ROLE OF BIOTECHNOLOGY IN PAPAYA *(Carica papaya)*

[*1] SHIVANI KUMARI, [*2] ARCHANA YADAV, [*3] AKHILESH KUSHWAHA, [*4] ATUL KUMAR SINGH

[*1] & [*2], M.Sc. Scholar Fruit Science, [*3] & [*4] , Ph.D Scholar Fruit Science,

Department of Horticulture,
Sam Higginbottom University of Agriculture, Technology and Sciences, Naini, Prayagraj.
Correspondence email: shivanikri.6250@gmail.com

Abstract

Papaya (*Carica papaya L.*) holds significant importance as a fruit crop in tropical and sub-tropical regions. In India, it gives earnings rivaling (higher yield per hectare) 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 within this chapter, our focus will be directed towards an exploration of diverse methodologies 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, attributed to the papaya ring spot virus (PRSV), is the subject under consideration 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:

The plant *Carica papaya* is a member of the Caricaceae family within 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. This fruit is commonly recognized by various names, including Tree melon, Backyard fruit, Pawpaw, Papau, Kapaya, Lapaya, Papyas, Papye, Tapayas, Fan mu gua, and Breakfast fruit. This holds significance as a crucial fruit crop in tropical and sub-tropical regions.

2. DISTRIBUTION OF PAPAYA:

Cultivation of this crop spans across different global regions, including India, Brazil, Indonesia, Mexico, Nigeria and many more countries. The majorly papaya-producing states in India encompass 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 additionally recognized as a nutraceutical fruit because of its diverse medicinal attributes. Its pharmacological properties include Anti-inflammatory, Wound healing, Anti-fertility, Antihelmintic, Anticancer, Antifungal, Antibacterial, Anti-hypertensive, Anti-amoebic, Immunomodulatory and Anti-sickling activities. From a phytochemical perspective, the entire plant comprises enzymes (such as Papain), lycopene, carotenoids, alkaloids, monoterpenoids, flavonoids, minerals, 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 approach presents numerous drawbacks, including issues like embryo abortion, suboptimal seed viability, and sterility in subsequent generations. All these limitations gets overcomed by biotechnological methods. Genetic engineering plays a crucial role in the biotechnological approach for enhancing the papaya crop, allowing for the modification of specific traits while preserving the original characteristics. Through rigorous breeding efforts on a global scale, enhanced papaya cultivars showcasing superior attributes such as heightened yield and exceptional quality have been effectively generated, as evidenced by successful initiatives outlined in studies by Chan (2002) and Nakasone and Paull (1998).

Major problem in papaya in disease infestation specially papaya ringspot which is caused by the papaya ringspot virus (PRSV) and has the potentiality to induce 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 from Cornell University and Dr. Richard Manshardt from the University of Hawaii efforts to develop papaya ring spot virus resistant (PRSV – resistant) by using genetic engineering, they leds to the introduction of two transgenic varieties, Sunup and Rainbow, into commercial markets 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. Fundamental principles of Biotechnology:

The term "Biotechnology" was initially 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. Within the realm of agriculture, it involves the alteration of plants by extracting specific genetic information from an organism, manipulating it within a laboratory setting, and subsequently introducing it into a plant to modify particular traits.

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 facilitates the swift generation of consistent, pathogen-free planting material for high-quality papaya cultivars (Fitch, 2005).

Challenges faced during papaya micropropagation involve the occurrence of endophytic bacteria within cultures, limited performance of mature explant tissues, and diminished regenerative capacity after extended periods of cultivation (Drew, 1988; Drew and Smith, 1986; Litz and Conover, 1982; Thomas et al., 2007). The existence of endophytic bacteria impacts both shoot proliferation and rooting processes. Regular indexing of culture stocks aids in pinpointing these endophytes, allowing for their removal to enhance plant regeneration (Thomas et al., 2007). Additional methods encompass altering culture routines between liquid and solid mediums, as well as eliminating sucrose from the medium after shoot proliferation, to generate pathogen-free 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 witnessed the emergence of adventitious shoots from callus on a culture medium comprising MS basal media supplemented with plant growth regulators (PGRs) IAA and Kinetin (KIN), or solely with KIN. It was suggested that moving papaya callus from a medium designed for callus initiation 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 development of spontaneous roots from the callus formed on the midrib of papaya cotyledons.

4.2.3. EMBRYO RESCUE:

It is very significant in modern plant breeding. The cultivation of hybrid embryos in controlled *in vitro* environments is referred to as embryo rescue, a technique extensively employed for enhancing crop traits. In 1996, the article "Enhanced Protocol for Embryo-Rescue in a Carica Interspecific Hybrid" was authored by PM Magdalita, SW Adkins, ID Godwin, and RA Drew. An improved procedure for embryo-rescue was devised, initially focusing on embryos (90 days old) of Carica papaya L. (Clone 2001). This protocol was subsequently employed to efficiently generate interspecific hybrids of *C. papaya* × *C. cauliflora Jacq.* from embryos ranging from 90 to 120 days old. The method involved pre-incubating *C. papaya* embryos for 7 days on a germination medium containing half-strength De Fossard nutrients along 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), resulting in 100% germination success. Transferring these germinated embryos to a nutrient medium identical to the first but devoid of plant growth regulators led to substantial growth, yet triggered shoot etiolation and callus formation. By reducing the pre-incubation period on this medium to 5 days before shifting to the growth regulator-free medium, a high germination rate (96%) was maintained. This modification resulted in the production of high-quality seedlings devoid of unwanted callus and unetiolated shoots. For interspecific hybrids, a 5-day pre-incubation on a liquid medium was superior to a solid medium, fostering better growth and vigor for typically nonviable interspecific hybrid embryos. Utilizing this enhanced protocol, 1981 out of 2100 (94%) interspecific hybrid embryos, encompassing both single and multiple forms, were successfully germinated. In all instances, the germinating

multiple embryos underwent further embryogenesis, leading to the generation of 485 (25%) morphologically normal hybrid plants, cultivated in soil within a glasshouse environment.

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 the most notable rates of callus formation were achieved by culturing anthers at the uninucleate stage in darkness. An increased quantity of embryoids developed on the surface when embryoids derived from anthers were transferred to MS media containing 3% sucrose, without the addition of growth regulators.

4.2.5 SOMATIC EMBRYOGENESIS:

This technique has been devised for a range of purposes, spanning from the desire to establish a method for large-scale micropropagation (De Bruijne et al. 1974; Yie and Liaw 1977; Chen et al. 1987; Chen 1988a,b) to the necessity of obtaining suitable host tissues for gene transfer applications (Fitch and Manshardt 1990; Fitch 1993). Successful somatic embryogenesis in papaya was reported in 1974 by De Bruijne et al. initiated somatic embryos from sections of petioles, which were cultured using a multistep protocol on Murashige and Skoog (MS) (1962) as well as White (1963) media. Numerous studies documented papaya embryogenesis and plant regeneration, including research conducted 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) as well as Litz (1984) suggested employing protoplasts to generate virus-free papaya plants, their efforts to cultivate plantlets from calli derived from protoplasts were ultimately unsuccessful. Jordan et al, practiced successful fusion of two sexually incompatible species viz. *Vasconcella cundinamarcensis* and *Carica papaya*. Complete papaya plants were regenerated through the fusion of protoplasts which were isolated from

embryogenic suspension cultures of *Vasconcella cundinamarcensis* and *Carica papaya*. Chen et al. (1991) and Chen and Chen (1992), as summarized by Chen (1994), effectively isolated protoplasts from vigorously regenerating suspension cultures derived from interspecific crosses involving zygotic embryos of *C. papaya* × *C. cauliflora*. 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 abortion OECD (2005). Further *C. papaya* and *Vasconcellea quercifolia* hybridized and produced fertile resistant hybrids.

The neomycin phosphotransferase II (npt II) gene, which imparts resistance to the antibiotic kanamycin, stands out as the preferred and extensively utilized selectable marker in papaya transformation, as highlighted by the research of Dhekney et al. in 2007. Cells that have undergone genetic modification to express nptII exhibit preferential growth on culture media containing kanamycin, effectively suppressing the growth of unaltered cells.

Foreign gene introduction is achieved through two main methods: biolistic techniques, also known as bombardment, and *Agrobacterium*-mediated transformation (Fitch et al., 1990; 1993).

Particle bombardment procedures involve enveloping microcarriers or particles made of gold or tungsten with plasmid DNA. These plasmids comprise the desired genes of interest in addition to a reporter gene and a selectable marker gene**.** Next, processed microcarriers are used to bombard embryogenic culturesSubsequent to the bombardment process, the cultures are relocated to an induction medium enriched with a selective agent to facilitate the growth and multiplication of transgenic cells. Embryogenic cultures that have undergone transformation yield somatic embryos upon transfer to a development medium devoid of hormones. The regenerated transgenic plants are then subjected to screening for the presence and copy number of the transgene using techniques like PCR and Southern blot hybridization (Fitch et al., 1993).

The process of ***Agrobacterium-mediated*** transformation entails co-cultivating embryogenic cultures with modified Agrobacterium tumefaciens strains harboring vectors carrying the desired genes. An often incorporated phenolic compound, acetosyringone, is introduced into the bacterial suspension to augment the virulence of the vir gene, thereby enhancing the efficiency of transformation (Ying et al., 1999).

To impede the proliferation of bacteria, a technique involving co-cultivation is employed, spanning a period of 24 to 72 hours under light-deprived conditions. During this process, embryogenic cultures undergo a thorough rinse in a liquid induction medium infused with the antibiotics carbenicillin and cefotaxime. Subsequently, the cultures are transposed into an induction medium that contains not only carbenicillin and cefotaxime but also a discriminating agent such as kanamycin. The aim is to encourage the growth of transgenic plants from these embryogenic cultures through a regenerative process.

Enhancing the efficiency of transformation can be achieved by employing methods such as causing controlled injuries to embryogenic cultures using substances like carborundum or tungsten before initiating co-cultivation, as documented in studies by Cheng et al. (1996) and Ying et al. (1999). In either distilled water or liquid medium, Somatic embryos experience injury through agitation via vortex mixing, utilizing either 600 mesh carborundum or tungsten M-15. Embryogenic cultures are co-cultivated with *Agrobacterium* and subsequently placed onto induction medium supplemented with antibiotics. This step serves to select and enhance the growth of transgenic cells. The transgenic cells are then transitioned to a development medium, where they give rise to somatic embryos. These embryos are nurtured into germination, leading to the successful cultivation of transgenic plants.

Challenges Encountered in *Agrobacterium*-Mediated Transformation– Controlling bacterial growth after co-cultivation poses challenges, as the efficiency of transgene insertion and integration into embryogenic cultures, as well as the recovery of transgenic plants, is suboptimal. Approaches like wounding with carborundum can lead to abnormal growth and hinder successful recovery of transgenic plants (Carlos-Hilario and Christopher, 2015). All these problems can be overcome by various genetic engineering methods like employing meticulously cultivated suspension cultures in thin layers, Co-cultivation is conducted using exceedingly low densities of

bacterial cells, and the duration of co-cultivation is reduced from 72 hours to 24 hours (Carlos-Hilario and Christopher, 2015).

Genetic engineering for Papaya Ringspot Virus (PRSV):

It's a best remedy for Papaya Ringspot Virus (PRSV). The virus was initially documented in Hawaii in the 1940s, and by 1992, it had become a significant menace to the papaya industry (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, moist or oily streaks appearing 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 notion of utilizing pathogen-derived resistance was efficiently harnessed to create PRSV resistance. Transgenic plants resistant to Papaya Ringspot Virus (PRSV) were created through the introduction of the coat protein gene from a modified, less severe strain of PRSV. This genetic modification was achieved using particle bombardment on papaya embryogenic cultures (Fitch et al., 1992). SunUp and Rainbow, two transgenic papaya cultivars, trace their origins back to the transgenic line 55-1 as established by Manshardt in 1998. The "SunUp" variety exhibits a homozygous trait for the coat protein (CP) gene and 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 papaya plants, both in early and mature growth stages, were subjected to a comprehensive assessment encompassing diverse parameters influencing their resistance. These plants were subsequently exposed to PRSV isolates from different regions including Hawaii, Brazil, Jamaica, and Thailand. Among these, Hawaiian isolates exhibited nucleotide sequence similarities ranging from 96.7% to 99.8% with the coat protein transgene, while the remaining isolates displayed sequence homologies between 89.5% and 92.5%. Resistance is influenced 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 Among the Rainbow plants, only those in a more advanced growth stage exhibited resistance to the additional Hawaiian isolates with a homology of 96.7%. Nevertheless, all the Rainbow plants

subjected to inoculation succumbed to PRSV isolates originating 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, the findings indicated that the resistance of transgenic papaya was attributed to post-transcriptional gene silencing (PTGS) (Tennant et al., 2001).

Transgenic papaya varieties resistant to different strains of PRSV have been cultivated in regions such as Asia, the US Virgin Islands, South America, and the Caribbean. Efforts to grant regulatory approval for these modified papaya lines are currently in progress (Gonsalves, 2004; Zimmerman et al., 2007).

Transgenic papaya for fungal disease:

Carica papaya L. is vulnerable to diverse fungal ailments like root rot, stem rot, and fruit rot, induced by *Phytophthora palmivora.* Various efforts are made to develop fungal disease resistance with transgenic papaya. Mycelium growth is inhibited by resveratrol. The inherent resistance of papaya against the *Phytophthora palmivora* pathogen can be enhanced through genetic transformation using the grapevine stilbene synthase construct pVst1. This construct harbors the Vst1 gene along with its promoter that is responsive to pathogen presence. Plant lines were induced by resveratrol glycoside and RNA transcripts from stilbene synthase were introduced into the plant, and the grapevine pVst1 construct was transformed shortly after the introduction of the pathogen. Consequently, the papaya lines that underwent transformation exhibited increased resistance to the *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. The process of papaya fruit ripening involves a rapid increase in the production of ethylene, which then activates subsequent genes responsible for the softening of the fruit (Lelie`vre et al., 1997). Enhancing the post-harvest duration of fruits can be achieved through the utilization of 1-methylcyclopropane

(1-MCP), an ethylene inhibitor. When applied to mature fruit, this compound effectively reduces ethylene production, leading to enhanced firmness and delayed ripening while in storage. This application of 1-MCP results in a considerable extension of the post-harvest lifespan of fruits (Manenoi et al., 2007). Transgenic approaches aimed at enhancing the post-harvest shelf life of papaya focus on either modifying genes associated with the production of ethylene during fruit ripening, or altering the receptors responsible for sensing ethylene. These modifications subsequently induce the activation of genes related to fruit ripening and softening (Stearns and Glick, 2003). The enhancement of post-harvest shelf life is currently under investigation through various methods, including RNA interference (RNAi) and the inhibition of ethylene receptors, as explored by Sekeli et al. in 2014.

4.2.8 PAPAPYA GENOMICS:

Carica papaya represents a good fruit exemplar for genomic analysis and this is because of their short duration of juvenility, profuse blossoming and yielding fruit, with multiple seeds produced by each individual fruit. In 2004 the initiation of the papaya genome project took place in Hawaii to ascertain the information regarding the 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 transforming RAPD markers into SCAR markers enables rapid sex identification. Among various dioecious and gynodioecious genotypes SCAR marker was validated in W11. In the case of papaya, markers such as RAPD and

microsatellites associated with sex determination have been documented (Sondur et al. 1996). Parasnis et al. (1999) employed an oligonucleotide (GATA)4 microsatellite probe.

In comparison to other species, the genetic mapping of papaya has lagged behind due to the limited level of polymorphism within the existing germplasm (Sharon et al. 1992; Stiles et al. 1993; Kim et al. 2002). To establish a high-density genetic profile of papaya, Ma et al. (2004) generated 54 F2 plants from 'Kapoho' and 'SunUp' cultivars, incorporating 1501 markers, including 1498 amplified fragment length polymorphism (AFLP) markers, the PRSV cp marker, morphological sex type, and fruit flesh color. These markers were mapped into 12 linkage groups with a recombination frequency of 0.25. This study unveiled a significant reduction in recombination around the sex determination locus, with a total of 225 markers co-segregating with sex types. As a result, the high-density genetic map was recommended for endeavors such as the cloning of specific genes of interest, including the sex determination gene, and for integrating the 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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