The Journal of Animal & Plant Sciences, 28(5): 2018, Page: 1208-1226
ISSN: 1018-7081

REVIEW PAPER

BIOTECHNOLOGICAL METHODS AND LIMITATIONS OF MICROPROPAGATION IN PAPAYA (CARICA PAPAYA L.) PRODUCTION: A REVIEW

Baker Al-Shara[1], Rosna Mat Taha[*1], and Kamaludin Rashid[2]

[1]Institute of Biological Sciences, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia
[2]Biology Division, Center for Foundation Studies Building, University of Malaya, 50603 Kuala Lumpur, Malaysia
[*]Corresponding author: Rosna Mat Taha email: rosna@um.edu.my"

ABSTRACT

Carica papaya L. is considered one of the most important fruit, especially in tropical and subtropical regions. It generates flowers continuously throughout the year and has been conventionally cultivated by seeds. This review summarizes the morphological and evolutionary descriptions of *Carica papaya* L., the protocols used to achieve *in vitro* micropropagation of *Carica papaya* using different plant tissue culture techniques, as well as the problems faced by papaya micropropagation. While there are many problems encountered during *in vitro* propagation of papaya through somatic embryogenesis, this review focuses specifically on contamination, rooting processes and acclimatization. Solving these problems will enable a reproducible protocol to be established. Subsequently, high-quality papaya plants can be produced and strains that are resistant towards various diseases can be generated via molecular studies. Cryopreservation and synthetic seeds technology are also potential methods for large scale production and crop improvement of papaya.

Keyword: Papaya, Somatic embryogenesis, Acclimatization, Cryopreservation, Synthetic seeds.

INTRODUCTION

Carica papaya L. (*C. papaya*) is considered one of the most important fruit trees, especially for tropical and subtropical regions. The fruit can be eaten either raw and fresh or cooked. Ripe papaya fruits are commonly eaten fresh. Additionally, they can be made into candy, jelly or jam, and even be used as an element in fruit cocktails. The unripe fruits, which contain latex, are the primary source of papain (Mendoza *et al*. 2008). Papain is used in different industries for instance; the pharmaceutical industry, in beer industry, in leather industry, in the cosmetics industry and industry of candy and chewing gum (Nakasone and Paull 1998). Papayas have earlier productions and higher yields compared to other perennial plant crops. They can produce flowers four months after being germinated and can produce fruits eight months after being planted. Every papaya tree can provide about 30-40 fruits annually (Farzana *et al*. 2008).

Papaya fruits normally have short shelf lives, particularly in tropical countries where the temperature and humidity are always high. The quality of the fruits may be reduced through handling, storage, transportation, and sales, resulting in an unfortunate appearance, texture, flavor and overall acceptability (Mendoza *et al*. 2008).

The selection of sex type of papaya is useful for commercial planting, sex identification, propagation, higher fruit production and to increase profitability. Honey Gold and Richter Gold are popular dioecious papaya varieties spread out in Africa and Australia respectively, because of their high output (Allan 1981). On the other hand, in Malaysia and Philippines, hermaphrodite papaya varieties are preferred for cultivation because of their high output and crop similarity over numerous generations (Chen *et al*. 1987).

Unfortunately, sex determination in papaya is impossible until the plant produces flowers (Magdalita and Mercado 2003). Predicting the sex type during the early developmental stages of papaya can be done using PCR amplification with primers. However, it is costly and not practical because all samples must be examined to detect sex type (Magdalita and Mercado 2003).

Caricaceae: In 2000, the Caricaceae classification was revised by Badillo to include six genera; *Carica, Jacaratia, Horovitzia, Jarilla, Vasconcella*, and *Cylicomorpha* (Badillo 2000). Of the 35 species belonging to the Caricaceae family, 32 are dioecious, two are trioecious (*V. cundinamarcensis* and *C. papaya*) and one is monoecious (*V. monoica*) (Badillo 2000; Ming *et al*. 2008). The *Carica* genus consists of only one species (*C. papaya*). The *Cylicomorpha* genus consists of two species found in tropical Africa, namely the *Cylicomorpha solmsii* found in West Africa and the *Cylicomorpha parviflora* found in East Africa. The Horovitzia genus consists of one species (*Horovitzia cnidoscoloides*). The *Jarilla* genus comprises of three species of perennial herbs. The *Jacaratia* genus has 83 species while the *Vasconcellea* genus has 20 species (Badillo 2000; Carvalho and Renner 2012).

In the past, scientists commonly supposed that papaya originated from Central America, specifically from Chile and Argentina to Mexico (Manshardt *et al*. 1992). This assumption was improved by Olson (2002).

Moringaceae is the sister group of Caricaceae. The DNA sequence in the chloroplast gene *rbc L* props in Caricaceae differs from that in Moringaceae (Olson 2002). The deepest split in the Caricaceae took place during the Late Eocene (Carvalho and Renner 2012). Recently, it was discovered that the *C. papaya* is related to *Jarilla* and *Horovitzia*, and that all three were spread from South America in the Oligocene around 22–33 Ma ago. The discovery that *C. papaya* is the closest to a herbaceous species has presented a better substitute for plant breeders who have so far only used woody highland papayas (*Vasconcellea*) to cross with *C. papaya* (Carvalho and Renner 2012).

Carica papaya: *C. papaya* ~~L.~~ is the only species in the *Carica* genus. Papaya is dicotyledonous and is considered a polygamous species; the *C. papaya* plant has three types of sex; male, female and hermaphrodite (Bhattacharya and Khuspe 2001; Liu *et al*. 2004).

Papaya cultivars are differentiated by various vegetative morphological traits such as; leaf shape, central leaf veins, the number of lobes, stomata type, wax structures in leaves, and the petiole color. It generates flowers unceasingly throughout the year and has been conventionally cultivated by seed (Bhattacharya and Khuspe 2001; Liu *et al*. 2004). The life cycle of papaya is short, it only takes between 8 to 12 months from seed to ripe fruit (Storey 1953). Pistillate and hermaphrodite papaya plants produce fruit all year-round in tropical regions while staminate papaya plants usually do not produce fruit (Storey 1953). Consumers prefer papayas that have flavor and fragrance, flesh color, shape, standards, firmness and size.

Papaya seeds consist of many layers namely, the seed coat that is derived from the multiple outer epidermises of the outer integument of the ovule. The outer region of the seed layer of *C. papaya* is fleshy and becomes a gelatinous sarcotesta at maturity. The mesotesta of papaya seeds is compact and hard at maturity, consisting of a series of sculptured, soft, and hydroscopic longitudinal folds, derived from sub-epidermal layers of the outer integument. Endosperm consists of superfine walled cells. The embryo is white with ovoid cotyledons (Fisher 1980).

Morphologically, the papaya stem is unbranched, blank and contains leaf scars. It rarely has branching stems without damaging the apical meristem. The wood of the trunk and tap root is soft and succulent. Internodes are hollow (Storey 1953; Badillo 2000). The leaves of papaya are spirally arranged and clustered at the crown. Mature papaya leaves are palmate with broad pinnatifid lobes. The petioles are green or purple in color, and are lengthy and blank (Storey 1953; Campostrini and Yamanishi 2001).

Papaya has three kinds of flowers: staminate, pistillate and hermaphrodite. Staminate inflorescences are cymose, lateral, long, pendulous and have many branching inflorescences. The staminate flower consists of solid pedicel, and the calyx cup is five toothed. The corolla tubular consists of five light yellow lobes. There are ten stamens arranged in two whorls. The first whorl alternates with petals from the second whorl. The filaments are long, and the anthers have four locules (Fisher 1980). The pistillate flowers are solitary, the pedicel is solid, and the calyx cup is also five toothed. The corolla in pistillate flowers consist of five petals that are twisted and fused at the base. The ovary is inferior with parietal placentation containing five carpellate that have numerous ovules and a fan-shaped stigma. The style canal is short and lined with stigmatic hairs and mucilage (Fisher 1980). The hermaphrodite flower is intermediate in size compared to the staminate and pistillate flowers. There are two types of hermaphrodite flowers: elongated and pentandria. The elongated type consists of short pedicles with a corolla that has united lobes, one-third its length. The ten stamens have the same arrangement as in male flowers. The pistil is functional and elongated. On the other hand, the pentandria type is similar to female flowers but has five stamens with long filaments attached to the base of the ovary. The pistil is functional and not elongated (Fisher 1980).

Papaya fruit is a fleshy berry. Its shape varies according to sex; female flowers produce spherical shape fruits, hermaphrodite flowers provide pyriform to cylindrical shape fruits (Fisher 1980). While the immature fruit is green and rich in white latex, mature fruits are yellow-orange in color and do not have latex. They contain plenty of grey-black ovoid seeds attached by soft, white, fibrous tissue with flesh. The shape of the fruit is a discriminatory characteristic for sex determination. The fruits from pistillate flowers vary from sphere-shaped to ovoid, while the fruits from hermaphrodite flowers are elongated, cylinder-shaped or pyriform. The color of ripe fruits are yellow, orange or red depending on the carotenoids present in the papaya (Aikpokpodion 2012; Paull *et al*. 2008).

Conventional propagation: The bulk of papaya plantations have been based on seed propagation. The spread of *C. papaya* by seed is a preferable method for farmers to grow papaya because it is relatively cheap (Bhattacharya and Khuspe 2001). Employing tissue culturing techniques are easily accessible, however, they are exhausting and costly especially when used for small farms (Magdalita and Mercado 2003). In some countries, such as South Africa, vegetative propagation of papaya is achieved by grafting, budding, and root cutting but these methods are not used widely for papaya propagation as

not all papaya varieties have the same tendency to produce multi-branches. Also, there is a limited number of plants produced per mother plant (Farzana *et al*. 2008).

Papaya is classified as a perennial, but common parasitic infestation often prevents farmers from growing papayas annually. This results in the reduced productivity of papaya trees after 2-3 years. Farmers have a tendency to produce low quality seeds from fruits grown in the orchard. This conventionalism can spread severe diseases in papaya plantations. Papaya fruits from hermaphrodite plants are mostly preferred by producers because they are highly marketable. In conventional propagation, two seeds or seedlings are usually planted together and when the flower sexes are determined, only the preferred sexes are kept (Wu *et al*. 2012).These practices are very costly for farmers and subsequently consumers (Schmildt *et al*. 2015). Many problems such as high variability in agronomic characteristics resulting from cross-pollination, dioecious nature, and susceptibility to a large number of diseases hamper papaya cultivation (Kavitha *et al*. 2010; Paull *et al*. 2008). The absence of complete similarity to the mother strain, prevalent occurrence of diseases, high percentages of unwanted male plants, and significant genetic variability are the major limitations of papaya propagation in commercial quantities (Farzana *et al*. 2008).

Heterogeneity caused by cross-pollination has many disadvantages. Seeds derived from open pollinated flowers can produce plants with an extensive difference in sex types and variation in fruit quality and type, in addition to the higher possibility of polyploidy, aneuploidy or even chromosomal aberrations.

***In vitro* propagation:** Plant cell cultures can manifest in three different ways: the production of secondary metabolites, micropropagation, and the study of a plant cell in different aspect levels (Zhong 2001). Cell culture propagation systems are more suitable compared to the conventional spread of whole plants. Since plants propagated in cell cultures are under controlled conditions, they are independent of environmental conditions. Therefore, the cells can proliferate at higher growth rates, and are free of microbes and insects (Vanisree *et al*. 2004).

Propagation by shoot tips and axillary buds: Vegetative propagation from carefully chosen clones is in high demand. Propagation of preferred genotypes to obtain high similarity plants on a large scale is one of the many applications of plant tissue cultures (Hossain *et al*. 1993; Panjaitan *et al*. 2007).

Commercial *in vitro* mass propagation of *C. papaya* has not yet been established. This is due to the various difficulties in micropropagation such as: microbial contamination by endogenous bacteria, especially when mature plants are grown in the field and used as explants, low proliferation rate during subculturing and difficulty in establishing normal roots and acclimatization (Wu *et al*. 2012). According to Litz and Conover (1981), maintenance of proliferating cultures is inadequate during the time. Also, apical dominance in shoots are affected with time on the multiplication medium (Rajeevan and Pandey 1986).

In vitro propagation of *C. papaya* is generated using a single node in a modified De Fossard medium supplemented with 0.5 μM of both benzyl adenine (BA) and α- naphthaleneacetic acid (NAA) for shoot induction and development, 10 μM indole 3- butyric acid (IBA) for root induction and is then transferred to a hormone-free Drew- Smith medium for development (Drew 1992). Dioecious papaya can be propagated *in vitro* by using axillary buds as explants. Axillary buds are cultured in MS basal salt with full strength vitamins supplemented with 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for establishment and proliferation. Then, it is transferred to a MS medium supplemented with 1.0 mg L^{-1} kinetin and 0.05 mg L^{-1} NAA for the elongation stage and finally, half-strength macronutrient of MS medium with full strength of other components is supplemented with 1.0 mg L^{-1} IBA for rooting (Reuveni *et al*. 1990). Chan and Teo (1994), applied the clonal propagation method to induce the *in vitro* direct establishment of multiple shoots from field grown trees explants. These shoots could then be rooted and grown in the field (Chan and Teo 1994). Other micropropagation of papaya studies are summarized in table 1.

Organogenesis: There are limited studies on the propagation of papaya through organogenesis. Anandan *et al*. (2011) developed a protocol for micropropagation of Indian *C. papaya* L. var. Co7. using epicotyl segments by direct organogenesis from *in vitro* papaya seedling plants. Hossain *et al*. (1993) generated a high efficacy protocol for plant regeneration from petiole of *C. papaya* L. cv. “Rajshahi-red” by using indirect organogenesis. The callus cultures obtained from MS medium augmented with ~~α- naphthaleneacetic acid~~ NAA (0.5-10.5 μM) in combination with ~~benzyl adenine~~ BA (0.5-5 μM) were the best callus observed at a low ratio of cytokinin and auxin which resulted in hard, green and nodular callus. Then, shoots were regenerated in MS medium supplemented with 100 mg L^{-1} casein hydrolysates 2 μM BA and 0.1 μM NAA. For shoot elongation, the regenerated shoots were transferred to the medium without plant growth regulators. Finally, for root regeneration, the elongated shoots were subcultured in ½ MS medium supplemented with 3 μM NAA and 0.5 μM Gibberellic acid (GA_3).

Somatic embryogenesis: The cultivation of papaya by

Table. 1 Summary of micropropagation studies of *C. papaya.*

Authors	Cultivar and explant	Shoot development hormones concentration and culture condition	Root development concentration and culture condition
(Litz & Conover, 1977)	*C. papaya* Shoot apex and small petioles	**Establishment medium** 50 µM kinetin + 10 µM NAA **Proliferation medium**: 2.0 µM BA + 0.5µM NAA Difco Bacto agar 8 g L^{-1}, 28 ºC with 16 hr. light (3500 lux) and 8 hr. darkness. Then, transferred to free hormone media	**Root medium**: Rooting was induced in media supplemented with 0.5 µM NAA
(Reuveni *et al*. 1990)	Open pollinated dioecious papaya, axillary buds	MS medium supplemented with 1.0 mg L^{-1} Kin and 0.05 mg L^{-1} NAA.	Half-strength macro-elements of MS basal medium supplemented with 1 mg L^{-1} IBA.
(Drew, 1992)	Female *C. papaya* seedling of "Hybrid 14", nodal segment	A modified De Fossard *et al*. (1974) medium containing high concentrations of minerals supplemented with 0.5 µM of both BAP and NAA, 2% sucrose and 0.8% Difco Bacto-agar. Culture condition at 25 ± 1 ºC with cool-white fluorescent tubes	**Rooting medium** A modified De Fossard *et al*. (1974) containing intermediate concentrations of minerals supplemented with 10 µM IBA, 2% sucrose,0.8% Difco Bacto-agar Culture condition at 25 ± 1ºC with cool-white fluorescent tubes
(Panjaitan *et al*. 2007)	*C. papaya* cv. Eksotika Shoot tips of field grown hermaphrodite	**Shoot induction medium**. MS medium supplemented with1.0 mg L^{-1}BAP and 0.05 mg L^{-1}NAA, 500 mg L^{-1} casein hydrolysate and 30 g L^{-1} sucrose **Proliferation medium**: MS medium without plant growth regulators for one week	**Root medium** MS medium supplemented with1.0 mg L^{-1} IBA **Acclimatization** MS medium with or without vermiculite for further root development.
(Anandan *et al*. 2011)	Indian *C. papaya* L. var. Co7. Shoot bud from epicotyl segments	**Induction medium** MS medium supplemented with 2.5 µM Thidiazuron (TDZ) and 30 g L^{-1} sucrose **Shoot and multiplication medium:** MS medium supplemented with B5 vitamins, 5.0 µM BAP and 0.05 µM NAA and 30 g L^{-1} sucrose **Shoot elongation medium** half strength MS basal salts supplemented with B5 vitamins, 400 mg L^{-1} L-glutamine 1.5 µM GA_3 and 30 g L^{-1} sucrose	**Root induction medium.** ½ MS medium supplemented 2.5 µM IBA, and 30 g L^{-1} sucrose **Acclimatization** 1/4 MS salts without sucrose for 24-48 h and then transferred to pots containing autoclaved soil and soilrite (1:1, w/w) and covered with polybags.
(Wu. *et al*. 2012)	*C. papaya.* Hermaphrodites cv. Meizhonghong. Hybrid of Sunrise with Shuizhonghong Shoot buds and axillary	**Shoot initiation medium:** MS medium supplemented with 0.5 mg L^{-1}BA and 40 g L^{-1} sucrose. **Proliferation medium:** MS medium supplemented with 0.25 mg L^{-1} BA and 40 g L^{-1} sucrose. **Shoot elongation medium:** MS medium supplemented with 0.25 mg L^{-1} BA 1.0 mg L^{-1} GA_3 and 40 g L^{-1} sucrose.	**Root medium** The 3/2 MS medium supplemented with 500 mg L^{-1} activated charcoal and 5 g L^{-1} sucrose.

seeds is hampered by many problems such as considerable variability in a commercial population resulting from open pollination, dioeciously nature, and susceptibility to a large number of diseases (Kavitha *et al*. 2010; Paull *et al*. 2008; Litz and Conover 1977). Somatic embryogenesis has been employed in papaya crops to solve these problems. Elite plantlets with desirable characteristics and tolerance to distortion ringspot are multiplied and maintained (Anandan *et al*. 2012; Fitch 1993; Castillo *et al*. 1998a; Litz and Conover 1977). Somatic embryogenesis is an appropriate micropropagation technique in *C. papaya.* This technique helps to address the difficulties that occur during conventional seed proliferation as well as the absence of an actual method for early sex determination (Abreu *et al*. 2014). Chen *et al*. (1987) generated a high efficacy protocol for plant regeneration through somatic embryos initiated from root callus. This protocol generated more than 100 plants per explant.

Tissue culture techniques are considered useful tools for overcoming incompatibility that arises between cross pollination of the *C. cauliflora* and *C. papaya*. Chen *et al*. (1991) induced somatic embryogenesis from interspecific hybridization generated from cross breeding between *C. papaya* and *C. cauliflora*. The result indicated that the somatic embryos can proliferate continuously and can induce plant growth by manipulating plant growth hormones in the culture medium (Chen *et al*. 1991). Plant regeneration through plant tissue culture techniques, especially somatic embryogenesis, is necessary for many applications of biotechnology such as synseeds, transgenic plants, and micropropagation (Bukhori 2013). Somatic embryogenesis can be applied for producing synseeds (artificial seeds) which can be moved easly like regular seeds, preserved and planted. Similarly, the somatic embryo is suitable for long-range stockpiling methods such as cryopreservation and is an ideal model for genetic transformation research.

A light microscopic study of somatic embryogenesis revealed that the daughter embryos produced during somatic embryogenesis originated from single cells found on the external surface of parent embryos. Somatic embryo cultures of cross-pollinated *C. papaya* with *C. cauliflora* are considered an appropriate system for the study of somatic cell genetics due to many reasons. Firstly, the origin of the somatic embryo is a single cell on the external surface of the parent embryos. Next, there is potential for frequent subculturing. Finally, the ability of the plant to regenerate is another reason (Chen *et al*. 1991). Somatic embryogenesis addresses the problem of rooting caused by micropropagated plantlets and the bipolar structures of embryos containing both shoot and root apices. Also, embryogenic cultures have the ability to produce scores of embryos per culture, especially when grown in liquid mediums. The embryos in somatic embryo callus separated mechanicaly by agitation and float freely in the medium, therefore, manual separation and mechanical handling is not needed in lquid medium (Farzana *et al*. 2008).

Numerous protocols based on somatic embryogenesis have been presented for the propagation of papaya *in vitro*. Malabadi *et al*. (2011) induced somatic embryogenesis via immature zygotic embryos of thirteen popular varieties of *C. papaya* grown in India. The results demonstrated that a lower concentration of TDZ (2.27 μM) was optimal for inducing the highest percentage of somatic embryogenesis in all the tested varieties of *C. papaya*. Also, the ability to induce somatic embryogenesis is varied for different varieties of papaya.

Casein hydrolysate was significant and suitable for the maturation of somatic embryogenesis generated from zygotic embryos or hypocotyl explants of *C. papaya* L. cv. Rathna (Farzana *et al*. 2008). Somatic embryogenesis protocol generated from leaves of hermaphrodite *C. papaya* plants in culture mediums supplemented with 2,4-Dichlorophenoxyacetic acid (2,4-D) demonstrated asynchronicity, with new globular embryos continuously forming from the friable embryonic callus (Koehler *et al*. 2013). Somatic embryos of papaya faced complications to develop a reproducible procedure to obtain normal *papaya* plants. Complications included low germination, callus production, and unsuccessful acclimatization due to poor root quality (Ascencio-Cabral *et al*. 2008). Other somatic embryogenesis protocols for various purposes are described in table 2.

Factors affecting plant tissue culture of *C. papaya.*

Variety: Different papaya varieties respond differently to *in vitro* cultures. Also, the age or degree of maturity of the plant causes different responses. This is due to the fact that the physiological state relating to the endogenous hormonal concentration of the explant at various ages differs significantly. The *C. papaya* (Taiping) produces 5–6 times more shoots in cultures compared to the *C. papaya* (Eksotika) even when the medium is controlled for. Also, the continuous usage of papaya cultures to generate shoots in liquid media produced abnormal shoots. The Eksotika variety was more susceptible to abnormality than the Taiping variety (Chan and Teo 1993). The callus produced from cross-pollinated *C. papaya*, and *C. cauliflora* did not have the ability to produce embryogenic calli. Only the *C. goudotiana* was able to produce embryogenic calli. Also, the media constituent and the size of the flower bud had a substantial effect on callus induction in anther culture (Azad *et al*. 2013). Callus induction for somatic embryogenesis is affected by papaya varieties. For instance, the 'Solo' variety produced more callus than the explants from the Sunrise variety in the same type of media (Chen *et al*. 1987). The different varieties

Table. 2 Summary of protocol used for propagation of *C. papaya* using somatic embryogenesis.

Authors and years	Papaya variety and Organs	Initiation Media	Induction Media	Maturation Media	Regeneration
(Chen *et al*. 1987)	*C. papaya* Sunrise and 'Solo.' Somatic embryo from the stem, shoot tip, leaf,cotyledon, and root	½ MS medium supplemented with 160 mg L^{-1} adenine sulfate, and 1.0 mg L^{-1} NAA, 0.5 mg L^{-1} kinetin and 1.0 mg L^{-1} GA_3	½ MS with full strength vitamins supplemented with casein hydrolysate, I00 mg L^{-1} myo-inositol, 160 mg L^{-1} adenine sulfate, 30 mg L^{-1} sucrose, 1.0 mg L^{-1}NAA, 0.5 mg L^{-1} kinetin and 1.0 mg L^{-1} GA 3 and 8 mg L^{-1} agar.		**Germinated medium** MS medium supplemented with NAA only 16 h light (2000 lux) and eight h darkness **Acclimatization**: mixture of sand: soil (1:1: v/v). One percent IAA was sprayed on the plantlets for two weeks.
(Fitch and Manshardt 1990)	*C. papaya* hybrid Sunrise', 'Sunset,"Waimanalo,' and 'Kapoho.' Hermaphroditic, Immature zygotic embryos from open pollination and self-90 to 114 days		MS medium Supplemented with 20% coconut water and 2 mg L^{-1} 2,4-D. Also, different combinations of plant growth hormones, 0.4 mg L^{-1} BA, 0.02 mg L^{-1} TZD, 1 mg L^{-1} 2,4-D 0.5 mg L^{-1} picloram.)	**Germination somatic medium** MS basal salts supplemented with, 5 mg L^{-1} Kinetin, 100 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose and 1% Difco Bactoagar	**Germination medium** Same somatic embryo germination medium without Kinetin
(Chen *et al*. 1991)	*C. papaya* hybrid Between Costarica Red *C. papaya* as female With male cauliflora .60 days after pollination.		½ MS medium with full strength vitamin, supplemented with 0.001 mg L^{-1} BA, 1 mg L^{-1} casein hydrolysate, 20 mg L^{-1} sucrose and 0.6% agar. 25 ± 2°C with 16 h/8h light dark condition	**Proliferation Medium:** same induction medium but without ABA	**Germination medium:** MS medium without growth hormones.
(Castillo *et al*. 1998b)	*C. papaya* Hermaphroditic Solo self-pollinated Immature zygotic embryo (65–70 days post-Anthesis)		MS basal salts supplemented with 10 µM 2,4-D, 50 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose, and 0.7% Difco Bacto-agar incubated in the dark at 27°C	Similar to induction medium but with 60 g L^{-1} sucrose and without plant growth regulators. Dark condition **Liquid maturation medium**: Supplemented with 0.5 µM ABA. At 120 rpm under 40 µmol m–2 s–1 constant irradiance.	

(Vilasini *et al*. 2000)	*C. papaya* hermaphrodite Eksotika Immature zygotic embryo from 90–100 days old after Anthesis.		½ MS medium with full strength vitamins, supplemented with 10 mg L^{-1} 2,4-D, 50 mg L^{-1} myo-inositol, 45 mg L^{-1} adenine sulphate, 100 mg L^{-1}glutamine, 60 g L^{-1} sucrose and 1% Difco Bacto agar. At 25–27 °C in dark condition	MS medium without plant growth regulators Light condition.	MS medium supplemented with a 0.1 mg L^{-1} NAA and 0.1 mg L^{-1} 6-BAP, 30 g L^{-1} sucrose **Root medium** according to Drew *et al*. (1991) in the light **Acclimatization**: soil: sand: vermiculite mixture (1:1:1).
(Fernando *et al*. 2001)	*C. papaya* Sunrise Solo Mature zygotic embryos	.	MS medium supplemented with 2 mg L^{-1} 2,4-D, 0, 7% agar. at 25+2 ºC under dark condition		
(Yu *et al*. 2003)	*C. papaya* L. Tainung No. 2 Root segment	MS basal salts supplemented with B vitamins, 4.5 µM 2, 4-D, 0.45 µM BA, 30 g L^{-1} sucrose, and 0.8% agar. At 28ºC under dark condition		**Germination medium**: MS medium supplemented with 0.1µM NAA and 0.8 µM BA Under light condition	**Root medium**: MS salts supplemented with B vitamins, 2.5 mM IBA, 30 g L^{-1} sucrose and 0.8% agar.
(Clarindo *et al*. 2008) As recommend by (Castillo *et al*. 1998b)	*C. papaya* Hermaphroditic 'Golden.' Immature zygotic embryo 90 to114 days post-Anthesis		½ MS basal salts with full strength vitamins supplemented 9.05 mM 2,4-D, 0.55 mM myo-inositol, 2.75 mM L-glutamine, 60 g L^{-1} sucrose and 0.28% Phytagel. At 27ºC under dark condition.	**Maturation medium**: ½ MS basal salts with full strength vitamins supplemented with 0.5 µM ABA, 0.55 mM myo-inositol, 2.75 mM L-glutamine, and 60 g L^{-1} sucrose. At 27ºC in a 16/8 h light/dark condition	**Germination medium**: MS medium supplemented with 0.55 mM myo-inositol, 30 mg L^{-1}sucrose and 0.7% agar under dark condition. **multiplication medium** MS medium supplemented with 0.88 µM BAP, 0.11 µM NAA, 0.55 mM myo-inositol, 30 g L^{-1} sucrose, and 0.7% agar. At 27ºC under a 16/8 h light/dark condition
Ascêncio *et al*. 2008)	*C. papaya* Maradol Hermaphrodite zygotic embryos	½ MS medium supplemented with 0.02 mg L^{-1} 2,4-D, 0.2 mg L^{-1} kinetin, 0.02 mg L^{-1} ABA,		**Maturation and Germination medium:** ½ MS medium supplemented with	**Root medium:** ½ MS medium without plant growth regulators supplemented with 3.0 g L^{-1} activated charcoal

		100 mg L^{-1} L-glutamine, 40 g L^{-1} sucrose and 7.5 % Difco1 Bacto agar. At 27- 28 ºC under light condition.		Chen vitamins (Chen *et al*., 1987), 100 mg L^{-1} L-glutamine, 68 mg L^{-1} adenine hemisulphate, 16 g L^{-1} sucrose, and 0.35 mg L^{-1} GA3.	**Acclimatization:** Mixture (70:30:10 of peat moss: vermiculite: pine bark)
(Malabadi *et al*. 2011)	*C. papaya* Coorg Honey dew, Honey dew, Washington, Pusa nanha, Pusa delicious, Taiwan 785,Taiwan 786, Sunrise, Solo, Co-1, Co-3, Co-7, Immature zygotic embryos.		MS medium supplemented with 4.52 µM 2, 4-D, 2.27 µM TDZ, 0.5 mg L^{-1} myo-inositol, 1.0 mg L^{-1} casein hydrolysate, 0.5 mg L^{-1} glutamine, 250 mg L^{-1} peptone, 0.2 mg L^{-1} p-aminobenzoic acid,0.1 mg L^{-1} biotin, 30 g L^{-1} sucrose and 0.7% agar. Under dark condition	MS medium supplemented with 30 g L^{-1} sucrose, 5 µM ABA, and 0.8% agar Dark condition	½ MS medium with 0.7% agar without plant growth regulators
(Anandan *et al*. 2012)	*C. papaya* Co7 Immature zygotic embryos	½ MS medium supplemented with 2mg L^{-1} 2,4-D, 400 mg L^{-1} glutamine, 1.0% activated charcoal, 60 g L^{-1} sucrose and 4% phytagel. At 25 ºC under dark condition		**Maturation liquid phase**: liquid MS medium supplemented with 10.0 mg L^{-1}ABA and 10 g L^{-1} sucrose. At 25 ± 2ºC Agitated at 110 rpm under a 16 h light condition. **Maturation solid phase**: containing same initiation media with 4% phytagel and 10 g L^{-1} sucrose	MS medium supplemented with 0.4 mg L^{-1} BAP, 0.02 NAA, 100 mg L^{-1} casein hydrolysate, 100 mg L^{-1} malt extract, 30 g L^{-1} sucrose and 4 % phytagel. at 25 ºC day/22 ºC night
(Azad *et al*. 2012)	*C. papaya* hybrid between Shahi and *C. cauliflora* immature hybrid embryos	½ MS medium supplemented with 5 mg L^{-1} 2,4-D, 100 mg L^{-1} casein, 100 mg L^{-1} glutamine, hydrolysate and 60 g L^{-1} sucrose	MS medium without plant growth regulator	½ MS medium supplemented with 0.5 mg L^{-1} BAP, 0.2 mg L^{-1} NAA, and 60 g L^{-1} sucrose at 24 °C ± 2 °C under 16 h photoperiod	MS medium without plant growth regulator **Acclimatization** amixture of autoclaved cocopeat, sand and garden soil (1:1:1).
(Bukhori 2013)	*C. papaya* Eksotika hermaphrodite 90-100 days old immature zygotic embryo		½ MS with full strength vitamins Supplemented with 10 mg L^{-1} 2,4-D, 50 mg L^{-1} Myo-insitol,	**Germination medium** MS basal salts supplemented with 0.2 mg L^{-1} of both BAP	**Regeneration medium** MS medium supplemented with 1 mg L^{-1} GA3, 0.5 mg L^{-1} IBA, and 3.76 mg L^{-1}

			45 mg L^{-1} adenine sulphate, 250 mg L^{-1} carbenicillin, 100 mg L^{-1} L-glutamine 60 g L^{-1} sucrose, and 0.195% Phytagel. At ±25ºC under dark condition. **Liquid multiplication medium** same induction medium, but decreasing the concentration of 2,4-D to 2 mg L^{-1}.	and NAA, 108 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose and 0.195% phytagel. Under light condition	riboflavin **Rooting medium** MS medium supplemented with 2.0 mg L^{-1} IBA. At ±25ºC, under a 16 photoperiod
(Abreu *et al*. 2014)	*C. papaya* Golden Hermaphroditic ': Immature zygotic embryos 90-114 day	½ MS with full strength vitamin supplemented with 9.05 µM 2,4-D, 0.1 g L^{-1} Myo-insitol, 0.4 g L^{-1}-glutamine, 0.04 g L^{-1} Cysteine 30 g L^{-1} sucrose and 3 % phytagel	Liquid media ½ MS with full strength vitamin supplemented with 9.05µM 2,4-D, 2.25 µM BAP,0.1 g L^{-1} Myo-insitol, 0.4 g L^{-1} L-glutamine, 0.04 g L^{-1} Cysteine, 0.1 g L^{-1} Malt extract, and 3% sucrose. At 100 rpm and maintained at 27°C under a 16/8 h light/dark condition	½ MS with full strength vitamin supplemented with 0.5 µM ABA , 0.1 g L^{-1} Myo-insitol, 0.4 g L^{-1} L-glutamine, 0.04 g L^{-1} L-Cysteine, 0.1 g L^{-1} Malt extract, and 30 g L^{-1} sucrose. at 100 rpm and maintained at 27°C under a 16/8 h light/dark condition	MS medium supplemented with 0.5 µM GA, 0.1 g L^{-1} Myo-insitol and 30 g L^{-1} sucrose. At 27°C under dark condition **Acclimatization:** Vermiculite: peat moss (2: 1).
(Razak *et al*. 2015)	*C. papaya* L. 'Eksotika' Immature zygotic embryos		½ MS medium with full strength vitamins supplemented with 45.2 µM 2,4-D, 50 mg L^{-1} myo-inositol, 0.14 mg L^{-1} adenine hemisulphate, 400 mg L^{-1} glutamine, 250 mg L^{-1} carbenicillin, 60 g L^{-1}sucrose and 3.2 mg L^{-1} gelrite. At ± 25ºC under light condition **Maturation medium** De Fossard (De Fossard, 1974) without growth regulator	De Fossard medium supplemented with 0.89 µM 6- BA 1.1 µM NAA and 150 mL coconut water under a light photoperiod at 26 ± 2^{O}C	
(Heringer *et al*. 2013)	*C. papaya* hybrid UENF/CALIMAN 01 Immature zygotic embryos 120 and 130d		**Induction medium**: MS medium supplemented with 20 µM 2,4-D 30 g L^{-1} sucrose, and 2 % Phytagel. At 25±2°C under dark condition.		

		Maturation medium: MS medium supplemented with 0.05 g L^{-1} myo-inositol, 6 % PEG MW 335030 g L^{-1} sucrose and 2% Phytagel. At 25±1°C under dark condition for the first week then light condition	
(Koehler *et al*. 2013)	*C. papaya* hermaphrodite plants Young leaves	**Induction media**: ½ MS full strength vitamins supplemented with 9.05 µM 2,4-D, 0.55 mM Myo-insitol, 2.75 mM glutamine, 87.6 mM sucrose and 0.28% (w/v) Phytagel. At 27°C under dark condition. **Maturation medium:** ½ MS with full strength vitamins supplemented with 5 µM abscisic acid, 100 mg L^{-1} malt extract, 5% polyethylene glycol 2000, 0.2% activated charcoal, and 0.55 mM Myo-insitol, 2.75 mM L-glutamine, 87.6 mM sucrose and 0.28% (w/v) Phytagel. at 16/8h light/dark	**Germination medium**: MS basal medium, supplemented with 0.55 mM myo-inositol, 58.4 mM sucrose and 0.65% (w/v) agar. at 27°C under a 16/8h light/dark photoperiod,

of *C. papaya* responded differently during embryogenesis that took place in the same concentrations of 2,4-D (Fitch and Manshardt 1990).

Explant: The source of explants is vital for the successful establishment of papaya in cultures (Reuveni *et al*. 1990). During *in vitro* micropropagation, the age of explants played a powerful role in determining successful propagation. This is due to the fact that immature or less differentiated plants are easier to sterilize and initiate cultures. They also have the ability to multiply and form roots better than explants from mature plants (Anandan *et al*. 2012). According to Wu *et al*. (2012), the induction of shoot buds and proliferation varied with the age of explants. Chen *et al*. (1987) studied the ability of different papaya organs (i.e. stem, leaf, root, shoot tip and cotyledon explants) and different papaya cultivars (Solo and Sunrise) to generate somatic embryogenesis. It was found that the shoot tips and stems of these two papaya varieties were most appropriate for forming a callus, whereas cotyledons, leaves, and roots were difficult to induce callus. Zygotic embryos of *C. papaya* were high in embryogenic potential and responded quickly to tissue culture conditions (Anandan *et al*. 2012). A large number of embryogenic calluses can be generated from an immature zygotic embryo (110 to 120 days old) in MS medium augmented with 2,4-D (2.0 mg L^{-1}) after 6–8 weeks of culturing (Anandan *et al*. 2012).

Plant Growth Regulators: Phytohormones were one of the essential factors for callus induction from petiole explants (Hossain *et al*. 1993), whereas, auxin was the key to the initiation and continuous growth of callus. Conversely, kinetin and gibberellic acid were not essential for the induction of callus (Arora and Singh 1978). Somatic embryos initiated in culture mediums supplemented with picloram, benzyl adenine, thidiazuron, and coconut water, showed inhibitory effects to somatic embryogenesis. On the other hand, zygotic embryos remained inactive or terminated in phytohormone-free media (Fitch and Manshardt 1990).

The 2,4-D hormone was appropriate for prompting somatic embryogenesis in papayas (Clarindo *et al*. 2008). Low concentration levels of the 2,4-D hormone were substantial for the initiation of somatic embryos in papaya, especially from immature zygotic embryos. Higher concentration levels tended to induce genetic changes and caused variations between the *in vitro* propagated plants (Anandan *et al*. 2012).

Abscisic acid (ABA) played a substantial role in regulating the *in vitro* somatic embryogenesis in papaya. ABA allowed direct somatic embryogenesis without callus formation. ABA could initiate growth of the early embryo and set the accumulation of storage proteins. Induced somatic embryos from immature zygotic embryos of *C. papaya* x *C. cauliflora* in the absence of ABA generated a small number of abnormal embryos (Chen *et al*. 1991; Anandan *et al*. 2012). The presence of BAP and NAA in the medium enhanced the number of shoots produced from shoot tip explants. The BAP hormone helped to prevent inertness and to begin shoot initiation, while NAA increased elongation of meristem cells (Panjaitan *et al*. 2007).

Solidifying agent: Gelling agent had considerable effects compared to light and phloridzin. Solidified media with bacto Agar achieved a higher germination percentage with lesser genetic variation. On the other hand, phytagel and agar produced genetic variations in the callus and plantlet (Ascencio-Cabral *et al*. 2008).

Light: Light is one of the physical factors that affects many physiological processes of *in vitro* plantlets, for instance, photomorphogenesis and photosynthesis. The light quality, quantity, and exposure duration can affect plant growth and development (Gupta and Jatothu 2013). Ascencio-Cabral *et al*. (2008) studied the effects of light quality on the germination of *C. papaya* somatic embryos to promote efficient and reproducible protocol for propagation. They found that light quality had significant effects on the development of somatic embryogenesis and plant growth. The plant exposed to gro-lux lamps showed higher percentages of survival without hyperhydricity.

Gaseous atmosphere: The growth and development of *in vitro* plants depend on the constituents of the culture medium and the constituents of the gaseous atmosphere. The ventilation of culture flasks has an important effect on the oxygen and ethylene concentration inside the culture flasks, which subsequently affects the number of shoots and leaves as well as leaf expansion. Moreover, plants grown in non-aerated flask media for an extended duration demonstrated growth retardation (Lai *et al*. 2000). The type of culture flask is an important factor for *in vitro* culturing. The ethylene and carbon dioxide concentration increases when light is absent in smaller-sized airtight culture flask. It was proposed that incubating nodal cultures of papaya with different ethylene levels caused poor performance (Magdalita *et al*. 1997). On the other hand, the addition of some chemicals to reduce ethylene such as aminoethoxy vinyl glycine (AVG), and silver thiosulphate (STS), significantly improved nodal culture growth and leaf area production as well as reduced leaf deterioration (Magdalita *et al*. 1997).

Sucrose: Plants in *in vitro* culture conditions need an exogenous carbohydrate source because most of the plants grown *in vitro* tend to shift from autotroph to heterotroph. Selecting the best carbohydrate source and concentration in culture media depends on the plant species and the micropropagation phase. Most papaya species induced somatic embryogenesis in media supplemented with 6% (w/v) sucrose, which promoted a high frequency of embryogenesis. Also, under similar

concentrations and mediums, sucrose is better than maltose in inducing embryogenic callus (Vilasini *et al*. 2000). Sucrose plays an important role and affects the formation of somatic embryogenic callus. In *C. papaya* var. Eksotika, 60 g L^{-1} sucrose produced the highest frequency of somatic embryo callus from immature zygotic embryo of hermaphrodite papaya (Razak *et al*. 2015). On the other hand, decreasing or sucrose absent media reduced the risk of contamination, enhanced the photosynthetic capacity of the plant and also, improved acclimatization of plants propagated *in vitro* (Xiao *et al*. 2011).

Other culture media components: Induction of embryogenic callus is affected by the nutrient media and plant growth regulators used (Jordan 1986). Adenine hemisulfate is an example of supplements that can improve the capability of papaya shoots to regenerate from calluses (Drew 1987). MS medium containing 160 mg L^{-1} adenine sulfate was found to be optimal for the propagation rate of papaya (Reuveni *et al*. 1990). Activated charcoal is mostly used in root media and can be employed for regulating phenolic oxidation during embryogenic callus formation and embryo induction (Anandan *et al*. 2012). The addition of casein hydrolysate to the medium improves the shoots number and when combined with activated charcoal has a favorable effect on shoot growth as well as prevents leaf abnormality. Also, adding a combination of urea and activated charcoal to the medium enhances shoot elongation (Roy *et al*. 2012). Polyethylene glycol has a significant effect on the maturation of somatic embryo of papayas (Heringer *et al*. 2013). The addition of coconut water to the media does not give significant results when compared with media without coconut water (Fitch and Manshardt 1990).

Boron is one of the micronutrients that plants need a ~~high~~ low concentration of as it plays a significant role in phenylpropanoid metabolism and lignin biosynthesis. Boric acid is a source of boron in plant tissue culture media. Renukdas *et al*. (2003) demonstrated that adding boric acid (62 mg/dm^3) to MS media supplemented with B5 vitamin, 2 mg L^{-1} 2,4,D resulted in a maximum number of somatic embryos initiated from immature zygotic embryo of *C. papaya*. On the other hand, increasing the concentration of boric acid in the medium to more than 100 mg/dm^3 completely inhibited the induction of somatic embryos from the zygotic embryo explant.

In tissue culture media, combinations of vitamins with other media constituents, directly and indirectly affects all phases of somatic embryogenesis from induction to rooting. Adding components of cytokinin with thiamine positively affect the induction of callus growth and rooting. Also, a combination of Biotin and Riboflavin play a role in the development of callus. Furthermore, riboflavin can positively affect the plant rooting of papaya. On the other hand, vitamin D improves cell elongation and meristematic cell division, which enhances shoot and rooting growth (Abrahamian and Kantharajah 2011).

The culture media constituent has a significant effect on callus induction. The ½ MS medium with full strength Na-Fe-EDTA supplemented with NAA (2.0 mg L^{-1}), BAP (1.0 mg L^{-1}) produced the highest percentage of callus in three *Carica* species: *C. papaya, C. cauliflora*, and *C. goudotiana* (Azad *et al*. 2013).

Root formation: The possibility of large scale propagation via *in vitro* protocols depends on the capability to produce high numbers of plants with low prices. Also, the ability of propagated plants to adapt to the *ex vitro* conditions to ensure high quality and survival rates is another factor to be considered (Hazarika 2006). Riboflavin and IBA promoted root initiation. Riboflavin and IBA concentrations decreased rapidly in media exposed to light. So, dark conditions are recommended when IBA and riboflavin are added in the media. On the other hand, increasing concentrations of riboflavin in the media quickened the reduction of IBA (Drew *et al*. 1991). The use of riboflavin injections instead of transferring to hormone-free mediums saved time and cost of subculturing (Drew *et al*. 1991; Drew *et al*. 1993). IBA is better than other plant growth regulators, such as IAA, NAA or PCPA for root initiation of *C. papaya*. Moreover, the exposure of shoots to a medium containing 10 µM of IBA for two days after being transfered to free media with 31 µM riboflavin under dark condition produced the best result for root initiation (Drew *et al*. 1993). Apart from incurring a lower cost, it is also an easy and economical protocol to acclimatize root systems of *C. papaya* L which promotes large-scale micropropagation. This protocol depended on root induction in mediums supplemented with low concentrations of IBA followed by root development in ~~half strength~~ ½ MS medium supported with vermiculite under ventilated conditions (Yu *et al*. 2000).

Problems associated with root development and acclimatization: A plant that is grown *in vitro* differs from those produced *in vivo*. Plants grown *in vitro* are heterotrophic, whereas plants grown *in vivo* are photoautotrophic. The gaseous, light, and nutrition varies between *in vitro* and *in vivo* (Kadleček *et al*. 2001). Plant growth *in vitro* is classified into three types according to carbon and energy source. The first type, photoautotrophic growth, occurs when the plant is dependent on photosynthesis in *in vitro* culture. The second type, heterotrophic growth, occurs when the plant is dependent on sugar found in the culture medium. The third type, photo mixotrophic growth, occurs when the plant depends on photosynthesis and sugar in common cultures (Kozai *et al*. 2005). Micropropagation of the

plant in culture mediums without a carbon source is called photoautotrophic micropropagation (Zobayed *et al*. 2001; Kozai *et al*. 2005). Photoautotrophic can be stimulated *in vitro* by eliminating carbohydrates from the culture medium and increasing gas exchange in the culture flask (Xiao *et al*. 2011).

Many factors influence the development of roots in *in vitro* cultures such as auxin type and concentration, shoot quality, donor age, and temperature (Mohammed & Vidaver, 1988). The germination of somatic embryos suffered problems associated with root development due to the accumulation of calluses at the base end of somatic embryos which prevented the proper development of roots and weakened the joining of roots to the stem (Fitch and Manshardt 1990; Sekeli *et al*. 2012).

Induction of roots *in vitro* from young papaya leaves was possible after being exposed to several auxins such as a 2,4,5-trichlorophenoxyacetic acid; IBA; α-NAA. Recently, Pérez *et al*. (2015) studied the effects of phloroglucinol in rooting. Phloroglucinol stimulated construction of new roots and enhanced root elongation, especially when added to ½ MS medium without sucrose, fortified with IBA (9.8 μM). This resulted in excellent rooting development with 100% rooting and a larger number of roots for each plant.

One of the biggest problems associated with somatic embryogenesis of *C. papaya* is the acclimatization of regenerated plants, whereby over 2/3 of the plants are lost before being moved to the field (Malabadi *et al*. 2011) due to the inability to adapt to the new environment. Reducing the relative humidity and increasing the ventilation inside the culture containers seems to have a larger influence on the adaptation and increasing plant survival of papaya under *ex vitro* acclimatization (Pérez *et al*. 2015). Acclimatization is a critical stage in all *in vitro* propagation protocols. In this phase, the relative humidity should be gradually decreased to improve stomata function, enhance cuticle development, and decrease water loss. Acclimatization of *C. papaya* using ½ MS medium supplemented with IBA without sucrose and with zeolite as supporting material can also increase ventilation, improve root quality and increase the survival rate of plants (Pérez *et al*. 2015). Poor photosynthetic rate of *in vitro* plants is associated with CO_2 concentration. So, improving CO_2 concentration and light intensity inside the culture flask and decreasing relative humidity will improve the photosynthetic rate (Kozai *et al*. 2005).

Somaclonal variation: Plant tissue culture technique causes genetic variation. This variation can prevent and block some stages of *in vitro* cultures, and generate undesired characteristics especially during genetic transformation. However, it can also cause useful genetic variability for crop improvement (Clarindo *et al*. 2008). There are many problems associated with somatic embryos of papaya in achieving a reproducible and secure protocol to produce healthy plants without genetic changes. Examples include decreased germination rate, accumulation of callus that prevents root germination, the occurrence of abnormal plantlets and unsuccessful acclimatization (Ascencio-Cabral *et al*. 2008).

The occurrence of polyploidy is one of the factors that generates abnormal plants during somatic embryogenesis, especially when used for propagation. In the past, most scientists used conventional chromosome counting to detect ploidy level whereas now, flow cytometry is usually used. For instance, Clarindo *et al*. (2008) used flow cytometry to detect polyploidy of somatic embryogenesis of papaya derived from immature zygotic embryos. There were no changes in ploidy level recorded in all papaya plantlets in the multiplication medium (Clarindo *et al*. 2008). The flow cytometry level and cytogenetic analyses are necessary for assessing the DNA amount (ploidy level), investigating chromosomal stability and providing a rapid, reliable and final assessment of the genetic constancy of *C. papaya* plantlets restored by somatic embryogenesis, (Abreu *et al*. 2014).

Cell suspension culture: Cell suspension culture involves growing friable portions of a callus in a liquid medium under appropriate conditions of light, temperature, agitation, aeration, and other aspects (Chattopadhyay *et al*. 2002). It is advantageous to use a liquid system medium compared to a solid system medium for *in vitro* propagation. The fluid system increases the probability of every plant cell to uptake plant growth regulators and nutrients. The liquid MS medium improves development and enhances the number of embryos (Anandan *et al*. 2012). The tissue culture depending on fluid system significantly saved ~~consumes~~ time and cost of propagation (Abreu *et al*. 2014). Plant cell suspension culture is a sterile system available for numerous plant studies. For instance, experiments involving *in vitro* selection of cold and salt tolerance, disease resistance, and genetic transformation.

Cell suspension culture can be applied in *Carica candamarcensis* to improve asexual propagation rates (Jordan 1986). Furthermore, other scientists found that the liquid medium was better than a solid medium for the conversion of a higher number of somatic embryos during early stages (Anandan *et al*. 2012).

Chan and Teo (1994) cultured papaya axillary buds obtained from mature trees in solid MS supplemented with BA (0.1 mg L^{-1}), casein hydrolysate (500 mg L^{-1}) and riboflavin (0.38 mg L^{-1}). They found that 2 to 18 weeks were needed to produce less than two shoots per explant. However, when the explants were cultured in solid medium followed by liquid medium for ten weeks each, 82 times more shoots were produced compared with culturing in a purely solid medium.

Additionally, it was possible to produce many shoots rapidly when cultured in liquid media for a duration of less than ten weeks (Chan and Teo 1994). Bioreactors are used to maintain plant cell cultures on a large scale production of high-quality cell lines. Growing cell cultures in bioreactors are beneficial to control growth parameters that cause variations and affect product quality. It also allows genetic manipulation through genetic engineering (Khalid *et al*. 2010).

Cryopreservation: Cryopreservation can be used for the conservation of germplasm as well as the storage of embryogenic cultures and somatic embryos for micropropagation, genetic transformation, and selection studies (Fitch 2005). Cryopreservation can ~~affect~~ affected by incubation media, time of incubation, vitrification, exposure time, concentration of vitrification, temperature of vitrification condition and explant type for instance. Vitrification solutions (PVS2) contain large concentrations of highly toxic material that harms plant tissue. Therefore, minimum exposure to the solution is recommended to obtain sufficient dehydration and to prevent toxic effects (Azimi *et al*. 2005). Desiccated *C. papaya* seeds differing in moisture content (i.e. ranging from 5% to 40%) and frozen in liquid nitrogen demonstrated a significant increase in germination rate (48%) at 10% moisture content. On the other hand, the germination rate decreased for cryopreserved seeds when the moisture content was less than 10% (Azimi *et al*. 2005). According to Perveen *et al*. (2007), temperature and humidity were the main important factors in pollen viability. Decreasing the temperature and humidity helped to increase the period of viability. Pollen stored at –60^{o}C demonstrated the best result with 60% viability after being preserved for 48 weeks.

Azimi *et al*. (2005) utilized two procedures for storing papaya germplasm namely, desiccation and cryopreservation of seeds and vitrification-based cryopreservation of shoot tips. Results demonstrated that shoot tips were successfully cryopreserved by incubation for 1 to 4 days before vitrification. 100% PVS2 for 20 minutes of exposure to vitrification at 0^{o}C gave 70% recovery from the shoot tips.

Cryopreservation technique based on vitrification was used to store 12 genotypes of papaya. Where the shoot tips were used as explant, after that the genetic stability was examined for screened the genetic and epigenetic changes. Results indicated that the regenerated plantlets were abnormal with numerous genetic variations. In addition, the exposure of shoot tips to cryoprotectants reduced recovery growth rates and induced genetic variation by inducing methylation and DNA changes (Kaity *et al*. 2008).

Randomly amplified DNA fingerprinting (RAF) is a technique used for screening changes in genomic DNA. This technique is useful and easy since information regarding DNA sequence is not necessary. Additionally, it does not require a restriction template but instead only requires one primer and amplification reaction. Therefore, it is possible to obtain a rapid and reproducible amplification result and to determine the markers from any organism. Furthermore, this technique has the capacity to generate identical profiles regardless of the concentrations, as well as the ability to create profiles using small samples (Kaity *et al*. 2008).

Encapsulation: The production and encapsulation of somatic embryos allow mass propagation of genetically engineered papaya (Castillo *et al*. 1998b). The occurrence of regeneration from encapsulated embryos of *C. papaya.* L. is affected by many factors, such as the presence or absence of nutrient salts in the capsule, the concentration of sodium alginate, and finally, the duration of exposure to calcium chloride. Castillo *et al*. (1998b), presented a protocol for the alginate encapsulation of leaves or leaf-derived callus (soft or hard) from two papaya cultivars, Rainbow and Sunrise Solo. The purpose was to preserve valuable germplasm using cryopreservation. The ability to store these encapsulated alginate beads at low temperatures and to cryopreserve them upon their successful regeneration provides a useful and innovative mid- to long-term method of preserving *C. papaya* germplasm.

Castillo *et al*. (1998b) also achieved an optimized protocol for encapsulation that ensured proper regeneration of *C. papaya* using a sodium alginate (2.5%) concentration in a half-strength MS with short exposure time to $CaCl_2$ (less than 10 min).

Papaya of Malaysia: The history of papaya in Malaysia started when Spaniards brought the plant to the Philippines in 1598 and then transferred and grown in Malaya (Schery 1952: cited by Chan & Theo, 2000).

Papaya is listed as one of the most important fruit exports in Malaysia. It is mainly cultivated in Johore, Perak, and parts of Selangor. The most popular grown cultivar of *C. papaya* in Malaysia is 'Eksotika.' This variety resulted from a cross breed between Subang 6 and the Hawaiian Sunrise Solo (Chen *et al*. 1987; Chan *et al*. 2015). The Eksotika fruit is superior to Sunrise Solo fruit in size, weights and firm texture (Chan 2009). The 'Eksotika' papaya cultivar, grown from seeds, tends to produce hermaphrodite and female populations in a (3:1) proportion respectively, with a 100% fruit-producing population (Chan and Teo 1993). The Eksotika is very sensitive to many diseases especially the Papaya Ringspot Virus (PRSV) which infected papaya crops in Johor in 1991 (Vilasini *et al*. 2000). The PRSV disease and rapid damage to the fruit are two major restriction challenges for the papaya industry in Malaysia (Chan *et al*. 2015; Chan 2002). The Malformed Top Disease (MTD) caused by *Cladosporium*- thrip complex is another challenge. The *C. papaya* Eksotika and Solo

cultivars are considered the most susceptible to this disease. It is very destructive especially to developing vegetation. Trees infected with this fungus shows many symptoms such as; the leaves display typical 'shot-hole' symptom and trees will be weakened (Chan and Mak 1993). Chan and Teo (1993), reported that 99–100% of the explants obtained from mature field-grown trees were contaminated with fungus and bacteria.

There is a keen rising interest in plant cell cultures that can help rapid propagation of selected plants. In Malaysia, there is an urgent need for sufficient supply to meet the significant demand for seedlings, which are an important plantation and cash crop (Khalid *et al*. 2010). Plantlet regeneration using shoot tips of hermaphrodite papaya (Eksotika) was achieved by culturing in MS medium supported with combinations of BAP (1.0 mg L^{-1}) and NAA (0.05 mg L^{-1}). The result demonstrated that the maximum rooting percentage was obtained when shoots explants were cultured in media augmented with IBA (1.0 mg L^{-1}) (Panjaitan *et al*. 2007). Unfortunately, the roots generated were abnormal, when ½ MS medium, with full-strength vitamins and 2,4-D (10 mg L^{-1}) were used for induction of somatic embryogenesis from immature zygotic papaya (Eksotika) (Vilasini *et al*. 2000). Bukhori (2013), generated an efficient *in vitro* protocol for the production of papaya (Eksotika), using immature zygotic embryo grown in different culture media. Induction media cultures consisted of ½ MS medium supplemented with carbenicillin (250 mg L^{-1}) and 2,4-D (10 mg L^{-1}). Germination media consisted of MS supplemented with BA and NAA (0.2 mg L^{-1}), whereas rooting media consisted of MS fortified with IBA (0.5 mg L^{-1}).

Eksotika resistant to papaya ringspot virus were generated via microprojectile bombardment of coat protein gene isolated from the local virus. The same variety was also engineered for fruits with improved shelf life (Vilasini *et al*. 1998). The two major difficulties faced in developing transgenic Malaysian Eksotika papaya plants are low efficiency of regenerated roots from shoots and low acclimatization rate of rooted transgenic papaya plants in the field. Therefore, rooting efficiency with high-quality roots formation are critical in ensuring successful and continuous production of transgenic Eksotika papaya (Sekeli *et al*. 2014). It is also possible to develop new papaya varieties with enhanced performance and disease resistance using irradiation. Irradiation-induced mutation is progressively being used as a complementary tool in plant breeding. It is most suitable for developing one or two easily identifiable traits and well-accepted breeding lines. There is an excellent response regarding selecting dwarf trees, lower fruit bearing stature, higher total soluble solids in fruits and for resistance to MTD. Unfortunately, irradiation did not seem to be favorable for developing PRSV resistance in Eksotika papayas (Chan *et al*. 2007).

Inter-Retrotransposons Amplified Polymorphism (IRAP) molecular markers are used to simplify the chosen papayas that have resistance to PRSV in breeding programs. The result showed significant polymorphism in DNA banding patterns to determine parental-progeny relations and categorized progenies based on their resistance to PRSV. The best primers regarding inheritance pattern based on the PRSV resistance trait was a primer combination between LTR 6150 and Nikita primers (Rashid *et al*. 2014). Razak *et al*. (2015) developed marker-free positive selection of transgenic papaya plants using phospho-mannose isomerase (pmi) genes. Only transformed cells can employ mannose as a carbon source to grow. They also evaluated the effect of mannose on the growth and development of embryogenic 'Eksotika' papaya callus. Mannose at 30 g L^{-1} was found to be effective for screening transformed embryogenic calli.

Conclusions: *C. papaya* is listed as one of the most important economic export fruits in tropical and sub-tropical countries. The bulk of papaya plantations is based on seed propagation. The spread of *C. papaya* by seed is a preferable method for farmers to grow papaya because it is cheap. However, this conventionalism can spread severe diseases in papaya plantations. Commercial *in vitro* mass propagation of *C. papaya* has not been established because of many difficulties in micropropagation. For instance, the multiplication and maintaining scores of elite plantlets with desirable characteristics, retaining tolerance to distortion ringspot and the lack of standardized protocol for *in vitro* propagation. Somatic embryogenesis has been employed in papaya crops to solve these problems. On the other hand, *in vitro* propagation of papaya by using somatic embryogenesis faces many challenges, especially contamination, rooting, and acclimatization. Solving these issues will help to develop a reproducible protocol that will produce high-quality papaya plants and aid in molecular study to generate resistance strains against various diseases. So, focusing on the optimize different steps in somatic embryogenesis, synchronized stages of somatic embyogenesis, and enhaned root formation by knowing the causes behined callus formation at the base end of embryos will improve the profficient reproduciable protocols.

Acknowledgment: The authors thank the University of Malaya for all the facilities provided and the postgraduate grant (IPPP: PG204-2014B).

REFERENCES

Abrahamian, P. and A. Kantharajah (2011) Effect of vitamins on *in vitro* organogenesis of plant. Am J Plant Sci. 2 (05):669-674.

Abreu I.S., C.R. Carvalho, and W.R. Clarindo (2014). Massal induction of *Carica papaya* L.'Golden'somatic embryos and somaclone screening by flow cytometry and cytogenetic analysis. Cytologia. 79(4): 475-484

Aikpokpodion, P.O. (2012) Assessment of genetic diversity in horticultural and morphological traits among papaya (*Carica papaya)* accessions in Nigeria. Fruits. 67 (3):173-187

Allan, P. (1981) Clonal 'Honey Gold'papaws-a horticultural and commercial success. Citrus Subtrop Fruit J. 575:19-23

Anandan, R., D. Sudhakar, P. Balasubramanian and A. Gutieirrez-Mora (2012) *In vitro* somatic embryogenesis from suspension cultures of *Carica papaya* L. Sci Hort. 136:43-49.

Anandan, R., S. Thirugnanakumar, D. Sudhakar, and P. Balasubramanian (2011) *In vitro* organogenesis and plantlet regeneration of (*Carica papaya* L.). J. Agri. Tech. 7 (5):1339-1348

Arora, I.K., and R.N. Singh (1978) Growth hormones and *in vitro* callus formation of papaya. Sci Hort. 8 (4):357-361.

Ascencio-Cabral A., H. Gutierrez-Pulido, B. Rodriguez-Garay, and A. Gutierrez-Mora (2008) Plant regeneration of *Carica papaya* L. through somatic embryogenesis in response to light quality, gelling agent and phloridzin. Sci Hort. 118 (2):155-160.

Azad, M.A., M.G. Rabbani, and L. Amin (2012) Plant Regeneration and Somatic Embryogenesis from Immature Embryos Derived through Interspecific Hybridization among Different Carica Species. Int J Mol Sci. 13 (12):17065-17076.

Azad, M.A.K., M.G. Rabbani, and L. Amin (2013) Effects of different culture media and flower bud size on haploid plant production through anther culture of three Carica species. J. Food, Agri. Environ. 11 (2):296-300

Azimi, M., C. Brien, S. Ashmore, and R. Drew (2005) Cryopreservation of papaya germplasm. Acta Hortic. 692:43-50

Badillo, V. (2000) Carica L. vs. Vasconcella St. Hil.(Caricaceae) con la rehabilitación de este último. Ernstia. 10 (2):74-79

Bhattacharya, J. and S.S. Khuspe (2001) *In vitro* and *in vivo* germination of papaya (*Carica papaya* L.) seeds. Sci Hort. 91 (1-2):39-49.

Bukhori, M.F.M. (2013) Improved protocol for high frequency plant regeneration through somatic embryogenesis in *Carica papaya*. Research in Biotechnology 4 (5): 9-19

Campostrini, E. and O.K. Yamanishi (2001) Estimation of papaya leaf area using the central vein length. Sci. Agric (Piracicaba, Braz.). 58 (1):39-42

Carvalho, F.A., and S.S. Renner (2012) A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. Mol Phylogenet Evol. 65 (1):46-53

Castillo, B., M.A.L. Smith, and U.L. Yadava (1998a) Liquid system scale up of *Carica papaya* L. somatic embryogenesis. J Horticult Sci Biotechnol. 73 (3):307-311

Castillo, B., M.A.L. Smith, and U.L. Yadava (1998b) Plant regeneration from encapsulated somatic embryos of *Carica papaya* L. Plant Cell Rep. 17 (3):172-176

Chan, L. and C. Teo (1993) *In vitro* production of multiple shoots in papaya as affected by plant tissue maturity and genotype. MARDI Res J. 21:105-111

Chan, L.K. and C.K. Teo (1994) Culture of Papaya Explant in Solid-Liquid Media Sequence as a Rapid Method for Producing Multiple Shoots. *Pertanika* J. Trop. Agric. Sci. 17 (2):103-106

Chan, L.K. and C.K.H. Teo (2000, November). Micropropagation of Eksotika, a malaysian papaya cultivar, and the field performance of the tissue culture derived clones. In International Symposium on Tropical and Subtropical Fruits 575 (pp. 99-105).

Chan, Y. K. (2009). Breeding papaya (*Carica papaya* L.). In Breeding plantation tree crops: Tropical species (pp. 121-159). Springer, New York, NY.

Chan, Y. (2002) Breeding papaya for resistance to ringspot virus disease in Malaysia. In: XXVI International Horticultural Congress: Citrus and Other Subtropical and Tropical Fruit Crops: Issues, Advances and 632. Agust, 2002. pp 57-64

Chan, Y.K., H.K. Lee, and I. Rusna (2007) Irradiation-induced variations in M2 populations of Eksotika papaya. J Trop Agric Food Sci. 35 (1): 49–57

Chan, Y.K and C. Mak (1993) Resistance of six papaya varieties and their hybrids to malformed top disease. MARDI Res J. 21:7-13

Chan, Y.K., H.K. Lee, and R. Isa (2015) Irradiation-induced variations in M2 populations of Eksotika papaya (Variasi iradiasi-aruhan dalam populasi M2 betik Eksotika). J Trop Agric Food Sci. 35:49-57

Chattopadhyay, S., S. Farkya, A.K. Srivastava, and V.S. Bisaria (2002) Bioprocess considerations for production of secondary metabolites by plant cell suspension cultures. Biotechnol Bioprocess Eng. 7 (3):138-149

Chen, M., C. Chen, D. Wang, and F. Chen (1991) Somatic embryogenesis and plant regeneration from immature embryos of *Carica papaya*×

Carica cauliflora cultured *in vitro*. Can J Bot. 69 (9):1913-1918

Chen, M., P. Wang, and E. Maeda (1987) Somatic embryogenesis and plant regeneration in *Carica papaya* L. tissue culture derived from root explants. Plant Cell Rep. 6 (5):348-351

Clarindo, W.R., C.R. de Carvalho, F.S. Araujo, I.S. de Abreu, and W.C. Otoni (2008) Recovering polyploid papaya *in vitro* regenerants as screened by flow cytometry. Plant Cell Tissue Organ Cult. 92 (2):207-214.

Drew, R.A. (1987) The Effects Of Medium Composition And Cultural Conditions On *In vitro* Root Initiation And Growth Of Papaya (*Carica Papaya* L). J Hortic Sci. 62 (4):551-556

Drew, R.A. (1988) Rapid Clonal Propagation Of Papaya *In vitro* From Mature Field-Grown Trees. HortScience. 23 (3):609-611

Drew, R.A. (1992) Improved Techniques For *In vitro* Propagation And Germplasm Storage Of Papaya. HortScience. 27 (10):1122-1124

Drew, R.A., J.A. McComb, and J.A. Considine (1993) 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 Cult. 33 (1):1-7

Drew, R.A., B.W. Simpson, and W.J. Osborne (1991) Degradation Of Exogenous Indole-3-Butyric Acid And Riboflavin And Their Influence On Rooting Response Of Papaya *In vitro*. Plant Cell Tissue and Organ Cult. 26 (1):29-34.

Farzana, A.R.F., P. Palkadapala, K. Meddegoda, P.K. Samarajeewa, and J.P. Eeswara (2008) Somatic embryogenesis in papaya (*Carica papaya* L. cv. Rathna). J.Natn.Sci.Foundation Sri Lanka. 36 (1):41-50

Fernando, J.A., M. Melo, M.K.M. Soares, and B. Appezzato-da-Gloria (2001) Anatomy of somatic embryogenesis in *Carica papaya* L. Braz Arch Biol Technol 44 (3):247-255.

Fisher, J.B. (1980) The vegetative and reproductive structure of papaya (*Carica papaya*). Lyonia : occasional papers of the Harold L. Lyon arboretum. 1 (4): 191-208

Fitch, M.M. (2005) 6.1 *Carica papaya* Papaya. General Editor: Gabrielle J Persley, The Doyle Foundation, Glasgow, Scotland:174

Fitch, M.M., and R.M. Manshardt (1990) Somatic embryogenesis and plant regeneration from immature zygotic embryos of papaya (*Carica papaya* L.). Plant Cell Rep. 9 (6):320-324

Fitch, M.M.M. (1993) High-Frequency Somatic Embryogenesis And Plant-Regeneration From Papaya Hypocotyl Callus. Plant Cell Tissue Organ Cult. 32 (2):205-212.

Gupta, S.D., and B. Jatothu (2013) Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. Plant Biotechnol Rep. 7 (3):211-220

Hazarika, B. (2006) Morpho-physiological disorders in *in vitro* culture of plants. Sci Hortic. 108 (2):105-120

Heringer, A.S., E.M. Vale, T. Barroso, C. Santa-Catarina, and V. Silveira (2013) Polyethylene glycol effects on somatic embryogenesis of papaya hybrid UENF/CALIMAN 01 seeds. Theor. Exp. Plant Physiol. 25 (2):116-124

Hossain, M., S.M. Rahman, R. Islam, and O.I. Joarder (1993) High-Efficiency Plant-Regeneration From Petiole Explants Of *Carica papaya* L Through Organogenesis. Plant Cell Rep 13 (2):99-102

Jordan, M. (1986) Somatic embryogenesis from cell suspension cultures in *Carica candamarcensis.* Plant Cell Tissue Organ Cult 7 (3):257-261

Kadleček, P., I. Tichá, D. Haisel,. V. Čapková, and C. Schäfer (2001) Importance of *in vitro* pretreatment for *ex vitro* acclimatization and growth. Plant Sci. 161 (4):695-701

Kaity, A., S. Ashmore., R.A. Drew, and M. Dulloo (2008) Assessment of genetic and epigenetic changes following cryopreservation in papaya. Plant Cell Rep. 27 (9):1529-1539

Kavitha, P.S., N. Chezhiyan, and N. Kumar (2010) Regeneration of Papaya Embryos under *In vitro* Condition. Acta Hortic. (851): 213-217

Khalid, N., W.W. Chee, F. Bokhari, T.S. Kiat, F.D. Idris, M. Mohamad, H. Ibrahim, S. Annuar, R.Y. Othman, N.A. Rahman (2010) Banana, gingers and papaya cell cultures for high throughput agriculture. Asia Pac. J. Mol. Biol. Biotechnol. 18(1), 73-74.

Koehler, A.D., C.R. Carvalho, I.S. Abreu, and W.R. Clarindo (2013) Somatic embryogenesis from leaf explants of hermaphrodite *Carica papaya:* a new approach for clonal propagation. Afr. *J.* Biotechnol. 12 (18):2386

Kozai, T., Y. Xiao, Q.T. Nguyen, F. Afreen, and S.M. Zobayed (2005) Photoautotrophic (sugar-free medium) micropropagation systems for large-scale commercialization. Propagation of ornamental plants 5 (1):23-34

Lai, C.C., S.D. Yeh, and J.S. Yang (2000) Enhancement of papaya axillary shoot proliferation *in vitro* by controlling the available ethylene. Bot. Bull. Acad. Sinica. 41 (3):203-212

Litz, R.E., and R.A.Conover (1977) Tissue culture propagation of papaya. In: Proceedings of the Florida State Horticultural Society. 90, 245-246

Litz, R.E., and R.A.Conover (1981) Effect Of Sex Type, Season, And Other Factors On *In vitro*

Establishment And Culture Of *Carica papaya* L Explants. J Am Soc Hort Sci. 106 (6):792-794

Liu, Z., P.H. Moore, H. Ma, C.M. Ackerman, M. Ragiba, Q. Yu, H.M. Pearl, M.S. Kim, J.W. Charlton, and J.I. Stiles (2004) A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature. 427 (6972):348-352

Magdalita, P.M., I.D. Godwin, R.A. Drew, and S.W. Adkins (1997) Effect of ethylene and culture environment on development of papaya nodal cultures. Plant Cell Tissue and Organ Cult. 49 (2):93-100

Magdalita P.M. and C.P. Mercado (2003) Determining the sex of papaya for improved production. Food and Fertilizer Technology Center, for the Asian and Pacific Region. University of Philippines, Los Baños (Filipinas) 6 p

Malabadi, R.B., S.V. Kumar, G.S. Mulgund, and K. Nataraja (2011) Induction of somatic embryogenesis in papaya (Carica papaya). Res J Biotechnol. 2 (5): 40-55

Manshardt, R., F. Hammerschlag, and R. Litz (1992) Papaya. Biotechnology of perennial fruit crops. CAB International. 489-511

Mendoza, E.M.T., A.C. Laurena, and J.R. Botella (2008) Recent advances in the development of transgenic papaya technology. Biotechnol Annu Rev. 14:423-462

Ming, R., S. Hou, Y. Feng, Q. Yu, A. Dionne-Laporte, J.H. Saw, P. Senin, W. Wang, B.V. Ly, and K.L. Lewis (2008) The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). Nature. 452 (7190):991-996

Mohammed, G. H. and W. E.Vidaver (1991). Plantlet morphology and the regulation of net water loss in tissue-cultured Douglas-fir. Physiol Plant. 83(1), 117-121.

Nakasone, H.Y. and R.E. Paull (1998) Tropical fruits. C.A.B Int. Wallingford, U.K. p 445.

Olson M.E. (2002) Intergeneric Relationships within the Caricaceae-Moringaceae Clade (Brassicales) and Potential Morphological Synapomorphies of the Clade and Its Families. Int J Plant Sci. 163 (1):51-65

Panjaitan, S., M. Aziz, A. Rashid, and N. Saleh (2007) *In-vitr*o plantlet regeneration from shoot tip of field-grown hermaphrodite papaya (*Carica papaya* L. cv. Eksotika). Int J Agric Biol. 9 (6):827-832

Paull, R.E., B. Irikura, P. Wu, H. Turano, N.J. Chen, A. Blas, J.K. Fellman, A.R. Gschwend, C.M. Wai, and Q. Yu (2008) Fruit development, ripening and quality related genes in the papaya genome. Trop Plant Biol. 1 (3-4):246-277

Pérez, L.P., Y.P. Montesinos, J.G. Olmedo, R.R. Sánchez, O.N. Montenegro, R.B. Rodriguez, O.H. Ribalta, R.C.R. Escriba, D. Daniels, and R. Gómez-Kosky (2015) Effects of different culture conditions (photoautotrophic, photomixotrophic) and the auxin indole-butyric acid on the *in vitro* acclimatization of papaya (*Carica papaya* L. var. Red Maradol) plants using zeolite as support. Afr. *J*. Biotechnol. 14 (35):2622-2635

Perveen, A., R.A. Alikhan, and R. Abid (2007) Maintenance of pollen germination capacity of C*arica papaya* L.(Caricaceae). Pak J Bot. 39 (5):1403-1406

Rajeevan, M.S. and R.M. Pandey (1986) Lateral Bud Culture Of Papaya (*Carica papaya* L) For Clonal Propagation. Plant Cell Tissue and Organ Cult. 6 (2):181-188

Rashid, K., R.Y. Othman, B.S.B.K.S Ali, Y.M. Yusof, and A. Nezhadahmadi (2014) The Application of Irap Markers in the Breeding of Papaya (*Carica* papaya L.). Indian J Sci Technol. 7 (11):1720

Razak, R.A., R. Sekeli, N.A. Shaharuddin, and J.O. Abdullah (2015) Using Mannose As A Positive Selection Of Transformed *Carica papaya* L. Var 'Eksotika'. J Teknol. 77 (31)

Renukdas, N., M.L. Mohan, S.S. Khuspe, and S.K. Rawal (2003) Influence of boron on somatic embryogenesis in papaya. Biol Plant. 47 (1):129-132

Reuveni, O., D.R. Shlesinger, and U. Lavi (1990) *In vitro* Clonal Propagation Of Dioecious *Carica papaya*. Plant Cell Tissue and Organ Cult. 20 (1):41-46.

Roy, P.K., S.K. Roy, and M.L. Hakim (2012) Propagation Of Papaya (*Carica papaya* L.) Cv. Shahi Through *In Vitro* Culture. Bangladesh J Bot. 41 (2):191-195

Schmildt, O., A.T. Netto, E.R. Schmildt, V.S. Carvalho, W.C. Otoni, and E. Campostrini (2015) Photosynthetic capacity, growth and water relations in 'Golden'papaya cultivated *in vitro* with modifications in light quality, sucrose concentration and ventilation. Theor. Exp. Plant Physiol. 27 (1):7-18

Sekeli, R, J.O. Abdullah, P. Namasivayam, P. Muda, and U.K. Abu Bakar (2012) Better rooting procedure to enhance survival rate of field grown Malaysian Eksotika papaya transformed with 1-Aminocyclopropane-1-carboxylic acid oxidase gene. ISRN Biotechnol. 2013(Article ID 958945) 10 pages

Sekeli, R., J.O. Abdullah, P. Namasivayam, P. Muda, U.K. Abu Bakar, W.C. Yeong, and V. Pillai (2014) RNA Interference of 1-Aminocyclopropane-1-carboxylic Acid Oxidase (ACO1 and ACO2) Genes Expression Prolongs

the Shelf Life of Eksotika (*Carica papaya* L.) Papaya Fruit. Molecules. 19 (6):8350-8362.

Storey, W.B. (1953) Genetics of the papaya. J Hered. 44 (2):70-78

Vanisree, M., C.Y. Lee, S.F. Lo, S.M. Nalawade, C.Y. Lin, and H.S. Tsay (2004) Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. Bot Bull Acad Sin. 45 (1):22

Vilasini, P., Y. Chan, M. Hassan, P. Lam, C. Ong, C. Tan, and A. Umi Kalsom (1998) Development of improved variety of Eksotika papaya using conventional and non-conventional methods. In: Proceedings of the 4th Asia-Pacific Conference on Agricultural Biotechnology. Fyshwick, Canberra, Australia: CPN Publications, July 1998. pp 359-361

Vilasini, P., Z. Latipah, and A. Salasiah (2000) Induction of somatic embryogenesis and plant regeneration from immature embryos of Eksotika papaya (*Carica papaya*). J Trop Agric Food Sci. 28:121-126

Wu, K.L., S.J. Zeng, Z.L. Chen, and J. Duan (2012) *In Vitro* Mass Propagation Of Hermaphroditic *Carica papaya* Cv. Meizhonghong. Pak J Bot. 44 (5):1669-1676

Xiao, Y., G. Niu, and T. Kozai (2011) Development and application of photoautotrophic micropropagation plant system. Plant Cell, Tissue and Organ Cult. 105 (2):149-158

Yu, T.A., S.D. Yeh, Y.H. Cheng, and J.S. Yang (2000) Efficient rooting for establishment of papaya plantlets by micropropagation. Plant Cell Tissue and Organ Cult. 61 (1):29-35.

Yu, T.A., S.D. Yeh, and J.S. Yang (2003) Comparison of the effects of kanamycin and geneticin on regeneration of papaya from root tissue. Plant Cell Tissue Organ Cult. 74 (2):169-178.

Zhong, J.J. (2001) Biochemical engineering of the production of plant-specific secondary metabolites by cell suspension cultures. In Plant cells (pp. 1-26). Springer, Berlin, Heidelberg.

Zobayed, S., F. Afreen, and T. Kozai (2001) Physiology of Eucalyptus plantlets grown photoautotrophically in a scaled-up vessel. *In Vitro* Cell Dev Biol Plant. 37 (6):807-813.