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Review Article

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Recent Advances in Vegetative Propagation of Papaya

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Abstract

Worldwide, the papaya (*Carica papaya* L.) fruit tree is of enormous commercial and nutritional appeal. Its propagation is possible in both sexual and asexual ways. The micropropagation method in vegetative propagation has received a lot of attention. Grafting has been used in research development as well. The effort required for breeding new plant varieties can be reduced dramatically by producing haploids through anther culture. Over the past few years, the way papaya are grown and cultivated has become a model for other fruit plants, showing how biotechnological methods, such as plant genetic engineering and germplasm conservation, can be used. This has resulted in the advancement of techniques used to grow and preserve different types of fruit plants. However, growth of papaya is limited by various factors such as being dioecious (having separate male and female plants), being vulnerable to numerous viral diseases, and inherent heterozygosity. Since 1970s scientists have conducted extensive research on papaya's tissue culture, micropropagation and somatic embryogenesis techniques. This review summarizes the documented methods used to overcome the constraints of papaya cultivation by means of vegetative propagation.

Keywords

Papaya, Micropropagation, Grafting, Anther culture, Somatic embryogenesis

Introduction

Papaya (*C. papaya* L.) is an herbaceous plant having bright white or yellow flowers, oval, melon-like fruits, and deeply lobed leaves. It is considered as the "wonder fruit of tropics" [1]. It belongs to the Caricaceae family and chromosome number is 2n = 18. This tropical fruit holds significant commercial value, due to its excellent nutritional and therapeutic value. It originated from the tropical America. Its cultivation dates back more than 500 years. In the 16th century, the Spanish were the first to bring it to Asia. The fruit was already being grown in all tropical regions of the earth by the 19th century. India is at the forefront of papaya production, with the fruit being predominantly grown in states such as Andhra Pradesh, Karnataka, Gujarat, Orissa, West Bengal, Assam, Kerala, Madhya Pradesh, and Maharashtra. India is the world's top producer of papaya. Following closely is Gujarat in second place, with a production of 1,107.88 (000 MT) and a share of 19.29%. Maharashtra and Karnataka secure the third and fourth positions, respectively, with production quantities of 496.12 (000 MT) and 491.95 (000 MT), accounting for 8.64% and 8.56% of the total production [2].

It is a short-lived perennial crop. Its stem apex is a hollow cylinder trunk, which bears the plant's leaves (like an umbrella). The plant can reach a height of 5 to 20 feet depending on the cultivar. Papaya fruits grow in clusters at the tip of the plant trunk, under the leaf canopy. Milky latex from the plant is high in cysteine endopeptidases (papain, chymopapain, glycyl endopeptidase, and car-

icain) [3]. The plant is trioecious, as it has three sexes: male, female, and hermaphrodite (self-pollinating). Hermaphroditic trees are usually preferred for commercial papaya production since they all yield fruit, whereas female trees require at least 6-10 percent male trees for pollination and fruit production. The plants require a hot and humid climate with a temperature that ranges from 21-33 ˚C.

C. papaya has a wide range of uses in value addition includes fresh fruit, drinks, jams, jellies, ice cream, pies, and dried fruit [4]. Whole plant parts like fruit, roots, bark, peel, seeds, and pulp are all known to have therapeutic characteristics, in addition to being delicious and healthful [5]. The ripe fruit is a rich source of calcium, vitamins A, C and E. A 100 g serving of ripe papaya fruit has a nutritional value of energy (163 kJ), protein (0.6 g), fat (0.1 g), minerals (0.5 g), fiber (0.8 g), carbs (7.2 g), beta-carotene (888 m), total carotene (2740 m), salt (3 mg), iron (0.10 g), vitamin A (1094 IU), vitamin E (0.73 mg), niacin (3 mg), and water (89%) [6]. The nutrients present in papaya help to maintain cardiovascular health, prevent heart diseases, heart attacks, and strokes, and prevent colon cancer, dengue.

Across the world, papaya (*C. papaya* L.) is a fruit-bearing tree that holds significant economic and nutritional value. However, the dioecious nature of papaya presents a challenge for its cultivation. Although papaya can be propagated using seeds, vegetative propagation through *in vitro* and *ex vitro* culture has become a more prevalent practice.

Sexual propagation

The propagation of plants by seeds is referred to as sexual propagation. Papaya seedlings produced sexually through seed germination are commercially available and have a wide range of features. According to Geneve et al. [7], seed propagation is the most feasible method for propagating papaya. Seed propagation has several disadvantages, including the production of non-true-to-type planting materials. This occurs due to the segregation of offspring at the second generation, which is caused by the plant's inherent heterozygosity and dioecious nature. Additionally, the seeds of open-pollinated flowers can vary significantly in shape, size, and flavor, as well as disease susceptibility. Another challenge with seed propagation is that sex cannot be identified until the mid-development stage. Therefore, each planting position is seeded with three seedlings until blossoming, which can be time-consuming and require more resources [8].

Vegetative propagation

Vegetative propagation helps to solve the challenges of papaya sexual propagation as it ensures genetic uniformity and can preserve the identity of an elite clone or cultivar. As a result, developing true-to-type progenies requires an effective vegetative propagation strategy [9]. 100% hermaphrodite plants can be produced by vegetative propagation. Plants that are propagated asexually have the same physical appearance as their parent plants. As outlined above, there are limitations for cultivation of papaya by seed, interest in vegetative propagation has grown as researchers investigate the method's suitability for producing seedlings for commercial plantations [10]. The mother plant characteristics are maintained by means of vegetative propagation as well as various benefits such as fruit height and yield (Table 1).

The asexual propagation of papaya shoots results in improved uniformity, shorter fruiting period, lower fruit height, and higher yield per productive cycle [11]. This method allows growers to selectively multiply plants with desirable properties, such as hybrids or hermaphrodite plants that are pest and disease-resistant. Furthermore, plants propagated by this method begin flowering soon after planting, and hence fruiting happens sooner than plants propagated by seed. Papaya seeds have low viability. The percentage of seeds that germinated decreased significantly with age, with no seed germination after three months [12]. Vegetative propagation can be used instead of seed propagation for these constraints. Vegetative Propagation in papaya can be achieved by number of methods like

- Micropropagation
- Grafting
- Anther culture
- Somatic embryogenesis

Micropropagation

Micropropagation is a tissue culture technique that involves asexual or vegetative propagation of whole plants. This method is also known as *in vitro* propagation, and it involves the cultivation of plant cells, tissues, or organs in a nutrient-rich, artificial environment under sterile conditions. This technique is a relatively novel technology that is primarily utilized for large-scale production and quick multiplication of a variety of commercial plant species. Plants are propagated *in-vitro* by asexual reproduction or vegetative propagation in this type of technique. *In-vitro* papaya clones are homogeneous and yield high-quality fruits that are identical to those produced by their mother plants. Micropropagation is the only cost-effective approach to produce consistent planting materials of known sex on a continuous basis [13]. Micropropagation, as compared to other tissue culture techniques like embryogenesis and organogenesis, is more effective in producing a high percentage of clones of an elite variety or lineage, germplasm conservation (Figure 1).

Micropropagation involves a series of steps that include (1) aseptic cultures, (2) inducing multiple shoots, (3) elongating and proliferating the shoots, and (4) rooting, hardening, and conducting field trials. By using tissue culture has been used to propagate papaya species and has shown to be more effective than many other traditional approaches. This observation emphasizes the necessity of developing efficient methods for preserving and recovering high-quality plants from selected lines of genetically modified papaya over longer durations [14]. Because of their morphological, agronomic, and industrial characteristics, as well as cytological, iso-enzymatic, and molecular analyses, it has been demonstrated that micropropagated plants are genetically stable through the proliferation of axillary shoots and meristems, and it is the most appropriate system of papaya clone multiplication [15]. The *in vitro* culture of papaya axillary or apical buds has gained significant popularity and widespread adoption in both research and pri-

Table 1: Effect of different media compositions used for shoot and root initiation of different cultivars under micropropagation.

Cultivar	Explant	Shoot initiation hormone and media used	Root initiation hormone and media used	Ref.
Surya	Shoot tip	Murashige and Skoog (MS) medium with 2.0 mg/L BAP (benzyladenine purine) + 1.0 mg/L NAA (naphthaleneacetic acid).	MS medium + 3 mg/L NAA.	[46]
Shahi	Lateral buds and young leaves	The MS medium is combined with 1.0 mg/L of zeatin and 0.2 mg/L of NAA, it promotes the growth of shoots. Moreover, adding 200 mg/L of casein hydrolysate to the medium further enhances the growth of shoots in the culture.	Half-strength MS medium + 4.0 mg/L IBA.	[47]
Eksotika	Shoot tips	500 mg of casein hydrolysate, 1.0 mg/L BAP, 0.05 mg/L NAA, and 30 g/L sucrose were added to MS medium.	MS medium +1.0 mg/L	[48]
CO-5	Apical buds and lateral buds	The highest number of shoots and longest shoots were achieved under light conditions using full strength MS medium with 30.00 g/L sucrose and 6.50 g/L agar.	A combination of activated charcoal at 0.05%, 3.00 mg/L IBA, and 30.00 g/L sucrose in full strength MS medium was used.	[13]
Rainbow	Shoot-tip	The SMM contained a combination of MS basal medium with vitamins (Murashige and Skoog 1962) (Phytotech Labs®, product M519), along with 4% sucrose, 3 mg/L BAP, and 2.8 g/L Phytagel® (a gelling agent).	Full-strength MS basal medium + 1 mg/L of IBA, 2.8 g/L of Phytagel®.	[10]
Co7	Shoot bud	Shoot multiplication medium: MS medium with B5 vitamins, 5.0 µM BAP, 0.05 µM NAA, and 30 g/L sucrose Shoot elongation medium: ½ strength MS basal media with B5 vitamins + 400 mg/L, L-glutamine 1.5 µM GA3 (Gibberellic acid) and 30 g/L sucrose.	Half-strength MS medium + 2.5 µM IBA, and 30 g/L sucrose.	[49]
Solo	Micro shoots from somatic embryos and axillary buds	MS basal medium with 0.1 mg/L of NAA, Kin of 0.5 mg/L and 1 mg/L of GA_3.	Half-strength MS medium + IBA (1 mg/L).	[15]
Meizhonghong	Shoot buds and axillary buds	Shoot initiation: MS medium with BAP of 0.5 mg/L and 40 g/L sucrose. Shoot elongation: MS medium with BA 0.25 mg/L, 1.0 mg/L GA_3 and 40 g/L sucrose.	The medium used contained 1.5 times the macro-elements of MS, 500 mg/L of activated charcoal, and 5 g/L of sucrose and is referred to as 3/2 MS medium.	[50]
Maradol	Shoot buds	Shoot initiation and proliferation : achieved using MS medium containing 1 mg/l of BAP + 0.5 mg/L of NAA, highest number of shoots was obtained using MS medium with 1.0 mg/L of BAP + 0.5 mg/L of NAA.	MS media contained 1.5 mg/L IBA.	[21]

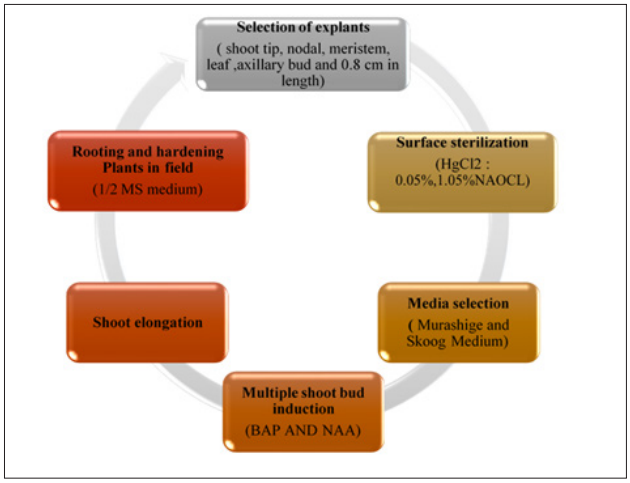

Figure 1: Steps followed in micropropagation.

vate seedling production. This technique serves as a well-established and widely utilized strategy for cloning purposes, offering a range of versatile applications [16].

Papaya micropropagation began three decades ago. Papaya has not been able to be propagated vegetatively using traditional procedures. *In vitro* propagation of papaya is used to overcome these problems. Two primary viral infections that endanger papaya farming across the world are papaya ring spot and papaya leaf curl. For papaya enhancement (viral resistance) using recombinant DNA technology, an effective *in vitro* regeneration process is required [17, 18].

In vitro axillary or apical bud culture for papaya cloning is a well-established and widely utilized approach in research and seedling production enterprises for a variety of purposes. This method was chosen because clonal propagation is quick, inexpensive, and produces good genetic and phytosanitary quality [10, 19]. Many studies have found that BAP and NAA are the most often utilized and effective plant regulators in the micropropagation of shoots. The combined action of two plant regulators, namely BAP and NAA, is directly connected to improve efficiency in induction time, frequency, and shoot growth in micropropagation. During the rooting induction phase, the culture media containing MS supplemented with

IBA at doses ranging from 1 to 2 mg/L are utilized depending on the genotype used, with the procedures with most promising outcomes in terms of rooting duration and frequency [20].

Micropropagation procedures for hermaphroditic papaya cultivars might lower the number of plants required per hectare from 6,000 to 2,000 (Table 2). The plants which are produced from micropropagation of buds were uniform and retained 100% hermaphroditism and 75% of seeds were conserved, 25% grew round fruits are marketable [21].

Caple and Cheah [10], stated that explants are collected from fifty-three, 2-month-old hermaphroditic 'Rainbow' seedlings. Shoot size and IBA exposure appear to have a big impact on rooting success. Based on 20 shoots with a length of 2 cm, root initial induction was achieved at a rate of 95%. In vermiculite, however, just 54% of plantlets formed strong root systems.

Grafting

Grafting is a technique of propagating plants by physically joining two sections of plant tissue, the scion at the top and the rootstock at the bottom, so that they can unite and grow together as a complete plant. Most grafting occurs during the winter and early spring when the scion and rootstock are both in a dormant state. Through grafting, true-to-type plants are produced in terms of cultivar genotype preservation in any crop [1]. The roots of plantlets produced by the grafting method (Figure 2) are stronger than those produced by cutting method, and they are larger than plantlets propagated by micropropagation [22]. The rootstock should possess the following characteristics: adaptability to local soil and weather conditions, resistance to pests and diseases, easy propagation, compatibility with the scion, and ability to promote early formation of the cambium layer. On the other hand, the scion should have wood from the previous season but not from plants older than a year, well-developed and healthy vegetative buds, and should be taken from trees in orchards with a good performance history.

Grafted plants are true-to-type and yield early, as well as being able to be multiplied and conserved. According to Airi et al. [23] scion shoots obtained from the CVS, namely Co-1 and Honey Dew, were successfully cleft grafted onto already grown seedlings. Some cultivators in Malaysia employed grafting to get rid of gynecious fruiting trees of the cv. Eksotika [19]. Grafting offers several advantages such as improving the local variety of older plants to a superior variety through top working. Additionally, grafting allows for the enhancement of resistance, vigor, and quality through rootstock selection. New varieties cannot be produced. Grafting using contaminated equipment or propagation material can cause freshly propagated plants to become infected are disadvantages of grafting.

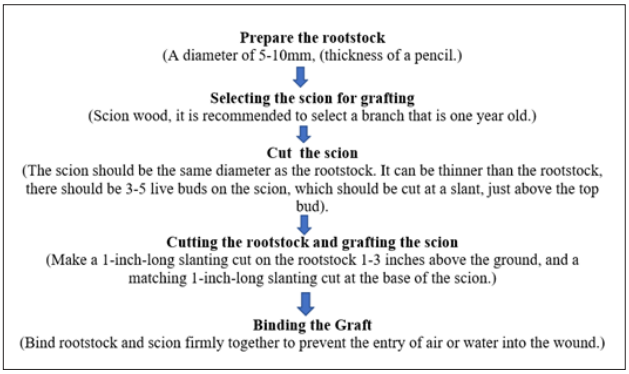

Figure 2: Flowchart for steps of grafting.

Grafting technique is the most effective and successful asexual propagation strategy for papaya. As a result, desirable sex forms, such as pistillate or staminate plants, can be generated in dioecious varieties using the grafting procedure by harvesting scions from the relevant plants soon after blooming. Grafting is a possible method for producing seed in dioecious papaya by growing male and female plants separately [24]. Grafting *C. papaya* plants has various advantages in terms of productivity, phytosanitary, and sexing [25]. The yield and fruiting in papaya is increased by grafting. Grafting has the potential to enhance the whole papaya production [26]. The higher yield, low fruiting height, 100 percent hermaphrodite plants with dwarf stature and longer economic lifespan was achieved by grafted papaya trees [1, 22].

Ramkhelawan et al. [27], concluded that the terminal

Table 2: Effectiveness of different grafting methods and ages on shoot grafting success in different cultivars.

Variety	Graft type	Age of rootstock	Age of scion	Success percentage	Ref.
Red Lady	Cleft grafting	1 month old rootstock	1 month old scion	93.33%	[23]
Eksotika	Cleft grafting	Nursery stage	Nursery stage	80%	[51]
Tainung No. 2	Cleft grafting	1 month old rootstock	-	96.70%	[52]
Maradol	Modified tongue approach grafting	1 month old rootstock	1 month old scion	92.50%	[26]
Kenyan papaya lines	*In vitro* grafting (wedge grafting)	3 months old rootstock	3 months old scion. Shoots of length 20 mm length shoot was used, the upper 10 mm of the tips used as scion, remaining portion as rootstock	80%	[53]
Papaya	Terminal wedge grafting	-	-	100%	[27]
LD-1999	Top grafting	-	-	83.91%	[1]

wedge grafting technique was more effective than both budding and side grafting techniques. This was demonstrated by achieving a 100% success rate in the field and observing growth progress. Hassan et al. [28], conducted an experiment on grafted papaya seedling adopting the Viorica variety as a highly tolerant rootstock can be used to control papaya dieback disease. The result showed that Eksotika/Eksotika, Eksotika/Viorica had disease severity of 86.7%, 71.0% and a disease score of 4.34, 3.55 respectively and the Viorica/Viorica has the lowest disease severity of 34.3% and a disease score of 1. 72. As a result the papaya Viorica is highly tolerant to papaya dieback disease than Eksotika/Eksotika and Eksotika/Viorica.

Allan et al. [29], stated that to overcome problems of bacterial infections that occur during grafting, using 5 or 10% household bleach (containing 3.5% sodium hypochlorite) is the convincing method for surface sterilization. The result showed that the side grafted into properly maintained and healthy rootstocks, the success rate was 81 percent after 15 weeks.

Sarip et al. [30], evaluated 6 different grafting combinations, the results indicated that Viorica onto Viorica was established by a sufficient grafting technique, with a success rate of over 90%. Viorica is a PDD-resistant rootstock that might be utilized to reduce elite scion susceptibility.

Anther Culture

Anther culture is a method in which unopened flower buds are used to extract developing anthers which are then placed on a nutrient medium for cultivation. The microspores within the anthers eventually develop into callus tissue or embryoids, leading to the production of haploid plants through organogenesis or embryogenesis [31]. Androgenesis is a term that is frequently used to describe anther culture. Litz and Conover [32, 33] were the first to try to employ anther culture in the breeding of papaya. Anther culture is a promising technique that can be used to speed up the breeding process by generating haploid plants [34].

Gyanchand et al. [35] reported the use of anther culture (Figure 3), specifically in *Datura innoxia* plants at the Botany Department of the University of Delhi's South Campus. Androgenesis refers to the generation of haploids using male gametes such as anthers, microspores, or pollen, whereas gynogenesis refers to the production of haploids using female gametes such as ovules [36].

Sopory and Munshi [37], explain that the effectiveness of haploid induction in anther culture is influenced by various factors, such as the donor plant's genotype and physiological state, properties of the media used, pollen developmental stages, and pre-culture treatments. Traditional papaya breeding methods are time-consuming, but anther culture can potentially reduce the breeding cycle. Papaya staminate flowers are used for anther culture investigations, and previous studies have reported the development of haploid and double haploid plants in Caricaceae using anther culture [32, 33, 38, 39]. Rimberia et al. [34], noted that anther culture plants exhibited diverse phenotypes and varying fruit yields, and they suggested that these plants have significant economic potential and

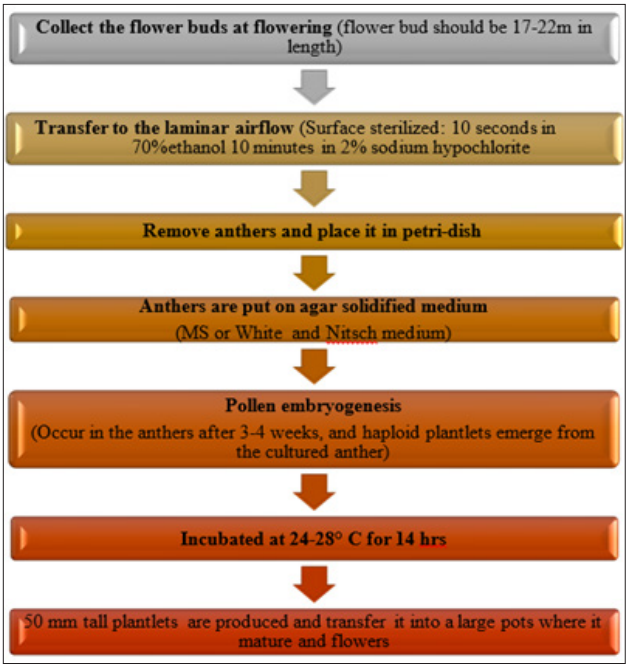

Figure 3: Steps involved in anther culture technique.

may be used in breeding projects.

In Litz and Conover [32], were the first to report the production of haploid papayas using a stationary liquid MS medium that contained 3.0% sucrose, 1.0% activated charcoal, 2.0 m BAP, and 0.5 m NAA, although they did not specify the rate of success. However, in 1979, they were able to demonstrate the generation of haploids through anther culture with a success rate of 0.4 percent.

According to Tsay and Su [38], anthers of *C. papaya* containing microspores in tetrads to early-binucleate stages were cultured successfully in a half-strength MS medium supplemented with 2 mg/L of NAA, 1 mg/L of BAP, and 6% sucrose for callus formation. The original embryoids were transferred to an MS medium with 3% sucrose and no growth regulators, resulting in the production of a large quantity of embryoids (Table 3).

Somatic Embryogenesis

Somatic embryogenesis is the term used to describe the development of embryo-like structures from somatic tissues, which eventually grow into fully formed plants. Many monocots and dicots adopt somatic embryogenesis for regeneration. This process involves totipotent cells following an embryogenic pathway to create somatic embryos, which can then be used to generate whole plants. The first discovery of this process occurred in carrots (*Daucus carota*), where single cells were found to grow into bipolar embryos [40].

The growth of somatic embryogenesis is dependent on the presence of auxin. Auxin induction results in the production of embryogenic clumps or proembryogenic calluses (induction medium), while mature embryos are formed when auxin are removed (maturation medium). During the early stages of cell division in somatic embryos, there is no established pattern, unlike in zygotic embryogenesis (Figure 4). However, later

Table 3: Embryo induction success rates and incubation durations in various nutrient media under anther culture.

Media	Nutrient source	Success rate	Incubation (Duration)	Ref.
Agar medium then incubated in MS media	The nutrient solution contains 0.1 mg/L of BAP, 0.1 mg/L of NAA, and 2.0% sucrose.	Embryo induction rate (8.0%)	7 days at 35 °C	[36]
MS liquid medium	2.0% sucrose.	Embryo induction rate 4.0%	35 °C for 1–5 days	[40]
MS Agar medium	The solution contain of 0.01 mg/L CPPU and 0.1 mg/L NAA.	Embryo induction rate of 13.8%	-	[54]

stages of somatic embryogenesis resemble the dicot pattern of zygotic embryogenesis, including the formation of multicellular globular structures, bilateral symmetry at the heart-shaped stage, and the emergence of the first cells of the shoot/root meristem in the torpedo-shaped stage [41].

Bhattacharya and Khuspe [42] developed conventional methods for improving the quality of papaya fruit through breeding techniques. Papaya is an allogamous species that faces challenges in large-scale propagation due to low seed production and poor germination percentages in certain commercial hybrid parents [43]. Somatic embryogenesis provides a solution to this problem by allowing for high-rate propagation of only hermaphrodite plants of specific genotypes. However, somatic embryogenesis in papaya, as with other plants, is genotype-dependent and may require certain conditions such as explant type, medium supplements, and culture conditions for successful induction and expression of somatic embryos [44]. According to Posada-Pérez et al. [45], somatic embryogenesis involves two steps: induction and expression. In the induction phase, auxins such as 2,4-D are commonly used to trigger the transition of cells from somatic to embryogenic stages. In the

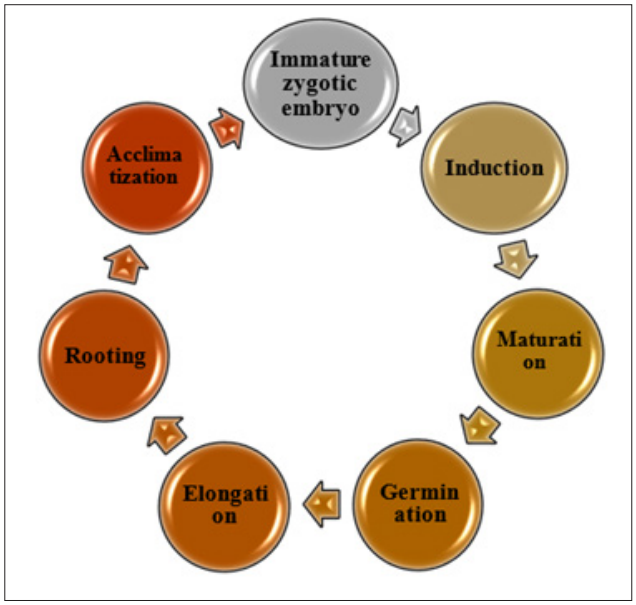

Figure 4: Steps in somatic embryogenesis.

Table 4: Embryo induction success and duration in different cultivars: explants and media effect in somatic embryogenesis.

Cultivar	Explant	Media	Nutrient source	Duration	Result	Ref.
Taiwan-786	Unripe fruits	Full strength MS (1962) basal medium)	The induction medium consisted of 4.52 micromoles per liter of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.27 micromoles per liter of thidiazuron (TDZ).	After 4-6 weeks embryogenic callus formed.	87.0 ± 4.2	[55]
P-7-9	Immature green papaya fruits seed	MS media	The treatment consisted of 7.0 mg/L of 2,4-D followed by a combination of 4.0 mg/L of 2,4-D + 1.0 mg/L of picloram.	Embryogenic calli, which were 8-9 weeks old, exhibited a high rate of proliferation andmaturation.	80% successful induction rate for somatic embryogenesis.	[56]
Co7	Fruits (110-120 days)	Half-strength MS	30 g/L sucrose and 1.0% activated charcoal.	Somatic embryos began to form embryogenic calluses after 6 weeks of growth.	68.35% somatic embryognenesis.	[57]
Eksotika	Immature fruits (90-100 days)	MS medium (Half-strength)	Full-strength vitamins and 10 mg/L of 2,4-D.	-	Embryogenic callus was produced from 78% of zygotic embryos.	[58]
Sunrise	Root	1/2 strength MS inorganic salts	The medium contained 160 mg/L of adenine sulfate, 1.0 mg/L of NAA, 0.5 mg/L of kinetin, and 1.0 mg/L of GA_3.	Three months.	Embryos were regenerated from 30% of the root cultures.	[59]

Hermaphrodite *C. papaya*	Leaf	Half-strength MS basal salts	This nutrient medium contains 100 mg/L malt extract, 5% polyethylene glycol, 2 g/L activated charcoal, and 5 µM abscisic acid.	The formation of somatic embryos were observed after 5 months cultured in embryogenic induction media.	Somatic embryos were observed at globular, heart-shaped, torpedo and early cotyledonary.	[60]
Eksotika	Fruit 90 to 100 days	Half-strength MS media	10 mg/L 2,4-D and 250 mg/L carbenicillin.	It took 3 months for 100% germination of somatic embryos.	77.5% success rate for formation of embryogenic callus and somatic embryos (100%) germinated.	[61]
Ratna	Hypocotyl and leaf	MS medium without hormone	Supplemented medium with 0.02 mg/L NAA and 0.5 mg/L BAP	Somatic embryos observed within 4 weeks.	In the plantlets, leaves were prominent, while shoots and roots equally sprouted and expanded. 60% plantlets were able to adapt to coir dust:sand 1:1 medium.	[62]
Hybrid UENF/ CALIMAN 01	Immature papaya seeds	Induction of embryogenic cultures: MS culture medium	30 g/L sucrose with 20 µM 2,4-D.	In between 20th and 25th days of induction emergence of somatic embryos was observed after 50 days of incubation.	46.7% highest induction rates and 8.8 mm largest callus diameters. Great potential to form somatic embryos	[63]
		Maturation: MS medium	60 g/L polyethylene glycol		Highest 83.3% maturation and 78% somatic embryos somatic embryos are converted into plantlets.	
Kaekdum	Ripe fruit seeds	Modified half-strength MS basal medium	2 mg/L 2,4-D and 60 g/L sucrose.	3–4 weeks.	Somatic embryos started to develop on this friable callus.	[64]
				After 4 weeks in germination medium.	When transfer to germination medium. 60% of the plantlets were found to survive.	
Honey Dew and CO2	Immature fruits	MS medium	3.0 mg/L 2,4,5-T.	3-6 weeks (incubated).	79.33% and 71.00% embryos of both cultivar showed the swelling and formation of loosely arranged globular embryos.	[65]

expression phase, cytokinin's like BAP and kinetin have been effectively employed at concentrations of 0.9 and 18.6 M, respectively, to enhance the germination of papaya somatic embryos [17] (Table 4).

Conclusion

Papaya is a fruit tree that holds significant economic and food value worldwide. It can be propagated through sexual and asexual means, with seed-based propagation being the traditional approach. However, vegetative propagation using *in vitro* and *ex vitro* culture techniques has also been widely explored. It has been demonstrated that papaya clonal propagation has potential for usage on a commercial scale, which might help the productive system in several ways. Grafting techniques have been found to be less economically viable than asexual propagation through tissue culture, which is limited by low *ex vitro* regeneration rates and the unreliability of procedures. The rooting and acclimation stages of regenerated plants are a major challenge in *in vitro* regeneration techniques, including embryogenesis, micropropagation, and organogenesis in papaya culture. Tissue culture-based micropropagation has emerged as a more efficient alternative to traditional approaches for propagating papaya species.

Acknowledgements

None.

Conflict of Interest

None.

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