**ROLE OF BIOTECHNOLOGY IN PAPAY**A *(Carica papaya)***

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***Abstract***

Papaya (*Carica papaya* L.) is an important tropical and sub-tropical fruit crop. In India, it gives higher production of fruits per hectare and income next to banana **(Singh, 1990)**. Papaya is becoming more popular among farmers because of its high productivity with high net returns. It is a fast growing herbaceous plant and also used as a filler plant in orchards. It can be consumed in ripened form and in unripened form which is also a source of papain and this papain has various uses like this can be used in food industries and pharmaceutical. And in this chapter we are going to discuss about the various methods of unconventional and biotechnological approaches in papaya which include micropropagation, organogenesis, embryo rescue, anther culture, somatic embryogenesis, protoplast culture for improvement of papaya. Most important topic we are covering here is genetic engineering. Severe loss causing disease of papaya is papaya ring spot which is caused by papaya ring spot virus (PRSV) and for this virus control several transgenic plant had been developed which is based on coat protein (CP) and replicase mediated resistance. In Hawaii, papaya industry was saved by transgenic PRSV resistant Rainbow and SunUp papaya cultivars. For future suitable method to control PRSV will be post-transcriptional gene silencing (PTGS). Generally farmers use conventional methods rather than nonconventional or biotechnological method because they don’t want to take risk and they don’t have trust on these PSRV transgenic papaya. But in future our modern farmers will definitely understand this.

***Keywords:***Micropropagation, organogenesis, embryo rescue, anther culture, somatic embryogenesis,

**1. INTRODUCTION:**

Papaya *(Carica papaya)* belongs to the family *Caricaceae* in the order Brassicales and which is diploid (2n=18) in nature. Family Caricaceae contains six genera including *Carica, Vasconcella, Horovitzia, Jarilla, Cylicomorpha and Jacaratia*. Its native origin is from southern Mexico (encompassing all Central America) until Colombia and Venezuela. It is popularly known as Tree melon, Backyard fruit, Pawpaw or Papau, Kapaya, Lapaya, Papyas, Papye, Tapayas, Fan mu gua and Breakfast fruit. It is an important tropical and sub-tropical fruit crop.

**2. DISTRIBUTION OF PAPAYA:**

It is grown in various parts of the World including India, Brazil, Indonesia, Mexico, Nigeria and many more countries. In India major papaya producing states are Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Himachal Pradesh, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand and West Bengal. During, 2015-16, more than 12.89 million tons (mt) of fruit were produced over about 0.44 million ha (Horticulture statistics 2015). Out of which, India produced more than 49.7%.

**3. NATURE AND PROPERTIES:**

It is a fast growing herbaceous plant and also used as a filler plant in orchards. Each and every part of papaya has their own value like unripe fruits are consumed in the form of vegetables or salad while ripe fruits are consumed fresh. Papaya is also considered as nutraceutical fruit because of its multifaceted medicinal properties. Its pharmacological properties include Anti-inflammatory, Wound healing, Anti-fertility, Antihelmintic, Anticancer, Antifungal, Antibacterial, Anti-hypertensive, Anti-amoebic, Immunomodulatory and Anti-sickling activities. Phytochemically, the whole plant contains enzymes (Papain), lycopene, carotenoids, alkaloids, monoterpenoids, flavonoids, mineral and vitamins. Papain extracted from unripe papaya.

By using conventional breeding it is quite difficult to produce papaya which is stress tolerance and having qualitative traits. Major breeding objectives include dwarf variety, high yield, resistant to biotic and abiotic stresses, wider adaptability, big fruit size, small ovary and less number of seeds, all these qualities gets introduced by conventional method also but this method have several disadvantages such as embryo abortion, poor viability of seeds and sterility in progeny. All these limitations gets overcomed by biotechnological methods. Genetic engineering is an important part of biotechnological method n papaya crop, which is use for modifying one or more traits without altering the existing characters. Papaya cultivars with improved traits such as high yield and quality have been successfully developed through intensive breeding programs worldwide (Chan, 2002; Nakasone and Paull, 1998).

Major problem in papaya in disease infestation specially papaya ringspot which is caused by papaya ringspot virus (PRSV) which can cause losses upto 100% and post harvest losses of upto 30% - 40%. This problem can be cured by the development of resistant varieties. Dr. Dennis Gonsalves of Cornell University and Dr. Richard Manshardt of the University of Hawaii efforts to develop papaya ring spot virus resistant (PRSV – resistant) by using genetic engineering, they resulted in the commercialization of two transgenic cultivars- Sunup and Rainbow in 1998.

Another big problem with papaya fruit is shorter self life. Their appearance, flavor and texture get affected during harvesting and handling, storage, transport and marketing. Their adaptability and the self life increased by using modern biotechnological methods.

**4. General concepts of Biotechnology:**

The term “Biotechnology” was first coined by Karl Ereky in 1919. It is a technical application that uses living organisms or derivatives for the modification of products or processes for specific use. In agriculture it is a process of plant modification by removing some genetic information from an organism, manipulating it in the laboratory and transferring it into a plant to change certain of its characters.

**4.1 Role of biotechnology in papaya:**

* Helps in crop improvement such as dwarf varieties, resistant to biotic and abiotic stresses, improved color, flavor and quality, develop seedless variety.
* Improve their handling quality, self life, taste and nutrition.
* In reference of pharmaceuticals produce edible vaccines.
* Plants which produce fuels and other products.

**4.2 Technologies used in biotechnology are:**

Few common technologies for propagation include:

**4.2.1 MICROPROPAGATION**:

Earlier, papaya is usually grown from seeds or vegetative propagation and various improvements practiced in conventional methods. Micropropagation technique, which is a part of tissue culture has expanded their potential for commercial production. As compared to conventional methods the size of the tissue is very small in micropropagation. It is well known for producing millions of identical clones in shorter period of time, under aseptic conditions. Micropropagation enables rapid production of uniform, disease-free planting material of elite papaya cultivars (Fitch, 2005).

Limitations encountered during papaya micropropagation include the presence of endophytic

bacteria in cultures, poor response of mature explant tissues and loss of regeneration potential

following long-term culture (Drew, 1988; Drew and Smith, 1986; Litz and Conover, 1982;

Thomas et al., 2007). Presence of endophytic bacteria affects shoot proliferation and rooting.

Frequent indexing of cultures stock assists in the identification endophytes, which can be

eliminated to improve plant regeneration (Thomas et al., 2007). Other techniques including

alternating culture regimes in liquid and solid medium, and eliminating sucrose in the medium

following shoot proliferation to produce clean plants (Drew, 1988; Fitch et al., 2003).

Various culture media are available for micropropagation of papaya plant tissues such as White’s medium and Murasigue and SKoog (MS) medium but most common medium is MS medium. Hence, this is the most common method to ensure uniformity of papaya plants and improvement for their production.

**4.2.2 ORGANOGENESIS**:

In this plant organs such as shoot, flower and root system are developed from either an ex-plant or from the callus of culture. There are several works done on the organogenesis of papaya varieties. Firstly, Yie and Liaw observed adventitious shoots from callus in a culture medium containing MS basal media supplemented PGRs IAA and Kinetin (KIN) or with KIN only. It was suggested that transfer of papaya callus from a callus induction medium to a root induction medium supplemented with KIN and NAA where they produced roots from the callus. MS medium with NAA and BAP also showed formation of adventitious roots from midrib callus of papaya cotyledons.

**4.2.3. EMBRYO RESCUE**:

It plays an important role in modern plant breeding. This culturing of embryo (hybrid) in *in vitro* conditions is known as embryo rescue and is widely used for crop improvement. In 1996 article “An improved embryo-rescue protocol for a Carica Interspecific hybrid’’ was written by PM Magdalita, SW Adkins, ID Godwin and RA Drew An improved embryo-rescue protocol was developed for embryos (90 days old) of *Carica papaya L*. (Clone 2001), and subsequently was utilised for efficient production of interspecific hybrids of *C. papaya × C. cauliflora*Jacq. from 90- to 120-day-old embryos. Pre-incubation of *C. papaya* embryos for 7 days on a germination medium containing half-strength De Fossard nutrients supplemented with gibberellic acid (10 μM), 6-benzylamino-purine (0.25 μM), alpha-naphthalene-acetic acid (0.25 μM). sucrose (58 mM) and agar (8 g L-1) supported 100% germination. Subsequent transfer of germinated embryos to a nutrient medium that was identical, except that it was free of plant growth regulator, allowed good growth but induced shoot etiolation and callus production. Reducing the pre-incubation of *C. papaya* embryos on this medium to 5 days before transfer to the medium free of plant growth regulator produced similarly high germination (96%), but allowed for the production of good quality seedlings that were unetiolated and free of unwanted callus. For interspecific hybrids, a 5-day pre-incubation of the embryos on a liquid formulation was better than the solid formulation as it promoted better growth and vigour of the normally abortive interspecific hybrid embryos. Using the improved protocol, 1981 of 2100 (94%) interspecific hybrid embryos consisting of single and multiple forms were germinated. In all cases, the germinating multiple embryos underwent further embryogenesis that allowed for the production of 485 (25%) morphologically normal hybrid plants grown in soil in the glasshouse.

**4.2.4 ANTHER CULTURE:**

It means regeneration from the haploid microspore cells for attaining haploid and diploid plant production. Anther culture in papaya were 1st cultured during 1978. IN 1985, a work was done on anther culture which report that highest frequencies of callus induction were obtained when anthers at the uninucleate stage were cultured in the dark. More quantity of embryoids formed on the surface when anther derived embryoids were transferred to MS media with 3% sucrose without growth regulators.

**4.2.5 SOMATIC EMBRYOGENESIS**:

This has been developed for various reasons, ranging from interest in method for mass micropropagation (De Bruijne et al. 1974; Yie and Liaw 1977; Chen et al. 1987; Chen 1988a,b) to a requirement for recipient tissues for gene transfer technology (Fitch and Manshardt 1990; Fitch 1993). Successful somatic embryogenesis in papaya was reported in 1974 by De Bruijne et al. who induced somatic embryos from petiole sections cultured on Murashige and Skoog MS (1962) and White (1963) media in a multistep protocol. Various work were reported on papaya embryogenesis and plant regeneration by Yie and Liaw (1977), Mehdi and Hogan (1979), Chen et al. (1987), Chen (1988b), and Fitch and Manshardt (1990).

Embryonic calli developed from various plant parts such as in zygotic embryo, hypocotyl sections, cotyledons, roots and shoots by using various growing media, this is also reported by Fitch M.M.M. (1995) by using different media.

**4.2.6 PROTOPLAST CULTURE:**

Litz and Conover (1978b, 1979) and Litz (1984) proposed the use of protoplasts as a means of producing virus-free papaya plants, they were unsuccessful in attempts to regenerate plantlets from protoplast-derived calli. Jordan et al, practiced successful fusion of two sexually incompatible species viz. *Vasconcella cundinamarcensis* and *Carica papaya*. Whole papaya plants regenerated from fused protoplasts which were isolated from embryogenic suspension cultures of *Vasconcella cundinamarcensis* and *Carica papaya*. Chen et al. (1991) and Chen and Chen (1992) (summarized by Chen 1994) who successfully isolated protoplasts from highly regenerable suspension cultures from interspecific crosses of *C. papaya × C. cauliflora* zygotic embryos. Hence, protoplast culture can be use for papaya improvement.

**4.2.7 GENETIC ENGINEERING:**

This technique overcome the limitations of conventional breeding and is practiced by introducing foreign gene. In earlier attempts, crosses between wild relatives (Vasconcellea) and Carica papaya had been failed because the PRSV-P-resistant parent species *V.cauliflora* was genetically distant from *Carica papaya* and ultimately cause embryo abortionOECD (2005). Further *C. papaya* and *Vasconcellea quercifolia* hybridized and produced fertile resistant hybrids.

Most widely used selected marker in papaya transformation is the neomycin phosphotransferase II (npt II) that confers resistance to kanamycin antibiotic (Dhekney et al., 2007). Transgenic cells expressing nptII selectively grow on culture medium containing kanamycin, while inhibiting the growth of non-transformed cells.

Introduction of foreign gene is practiced by bombardment which is known as biolistic techniques and Agrobacterium-mediated transformation (Fitch et al., 1990; 1993).

**Particle bombardment protocols** consist of coating gold or tungsten microcarriers/particles with plasmid DNA containing the genes of interest along with a reporter and selectable marker gene. Then embryogenic cultures are bombarded by the processed microcarriers. After bombardment cultures are transferred to induction medium containing a selective agent for promoting growth and proliferation of transgenic cells. Embryogenic cultures which were transformed produce somatic embryos following transfer to hormone-free development medium. Transgenic plants which are regenerated, screened for transgene presence and copy number using PCR and Southern bolt hybridization (Fitch et al., 1993).

***Agrobacterium*-mediated transformation** involves co-cultivation of embryogenic cultures with disarmed Agrobacterium tumefaciens containing the vector with genes of interest. A frequently added phenolic compound-Acetosyringone, which is added to the bacterial suspension for enhancing the virulence of vir gene and improve transformation efficiency (Ying et al., 1999).

For inhibiting bacterial growth, co-cultivation followed for 24-72 hrs in darkness where embryogenic cultures are washed in liquid induction medium containing carbenicillin and cefotaxime antibiotics. growth. Cultures are then transferred to induction medium containing carbenicillin, cefotaxime and a selective agent (e.g. kanamycin). Transgenic plants are regenerated from embryogenic culture.

Techniques to improve transformation efficiency include wounding embryogenic cultures with carborundum or tungsten prior to co-cultivation (Cheng et al., 1996; Ying et al., 1999). In either distilled water or liquid medium, somatic embryos are wounded by vortexing with 600 mesh carborundum or tungsten M-15. Co-cultivation of embryogenic cultures with *Agrobacterium* and transferred to induction medium containing antibiotics for selection and proliferation of transgenic cells. Transfer of transgenic cells to development medium produce somatic embryos, which are germinated to obtain transgenic plants.

Problems encountered in *Agrobacterium-*mediated transformation – It’s difficult to control bacterial growth following co-cultivation, bad efficiency of transgene insertion and integration in embryogenic cultures and transgenic plant recovery. Techniques such as wounding with carborundum can produce not normal growth and bad transgenic recovery of the plant (Carlos-Hilario and Christopher, 2015). All these problems can be overcome by various genetic engineering methods like the use of well developed suspension cultures in thin layers, co-cultivation practiced with extremely low bacterial cell densities and time of co-cultivation decreases by 72 hrs to 24 hrs (Carlos-Hilario and Christopher, 2015).

**Genetic engineering for Papaya Ringspot Virus (PRSV):**

It’s a best remedy for Papaya Ringspot Virus (PRSV). This virus was 1st reported in Hawaii during 1940s and posed major threat to the papaya industry by 1992 (Gonsalves, 2004). Papaya Ringspot Virus majorly effects the production of papaya. This disease is transmitted by various species of aphids to papaya and cucurbits. Symptoms caused by this virus is mosaic and chlorotic spots on leaves, vain clearing, yellowing of leaves, water-soaked oily streaks on the petioles and on the upper part of trunk, ringspot on papaya fruits. Based on their infectivity, type P and type W are two subtypes. Type P is major problem for papaya.

The concept of pathogen-derived resistance was effectively exploited to develop PRSV. PRSV developed by transgenic plants which were produced by particle bombardment of papaya embryogenic cultures with the coat protein gene of a mutant mild PRSV strain (Fitch et al., 1992). Two transgenic papaya varieties, SunUp and Rainbow were derived from transgenic line 55-1(Manshardt, 1998). “SunUp” variety is homozygous for the coat protein (CP) gene and exhibited broad spectrum resistance against several isolates along with HA isolate while “Rainbow” variety is hemizygous for the coat protein gene and exhibited susceptibility to the other isolates but resistance to Hawaii isolate. SunUp and Rainbow plants at younger and older stages were checked with various parameters that affected the resistance of them, which were inoculated with PRSV isolates from Hawaii, Brazil, Jamaica, and Thailand. Hawaiian isolates shared nucleotide sequence homology of 96.7–99.8% to the coat protein transgene, and the other isolates shared sequence homology of 89.5–92.5%. Resistance is affected by amount of coat protein dosage, plant developmental stages and coat protein sequence homology of the challenged isolates. Younger and older Rainbow plant which is hemizygous were resistance to the homologous PRSV HA isolates (99.8% homology to CP transgene) while only older Rainbow plants were resistant to the other Hawaiian isolates (96.7% homology). However, all inoculated Rainbow plants were susceptible to PRSV isolates collected from Jamaica, Brazil, and Thailand. Except Thailand, SunUp was resistance to all PRSV isolates. 89.5% homology to the transgene shares by resistance to the Thailand isolate was observed only with older stage SunUp plants. Hence, there results suggested that transgenic papaya resistance was based on post-transcriptional gene silencing (PTGS) (Tennant et al., 2001).

In Asia, the US Virgin islands, South America and the Caribbean developed transgenic papaya lines with resistance to other strains of PRSV and affects to deregulate them are ongoing (Gonsalves, 2004; Zimmerman et al., 2007).

**Transgenic papaya for fungal disease:**

*Carica papaya L.* is susceptible to various fungal diseases such as root rot, stem rot and fruit rot which is caused by *Phytophthora palmivora*. Various efforts are made to develop fungal disease resistance with transgenic papaya. Mycelium growth is inhibited by resveratrol. Resistance nature of papaya from *Phytophthora palmivora* pathogen can be transformation with the grapevine stilbene synthase construct pVst1, containing the Vst1 gene and its pathogeninducible promoter. Plant lines were induced by resveratrol glycoside and RNA transcripts of stilbene synthase and transformed with the grapevine pVst1 construct shortly after pathogen inoculation. Hence, transformed papaya lines show more resistance to *Phytophthora palmivora* pathogen (Zhu et al., 2004b).

**Post harvest effect:**

Papaya has poor self-life and when stored at low temperature it cause chilling damage, which affect the long-distance transportation and marketing (Chen and Paull, 1986). During storage and transit it causes physical damage of fruit and high loss caused by storage rot (Paull et al., 1997). By the use of genetic engineering techniques we can improve or increase the self-life of papaya. Papaya is a climacteric fruit means it emit ethylene after harvesting also. Fruit ripening in papaya is accompanied by a sudden burst in ethylene biosynthesis, which triggers downstream genes responsible for fruit softening (Lelie`vre et al., 1997). Post-harvest life can be improve by the application of 1-methylcyclopropane (1-MCP) which is a ethylene inhibitors to mature fruit suppresses ethylene biosynthesis, improve firmness, and delays ripening during storage thereby improving post-harvest life (Manenoi et al., 2007). Transgenic strategies for improving post-harvest shelf life of papaya target either genes involved in ethylene biosynthesis during fruit ripening, or the receptors that perceive ethylene and trigger expression of fruit ripening- and softening-related genes (Stearns and Glick, 2003). Self-life of post harvest can also be improved by using RNA interference (RNAi) and blocking ethylene receptors are currently being investigated (Sekeli et al., 2014).

**4.2.8 PAPAPYA GENOMICS:**

*Carica papaya* is a good fruit model for genome analysis and this is because of their short juvenile period, profuse flowering and fruiting, and each fruit produces several seeds. In 2004 papaya genome project was initiated in Hawaii to determine the information on DNA sequence for improve overall productivity, their quality and various disease resistance. It contains male, female and hermaphrodite (complete) all the three types of flowers but for commercial use hermaphrodite is best because of their pyriform shape and female plants are important for papain production. Male plants do not produce fruits so that their importance is low. For determining their sex type farmers need to wait for 3-4 months because farmers are unable to identify them in early stage and because of this input cost increase (labour cost and others). For saving the cost it is important for farmers to know about the sex before transplanting. And for overcoming this problem various molecular markers were used for identification of sex, few of them are Random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), inter simple sequence repeat (ISSR) and single nucleotide polymorphism (SNP). Several male linked RAPD markers were developed such as OPY7 (900 bp), OPF (800 bp), OPY (369 bp) and this RAPD marker specific to male and hermaphrodite (Urasaki et al., 2002a, 2002b). SCAR markers which were converted by male-hermaphrodite specific RAPD markers and this conversion of RAPD to SCAR marker allow quick sex identification. Among various dioecious and gynodioecious genotypes SCAR marker was validated in W11. In papaya, RAPD and microsatellite markers linked to sex have been reported (Sondur et al. 1996). Parasnis et al. in 1999 utilized oligo-nucleotide (GATA)4 microsatellite probe.

In comparison of others, genetic mapping of papaya lagged behind because of low level of polymorphism among existing germplasm (Sharon et al. 1992; Stiles et al. 1993; Kim et al. 2002). To develop high-density genetic makeup of papaya Ma et al. (2004) constructed 54 F2 plants derived from cultivars ‘Kapoho’ and ‘SunUp’ with 1501 markers including 1498 amplified fragment length polymorphism (AFLP) markers, the PRSV cp marker, morphological sex type and flesh color of fruit. They mapped those markers into 12 linkage groups with a recombination frequency of 0.25. This study was revealed that severe suppression of recombination around the sex determination locus with a total of 225 markers co-segregating with sex types. Therefore, the high density genetic map was recommended for the cloning of specific genes of interest such as the sex determination gene and for the integration of genetic and physical maps of papaya.

**CONCLUSION:**

Papaya crop has various limitations from sowing to post harvesting and through conventional methods it is quit tough to overcome all those problems. But with the help of biotechnological methods or we can say modern methods which includes micropropagation, organogenesis, embryo rescue, anther culture, somatic embryogenesis, protoplast culture, genetic engineering and with the help of genomes we can overcome all those obstacles. One of the major threat in papaya is Papaya ring spot virus (PRSV) and in this chapter we already discussed about various methods to overcome this problem like by developing varieties which is resistant to PRSV {use coat protein(CP) / RP gene} and overcome fungal diseases by developing transgenic papaya. Also by the use of genetic engineering techniques we can improve or increase the self-life of papaya. Several molecular markers were used for identification of sex, few of them are Random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), inter simple sequence repeat (ISSR) and single nucleotide polymorphism (SNP). But the acceptance of these biotechnological methods is quit low because farmers do not want to take risk with their crop and they use conventional methods, so still they need more awareness regarding biotechnological methods.

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