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REVIEW ARTICLE

Clonal propagation for sustainable production in papaya: A review

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Abstract

Papaya (*Carica papaya* L.) is a dicotyledonous, polygamous fruit crop bearing staminate, pistillate and hermaphrodite flowers and valued for its ability to produce fruits year-round. Despite its economic significance, conventional seed propagation is constrained by variability in sex expression, yield and fruit quality, largely attributed to the crop's inherent heterozygosity. These challenges hinder the production of uniform and true-to-type planting material, limiting the efficiency and predictability of commercial cultivation. Clonal propagation has emerged as a promising alternative, enabling the rapid multiplication of genetically uniform, disease-free plants while reducing reliance on natural seed resources. Its use of controlled environments minimizes land requirements, conserves elite germplasm and supports consistent plant quality, making it a more sustainable and scalable approach for papaya production. Asexual techniques such as cuttings, grafting and micropropagation not only address the limitations of seed-based propagation but also facilitate the preservation of desirable traits essential for industry advancement. This review synthesizes current knowledge on these clonal propagation methods, evaluating their advantages and constraints. Additionally, recent advancements, including optimized somatic embryogenesis protocols, bioreactor-based multiplication and improved acclimatization strategies, have further enhanced the efficiency, reliability and commercial viability of clonal papaya production.

Keywords: cuttings; grafting; micro propagation; papaya; propagation; true-to-type plants

Introduction

Papaya (*Carica papaya* L.), (2n = 18), is the only cultivated and economically important species in the family, Caricaceae and in the genus, *Carica*. It is widely cultivated in the tropical and sub-tropical regions of the world and is recognized for its significant nutritional composition, along with its medicinal and industrial uses. Area under commercial cultivation of papaya is increasing because of its fast-growing habit, ability to bear fruits throughout the year, high productivity and its potential for huge economic returns. Nutritionally, the fruit is rich in vitamin A (1094 IU 100 g^{-1}) and vitamin C (61.8 mg 100 g^{-1}), besides folate, lycopene, dietary minerals and dietary fibre (1). The fruit is primarily consumed as a dessert and also utilized for the preparation of various value-added products. Green fruits are also often cooked as vegetables and are also used in the preparation of 'tutti-frutti'. In addition, the mature unripe fruit also serves as the major source of proteolytic enzymes, including papain, chymopapain and carpin, among which papain has high industrial significance (2). The fruit and seed extracts have noticeable antibacterial activity (3) and the leaf extracts are used for curing dengue fever (4).

Though this crop originated in Central America, it was introduced into India during the 17th century and its high productivity along with nutritive value contributed to its widespread acceptance and preference among consumers in India. India ranks as the foremost producer of papaya globally, accounting for about 6.1 million tonnes cultivated over 1.3 lakh ha, followed by Brazil, Mexico, Indonesia and Nigeria (5). Papaya is a dicotyledonous and polygamous plant characterized by three distinct sex forms, *viz*., staminate, pistillate and bisexual plants. The staminate and bisexual sex forms in papaya are highly influenced by temperature and hence intermediate sex types have also been documented (6). Based on sex distribution within populations, papaya can be categorized as dioecious or gynodioecious.

Commercially, papaya is propagated through seeds (7) and this method presents the limitation of segregation of female and male plants (1:1) and female and bisexual plants (1:2) in dioecious and gynodioecious varieties respectively (8). In papaya, the determination of sex is not feasible until the plant flowers, due to the absence of morphological distinction. Though molecular markers have been reported for determining the sex of the papaya plants before flowering but it is yet to be commercially viable (9, 10). Although gynodioecious cultivars have the advantage of producing all fruit setting plants, their productivity is hampered due to poor fruit set in bisexual plants, especially in the summer season due to high temperatures.

In addition, all the gynodioecious varieties are also highly susceptible to papaya ring spot virus (PRSV) (11). The dioecious varieties are comparatively tolerant of PRSV than gynodioecious varieties, but have the disadvantage of segregation into female and male (1:1) when propagated by seeds. As a result, excess seedlings must be maintained in each pit until the onset of flowering, after which thinning excess male and female plants in dioecious and gynodioecious varieties respectively is to be done. In dioecious cultivars, standard practice involves retaining 4-6 seedlings per pit in the main field until flowering, which facilitates the establishment of an optimum proportion of female plants in commercial orchards. However, due to cross-pollination and its heterozygous nature, maintaining genetic purity is difficult, often resulting in non-true-to-type plants with significant differences in yield, fruit quality and tolerance to pests and diseases (12, 13). These limitations reduce the reliability of seed propagation and have encouraged the adoption of clonal propagation or asexual propagation techniques.

Clonal propagation offers an effective alternative to seed-based methods by enabling large-scale production of genetically uniform and true-to-type plants. Techniques such as cuttings, layering, grafting, budding and micropropagation have been widely employed across horticultural crops to ensure varietal fidelity, with micropropagation being especially valuable for producing disease-free plants with high genetic stability (14). By facilitating the rapid multiplication of genetically identical and pathogen-free propagules, micropropagation has become a critical tool in the commercial cultivation of fruit crops such as banana, papaya, pineapple, apple and date palm (14-16). Building on these successes, this review synthesizes recent advances in clonal propagation of papaya, highlighting the distinct advantages and limitations of each method. It further explores the challenges involved and outlines opportunities for improving these vegetative propagation techniques to enhance their commercial adoption and support sustainable papaya cultivation.

Seed propagation

Papaya is predominantly propagated through seeds (17); however, seed germination is characteristically slow, erratic and influenced by several physiological and environmental factors. Approximately 20 % of seeds are devoid of embryos, contributing to poor germination rates (18). In addition, papaya seeds exhibit intermediate storage behaviour and can withstand partial loss of moisture without loss of viability (19) and dormancy induced by a hard seed coat. Germination efficiency is further reduced by the presence of aril-associated inhibitory compounds and suboptimal seed handling practices (20). Moreover, seed propagation generates considerable genetic variability in sex expression and fruit characteristics (21). Seeds derived from open-pollinated orchards fail to maintain parental characters, thereby compromising fruit quality and uniformity (22). In papaya, breeding programs are inclined towards the evaluation of disease-resistant cultivars, but it becomes highly complicated due to limited and unpredictable seed germination. These limitations, particularly pronounced in hybrids with low seed production, emphasize the necessity for alternative propagation strategies to ensure breeding efficiency, genetic stability and uniformity in papaya cultivation. Hence, propagation through seeds becomes problematic in papaya hybrids where the seed yield is very low. Therefore, clonal propagation techniques offer a possible solution for the multiplication of true-to-type plants in hybrids with low seed production.

Clonal propagation

Clonal propagation has gained substantial attention in recent years owing to the growing demand for genetically uniform, true-to-type planting material and the inherent limitations of seed-based propagation in papaya. Across fruit crops, vegetative propagation offers a decisive advantage by enabling the production of plants with predetermined and stable sex expression. Initial efforts in papaya were reported in grafting (23), cuttings (24) and budding (21, 25) and are primarily aimed at reducing variation to facilitate reliable performance evaluation. Among budding techniques, higher success rates were reported in patch budding compared to shield budding, which is limited by the difficulty of lifting the bark of papaya rootstocks (26). Long-term success with vegetative propagation has been demonstrated in the cultivar ‘Honey Gold’, maintained in South Africa for over 2 decades through leafy cuttings and grafting, with similar approaches applied for regionally adapted clones (27). Compared with seed-propagated plants, vegetatively propagated papaya exhibited prolonged productivity and improved clonal uniformity (Fig. 1).

Propagation by cuttings

During the early 1960s, a significant breakthrough in the propagation of dioecious papaya varieties emerged. The first instance of clonal propagation through cuttings in papaya was documented in South Africa and in the dioecious cultivar "Hortus Gold". The cuttings from the female plants of the variety were used for the multiplication of true-to-type planting materials (28). Subsequently, the same method was adopted for multiplication of planting material in the cultivar ‘Honey Gold’, a selection derived from ‘Hortus Gold’ with higher sugar content and anthracnose resistance. Extensive studies for propagation through cuttings have been undertaken in ‘Honey Gold’ over several decades in South Africa (25, 29-31). During 1990s, papaya propagation through cuttings was expanded globally beyond South Africa and in Israel, high rooting success (85-100 %) in semi-hardwood cuttings was documented in 3 different clones of papaya (9/13, 15/1, 15/8) treated with talc containing 1 % benomyl (Benlate) and 1 % potassium salt of indole-3-butyric acid (IBA), placed in an aerated medium under mist with bottom heat maintained at 30 °C (32). In Brazil, the successful propagation of Tainung 01 and Improved Sunrise Solo Line 72/12, without using heated rooting beds, unlike earlier studies, was reported (33). Cutting-derived plants exhibited vigorous growth, earlier fruiting at lower nodes, a shortened vegetative phase and produced marketable fruits within 7 months of transplanting, emphasizing their relevance for commercial exploitation. Propagation through cutting was also successfully adopted for the cultivar ‘Taino 02’ in Japan and for the transgenic hybrid resistant to papaya ringspot virus ‘Rainbow’ in the United States (34, 35).

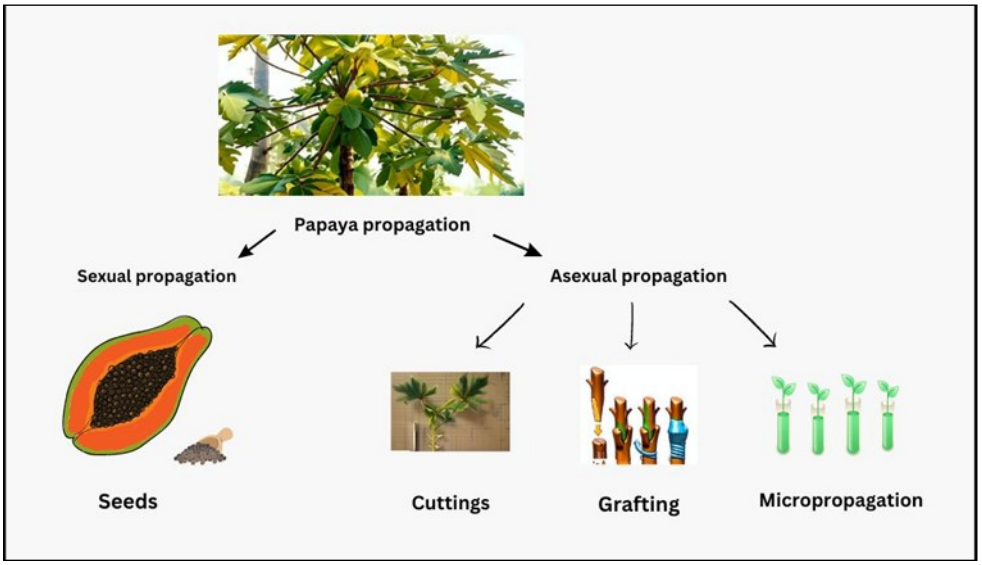

Fig 1. Propagation methods in papaya.

Rooting success in papaya is largely determined by the method of preparation of cuttings and their size. Initially, optimal rooting was obtained in cuttings of 12.7-30.5 cm length and 2.54 cm diameter (36). Later success was achieved with cuttings of smaller size (5-15 cm, 0.8-1.2 cm) and recently, mini cuttings (< 10 cm) are efficient for nursery management. In female "Honey Gold" papaya plants, macro-cuttings treated with 3000 mg L^{-1} IBA achieved a rooting success of 75-95 % (37). Cuttings are prepared from vigorous side shoots, preferably including the proximal swollen base (phellem tissue), with cuts made midway along the swollen tissue to maintain reserves. While preparing for cutting, the leaf lamina is removed and petioles are retained to minimize water loss, though in some protocols only the apical tip is left (38).

Auxin applications enhance rooting in cuttings, with IBA and 1-naphthaleneacetic acid (NAA) being most effective in papaya (Table 1). Optimal responses are observed with IBA at 1.25-2.5 µM for about 1 week, though higher doses over shorter periods can also be beneficial (36). Advanced gel formulations incorporating IBA, antimicrobials, vitamins and fungicides further improve rooting and cutting survival (38). Papaya cv. Eksotika II recorded 100 % rooting when cuttings were established in a sand layer with a peat moss: sand mixture (1:1) (39) while complete success was also obtained using rooting gels in perlite-coconut coir media, where roots developed within 3-5 weeks and plants flowered within 3 to 4 months, about 7 to 20 weeks earlier than those grown from seed (38).

Cutting propagation in papaya provides distinct advantages over seed propagation, particularly in ensuring genetic fidelity, guaranteed sex expression and uniformity of plant traits. Plants derived from cuttings flower earlier at shorter heights and reach harvest sooner, offering a significant reduction in the juvenile phase and accelerating both conventional and non-conventional breeding programs. This method also allows the maintenance and rapid multiplication of elite mother plants with desirable characteristics. However, its effectiveness relies heavily on the use of disease-free, healthy mother plants and well-managed production systems. Despite challenges such as potential disease transmission, reduced genetic variability and comparatively higher establishment costs, cutting propagation remains a valuable approach for achieving uniform, early-bearing and superior papaya plants.

Propagation by grafting

The art of grafting consists of joining 2 pieces of living plant tissue together so that they will unite and grow and develop into a composite plant. The primary purpose of grafting is to preserve and propagate desirable traits. Initial reports included grafting female branches onto male plants of dioecious varieties, as well as successful field trials in cultivars like 'Coorg Honey Dew' and 'CO 1'. The CO series represents Coimbatore, as these varieties are released from TNAU (Tamil Nadu Agricultural University), Coimbatore (40). Tongue, approach and cleft grafting are considered the most suitable techniques, with outcomes influenced by plant vigour, compatibility and post-grafting care.

A key challenge in papaya grafting is the limited lateral shoot production caused by strong apical dominance and this constraint can be mitigated by the application of hormones, viz., benzyladenine (BA) and gibberellic acid (GA), which promote side shoot development and provide suitable scions for grafting (41). Application of BA (500 mg L^{-1}) and GA (1000 mg L^{-1}) in lanolin paste to mature plant parts significantly increased axillary bud formation, while foliar application of BA (500 mg L^{-1}) and GA (100 mg L^{-1}) thrice a week also proved effective (32). For quality scion production, mother plants are to be maintained under insect-proof net houses and growth regulator treatments with auxins will aid in enhanced production of lateral shoot (42). Rootstocks play a vital role in determining scion vigor, water transport and graft compatibility (43). One month old 'Red Lady' rootstocks showed

Table 1. Rooting response of papaya cultivars as influenced by IBA application

Cultivar/Clone	IBA concentration/treatment	Rooting percentage (%)	Country
Hortus Gold	Not reported	Successful	South Africa
Honey Gold	3000 mg L^{-1} IBA	75-95 %	South Africa
9/13, 15/1, 15/8	1 % IBA + 1 % benomyl	85-100 %	Israel
Tainung 01; Sunrise Solo Line 72/12	Not reported	High success	Brazil
Taino 02	Not reported	Successful	Japan
Rainbow (PRSV-resistant hybrid)	Not reported	Successful	USA
Eksotika II	IBA	100 %	Malaysia

good graft success in both summer and autumn, while 'Viorica' demonstrated tolerance to papaya dieback when grafted with susceptible scions like 'Eksotika'. Similarly, 3 month old CO 2 rootstocks grafted with 9-1(D) gave superior results (44). Intervarietal grafts, such as TNAU Papaya CO 8 scion on TNAU Papaya CO 8 rootstock, improved chlorophyll content and soluble protein levels, reflecting enhanced physiological performance (45).

Several grafting methods have been explored for papaya propagation, with cleft grafting proving most reliable, showing up to 96 % success in varieties like CO 2 (46) and 80 % in 'Eksotika' (47). Cleft grafted plants also exhibited desirable traits, *viz.,* hermaphroditism, early fruiting at lower heights and longer productive life in papaya cv. Eksotika (47). Side grafting and wedge grafting have also been effective, with success rates around 80-81 %, though side grafting often suffers from bacterial scion rot (48). Softwood grafting exhibited higher success than cleft and side grafting, while tongue and approach grafting show moderate success but are more labour-intensive (49).

Micrografting in papaya

Micrografting techniques further expand possibilities for precision propagation. Micrografting is primarily employed to obtain virus-free and disease-free plant material, breeding specific genotypic combinations and for studying graft incompatibility between scions and rootstocks (50). Compared to traditional grafting, micrografting has several potential advantages including rapid production with reduced space requirements and the production of disease-free, genetically uniform, healthy, early-bearing and wind-resistant plantlets (51). Grafting combines the desirable fruit quality traits of the scion with the adaptability of the rootstock. In a study on three Kenyan papaya lines, *in vitro* micrografting was performed using shoot tips excised from seedlings cultured on MS medium with 0.1 mg L^{-1} 6-benzylaminopurine (BAP) and 0.05 mg L^{-1} NAA. After 28 days, the highest success was recorded in self-grafted combinations, with papaya line 1 and line 2 achieving 75 % and 80 % grafting success respectively, indicating superior compatibility when grafted onto their own rootstocks (51). Despite their effectiveness, grafting methods may influence gene expression across vegetative generations through mobile genetic elements and epigenetic changes, though the nuclear genome remains unchanged (52). Hence, long-term monitoring of grafted papaya plants is essential to ensure stability of traits like yield, stress tolerance and disease resistance.

Advantages of papaya grafting

Grafting is an effective method of papaya propagation that offers several advantages, such as improved disease tolerance/resistance by using rootstocks tolerant/resistant to soil-borne pathogens, enhanced plant vigor and higher yields. It enables genetic uniformity and consistent fruit quality, making it valuable for commercial cultivation. Additionally, grafted papaya plants often flower and fruit earlier, with dwarf stature suited for high-density planting systems. However, the technique requires skilled labour and meticulous care than seed propagation and may be affected by graft incompatibility or bacterial infections in scions (53). These limitations mean that while grafting is promising, careful management and protocol refinement to achieve a higher success rate with reduced cost of production are needed for large-scale adoption.

Micropropagation

Compared to traditional propagation methods such as seedling production and grafting, micropropagation through tissue culture offers a more appropriate approach for papaya, particularly for the rapid and large-scale production of disease-free, true-to-type planting material. In papaya, tissue culture-based techniques like organogenesis, somatic embryogenesis and shoot tip culture are especially valuable for multiplying elite lines with desirable traits such as high yield, improved fruit quality and tolerance to papaya ringspot virus. In addition to commercial clonal propagation, papaya tissue culture is widely used in studies on plant pathogen interactions, production of valuable biochemical compounds such as proteolytic enzymes (papain and chymopapain), genetic improvement through *in vitro* breeding and transformation and long-term conservation of superior germplasm (54). India is now emerging as one of the leading producers of tissue-cultured plants comprising around 200 commercial tissue culture laboratories with a production capacity of 500 million plantlets per year (55). Propagation of papaya through tissue culture has been reported in many studies (56-62). Although tissue culture protocols have been reported earlier in different cultivars, are not yet available to growers commercially. *In vitro* propagation of papaya primarily relies on micropropagation, organogenesis and somatic embryogenesis, which serve as key methods for plant regeneration (Fig. 2). These approaches are widely applied to facilitate classical genetic improvement, enabling hybridization among different Caricaceae species. In papaya, asexual propagation through cuttings and grafting has shown only partial success and is not widely adopted for commercial use (61). Moreover, these methods fail to prevent the sap-mediated transmission of PRSV, often leading to significant economic losses. Similarly, in papaya, *in vitro* regeneration has been successfully established using different approaches: organogenesis (2, 17, 60, 63-65), somatic embryogenesis (66-68) and suspension cultures (63, 69). These tissue culture-based methods not only enable efficient clonal multiplication but also provide a platform for genetic improvement and virus-free plant production.

Organogenesis

Organogenesis is one of the *in vitro* regeneration techniques in which adventitious buds develop either directly from explants or indirectly through callus formation (70). Its success is influenced by several factors, including the plant species, genotype, explant source, culture medium composition, hormonal balance and environmental conditions. Different types of explants have been successfully utilized for *in vitro* regeneration across fruit crops. For instance, nodal segments and shoot tips in banana (71), axillary buds and shoot tips in grapes (72), leaf discs, shoot tips, axillary shoots in aonla (73) and nodal segments in jamun (74, 75). Similarly, Table 2 below summarizes the different types of explants employed in papaya propagation.

Direct organogenesis and indirect organogenesis

Direct organogenesis occurs when shoots or buds arise directly from the explant tissues (e.g., from nodal segments, leaf bases, or hypocotyls) without an intervening callus phase. It is usually faster, genetically more stable and preferred for clonal propagation because the risk of soma clonal variation is low. Indirect organogenesis, on the other hand, involves two steps: first, the explant forms a callus mass and then shoots/buds differentiate from this callus. Although it can be more flexible and allows regeneration

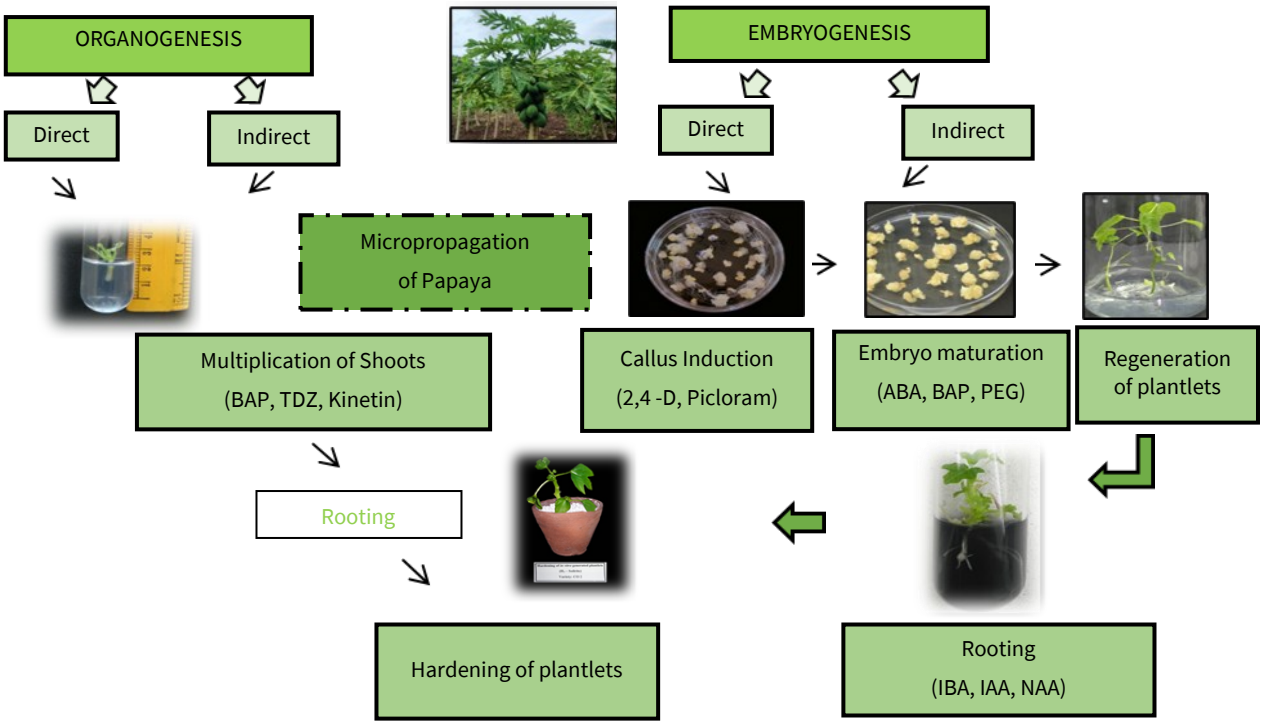

Fig. 2. Micropropagation methods in papaya.

Table 2. *In vitro* propagation of papaya through organogenesis

S. No.	Explant used	Cultivar/genotype	Reference
1.	Stem bits	*Carica papaya*	(83)
2.	Auxiliary buds	*C. papaya*	(84)
3.	Shoot tips	Rajshahi Red	(79)
4.	Shoot tips	Taiping	(85)
5.	Shoot tips	Red Lady	(86)
6.	Shoot tips	Rainbow	(62)
7.	Shoot tips	Callina	(87)
8.	Shoot tips	CO 2 and CO 8	(65)
9.	Shoot tips	Pusa Dwarf	(82)
10.	Multiple shoots	*C. papaya*	(88)
11.	Nodal segments	Red Lady	(89)
12.	Lateral bud culture	Pusa Dwarf	(57)
13.	Petioles	Rajshahi Red	(79)
14.	Root segments	Tainung No. 2	(90)
15.	Epicotyl segments	CO 7	(60)

from a wider range of explants or even highly dedifferentiated tissues, it is slower and more prone to genetic variation and off-types due to the callus phase.

The initial phase of organogenesis involves the initiation of adventitious buds from the explant used. The role of auxins and cytokinins (NAA and BAP) in regeneration and callus induction is widely used in the induction and regeneration of adventitious buds. NAA plays an essential role in innumerable physiological processes such as cell elongation, division, vascular tissue development and root initiation (76, 77). BAP stands out as the 1st synthetic cytokinin known for its role in stimulating plant growth and development and fruit setting. Moreover, it enhances fruit richness by initiating cell divisions (78). Several studies have shown that varying combinations and concentrations of BAP and NAA are highly effective in inducing and proliferating adventitious buds from diverse explant sources (60, 65). A micropropagation protocol was established for papaya variety CO 7 using epicotyl segments from *in vitro* derived seedlings through direct organogenesis (60). Similarly, an efficient regeneration method via indirect organogenesis from petiole explants of papaya cv. Rajshahi Red was also developed (79). Optimal callus formation was achieved on Murashige and Skoog (MS) medium supplemented with NAA (0.5-10.5 µM) and BA (0.5-5 µM), resulting in firm, green and nodular callus. Subsequent shoot regeneration was obtained on MS medium containing 0.1 µM NAA and 100 mg L^{-1} casein hydrolysate, after which shoots were transferred to a growth regulator-free medium to facilitate elongation. Increased concentrations of auxins and cytokinins were found to reduce shoot proliferation (61) while higher levels of thidiazuron (TDZ) caused a decline in callus formation in cultures (80).

Efficient *in vitro* seed germination and clonal propagation techniques have been standardized for cultivars CO 2 and TNAU Papaya CO 8. Surface sterilization with 5 % sodium hypochlorite for 1 min proved most effective in reducing contamination, while seed coat removal enhanced germination further. Pre-soaking treatments demonstrated that 500 ppm GA3 for 12 hr significantly improved germination percentage, seedling height and vigour index, whereas 10 % potassium nitrate (KNO_3) promoted early seedling emergence. The *in vitro* raised seedlings served as axenic sources of explants, enabling the development of clonal propagation protocols using shoot tip cultures. Multiple shoot induction was achieved on MS medium with 0.25 mg L^{-1} TDZ, followed by enhanced proliferation on MS supplemented with 1 mg L^{-1} BAP and 0.1 mg L^{-1} NAA. Shoot elongation was promoted by 3 mg L^{-1} GA3, while rooting was optimized on ½ MS medium with 2.5 mg L^{-1} IBA and activated charcoal. Acclimatized plants recorded survival rates above 70 % (65).

Inoculated shoot tips of the Horana Papaya hybrid 01 cultured in 1.0 mg L^{-1} BAP effectively produced an average of 4.8 shoots per explant (81). These proliferated shoots were subsequently cultured on a 1.5 strength MS medium supplemented with 0.25 mg L^{-1} BAP and varying concentrations of gibberellic acid (0, 0.15, 0.30 mgL^{-1}) to promote further elongation. The earliest shoot initiation was observed at 11.20 days in MS medium supplemented with 100 ppm glycine, 5 ppm casein hydrolysate and 0.05 % activated charcoal (82). Additionally, the longest shoot length of 3.25 cm was achieved in cv. Pusa Dwarf using MS medium supplemented with 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA, highlighting the significant role of optimized growth regulator combinations in enhancing papaya micropropagation.

Somatic embryogenesis: Explant sources and callus formation

Somatic embryogenesis offers a promising alternative for clonal propagation, as somatic embryos are bipolar in nature, enabling the simultaneous formation of both shoot and root apices. This bypasses the need for additional aseptic manipulations. This technique also supports large-scale multiplication and long-term conservation of elite papaya genotypes, including those with tolerance to PRSV-P strain, the predominant strain infecting papaya in India (56, 69, 91). Somatic embryogenesis can be established either through direct or indirect pathways. Since its 1st successful attempts in the 1980s, numerous protocols have been developed using different explant sources, as outlined in Table 3. Among these, immature zygotic embryos have proven to be the most effective and widely utilized explants for inducing somatic embryogenesis (66, 68, 69, 92-96). The first report of somatic embryo induction in papaya through indirect organogenesis was achieved using peduncles of *V. stipulata* as explants (97). Subsequently, somatic embryogenesis was obtained via interspecific hybridization between *C. papaya* and *C. cauliflora*, 2 species incompatible through grafting (98). Several protocols for somatic embryogenesis in papaya have been developed, including successful induction from immature zygotic embryos of widely cultivated *C. papaya* varieties (94). Their results demonstrated that a lower concentration of TDZ (2.27 µM) was most effective, producing the highest embryogenic response across all tested varieties. Notably, the ability to induce somatic embryogenesis was found to vary among cultivars, highlighting the role of genotype in regeneration efficiency.

Several studies have demonstrated that auxins, particularly 2,4-D, picloram and NAA, are highly effective in promoting embryogenic callus induction in papaya. Somatic embryogenesis was induced from immature zygotic embryos of several *C. papaya* varieties, including Coorg Honey Dew, Washington, Honey Dew, Pusa Delicious, Pusa Nanha, Taiwan 786, Taiwan 785, Sunrise Solo, CO 1, CO 7 and CO 3. Embryogenic callus was obtained within 4-6 weeks on MS medium supplemented with 4.52 µM 2,4-D and 2.27 µM TDZ, while no response occurred on hormone-free medium. Genotypic variation was observed, with the highest embryogenesis in Taiwan 786 (87.0 %), followed by Taiwan 785 (85.0 %) and Coorg Honey Dew (81.0 %) (94).

A rapid *in vitro* method for multiple shoot regeneration from immature embryo axis explants of *C. papaya* cv. Honey Dew, Washington and CO 2 were developed using modified MS medium. The highest shoot regeneration was obtained with 2.25 µM TDZ or a combination of 4.4 µM BAP and 0.5 µM NAA, while higher TDZ concentrations produced stunted shoots that elongated on 5.7 µM GA3. Rooting was induced with IBA (4.92-19.68 µM) and the regenerated plantlets were successfully hardened and transferred to pots (58). Similarly, the effect of varying boric acid levels (30 to 500 mg L^{-1}) on callus development from immature zygotic embryos of *C. papaya* cv. Honey Dew was assessed using MS medium supplemented with B5 Gamborg vitamins, 30 g L^{-1} sucrose and either 2 mg L^{-1} 2,4-D or 1 mgL^{-1} picloram (64). The study reported that 62 mg L^{-1} boric acid significantly improved callus induction, independent of the auxin used. Further investigations assessing a broad range of plant growth regulators, including auxins, cytokinins, alternative plant growth regulators (PGRs), inhibitors and retardants, highlighted the superior response of auxins such as 2,4-D, picloram and dicamba in inducing callus from explants at different developmental stages. Papain preparations with specific activities of ≥ 10-30 TU mg^{-1} protein are already exploited at industrial scale as meat tenderizers in food, detergent, leather and pharmaceutical formulations, while chymopapain has been used clinically in chemonucleolysis of herniated lumbar discs at doses of approximately 2-4 nanokatals per disc, underscoring the biotechnological and industrial relevance of developing highly embryogenic, protease-rich papaya lines (61).

Maturation, germination and rooting challenges in papaya somatic embryos

Somatic embryo maturation and germination are encouraged by various compounds, viz., polyethylene glycol (PEG), Abscisic acid (ABA), phloroglucinol (PG) (67). According to research, somatic embryo maturation in papaya exhibits a positive correlation in response to high doses of ABA (69). When ABA is lacking during maturation, somatic embryos become malformed and frequently aberrant, with little potential for germination and seedling development (109). Desiccation induced by PEG may promote endogenous ABA production, an essential hormone for creating reserve compounds during embryonic development. The maturation of calli was achieved using different concentrations of ABA (5-40 µM) along with ½-strength MS salts

Table 3. *In vitro* propagation of papaya through somatic embryogenesis

S. No.	Explant used	Cultivar/genotype	Reference
1.	Immature zygotic embryos 90 to 114 days old	Sunrise, Sunset, Waimanalo and Kapoho	(66)
2.	Immature zygotic embryo (60 days)	*C. papaya* hybrid between Costarica Red *C. papaya* as female with male *C. cauliflora*	(98)
3.	Immature zygotic embryos	CO 7	(69)
4.	Immature zygotic embryos	Eksotika	(99)
5.	Immature zygotic embryos	Red Maradol	(67)
6.	Immature zygotic embryos, hypocotyl and root apices	Pusa Delicious, CO 7 and Red Lady	(100)
7.	Immature zygotic embryos, leaf bits and hypocotyl	CO 7, TNAU Papaya CO 8, Red Lady	(68)
8.	Mature zygotic embryos	Sunrise Solo	(101)
9.	Young leaf segments	Shahi	(102)
10.	Nodular cultures	CO 7	(103)
11.	Apical shoots and cotyledonary leaves	THB Papaya	(104)
12.	Leaf and petiole	Pusa Nanha	(105)
13.	Anther	*Vasconcellea pubescens*	(106)
14.	Ovule	*Carica papaya*	(107)
15.	Leaves, roots, hypocotyls and mature zygotic embryos	Coorg Honeydew	(108)

and vitamins, 400 mg L^{-1} glutamine, 10 g L^{-1} sucrose and 4 g L^{-1} phytagel, with maximum maturity percentage observed at 40 µM ABA in liquid medium (110). In papaya cv. Pusa Delicious, somatic embryo maturation was further studied under the influence of mannitol, sorbitol and PEG at 15, 30 and 45 mg L^{-1} in MS medium supplemented with 75 mg L^{-1} kanamycin, 0.5 mg L^{-1} BAP and 20 % sucrose (111). Among the 3 osmotic agents utilized for embryo dehydration, 45 mg L^{-1} PEG demonstrated the highest effectiveness, resulting in the maximum conversion of embryos into micro shoots (81 shoots/culture). Casein hydrolysate was important and appropriate for the development of somatic embryogenesis produced from zygotic embryos or hypocotyl explants of papaya cv. Rathna (112). The highest globular embryo maturation was achieved in MS media supplemented with 1.5 mg L^{-1} ABA and 0.4 mg L^{-1} BAP, with 53.33 % in TNAU Papaya CO 8, 58.33 % in CO 7 and 56.67 % in Red Lady (82). Somatic embryo germination in papaya cv. Ekostika showed increased regenerative potential when cultured in MS medium supplemented with 30 g L^{-1} sucrose, 0.1 mg L^{-1} NAA and 0.1 mg L^{-1} BAP (113).

Papaya culture regenerated via embryogenesis, micropropagation, or organogenesis currently faces a significant hurdle in rooting and *ex vitro* acclimatization of regenerated plants. During acclimatization, plantlets often tend to decline and their growth after transplantation may be suboptimal in the field (114). In recent studies, several researchers emphasized that the physical and chemical nature of the rooting substrates affects the rooting nature (115, 116). Sometimes morphological abnormalities are found in roots. Auxins such as IBA, IAA (Indole -3-Acetic acid) and NAA are crucial for effective root induction. Auxins are essential for root formation, with the synthetic auxin IBA being particularly effective and consistent in promoting root development (98). However, prolonged exposure to elevated levels of IBA for more than a week may lead to inhibition of root development and also cause chlorosis in shoots and form thickened roots due to excessive dehydration. In papaya, rooting was very difficult under *in vitro* because of the formation of callus at the base of the micro shoots. Further, the roots formed with callus possess a low survival rate under field conditions (117).

Somaclonal Variations

Somaclonal variation represents a major limitation in papaya tissue culture, as prolonged *in vitro* subcultures and culture conditions often lead to genetic instability, resulting in non-uniform growth and reduced fruit quality (118, 119). Although somaclonal variation can generate useful genetic diversity for breeding, its practical benefits are limited due to unpredictable and often undesirable mutations (120). The risk of variation is further influenced by tissue origin, with differentiated tissues such as leaves and roots producing higher variability than meristematic explants (120). Moreover, exogenous PGRs, particularly higher concentrations of 2,4-D, can disrupt the cell cycle and induce oxidative stress, contributing to chromosomal anomalies, including euploidy and aneuploidy (69, 121). Cryopreservation followed by *in vitro* regeneration may also trigger somaclonal changes, underscoring the importance of optimizing growth regulators, tissue selection and culture duration to maintain genetic fidelity (119).

Conclusion

A clonal propagation system for papaya may bring several benefits to the productive system; however, protocols still need to be enhanced to make it feasible for commercial use. Propagation through cuttings and grafting has been partially successful but is still not commercially practiced due to the limited multiplication rate and further, these methods could spread the sap transmissible PRSV disease. One of the main hindrances to *in vitro* regeneration techniques is the lower survival rate of the roots and *ex vitro* acclimatization stages of regenerated plants, whether through embryogenesis, micropropagation, or organogenesis in papaya culture. Many biotechnology applications, including synseeds, transgenic plants and micropropagation, depend on plant regeneration using plant tissue culture techniques, particularly somatic embryogenesis. Similarly, somatic embryos make excellent platforms for genetic transformation studies and are appropriate for long-term storage techniques like cryopreservation.

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Authors' contributions

SC collected the literature and wrote the original draft. KC reviewed and edited the manuscript. GM, KKK, BRP, MSK and AJ reviewed the manuscript with valuable inputs. All authors read and approved the final manuscript.

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References

1. Chan HT Jr, Tang C-S. The chemistry and biochemistry of papaya. Tropical foods. 2012;1:33-53. https://doi.org/10.1016/B978-0-12-370901-1.50007-6
2. Kanth N, Singh AK, Syamal M. Effect of media pH on shoot proliferation of papaya (*Carica papaya L.*). International Journal of Current Microbiological Applied Science. 2017;6(10):1633-7. https://doi.org/10.20546/ijcmas.2017.610.196
3. Emeruwa A. Antibacterial substance from *Carica papaya* fruit extract. Journal of Natural Products. 1982;45(2):123-7. https://doi.org/10.1021/np50020a002
4. Singh SP, Kumar S, Mathan SV, Tomar MS, Singh RK, Verma PK, et al. Therapeutic application of *Carica papaya* leaf extract in the management of human diseases. DARU Journal of Pharmaceutical Sciences. 2020;28(2):735-44. https://doi.org/10.1007/s40199-020-00348-7

5. Food and agricultural organization corporate statistical database. 2024.
6. Storey WB. Genetics of the papaya. 1953. https://doi.org/10.1093/oxfordjournals.jhered.a106358
7. Hartmann HT, Kester DE. Plant propagation: principles and practices. 1975.
8. Storey WB. Pistillate papaya flower: a morphological anomaly. Science. 1969;163(3865):401-5. https://doi.org/10.1126/science.163.3865.401
9. Rimberia FK, Ombwara FK, Mumo NN, Ateka EM. Genetic Improvement of papaya (*Carica papaya L.).* Advances in plant breeding strategies: Fruits: Volume 3: Springer; 2018. p. 897-928. https://doi.org/10.1007/978-3-319-91944-7_21
10. Manoharan L, Chinnasamy K. 'Validation of sex expression in papaya using molecular markers'. Research Journal of Agricultural Sciences. 2018;9(6):1219-22.
11. Thirugnanavel A, Balamohan T, Karunakaran G, Manoranjitham S. Effect of papaya ringspot virus on growth, yield and quality of papaya (*Carica papaya*) cultivars. Indian Journal of Agricultural Sciences. 2015;85(8):1069-73. https://doi.org/10.56093/ijas.v85i8.50852
12. Hazarika B, Gogoi S, Das RT, Bordoloi A. Studies on seed germination, growth and vigour of papaya seedlings cv."Sapna" as influenced by growth regulators and propagation media. The Pharma Innovation Journal. 2023;12(11):2387-91.
13. Lanjhiyana R, Sahu G, Panigrahi H, Katiyar P. Role of pre-sowing seed treatment on germination behavior and seedling vigour of papaya (*Carica papaya* L.). Journal of Pharmacognosy and Phytochemistry. 2020;9(5):3039-42. https://doi.org/10.22271/phyto.2020.v9.i5ap.12803
14. Mishra M, Rajan S, Damodaran T. New paradigm shifts in micropropagation of fruit crops through bioreactors-a review. Indian J Hort. 2024;81:1-10. https://doi.org/10.58993/ijh/2024.81.1.1
15. Navarro L, Moreno B, Quiroga S, Pétiard V, Tanksley S, Casa A. Papaya (*Carica papaya* L.) micro-propagation and breeding. V International Symposium on Papaya 1250; 2017.
16. Kaluram, Vasugi C, Thomas P, Dinesh M, Nandeesha P. A comparative evaluation of micro-propagated and seed derived plants of intergeneric papaya hybrids. 2022. https://doi.org/10.5958/0974-0112.2022.00065.2
17. Bhattacharya J, Khuspe S. *In vitro* and *in vivo* germination of papaya (*Carica papaya* L.) seeds. Scientia Horticulturae. 2001;91(1-2):39-49. https://doi.org/10.1016/S0304-4238(01)00237-0
18. Furutani SC, Nagao MA. Influence of temperature, KNO3, GA3 and seed drying on emergence of papaya seedlings. Scientia Horticulturae. 1987;32(1-2):67-72. https://doi.org/10.1016/0304-4238(87)90017-3
19. Vozzo JA. Tropical tree seed manual: US Department of Agriculture, Forest Service; 2002.
20. Campos JCC, Ribeiro JC, Couto L. Emprego da distribuição diamétrica na determinação da intensidade de corte em matas naturais submetidas ao sistema de seleção. Rev Arvore. 1983;7 (2):110.
21. Sookmark S, Tai E, editors. Vegetative propagation of papaya by budding. III Africa Symposium on Horticultural Crops 49; 1973.
22. Senthilkumar S, Kumar N, Soorianathasundaram K, Jeyakumar P. Effect of plant bio-regulators in induction of lateral shoots in papaya cv. CO. 2. 2014.
23. Hancock W. Grafting male papaw trees. 1940.
24. Allan P. Pawpaw grown from cuttings-are more true to type-bear earlier-lower and longer. Farming in South Africa. 1964;101:1-6.
25. Allan P. Clonal "Honey Gold" pawpaws. A horticultural and commercial success. 1981.
26. Singh R, Singh G, Rao O. Vegetative propagation of papaya through budding. Indian Journal of Horticulture. 1986;43(1 and 2):1-8.
27. Allan P, editor. Propogation of 'Honey Gold' papayas by cuttings. International Symposium on Tropical Fruits 370; 1993.
28. Allan P. Papaws grown from cuttings. 1964.
29. Allan P, editor. Vegetative propagation and production of 'Honey Gold' papayas. Symposium on Tropical Fruit in International Trade 269; 1989. https://doi.org/10.17660/ActaHortic.1990.269.13
30. Allan P. Clonal pawpaws prove successful. 40 year old clone continues to produce well. SA Fruit Journal (South Africa). 2003;2(5).
31. Allan P, editor. Phenology and production of *Carica papaya* 'Honey Gold' under cool subtropical conditions. I International Symposium on Papaya 740; 2005.
32. Reuveni O, Shlesinger D, editors. Rapid vegetative propagation of papaya plants by cuttings. International Symposium on the Culture of Subtropical and Tropical Fruits and Crops 275; 1989. https://doi.org/10.17660/ActaHortic.1990.275.36
33. Katoh F, Ooishi A. A study on juvenility and nodal aging of papaya by vegetative propagation. Journal of the Japanese Society for Horticultural Science. 2003;72(2):93-8. https://doi.org/10.2503/jjshs.72.93
34. Fitch M. *Carica papaya* papaya. Biotechnology of fruit and nut crops: CAB International Wallingford UK; 2005. p. 174-201. https://doi.org/10.1079/9780851996622.0174
35. Schmildt O, Netto AT, Schmildt ER, Carvalho VS, Otoni WC, Campostrini E. Photosynthetic capacity, growth and water relations in 'Golden' papaya cultivated *in vitro* with modifications in light quality, sucrose concentration and ventilation. Theoretical and Experimental Plant Physiology. 2015;27(1):7-18. https://doi.org/10.1007/s40626-014-0026-y
36. Nguyen V-H, Nguyen M-T, Nguyen T-T, Tran D-H. Rooting media and growth regulators affect propagation from stem cuttings of *Carica papaya L* cv 'Tainung No. 2' and 'Red Lady'. Int J Res Agric Sci. 2022;9:24-35.
37. Allan P, Carlson C. Progress and problems in rooting clonal *Carica papaya* cuttings. South African Journal of Plant and Soil. 2007;24 (1):22-5. https://doi.org/10.1080/02571862.2007.10634776
38. Montás W, Moon P, Crane JH, editors. Propagation of Papaya (*Carica papaya* L) with Large-sized Cuttings. Proceedings of the Florida State Horticultural Society; 2016.
39. Muhamad Hafiz M, Hartinee A, Ab Kahar S, Zabedah M. Effect of different media use on rooting and growth of Eksotika II papaya cuttings (*Carica papaya* L.). 2013.
40. Airi S, Gill S, Singh S. Clonal propagation of papaya (*Carica papaya* L.). 1986.
41. Senthilkumar S, Kumar N, Soorianathasundaram K, Kumar PJ. Aspects on asexual propagation in papaya (*Carica papaya* L.)-a review. Agricultural Reviews. 2014;35(4):307-13. https://doi.org/10.5958/0976-0741.2014.00919.2
42. Meichander P, Vidhya D, Auxcilia J, Djanaguiraman M, Renukadevi P, Muthuvel I. Papaya grafting: Advance, mechanisms and future prospects. Plant Science Today. 2025. https://doi.org/10.14719/pst.8154
43. Rasool A, Mansoor S, Bhat K, Hassan G, Baba TR, Alyemeni MN, et al. Mechanisms underlying graft union formation and rootstock scion interaction in horticultural plants. Frontiers in Plant Science. 2020;11:590847. https://doi.org/10.3389/fpls.2020.590847
44. Nguyen V-H, Yen C-R. Rootstock age and grafting season affect graft success and plant growth of papaya (*Carica papaya* L.) in greenhouse. Chilean Journal of Agricultural Research. 2018;78(1):59-67. https://doi.org/10.4067/S0718-58392018000100059
45. Akino A, Auxcilia J, Soorianathasundaram K, Muthulakshmi P. Evaluation of seedlings and intervarietal grafts on biochemical properties and biotic stress tolerance enzymes activities of papaya

(*Carica papaya* L.). IJCS. 2018;6(4):2333-4.

46. Senthilkumar S, Kumar N, Soorianathasundaram K, Jeyakumar P. A histological investigation on graft formation in papaya (*Carica papaya* L.). Eco Env & Cons. 2016;22(Suppl):69–72.
47. Chong S, Prabhakaran R, Lee H, editors. An improved technique of propagating 'Eksotika' papaya. International Workshop on Tropical and Subtropical Fruits 787;2007. https://doi.org/10.17660/ActaHortic.2008.787.30
48. Hartmann HT, Kester DE. Plant propagation: principles and practices. 1959.
49. Satisha J, Vincent L. Vegetative propagation of papaya (*Carica papaya* L.) through grafting. Current Science (00113891). 2023;124 (2). https://doi.org/10.18520/cs/v124/i2/239-244
50. Navarro L, editor. Application of shoot-tip grafting *in vitro* to woody species. International Symposium on Vegetative Propagation of Woody Species 227;1987. https://doi.org/10.17660/ActaHortic.1988.227.2
51. Mumo N, Rimberia F, Mamati E, Kihurani A. Micrografting of selected Kenyan papaya (*Carica papaya* L.) lines. 2017.
52. Conceição CS, Felix KCS, Mariano RL, Medeiros EV, Souza EB. Combined effect of yeast and silicon on the control of bacterial fruit blotch in melon. Scientia Horticulturae. 2014;174:164-70. https://doi.org/10.1016/j.scienta.2014.05.027
53. Dogra K, Kour K, Kumar R, Bakshi P, Kumar V. Graft-incompatibility in horticultural crops. International Journal of Current Microbiology and Applied Sciences. 2018;7(2):1805-20. https://doi.org/10.20546/ijcmas.2018.702.218
54. Hartmann HT, Kester DE. Plant propagation: principles and practices. 1975.
55. Kadam DD, Chhatre AA, Lavale SA, Shinde NA. Low-cost alternatives for conventional tissue culture media. International Journal of Current Microbiology and Applied Sciences. 2018;7(4):2523-9. https://doi.org/10.20546/ijcmas.2018.704.288
56. Litz RE, Conover RA, editors. Tissue culture propagation of papaya. Proceedings of the Florida State Horticultural Society; 1977.
57. Rajeevan M, Pandey R. Lateral bud culture of papaya (*Carica papaya* L.) for clonal propagation. Plant Cell, Tissue and Organ Culture. 1986;6(2):181-8. https://doi.org/10.1007/BF00180803
58. Bhattacharya J, Renukdas N, Khuspe S, Rawal S. Multiple shoot regeneration from immature embryo explants of papaya. Biologia Plantarum. 2003;47(3):327-31. https://doi.org/10.1023/B:BIOP.0000023873.55327.fe
59. Agnihotri S, Singh S, Jain M, Sharma M, Sharma A, Chaturvedi H. *In vitro* cloning of female and male *Carica papaya* through tips of shoots and inflorescences. 2004.
60. Anandan R, Thirugnanakumar S, Sudhakar D, Balasubramanian P. *In vitro* organogenesis and plantlet regeneration of (Carica papaya L.). Journal of Agricultural Technology. 2011;7(5):1339-48.
61. Setargie A, Mekbib F, Abraha E. *In vitro* propagation of papaya (*Carica papaya* L.). World Journal of Agricultural Sciences. 2015;11 (2):84-8.
62. Caple AD, Cheah KT. Micropropagation of hermaphrodite Carica papaya L.'Rainbow'seedlings via axillary bud pathway. Biotechnology. 2016;12:1-5.
63. Litz RE, Stephen K, Conover RA. *In vitro* growth of *Carica papaya* L. cotyledons. Scientia Horticulturae. 1983;19(3-4):287-93. https://doi.org/10.1016/0304-4238(83)90076-6
64. Da Silva JAT. *In vitro* response of papaya (Carica papaya) to plant growth regulators. Nusantara Bioscience. 2016;8(1). https://doi.org/10.13057/nusbiosci/n080114
65. Bowiya K, Soorianathasundaram K, Kavitha C, Hemaprabha K. Effect of presoaking treatments on *in vitro* seed germination of papaya to facilitate axenic explant production. Int J Curr Microbiol Appl Sci. 2019;7(4):50-5.
66. Fitch MM, Manshardt RM. Somatic embryogenesis and plant regeneration from immature zygotic embryos of papaya (*Carica papaya* L.). Plant cell reports. 1990;9(6):320-4. https://doi.org/10.1007/BF00232860
67. Pérez LP, Montesinos YP, Olmedo JG, Rodriguez RB, Sánchez RR, Montenegro ON, et al. Effect of phloroglucinol on rooting and *in vitro* acclimatization of papaya (Carica papaya L. var. Maradol Roja). *In Vitro* Cellular & Developmental Biology-Plant. 2016;52(2):196-203. https://doi.org/10.1007/s11627-015-9733-6
68. Rajesh C, Kumar K, Kavitha C, Karthikeyan G, Soorianathasundaram K. Differential influence of growth regulators during somatic embryogenesis of gynodioecious Papaya Varieties "CO. 7" and'Red Lady'. Adv Res. 2020;196:10-8. https://doi.org/10.9734/air/2020/v21i430196
69. Anandan R, Sudhakar D, Balasubramanian P, Gutiérrez-Mora A. *In vitro* somatic embryogenesis from suspension cultures of *Carica papaya* L. Scientia Horticulturae. 2012;136:43-9. https://doi.org/10.1016/j.scienta.2012.01.003
70. Grattapaglia D, Machado MA. micropropagação. Cultura de tecidos e transformação genética de plantas. 1998;1:183-260.
71. Kavitha C, Surakhshitha N. *In vitro* plant regeneration of grape cv.'Red globe' through direct organogenesis. 2023. https://doi.org/10.5958/0974-4517.2023.00026.5
72. Theivanai M, Kavitha C, Ganga M, Hemaprabha K. Standardization of micropropagation protocol for grape rootstock Dog Ridge. Indian Journal of Horticulture. 2020;77(4):582-8. https://doi.org/10.5958/0974-0112.2020.00084.5
73. Mishra M, Pathak R. Effect of nodal position and season on *in vitro* shoot proliferation in aonla (Emblica officinalis Gaertn.). Journal of Applied Horticulture. 2001;3(2):103-4. https://doi.org/10.37855/jah.2001.v03i02.11
74. Yadav U, Lal M, Jaiswal VS. *In vitro* micropropagation of the tropical fruit tree Syzygium cuminii L. Plant cell, tissue and organ culture. 1990;21(1):87-92. https://doi.org/10.1007/BF00034498
75. Jain N, Babbar S. Recurrent production of plants of black plum, Syzygium cuminii (L.) Skeels, a myrtaceous fruit tree, from *in vitro* cultured seedling explants. Plant Cell Reports. 2000;19(5):519-24. https://doi.org/10.1007/s002990050766
76. Al-Duraid M, Al-Taey K, Al-Kikhani AH, editors. Effect of phenylalanine and naphthalene acetic acid on growth, yield and antioxidant activity of Fenugreek Trigonella foenum-graecum. IOP Conference Series: Earth and Environmental Science; 2019: IOP Publishing. https://doi.org/10.1088/1755-1315/388/1/012073
77. Safana HS, Ibrahim MA-HI, Abd A-KM. Effect of NAA and Chitosan in rooting branches resulting from stem nodes plantation of Kumquat (Citrus japonica) *in vitro*. Journal of Kerbala for Agricultural Sciences. 2022;9(1):7-18. https://doi.org/10.59658/jkas.v9i1.924
78. Hamza OM, Al-Taey DK. A study on the effect of glutamic acid and benzyl adenine application upon growth and yield parameters and active components of two broccoli hybrids. Int J Agricult Stat Sci. 2020;16(2):1163-7.
79. Hossain M, Rahman S, Islam R, Joarder O. High efficiency plant regeneration from petiole explants of *Carica papaya* L. through organogenesis. Plant Cell Reports. 1993;13(2):99-102. https://doi.org/10.1007/BF00235299
80. Waidyaratne S, Samanmalie L. *In vitro* propagation of Carica papaya L. Variety 'horana papaya hybrid 01' using shoot tip. World. 2022;10 (1):15-9. https://doi.org/10.12691/wjar-10-1-3
81. Kumari R, Kundu M, Mir H, Kumar P. *In vitro* regeneration technique of papaya (Carica papaya L.) Cv. Pusa Dwarf through shoot tip culture. National Academy Science Letters. 2023;46(1):1-5. https://doi.org/10.1007/s40009-022-01186-8
82. Fitch MM, Manshardt RM, Gonsalves D, Slightom JL. Transgenic

papaya plants from Agrobacterium-mediated transformation of somatic embryos. Plant cell reports. 1993;12(5):245-9. https://doi.org/10.1007/BF00237128

83. Gonsalves D. Control of papaya ringspot virus in papaya: a case study. Annual review of phytopathology. 1998;36(1):415-37. https://doi.org/10.1146/annurev.phyto.36.1.415

84. Renukdas N, Mohan M, Khuspe S, Rawal S. Influence of boron on somatic embryogenesis in papaya. Biologia plantarum. 2003;47 (1):129-32. https://doi.org/10.1023/A:1027353520043

85. Malabadi RB, Kumar SV, Mulgund GS, Nataraja K. Induction of somatic embryogenesis in papaya (*Carica papaya*). Research in Biotechnology. 2011;2(5):40-55.

86. Heringer AS, Vale EM, Barroso T, Santa-Catarina C, Silveira V. Polyethylene glycol effects on somatic embryogenesis of papaya hybrid UENF/CALIMAN 01 seeds. Theoretical and Experimental Plant Physiology. 2013;25:116-24. https://doi.org/10.1590/S2197-00252013000200004

87. Abreu IS, Carvalho CR, Clarindo WR. Massal induction of Carica papaya L.'Golden' somatic embryos and somaclone screening by flow cytometry and cytogenetic analysis. Cytologia. 2014;79(4):475-84. https://doi.org/10.1508/cytologia.79.475

88. Litz RE, Conover RA. Somatic Embryogenesis in Cell Cultures of Carica stipulata1. HortScience. 1980;15(6):733-4. https://doi.org/10.21273/HORTSCI.15.6.733

89. Chen M, Chen C, Wang D, Chen F. Somatic embryogenesis and plant regeneration from immature embryos of *Carica papaya* $\times$Carica cauliflora cultured *in vitro*. Canadian Journal of Botany. 1991;69(9):1913-8. https://doi.org/10.1139/b91-240

90. Da Costa AdFS, Abreu EFM, Schmildt ER, da Costa AN, Schmildt O. Advances observed in papaya tree propagation. Rev Bras Frutic. 2019;41:1-15. https://doi.org/10.1590/0100-29452019036

91. Anandan R, Soorianathasundaram K, Kumar N, Thirugnanakumar S, Sudhakar D, Balasubramanian P, editors. Somatic embryogenesis in *Carica papaya* through zygotic embryo derived callus culture. II International Symposium on Papaya 851; 2008.

92. Mishra M, Shukla N, Chandra R. Role of polyethylene glycol in maturation and germination of transformed somatic embryos of papaya (*Carica papaya* L.). Indian Journal of Biotechnology. 2010. https://doi.org/10.17660/ActaHortic.2010.851.33

93. Farzana A, Palkadapala P, Meddegoda K, Samarajeewa P, Eeswara J. Somatic embryogenesis in papaya (*Carica papaya* L.) cv. Rathna. Journal of the National Science Foundation of Sri Lanka. 2008;36(1). https://doi.org/10.4038/jnsfsr.v36i1.132

94. Vilasini P, Latipah Z, Salasiah A. Induction of somatic embryogenesis and plant regeneration from immature embryos of Eksotika papaya (*Carica papaya*). Journal of Tropical Agriculture and Food Science. 2000;28:121-6.

95. De Winnaar W. Clonal propagation of papaya *in vitro*. Plant cell, tissue and organ culture. 1988;12(3):305-10. https://doi.org/10.1007/BF00034373

96. Chow P, Kohashi C, Vangundy R, Murashige T. Effects of common components on hardness of culture media prepared with gelrite™. *In Vitro* Cellular & Developmental Biology-Plant. 1995;31(2):84-9. https://doi.org/10.1007/BF02632242

97. Huang L-C, Kohashi C, Vangundy R, Murashige T. Effects of common components on hardness of culture media prepared with gelrite™. *In Vitro* Cellular & Developmental Biology-Plant. 1995;31(2):84-9. https://doi.org/10.1007/BF02632242

98. Drew RA, McComb JA, Considine JA. Rhizogenesis and root growth of *Carica papaya* L. *in vitro* in relation to auxin sensitive phases and use of riboflavin. Plant Cell, Tissue and Organ Culture. 1993;33(1):1-7. https://doi.org/10.1007/BF01997591

99. Sekeli R, Abdullah JO, Muda P, Namasivayam P, Abu Bakar UK, Shin SY. 1-Aminocyclopropane-1-carboxylate oxidase 2 reduction effects on physical and physiological responses of Eksotika papaya. Journal of Crop Improvement. 2013;27(5):487-506. https://doi.org/10.1080/15427528.2013.795205

100. Joshi A, Tripathi A, Sharma N, Tailor A. Somaclonal variation for the improvement of tree species. Biotechnological approaches for sustaining forest trees and their products: Springer; 2024. p. 77-102. https://doi.org/10.1007/978-981-97-4363-6_4

101. Encina CL, Granero ML, Regalado JJ. *In Vitro* Long-Term Cultures of Papaya (*Carica papaya* L. cv. Solo). Horticulturae. 2023;9(6):671. https://doi.org/10.3390/horticulturae9060671

102. Krishna H, Alizadeh M, Singh D, Singh U, Chauhan N, Eftekhari M, et al. Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech. 2016;6(1):54. https://doi.org/10.1007/s13205-016-0389-7

103. Al-Shara B, Rosna M, Kamaludin R. Biotechnological methods and limitations of micropropagation in papaya (*Carica papaya* L.). Journal Animal and Plant Sciences. 2018;28(5):1208-26.

104. Yie S-T, Liaw S. Plant regeneration from shoot tips and callus of papaya. In Vitro. 1977;13(9):564-8. https://doi.org/10.1007/BF02627852

105. Drew R. Rapid clonal propagation of papaya *in vitro* from mature field-grown trees. HortScience. 1988;23(3):609-11. https://doi.org/10.21273/HORTSCI.23.3.609

106. Teo C, Chan L. The effects of agar content, nutrient concentration, genotype and light intensity on the *in vitro* rooting of papaya microcuttings. Journal of Horticultural Science. 1994;69(2):267-73. https://doi.org/10.1080/14620316.1994.11516454

107. Patel H, Krishnamurthy R. Elicitors in plant tissue culture. Journal of Pharmacognosy and Phytochemistry. 2013;2(2):60-5.

108. Efendi D, PutraB MR. Optimation of *in vitro* lateral shoots multiplication of papaya (*Carica papaya* L.) "Callina" with BAP and NAA. J Trop Crop Sci. 2017;4(3). https://doi.org/10.29244/jtcs.4.3.102-107

109. Chan LK, Teo C, editors. Micropropagation of Eksotika, a Malaysian papaya cultivar and the field performance of the tissue culture derived clones. International Symposium on Tropical and Subtropical Fruits 575; 2000.

110. Nguyen V-H, Yen C-R, Hsieh C-H. Effect of nutritional and growth hormonal factors on *in vitro* regeneration of papaya (*Carica papaya* L. cv. Red Lady). Journal of the National Science Foundation of Sri Lanka. 2018;46(4). https://doi.org/10.4038/jnsfsr.v46i4.8631

111. Yu T-A, Yeh S-D, Yang J-S. Comparison of the effects of kanamycin and geneticin on regeneration of papaya from root tissue. Plant Cell, Tissue and Organ Culture. 2003;74(2):169-78. https://doi.org/10.1023/A:1023906309446

112. Razak RA, Sekeli R, Shaharuddin NA, Abdullah JO. Using mannose as a positive selection of transformed *Carica papaya* L. var 'Eksotika'. Jurnal Teknologi (Sciences & Engineering). 2015;77(31). https://doi.org/10.11113/jt.v77.6889

113. Modi A, Suthar K, Thakkar P, Mankad MC, Kumari S, Narayanan S, et al. Evaluation of sex specific RAPD and SCAR markers linked to papaya (*Carica papaya* L.). Biocatalysis and Agricultural Biotechnology. 2018;16:271-6. https://doi.org/10.1016/j.bcab.2018.08.004

114. Fernando JA, Melo M, Soares MK, Appezzato-da-Glória B. Anatomy of somatic embryogenesis in *Carica papaya* L. Brazilian Archives of Biology and Technology. 2001;44:247-55. https://doi.org/10.1590/S1516-89132001000300005

115. Roy P, Roy S, Hakim L, Mamun A. Somatic embryogenesis and plant regeneration of papaya (*Carica papaya* L. cv. Shahi). Nucl Sci App. 2016;25.

116. Anandan R, Deenathayalan T, Kumar NS, Deepak K. An alternative *in vitro* plant regeneration system in papaya (*Carica papaya* L.) through callus derived nodular cultures. Meta Gene. 2018;17:147-52. https://doi.org/10.1016/j.mgene.2018.06.007

117. Cipriano JL, Cruz ACF, Mancini KC, Schmildt ER, Lopes JC, Otoni WC. Somatic embryogenesis in *Carica papaya* as affected by auxins and explants and morphoanatomical-related aspects. Anais da Academia Brasileira de Ciências. 2018;90(01):385-400. https://doi.org/10.1590/0001-3765201820160252

118. Malik N, Sengar RS, Yadav MK, Singh SK, Singh G, Kumar M. Effect of different plant growth regulators on *in-vitro* callus Induction in *Carica papaya* (cv. Pusa Nanha). Int J Curr Microbiol Appl Sci. 2019;8 (7):1217-25. https://doi.org/10.20546/ijcmas.2019.807.145

119. Chong-Pérez B, Carrasco B, Silva H, Herrera F, Quiroz K, Garcia-Gonzales R. Regeneration of highland papaya (*Vasconcellea pubescens*) from anther culture. Applications in Plant Sciences. 2018;6(9):e01182. https://doi.org/10.1002/aps3.1182

120. Litz R, Conover R. *In vitro* somatic embryogenesis and plant regeneration from *Carica papaya* L. ovular callus. Plant Science Letters. 1982;26(2-3):153-8. https://doi.org/10.1016/0304-4211(82)90086-4

121. Rathi U, Gupta A, Pradhan PJ, Choudhury A, Patil BL, Mazumdar-Leighton S. An improved plant regeneration protocol for a popular Indian Madhubindu variety of papaya (*Carica papaya* L.) via somatic embryogenesis. Vegetos. 2025;38(1):250-9. https://doi.org/10.1007/s42535-024-00971-8

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