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Micropropagation of *Tamarindus indica* L. - An underutilised tree: A critical review on methods and challenges

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Abstract

Tamarind (*Tamarindus indica* L.) is a tropical, multipurpose fruit-bearing tree that remains underutilised despite its nutritional, industrial and economic benefits, as well as its agroforestry value. Micropropagation offers a viable alternative to conventional propagation methods to derive true-to-type plantlets of elite tree species on a large scale. This review provides a comprehensive overview of tamarind micropropagation methods and their success rates, which are mainly influenced by explant type and age, media composition and combinations of specific plant growth regulators (PGRs) required at different culture stages. Based on the available reports, seedling-derived explants from < 20 days old (cotyledons, cotyledonary nodes and hypocotyl) and shoot tips or nodal segments of young shoots are highly suitable for clonal propagation, while somatic embryogenesis is the least responsive and remains challenging for tamarind micropropagation. Murashige and Skoog (MS) medium with different PGRs, either alone or in combinations of 6-benzylaminopurine (BAP), naphthaleneacetic acid (NAA), kinetin (KN) and indole-3-acetic acid (IAA), is most effective for shoot or root induction and multiplication from seed-based and nodal explants of tamarind. For callus induction from tamarind explants, MS medium supplemented with KN, BAP and NAA, as well as PGR-free woody plant medium (WPM), are promising. Currently, the root induction and acclimatization protocols and strategies required for the successful ex vitro plantlet establishment and survival of tamarind are limited. Further research is required to optimise rooting and acclimatization processes for the efficient acclimatization, *ex vitro* establishment and commercial-scale propagation of tamarind.

Keywords: micropropagation; seed-based explants; shoot tip and nodes; tamarind; tissue culture; woody fruit tree

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1. Introduction

Tamarind (*Tamarindus indica* L.), known as Indian tamarind or imli, is an evergreen or semi-evergreen multipurpose fruit-bearing tree belonging to the Fabaceae family. Tamarind is a dryland fruit crop that is native to tropical Africa and widely distributed and naturalised in tropical and subtropical regions worldwide, including Central America, South Asia, Southeast Asia and Oceania (1). India and Thailand are leading global producers of tamarind on a commercial scale. In Asian and African nations such as India, Sri Lanka, Thailand, Nigeria, Kenya and Sudan, the majority of tamarind producers are smallholder farmers. Tamarind can grow in a wide range of soils and can adapt to adverse conditions, including alkaline and saline environments. It can withstand prolonged drought and heat (1, 2). These characteristics of tamarind make it ideal for sustainable agriculture, economic benefits and profitability of small-scale farmers and rural families in semi-arid and dryland conditions (3, 4). In addition, the tamarind tree holds significant value for dryland and wasteland development due to its capacity to withstand various adverse climatic conditions.

The pulp, leaves and seed have nutritional, medicinal and industrial values. There is a growing demand for tamarind (mainly pulp and seed) in food, beverages, pharmaceutical and cosmetic industries due to increasing consumer preference for and awareness of natural, healthy and plant-based products (5, 6). Tamarind seed polysaccharides, a natural non-ionic biopolymer, have been used in the food industry and pharmaceutical industry as well as in therapeutic drug delivery systems (7). Tannins from the tamarind seed coat are considered eco-friendly natural dyes and bio-mordants with a vast potential for application in textile and leather industries (8, 9). The tamarind fruit is its most valuable component; it is widely utilised as a food additive due to its uniquely tangy and sweet flavour and it serves as a natural preservative in the food industry (10). Recent market analyses project substantial growth in global demand for tamarind-derived products, including fruit concentrate, seed powder and seed polysaccharides (11–13). Therefore, the growing demand for tamarind products and the resultant market dynamics are driving the expansion of tamarind cultivation and orchards. However, tamarind remains an underutilised fruit tree

crop. This is largely due to difficulties in selecting and multiplying high-yielding, uniform genotypes at a commercial scale using conventional breeding and vegetative propagation, a complication resulting from the high genetic diversity and phenotypic variability amplified by its cross-pollinating nature. In addition, the establishment of commercial plantations is hindered by the slow and time-consuming nature of conventional breeding methods, the prolonged juvenile phases of seed-grown trees (typically 8–10 years and up to 12 years before first fruiting), lack of nurseries for quality-controlled seedlings and plants, inadequate harvesting and processing facilities, vulnerability to post-harvest losses and a weak or fragmented market chain (14–16).

The vegetative/asexual propagation methods such as cleft grafting, veneer grafting and air layering are widely used for commercial propagation of tamarind due to the ability to maintain genetic consistency with the parent plant, faster maturity and earlier fruit production than plants from seeds (17–19). However, the success rate of these propagation methods is greatly influenced by several key factors such as restriction to specific plant sizes or plant parts, the requirement for skilled labour, graft failure stemming from improper techniques, seasonal and environmental constraints and susceptibility to abiotic and biotic stress (such as insect and microbial invasions), which collectively hinder large-scale commercial propagation (18–20). Therefore, the adoption of improved clonal propagation methods is essential to overcome these limitations and unlock the commercial potential of tamarind.

Micropropagation, commonly known as *in vitro* propagation or tissue culture, is an important and key biotechnological tool for the propagation of tree crops, including tamarind. Micropropagation has driven the commercial revolution in the food and plant-based industries through overcoming the major limitations of conventional propagation methods, mainly ensuring identical genetic traits (genetic homogeneity), cultivation of pathogen/disease-free plants, enabling year-round production of plantlets and facilitating large-scale production in a cost-effective and efficient manner (21, 22). In this review, we summarise and consolidate the current knowledge of micropropagation of tamarind and its efficiency, limitations and scope for commercial propagation, agricultural expansion and improving the economic value of tamarind.

Review methodology

The literature review was conducted following the PRISMA 2020 guidelines, encompassing the stages of identification, screening, eligibility assessment and inclusion (Fig. 1).

Identification

This review focuses on the major aspects of micropropagation of *T. indica*, including explant selection, culture media, plant growth regulators (PGRs), regeneration pathways and acclimatization. The earlier works focus on fundamental aspects of micropropagation in tamarind. Recent research focuses on successful micropropagation using various explants, growth hormones and callus induction as well as somatic embryogenesis.

The initial phase of article identification was based on searching online databases such as Google Scholar, ResearchGate, Centre for Agriculture and Bioscience International (CABI) and ScienceDirect. Advanced title searches were conducted with the following keywords: "Tamarindus" OR "Tamarind" AND "micropropagation" OR "clonal propagation" OR "*in vitro* regeneration" OR "*in vitro* propagation" OR "tissue culture" AND "cotyledon" OR "Hypocotyl" OR "somatic embryogenesis" OR "axillary bud" OR "nodal" OR "shoot tip" OR "cotyledonary" OR "explant" OR "sterilisation" OR "Organogenesis" OR "acclimatization". Publications between 1990 and 2026 were considered to capture the historical development and recent advances in tamarind micropropagation. A total of 1870 articles were identified in this search when Boolean operators such as OR and AND were used to identify appropriate articles, excluding theses. The document types included research articles, reviews, book chapters and conference papers.

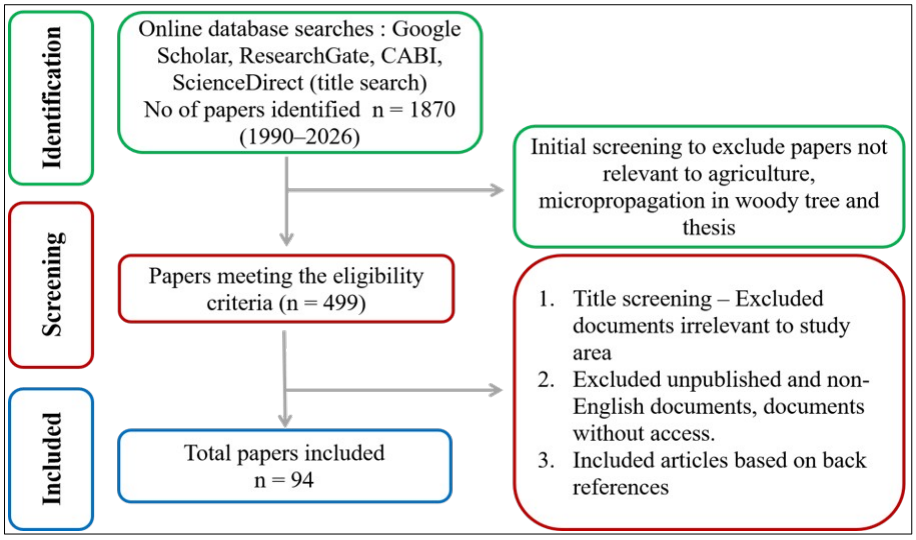


Fig. 1. PRISMA framework for selection of relevant publication.

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Screening

The retrieved articles were screened for relevance to tamarind micropropagation. From the initial identification, articles were mined for relevance to study the micropropagation of tamarind. Articles that were unrelated to plant tissue culture, micropropagation or tamarind biology were excluded during the initial screening stage. This resulted in 499 eligible articles. Further screening resulted in the exclusion of papers that were not related to this review and lacked relevant data. Restricted access, unpublished and non-English articles were also excluded from the review. Manual screening of each article was done by examining the title of the paper and the focus area of the paper. From the references, relevant articles were identified and included in the study. This resulted in a total of 94 papers included in this study.

Reporting

In this final phase, the key results of the articles were synthesised. The findings of these research publications were comparatively analysed with micropropagation studies in other tree crops wherever relevant. Consequently, a total of 94 articles were utilised for this critical review, highlighting the generally available micropropagation methods in tree crops and specifically covering the type of explants used, media composition, growth hormone composition, sterilisation and hardening techniques for successful tamarind micropropagation.

Major micropropagation methods of fruit tree species

Micropropagation is an indispensable tool for sustainable advancement and large-scale cultivation of woody plants, in particular fruit trees. This technique not only enhances the rapid multiplication and breeding of selected genotypes or cultivars that are difficult to propagate by traditional methods, but is also vital for the elimination of pathogens, germplasm conservation and preservation of endangered/elite tree species (23, 24). The efficiency of this process depends on optimising several key stages such as explant selection, sterilisation, culture medium and PGRs, shoot multiplication, rooting and final acclimatization (24–26).

Micropropagation technique is broadly classified based on explant types (meristematic culture or shoot/root apical meristem culture, shoot/root tip culture, nodal segment or node culture, leaf or cotyledon culture, zygotic or somatic embryo culture, anther culture and callus culture) and regeneration pathway (organogenesis or somatic embryogenesis) (23, 24, 27). However, the choice and use of appropriate micropropagation methods primarily rely on the specific characteristics of tree species and the purpose of propagation. *In vitro* micrografting, which combines tissue culture with conventional grafting technique, is particularly useful for woody species exhibiting poor rooting ability (28).

Axillary bud proliferation (a type of nodal culture), a direct organogenesis method that primarily uses shoot tip or nodal segments (pre-existing meristem) from healthy plants, remains a common and reliable method for commercial propagation of woody fruit trees due to a high success rate (up to 90 %), genetic stability and true-to-type propagation (29, 30). In addition, the adventitious shoot formation method (a type of organogenic culture), in which shoots are generated from leaves, internode segments, roots or callus tissue, is indispensable for commercial propagation of tree species with recalcitrant seeds and/or poor rooting ability, including mango, apple, peach and apricot. Under optimised procedures with diminished phenolic browning, the success rate of this method is up to 80 % (depending on species and explant type) accompanied by high multiplication potential, ensuring pathogen/disease elimination and genetic uniformity (31–34).

Meristem culture, involving *in vitro* culture of shoot apical or axillary meristems which are naturally pathogen-free plant parts, is widely used for production of virus-free planting materials. Despite its technical complexity, such as highly skilled labour requirements, high cost and low initial establishment rate, meristem culture is an ideal method facilitating rapid multiplication of disease-free true-to-type plantlets for commercial propagation of fruit trees such as apple, citrus and stone fruits that are more susceptible to viral infection, germplasm conservation and rescue of endangered or rare species (35–37).

Somatic embryogenesis is an advanced *in vitro* propagation method, mimicking zygotic embryogenesis, but inducing embryo formation from somatic cells of leaves, stem sections, immature seeds and immature zygotic embryos (38). It is a powerful and highly valuable technique for large-scale clonal propagation, genetic improvement, synthetic seed production and conservation of germplasm of woody fruit trees such as mango, guava, date palm and citrus, which are often facing difficulties in conventional propagation methods due to recalcitrance and long juvenile period (39–42) (Table 1). Among woody fruit species, tamarind presents unique challenges for *in vitro* propagation because of its phenolic-rich tissues, prolonged juvenile phase and limited success of conventional propagation methods. Therefore, optimisation of micropropagation protocols is essential for its large-scale multiplication and conservation.

Table 1. Major micropropagation methods of woody fruit trees

Micropropagation method	Explants used	Key findings	Success rates	Limitations	References
Axillary bud proliferation (nodal culture)	Shoot tips and nodal segments (pre-existing meristems) from healthy plants	High genetic stability and true-to-type. Propagation established commercial method.	Up to 90 %	Limited multiplication rate versus indirect methods. Species-specific responses.	(29, 30)

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				Requires vigorous donor plants.	
Adventitious shoot formation (organogenesis)	Leaf, Internode, root or callus explants	Enables propagation of species with recalcitrant seeds or poor rooting. High multiplication potential; pathogen elimination.	Up to 80 % (species- and explant-dependent)	Phenolic browning. Somaclonal variation risk. Genotype-dependent response.	(31-34)
Meristem culture	Shoot apical or axillary meristems	Produces virus-free and genetically uniform plantlets. Ideal for germplasm rescue and elite cultivar propagation.	Variable; Often low initial establishment.	Technically complex. Labor-intensive. High cost and time requirements. Low establishment rates.	(35-37)
Somatic embryogenesis	Leaf/stem sections, immature seeds, immature zygotic embryos	Mimics zygotic embryogenesis. Supports large-scale clonal propagation. Synthetic seed production Germplasm conservation.	Species- and protocol-dependent. High potential.	Genotype-dependent induction. Risk of abnormal embryos. Low embryo-to-plantlet conversion. Somaclonal variation; technical demands.	(39-42)

Micropropagation of tamarind

Selection of explant

The explant type, size of explant, its age and orientation of the explant in culture media are most important aspects for the initial establishment of *in vitro* tissue culture and successful regeneration of plantlets. In phenol-rich plants such as tamarind, the explants from juvenile and young plants have higher regenerative capacity, due to low phenolic content, than old trees (43). The germinating seeds are the ultimate source for explants such as cotyledons, cotyledonary nodes and hypocotyl segments (Fig. 2a–2c). To obtain the explants from seedlings, the seeds should be aseptically prepared and grown in laboratory conditions (44, 45). Most importantly, the tamarind seeds developing a wrinkled/cracked seed coat after the sterilisation procedure should be eliminated and seeds only with a smooth seed coat should be used for germination. The seeds with a wrinkled seed coat are often prone to microbial infection and reduce the vigour of seedlings by hindering the germination process under *in vitro* conditions (46). Thus, the selection of healthy seeds is a key determinant in the successful establishment of *in vitro* culture from seed-based explants.

Under optimised conditions, 100 % shoot induction with high proliferation rate (~9 shoots/per culture) and > 90 % shoot elongation and root induction can be achieved using the cotyledonary node of tamarind as an explant. Most importantly, the cotyledonary node should contain a part of the hypocotyl (~5 mm) below the cotyledons but without a terminal bud and half to a third of the distal part of the cotyledons (44). A cotyledon node from a developing tamarind seedling, specifically at the stage where the cotyledons have completely emerged from the seed coat but the first true leaves have not yet expanded (Fig. 2a), is the best suited and most competent explant for achieving 100 % induction and a high proliferation coefficient in tissue culture. However, the duration taken for the emergence until reaching this developmental stage varies between 12 and 20 days after sowing (DAS), depending on the genotype and habitat of the mother plant (44). In addition, the cotyledons of tamarind generally respond well to callus induction, but shooting from cotyledonary callus is limited in comparison with shooting from leaf callus when the shoot induction media include kinetin (KN) and 6-benzylaminopurine (BAP) as growth regulators (45).

Direct organogenesis using cotyledons of 12–15 days old tamarind seedlings offers 100 % regeneration (producing 2–3 shoots/explants) and generates a greater number of shoots with high rooting ability (~90 %) in culture medium under optimal conditions (47). The cotyledonary nodes of 5–8 days old seedlings also exhibit remarkable shoot proliferation (86.6 %) and multiplication ability (48) (Fig. 2b and 2c). In contrast, cotyledonary nodes of 4-weeks old seedlings only have 68 % shooting efficiency and 82 % rooting ability (49). The hypocotyl segment of 12-day old tamarind seedlings showed considerable regeneration of shoot buds (bud formation from > 66 % of hypocotyl explants) and roots (50). These findings reveal that regenerative response, in particular shoot formation, is high and limited to cotyledonary nodal tissues from young seedlings of tamarind. Moreover, the stage and age of the cotyledon node is important for maximal efficiency of micropropagation.

The size and orientation of the cotyledon is also an important determinant of regenerative response. The proximal halves of transversely sliced cotyledons and the adaxial surface have high regeneration capacity (100 %), while complete cotyledons exhibit only 80 % regeneration *in vitro*. But the transverse distal halves or longitudinal halves of cotyledon segments do not have any regenerative response (51). Thus, the whole cotyledons or transverse proximal halves of cotyledons of tamarind

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are the best choice for the plantlet generation *in vitro* (44, 51). For the best/maximum regeneration frequency, the proximal part of the cotyledonary node should be kept upward and obliquely in culture medium (44). The morphogenic response and regenerating frequency of tamarind cotyledon explants can also be enhanced by placing the adaxial surface of cotyledons on culture medium. In contrast, the regenerating response and shoot forming capacity of cotyledons are radically reduced when cotyledons are kept longitudinally or the distal end or abaxial surface is embedded in the culture medium (51).

Zygotic embryo culture is an important technique for woody and recalcitrant tree species, particularly for overcoming physical and biotic challenges associated with breaking dormancy in traditional seed germination. This method is useful for preserving valuable genetic combinations through rescuing and enabling development/growth of hybrid embryos (52, 53). The zygotic embryos of tamarind have 50–60 % germination response in the culture medium supplemented with an appropriate concentration of nutrient salts (25–50 %). A healthy and intact zygotic embryo can be easily extracted from tamarind seeds by imbibing them in sterile deionised water for 48 hr (54). In many species of the Fabaceae family, the cotyledons not only serve as a nutrient reserve for the initiation of germination but are also indispensable for hormonal and moisture balance by forming vascular connections. Thus, zygotic embryo without cotyledons generally lacks germination potential and has a low percentage of development of shoot/root segments *in vitro* (54, 55). For the best caulogenic response, the longitudinal section of the embryo axis should contain cotyledons and the adaxial side of the cotyledon should directly be in contact with the culture medium, wherein the cotyledon turns green and elongate within 4–6 days and a shoot bud appears within 3 weeks. However, the embryonic axis without cotyledons fails to respond in the culture medium (55). Under optimal conditions, the mature zygotic embryo axis, consisting of embryonic shoot apex and radicle (embryonic root), from tamarind seed can efficiently regenerate plantlets through direct adventitious bud formation, a type of direct organogenesis method, without callus induction. In this method, up to 85 % of shooting induction and 70 % rooting efficiency can be achieved under optimal conditions (55).

Somatic embryogenesis, producing embryos from somatic cells utilising cellular totipotency, is an efficient morphogenic method for producing new plants through supplementation of adequate plant growth regulators, culture medium and incubation conditions. There are direct or indirect embryogenic pathways in which embryos are formed without callus formation from isolated tissue or by forming callus prior to somatic embryo formation respectively (56). In general, the induction of somatic embryogenesis is feasible and easier from seed/seedling-based explants, including immature zygotic embryos (IZEs), having higher embryogenic potential, in most of the hardwood species (39, 57, 58). An attempt to regenerate tamarind plantlets via somatic embryogenesis, using immature zygotic embryos (IZEs) from green and tender pods (12–16 weeks old) resulted in a relatively low (maximum 26.7 %) and inconsistent embryogenic response (59). The somatic embryos (SEs) primarily originate from epidermal and subepidermal tissues of the apical region of IZEs. The size of IZE explant, gelling agent in nutrient media and light/dark period greatly influence the somatic embryogenic responses of IZE of tamarind. IZEs with a size of less than 6 mm exhibit morphogenic potential; specifically, IZEs of 3 mm in size possess the highest SE formation potential. But IZEs with a size of more than 6 mm do not respond in culture even after extended incubation (9 weeks) and exhibit browning of explants. In addition, minimal response in SE formation (26.7 %) was found when cultured in nutrient media containing phytagel and maintaining the culture under dark condition and even lower response of 17.5 % embryogenic response, when 16 hr light period was provided. In addition, the maturation and proliferation of SEs, upon subculturing through repetitive embryogenesis, is slow. Their development and morphogenic responses are poor (less differentiation of shoot and sporadic rooting) and conversion into plantlets is poor due to failure of shoot differentiation and thickening and stunting of roots. Above all, the embryogenic response and frequency of developmental response of SE in tamarind are genotype-dependent, which is common in leguminous tree species (59). Current studies suggest that somatic embryogenesis in tamarind remains challenging and requires further optimisation before large-scale commercial application.

The shoot tip and nodal segments of tamarind can also be used as explants. The nodes from young shoots/branches of adult trees respond better in adventitious shoot proliferation or axillary bud formation methods of micropropagation. The apical or floral meristems are suitable for adventitious shoot proliferation or axillary bud formation. However, the pre-existing lateral bud in the nodal segment of young shoots from adult trees responds better with 59 % shooting initiation and 62 % rooting ability when compared to explants from mature shoots (survival rate < 40 %) (60). Seedlings that are 40–45 days old yield up to 5 nodal segments and are a suitable explant for *in vitro* culture of tamarind (54, 61). This explant can produce friable calluses with sprout induction efficiency about 70–80 % and survival rate about 70–85 % in culture medium containing appropriate concentration of salt and charcoal (54). The plantlets produced/generated, using *in vitro* tissue culture, from axillary buds of 2–4 months old tamarind seedlings (Fig. 2d) have superior growth (early vigour), uniform growth without phenotypic abnormalities, enhanced biomass, early maturity and flowering (within 3–4 years) after planting, greater yield compared to tamarind trees grown from seeds (43). In addition, the shoot tip from a mature tamarind tree has higher regenerative efficiency (up to 85 %) compared to nodal segments (approximately 70 %), with rooting efficiency > 75 % under optimal conditions. In addition, shoot tips from 6–8 months old seedlings have a high frequency of shoot induction

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(96.7 %) and rooting efficiency (88.5 %) (62). Collectively available studies indicate that cotyledons, cotyledonary nodes and hypocotyls derived from young seedlings (< 20 days old) exhibit the highest regenerative competence. Shoot tips and nodal segments from juvenile plants also perform well and are suitable for clonal propagation and early fruit production (Table 2).

Sterilization of explants

The surface sterilisation of explants is the foremost step determining the outcome of *in vitro* plant propagation. An effective sterilisation is critical for the establishment of contamination/pathogen-free explants. The choice of sterilising agent, its concentration and exposure duration largely determine both explant survival and decontamination efficiency (63, 64). A final gentle rinsing thoroughly with sterile distilled/deionised water is required for completely eliminating sterilising agents and avoiding phytotoxicity (63, 65). The disinfection of tamarind seeds is a prerequisite to obtaining healthy and pathogen-free explants such as cotyledons, cotyledonary nodes and hypocotyl segments. Most successful protocols employ sequential washing with detergent or Tween-20, ethanol treatment, followed by either mercuric chloride ($HgCl_2$) or sodium hypochlorite (NaOCl) disinfection and multiple sterile-water rinses, resulting in high seed germination and explant survival rates (44, 46, 47, 49, 51, 54, 61, 64) (Table 3).

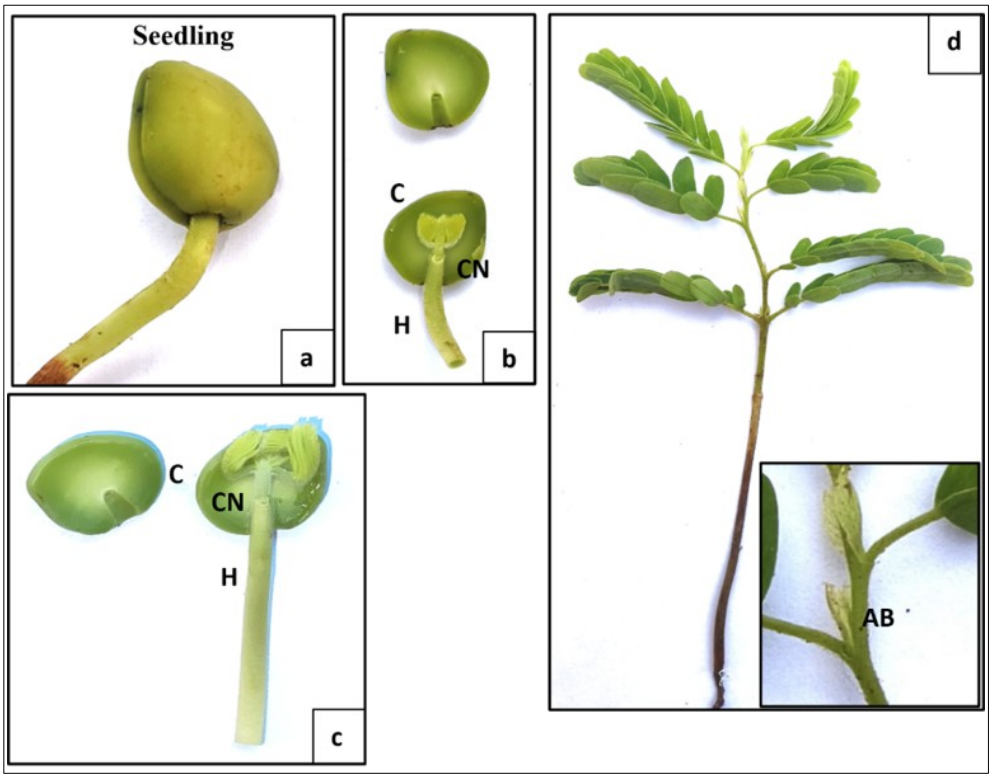


Fig. 2. Explants of tamarind.
AB: axillary bud; C: cotyledon; CN: cotyledonary node; H: hypocotyl region.

Table 2. Tamarind explants and their micropropagation success rate

Source of explants	Age of explant sources	Shooting success	Rooting success	Acclimatizatio n/ survival	Key findings	References
Cotyledon node	12–20 days old seedling	> 90 %	> 90 %	87 %	Best suited for 100 % shoot induction, high proliferation (~9 shoot per culture)	(44)
Cotyledon (adaxial surface)	12-day-old seedlings	80 %	NA	70 % (Soil establishment)	100 % regeneration, 95 shoots per explants in 16 weeks	(51)
Cotyledon (proximal halves)	12-day-old seedlings	55.5 %	NA	NA	100 % regeneration	(51)
Cotyledonary node	5–8 days old seedlings	86.6 %	NA	NA	Remarkable shoot proliferation	(48)

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Cotyledonary node	4-week-old seedlings	68 %	82 %	NA	Moderate shooting efficiency	(49)
Hypocotyl segment	12-day-old seedlings	66 %	Up to 87 %	NA	Shoot regeneration (> 66 %)	(50)
Nodal segments (young shoots)	Young branches of adult trees	59 %	62 %	High (rate not specified)	Younger nodes have better responsiveness than mature shoots, shoot induction	(60)
Nodal segments	40 days old seedlings	75 % sprouting (WPM medium)	NA	NA	Produces friable calluses; 70–80 % callus induction (WPM medium); Sprout elongation (MS with 75 % salt conc.)	(54, 61)
Axillary buds	2–4 months old seedlings	-	-	High survival and vigor	Superior growth, early flowering (3–4 years), higher yield	(43)
Zygotic embryo (intact)	Mature seeds	NA	NA	NA	50–60 % Germination response (radicle and plumule development) in WPM medium	(54)
Mature zygotic embryo axis	Mature seeds	85 % adventitious buds	70 %	>70 % acclimatization, 50 % hardening	Direct adventitious bud formation (no callus)	(55)
Shoot tip	Mature trees 6–8 months old seedlings	85 % 96.7 %	>75 % 88.5 %	70 % 78 %	Higher regeneration than nodal segments High shooting and rooting efficiency	(62)
IZE	12–16 weeks old (with green pods)	NA	NA	NA	Less embryogenic response, genotype-dependent	(59)

IZE: immature zygotic embryos; MS: Murashige and Skoog; SE: somatic embryo; WPM: woody plant medium, NA: not available/reported.

Table 3. surface sterilisation methods and regeneration outcomes

Explants and source	Sterilisation steps, concentration and exposure time	Regeneration or survival	References
Mature seeds (for cotyledonary nodes)	Tap water with few drops of Tween-20 rinse (15 min); 70 % EtOH (30 sec); 0.1 % $HgCl_2$ (8–10 min rinse); 4–5 sterile distilled H_2O rinses.	> 90 % shoot and rooting from Cotyledonary nodes.	(47)
Mature seeds (for cotyledons and hypocotyls)	70 % EtOH (1 min rinse); 0.1 % $HgCl_2$ (20 min soak); 4–5 sterile distilled H_2O rinses.	100 % shoot bud formation from Cotyledons. 66 % shoot buds and callus from Hypocotyls.	(51) (50)
Mature seeds (for zygotic embryo axis) Tender pods (for immature zygotic embryos)	Mild detergent wash in tap water; 0.1 % $HgCl_2$ (15 min soak); Multiple washes with sterile distilled H_2O. For mature seeds: Additional 4–5 hr initial soaking in sterile distilled H_2O.	> 85 % shoot formation. Up to 26.7 % somatic embryo formation.	(55) (59)
Mature seeds (for cotyledonary nodes, hypocotyls)	70 % EtOH rinse (30 sec); 2 % NaOCl (15 min soak); 3–5 sterile distilled H_2O rinses; 24 hr sterile distilled H_2O soak.	100 % germination; > 93 % regeneration from Cotyledonary nodes and hypocotyls.	(44)
Mature seeds (for callus)	10 % NaOCl pre-soak (24 hr); multiple sterile H_2O rinses; 70 % EtOH + few drops of Tween-20 (1 min rinse); 95 % EtOH (10 min immerse); multiple sterile distilled H_2O rinses.	> 90 % germination; 80–100 % callus from explants.	(45)
Nodal segments from seedlings, mature seeds (for zygotic embryos)	70 % EtOH (1 min rinse); 1 % NaOCl + Tween-20 (30 min soak); sterile distilled H_2O rinses (3 times).	80 % callus and 75 % sprouting from nodes; 50–60 % viability of zygotic embryos	(54, 61)
Nodal segments from seedlings	70 % EtOH (1 min rinse); 1 % NaOCl + Tween-20 (30 min soak); sterile distilled H_2O rinses (3 times).	Contamination: MS 25 or 50 % salt + AC No contamination: MS 74 or 100 % salt + AC	(59)

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Lateral and terminal buds (from young and mature shoots)	Tap water wash (2–3 hr); 0.05 % $HgCl_2$ (15 min soak); 3–4 sterile distilled H_2O rinses.	59–62 % shoot and root induction.	(60)
Shoot tips (6–8 week seedlings)	Tap water wash (30 min); 70 % EtOH (30 s rinse); 0.1 % $HgCl_2$ (8 min soak); 4–5 sterile distilled H_2O rinses.	82 % shoot formation.	(62)
Shoot tips, axillary buds, stems, leaves (from young and mature shoots)	0.1 % Tween-20 and 0.2 % Bavistin prewash; wash 4–5 times with tap water; 3–4 sterile distilled H_2O rinses; 0.1 % $HgCl_2$ (10 min soak); 2–3 sterile distilled H_2O rinses.	100 % decontamination and 100 % survival of explants.	(64)
	0.1 % Tween-20 and 0.2 % Bavistin prewash; 4–5 tap water wash; 3–4 sterile distilled H_2O rinses; 0.2 % NaOCl (10 min soak); 2–3 sterile distilled H_2O rinses.	57 % decontamination and 53 % survival survival of explants.	

EtOH: ethanol; $HgCl_2$: mercuric chloride; H_2O: water; NaOCl: sodium hypochlorite; AC: activated charcoal

Several studies reported that 0.1 % $HgCl_2$ applied for 10–20 min, following detergent and ethanol pretreatments, consistently achieved high decontamination rates while maintaining seed viability and regenerative potential (48, 50, 55, 59). In recent studies, NaOCl is widely used to evade the risk of $HgCl_2$ toxicity. The NaOCl has the capability to break seed dormancy in addition to eliminating microbial contaminants (66, 67). NaOCl-based sterilisation protocols, typically involving 0.78–2 % NaOCl following ethanol pretreatment, generally provide high germination and regeneration frequencies comparable to $HgCl_2$-based methods while offering a safer alternative for routine laboratory use (44–46, 49). Sterilisation requirements vary according to explant type. Juvenile shoot tips and nodal segments generally exhibit higher aseptic survival and regeneration frequencies than explants obtained from mature tissues, reflecting lower microbial loads and reduced phenolic exudation (58, 60, 62). Although several studies reported slightly higher decontamination efficiency with $HgCl_2$ than NaOCl, the latter remains a preferred disinfectant because of its lower toxicity and environmental risk (54, 60–62, 64).

Overall, an optimal and effective sterilisation protocol involves sequential treatment with tap water/distilled water and Tween-20 (0.1 %), 70 % ethanol immersion (30 sec to 3 min) and disinfection with either 0.1 % $HgCl_2$ or 2 % NaOCl, followed by sterile water rinsing. This approach ensures contamination-free explants and consistently supports > 80 % shoot and root regeneration across most explant types. Notably, the regeneration efficiency varies slightly depending on the explant type under the same sterilisation conditions; for example, cotyledon explants exhibit a higher frequency of direct shoot induction rates than hypocotyl-derived explants. Despite the protocols utilising $HgCl_2$ demonstrating marginally higher decontamination efficiency and regeneration consistency, NaOCl is a safer alternative that maintains comparable regeneration success as well as addressing biosafety, health and environmental concerns associated with mercury compounds.

Media composition and growth regulators for clonal propagation of tamarind

Culture medium composition is a critical determinant of regeneration efficiency and successful micropropagation in tamarind. The *in vitro* regeneration of plantlets from explants is a multistep process requiring different combinations of culture media and PGRs, which provide essential nutrients and physical conditions for the development and growth of shoot, leaf and root. In plant tissue culture, cytokinins are indispensable for directly or indirectly promoting shoot formation. However, their effects vary depending on the type of culture and source of the explant (48). The synthetic cytokinins such as BAP or 6-BA and 6-furfurylaminopurine or KN are widely used for shoot initiation and regeneration. BAP and KN are commonly referred to as shoot proliferation hormones. In contrast, synthetic auxins such as naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) are primarily involved in root induction and development.

Tissue culture medium for tamarind germination *in vitro*

For *in vitro* seed culture and establishment of seed-derived explants of tamarind, full-strength Murashige and Skoog (MS) medium or woody plant medium (WPM), supplemented with PGRs (BAP and NAA), is most useful (Table 4). Generally, germination from seed or zygotic embryos and growth rate of tamarind plantlets are remarkably higher in MS medium than in WPM medium. This might be closely associated with the composition of MS medium, which has adequate primary macronutrients such as potassium, nitrogen and phosphorus, which are required for photosynthesis and energy production, vegetative growth and root development, respectively. But the levels of these nutrients are comparatively very low in WPM medium (68). Tamarind seeds inoculated in MS culture medium consisting of sucrose (25.7–30 g/L) and agar (8 g/L) or carrageenan (7 g/L) or phytagel (2.285 g/L) at pH 5.8 and 25 ± 2 °C temperature with a 16/8 hr light/dark cycle (photoperiod) have shown 100 % germination and fully grown plantlets within 15–20 days (44–47). In addition, a solidified half-strength MS medium consisting of 2 % sucrose and 7 g/L agar also results in germination within 15 days of culture (48). Hence, MS medium is the standard choice and most widely used culture medium for *in vitro* germination and aseptic generation of seed-based explants of tamarind. The gelling agents such as carrageenan and phytagel are suitable alternatives to agar in plant tissue culture.

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Table 4. *In vitro* germination of seed and embryonic zygote

Explant type	Optimal culture medium and supplements	Induction rate
Seeds	MS + sucrose (25.7–30 g/L) + agar (8 g/L) or carrageenan (7 g/L) or phytagel (2.285 g/L).	100 % germination (15–20 days).
Seeds	Half-strength MS + 2 % sucrose + agar (7 g/L).	Germination (within 15 days).
Zygotic embryos from mature seeds	MS or WPM with 25 % salt + sucrose (30 g/L) + agar (4 g/L).	50–55 % germination.
	WPM with 50 % salt + sucrose (30 g/L) + agar (4 g/L) + 2 % AC.	Upto 60 % germination (30 days)

AC: activated charcoal; MS: Murashige and Skoog medium; WPM: woody plant medium.

Murashige and Skoog medium or WPM medium containing 25 % salt and supplemented with sucrose (30 g/L) and agar (4 g/L) is suitable for the germination of zygotic embryos (55 % germination percentage), whereas adding 2 % activated charcoal to WPM medium with 25 or 50 % salt and the same concentration of sucrose and agar slightly increases the germination percentage to 60 %. The development of plumule and radicle is an indicator of the germination of the embryo (54). Addition of activated charcoal (2 g/L) in WPM medium increases the length of plantlets originating from zygotic embryos of tamarind *in vitro*, despite its slow growth. After subculture, the growth of plantlets is found to be associated with increased salt concentration (75–100 %) in either MS or WPM medium with or without activated charcoal (54).

Tissue culture medium for shoot induction from tamarind explants

Murashige and Skoog medium provides a well-balanced supply of macro- and micronutrients required for shoot induction, elongation and subsequent root induction when supplemented with suitable plant growth hormones (68), whereas WPM has also been successfully utilised. When 75 % MS medium is used with or without activated charcoal, it induces 50–60 % shoots and promotes better shoot elongation, possibly because of its relatively high nitrogen content. Whereas, the full-strength WPM without activated charcoal induces 75 % shoot from nodal segments within 30 days but addition of 2 % activated charcoal in the same medium markedly reduces shoot induction (54).

Murashige and Skoog medium or WPM media supplemented with various combinations of growth regulators, including BAP, NAA, KN, TDZ, IBA and IAA have produced variable results (Tables 5 and 6). The MS medium with 0.5 mg/L (~2.5×10^{-6} M) to 1.126 mg/L (5×10^{-6} M) of BAP effectively induced shoot regeneration from cotyledonary node (from 8–12 days old seedlings) and hypocotyl segment (12 days old seedlings) (48, 50, 51) as well as 100 % callus induction from proximal end of hypocotyl explants (50). In this culture condition, 3–4 weeks is required for the shoot development and multiple bud formation from cotyledon (48, 51) and from hypocotyl segments 8 weeks is required (50) respectively. Kinetin, a shoot-inducing cytokinin, is comparatively less effective than BAP in induction and multiplication of shoot, from cotyledon, cotyledonary node and hypocotyl segments (48, 50, 51). 1 mg/L KN induces 65 % shoot formation from cotyledonary nodes. However, increasing concentrations of BAP or KN to 2 or 3 mg/L reduces shoot induction and proliferation (48).

A low auxin to cytokinin ratio generally favours shoot multiplication. Murashige and Skoog medium medium containing 0.5 mg/L BAP and 0.1 mg/L NAA is optimal for enhancing shoot induction (86.7 %) and multiplication rate (100 %) in 5–8 days old cotyledonary nodes. NAA concentration above 0.2 mg/L in the presence of 0.5 mg/L BAP suppressed shoot formation and promoted callus formation (48). In another study, 3 mg/L BAP and 0.5 mg/L NAA induced adventitious bud more effectively in WPM than in MS, with a response of 93 % and 80 % respectively. Both lower and higher concentrations reduced bud induction, indicating that WPM with 3 mg/L BAP and 0.5 mg/L NAA is suitable for bud induction from cotyledonary nodes (44). Indole-3-acetic acid (IAA), a synthetic auxin, in combination with BAP enhances shoot multiplication from cotyledons by two to three-fold compared to BAP alone (51). While 1 mg/L KN with 0.1 mg/L NAA produced 68 % shoot induction and 90 % multiplication (48). Low concentrations of BAP and KN promote shoot-bud formation from hypocotyls or callus, although their effectiveness depends on the explant and culture stage (45, 50).

Table 5. Culture medium and growth regulators for shoot and root induction from explants

Explant type and source	Culture medium and key PGRs	Induction and regeneration rate	References
Cotyledonary node and hypocotyl (8–12 days old)	MS + BAP (0.5 mg/L or 1.126 mg/L)	Shoot regeneration: 66–100 % Callus induction (proximal hypocotyl): 100 %	(48, 50, 51)
Cotyledonary Node (5–8 days old)	MS + KN (1 mg/L)	Shoot induction: 65 %	(48)
	MS + KN (1 mg/L) + NAA (0.1 mg/L)	Shoot induction: 68.7 %, multiplication: 90 %	
	MS + BAP (0.5 mg/L)	Shoot induction: 85 %, multiplication: 100 %	
	MS + BAP (0.5 mg/L) + NAA (0.1 mg/L)	Shoot induction: 86.7 %, multiplication: 100 %	
Cotyledon (Seedling)	MS + BAP (1.126 mg/L) + IAA (0.88 mg/L)	Shoot multiplication: 2–3 folds increase	(51)
	Basal MS medium	Root induction: 50 %	

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	MS + IAA (0.88 mg/L)	Root induction: 83.3 %	
Hypocotyl (12 days seedlings)	MS + BAP (1.126 mg/L)	Optimal for shooting	(50)
	MS + IBA (1.02 mg/L)	Optimal for Rooting: 62–87 %	
	MS + IAA (0.88 mg/L) with or without 0.2 % AC	Root induction: 50–58 %	
Mature embryo axis with cotyledons (longitudinal section)	Initiation: MS + BAP (10 mg/L) + NAA (0.5 mg/L) + 4 % sucrose + 0.5 % agar.	Up to 12 shoots /explant	(55)
	Elongation: Liquid MS + BAP (0.5 mg/L) + Zeatin (0.2 mg/L) + calcium pantothenate (0.195 mg/L) and biotin (0.1 mg/L).	Enhanced shoot elongation and inhibition of callusing (by zeatin)	
	Root: half-strength liquid MS + mixture of IAA, IBA, NAA and 3-propionic acid (2 mg/L) for 3 days and half-strength liquid MS for 4 weeks	Vigorous root formation.	
Cotyledonary Node	Bud and shoot: WPM or MS + BAP (3 mg/L) + NAA (0.5 mg/L)	Bud induction: 80 % (MS) and 93 % (WPM)	(44)
	Rooting: half-strength MS + IBA (2 mg/L) for 2 weeks in dark; half-strength MS alone next 2 weeks.	Root induction: 83 % (multiple roots and robust growth)	
Leaf-derived callus (12 days old seedlings)	MS + BAP (0.1 mg/L) + KN (0.05 mg/L)	Shoot induction within 4 weeks.	(45)
Nodal segment (young shoots)	Initiation: MS + BAP (2 mg/L) + KN (2 mg/L) + 3 % sucrose + 0.5 % AC.	Lateral bud sprouting: 59 % (in 4–5 weeks).	(60)
	Multiplication: MS + BAP (1 mg/L) + KN (0.5 mg/L) + 3 % sucrose.	Multiple shoots in 3 weeks.	
	Root: Half-strength MS + IBA (5 mg/L) + 3 % sucrose.	Root induction: 62 %	
Nodal segments	WPM (100 % or 75 % nutrient salts)	Shoot induction: 75 %	(54)
	MS (75 % nutrient salts)	Shoot elongation (during subculture)	
Cotyledonary node, epicotyl node, shoot tip	Shoot: MS + TDZ (0.035 mg/L) + IBA (0.265 mg/L) or BAP (1.48 mg/L) + NAA (0.037 mg/L); sucrose (30 mg/L), agar (8 mg/L).	Cotyledonary node: up to 5 shoots /explant; epicotyledonary node: up to 3 shoots/explant; shoot tip: 1–2 shoots/explant.	(46)

AC: activated charcoal; BAP: 6-benzylaminopurine; IAA: indole-3-acetic acid; IBA: indole-3-butyric acid; KN: - kinetin; MS: Murashige and Skoog medium; NAA: naphthaleneacetic acid; PGR: plant growth regulator; WPM: woody plant medium.

Table 6. Shoot multiplicate rates of tamarind explants in different media composition

Explant type	Media composition	Shoot multiplication rate (No.of shoots/explant)	Reference
Cotyledonary nodes	MS + BAP 0.5 mg/L + NAA 0.1 mg/L	2.5	(48)
Cotyledonary node	WPM + BAP (3 mg/L) + NAA (0.5 mg/L)	4.1 (bud proliferation coefficient).	(44)
Hypocotyl segment	MS + BAP (1.13 mg/L)	3.1	(50)
Cotyledon (Proximal end)	MS + BAP (1.126 mg/L) + IAA (0.01 mg/L)	15	(51)
Cotyledon (Proximal end)	MS + BAP (1.126 mg/L)	3.6	
Cotyledon (Adaxial surface)	MS + BAP (1.126 mg/L)	30	

BAP: 6-benzylaminopurine; IAA: indole-3-acetic acid; NAA: naphthaleneacetic acid; MS: Murashige and Skoog medium; WPM: woody plant medium.

Regeneration from mature embryo axis involves a multistep method with various combinations of MS media (55). Clusters of adventitious buds are initiated in MS medium containing 10 mg/L BAP, 0.5 mg/L NAA, 4 % sucrose and 0.5 % agar. After 4 weeks, the buds are transferred to liquid MS medium containing 0.5 mg/L BAP, 0.2 mg/L zeatin, calcium pantothenate, biotin and 2 % sucrose. This treatment produced up to 12 asynchronously elongated shoots per explant. However, the shoot formation and proliferation were reduced in subsequent passages (55).

For nodal explants from mature trees, MS medium containing 2 mg/L BAP, 2 mg/L KN and 0.5 % activated charcoal initiated 50 % nodes from young shoot explants but only 25 % of nodes from mature shoots within 4–5 weeks (60). This low response in mature tissues might be due to greater phenolic exudation. Activated charcoal, extensive washing and partial drying of explants may reduce tissue browning (60, 69). Established shoots can be multiplied on MS medium with 1 mg/L BAP and 0.5 mg/L KN and 3 % sucrose which yields multiple shoots in 3 weeks. Once established, the age of explants has little influence on the shoot

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proliferation rate (60). Shoot multiplication rates range from 2.5–30 shoots per explant depending on different explants and growth regulators used (Table 6). Murashige and Skoog medium or WPM is known to induce shoot in various explants. Hence, MS medium or WPM with growth regulators, BAP either alone or in combination with a low concentration of NAA, is an effective treatment for shoot induction and multiplication. The use of other growth regulators such as KN and IAA shows limited success.

Tissue culture medium for callus induction from tamarind explants

The different combinations and concentrations of PGRs, in particular, the combination of auxins and cytokinins, have a great influence on callus induction from various explants. In addition, the proficiency and differential effects of PGRs on callus induction are mainly due to the type and age of explants (70–72) (Table 7). Kinetin either alone at the concentrations of 5×10^{-5} to 5×10^{-6} M (1.17-11.17 mg/L) (50) or in combination with 0.5 mg/L BAP and 0.25 mg/L NAA (45) is well suited for mass production and healthy callus induction (80–100 %) from a 12-day-old hypocotyl segment (50) and from cotyledon, leaf and root tissues of 14-day-old seedlings (45) respectively. However, increasing the concentration of these growth regulators (KN, BAP and NAA) above this level was found to reduce callus formation. Similarly, MS medium containing 0.035 mg/L thidiazuron (TDZ) or 0.265 mg/L IBA with 1.48 mg/L BAP and 0.037 mg/L NAA also induces callus formation from tamarind cotyledon without any shoot regeneration (46).

The combination of 10 mg/L BAP and 0.5 mg/L NAA and 2 % sucrose in MS medium induces callus development in 34 % of explants. Further increasing sucrose concentration up to 4 % significantly increases the callogenic response in 48 % of embryonic axis whereas 6 % sucrose caused medium browning and inhibiting (55). For callus induction from nodal segments (containing one bud) of 40-day-old seedlings of tamarind, the supplementation of sucrose (30 g/L) and agar (4 g/L) in MS medium containing nutrient salt (mixture of macro- and micro-nutrients) concentration of 25 % or WPM medium containing 25–75 % of salt, produces ≥ 70 % callus. Among different salt concentrations of MS and WPM medium, the highest callus formation (> 95 %) was shown when using the base of nodal segments as an explant in WPM medium with 50 % and 75 % salt concentrations, without addition of activated charcoal (54). The addition of activated charcoal (2 g/L) to MS or WPM medium considerably reduces callus formation but does not affect sprout formation (54).

Indirect organogenesis is useful for recalcitrant genotypes and explants facing difficulties in directly regenerating plantlets. Due to the increased incidence of genetic changes and somaclonal variations in plants propagated from callus, this method is only suitable when developing plants for novel and improved traits rather than genetic fidelity, which is required for commercial propagation (54, 73). Murashige and Skoog medium with KN, BP and NAA combination as well as WPM medium without growth regulators shows a better callus induction in tamarind explants. But further standardisation by comparative studies on different growth regulator combinations with WPM and MS medium will provide the best combination of media for callus induction in tamarind.

Culture medium for somatic embryos from immature zygotic embryos of tamarind

In somatic embryogenesis, the initiation/induction and maturation (development or expression) of SE is generally carried out in two-step procedures for most of the hardwood plants, with two different culture media (induction and expression/maturation medium), irrespective of the type/source of explants. The induction media for SE typically contain a high concentration of auxins with or without cytokinins, whereas maturation media have low levels of PGRs or are free of PGRs (58). The basal MS medium containing 60 g/L sucrose and 2.2 g/L phytagel and supplementation with synthetic auxins such as 1–5 mg/L dicamba or 0.5–1 mg/L picloram or 0.5 or 1 mg/L 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) is suitable for the induction of somatic embryogenesis from IZE of tamarind. Among them, dicamba is most effective for direct induction of SE, without callus formation, from IZE of tamarind. The highest SE response (up to 17.5 %) and the frequency of multiple SE formation can be seen in MS medium supplemented with 5 mg/L dicamba. However, SE responses decrease when the concentration of dicamba is increased further. Similarly, a higher concentration of 2,4,5-T and picloram was ineffective in SE response (59). Hence, it is ideal to supplement the culture medium with 5 mg/L dicamba and solidify it with phytagel for better embryogenic response of IZE when incubated in the dark for 60 days.

Table 7. Culture medium and growth regulators for callus induction

Explant type and source	Culture medium and key PGRs	Callus induction rate and regeneration	References
Hypocotyl (12-day-old seedling)	Basal MS + KN (1.07–10.76 mg/L) Basal MS + BAP (1.126 mg/L)	80–90 % callus induction 100 % callus along with shoot induction.	(50)
Cotyledon, leaf, root (14-day-old seedling)	MS + KN (0.2 mg/L) + BAP (0.5 mg/L) + NAA (0.25 mg/L)	100 % callus from cotyledon. 80 % callus from leaf and root.	(45)
Cotyledon	MS + TDZ (0.035 mg/L) + IBA (0.265 mg/L) or BAP (1.48 mg/L) + NAA (0.037 mg/L) + sucrose (30 g/L) + agar (8 g/L)	Callus formation (no shoots).	(46)

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Embryonic axis (with or without cotyledons)	MS + BAP (10 mg/L) + NAA (0.5 mg/L) + 2 % sucrose MS + BAP (10 mg/L) + NAA (0.5 mg/L) + 4 % sucrose	34 % 48 %	(55)
Nodal segments (40-day-old seedling)	Basal WPM (50–75 % nutrient salts) + 30 g/L sucrose + 4 g/L agar (without AC)	>95 %	(54)

AC: activated charcoal; BAP: 6-benzylaminopurine; IBA: indole-3-butyric acid; KN: kinetin; MS: Murashige and Skoog; NAA: naphthaleneacetic acid; PGR: plant growth regulator; WPM: woody plant medium.

The initiation and development of somatic embryogenesis, indicated by a shining mass or swelling at the plumule end of IZE whose structure resembles torpedo or cotyledonary-stage SE, can be seen within 30 –40 days (59). For further multiplication of SEs, IZEs with SEs should be transferred to a fresh basal MS medium supplemented with 60 g/L sucrose, 5 mg/L dicamba and 2.2 g/L phytagel once every 60 days. Initially, the base of torpedo or cotyledonary-like structures is constrained near the cotyledonary node and swelling can be seen around the middle region. Upon extending the duration of incubation for 30–40 days, multiple SE-like structures arise from both the cotyledonary nodal region and the swollen plumular region of IZEs. The primary SEs also transform into glistening masses of new SEs called secondary SEs (SSEs). These SSEs sustain their regenerative and proliferation ability for more than 2 years when the culture is maintained under optimal conditions (59).

The maturation of SSE can be achieved in half-strength MS medium with activated charcoal, 10 % sucrose and low concentrations of dicamba (mostly 0.5 or 1 mg/L) and abscisic acid (ABA) (59). It is well defined that ABA promotes maturation of SE and improves the quality and yield of SE by preventing cell death, promoting seed reserve synthesis, increasing desiccation tolerance and preventing premature germination (58, 74, 75).

Germination of SSE can be achieved in PGR-free half-strength MS medium with 0.25 % activated charcoal (59) (Table 8). The activated charcoal adsorbs excessive PGRs and other inhibitory compounds released, thereby aiding germination and plantlet development, while creating a stable environment and protecting against reactive oxygen species (ROS)-induced cell damage (58, 76).

Shoot differentiation and development are difficult or not achievable in typical basal medium with/without activated charcoal, basal medium with 10 % sucrose, basal medium with ABA or low concentrations of dicamba or basal medium with a single or combination of different growth regulators such as KN, TDZ, BAP and zeatin (59). Sporadic, think or stunted roots from somatic embryos are achieved on MS basal or half-strength MS basal media containing activated charcoal or 10 % sucrose or 0.1 mg/L ABA or 10 mg/L NAA or 0.3 % phytagel. Hence, these protocols do not favour the development and yield of complete plantlets from SE of tamarind IZE even when considerable germination can be achieved.

Limited embryo maturation and morphological abnormalities are major constraints to plantlet conversion in woody legumes. There are no standard or good indicators to determine the appropriate maturation level of SEs, that can be used for germination and plantlet conversion. As a result, the germinated SEs produce only shoot or root in many cases of hardwood and/or legume species (58, 77) and the success of SE is limited, though recent studies focus on newer approaches to achieve success in SE (78).

Potential improvement includes extending the maturation phase, supplementing maturation media with high concentration of sucrose and including osmotic agents such as D-sorbitol or polyethylene glycol (PEG) and amino acids (79, 80) or adding gibberellic acid (GA_3) in germination medium (58, 81, 82). Gibberellic acid, either alone or in combination with other PGRs, is widely used to break dormancy and to increase germination and shoot development from mature SE originating from IZE (57, 82, 83). Currently, only a few reports are available for the efficient mass propagation of tree legumes through direct somatic embryogenesis from various explants, including IZE. Further optimisation of protocols for SE maturation, germination and development of plantlets is required for the successful large-scale tamarind propagation.

Table 8. Culture medium and growth regulators for somatic embryogenesis (SE) from explants

Explant type/source	Culture medium and supplements	Percentage induction or regeneration	References
Immature zygotic embryo (IZE) from tender pods	Induction: Basal MS + 60 g/L sucrose + 2.2 g/L phytagel + 5 mg/L Dicamba; pH 5.8. Keep in a culture/test tube. Proliferation/multiplication: Same medium composition and subculture every 60 days. Maturation: Half-strength basal MS + 10 % Sucrose + 0.5 or 1 mg/L Dicamba + ABA + 0.25 % AC. Germination: Half-strength basal MS (PGR-free) + 0.25 % AC. Rooting: Two-step method: Half-strength basal MS (PGR-free) and then half-strength basal MS + 0.25 % AC	SE generative response 17.5 %. High frequency of multiple SEs. Proliferation sustained for > 2 years. Sporadic root formation	(59)

AC: activated charcoal; ABA: abscisic acid; BAP: 6-benzylaminopurine; IAA: indole-3-acetic acid; IBA: indole-3-butyric acid.

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Tissue culture medium for root induction

Well-developed shoots are transferred to a medium with rooting hormones for root induction. Auxins such as IAA, IBA, 2,4-D and NAA are commonly used to induce high-quality roots *in vitro*. A study with full-strength basal MS medium containing different auxins such as IBA, NAA and IAA found that IBA (5×10^{-6} M) is optimal for rooting of shoots from hypocotyl explants. IAA (5×10^{-6} M) also considerably induces rooting (50–58 %) but NAA is not effective in inducing rooting of shoots generated from hypocotyl explants (50). Indole-3-acetic acid (5 × 10-6 M) alone or in combination with 0.2 % activated charcoal induces adventitious root formation without callusing at the cut ends, which is essential for the survival of plantlets after transplantation (50). In general, the shoots and roots are directly connected by a vascular network composed of vascular bundles and vascular cambium. In plant tissue culture, some auxins induce callus formation at the shoot end, which subsequently leads to the death of plantlets by interrupting the vascular connection and affecting the transportation (50). Indole-3-acetic acid at approximately 5.7×10^{-6} M induces roots from cotyledons and resulted in 70 % plant establishment in soil (51). In some cases, PGR-free MS medium induces 50 % rooting (51).

Several studies demonstrated a two-step rooting protocol to avoid the adverse effects of growth hormone toxicity. Root primordia are initiated by culturing the regenerating buds on a half-strength MS medium with 2 mg/L IBA in darkness for two weeks followed by culture on auxin-free half-strength MS medium for another two weeks. This treatment resulted in 83 % rooting, whereas 1–3 mg/L IBA is less effective (44). For the shoots derived from mature embryonic axis, a similar two-step process involving initial transfer of shoot to a half-strength liquid MS medium comprising a mixture of 2 mg/L each of IAA, IBA, NAA and 3-propionic acid and incubated for 72 hr followed by transferring them to PGR-free half-strength liquid MS medium incubated in the dark for 4 weeks. Roots appear in about 10–15 days and a vigorous root system develops over the next 15–20 days (55). Thus, IAA or IBA can be used effectively in either a single-step method or a two-step rooting system (Table 5).

Ex vitro rooting is a cost-effective alternative to improve field survival of phenol-rich woody trees such as tamarind, as it combines rooting and acclimatization phases. This method reduces the systemic browning that occurs during *in vitro* root induction, poor vascular connections and transplantation loss. Successful *ex vitro* rooting has been reported in papaya, Ceylon olive and sea grape. For *ex vitro* root induction, once sufficiently sized plantlets are formed through micropropagation, they can be given a brief treatment with phloroglucinol, IBA or NAA, respectively, before planting in soil. Dipping the papaya plantlets in 1000 mM phloroglucinol before planting in a sterile potting medium induces rooting effectively and provides 82 % survival during hardening (84). Similarly, pulse treatment of Ceylon olive micro-shoots with 2 mM IBA for 5 min enhances rooting (up to 86.11 %) and survival (85). Treatment of *in vitro*-regenerated sea grape shoots with 400 mg/L NAA for 3–7 min induces 100 % rooting response and 93.8 % plant survival compared to 75 % survival of *in vitro*-rooted plantlets (86). *Ex vitro* rooting method not only reduces the costs associated with *in vitro* rooting but also enhances successful plant establishment and survival in the field. Similar approaches should be evaluated in tamarind to improve acclimatization, field survival and promote the commercial feasibility of micropropagation.

Acclimatization

Acclimatization serves as the pivotal final stage in micropropagation protocols, requiring a controlled transition from the optimised, sterile conditions of *in vitro* culture vessels to the unpredictable *ex vitro* environment (81, 87). A major constraint to large-scale micropropagation is the high mortality of *in vitro*-raised plantlets during their transfer from laboratory to field conditions. This transition exposes plants to abrupt *ex vitro* stresses, including altered temperature, increased light intensity and water deficits, which starkly contrast with the controlled *in vitro* environment. Therefore, a dedicated acclimatization (hardening) phase is essential to minimise physiological shock, reduce desiccation risk and improve establishment and survival, particularly in phenol-rich woody fruit trees and plants, where acclimatization frequently remains a bottleneck (81, 87). Studies have reported high survival rates of regenerated tamarind plants in soil. About 70 and 75 % survival rates have been reported for plantlets derived from cotyledon and hypocotyl explants, respectively (50, 51). Tissue culture-derived tamarind trees established successfully under field conditions and also exhibited superior traits to seed-propagated trees, indicating successful field transfer of acclimatised tamarind micropropagules (43). These *in vitro*-derived trees are comparatively shorter with increased branching and an early flowering habit (flowering within 3–4 years) than the seed-derived trees.

An improved environmental management *in vitro*, particularly through carbondioxide (CO_2) enrichment, regulation of light and humidity, promotes photosynthetic competence and enhances survival during acclimatization. Accordingly, gradual exposure of plants to *ex vitro* conditions by reducing the humidity and increasing the light in a phased manner in controlled facilities such as a green house or mist chamber can enhance the survival of plants (81). In this context, maintaining high humidity by covering the pot-transferred plantlets of tamarind with a glass beaker or polythene bag during the initial 15 days after transfer has been shown to improve survival rates by 70–75 % (50, 51). Furthermore, the developmental stage at which the shoots are transferred to soil plays an important role in survival during acclimatization. Shoots of *in vitro*-derived tamarind plantlets bearing unopened leaves are unable to adapt effectively to the external environment and their shoot tips tend to dry out and deteriorate rapidly when transferred to soil. In contrast, plantlets with partially or fully expanded leaves have higher survival rates and better stabilisation during acclimatization (55).

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Cotyledon-derived plantlets possessing a well-developed shoot, axillary branches and root displayed better adaptation, as indicated by survival and resumption of growth in about 70 % of plantlets after successful establishment in soil. These observations suggest that *ex vitro* hardening is feasible when plantlets are adequately developed, properly rooted, as well as handled carefully during transfer (51). Similarly, well-developed plantlets generated through hypocotyl-derived organogenesis achieved approximately 75 % survival following transplantation into pots (50). Collectively, these findings indicate that the developmental stage at which plantlets are transferred to hardening is a critical determinant of successful establishment and survival in the outside environment. Successful acclimatization is associated with adequate leaf and shoot development and expansion at the time of transplanting, indicating sufficient photosynthetic capacity and structural ability of the plant to withstand the *ex vitro* conditions.

The choice of appropriate potting mixture, in addition to gradual hardening, is crucial for enhancing the survival and vegetative growth in fruit trees. The *in vitro* plantlets of tamarind are prepared for hardening by removing the residual agar by gentle washing before transplanting into a suitable potting medium (51). Establishment of tamarind plantlets in sterile vermiculite under high humidity for the first few days, followed by transfer to soil after 15 days, resulted in 70 % success in plant establishment. Similarly, up to 75 % plant establishment success was achievable from hypocotyl-derived plantlets when transplanted into a mixture of soil, sand and vermiculite (2 : 1 : 1 ratio) and maintained under high humidity during the first few days by covering them with a polythene bag (50).

Currently, strategies and potting mixtures for the acclimatization of micropropagated tamarind, to improve and enhance their survival, are limited and remain largely unexplored. Nevertheless, it is possible to utilise the most common and successful potting media that have been used for other phenol-rich woody trees and fruit trees, to improve tamarind acclimatization. The successful and effective potting mixtures in other fruiting trees include combinations of vermiculite, sand and peat moss at different ratios. A balanced substrate comprising of vermiculite, sand and peat moss (1 : 1 : 1) has emerged as one of the most suitable combinations, promoting robust rooting and establishment under controlled conditions (25). For example, peat moss and perlite (2 : 1) work well for the establishment of date palm cv. Maktom (88), while a sand and peat moss mixture (1 : 1) and perlite, vermiculite and cocopeat (1 : 1 : 2) are more suitable for acclimatization of coffee (89) and papaya (84), respectively. These findings imply the efficacy of lightweight, well-draining, moisture-retentive media incorporating peat moss, vermiculite, cocopeat, sand, or perlite in the potting mixture. In addition, variations in mixtures like 1 : 1 : 1 (vermiculite : sand : peat moss) and 1 : 1 : 2 (perlite, vermiculite and cocopeat) consistently outperform others by balancing aeration, water retention and nutrient availability, reducing transplant shock in many fruit trees including date palm, coffee and papaya. Similarly, soil, sand, vermicompost and farm yard manure are known to have an optimal effect for successful hardening in a number of tree species as well as other plants (90). Hence, these combinations, along with providing high humidity in the initial few days, by covering them with a polythene sheet or placing them in a plastic tunnel can result in successful acclimatization of tamarind in the *ex vitro* condition.

Innovative approaches integrating environmental gradients, microbial biostimulants and tailored growth substrates can further reduce transplant shock while enhancing survival and commercialisation potential across diverse plant species (90). In particular, biohardening with beneficial microorganisms such as *arbuscular mycorrhizal fungi* (AMF; symbiotic fungi), *Piriformospora indica* (a root endophytic fungus) and plant growth-promoting rhizobacteria such as (*Pseudomonas* sp. and *Bacillus* sp.), can help micropropagated plants overcome transplant shock by neutralising phenolic oxidative stress, compensating for a defective or poorly developed root vascular system, producing auxins and mobilising nutrients, thereby enhancing growth and survival (91–93). As the tamarind root microbiome is naturally rich in an active symbiotic network of AMF and rhizobacteria (94), inoculation of these microorganisms in the pot mixture during acclimatization may improve and enhance the growth of micropropagated tamarind plantlets. For instance, inoculation with AMF (*Grammatophyllum margarita* and *Grammatophyllum albida*) in a potting mixture (sand : soil, 1 : 1) during the acclimatization of Fegra fig plantlets greatly improved plantlet growth and *ex vitro* survival (100 %) (92). Similarly, in fruit trees such as banana, root inoculation with auxin-producing bacteria such as *Bacillus* improves the quality of the hardened plants and increases plantlet survival (93). Collectively, these strategies enhance acclimatization success and pave the way for large-scale commercialisation of elite woody propagules such as tamarind. Further research is needed to assess the potential of AMF and microorganisms to improve the *ex vitro* acclimatization of micropropagated tamarind plantlets.

2. Conclusion

Tamarind micropropagation holds strong potential for the mass production of elite planting material and for the expansion of tamarind cultivation across tropical and subtropical regions. Based on the studies, young seedling-derived explants (up to 20 days after germination) of tamarind typically exhibit higher regeneration success, whereas zygotic embryos tend to have lower regeneration efficiency. Appropriate nutrient media supplemented with growth regulators such as 6-benzylaminopurine (BAP) and kinetin (KN) promote shoot induction, while a combination of BAP and naphthaleneacetic acid (NAA) induces callus formation. A successful root induction is achieved by using indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA).

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However, the concentrations of these plant growth regulators (PGRs) are very critical in determining the success of tamarind micropropagation. At present, relatively few studies have compared the regeneration efficiency and survival of micropropagated tamarind derived from different explants. Currently observed variability in the success rate may be attributed to differences in culture media composition, PGR combinations, *in vitro* culture protocol and acclimatization practices.

A complete and reliable propagation system for tamarind will be achieved only when both *in vitro* regeneration and *ex vitro* establishment are optimised. Therefore, further studies should focus on standardising and optimising micropropagation protocols to improve morphogenetic response, multiplication rate and acclimatization efficiency, thereby enhancing the survival and field establishment of *in vitro*-propagated tamarind plantlets. Moreover, future research should prioritise the optimisation of somatic embryogenesis, the improvement of acclimatization protocols, the evaluation of genetic fidelity using molecular markers and the development of cost-effective systems for commercial-scale propagation of tamarind.

Authors' contributions

MRPS, AAK, RM and RS formulated the concept, collected the data and prepared the manuscript. RS critically reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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