAP	0.5 mg/L + 2.0 mg/L	Optimal regeneration medium	*E. guineensis*	(88)
BAP (Cytokinin)	0.5–1.5 mg/L	Shoot initiation and proliferation	*E. guineensis*	(89)
Kin (Kinetin)	0.5–1.0 mg/L	Enhances shoot proliferation	*E. guineensis*	(41)
GA_3 (Gibberellic Acid)	0.1–0.5 mg/L	Promotes shoot elongation	*E. guineensis*	(41)
NAA (Auxin)	0.1–1.0 mg/L	Root initiation in regenerated plantlets	*E. guineensis*	(42)
BAP (Cytokinin)	1–5 mg/L	Shoot initiation and proliferation	*C. nucifera*	(90)
Thidiazuran (TDZ)	22-44mg/L	Shoot induction and regeneration	*C. nucifera*	(54, 59)
IBA (Auxin)	0.5–1.5 mg/L	Root elongation and development	*C. nucifera*	(91)
Kin (Kinetin)	0.5–1.0 mg/L	Stimulates shoot proliferation	*C. nucifera*	(59)
Abscisic acid	0.5- 2.0 mg/L	Induces somatic embryo maturation	*C. nucifera*	(92)
Brassinosteroids	1.0 mg/L	Enhances callus formation	*C. nucifera*	(92)
Activated Charcoal	1–3 g/L	Reduces phenolic browning and oxidative stress	*C. nucifera*	(93)
2,4- Dichlorophenoxyacetic acid (2,4-D)	5.0mg/L	Stimulates callus induction	*C. nucifera*	(94)
Gibberellins	2 mg/L	Improved shoot formation	*Areca catechu*	(94)
Cytokinin (MS+BA)	20µM	Germination of Somatic embryo (74.02%)	*Areca catechu*	(95)
MS+NAA	1mg/L	95% of embryos developed into plantlets	*Areca catechu*	(72)
MS+GA3	3ppm	50% of shoots germinated	*Areca catechu*	(96)

propagation (Table 1).

Somatic embryogenesis

The development of elite oil palm plants and large-scale clonal replication are made possible through somatic embryogenesis, which is considered the best method for generating true-to-type oil palm plantlets (42). A study on oil palm somatic embryos identified three developmental stages—globular, scutellar, and coleoptilar. Techniques spanning somatic embryogenesis to synthetic seed production were employed, including the maturation of somatic embryos, the generation of friable embryogenic callus and somatic cell suspension. The maturation process involved the use of glutamine and arginine for storage protein accumulation, followed by abscisic acid to induce desiccation tolerance. The dried embryos were then used to create synthetic seeds by encapsulating them in alginate and calcium chloride solutions. After a month on germination media, the synthetic seeds sprouted (36). Somatic embryogenesis enables the production of large quantities of genetically identical embryos, thereby providing the oil palm industry with elite varieties characterized by high oil yield and enhanced disease resistance. The genetic uniformity achieved through tissue culture and clonal propagation offers significant advantages over seed-based propagation in terms of efficiency and cost-effectiveness. While the initial setup costs for tissue culture laboratories may be higher, genetic uniformity reduces long-term costs by minimizing losses due to disease and underperforming plants. Moreover, uniform crops optimize resource utilization, including irrigation and pest management, unlike seed-based plantations that require tailored inputs to accommodate the diverse needs of different plants (36). This technology offers significant advantages over traditional breeding, contributing to sustainability, enhanced oil production, and disease resistance (37). The future prospects of somatic embryogenesis in oil palm are promising due to potential improvements in genetic variation and disease resistance.

Arecanut (*Areca catechu*)

Propagation of arecanut remains challenging due to limited research and high dependency on seed-based methods, which can lead to variation in yield and quality due to factors such as genetic segregation, heterozygosity and environmental interactions. Genetic segregation occurs because seeds are the product of sexual reproduction, combining genetic material from two parent plants. Environmental factors during seed development and germination can further contribute to variability by affecting seedling vigor. Examples includes arecanut, date palm and oil palm. In arecanut, this method results in variations in nut size, yield and susceptibility to diseases like Yellow Leaf Disease (YLD), whereas tissue culture ensures uniformity and improved performance. In date palms, seed propagation results in variability in fruit size, sugar content and ripening time, making clonal propagation *via* offshoots a more reliable method. In oil palm, seed-based method produces significant variation in yield and oil quality due to genetic segregation, making clonal propagation through tissue culture the preferred method for uniformity and enhanced productivity (38). Embryo rescue techniques have been explored to improve germination rates and achieve better success *in vitro* propagation, particularly in challenging environments (17). Efforts are underway to develop cost-effective and scalable methods for arecanut micropropagation, as commercial viability remains a significant barrier. Standardizing these techniques will be crucial for achieving large-scale production and supporting the agricultural needs of regions heavily reliant on arecanut farming (43).

Somatic embryogenesis

The ICAR-Central Plantation Crops Research Institute in Kasaragod developed a repeatable protocol for arecanut somatic embryogenesis and plantlet regeneration from immature inflorescence explants. This enabled the rapid multiplication of elite genotypes, including palms that are resistant to Yellow Leaf Disease (YLD), Hirehalli Dwarf (HD), a naturally occurring short-statured mutant, and dwarf hybrids VTLAH–1 and VTLAH–2. Their resistance contributes to yield stability, allowing farmers to maintain consistent production even in disease- prone areas and it also supports long term sustainability by reducing the need for chemical inputs like fungicides and pesticides. Additionally, these palms may exhibit tolerance to environmental stresses, further enhancing the resilience of arecanut cultivation. In order to facilitate mass multiplication, immature inflorescence rachillae were cut into explants measuring 1-2 cm in size. These were then implanted in Y3 basal medium for somatic embryogenesis. The next culture step showed the induction of calli from higher auxin concentrations to lower ones. Hormone-free Y3 medium was used to create somatic embryos, and cytokinin supplementation resulted in germination. Subsequent plantlet development was accomplished in regeneration media with a 16-hour photoperiod in a light chamber (5).

Arecanut pollen collection and cryopreservation

There are several standardized steps in arecanut pollen cryopreservation, including pollen collection and desiccation, *in vitro* germination, viability, and fecundity investigations. Fresh and dehydrated pollen from three arecanut genotypes—Sumangala, Hirehalli Dwarf, and Hirehalli Dwarf x Sumangala—were used in *in vitro* viability assays. The dried pollen was directly submerged in liquid nitrogen for varying lengths of time (24 hours to 2 years) to cryopreserve it. Studies on fertility and viability were carried out with cryopreserved pollen. Male flowers that had fully opened were desiccated at room temperature (33 –34°C) to harvest pollen. Among the several sucrose media examined, 2.5 g/L of sucrose was determined to be the most effective for *in vitro* germination at room temperature. There was no discernible change in germination between desiccated and cryopreserved pollen, although pollen tube length decreased significantly in cryopreserved pollen in Hirehalli Dwarf and Sumangala. Cryopreserved arecanut pollen from HD x Sumangala was used in fertility trials at varying time periods (one month,

one year, and two years), yielding setting percentages of 70, 43, and 62, respectively. Using cryopreserved pollen, a typical nut set was observed. Setting percentages are a proportion of flowers or ovules that develop into fruit after pollination. Higher setting percentages directly relate with successful pollination, which leads to increased fruit and seed production, this reflects the fertility and productivity of the crop (44).

Coconut (*Cocos nucifera*)

Coconut propagation traditionally relies on seed propagation, which limits the preservation of genetic diversity and leads to variability in growth and productivity (45). Somatic embryogenesis has also been employed for mass propagation, although it requires precise conditions to avoid somaclonal variation, a common issue in coconut micropropagation (46). Recent advances in cryopreservation techniques have made it possible to store coconut embryos for extended period, safeguarding genetic resources and providing a means for preserving endangered coconut varieties (47). Addressing somaclonal variation remains a key focus, with recent research exploring methods to enhance genetic stability, such as optimizing growth media and environmental conditions *in vitro* (48).

Somatic embryogenesis

Numerous factors, including the genotype of the donor plant, the type of explants, the composition of the media, plant growth regulators, polyamines and DNA methylation levels, influence somatic embryogenesis (49). Somatic embryogenesis can also be induced by stress-related variables such as nutrients deficiency, wounding, extreme temperatures (heat or cold), osmotic shock, water scarcity, heavy metals, mild dehydration, UV light, and pH (50). Somatic embryogenesis in coconut palms is supported by a various tissue, including unfertilized ovaries, plumules, rachillae, seedlings, stem sections from early leaves, and immature embryos (51).

Axillary bud culture

Apical meristems, which are influenced by apical dominance, often result in axillary meristems that were induced from pre-existing meristematic cells becoming inactive. The growth of axillary buds can be directly favored by phytohormones such as cytokinin. Two distinct protocols (cut and meristem) were developed for inducing axillary shoot production *in vitro* by means of meristem proliferation in various coconut varieties. In the cut procedure, the induction of proliferating meristems from the initial explants were recorded at a rate of 30.6%, while in the meristem protocol, the rate was 18.8%. Out of every regenerated meristem clump, 5 to 18 *in vitro* plantlets were produced (44, 52). The "cut" treatment with 1 µM TDZ significantly promotes apical meristem proliferation, achieving a 33.3% initiation rate in coconut palms. This method offers an efficient alternative to somatic embryogenesis, enabling indefinite multiplication with lower risks of off-type plants. It addresses the industry's demand for high-quality planting material, supporting sustainable coconut production (53).

Shoot tip culture

Shoot tip culture offers significant advantages over somatic embryogenesis and other indirect methods, primarily due to its reduced risk of somaclonal variation. Plantlets generated through direct organogenesis methods, such as shoot tip culture, are typically true to type. Furthermore, embryo-derived shoot tip culture eliminates the need to destroy mature coconut plants. Sliced shoot tips have the potential to overcome apical dominance. While somatic embryogenesis is often regarded as the most effective approach for coconut micropropagation, it is associated with risks such as somaclonal variation and fidelity issues. The study recorded shoot regeneration in Y3 media enriched with 200 µM TDZ using whole shoot tip, two and four sliced shoot tips (embryo-derived shoot tips) (84%, 164% and 325%) respectively. It also produces faster results than alternative techniques (54). The TDZ concentration for shoot tip culture and embryo-derived shoot tip culture in coconut is represented in Table 1 & Fig. 2.

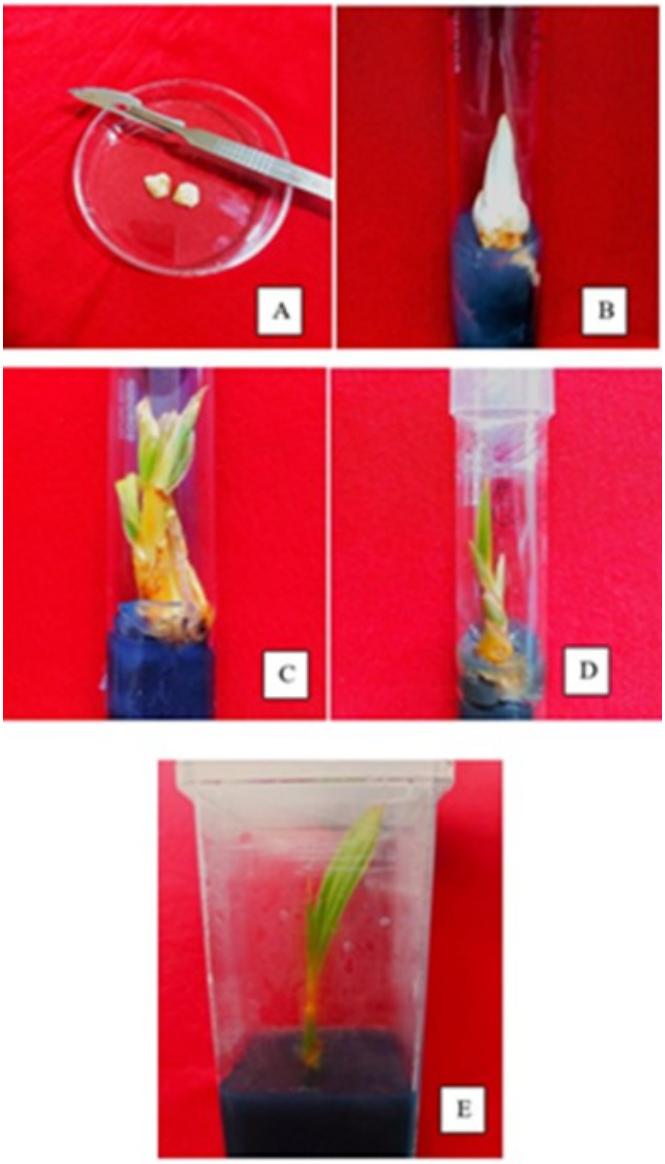

Fig. 2. Embryo-derived shoot tip culture in coconut (A) Sliced shoot tip (B) 30 DAI (C) 60 DAI (D) 120 DAI (E) 150 DAI as [adapted from (54)].

Sliced embryo culture

Three distinct types of proliferative responses, including whitish nodular callus, yellowish rounded callus, and whitish dome-like mass, were observed in coconut embryo tissues. These tissues were transversely cut and cultured in media enriched with 100 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.25% of activated carbon (55). The process of embryo splitting was used to double the output of Macapuno seedlings (56). The meristem region of the embryo was cut, and after four weeks of culture, the embryos were divided in half and placed in MS media with 2 µM IBA and 15 µM kinetin for recovery. This procedure resulted in 30 zygotic embryos producing 56 shoots (57). When inoculated in Y3 media supplemented with 150 µM TDZ, sliced embryos produced the highest rates of shoot initiation (75%) and regeneration (66.6%) in the East Coast Tall variety (ECT) of coconut (58). When cultivated in Y3 media supplemented with 150 µM TDZ, longitudinally sliced embryo explants of the Chowghat Orange Dwarf (COD) coconut cultivar showed significant shoot induction (90.9%) and regeneration as given in Table 1 (59). When the embryo was cut into four pieces and cultivated in Y3 medium supplemented with 200 µM TDZ, there was a higher induction and regeneration of shoots (44).

Cryopreservation

A traditional cryopreservation protocol was used in the first attempt to cryopreserve coconut tissues. This protocol entailed gradual freezing and chemical dehydration of premature zygotic embryos. Cryopreservation of zygotic embryos is an effective technique for conserving coconut germplasm. The study compared the seedlings recovered from cryopreserved zygotic embryos with their non-cryopreserved counterparts and found no morphological, cytological, or molecular differences (55). A 60% recovery rate was reported after mature zygotic embryos were desiccated for 10 hours and then cryoprotected for 15 hours in a solution containing 15% glycerol (56). Applying an 18-hour silica gel desiccation pretreatment to mature embryos resulted in a high recovery rate of plantlets (55). Zygotic embryos subjected to chemical dehydration with plant vitrification solutions achieved a survival rate of 70 to 80% and a plant recovery rate of 20 to 25% (60). In order to preserve germplasm, the Central Plantation Crops Research Institute in Kasargod has developed a zygotic embryo culture procedure (61).

Technological innovations in palm micropropagation

use of bioreactors for large-scale propagation

The use of bioreactors has revolutionized large-scale palm micropropagation, providing an efficient and scalable method for producing high volumes of uniform, high-quality plants. Bioreactors create controlled environments that support the rapid multiplication of somatic embryos or organogenic cultures, significantly reducing the risk of contamination and the labor required compared to traditional tissue culture methods. In India and Southeast Asia, for instance, bioreactors have allowed for mass production of tissue cultured bananas, which have found a substantial place in the agricultural economy and market of the region, owing to yield and quality improvements enabled at low cost. Such examples highlight the economic implications and practical applications of bioreactor technology (62). Temporary immersion bioreactors (TIBs) have been especially successful in oil palm and date palm micropropagation, allowing for precise control over nutrient supply and gaseous exchange, thereby enhancing growth rates and reducing production costs (63). This method has proven essential in meeting the commercial demand for uniform palm plantlets in regions where these species play a critical economic role.

Advances in cryopreservation techniques for long-term storage of germplasm

Cryopreservation has emerged as a powerful tool for the long-term storage of palm germplasm, providing a reliable way to preserve genetic material for conservation and breeding purposes. Cryopreservation involves storing plant tissues or embryos at ultra-low temperatures, typically in liquid nitrogen (-196°C), where metabolic processes are suspended, enabling indefinite storage without genetic deterioration (55). For palms, which are often difficult to conserve through traditional seed storage due to recalcitrant seed behaviour, cryopreservation of somatic embryos or embryogenic callus has proven effective, particularly in date palm and coconut (55). Recent advancements have improved the survival and regeneration rates of cryopreserved materials, ensuring that genetic resources remain viable and accessible for future research and propagation. Although this technique is very useful in maintaining a phyto diversity, it also presents significant challenges. Cryopreservation also requires considerable technical know-how to maintain the viability of preserved specimens, as well as careful manipulation during cryogenic operations. Moreover, the costs of the specialized equipment, cryoprotectants and facilities for long-term storage may be high, especially in resource-limited settings. These technological barriers are crucial to overcome in order to drive adoption of this technology across the wider agricultural system (64).

Role of molecular markers in assessing genetic stability

Molecular markers have become essential tools for assessing and ensuring genetic stability during the micropropagation process, particularly for long-lived species like palms. Techniques such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), and Inter-Simple Sequence Repeats (ISSR) enable researchers to monitor clonal fidelity and detect any genetic variation arising during *in vitro* culture (60). This approach has been instrumental in oil palm and date palm micropropagation, where even minor genetic variations can impact commercial traits like fruit yield and disease resistance (65). Therefore, molecular markers serve as a critical quality control mechanism, ensuring that only genetically stable plantlets advance to commercial cultivation (66). Microsatellite-based characterization of

coconut accessions was conducted for various regions, including the Andaman and Nicobar Islands (100 palms, 26 accessions), Amini and Kadmat Islands (56 palms, 9 accessions), Minicoy Island (67 palms, 4 accessions). The study also included tall germplasm (243 palms, 20 accessions) and dwarf germplasm (134 palms, 24 accessions) and 'MohachaoNarel' (28 palms) from Maharashtra. The study assessed the population structure and genetic diversity within these accessions. The characterization of Annur, Bedakam, and Kuttiyadi ecotypes, along with a comparison to the West Coast Tall (WCT) cultivar, revealed distinct genetic differences, suggesting the ecotypes' divergence from the WCT population (67).

Challenges and limitations

While micropropagation holds great promise for the mass production of high-quality, uniform palm plants, several challenges and limitations must be addressed to optimize its effectiveness. These limitations include a low multiplication rate, poor rooting and a high mortality rate of *in vitro*-grown plantlets during the transfer from the laboratory to the soil. These challenges are commonly encountered in date palm tissue culture micropropagation (68). Another difficulty is the inefficiency of seedling production and supply, particularly in situations when local populations' resistance to possible disease threats is unpredictable (69). Many methods and strategies have been investigated to address these issues, including the use of various explants, specific treatments for embryo maturation and the addition of polyamines and silver thiosulfate to the culture medium. Further research is needed to reduce the risk of somaclonal variation and improve the efficiency of tissue culture techniques, which are essential for producing large quantities of palms with desirable traits.

High costs associated with tissue culture and maintenance of sterile conditions

One of the primary limitations of micropropagation is the high cost associated with setting up and maintaining tissue culture facilities. Establishing a sterile environment is essential to prevent contamination, which can lead to significant losses in cultured material. The costs of equipment, culture media and energy needed to maintain sterile conditions are high, making micropropagation an expensive process compared to traditional methods (10). For large-scale commercial production, the need for specialized culture vessels, air filtration systems, and regular sterilization procedures further increases operational costs (70). High-quality culture media and consumables, including culture vessels, glassware, plasticware, cotton plugs and pipettes, are significant recurring expenses. Additionally, maintaining optimal growth conditions, such as controlled temperature, light and humidity, requires substantial energy resources. While micropropagation allows for rapid, disease-free plant development, it has greater upfront expenses due to specialized equipment, sterile conditions, and expert workers. In comparison, traditional procedures such as cuttings and grafting are less expensive but slower and more susceptible to pests and diseases, thus raising long-term expenditures. Understanding these trade-offs allows stakeholders to select the strategy that best meets their economic and production needs (14). All things taken into account, these expenses make micropropagation a far more expensive alternative to conventional propagation techniques, which may deter its adoption in agricultural applications (71).

Risk of somaclonal variations and genetic instability in *in vitro* propagated plants

Somaclonal variation, or unintended genetic changes that occur during tissue culture, is a major concern in the micropropagation of palms, as it can lead to phenotypic abnormalities and reduced uniformity in plant characteristics (72). This genetic instability is particularly problematic in commercially propagated species like oil palm and date palm, where consistency in yield and quality is essential (73). The presence of somaclonal variation can also compromise research and breeding efforts by introducing unintended traits, thus reducing the reliability of micropropagated plants.

Limited success rates in establishing mature, field-ready plants

Despite advances in tissue culture techniques, the transition of *in vitro* plants to the field remains challenging. Palm species often struggle with acclimatization, as the shift from controlled, sterile conditions to natural environments can lead to high mortality rates (74). Additionally, tissue-cultured plants may lack essential symbiotic relationships, such as with mycorrhizal fungi, which support nutrient absorption and improve growth in natural soils. Microbial treatments can enhance a plant's resistance to pathogens by competing with harmful microbes or inducing systemic resistance. Mycorrhizal fungi can help plants tolerate environmental stress such as drought and salinity and also assist plants in accessing essential nutrients like phosphorous and nitrogen more efficiently (14). These factors result in lower success rates in establishing mature, field-ready plants, which can limit the scalability of micropropagation for commercial agriculture.

Need for specialized equipment and expertise

Micropropagation is a technically demanding process that requires skilled personnel and specialized equipment, limiting its accessibility to research institutions and well-funded commercial operations. The high installation and operating costs of tissue culture facilities are one of the main obstacles to micropropagation. Acquiring specialized equipment such as sterilizing tools, autoclaves and laminar airflow cabinets can often be expensive, thus contributing to additional expenses. Techniques such as somatic embryogenesis, cryopreservation, and molecular marker analysis require not only precision but also expertise in plant tissue culture and genetic analysis, which are not readily available in all regions. Overcoming challenges with simplified culture techniques, such as temporary immersion bioreactors and cost-effective alternatives for culture media preparation could open

new doors for micropropagation, including cryopreservation and molecular marker analysis. However, emerging technology and economically viable solutions may be the key to overcoming the high costs and the need for specialized skills, infrastructure and experience required for advanced techniques like somatic embryogenesis. Open-source designs for crucial equipment, such as laminar airflow hoods and sterilizing systems, can help cut costs. Creating community labs and collaborating with universities and businesses can help make advanced plant tissue culture technology more accessible. These strategies can increase the efficiency and accessibility of plant tissue culture globally (75). The shortage of trained personnel and high costs of equipment, remain barriers to the widespread adoption of micropropagation, particularly in developing regions where resources are limited (76).

Future Prospects

The future of palm micropropagation is poised for exciting advancements, driven by emerging technologies and collaborative efforts. One promising avenue is the application of molecular and gene-editing techniques to introduce targeted genetic modifications in palm species. These techniques enable precise alterations to enhance desirable traits, such as increased yield, drought tolerance, or resistance to diseases like lethal yellowing in coconut and Fusarium wilt in oil palm (77). This genetic precision could significantly accelerate breeding efforts and lead to the production of robust palm varieties tailored to meet agricultural demands (78). Integrating artificial intelligence (AI) and machine learning (ML) into micropropagation processes represents another forward-looking approach. AI and ML algorithms can analyze data from various *in vitro* culture experiments to predict optimal growth conditions, adjusting factors such as nutrient composition, lighting, and temperature. This data-driven optimization can enhance growth rates, reduce contamination, and improve efficiency in laboratory settings, especially for complex protocols like somatic embryogenesis (60). AI-enabled systems could also facilitate monitoring and quality control, providing real-time feedback to minimize somaclonal variation and genetic instability. To address the high costs and scalability issues in palm micropropagation, developing cost-effective, high-throughput bioreactor systems will be crucial. Newer, modular bioreactor designs could allow for the production of large volumes of uniform plantlets while reducing resource consumption and labour costs (79).

Public-private partnerships will play a critical role in advancing micropropagation research. By combining government resources, research expertise, and industry funding, these collaborations can accelerate the development and commercialization of innovative technologies. Partnerships between universities, agricultural research institutions, and companies can help bridge resource gaps, providing essential infrastructure, training, and funding to support micropropagation initiatives. This collaborative approach is especially important for ensuring sustainable cultivation of major palm species, fostering both agricultural productivity and environmental conservation (80).

Conclusion

Advancements in micropropagation have revolutionised palm cultivation by enabling sustainable large-scale production of genetically uniform, high-quality plants through innovations like bioreactor systems, cryopreservation, and molecular markers. Despite these achievements, scalability remains limited by high costs, insufficient funding, and inadequate research infrastructure, underscoring the need for collaborative international efforts to share resources and expertise. Emerging technologies such as gene editing, artificial intelligence, and cost-effective culture systems have the potential to address these challenges by improving yields, disease resistance, and resilience to environmental stresses. Investment in partnerships and innovation will ensure the sustainability of economically vital palm species, strengthen global food security, and enhance local economies while conserving genetic diversity.

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Authors' Contributions

KP and RR contributed to writing the original draft. RR handled review and editing. RR and MBN were responsible for conceptualization, data curation, methodology and project administration. RV, SJ and RS provided resources, supervision and validation. All authors have read and approved the final manuscript.

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