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Review

Advances in papaya biotechnology

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ABSTRACT

Papaya (*Carica papaya* L.) is an important tropical fruit crop. The fruit is consumed fresh and used in the pharmaceutical, rayon and food industries. Papaya improvement for stress tolerance and qualitative traits using conventional breeding has been difficult due to the narrow germplasm pool in the *Carica* genus and sexual incompatibility problems encountered during intergeneric hybridization with other genera in the Caricaceae family. Genetic engineering is an important tool in papaya improvement for modifying one or more traits in elite cultivars without altering existing characteristics. Advances in genetic engineering have been facilitated by concerted efforts for genome sequencing of papaya, development of papaya regeneration systems and efficient gene insertion techniques for transfer of desirable traits.

Papaya regeneration via organogenesis and somatic embryogenesis has been refined during the past 3 decades. Early efforts to optimize gene insertion protocols utilized a number of reporter and selectable marker genes, viral- and bacterial-derived regulatory sequences and functional genes for biotic and abiotic stress tolerance. Transgenic plants were routinely produced with several cultivars. One of the best success stories in the commercialization of a genetically modified fruit crop has involved the development of transgenic papaya ring spot virus (PRSV) resistant Rainbow and SunUp cultivars, which saved the Hawaiian papaya industry. Additionally, genetically modified papayas with traits for disease resistance and extended shelf life have been extensively screened in field tests.

The papaya genome sequence was published in 2008 and has opened new avenues for papaya improvement by precision breeding, which involves the use of regulatory and functional gene sequences from related genera of the Caricaceae family, and is a logical extension of conventional breeding and genetic transformation. The application of precision breeding technology for papaya can pave the way for the development of consumer and eco-friendly cultivars that would be developed in ways similar to conventional breeding while causing fewer GMO-related concerns.

Contents

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1. Introduction

Papaya, *Carica papaya* L. is an important tropical fruit crop. Ripe fruits are consumed fresh while unripened fruits can be used in salads or as a vegetable and as a source of papain. Papaya fruits are a rich source of vitamins and minerals, low in sodium, fat and calories, and lack starch (Hewajulige and Dhekney, 2016). Papain, a proteolytic enzyme obtained from immature fruits is used in the pharmaceuticals, leather, wool and rayon industries (Seelig, 1970). Papaya is grown on 0.43 million ha with an annual production of 11 million tons. A majority of the production occurs in Asia and the Americas (FAOSTAT, 2013).

Papaya ($2n=2x=18$) is a member of the Caricaceae family, which consists of herbaceous plants (Badillo, 1993). The crop is believed to have originated in Central America in regions ranging from Mexico to Panama. The Caricaceae was originally comprised of 31 species in the *Carica*, *Jacartia* and *Jarilla* genera from Central America and the African *Cylicomorpha* genus (Nakasone and Paull, 1998). Taxonomic revisions resulted in some species being transferred from *Carica* to the *Vasconcella* genus (Badillo et al., 2000). Consequently, the *Vasconcella* genus now consists of 21 species followed by *Jacartia* with 7 species, while papaya is now the sole species in the *Carica* genus (Badillo, 1993).

Major goals of papaya improvement programs include increased yield and productivity, resistance to biotic and abiotic stress factors and improved quality characteristics. 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). Minisatellite and microsatellite markers have been explored to accelerate papaya genetic improvement, analyze phenotypic variation for traits of interest, and understand genetic relationships for efficient management and conservation of genetic resources (Oliveria et al., 2010; 2015a, 2015b). Papaya improvement for stress tolerance (abiotic and biotic) via hybridization with species from other genera of the Caricaceae family has been marginally successful. Limitations for transfer of useful traits are attributed to several post-zygotic incongruities including embryo abortion, poor seed viability and sterility in progeny obtained following hybridization between two genera (Horovitz and Jimenez, 1967; Manshardt and Wenslaff, 1989).

The limitations encountered in papaya improvement via conventional breeding can be overcome by biotechnological approaches such as embryo rescue and genetic engineering. Genetic engineering of papaya allows incorporation of specific traits into elite cultivars without potentially altering the existing phenotype. The process involves transfer of specific DNA sequences in cell cultures and their subsequent integration into the host genome. The prerequisites for successful gene transfer include efficient cell culture systems for plant regeneration and gene insertion techniques (Birch, 1997). Recent advances in papaya genomics along with refinement of cell culture and gene insertion protocols make genetic engineering as an important tool for studying gene function and expression in papaya and the development of improved cultivars. This chapter will review the progress in papaya cell culture, genetic engineering and genomics, and the successful application of this technology for papaya improvement.

2. Regeneration systems

The concept of totipotency, which is the ability of single cells to grow into entire plants, forms the basis of plant regeneration (Hansen and Wright, 1999). Plant regeneration of papaya via somatic embryogenesis, organogenesis and micropropagation is well documented (Fitch, 2005). Somatic embryogenesis and organogenesis occur through dedifferentiation and redifferentiation of explant cells. These events depend on the development of meristems from mature differentiated cells or undifferentiated callus tissues (Ziv, 1999).

2.1. Embryogenic cell culture system

The papaya embryogenic culture system involves the production of somatic embryos from a wide array of explant material. Embryogenic cultures are produced on induction medium via an indirect embryogenic pathway that involves a callus phase or a direct pathway where embryos are produced without an intervening callus phase. Somatic embryo development and maturation is observed when cultures are transferred to medium devoid of growth regulators. Embryogenic cultures are used as target tissues for inserting desired genes of interest and developing cultivars with improved traits (Fitch, 2005).

Papaya embryogenic cultures are obtained from hypocotyl, axillary bud, stem, ovule, zygotic embryo and root explants (Anandan et al., 2012; Ascencio-Cabral et al., 2008; Chen et al., 1987; Fitch and Manshardt, 1990; Fitch, 1993; Jordan and Velozo, 1997; Litz and Conover, 1980; Abreu et al., 2014; Razali and Drew, 2014). In most cases, cultures develop via indirect embryogenesis. Highly reproducible protocols for obtaining embryogenic cultures were reported by Fitch and Manshardt (1990) and Fitch (1993). Hypocotyl explants obtained from germinated seedlings produce embryogenic cultures on induction medium containing ½ strength MS salts and vitamins with 9.0 μM 2,4-D, 400 mg L^{-1} glutamine, 60 g L^{-1} sucrose and 7 g L^{-1} TC agar (Fig. 1a). Repeated transfer of embryogenic cultures on induction medium results in the development and proliferation of proembryonic masses or PEMs (Fig. 1b; c). Embryogenic cultures can be maintained by repeated transfer of PEMs to induction medium. Alternatively, immature zygotic embryos excised from fruits obtained 90–113 days after pollination also produce embryogenic cultures on induction medium (Fig. 1d). Cultures appear 4 weeks after induction and the production of somatic embryos (SE) occurs exclusively from the embryo axis (Fig. 1e). Such cultures proliferate on induction medium (Fig. 1f) and can be maintained up to 12 weeks. Somatic embryogenesis appears to be genotype-dependent with some genotypes exhibiting greater production of somatic embryos (Fitch, 1993). Embryogenic cultures have also been induced from ovule explants cultured on White's medium modified with the addition of 60 g L^{-1} sucrose, 400 mg L^{-1} glutamine, and 20% (v/v) filter-sterilized coconut milk (Litz and Conover, 1982). Other studies have indicated successful production of embryogenic cultures using 2,4-D in the induction medium (Bukhori et al., 2013; Razali and Drew, 2014).

Papaya embryogenic cultures can be maintained by transfer to semi-solid or liquid induction medium at 3 weeks intervals (Fitch, 1993; Litz and Conover, 1983; Mahon et al., 1996). Embryogenic cultures in liquid induction medium proliferate by repetitive budding of PEMs. Cultures growing in liquid medium can be synchronized by sieving to retain the smallest fraction of PEM, which is subsequently transferred to fresh medium for further proliferation. Such PEM effectively produce somatic embryos when transferred to growth regulator-free medium. The proliferation rate of PEMs is higher in suspension cultures after culture synchrony is attained (Von Arnold et al., 2002). Suspension cultures exhibit greater proliferation potential and higher plant regeneration rates compared to solid medium grown cultures and may be better suited as targets for gene insertion and the production of transgenic plants (Castillo et al., 1998; Ying. et al., 1999; Lines et al., 2002; Carlos-Hilario and Christopher, 2015).