Chapter 21
Genetic Improvement of Papaya (*Carica papaya* L.)

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Abstract Arising from a relatively isolated center of origin, papaya has spread throughout all tropical and subtropical countries through human intervention. This global dispersal has coincided with continuous improvement of the cultivated plants through breeding programs often designed to improve the agronomic characters and to address biotic and abiotic stresses that affect papaya production. Papaya production is threatened by a myriad of problems including devastating pests and diseases as well as the inability for both farmers and researchers alike to differentiate among the three sex types, male, female and hermaphrodite at the seedling stage, among others. Many attempts have been made by researchers over the years to resolve the problems through conventional and biotechnological techniques. Conventional plant breeding has given rise to varieties that are resistant to diseases as well as high yielders of quality fruits. However, conventional techniques require 12–14 years to develop new papaya varieties. Besides, devastating viral diseases like papaya ringspot virus (PRSV) have proved almost impossible to control through conventional means. The innovative technologies and growing understanding to manipulate the papaya phenotype at the molecular level provide new opportunities for the improvement of papaya. Through gene transfer technology, it is possible to develop transgenic papaya with pest and disease resistance as well as improved nutritional quality. This chapter provides insight into conventional breeding of papaya, the role of tissue and

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protoplast culture as well as molecular techniques in papaya improvement such as genetic transformation, mutation breeding and marker assisted selection and breeding. In addition, the potential of parthenocarpy as well as polyploidy and somaclonal variation in papaya breeding are discussed.

Keywords Aluminum tolerance · Anther culture · *Carica papaya* L. · Carmine spider mite · Dwarf papaya · Genetic transformation · Mating systems

21.1 Introduction

21.1.1 Origin and Distribution

Papaya *(Carica papaya* L.) is a cosmopolitan tropical fruit tree, believed to have originated in the Southern Mexico/Central American region. It is well adapted to a wide range of environmental conditions, and readily cultivated throughout the tropical and subtropical regions of the world (Villegas 1997). Its global distribution is reflected by the numerous synonyms used to describe it, some of which include pawpaw, tree melon (English), mamão (Portuguese), melonenbaum (German), malakor (Thai) and apoyo in parts of East Africa.

Papaya is cultivated in the Americas, Asia, Africa and Oceania. The plant requires relatively little water for its establishment and needs only minimal levels of mineral nutrients for growth and development, making it suitable for cultivation under suboptimal nutrient conditions (Saran and Choudhary 2013). The plant is readily found under cultivation anywhere between 36°N and 36°S latitudes, but the greatest proportion of papaya production occurs between 26°N and 26°S (Nakasone and Paull 1998).

World production of papaya was estimated at over 12.8 million mt of fruit in 2016. India, Brazil, Indonesia, Nigeria and Mexico were ranked the five largest papaya producers, in that order, cumulatively accounting for 76% of the global production, with India alone contributing nearly 44% of the total (FAOSTAT 2017).

21.1.2 Taxonomy and Morphology

Papaya is a flowering plant, which belongs to the family Caricaceae. The family has an amphi-Atlantic distribution, with two species indigenous to tropical Africa and approximately 33 species endemic to Central and South America (Carvalho and Renner 2012). The family comprises six genera, namely *Cyclimorpha, Jacaratia, Jarilla, Vasconcellea, Horovitzia* and *Carica*. In the genus *Carica*, *Carica papaya* L. represents the cultivated papaya (Badillo 2000; Milind and Gurditta 2011). It is a member of the order Brassicales and shares a common ancestor with *Arabidopsis*

(Wikström et al. 2001). *Vasconcellea* spp. are the closest relatives to *Carica papaya* (Van Droogenbroeck et al. 2004) and its members include many species with edible fruits and have great importance for breeding and genetic studies, with a few cultivated varieties (Badillo 2000; Scheldeman and Van Damme 2003). Species of *Vasconcellea* which produce edible fruits include *V. cundinamarcensis* (chamburo) and *V. pubescens* (ababai) grown in the Americas and the fruits are normally cooked and flavored by adding sugar before eating. *Vasconcellea pentagona* (babaco), which is grown in western South America, New Zealand, South Africa, Spain and Italy, is commercially cultivated for its large parthenocarpic fruits. *Vasconcellea chrysopetala* also known as higacho or toronche, is found in Ecuador and New Zealand (Scheldeman and Van Damme 2003). Some *Vasconcellea* species such as *V. cauliflora* and *V. quercifolia* are resistant to papaya ringspot virus, which affects *Carica papaya* L.

21.1.3 Plant Description

Papaya is a herbaceous tree, with a soft, woody stem topped by a crown of leaves clustered at the top of the trunk. The plant is predominantly single-stemmed but will occasionally branch due to wounding of the terminal shoot or as the plant ages. The stem is hollow, light green to deep purple in color, with prominent leaf scars. The leaves are large, palmately-lobed or deeply incised, with entire margins borne on long, hollow petioles about 125 cm long, and open in sequence up the stem as the tree grows (Milind and Gurditta 2011). The papaya root system comprises two main components; a vertical tap root for absorbing water; and lateral fine feeder roots for the absorption of nutrients.

The papaya is polygamous, having, male, hermaphroditic or female plants, each of which bears flowers of unique morphology reflecting the plant's sexual diversity (Eustice et al. 2008). The male papaya's numerous flowers are borne on extended, branched peduncles, often greater than 30 cm in length, while the hermaphrodite tree bears several bisexual flowers, with an occasional male flower due to sex reversal. The female tree bears a few female flowers exclusively on short peduncles. Individual flowers are white, cream-colored, yellow, or purple-tinged, and are borne in inflorescences on the trunk in the leaf axils. Both male and female trees must be present to produce fruit, while hermaphrodite trees are self-pollinating.

The plant flowers 3–9 months after planting and its fruits mature 5–15 months later, depending on the cultivar and prevailing temperatures (Milind and Gurditta 2011; Paterson et al. 2008). Commercial varieties normally flower 5–6 months after transplanting and the fruits ripen 5–6 months after flowering. Papaya has a moderately small genome of 372 megabases (Mb) (Arumuganathan and Earle 1991) and diploid inheritance with nine pairs of chromosomes (Bennett and Leitch 2005; Wikström et al. 2001).

The plant bears fruits throughout the year and fruits are either borne singly or in clusters, in the axils of the stem. The fruits are attached to the trunk by a peduncle attached to the upper trunk, below the old leaves, and arranged in an acropetal manner

whereby younger fruits arise above the older fruit on the trunk. The fruits vary in the size, shape and quality depending on the sex of the fruit (Milind and Gurditta 2011; Yogiraj et al. 2014). The female plants produce medium to large round-shaped fruits with a relatively large seed cavity, while the hermaphrodites produce small to medium elongated fruits with smaller seed cavity. The color of the fruit ranges from green when unripe to yellow or red orange when ripe and the color of flesh ranges from yellow-orange or pinkish orange at maturity. The fruit is climacteric, with ripening being controlled in part by the synthesis of ethylene (Magdalita et al. 2002).

21.1.4 Utilization

Various parts of the papaya plant have numerous applications. The young leaves and shoots and flowers are often cooked and consumed as a vegetable (Oloyede 2005; Saran and Choudhary 2013). The ripe papaya fruit is eaten as a fresh fruit or desert. When consumed ripe, papaya fruits are rich in vitamins A, B and C, various minerals including potassium, magnesium and boron, and dietary fiber (Ming et al. 2008; Yogiraj et al. 2014). The actual chemical composition of papaya fruits is dependent on various factors, such as the variety, climate, cultural practices employed and location at which the plants are grown (Imungi and Wabule 1990). Studies indicate that one medium-sized papaya with an edible portion of approximately 350 g, exceeds the dietary reference intakes (DRI) of 3000 IU for vitamin A and 90 mg for vitamin C as recommended by the U.S. Food and Nutrition Board for adult minimum daily requirements (Farzana et al. 2008; Ming et al. 2008). The ripe fruit is also often processed into a range of food products which include canned tropical fruit cocktails, juices, wines, purees and jam. The fruit is also often processed blanched and dried for consumption as a sweet condiment. Papaya seeds are dried, ground and used as a spice due to their peppery flavor (Aravind et al. 2013).

The green fruit is rich in potassium, calcium and phosphorous and is peeled, deseeded and cooked as a vegetable or used in a variety of savory Asian dishes, including pickles and chutneys and for canning in sugar syrup (Manshardt 1992; Nakasone and Paull 1998). However, due to the toxic nature of its latex, unripe papaya fruit should never be eaten raw (Saran and Choudhary 2013). The latex of the immature green papaya fruit is used as a natural source of the proteolytic enzyme papain. The latex is tapped manually from green fruits and air or oven dried, before being refined for use in the food processing, pharmaceutical, cosmetic and manufacturing industries (Ming et al. 2008). Papain production, being a manual process, is predominantly carried out in developing nations which include Tanzania and India, due in part to the abundance of relatively cheap labor (Sankat and Maharaj 1997).

Papaya is reported to have numerous medicinal uses, some of which include: the use of the bark in treatment of jaundice, the dressing of wounds using the leaves of the plant, the use of the plant latex to cure diarrhea, the use of root infusions which act as

diuretics (dehydrants) and the consumption of seeds which act as a vermifuge (Boshra and Tajul 2013; Krishna et al. 2008). The latex from papaya is used to treat warts, cancers, tumors, corns and skin defects (Saran and Choudhary 2013). Oloyede (2005) reported that fruit and seed extracts possess pronounced bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella flexneri*.

21.1.5 Production Constraints

The papaya industry faces two main problems; diseases and unreliable methods of differentiating among the three sex types, male, female and hermaphrodite, at seedling stage. Papaya is mainly propagated sexually. Prior to flowering, the seedlings look alike irrespective of their sex. As such producers have to wait until flowering time to differentiate among the sex types. The general recommendation has been to plant 3 seedlings per hole and destroy the extra plants at the onset of flowering. Since only a few males are required for pollination (ratio of 1 male: 9 females), this recommendation is very uneconomical for producers in terms of both capital and time investment.

Molecular markers that can differentiate papaya sex types were developed by Lemos et al. (2002) and Urasaki et al. (2002a, b), but these procedures have not been commercialized. Their commercialization will ensure that producers purchase only the desired sex of seedlings. However, molecular markers are currently very expensive and it might be impractical to apply such a procedure in commercial production right away, especially in resource-poor developing countries.

Furthermore, the widespread nature of papaya ring spot potyvirus (PRSV) in all major papaya-producing areas of the world (Manhsardt 1992) has not spared the Kenyan papaya industry. As a result, papaya is being wiped out at a fast rate, and the devastation is already becoming obvious in both research institutions and farmer fields. Effective control has remained elusive since resistant cultivars are not available and chemical control of insect vectors using insecticides is impractical since PRSV is non-persistently transmitted. Viral diseases, particularly the papaya ringspot disease (PRSD) which causes yield losses of up to 30–40%, has caused enormous devastation of papaya fields in various countries worldwide resulting in decreased fruit production.

Other challenges facing papaya production include bacterial dieback (*Erwinia papaya*), fruit brown blotch (*Colletotrichum gloeosporioides*), fruit fly (*Bactrocera papaya*) and oriental fruit scale (*Aonidiella orientalis*) among others. Several attempts have been made to resolve these challenges through conventional and biotechnological crop improvement strategies as outlined below.

21.2 Conventional Breeding

21.2.1 Flower Types

The somatic chromosome number in the dicotyledonous genus *Carica*, is 2n = 18. Most *Carica* species are dioecious, except for *Carica papaya* L. (commercially-produced papaya). *Carica papaya* L. is characterized by various flower types and polygamous sexual types, pistillate (female), staminate (male) and hermaphrodite. Staminate trees produce long pendulous male inflorescences bearing 10 stamens in each flower, pistillate trees bear 1 or 2 flowers at each leaf axil, with a round to oblong ovary, while hermaphrodite trees normally bear one to several bisexual flowers characterized by an oblong ovary and usually 10 stamens. Based on the sex segregation ratios which resulted from cross- and self-pollination studies of the three basic sex forms. Storey (1938, 1953) and Hofmeyr (1938) symbolized sex genes of the three sex types respectively as M1 m (male), mm (female), and M2 m (hermaphrodite), and hypothesized that dominant homozygotes (M1M1, M1M2 and M2M2) were lethal. This theory was confirmed through in vitro culture of papaya anthers which generated only female plants (Rimberia et al. 2006). The absence of males among the shoot samples was presumably related to the lethality of dominant homozygotes, as proposed by Storey (1938, 1953) and Hofmeyr (1938). Pollen grains with M1 may have been impotent for embryo differentiation or differentiated embryos may have died at an early stage of development (Rimberia et al. 2006).

21.2.2 Sex Determination

Recent developments in molecular-marker techniques have made it possible for papaya sex types to be accurately differentiated at an early developmental stage.

Sex-linked DNA markers have been developed in different parts of the world through DNA analysis using polymerase chain reaction (PCR) that identified papaya sex type at an early developmental stage. These include randomly amplified polymorphic DNA (RAPD) markers (Chaves-Bedoya and Nunez 2007; Lemos et al. 2002; Parasnis et al. 2000; Reddy et al. 2012; Urasaki et al. 2002a); sequence characterized amplified region (SCAR) markers, converted from RAPD (Chaves-Bedoya and Nunez 2007; Deputy et al. 2002; Parasnis et al. 2000; Urasaki et al. 2002a, b) and simple sequence repeat (SSR) markers (Costa et al. 2011; Parasnis et al. 1999).

Sex type of papaya (*Carica papaya*) is determined by the pair of sex chromosomes XX, XY and XY^h for female, male and hermaphrodite, respectively, in which there is a non-recombining genomic region in the Y and Y^h chromosomes. This region is presumed to be involved in determination of males and hermaphrodites; it is designated as the male-specific region in the Y chromosome (MSY) and the hermaphrodite-specific region in the Y chromosome (HSY) (Ueno et al. 2015). They further reported

that the study of *short vegetative phase (SVP)-like* transcripts revealed that the MSY allele encoded an intact protein, while the HSY allele encoded a truncated protein.

Tsai et al. (2016) developed a rapid and efficient method for detecting the male-hermaphrodite-specific marker to examine papaya sex type based on the loop-mediated isothermal amplification (LAMP) assays without prior DNA purification. The molecular-marker technology is accurate, but expensive and out-of-reach for ordinary farmers. Recently, Abreu et al. (2015) developed a probe for fluorescence in situ hybridization (FISH) protocol for differentiating between hermaphrodite and female papaya, which gave fluorescent signals in hermaphrodite nuclei isolated from leaves, but no detectable intensity fluorescence signal in female nuclei. They further confirmed that this protocol is superior to the molecular-marker technology in that it has potential for commercialization and automation.

Development of physiological and biochemical markers has also shown promise. Soni et al. (2017) reported that the hermaphrodite plants had higher leaf chlorophyll content than their male counterparts. They further reported significantly higher total phenols and stomatal conductance in the leaves of the female plants, followed by the hermaphrodite plants, at the seedling stage. Further research needs to be done on physiological and biochemical sex identification markers.

21.2.3 Genetic Diversity

Genetic diversity is the basis for crop improvement and maintenance. Papaya germplasm in different parts of the world has reportedly been characterized using morphological, isozyme, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and PCR-RFLP markers (Asudi et al. 2013; Calderón et al. 2016). The simple sequence repeat (SSR) markers have been used successfully for determining the genetic relationship between *Carica papaya* L. cultivars (Ocampo et al. 2006). Most studies showed limited genetic diversity in the common papaya. Asudi et al. (2010, 2013), using both morphological and SSR markers reported limited genetic diversity in Kenyan papaya, with accessions from the Coast and Rift Valley presenting the highest diversity compared to accessions from other parts of the country. Elsewhere, Saran et al. (2015) reported a high degree of variation among Indian papaya germplasm using morphological and two different types of molecular markers (RAPD) and inter-simple sequence repeats (ISSR). They further concluded that both morphological and molecular markers were useful for inferring genetic diversity and relatedness in papaya.

21.2.4 Conservation of Germplasm

Conservation of papaya germplasm is essential for future breeding programs. Papaya bears recalcitrant seeds which have been reported to lose viability below moisture content of 8–10% (Ellis et al. 1991). Ashmore et al. (2011) investigated seed storage behavior from liquid nitrogen to 15 °C/15% RH and optimized treatments to break dormancy and to ensure germination after seed storage. Thus, conditions for ex situ conservation of papaya through cryopreservation, including conditions for maximum regrowth and recovery of plantlets following cryopreservation have already been established (Ashmore et al. 2011; Dhekney 2004).

21.2.5 Mating Systems and Commercial Seed Production

Dioecious papaya varieties are forced cross-pollinators because of physical separation of the androecium and gynoecium. It was previously assumed that dioecious papaya is wind pollinated due to the long pendulous male inflorescence which readily sheds pollen into the breeze. However, it was later shown that very little papaya pollen was airborne. Many insects have been credited with pollinating papaya including honeybees, skipper butterflies and hawkmoth (*Hyles* sp.) (Chan 2009). Papaya pollen is not a favorite for pollinators, so it is assumed that the native vegetation and cultivated crops attract these pollinators and increase fruit production in papaya (Chan 2009).

In gynodioecious populations, the stamens are packed inside the corolla tube and seldom protrude prominently out of the flower. Many gynodioecious varieties such as Sunrise Solo, Kapoho Solo and Eksotika are self-pollinated and are, therefore, pure lines (Chan 2009). The hermaphrodite flowers are mostly cleistogamous; anthers dehisce and release the pollen to effect self-pollination prior to anthesis of the flower (Rodriquez-Pastor et al. 1990).

Papaya pollen production and viability depends on variety and season. For instance, between the two inbred parental lines 19 and 20 of F1 hybrid Eksotika II, line 20 consistently produced more pollen. Additionally, pollen production decreased during winter and early spring because of the degeneration of pollen mother cells. Under ideal storage conditions, pollen remains viable for 5–6 years. Stigma receptivity remains high throughout the year and both female and hermaphrodite flowers pollinated with viable pollen successfully set fruit even in winter (Chan 2009).

Hermaphroditic cultivars are commercially cultivated in the tropics, while female cultivars are predominantly used in the subtropical regions such as South Africa, Australia and Okinawa, Japan. This difference in cultural adaptation between both types of papaya may be due to sex reversal. Hermaphrodite and male papaya plants seasonally reverse their flower sex by stamen carpellody and female sterility, resulting in poor quality and low yield of fruits. On the other hand, the female is stable with respect to sex expression throughout the year (Rimberia and Adaniya 2010).

Commercial seed production in gynodioecious varieties such as Sunrise Solo and Eksotika, is achieved through selfing hermaphrodite flowers or hybridization of hermaphrodite flowers with hermaphrodite pollen. Seed derived from these cross-combinations will have twice the number of hermaphrodites compared with females. For dioecious varieties, the preferred combination for seed production is mm × Mm, namely, to use pollen from male flowers for crossing female flowers. A ratio of 1:1 male to female will be obtained. Crosses between females and hermaphrodites may be used for hybrid seed production because it obviates emasculation when female flowers are used as the maternal parent (Chan 2009).

21.2.6 Parthenocarpy in Papaya Improvement

Female papaya plants are usually inter-cropped with males to allow pollination and increase fruit yield. Intercropping consumes extra space in small enterprises, or where papaya is produced within a windproof screen or glass houses for instance in Japan to protect against diseases like papaya leaf distortion, papaya ringspot virus (PRSV), papaya mosaic virus diseases and damage by frequent typhoons (Ogata et al. 2016). An alternative to pollination might be found in promoting parthenocarpic fruit production.

Nakasone and Paull (1998) and Ray (2002) reported the occurrence of natural parthenocarpy in female papaya. Rodriquez-Pastor et al. (1990) reported variation in parthenocarpic ability among female cultivars. More recently, Rimberia and Adaniya (2010) and Rimberia et al. (2006b) reported that both female and hermaphrodite plants had parthenocarpic ability and that this ability varied among cultivars. They further demonstrated that female plants had significantly higher parthenocarpic ability than their hermaphrodite counterparts (Fig. 21.1). This intra-specific variation of this trait suggests a possibility of improving papaya genotypes with high parthenocarpic ability. Rimberia et al. (2007) reported much variation in parthenocarpic ability among the anther-culture derived strains. The dwarf strains with high fruit yield showed high parthenocarpic ability, but the strains with low fruit yield had low parthenocarpic ability. The phenotypic variation in dwarf nature and parthenocarpic ability among the microspore-derived plants indicate that the anther culture technique has potential to contribute to the systematic breeding of papaya.

21.2.7 Varietal Development

All breeding methods useful for the improvement of self-pollinating species are applicable to the breeding of hermaphrodite papaya, while population improvement methods are useful in both females and hermaphrodites (Manshardt 1992). True-bred lines of gynodioecious varieties such as Kapoho, Sunrise Solo, Waimanalo, Kamiya and Eksotika, among others, were established through inbreeding by means of pedi-

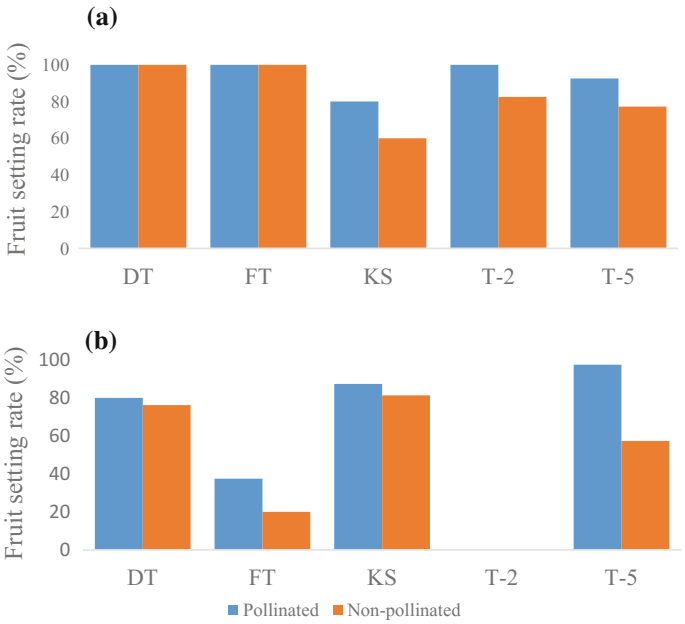

Fig. 21.1 Fruit setting rates of pollinated and non-pollinated female (**a**) and hermaphrodite (**b**) cultivars. DT: Dantesu, FT: Fruit tower, KS: Kansen, T-2: Taino-2, and T-5: Taino-5. *Source* Rimberia and Adaniya (2010), Rimberia et al. (2006b)

gree or backcross breeding methods (Chan 2009; Manshardt 1992). Self-pollination (inbreeding) for sustaining a variety is disadvantaged by inbreeding depression. Louw (2016) reported serious inbreeding depression resulting from continuous self-pollination, characterized by loss of productivity and poor or no seed germination.

Development of new varieties through inbreeding following selection has shown significant promise (Dinesh 2010). Four varieties, Pusa Delicious, Pusa Majesty (gynodioecious), Pusa Giant and Pusa Dwarf (dioecious) were developed through continuous sibmating and selection by conventional breeding in India (Singh et al. 2010), between 1966 and 1982. Commercial dioecious varieties like Hortus Gold, Honey Gold, Sunnybank, Hybrid No. 5, Cariflora, Co1 and Co2 were also developed through conventional plant breeding (Chan 2009).

Recently, Rimberia et al. (unpublished data) evaluated over 100 papaya accessions collected from within Kenya (both local and imported varieties), crossbred (controlled hand pollinated) those with potential for dwarfness, high yields and tolerance to viral diseases (Fig. 21.2), from 2008 to 2014. Four dioecious lines were successfully selected and are in the process of registration by the Kenya Plant Health Inspectorate Services (KEPHIS). Preliminary evaluation of the new Kenyan papaya varieties developed at Jomo Kenyatta University of Agriculture and Technology (JKUAT) indicate that they start flowering at a height of 58–79 cm, yield 13–20 kg of fruits per tree in the first season and have a mean Brix range of 11–12.

Fig. 21.2 Mature female JKUAT papaya varieties that are in the process of registration

21.3 In Vitro Applications

21.3.1 Tissue Culture

Papaya is commercially propagated by seed. Male, female and hermaphrodite papaya seedlings are indistinguishable before flowering. Farmers and researchers wait for about 6 months to differentiate among the sex types. Moreover, the papaya varieties cannot be maintained through sexual propagation due to the open pollination nature of the dioecious varieties and inbreeding depression among the hermaphrodite varieties (Louw 2016). Clonal propagation methods like tissues culture can overcome some of these difficulties in papaya cultivation and improvement. Additionally, tissue culture is useful for production of disease free planting materials.

The development of an efficient in vitro regeneration system for papaya represents remarkable progress for mass propagation and maintenance of uniform plants for both commercial and research purposes. The success of this technique depends on various factors such as (i) time of year, (ii) nature of the primary explant, (iii) the genotype and (iv) hormone component of the growth medium. Spring and early summer or tropical conditions offer the best natural conditions for rapid growth and development. Explants from actively growing papaya plants perform best under in vitro culture. Plant regeneration through tissue culture can be accomplished through shoot development from meristems, somatic embryogenesis and organogenesis.

21.3.2 Shoot Tip Meristems

The most reliable method of micropropagation in papaya is by shoot tip or axillary bud culture (Teixeira da Silva et al. 2007). Plants established from shoot tips have been shown to preserve the integrity of the parental genotype (Drew 1988).

Table 21.1 Axillary shoots induced from shoot tip explants of three papaya breeding lines cultured on MS media supplemented with different concentrations of BAP and NAA

BAP + NAA (mg/l)	Mean number of shoots formed per shoot tip		
	Line 1	Line 2	Line 3
0.0 + 0.0	0.8 ± 0.16^{h}	1.0 ± 0.44^{g}	0.6 ± 0.21^{h}
0.1 + 0.05	11.8 ± 0.91^{c}	8.8 ± 0.30^{c}	16.5 ± 1.54^{b}
0.5 + 0.05	13.8 ± 0.70^{b}	13.6 ± 0.95^{b}	16.8 ± 0.94^{b}
1.0 + 0.05	8.5 ± 0.22^{ef}	8.8 ± 0.47^{c}	6.8 ± 0.60^{efg}
2.0 + 0.05	6.5 ± 0.50^{fg}	7.8 ± 0.37^{cd}	6.5 ± 0.88^{efg}
0.1 + 0.1	11.0 ± 0.51^{cd}	12.3 ± 0.84^{b}	14.3 ± 0.91^{c}
0.5 + 0.1	24.3 ± 0.95^{a}	25.8 ± 2.08^{a}	19.3 ± 0.98^{a}
1.0 + 0.1	8.1 ± 0.47^{ef}	9.0 ± 0.25^{c}	10.6 ± 0.33^{d}
2.0 + 0.1	5.0 ± 0.36^{g}	7.6 ± 0.33^{cd}	5.8 ± 0.60^{efg}

Mean values ± SE within a column followed by the same letter are not significantly different by SNK ($P \leq 0.05$) within 12 weeks of subculture. *Source* Mumo et al. (2013)

In vitro regeneration of papaya is very much influenced by the genotype (Mishra et al. 2007). Reuveni et al. (1990) reported different rates of multiplication among 3 clones in the same media composition. Similarly, Mumo et al. (2013) demonstrated differences among 3 breeding lines in both rates of regeneration (Table 21.1) and elongation (Table 21.2) in the same media. Researchers from different parts of the world (Gatambia et al. 2016; Mumo et al. 2013; Panjaitan et al. 2007; Teixeira da Silva et al. 2007) established papaya shoots in vitro from shoot tip meristems of different genotypes of papaya, while Anandan et al. (2011) induced shoots from epicotyl segments.

Different phytohormones have been used successfully for shoot establishment, proliferation and elongation. Litz and Conover (1978) used solidified Murashige and Skoog (1962) (MS) basal medium with 50 μM kinetin and 10 μM naphthaleneacetic acid (NAA) for establishment, then subcultured into MS with 2 μM benzylaminopurine (BA) and 0.5 μM NAA for proliferation. All the media were solidified with 8 g/l Difco Bacto agar. Anandan et al. (2011) induced shoots on MS basal medium supplemented with 2.5 μM thidiazuron (TDZ), multiplied them in MS with 5 μM BAP and 0.05 μM NAA and elongated in 1.5 μM gibberellic acid (GA_3). All the media were fortified with 30 g-l sucrose and gelled with 0.7% agar. Caple and Cheah (2016), while using a Hawaiian variety Rainbow achieved initiation and multiplication of shoots in MS basal medium with vitamins, 3 mg/l benzyladenine purine (BAP) and elongation in 1.5X MS basal medium, 0.25 mg/l BAP, 0.3 mg/l GA3, maintaining the same concentration of sucrose (4% sucrose) and gelling agent (2.8 g/l Phytagel®). Mumo et al. (2013) used solidified MS with 0.5 mg/l 6-Benzyl Amino purine (BAP) and 0.1 mg/l NAA for both establishment and multiplication of 3 Kenyan papaya breeding lines through 3 weekly subcultures, followed by elongation in MS with

Table 21.2 Shoot length (cm) of shoot tip explants of three papaya breeding lines cultured on MS media supplemented with different concentrations of BAP and NAA

BAP + NAA (mg/l)	Mean shoot length (cm)		
	Line 1	Line 2	Line 3
0.0 + 0.0	1.31 ± 0.017^{g}	1.38 ± 0.031^{g}	1.58 ± 0.033^{f}
0.1 + 0.05	3.25 ± 0.085^{a}	3.30 ± 0.082^{a}	3.28 ± 0.070^{a}
0.5 + 0.05	2.63 ± 0.042^{b}	3.03 ± 0.105^{b}	3.01 ± 0.087^{b}
1.0 + 0.05	1.80 ± 0.037^{de}	2.05 ± 0.043^{d}	1.73 ± 0.042^{e}
2.0 + 0.05	1.76 ± 0.049^{e}	1.68 ± 0.031^{ef}	1.60 ± 0.037^{ef}
0.1 + 0.1	2.71 ± 0.048^{b}	2.78 ± 0.079^{c}	2.16 ± 0.033^{c}
0.5 + 0.1	2.26 ± 0.042^{c}	2.11 ± 0.031^{d}	2.13 ± 0.056^{c}
1.0 + 0.1	1.70 ± 0.026^{ef}	2.10 ± 0.058^{d}	2.13 ± 0.056^{c}
2.0 + 0.1	1.43 ± 0.033^{g}	1.83 ± 0.033^{e}	1.96 ± 0.071^{d}

Mean values ± SE within a column followed by the same letter are not significantly different by SNK ($P \leq 0.05$) within 12 weeks of subculture. *Source* Mumo et al. (2013)

0.1 mg/l BAP and 0.05 mg/l NAA. Both initiation/multiplication and elongation media had 30 g/l sucrose and solidified with 2.5 g/l gelrite.

Setargie et al. (2015) successfully used MS medium supplemented with 1 mg/l BAP and 0.5 mg/l NAA, solidified with 8 g/l agar for establishment, multiplication and elongation of hermaphroditic papaya cv. Maradol in Ethiopia. More recently, Gatambia et al. (2016) induced shoots from 3 local Kenyan papaya in MS basal media with 0.15 mg/l 2-Chloro-4-Pyridy-N Phenyl-urea (CPPU) with 30 g/l sucrose in a semi-solid/liquid double layer arrangement, solidified with 2.5 g/l gelrite mounted on an orbital shaker at a speed of 60 revolutions per minute (rpm). Multiplication and elongation of the shoots through bi-weekly sub culturing in the same media was achieved. CPPU, a synthetic cytokinin, has proved effective for shoot induction, multiplication and elongation. Cultures in liquid media on a shaker encourage close contact between the explant tissues and the culture medium, which facilitates the uptake of nutrients and phytohormones leading to better growth (Mehrotra et al. 2007). Additionally, continuous shaking improves aeration and eliminates apical dominance thus hastening the induction and proliferation of numerous axillary buds (Mehrotra et al. 2007). The semi-solid/liquid double layer technology has potential for automation of papaya micropropagation in bioreactors, as a strategy for reducing the cost of operation.

The shoots generated using solid media were successfully rooted in MS basal medium with 2.5 mg/l IBA (Anandan et al. 2011; Mumo et al. 2013; Teixeira da Silva et al. 2007), while those from solid/liquid bilayer were rooted in MS basal medium with 3 mg/l IBA (Gatambia et al. 2016). Different researchers (Mumo et al. 2013; Panjaitan et al. 2007; Yu et al. 2000) recommended the exposure of in vitro generated papaya shoots to MS basal medium with low concentration of IBA for 1 week, for root induction, followed by transfer to a medium supplemented with vermiculite (under

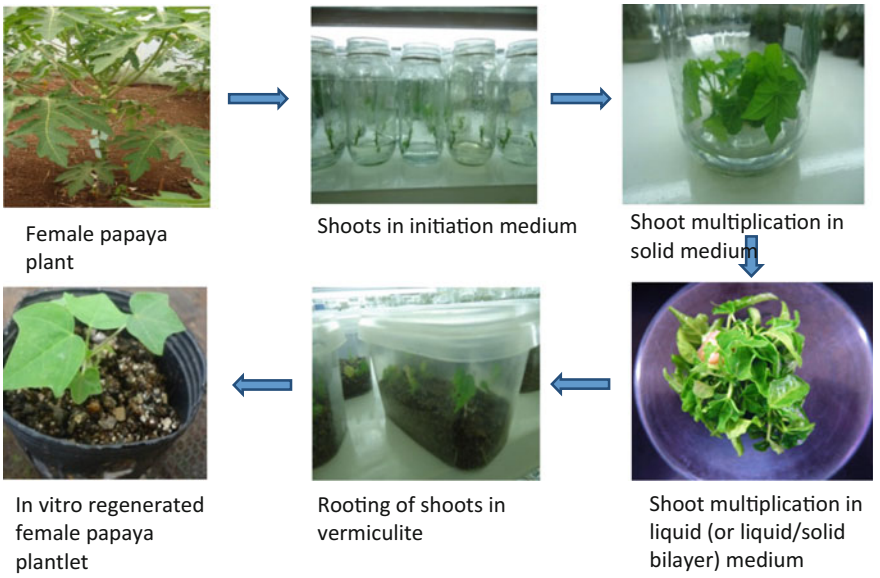

Fig. 21.3 Schematic representation of the processes involved in micropropagation of papaya plantlets

aerated conditions) for root development as being optimal for plantlet establishment. Fig. 21.3 illustrates the process of micropropagation of papaya plantlets of known sex using in vitro culture of shoot tip meristems. Since the shoots develop directly from the shoot meristem explant, which is genetically stable, somaclonal variation is eliminated. This makes micropropagation of papaya via meristem culture amenable for crop improvement, conservation of elite germplasm and commercial propagation.

21.3.3 Callus Induction and Somatic Embryogenesis

This method involves induction of embryogenic calli or embryos from which shoots are regenerated. Somatic embryos have been induced from different papaya explants such as the internode stem of seedlings, hypocotyl sections, seedling root explants, the midrib and lamina of cotyledons of seedlings, adventitious roots, immature zygotic embryos and immature seeds (Teixeira da Silva et al. 2007). Researchers have used different basal media with different concentrations and combinations of phytohormones to induce somatic embryogenesis such as MS basal medium with NAA and kinetin for callus induction, then MS with IAA and kinetin for embryo induction; MS with BA and NAA; Fitch's liquid medium with ½ MS and vitamins, myo-inositol, sucrose, 2,4-D and glutamine and de Fossard medium with BAP, NAA and GA3 (Abreu et al. 2014; Teixeira da Silva et al. 2007). Recently, Bukhori et al. (2013)

reported 77.5% embryogenic callus induction form immature zygotic embryos cultured on MS medium augmented with 10 mg/l 2,4-D and 250 mg/l carbenicillin. Auxins are critical for the initiation and subsequent growth of callus; NAA being the most effective, followed by 2,4-D and IAA.

Protoplast culture was attempted by Chen (1994) who successfully isolated protoplasts from highly regenerable suspension cultures from interspecific crosses of *Carica papaya* × *C. cauliflora* zygotic embryos. The somatic embryos proliferated and formed plantlets. The major challenge with somatic embryogenesis as a method of micropropagation is somaclonal variation which produces off-types and chimeras. However, it is an important procedure used in genetic transformation.

21.3.4 Anther Culture

This method is important for induction of haploid papaya plants which can be dihaploidized to generate homozygous parental breeding lines. The first attempt to produce haploids through anther culture in papaya was by Litz and Conover (1978), but did not specify the induction efficiency. A second attempt in 1979 (Litz and Conover 1979) managed a success rate of 0.4%. Tsay and Su (1985) also reported a low rate (0.67%) of embryo induction. Rimberia et al. (2005), while using anthers from Fl plants of a cross between a female cv. Wonder Blight and an unknown male strain reported an improved embryo induction rates in vitro to about 4.0% by pre-treating anthers on water or MS liquid medium with 2.0% sucrose at 35 °C for 1–5 days prior to culture on agar-solidified MS medium supplemented with 0.l mg/l BA, 0.1 mg/1 NAA and 3% sucrose. Gyanchand et al. (2015), used the same pretreatment and phytohormones to generate anther-derived embryos at a rate of 8.0% using papaya cv. Pusa Nanha. High temperature pretreatment improved the embryo induction rate from cultured anthers (Gyanchand et al. 2015; Rimberia et al. 2005), although the effects of nutrients and/or sucrose during the pre-treatment was ambiguous. The embryo induction rate was further improved to 13.8% by culturing anthers on agar-solidified MS medium with 0.01 mg/l CPPU and 0.1 mg/l NAA (Rimberia et al. 2006a). It appears that CPPU was more effective in embryo induction from anthers than high temperature pre-treatment. Additionally, genotypic differences have been demonstrated in embryo induction rates from cultured anthers (Gyanchand et al. 2015; Rimberia et al. 2005).

The sex diagnostic technique developed by Urasaki et al. (2002) and greenhouse evaluation were used to confirm that all the anther culture-derived papaya plants were female (Rimberia et al. 2007). This indicates that the plantlets were not derived from anther wall tissues but microspores. However, the optimum medium conditions for normal embryo growth was not defined because most of the embryos formed embryogenic calli (Fig. 21.4) (Gyanchand et al. 2015; Rimberia et al. 2005), that developed into fasciated shoots.

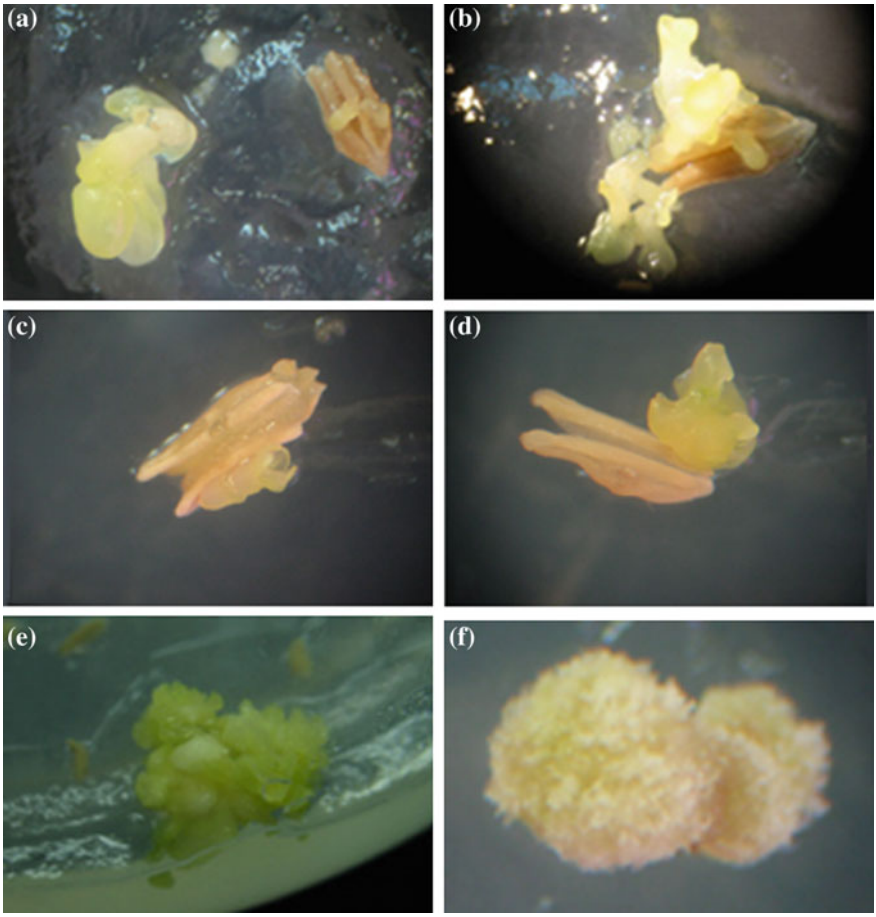

Fig. 21.4 Embryos and calli formed on anthers (**a**) Normally differentiated embryos, **b** multiple embryos, **c**–**e** Embryogenic calli, **f** Non-embryogenic callus *Source* Rimberia and Adaniya (2010)

21.3.5 Protoplast Culture

The first reports of successful protoplast culture in papaya were by Chen and Chen (1992) and Chen (1994), who isolated protoplasts from highly regenerable suspension cultures from interspecific crosses of *Carica papaya* × *C. cauliflora* zygotic embryos. Embryogenic cultures were digested with a mixture of cellulase R-10, macerozyme R-10 and driselase in 0.4 M mannitol, and plants were regenerated via somatic embryogenesis (Saksena 2013). Protoplast culture holds a lot of potential for somatic hybridization and genetic transformation.

21.3.6 Somaclonal Variation

Somaclonal variation usually accompanies in vitro plant regeneration via the callus phase and may be of great interest in crop improvement. Researchers over the years have managed to generate papaya plants of different ploidy levels using different explants. Clarindo et al. (2008) used immature papaya zygotic embryos to induce both somatic embryogenesis and somaclonal variants. The resulting plants were euploid (diploid, mixoploid, triploid and tetraploid) and aneuploidy that remained stable during the successive subcultures in multiplication medium. Sun et al. (2011) induced triploid papaya from immature endosperm with embryos. A total of 75% of the plants were triploids confirming their endosperm origin. Rimberia et al. (2006a) and Rimberia and Adaniya (2010) induced haploids, dihaploids, triploids, and tetraploids through in vitro culture of immature anthers. The triploids formed the bulk (87.5%) of the plantlets. Both triploids and tetraploids were evaluated in the greenhouse and they turned out to be females confirming their microspore origin. The triploids were variable in height and fruit bearing (Fig. 21.5). Some of the triplods were found to be dwarfs that produced seedless fruits of normal size (Rimberia and Adaniya 2010; Rimberia et al. 2007). The dwarf and parthenocarpic traits are important for the breeding of female papaya cultivars used for greenhouse cultivation. A system of propagation and maintenance of the triploids needs to be developed for them to be useful for commercialization.

21.3.7 Mutation Breeding

Mutation breeding is a useful tool for developing new varieties. In papaya, irradiation has been used to improve production (Chan et al. 2007; Hang and Chau 2010; Husselman et al. 2016; Kumar et al. 2017; Liao et al. 2017; Mahadevamma et al. 2012). In Malaysia, Chan et al. (2007) used gamma irradiation in variety Eksotika to produce several **M2** from which they screened for resistance to papaya ringspot virus (PRSV), dwarfness, lower fruit bearing stature and higher total soluble solids. No mutant was found to be resistant to PRSV (Chan et al. 2007). In Vietnam, Hang and Chau (2010) used gamma irradiation ranging from 10–60 Gy to improve precocity and reduction of plant height on a local papaya variety Dai Loan Tim. Four mutants were reported to be superior in plant height at first flower, plant vigor, fruit yield and quality compared to non-mutants. Irradiation has however been shown to result in poor germination and negatively affected the number of fruits and fruit quality compared to the control (Kumar et al. 2017). There is need for further research in this subject to develop resistance to devastating viral, bacterial and other diseases.

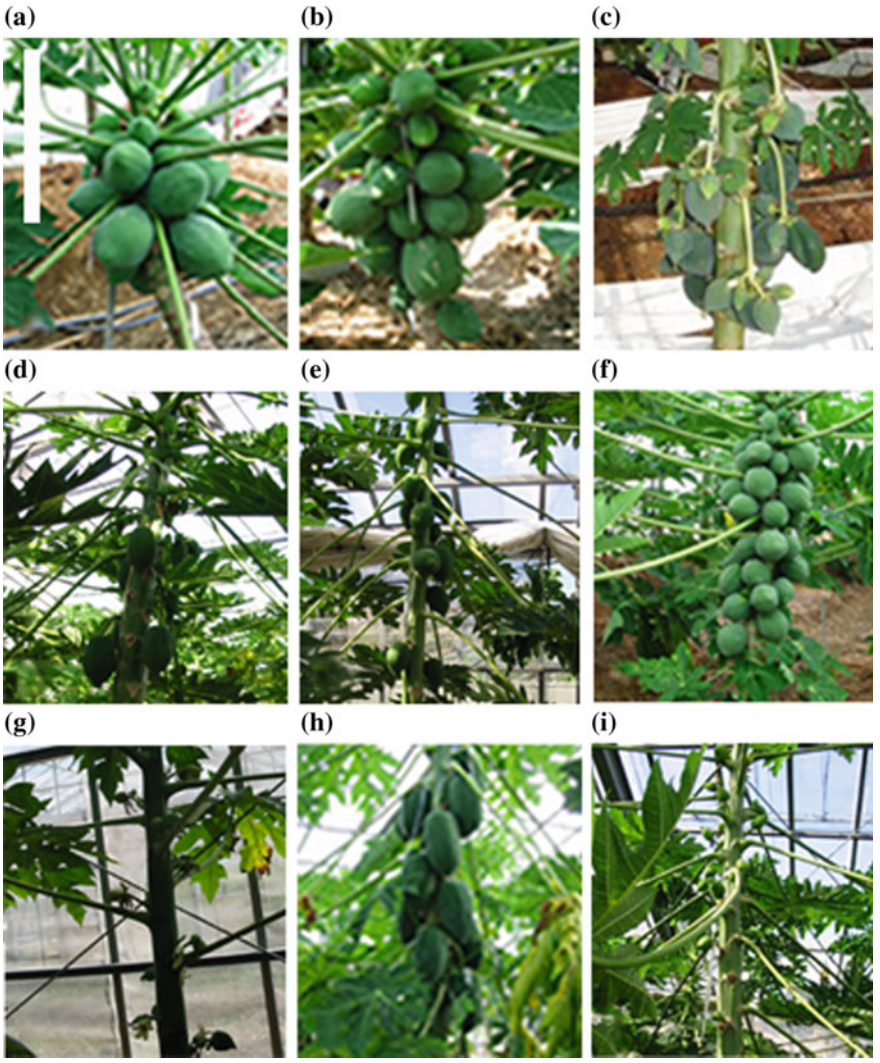

Fig. 21.5 Variation in fruiting habits among triploid female plants derived from anther culture. The strains shown are **a** EMB-1, **b** EMB-2, **c** EMB-7, **d** EMB-10, **e** EMB-15, **f** EMB-17, **g** EMB-19, **h** EMB-26, **i** EMB-30. Vertical bar indicates 50 cm *Source* Rimberia et al. (2007), Rimberia and Adaniya (2010)

21.4 Molecular Marker-Assisted Selection and Breeding

Molecular marker assisted selection and breeding have been applied in papaya, particularly in the selection for disease resistance. Although papaya ringspot virus (PRSV) is a major bottleneck of papaya production worldwide, no papaya cultivar is resistant

to the virus. Resistance has only been reported in wild relatives in the genus *Vasconcellea* such as *V. cauliflora*, *V. stipulata*, *V. pubescens* and *V. quercifolia* (Manshardt and Drew 1998). O'Brien and Drew (2009) showed that resistance to PRSV-p in *V. pubescens* was controlled by a single dominant gene (prsv-1) through successful backcrossing of *V. pubescens* to *V. parviflora* from F3 interspecific hybrids. The markers linked to prsv-1 have been used in breeding programs because of their dominant inheritance.

Carica papaya is also susceptible to other pathogens such as *Phytophthora palmivora*. Tolerance to *P. palmivora* has been identified and molecular markers linked to the tolerance developed using AFLPs (Noorda-Nguyen et al. 2010). This was identified from the F2 population achieved by crossing tolerant Hawaiian cv. Kamiya with highly susceptible cv. SunUp. Markers have also been used to select for other traits. For instance, Blas et al. (2010) found a major gene associated with yellow flesh of papaya which is dominant over the red flesh color in simple Mendelian fashion.

21.5 Genetic Engineering in Trait Improvement

21.5.1 Breeding for Resistance to Pests and Diseases

Papaya is mostly grown in the tropics and subtropics. The fruit like any other is affected by a couple of pathogens such as fungi, viruses and bacteria as well as insect pests such as aphids, leafhoppers, mites and nematodes (Nishijima 2002).

21.5.2 Breeding for Resistance to Carmine Spider Mite

Mites are some of the problematic pests in papaya due to various characteristics they possess such as being minute and their high fecundity. These mites colonize the papaya plant parts, and as a result of their feeding activity, they cause premature defoliation, reduced fruit yields and fruit-skin blemishes leading to reduced market value (Nishina et al. 2000).

Carmine spider mite (*Tetranychus cinnabarinus*) is of large economic importance due to its broad host range when compared with other *Tetranychidae* species because the mites are polyphagous and may be found on weeds and cultivated crops such as fruits, vegetables and ornamentals (Biswas et al. 2004). Genetically-transformed plants have shown to offer a long-term and sustainable control of pests such as the carmine spider mite and many other insect pests through targeting chitin metabolism (McCafferty et al. 2006). Chitin is an insoluble structural polysaccharide and forms part of the cell wall of fungi and cuticle of mites and nematodes (Kramer and Muthukrishnan 1997). The introduction of the insect chitinase gene into plants has shown

to improve resistance to insect attack. Tobacco budworm (*Heliothus virescens*) has been controlled using chitinase enzyme extracted from *Manduca sexta*; this caused a stunted growth of the larva (Ding et al. 1998).

A transgenic PRSV-resistant papaya cv. Rainbow which was bred from two parents SunUP (female) and Kapoho (male), both of which were susceptible to mites and leafhoppers, was enhanced to become tolerant to carmine spider mite. The cultivar was also transformed using chitinase from *Manduca sexta*. The chitinase activity was found to be high, up to 52%, in the transgenic leaf extracts when compared to the control. When tested under field conditions, there was a significant control of mites in the transformed lines when compared to the control (Kapoho). The chitinase activity was high in two lines in which high mite mortality was noted (McCafferty et al. 2006).

21.5.3 Breeding for Resistance to Phytophthora

Papaya is highly susceptible to root rot caused by *Phytophthora palmivora,* particularly in poorly-drained soils and during the rainy season. Papaya resistance to Phytophthora has been improved through the introduction of a defensin gene from *Dahlia merckii* through particle bombardment in embryogenic calli of papaya (McCafferty et al. 2006). Defensins are a family of peptides that occur in various plant species, *D. merckii* being one of them (Broekaert et al. 1995). These plant peptides have been shown to induce a response to fungal infection (Cociancich et al. 1993). Resistance to fungal attack has also been observed when the defensin gene was introduced to crops such as in the case of tobacco in a move to control *Alternaria longipes* (Terras et al. 1995).

To improve the resistance of papaya to *Phytophthora palmivora* (Zhu et al. 2007) used a gene extract containing the defensins gene from *Dahlia* which was driven from a CaMV 35Spromoter and nptII gene. This was under the nopaline synthase (NOS) promoter as a selectable marker. The papaya which had been transformed using this gene were found to contain total soluble protein of 0.07–0.14% and 0.05–0.08% in callus and leaves, respectively. The presence of this gene in genetically transformed papaya was able to inhibit mycelial growth of *P. palmivora* by up to 35–50% when leaf extract was used. Further tests in the greenhouse showed that papaya with the defensins gene had an improved resistance towards *P. palmivora* and this was associated with a decline in growth of the hyphae of the fungus on infected sites, thus indicating the capacity of the defensins to control the pathogen (Zhu et al. 2007).

21.5.4 Breeding for Resistance to PRSV

There are many economically important diseases of papaya: the most significant among them is papaya ring spot disease (Purcifull 1972). Papaya ringspot virus

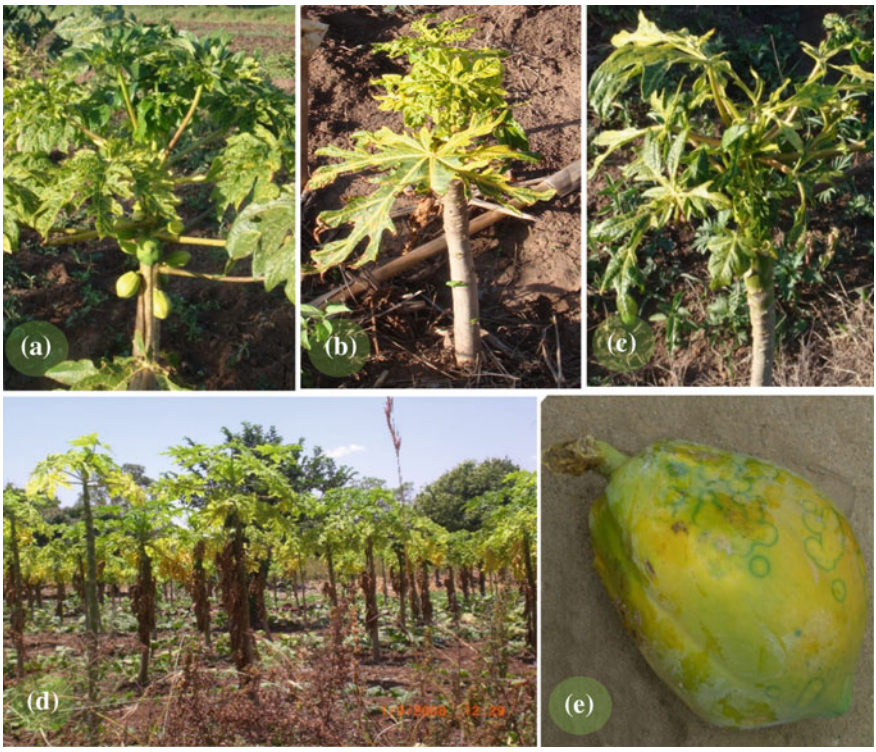

Fig. 21.6 Symptoms of PRSV infection in papaya. **a** Mosaic and leaf distortion, **b** Stunting and chlorosis as well as leaf distortion, **c** Stunting and leaf strapping, **d** A papaya orchard severely infected with PRSV, **e** Ringspot symptom on a papaya fruit

(PRSV) (genus *Potyvirus*, family Potyviridae) (Tripathi et al. 2008) infects papaya plants leading to symptoms including chlorotic leaves, deformation of leaves which resembles the damage of mites, ringed spots on the fruit (Fig. 21.6) and depressed fruit production which eventually leads to the death of the infected plants. In many parts of the world, PRSV is considered a serious threat to the papaya industry. The virus is transmitted by aphids in a non-persistent manner.

Management of PRSV through rouging of infected plants, quarantine regulations of restricting the plant movement, use of insecticides against insect vectors and cross protection, generally have not been effective in controlling the disease. Hitherto, no naturally-occurring resistance to PRSV has been identified in any papaya cultivar. Therefore, breeding for resistance to PRSV in papaya has only resulted in tolerant varieties (Conover 1976; Conover and Litz 1978). Large collections of papaya germplasm and cultivars representing the world's major production have been screened, but resistance has not been found. Because of the aforementioned, moderate levels of horizontal resistance were identified in papaya germplasm and used in papaya breeding programs with minimal success (Conover and Litz et al. 1978).

Breeding tolerant varieties with susceptible high yielding commercial varieties has improved yield under infectious conditions (Chan 2004).

It is common in plant breeding programs to obtain genes for plant disease resistance from wild relatives for introgression into acceptable high-yielding cultivars. Breeding of resistant varieties (by interspecific hybridization of *Carica papaya* with *Vasconcellea* sp.) therefore serves as a way of controlling PRSV. Numerous efforts have been made to incorporate the resistance genes from other genera in the Caricaceae namely, *Vasconcellea cauliflora*, *V. quercifolia*, *V. stipulate* and *V. pubescens*. Intergeneric hybrids between papaya and PRSV resistant species have been produced by a number of investigators with the aid of embryo rescue techniques (Horovitz and Jimenez 1967; Khuspe et al. 1980). However not much progress has been made.

In the mid-1980s, with the success in the development of genetically-modified crops such as cotton, maize and soybean, and the complete molecular characterization of PRSV (Yeh et al. 1992), scientists from Cornell University and the University of Hawaii initiated the development of PRSV-resistant papaya by genetic engineering. Transgenic papaya resistant to PRSV was developed and commercialized at Hawaii in 1998 (Gonsalves and Ferreira 2003). This was actualized through somatic embryogenesis and microprojectile transformation as one of the preferred methods of transformation; although the biolistic method of transformation also worked with high efficiency (Cai et al. 1999; Fitch et al. 1992).

Like numerous other dicotyledonous plant species, papaya can be transformed with *Agrobacterium tumefaciens* and regenerated into phenotypically normal appearing plants that express foreign genes. Yeh and Gonsalves (1994) developed a plant-expressible PRSV-cp gene construct from a Taiwanese PRSV strain. Transgenic plants expressing ß-glucuronidase (gus) were regenerated following co-cultivation of petiole explants with *A. tumefaciens*.

Pathogen-derived resistance is a phenomenon whereby transgenic plants containing genes or sequences of a pathogen are protected against adverse effects of the same or related pathogens. Coat protein mediated production is based on the phenomenon of cross-protection. Cross-protection is the term used for the phenomenon that a plant, when first inoculated with a mild strain of a given virus, becomes protected against the infection with a second, more severe strain of the same virus with which it has been infected (Fermin et al. 2010). Yeh et al. (2003) reported that the coat protein (cp) gene mediated transgenic resistance is the most promising approach for protecting papaya against the devastating effects of papaya ring spot viruses (PRSV). Viral cp gene imparting resistance against virus is known as coat protein mediated resistance (CPMR). Accumulation of the cp gene in transgenic crops has been proven to counter resistance to infection and/or disease development by the virus from which the cp gene was derived and by related viruses (Rosales et al. 2000).

21.5.5 Breeding for Aluminum and Herbicide Tolerance

Worldwide, up to about 40% of most agricultural lands are characterized by acidic soils. Aluminum is considered toxic to many plants. Organic acid excretion by plants is always associated with tolerance to aluminum. Therefore, overproduction of an organic acid in a plant either through genetic engineering or conventional breeding could address the problem of aluminum toxicity. De la Fuente et al. (1997) reported production of transgenic papayas by particle bombardment, with constructs driving the overexpression of the citrate synthase (cs) gene from *Pseudomonas aeruginosa*. There were lines expressing the cs gene that released 2–3 times more citrate than control plants. These transgenic lines were capable of forming roots and to grow in solutions containing up to 300 mM of aluminum, as compared to the control plants. The study indicated that excretion of the organic acid is a mechanism of aluminum tolerance in plants.

21.6 Conclusions and Prospects

Papaya is cultivated in all tropical and subtropical parts of the world where a majority of the people are poor and food insecure. It has therefore become a very important fruit crop with potential to alleviate human food and nutritional insecurity as well as provide some income. Compared to other fruit crops, papaya is one of the few fruit crops that bears fruits when less than 1 year old and continues to bear throughout the year under good management.

Papaya production is threatened by a myriad of problems including devastating pests and diseases, inability for farmers to differentiate among the three sex types, male, female and hermaphrodite at seedling stage, among others. Many attempts have been made by researchers over the years to resolve these problems through conventional and biotechnological techniques. Conventional plant breeding through introgression has given rise to many varieties with high yields and fruit quality.

Managing devastating viral diseases in papaya like PRSV requires efforts beyond conventional techniques as there is no known natural resistance available. Researchers in many parts of the world have managed to develop/introduce PRSV resistance to existing varieties through genetic transformation. However, genetically-modified fruits are still not accepted in most European countries and Japan. This notwithstanding, papaya plants tolerant to PRSV have been identified in Malaysia, USA (Florida) and recently Kenya. These tolerant plants hold important promise in resolving the devastating PRSV challenge although there is need for further development. On the other hand, tissue culture of papaya through shoot tip meristems has achieved a lot towards mass production of disease-free plantlets of known sex which will reduce the cost of production and enable growers to purchase the exact quantity of seedlings required. Somaclonal variation resulting from anther and embryo culture

derived calli hold huge potential for papaya improvement and needs to be explored further.

Dwarfness and parthenocarpy are two traits to be considered in the development of varieties for greenhouse production. Some varieties such as Kamiya Co 1 and Co 2 were released years ago. Where possible, the approach of developing varieties that combine dwarfness, parthenocarpy and PRSV tolerance needs to be adopted and encouraged to take advantage of greenhouse production as well as decreasing sizes of land available for production. Somaclonal variation probably holds considerable promise in research towards this direction.

A lot of effort has gone and continues to go into the improvement of papaya with each research group targeting different traits. It is widely agreed that the main challenge facing papaya productivity is PRSV which reduces orchard lifetime to about one year instead of three. Other priorities will include improvement of papaya for quality and yield. Whatever the location, the order of priorities may vary and therefore the priority of interventions may also change. Critical, however, is the choice of the most appropriate approach to solve the challenge.

Acknowledgements The authors thank the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Research Production and Extension (RPE) Division for funding the papaya varietal development project from 2008 to 2014, the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for funding papaya tissue culture project 2010–2012 and the Africa Union-African innovation-JKUAT and PAUSTI (Africa –ai-JAPAN) for funding agronomic evaluation of papaya varieties from 2015 to date.

Appendix 1: Research Institutes and Online Resources Relevant to Papaya Genetic Improvement Research

Country	Institution	Specialization	Research activities	Contact information
Australia	Griffith University	Teaching and Research institute	Biotechnology of the papaya	Prof. Dr. Roderick A. Drew Griffith Sciences, Logan Campus, Griffith University, Meadowbrook, QLD 4131 Australia Telephone: (61)733821291 Fax: (61)737357618 E-mail: r.drew@griffith.edu.au
Brazil	Capixaba Institute for Research, Technical Assistance and Rural Extension (INCAPER)	Research, technical assistance and rural extension	Germplasm selection and improvement	Eng Agr, D. Sc., Luiz Augusto Lopes Serrano INCAPER/CRDR Nordeste, C. P. 62, 29900-970, Linhares-ES. E-mail: lalserrano@incaper.es.gov

Country	Institution	Specialization	Research activities	Contact information
India	Indian Agricultural Research Institute Regional Station, Pusa, Bihar, India	Research institute	Breeding papaya varieties of uniform, high yielding with better quality for wider adaptability	Dr. Tapas Ranjan Das Phone: 06274-240232 Fax: 06274-240236 E-mail: head_bihar@iari.res.in
Indonesia	Indonesian Tropical Fruit Research Institute (ITFRI)	Research institute	Breeding and biotechnology	Jl. Raya Solok, Aripan Km. 8, PO Box. 5, Solok 27301, West Sumatra Phone: 0755-20137 Fax: 0755-20592 Email: rif@padang.wasantara.net.id; balitbu@litbang.pertanian.go.id
Kenya	Jomo Kenyatta University of Agriculture and Technology	Teaching and Research institute	Varietal Development and Evaluation	Dr. Fredah K. Rimberia, P.O. Box 62000-00200 Nairobi Kenya Phone: +254726856304 Email: frenda@agr.jkuat.ac.ke or fredawanza@yahoo.com
Malaysia	Felda Agricultural Services Sdn Bhd	Commercial Agribusiness firm	Agricultural extension and Disease management in Papaya	Menara Felda, Platinum Park, Persiaran KLCC, Kuala Lumpur, Malaysia 50088 +60 3-2859 0366 Email: feldabiotech@felda.net.my
Nigeria	The National Horticultural Research institute	Research institutes	Papaya Varietal development and evaluation	Ms. Olubunmi Ibitoye National Horticultural Research Institute, P. M. B. 5432 Jericho Reservation Area, IDI-ISHIN, Oyo, Ibadan, Nigeria Telephone: (234)8023629104 E-mail bunmiajisafe@yahoo.com
Philippines	Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines, Los Baños	Research institute	National biotechnology research center and repository for all crops	Office of the director, 4031 College, Laguna, Philippines Telephone: (049) 536-5287; (049) 543-9571 Email: ipb.uplb@up.edu.ph
South Africa	Neofresh (Pty) Ltd.	Commercial fruit growers and exporters	Selection, hybridization and production of high quality papaya fruit for supermarkets and export outlets	Dr Aart Louw (Chief Researcher and Plant Breeder) PO Box 201 Sonpark 1206 Mpumalanga RSA T +27 13 590 0947 adminmanager@neofresh.net www.neofresh.net

Country	Institution	Specialization	Research activities	Contact information
Thailand	East West Seed Company	Commercial Seed company	Breeding	Lamai Yapanan Business Development Manager, 7 Moo 8, Chiang Mai Praw Road, 50290, Chiang mai, Thailand Email: lamai.yapanan@eastwestseed.com
United States of America-Hawaii	Hawaii Agricultural Research Centre (HARC)	Research Centre	Tissue culture and transformation of papaya	94-340 Kunia Rd, Waipahu, HI 96797, USA Phone:+1 808-677-5541
United States of America-Hawaii	University of Hawaii		Papaya breeding	Dr. Dennis Gonsalves 789 Hoolaulea Street, Hilo Hawaii 96720 USA E-mail dennisgonsal@gmail.com

Appendix 2: Genetic Resources

Country	Cultivar	Sex type	Flesh color
Australia	Improved Petersen	Dioecious	Yellow
	Guinea Gold	Hermaphrodite	Yellow
	Sunnybank/S7	Dioecious	Yellow
	Richter/Arline	Dioecious	Yellow
America – Mexico	Verde	–	–
	Gialla	–	–
	Cera	–	–
	Chincona	–	–
USA – Florida	Cariflora	Dioecious	Yellow
	Betty	Dioecious	Yellow
	Homestead	Dioecious	Yellow
USA – Hawaii	Kapoho Solo	Hermaphrodite	Yellow
	Sunrise	Hermaphrodite	Red
	Waimanalo	Hermaphrodite	Yellow
	Rainbow	Hermaphrodite	Yellow
Venezuela	Paraguanera	–	–
	Roja	–	Red
Caribbean – Barbados	Wakefield	–	–
	Graeme 5, and 7	–	–
Cuba	Maradol	Hermaphrodite	Red
Trinidad	Santa Cruz Giant	–	–
	Cedro	–	–

Country	Cultivar	Sex type	Flesh color
Dominican Republic	Cartagena	Hermaphrodite	Yellow
Asia – India	Coorg Honey Dew	Hermaphrodite	Yellow
	Coimbitor 2	Dioecious	Yellow
Indonesia	Semangka	Hermaphrodite	Red
	Dampit	Hermaphrodite	Red
Malaysia	Eksotika	Hermaphrodite	Red
	Sekaki	Hermaphrodite	Red
Philippines	Cavite/Sinta	Hermaphrodite	Red
Taiwan	Tainung No. 5	Hermaphrodite	Red
Thailand	Sai-nampueng	Hermaphrodite	Red
	Khaek Dam	Hermaphrodite	Red
South Africa	Hortus Gold	Dioecious	Yellow
	Kaapmuiden	–	Yellow
	Honey Gold	Dioecious	Yellow

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