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Papaya (*Carica papaya* L.) Biology and Biotechnology

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ABSTRACT

Papaya (*Carica papaya* L.) is a popular and economically important fruit tree of tropical and subtropical countries. The fruit is consumed world-wide as fresh fruit and as a vegetable or used as processed products. This review focuses primarily on two aspects. Firstly, on advances in *in vitro* methods of propagation, including tissue culture and micropropagation, and secondly on how these advances have facilitated improvements in papaya genetic transformation. An account of the dietary and nutritional composition of papaya, how these vary with culture methods, and secondary metabolites, both beneficial and harmful, and those having medicinal applications, are discussed. An overview of papaya post-harvest is provided, while 'synseed' technology and cryopreservation are also covered. This is the first comprehensive review on papaya that attempts to integrate so many aspects of this economically and culturally important fruit tree that should prove valuable for professionals involved in both research and commerce.

Keywords: biolistic, papain, *Papaya ringspot virus*, postharvest management

Abbreviations: **½MS**, half-strength Murashige and Skoog (1962) medium; **2,4,5-T**, 2,4,5-trichlorophenoxyacetic acid; **2,4-D**, 2,4-dichlorophenoxyacetic acid; **2-iP**, 6-(γ,γ-dimethylallylamino)-purine; **AAC**, 1-aminocyclopropane-1-carboxylic acid; **ABA**, abscisic acid; **ACC**, 1-aminocyclopropane-1-carboxylic acid; ***ACS 1*** and ***ACS 2*** 1-aminocyclopropane-1-carboxylic acid synthase genes; **AFLP**, amplified fragment length polymorphism; **AVG**, aminoethoxyvinylglycine; **AVG**, aminoethoxyvinylglycine; **BA**, 6-benzyladenine; **BAP**, 6-benzylamino purine; **BC**, back-cross; **CAPS**, cleaved amplified polymorphic sequences; **$CaCl_2$**, calcium chloride; **CBF**, C repeat binding factor; **$CoCl_2$**, cobalt chloride; ***cp***, coat protein gene; **CPA**, *p*-chlorophenoxyacetic acid; ***CP-ACO1*** and ***CP-ACO2*** **1**, aminocyclopropane-1-carboxylic acid oxidase genes; **CPL**, *C. papaya* lipase; **CP_{NT}**, nontranslatable coat protein gene construct; **CP_T**, translatable coat protein gene constructs; ***CSb***, citrate synthase gene; **CW**, coconut water; **DAF**, DNA ampli-fication finger-printing; ***DmAMP*1**, *Dahlia merckii* defensin gene; **EFE**, ethylene forming enzyme; **EST**, expressed sequence tag; **GA_3**, gibberellic acid; **GFP**, green fluorescent protein; **GRAS**, Generally Regarded As Safe; **GUS**, β-glucuronidase; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **KNO_3**, potassium nitrate; **LED**, light emitting diode; **MA**, modified atmosphere; **Man**, mannose; **MSF**, methanol sub-fraction; **MSY**, male-specific; **Mt**, million tones; **NAA**, α-naphthaleneacetic acid; **NH**, Nivun Haamir; ***npt*II**, neomycin phosphotransferase II; **NSAIDs**, non-steroidal anti-inflammatory drugs; **PANV**, *Papaya apical necrosis virus*; **PBT**, Papaya bunchy top; **PCR**, polymerase chain reation; **PDB**, Papaya dieback; **PDNV**, *Papaya droopy necrosis virus*; **PM**, Papaya mosaic; **PMeV**, *Papaya meleira virus*; **PMI**, phospho-mannose isomerase; **PPT**, phosphinothricin; **PPT**, phosphinothricin; **PRSV HA 5-1**, mild strain of *Papaya ringspot virus*; **PRSV**, *Papaya ringspot potyvirus*; **PSDM**, papaya sex determination marker; **PYC**, Papaya yellow crinkle; **PLYV**, *Papaya lethal yellowing virus*; **RAF**, randomly amplified DNA fingerprint; **RAPD**, random amplified polymorphic DNA; ***RP***, viral replicase gene; **SCAR**, sequence characterized amplified region; **STS**, silver thiosulphate; **TDZ**, thidiazuron; **TIBA**, 2,3,5-triiodobenzoic acid); ***uid*A**, β-glucuronidase gene

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Review

INTRODUCTION

Geographic distribution and nomenclature

Papaya, *Carica papaya* L., is one of the major fruit crops cultivated in tropical and sub-tropical zones. Worldwide over 6.8 million tonnes (Mt) of fruit were produced in 2004 on about 389,990 Ha (FAO 2004). Of this volume, 47% was produced in Central and South America (mainly in Brazil), 30% in Asia, and 20% in Africa (FAO 2004; **Table 1**). The papaya industry in Brazil is one of the largest worldwide that continues to show rapid growth. do Carmo and Sousa Jr. (2003) reported on a 151% increase in total area cultivated over the past decade (16,012 ha in 1990 to 40,202 ha in 2000) and a 164% increase in the quantity produced during the same period (642,581 to 1,693,779 fruits from 1990 to 2000). In 11 years, the volume exported increased 560% from 4,071 t to 22,804 t in 2001 (SECEX-MDIC 2002) and 38,760 t in 2005 (FAO 2005). Although papaya is mainly grown (>90%) and consumed in developing countries, it is fast becoming an important fruit internationally, both as a fresh fruit and as processed products.

The classification of papaya has undergone many changes over the years. The genus *Carica* was previously classified under various plant families, including Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae. However it is presently placed under Caricaceae, a plant family incorporating 35 latex-containing species in four genera, *Carica*, *Cylicomorpha*, *Jarilla* and *Jacaratia* (Kumar and Srinivasan 1944). It is widely believed that papaya originated from the Caribbean coast of Central America, ranging from Argentina and Chile to southern Mexico (Manshardt 1992) through natural hybridization between *Carica peltata* and another wild species (Purseglove 1968). *Carica* consists of 22 species and is the only member of the Caricaceae that is cultivated as a fruit tree while the other three genera are grown primarily as ornamentals (Burkill 1966). *Cylicomorpha* is the only member of the Caricaceae that is indigenous to Africa, and consists of two species. *Jacaratia*, found in tropical America, consists of six species. *Jarilla*, from central Mexico consists of only one species. The mountain papaya (*C. candamarcencis* Hook. f.), is native to Andean regions from Venezuela to Chile at altitudes between 1,800-3,000 m (Morton 1987). The 'babaco', or 'chamburo' (*C. pentagona* Heilborn), is commonly cultivated in mountain valleys of Ecuador; plants are slender, up to 3 m high, and pentagonal fruits reach 30 cm in length (Morton 1987). Compared to the well known tropical papaya, *C. papaya*, fruits of the mountain papayas tend to be smaller in size and less succulent.

Recently, another taxonomic revision was proposed and supported by molecular evidence that genetic distances were found between papaya and other related species (Jobin-Décor *et al*. 1996; Badillo 2002; Kim *et al*. 2002). Some species that were formerly assigned to *Carica* were classified in the genus *Vasconcella* (Badillo 2002). Accordingly, the classification of Caricaceae has been revised to comprise *Cylicomorpha*, *Carica*, *Jacaratia*, *Jarilla*, *Horovitzia* and *Vasconcella*), with *Carica papaya* the only species within the genus *Carica* (Badillo 2002).

Table 1 Production of papaya by region.

Region	Area harvested (Ha)	Production (Mt)
Africa	128,807	1,344,230
Asia and the Pacific	157,203	2,063,352
Australia	403	5,027
Caribbean	9,179	179,060
Central America	28,966	1,057,024
North America	500	16,240
South America	65,546	2,120,370

Source: FAOSTAT, 2006

The history of papaya appears to be first documented by Oviedo, the Director of Mines in Hispaniola (Antilles) from 1513 to 1525, where he describes how Alphonso de Valverde took papaya seeds from the coasts of Panama to Darien, then to San Domingo and the other islands of the West Indies. The Spaniards gave it the name 'papaya' and took the plant to The Philippines, from where it expanded to Malaya and finally India in 1598 (Schery 1952). By the time papaya trees were established in Uganda in 1874, their distribution had already spread through most tropical and sub-tropical countries.

When first encountered by Europeans, papaya was nicknamed 'tree melon'. Although the term papaya is most commonly used around the world (Burkill 1966; Storey 1985), the fruit is also known as 'kapaya', 'kepaya', 'lapaya', 'tapayas' and 'papyas' in The Philippines, 'dangandangan' in Celèbes (Indonesia), or 'gedang castela' or 'Spanish Musa' in Bali. Malaysians and Singaporeans, primarily the Malays, refer to the fruit as 'betik', while in Thailand it is known as 'malakaw', 'lawkaw' or 'teng ton'. In Mexico and Panama, it is referred to as 'olocoton', the name having originated from Nicaragua. In Venezuela it is known as 'lechosa', as 'maman' in Argentina, and 'fruta bomba' in Cuba. In other Spanish-speaking countries the names vary as follows: 'melon zapote', 'payaya' (fruit), 'papayo' or 'papayero' (the plant), 'fruta bomba', 'mamón' or 'mamona', depending on the country. Portuguese-speaking countries (Portugal, Brazil, Angola, Mozambique, Cape Verde, East Timor) refer to the fruit as 'mamão' or 'mamoeiro'. In Africa, Australia, and Jamaica, the fruit is commonly termed 'paw-paw', while other names such as 'papayer' and 'papaw' are also heard. The French refer to the fruit as 'papaya' or to the plant as 'papayer', or sometimes as 'figuier des Îles'. For standardization, we refer to *C. papaya* as papaya throughout this manuscript. *Asimina triloba* (also commonly known as pawpaw, paw paw, papaw, poor man's banana, or hoosier banana) is indigenous to the USA. This genus and related species will not be covered in the review.

BOTANY AND CULTIVATION

Papaya is a fast-growing, semi-woody tropical herb. The stem is single, straight and hollow and contains prominent leaf scars. Papaya exhibits strong apical dominance rarely branching unless the apical meristem is removed, or damaged. Palmately-lobed leaves, usually large, are arranged spirally and clustered at the crown, although some differences in the structure and arrangement of leaves have been reported with Malaysian cultivars (Chan and Theo 2000). Generally, papaya cultivars are differentiated by the number of leaf main veins, the number of lobes at the leaf margins, leaf shape, stomata type, and wax structures on the leaf surface, as well as the colour of the leaf petiole.

The fruit is melon-like, oval to nearly round, somewhat pyriform, or elongated club-shaped, 15-50 cm long and 10-20 cm thick and weighing up to 9 kg (Morton 1987). Semi-wild (naturalized) plants bear small fruits 2.5-15 cm in length. The skin is waxy and thin but fairly tough. When the fruit is immature, it is rich in white latex and the skin is green and hard. As ripening progresses, papaya fruits develop a light- or deep- yellow-orange coloured skin while the thick wall of succulent flesh becomes aromatic, yellow-orange or various shades of salmon or red. It is then juicy, sweetish and somewhat like a cantaloupe in flavor but some types are quite musky (Morton 1987). Mature fruits contain numerous grey-black ovoid seeds attached lightly to the

Table 2 Commonly cultivated papaya varieties and their description.

Common Varieties	Description
Solo	High quality selection with reddish-orange flesh. Fruit weight is about 500 g. Commercially propagated in the Philippines. Pear-shaped.
Cavity special	A semi dwarf type that blooms 6-8 months after planting. Fruit is large, oblong and weighs from 3-5 kg. It has a star shaped cavity and the flesh is yellowish orange.
Red Lady papaya	Tolerant to *Papaya ringspot virus*, fruits are short-oblong on female plants and long shaped on bisexual plants, weighing about 1.5-2 kg.
Sinta	First Philippine bread papaya, moderately tolerant to ringspot virus, It is semi-dwarf, therefore easy to harvest. Fruit weighs about 1.2-2 kg.

Source Grow papaya: Mimeographed Guide. Bureau of Plant Industry, Manila.

flesh by soft, white, fibrous tissue. These corrugated, peppery seeds of about 5 mm in length are each coated with a transparent, gelatinous aril. 'Sunset Solo', 'Kapoho Solo', 'Sunrise Solo', 'Cavity Special', 'Sinta' and 'Red Lady' are commonly known Philippine varieties (**Table 2**).

Papaya grows best in a well drained, well aerated and rich organic matter soil, pH 5.5-6.7 (Morton 1987). Waterlogging of soils often results in the death of trees within 3-4 days (Storey 1985). The plants are frost-sensitive and can only be grown between latitudes 32′ N and S (Litz 1984), with optimal growth at 22-26°C and an evenly distributed rainfall of 100-150 cm. Some, however, are able to survive the high humidity of equatorial zones. Samson (1986) claimed that the best fruit develops under full sunlight in the final 4-5 days to full ripeness on the tree. Among five treatments, papaya intercropped with feijão-de-porco (*Canavalia ensiformis*) or mucuna-preta (*Stizolobium aterrinum*) improved the growth and yield of plants (Vieira Neto 1995).

Papayas are usually grown from seeds. Unlike the seed of many tropical species, papaya seed is neither recalcitrant nor dormant and are classified as intermediate for desiccation tolerance (Ellis *et al*. 1991). Germination occurs within 2-4 weeks after sowing. While seeds may be sowed directly in the orchard, some orchards are started with established seedlings (6-8 weeks after germination). Whether direct seeding or transplanting is practiced, a number of seeds or transplants are sown per planting site since the sex of a given plant cannot be determined for up to 6 months after germination (Gonsalves 1994), although molecular methods for detection are now available (Gangopadhyay *et al*. 2007). At this time, plants are thinned to achieve the desired sex ratio and to reduce competition between plants, which would later affect fruit production (Chia *et al*. 1989). For dioecious varieties, a ratio of one male to 8-10 female plants is recommended to maximise yield (Nakasone and Paull 1998; Chay-Prove *et al*. 2000) whereas one bisexual plant is left in each planting position.

Vegetative propagation of papaya is possible but is not widely practiced except in South Africa where rooting of cuttings is used to eliminate variability in some papaya varieties. Allan (1995) and Allan and Carlson (2007) showed how a female clone 'Honey Gold' could be vegetatively propagated, by rooting leafy cuttings, for over 40 years. These authors claimed that vigorous stock plants, strict sanitation, adequate bottom heat (30°C), and even distribution and good control of intermittent mist to ensure leaf retention, are crucial for success. Allan and Carlson (2007) also indicated that suitable rooting media consisted of either perlite or well composted, mature pine bark of varying air filled porosity (9-30%) and water holding capacity (58-82%). Up to 75-95% rooting of small to medium-sized leafy cuttings could be achieved in six to ten weeks during summer, but slow and poor rooting (20% after 16 weeks) occurred in certain bark media. The latter was attributed to insufficient bottom heat, different physiological conditions in spring, or toxic compounds other than high levels of tannin. Bacterial infection was also regarded a limiting factor to the success of the procedure. It was noted that well-rooted cuttings resulted in excellent production of uniform quality fruit that commanded premium prices in South Africa. Allan and MacMillan (1991) had, in earlier studies, reported on rooting of cuttings in a mist bed following immersion in a solution of fungicides (2 mg/L dithane and 1 g/L benlate), a 20-min drying period, and a dip in a commercial IBA rooting powder:captan:benlate mix at 9:2:2.

Papaya trees are fast-growing and prolific and can often result in widely-separated internodes; the first fruit is expected in 10-14 months from germination and in general the fruit takes about 5 months to develop. Soil application of paclobutrazol, a growth retardant, at 1000 mg/L resulted in reduced overall height and reduced height at which first flowers bud; it did not affect the start of production or yield (Rodriguez and Galán 1995). Fruit production may occur following either self-pollination or cross-pollination and is affected by pollinator efficiency or abundance. Honeybees, thrips, hawk moths have been reported as pollinators of papaya (Garrett 1995). Although the floral morphology in papaya plants suggests insect pollination, various authors have indicated that wind pollination may also be important (Naskasone and Paull 1998).

Details on planting distances and general agronomic practices can be found in Morton (1987).

PESTS AND DISEASES

As with many tropical crops, papaya is host to various species of pests and pathogens. In 1990, Singh reported that of the 39 arthropods that infest papaya, 4 insect and mite species are major pests of papaya. More important than mite and insect pests are pathogens that reduce plant vigour and affect fruit quality (OECD 2003). In most regions papaya, which is classified as a perennial, is grown as an annual given the reduction of productive years to 1-2 years because of parasitic infestations. A description of the major pests and diseases and strategies adopted for their management are reviewed.

The major pests that attack papaya foliage, fruit and roots include fruit flies, the two-spotted spider mite, the papaya whitefly (*Trialeuroides varibilis*), and nematodes (Morton 1987, Nishina *et al*. 2000).

Papaya fruit fly (*Toxotrypana curvicauda*) is the principal insect pest of *C. papaya* throughout tropical and subtropical areas. The insect deposits its eggs in the papaya fruit. After about 12 days, the larvae emerge and feed on the developing seeds and internal portions of the fruit. Infested fruits subsequently turn yellow and eventually fall from trees pre-maturely (Mossler and Nesheim 2002). However, the major problem affecting production is not the damage to the fruit but rather that fruits from regions with fruit flies cannot be exported to regions that do not have these pests unless they are previously given a postharvest hot-water treatment (Reiger 2006). Control with insecticides targeted to the adult fly is difficult. Mechanical protection can be achieved by covering young fruits with paper bags at an early stage (after the flower parts have fallen off). However this is not a feasible practice on large commercial orchards since it is a laborious procedure, requires regular monitoring and fruits can easily be damaged unless handled carefully. Work into the feasiblity of using parasitic wasps as biocontrol agents is being conducted (Nishina *et al*. 2000).

Feeding damage of mites has a major impact on the health and longevity of the papaya orchard. These pests, *Tetranychus urticae*, *Tetranychus kansawi* and *Brevipalpus californicus*, feed by penetrating plant tissue with their piercing mouth parts and are generally found on the under surface of leaves where they spin fine webs. Eventually small chlorotic spots develop at the feeding regions and with continued feeding, the upper surface of leaves exhibit a stippled bleached appearance. Uncontrolled infestations can initially result in yellow or bronze canopies and later in complete defoliation (Fasulo and Denmark 2000). Scarring of fruits

has also been documented, particularly during cool weather (Morton 1987). Applications of insecticides with miticidal properties are used to keep populations under control (Morton 1987; Nishina *et al*. 2000). Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (or BTH), a non-pesticidal chemical, could control *Phytophthora* root-rot and blight (or PRB) on *C. papaya* seedlings (Zhu *et al*. 2002).

The papaya whitefly, *Trialeuroides variabilis*, is also a major pest of the leaves of papaya trees. Damage to papaya caused by *T. variabilis* is similar to the damage commonly caused by whiteflies in other crops with heavy infestations; the leaves fall prematurely, fruit production is affected, and their secretions promote the growth of sooty mold on foliage and fruits (Reiger 2006). *T. variabilis* is widely distributed in the Americas from the USA to Brazil and is a pest of papaya in Florida (Culik *et al*. 2003), the Caribbean (Pantoja *et al*. 2002) and recently, Brazil (Culik 2004). Infested leaves are usually removed and appropriate pesticides applied to orchards.

The nematodes namely, *Rotylenchulus reniformis*, *Meloidogyne* spp., *Helicotylenchus dihysteria*, *Quinisulcius acutus*, and *Criconemella* spp. have been reported associated with the roots of papaya plants. However only two genera, *Meloidogyne* spp. and *Rotylenchulus reniformis*, appear to be economically significant to papaya production (El-Borai and Duncan 2005). Yield losses to these nematodes of up to 20% have been reported in Hawaii (Koenning *et al*. 1999). Affected trees typically exhibit stunting, premature wilting, leaf yellowing, and malformed roots (Pernezny and Litz 1993). Few reports on management of field infestations of nematodes are available. Generally, heavily infested lands are avoided and seedlings transplanted to raised mulched beds that have been fumigated (Nishina *et al*. 2000).

Other pests, that occasionally limit papaya production, include the Stevens leafhopper (*Empoasca stevensi*), scale insects *(Pseudaulacaspis pentagona*, *Philephedra tuberculosa*), mealy bugs (*Paracoccus marginatus*), thrips (*Thrips tabaci*) and papaya web-worm, or the fruit cluster worm (*Homolapalpia dalera*) (Morton 1987). The leafhopper induces phytotoxic reactions in papaya that is manifested as browning of leaf tips and edges. Mealy bugs, scale insects, and thrips produce scars on the skin of fruits. Papaya webworm eats into the fruit and stem and leads to infections with anthracnose. Cucumber fly and fruit-spotting insects also feed on very young fruits, causing premature fruit drop. Although aphids do not colonize papaya plants and are considered minor pests, they are a serious threat to papaya production given their ability to transmit virus diseases, in particular *Papaya rinspot virus*. Aphid species composition appears to be associated with the types of weeds as well as commercial crops growing in the vicinity of papaya orchards. *Myzus* spp and *Aphis* spp are generally prevalent.

Papaya is susceptible to more than a dozen fungal pathogens. Phytophthora (*Phytophthora palmivra*) root and fruit rot, anthracnose (*Collectricum gloerosporioides*), powdery mildew (*Oidium caricae*) and black spot (*Asperisporium caricae*) are, however, the more important fungal pathogens (Zhu *et al*. 2004).

Phytophthora rot or blight is a common disease of papaya particularly in rainy periods and in heavy, poorly-drained soils. *Phytophthora palmivora*, the etiological agent, attacks the fruit, stem, and roots of papaya plants. The first manifestations of root rot are seen in the lower leaves. These leaves turn yellow, wilt, and fall prematurely whereas the upper leaves turn light green. New leaves are generally smaller than usual and form a clump at the top of the plant. Germinating spores of *P. palmivora* also attack lateral roots, causing small reddish-brown lesions that spread and eventually result in a soft necrotic root system. Leaning or fallen plants with small tufts of yellow-green leaves are typical symptoms of *Phytophthora* rot. Stem cankers cause leaves and young fruit to fall prematurely. Infected fruits show water soaked lesions covered with mycelial and sporangial masses (Nishijima 1994). Fruit rot of papaya was first reported in 1916 in the Philippines and has since been attributed to root, stem and fruit rot in many countries including Australia, Brazil, Costa Rica, Hawaii and Malaysia. Measures of escape, exclusion and eradication are recommended for the control of *Phytophthora* rot.

Root-rot by *Pythium* sp. is very damaging to papayas in Africa, India (Morton 1987), Mexico (Rodriguez-Alvarao *et al*. 2001), and Brazil, to name a few. *P. ultimum* causes trunk rot in Queensland. Young papaya seedlings are highly susceptible to damping-off, a disease caused by soil-borne fungi, *Pythium aphanidermatum*, *P. ultimum*, *Phytophthora palmivora*, and *Rhizoctonia* sp., especially in warm, humid weather. Disease symptoms include the initial development of a watery spot in the region of the collar of plants which increases over time leading to lodging and eventually death. The disease occurs sporadically in nurseries and also in seedlings that have been recently transplanted in the field. Pre-planting treatment of the soil is the only means of prevention (Morton 1987). Collar rot in 8- to 10-month old seedlings, evidenced by stunting, leaf-yellowing and shedding and total loss of roots, was first observed in Hawaii in 1970, and was attributed to attack by *Calonectria* sp. *Rhizopus oryzae* is commonly linked with rotting fruits in Pakistan markets. *R. nigricans* injured fruits are prone to fungal rotting caused by *R. stolonifer* and *Phytophthora palmivora*. Stem-end rot occurs when fruits are pulled, not cut, from the plant allowing the fungus, *Ascochyta caricae*, to enter. Trunk rot is caused when this fungus attacks both young and older fruits. A pre-harvest fruit rot caused by *Phomopsis caricae papayae* was described in India in 1971 (Dhinga and Khare 1971). In Brazil, Hawaii and other areas, the fungus, *Botryodiplodia theobromae*, causes severe stem rot and fruit rot (Morton 1987). *Trichothecium* rot (*T. roseum*) is evidenced by sunken spots covered with pink mold on fruits in India. Charcoal rot, *Macrophomina phaseoli*, is reported in Pakistan.

Anthracnose, caused by *Colletotrichum gloeosporiodes* (Penz.), primarily affects papaya fruit and is an important postharvest disease in most tropical and subtropical regions. Disease symptoms begin as small water-soaked spots on ripening fruit. Over time, the spots become sunken, turn brown or black, and may enlarge to about 5 cm in diameter. Pinkish orange masses of mycelia and spores cover the central regions of older spots. The spots are frequently produced in a concentric ring pattern. The fungus can grow into the fruit, resulting in softening of the tissue and an off flavour of the pulp. Another lesion formation is also associated with *Colletotrichum* infection. Slightly depressed reddish brown irregular to circular spots ranging from one to 10 mm in diameter develop on fruits. These chocolate spots eventually enlarge to 2 cm and form the characteristic circular sunken lesions (Dickman 1994). Leaf infection can occur. Infection begins with the appearance of irregularly shaped small water-soaked spots. These eventually turn brown with gray-white centers which often fall out (Simone 2003). In addition to causing leaf spots and defoliation, stem lesions, collar rots, and damping off are also associated with *C. gloesporiodes*; resulting in severe papaya seedling losses (Uchida *et al*. 1996). Because anthracnose is such a potentially damaging disease, an effective fungicide spray program at the beginning of fruit set is initiated and continued during fruit production.

A disease resembling anthracnose but which attacks papayas just beginning to ripen, was reported in the Philippines in 1974. The causal agent was identified as *Fusarium solani* (Quimio 1976).

Powdery mildew, caused by three species of *Opidium*; *Oidium caricae* (the imperfect state of *Erysiphe cruciferarum* the source of mildew in the Cruciferae), *O. indicum*, and *O. caricae–papayae* has been reported in many papaya producing regions (Morton 1987; Ventura *et al*. 2004). Another powdery mildew caused by *Sphaerotheca humili* is reported in Queensland and by *Ovulariopsis papayae* in East Africa. Angular leaf spot, a form of powdery mildew, is linked to the fungus *Oidiopsis taurica*. The disease is ea-

sily recognized by the growth of white, superficial mycelia that gives a distinct powdery appearance on leaf surfaces. Initially, tiny light green or yellow spots develop on the surfaces of infected leaves. As the spots enlarge, the mycelia and spores of the fungus appear. Stem, flower pedicels, and fruit can also be affected. This common disease generally causes little damage or yield loss. However, serious damage to seedlings occurs during rainy periods (Ooka 1994). Management is generally achieved by the application of fungicides.

Black spot is a common disease occurring on the leaves and fruit of papaya. *Asperisporium caricae* (Speg.) Maubl., the etiological agent, has been reported in the USA, Central and South America, Asia, Africa, Oceania (EPPO 2005), and recently in the Phillipines (Cumagun and Padilla 2007). Symptoms of this disease are irregular dark brown to black fungal spots on the lower surfaces of older papaya leaves and round light-brown spots on upper leaf surfaces. Typically foliar damage by the fungus is minimal unless there is a heavy infection and or the infestation with other diseases and arthropods (e.g. powdery mildew and mites). Curling and drying of the lower leaves and defoliation can occur. Similar black spots have also been observed on the surface of fruits but at lower incidences than those found on the foliage. The lesions are epidermal and do not affect the fruit pulp. Although fruit damage is mainly cosmetic, the commercial value is reduced. Periods of wet weather may increase the development of black spot and necessitate the need for fungicides.

Of note, black spot disease of papaya should not to be confused with "black spot of papaya" caused by *Cercospora papayae*. Leaf spots of *C. papayae* are grayish white (Nishijima 1994) compared to the dark brown to black spots of *A. caricae*. Black spot, resulting from infection by *Cercospora papayae*, causes defoliation, reduces yield, and produces blemished fruit. Corynespora leaf spot, or brown leaf spot, greasy spot or "papaya decline" which induces spotting of leaves and petioles and defoliation in St. Croix, Puerto Rico, Florida and Queensland, is caused by *Corynespora cassiicola* (Morton 1987).

Transgenic strategies developed against some of the fungal diseases are dicussed in the transgenic section of the review.

Three bacterial diseases have been found associated with papaya since the mid 1950s. The diseases are, however, limited in distribution to Brazil and Hawaii and are not generally of any major global consequence to papaya production. More recently Papaya bunchy top (PBT) has been described. Various pathogens have been assumed responsible for PBT over the years; a virus, a mycoplasm-like organism, and in the late 1990s, a bacterium (Davis *et al*. 1996).

Bacterial leaf spot was first recorded in the state of Rio Janeiro, Brazil, in the mid 1950s and since then has been described in Hawaii and Australia (Cook 1975). Recent outbreaks in the state of Parana, Brazil, were described on nursery and field plants (Ventura *et al*. 2004). The causal agent, a gram negatuive, rod shaped bacterium *Pseudomonas carica-papayae* Robbs, is mainly a parasite of foliage where it induces small circular to angular dark green water soaked lesions on the lower surface of leaves. The lesions eventually coalesce into larger necrotic areas. Milky bacterial exudates are often visible during periods of high humidity. Despite sporadic occurrence, *Pseudomonas carica-papayae* Robbs can cause the death of plants particularly young nursery plants. Management of bacterial leaf spot is dependent on the use of clean seeds, copper-based sprays, removal of infected plant parts, and roguing.

Internal yellowing and Purple stain fruit rot are aptly named bacterial diseases of papaya that cause discoloration and rotting of ripening papaya fruits (Nishijima 1994). Internal yellowing has been described only in Hawaii whereas Purple stain fruit rot has been described in both Hawaii and Brazil.

Internal yellowing is caused by the Gram-negative, rod shaped, facultative anaerobe, *Erwinina cloacae* (Nishijima *et al*. 1987). Generally tissue around the seed cavity of infected fruits is soft, yellow in colour, and gives off an offensive rotting odor. No external fruit symptoms are however visible. In some cases the vascular tissue at the stem end is affected and also appears yellow. Jang and Nishijima (1990) showed that the oriental fruit fly, *Dacus dorsalis*, is attracted to the bacterium and is the likely vector. Presumably after transmission to papaya flowers, *E. cloacae* remains quiescent until symptom expression at full fruit maturity.

Purple stain fruit rot is also an internal fruit disease (Nishijima 1994). Typically, the pulp of ripening diseased fruits is soft and appears reddish purple without the expression of external symptoms. However, some reports note that infected fruit can be identified just before harvest as yellowing of the fruit skin is not uniform. Sporadic disease incidence is typically found but high incidences are reported during the cooler months of January and February. A vector has not been implicated in the spread of the causal agent. Management of both diseases, Internal yellowing and Purple stain fruit rot, focuses on the removal of infected fruits in the field and sanitation of thermal treatment tanks and installations at packing houses (Ventura *et al*. 2004).

Bunchy top (PBT) is a devastating disease of papaya in the American tropics (Davis 1994). PBT was first reported in Puerto Rico in the early 1930s (Cook 1931), Jamaica (Smith 1929) and the Dominican Republic (Ciferri 1930). Today, PBT can be found in many other Caribbean islands, from Grand Bahama in the north and southward in Trinidad and South America. Symptoms of PBT start with the faint mottling of the upper leaves of the canopy followed by chlorosis (especially in the interveinal regions) and reduced growth of leaves and petioles. Eventually the internodes shorten, petioles assume a horizontal position, and apical growth ceases, resulting in the trees exhibiting the characteristic the "bunchy top" appearance (Davis 1994). Of note, PBT is distinguishable from boron deficiency by the fact that the tops of affected plants do not ooze latex when wounded. Two leaf hoppers, *Empoasca papayae* Oman and *E. stevensi* transmit the PBT agent. *Empoasca papayae* is reported as the primary vector in Puerto Rico, the Dominican Republic, Haiti, and Jamaica, *E. papayae* and *E. dilitara* in Cuba, and *E. stevensi* in Trinidad (Morton 1987). In 1996, symptomatic papaya samples from 12 countries were tested by polymerase chain reaction (PCR) for the presence of 16S rRNA genes of phytoplasmas and transverse sections of petioles examined by epifluorescence microscopy (Davis *et al*. 1996). All samples were negative in PCR but rod-shaped, laticifer-inhabiting bacteria were consistently detected in infected materials and not healthy samples. Later studies showed that the PBT-associated bacterium is related to members of the *Proteobacteria* in the genus *Rickettsia* (Davis *et al*. 1998). This was the first example of *Rickettsia* as a plant pathogen. Rickettsias are small Gram-negative bacteria that are generally intracellular parasites.

Management of PBT currently involves the use of tolerant papaya varieties, removal of inoculum sources, topping of trees below the point of latex exudation, and vector control. Antibiotic therapy has proven effective only under experimental conditions (Davis 1994).

Viruses belonging to 6 taxonomic groups can infect and induce diseases of varying economical importance in papaya but *Papaya ringspot virus* (PRSV) is by far the most serious of the virus diseases (Fermin and Gonsalves 2003). Early literature reports PRSV in the Caribbean since the 1930s. In the 1940s, Jensen reported that the first papaya disease attributed to a virus was recognised by Smith in Jamaica in 1929 (Jensen 1948). Later accounts detail similar incidents between mid 1930s and 1940s in Trinidad, Cuba, and Puerto Rico (Jensen 1948). The virus has since been recognized in many tropical and subtropical areas including the USA, South America, Africa (Costa *et al*. 1969; Purcifull *et al*. 1984), India (Khurana 1975), Thailand, Taiwan, China, the Philippines (Gonsalves 1994), Mexico (Alvizo and Rojkind 1987), Australia (Thomas and Dodman 1993), Japan (Maoka *et al*. 1995), and the French Polynesia

and Cook Islands (Davis *et al*. 2005).

The disease in papaya is caused by the type p strain of PRSV (Purcifill *et al*. 1984). Typical symptoms of PRSV include mosaic and distorted leaves, stunted trees, drastically reduced fruit yield, and small fruits with ringspotting blemishes (Purcifull *et al*. 1984). Symptom expression is highly influenced by environmental conditions. Symptoms are more severely expressed during cooler months (Gonsalves and Ishii 1980).

PRSV is sap transmissible and reported to be vectored by many species of aphids, including *Myzus persicae*, *Aphis gossypii*, *A. craccivora*, and *A. maidis* in a non-persistent manner (Purcifull *et al*. 1984). This mode of transmission is characterised by a short acquisition period followed by rapid loss of infectivity (Purcifull *et al*. 1984). An entire papaya orchard can become completely infected with PRSV in three to four months (Gonsalves 1994, 1998). Losses up to 70% have been reported in some regions (Barbosa and Paguio 1982). Although transmission is widely shown to be by aphid vectors, one study in the Philippines reported seed transmission of PRSV (Bayot *et al*. 1990). Two of 1355 seedlings (0.15%) from fruit of an infected tree were reported to develop symptoms of PRSV six weeks after emergence.

Much of the characterisation of PRSV was done with strains from Hawaii (Quemada *et al*. 1990; Yeh *et al*. 1992). These strains have been completely sequenced. The virus is classified as a *Potyvirus*, in the family *Potyviridae* and consists of 800-900 nm-long filametous particles, with a ssRNA genome of about 10,326 nucleotides (Yeh and Gonsalves 1985).

Growing papaya presently involves a combination of quarantine and cultural practices aimed at reducing sources of PRSV infection. These include restricted movement of papaya seedlings, scouting of orchards and the prompt removal of infected trees. By adapting integrated crop manage-ment practices, Flores Revilla *et al*. (1995) showed how a complex set of strategies could increase yield from 17 ton/ha in control plots to 28 ton/ha in Mexico. These strategies were: 1) Seedbeds covered with an insect proof polypropylene mesh; 2) High density papaya plantings (2222 plants/ ha) which allowed roguing of diseased plants; 3) foliage and soil nutrients to improve plant vigor; 4) poisoned plant barrier (two lines of corn (*Zea mays*) and two of *Hibiscus sabdariffa* L.); 5) Two plastic strips, 5 cm wide and with a shiny gray-metallic color above each papaya row of plants; 6) Biweekly sprays with 1.5% mineral oil. However, these measures are only effective in regions where disease pressure is low. Cross protection was investigated in the 1980s as a potential method for managing the PRSV (Yeh and Gonsalves 1984; Yeh *et al*. 1988). The procedure essentially involves inoculating papaya seedlings with a mild strain prior to transplanting in orchards. A nitrous acid-induced mutant (PRSV HA 5-1) from Hawaii was developed as a protectant strain. Cross protection with PRSV HA 5-1 is highly successful in Hawaii but the procedure was moderately successful against PRSV strains in Taiwan and not successful in Thailand. Subsequent studies have verified that the level of protection with PRSV HA 5-1 is variable and dependent on the geographic region in which it is used. In greenhouse evalu-ations, 'Sunrise solo' seedlings previously challenged with PRSV HA 5-1 were challenged with PRSV from 11 geogra-phical regions (Tennant *et al*. 1994). Complete resistance, delay in symptom expression and symptom attenuation were observed againt virus from the Bahamas, Florida, and Mexico but a shorter delay in symptom development and no symptom attenuation with virus from Brazil and Thailand. It was, therefore, concluded that the method using PRSV HA 5-1 would not likely translate to significant protection under field conditions in other countries. Moreover, given the potential disadvantages of cross protection such as the adverse effects of the protectant strain on the host, dissemination to other crops, and the probability of revertants (Yeh and Gonsalves 1994), alternative methods of genetic resistance are considered more attractive.

Various PRSV tolerant papaya cultivars are available in Florida-'Cariflora' (Conover *et al*. 1986), Thailand – 'Thapra' (Prasartsee *et al*. 1995), and Taiwan-'Red Lady' and 'Known You No. 1' (Story 2002). Tolerant selections may become infected with the virus but remain symptomless or show mild symptom expression and produce economically useful yields (Gonsalves 1994). The horticultural characteristics of these tolerant selections vary from the small (0.5-0.75 kg) sweet yellow flesh fruits of 'Cariflora' to the larger (1-3 kg), light to deep yellow-fleshed fruits of 'Thapra' (Prasartsee *et al*. 1995; Gonsalves *et al*. 2005) and 'Known You No. 1', and red fleshed fruits of 'Red Lady' (Gonsalves *et al*. 2005). The reactions of tolerant varieties to PRSV isolates are also known to vary and depend on the challenge virus strain. In one study with tolerant germplasm and PRSV isolates from Jamaica, diverse reactions dependent on the challenge isolate and disease pressure were observed in infectivity assays under greenhouse conditions (Turner *et al*. 2004). Useful reactions of no symptoms or mild symptom expression were obtained with tolerant cultivars from Taiwan ('Red Lady'), Thailand ('Thapra') and Florida ('Cariflora'). In subsequent field evaluations, diverse reactions were observed and included no foliar or fruit symptom expression, mild foliar and some fruit symptom expression and severe symptom expression on both foliage and fruits. The varieties 'Thapra' and 'Red Lady' exhibited useful levels of tolerance and good agronomic characteristics, such as good skin and acceptable brixes (Turner *et al*. 2004).

Resistance against PRSV has not been found in *C. papaya*. However, much effort is being expended to introduce resistance genes from other genera in the Caricaceae even though the resistance appears to be variable and dependent on the geographic origin of the virus and environmental conditions (Gonsalves *et al*. 2005). In the 1960s and 1970s, monogenic resistance against PRSV was identified in several *Vasconcella* species; namely, *V. cundinamarcensis* (formerly *pubescens*), *V. stipulata*, *V. candicans*, *V. quercifolia*, and *V. heibornii nm pentagona* (Conover 1964; Mekako and Nakasone 1975). Later research in the 1990s in Hawaii involved interspecific crosses and employed *in vitro* embryo rescue or ovule culture techniques in an attempt to rescue hybrid embryos of nonviable seeds (Manshardt and Wenslaff 1989). Regenerated F_1s of *C. papaya* x *V. cundinamarcensis* showed excellent field resistance to PRSV while similarly grown commercial papaya were all infected with the virus. However, the F_1s were sterile and backcrosses resulted in sesquidiploids with reduced resistance. Similar studies in the 1990s in Australia have been conducted with local varieties and *V. cundinamarcensis* and *V. quercifolia* using refined protocols of hybridization and embryo rescue (Magdalita *et al*. 1996, 1997, 1998; Drew *et al*. 2006a). Seventy five to 100% of the hybrid progenies of *V. quercifolia and V. cundinamarcensis*, respectively, were resistant to PRSV. Backcross breeding was initiated with hybrid progeny of *V. quercifolia* and in 2006, the first report of a fertile backcross was published (Drew *et al*. 2006b). BC_1 and BC_2 were generated in Australia and the Philippines. Marketable fruits were obtained from BC_2 trees. As for the levels of resistance against PRSV, 13% of the BC_2 plants remained symptomless under greenhouse conditions and repeated inoculations with virus. On transfer to the field in Australia, the asymptomatic plants, however, developed symptoms of severe infection after 9 months. It was concluded that more than one gene is responsible for resistance in *V. quercifolia*. In later studies (Drew *et al*. 2007) using a bulked segregant analysis strategy, a polymorphic randomly amplified DNA fingerprint (RAF) marker was shown to be linked to the PRSV-P resistant phenotype and was shown to be present in other PRSV-P resistant *Vasconcellea* species. It mapped to within 6.3 cM of the predicted PRSV-P resistance locus. The RAF marker was converted into a co-dominant CAPS marker, diagnostic for resistance based on digestion with the restriction endonuclease *Psi*I.

Although considerable progress has been made in trans-

ferring natural resistance against PRSV from *Vasconcella* to commercial papaya varieties, it may be some time before a variety is available in commerce. The use of *Vasconcella* as the source of germplasm to introduce resistance against PRSV has added advantages. *Vasconcella* is also a source of resistance genes against *Phytophthora* in *V. goudotiana* and pawpaw dieback in *V. parviflora* (Drew *et al*. 1998), blackspot and cold-tolerance in *V. pubescens* (Manshardt and Wenslaff 1989). Despite the discovery of the latter in the form of cold-inducible sequences, Dhekney *et al*. (2007) believe that transformation of papaya with the C repeat binding factor (CBF) genes may not be a viable strategy for inducing cold-tolerance in papaya. Alternatively, the introduction of PRSV resistance in papaya and other traits by genetic engineering is being investigated. Details on genetic engineering of papaya involving the transfer and expression of PRSV coat protein gene and other genes in transgenic papaya are discussed later in the review.

After PRSV, three viruses, *Papaya lethal yellowing virus*, *Papaya droopy necrotic virus* and *Papaya meleira virus*, are considered important in papaya production (Ventura *et al*. 2004).

Papaya lethal yellowing virus was first described in Brazil in the early 1980s (Loreto *et al*. 1983). Since then, the virus has not been documented in other regions. PYLV was first described as a member of the family *Tombusviridae*, genus *Carmovirus* but Silva (2001) later suggested that the virus should be a member of the family *Sobemoviridae*, genus *Sobemovirus*. PYLV is an isometric virus with a diameter of 25-30 nm and a ssRNA genome (Silva 2001). Studies with 26 greenhouse species indicated that PLYV is strictly limited to the host *C. papaya* (Lima *et al*. 1994). Amaral *et al*. (2006) later showed that PLYV also infects *Vasconcellea cauliflora* (Jacq.) A. DC. (previously *Carica cauliflora* (Jacq.). Initial infection with the virus manifests as yellowing of the upper leaves of trees and later progresses to more severe symptoms of curled leaves, wilting and senescence. Green blemishes are commonly found on immature fruits and they turn yellow as the fruits mature (Lima *et al*. 2001). PLYV is transmitted mechanically and can be found in the soil (Camarco-Rosa *et al*. 1998). Management of the disease is limited to quarantine, roguing and sanitation.

Papaya apical necrosis virus (PANV), caused by a *Rhabdovirus*, was reported in Venezuela in 1981 (Lastra and Quintero 1981) and later in 1997 (Marys *et al*. 2000). Initial infections with PANV are yellowing of mature leaves followed by wilting of younger leaves, and necrosis and death of the apical portions of the tree (Zettler and Wan 1994). A similar *Rhabdovirus*, *Papaya droopy necrosis virus* (PDNV) occurs in Florida (Zettler and Wan 1994). Both viruses consist of ssRNA encapsidated in bacilliform particles of lengths between 230-254 nm. The viruses are documented as not being transmitted mechanically. Zettler and Wan (1994) reported that PANV is vectored by the leafhopper *Empoasca papayae*. Given the low field incidence, PANV and PADV are presently controlled by roguing diseased plants and isolating papaya plantings.

Papaya meleira virus (PMeV), causing papaya "sticky" disease, is a new and recently described virus disease of papaya (Rodrigues *et al*. 1989; Kitajima *et al*. 1993; Lima *et al*. 2001; Maciel-Zambolin *et al*. 2003). The disease was actually observed in Brazil by papaya producers in the 1970s but it was not considered a problem until the 1980s when considerable losses were reported in orchards in Bahia (Ventura *et al*. 2004). So far, the virus has only been described in Brazil. The disease is characterized by latex exudation from petioles, new leaves and fruits. Necrosis on the affected areas occurs following the oxidation of exuded latex. The silverleaf whitefly, *Bemisia argentifolii* Bell & Perring, also known as *B. tabaci* biotype B, has been associated with the transmission of PMeV under experimental conditions (Vidal *et al*. 2000). PMeV particles have been found in the latex and extract of leaves and fruit and are of isometric symmetry with a diameter of about 50 nm. The genome appears to consist of ds RNA molecules. Roguing of infected plants is currently recommended until more specific procedures are developed.

Three phytoplasma diseases are known to infect papaya; dieback (PDB), yellow crinkle (PYC) and mosaic (PM) (Simmonds 1965; Gibbs *et al*. 1996; Liu *et al*. 1996). PDB has been prevalent since the 1920s in Queensland, Australia, and for a long time the symptoms were considered to be the result of a physiological disorder (Glennie and Chapman 1976). It is currently widely accepted that the three diseases are associated with phytoplasmas (Gibb *et al*. 1996; Liu *et al*. 1996; Gibb *et al*. 1998). Phytoplasmas are similar to bacteria but they do not possess a rigid cell wall. The pathogens are limited to the phloem tissue of the plant (Siddique *et al*. 1998). The phytoplasmas associated with PYC and PM are genetically indistinguishable and have been identified as the tomato big bud and sweet potato little leaf vein which are closely related to phytoplasma diseases of faba bean. However, PDB is indistinguishable from Australian grapevine yellows and is more closely related to phytoplasma diseases of aster (Schneider *et al*. 1995; Gibb *et al*. 1998). PDB, PYC, and PM cause symptoms of stem death from the top downwards, a claw-like appreance of the crown, and yellowing and stunting, respectively (Persley 2003). *Orosius* spp., the brown leaf hopper, is the common vector of the pathogens in Australia (Padovan and Gibb 2001). Although plants infected with PDB can continue to produce fruits after they have been topped at the first sign of symptom development, the practice is not effective against PYC or PM. New growth usually develops symptoms and the trees are just as unproductive. Removal of these trees as soon as they be-come unproductive is recommended (Persley 2003).

Papaya plants grown in Israel were severely devastated by a disease named Nivun Haamir (NH) some years ago. Symptoms of NH infections were reported similar to those of PDB. Early observations of NH infected plants suggested the involvement of an airborne pathogen (Franck and Bar-Joseph 1992) but studies conducted later in 1995 using PCR demonstrated the presence of phytoplasma (Liu *et al*. 1996). An association between NH and a 'Ca. Phytoplasma australiense' isolate was recently demonstrated (Gera *et al*. 2005).

Both rickettsias and phytoplasmas have been implicated in recent outbreaks of PBT-like symptoms on papaya in Cuba (Arocha *et al*. 2003, 2006).

A comprehensive and updated list of pests and diseases can be found at the University of Hawaii homepage (http://www.extento.hawaii.edu/kbase/crop/crops/papaya.htm) and Fermin and Gonsalves (2003). Miscellaneous and abiotic diseases are covered by Ventura *et al*. (2004).

GENETICS, CONVENTIONAL AND MOLECULAR BREEDING

The somatic chromosome number in the dicotyledonous genus *Carica*, is 2n=18. Most *Carica* spp. are dioecious, except for *C. papaya* which is characterized by various flower types and three primary, polygamous sexual types, viz. pistillate (female; mm), staminate (male; M_1m) and hermaphrodite (M_2m). Intermediate types have also been described (Hofmeyr 1938; Storey 1938, 1953; Chan 1996).

The 5-petalled flowers of papaya are fleshy, waxy, cream to yellow in colour, and slightly fragrant. Flowers are borne singly or on cymose inflorescences in the leaf axils. Staminate trees produce long pendulous male inflorescences bearing 10 stamens in each flower, while pistillate trees bear one or two flowers at each leaf axil, with the absence of stamens and a large ovary with numerous ovules. Hermaphrodite trees normally bear one to several bisexual flowers characterized by an elongated, slender ovary and usually 10 stamens. Based on the sex form within a population, papaya can be grouped into either dioecious or gynodioecious, the former consisting of female and male trees, the latter of female and hermaphroditic trees. In the gynodioecious group, pollen for fertilization of the female flowers is derived from the bisexual flowers of the hermaphrodite

trees. Storey (1986) claimed that three sex forms exist in some papaya, and are thus classified as trioecious.

Segregation ratios established by the studies in the 1950s showed that males and hermaphrodites are heterozygous, females are homozygous but dominant homozygotes (M_1M_1, M_1M_2, M_2M_2) are lethal. Lethality is attributed to inert regions missing in M_1 and M_2 (Hofmeyr 1967). Essentially, there are two breeding systems in papaya (Aquilizan 1987; Manshardt 1992): a) The Hawaiian system with true-bred lines, e.g. 'Solo', established through inbreeding by pedigree or back-cross breeding; b) the Yarwun (Queensland) system in which homozygous female lines breed with inbred, ambivalent males.

In addition to the time-consuming nature of breeding in papaya, in which six generations are needed for homogenization of alleles for a particular trait (Ray 2002), there is also the problem of sex instability. Pistillate plants are generally stable while the staminate and hermaphroditic trees undergo frequent sex reversals, especially in the tropics (Storey 1976). The reversion of hermaphroditic trees to pistillate trees during heat and drought stress is particularly common (Nakasone 1967). Hofmeyr (1967) claimed that changes in photoperiod induced in sex reversal. Chemical treatment of male papaya trees with morphactin, ethephon (2-chloroethane phosphoric acid) and TIBA (2,3,5-triiodobenzoic acid) resulted in the conversion to female trees (Jindal and Singh 1976). Sex reversion was shown to be seasonal (Hofmeyer 1939; Storey 1953; Nakasone and Paull 1998; Ray 2002), and often accompanied by stamen carpellody and female sterility (Lange 1961; Nakasone and Paull 1998) and consequently poor fruit quality and low yields.

Given that the sex of papaya plants cannot be determined for up to 6 months after germination, the establishment of papaya orchards with appropriate sex ratios was a challenge up until the 1960s. Agnew, in 1968, recommended overplanting dioecious papaya seedlings and thinning seedlings at the flowering stage in order to obtain the desired male to female ratio, and reduce the unproductive male population. Chan and Teo (1992) improved on this idea by suggesting the use of papaya cultivars in which sex ratios can be predicted, such as 'Exotica'. 'Exotica' is a gynodioecious papaya cultivar in which stands grown from seeds can produce a 70.9% hermaphrodite and a 29.1% female population, thus potentially guaranteeing a 100% fruit-producing population. But since only hermaphroditic fruits are in demand for export, three seeds should be planted together, and female plants culled at the flowering stage. Magdalita *et al*. (1997) reported that one male to every 10-20 vigorous females are usually planted. Seed propagation can therefore be costly to producers given that plantations need to be renewed every three years to ensure the production of high quality fruit (Samson 1986). In South Africa (Allen 1976), Australia (Queensland; Aquilizan 1987; Drew 1988) and Okinawa, Japan female cultivars are predominantly used while hermaphrodite cultivars are used in tropical market countries, in order to avoid sex reversion. The breeding of females has its downside; there is the challenge of maintaining and propagating pure-bred cultivars (Aquilizan 1987) and the need for male plants as pollenizers (Nakasone and Paull 1998).

In addition to the variability derived from seed-derived populations, there is a high possibility of polyploidy (**Fig. 1**), aneuploidy or even chromosomal aberrations.

Somsri *et al*. (1998) first attempted the identification of molecular markers that coded for sex in papaya. They used random amplified polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF) to identify male-specific bands. Although the latter were more informative, there were difficulties in converting to SCAR markers. Using bulk segregant analysis, however, Somsri *et al*. determined that these markers were reasonably closely linked to the sex-determining alleles. Recent studies by these authors (Somsri and Bussabakornkul 2007) in which a total of 52 primers were used in bulk segregate analysis (BSA) against male, female and hermaphroditic plants. The OPA 06 (5′-GGTCCC

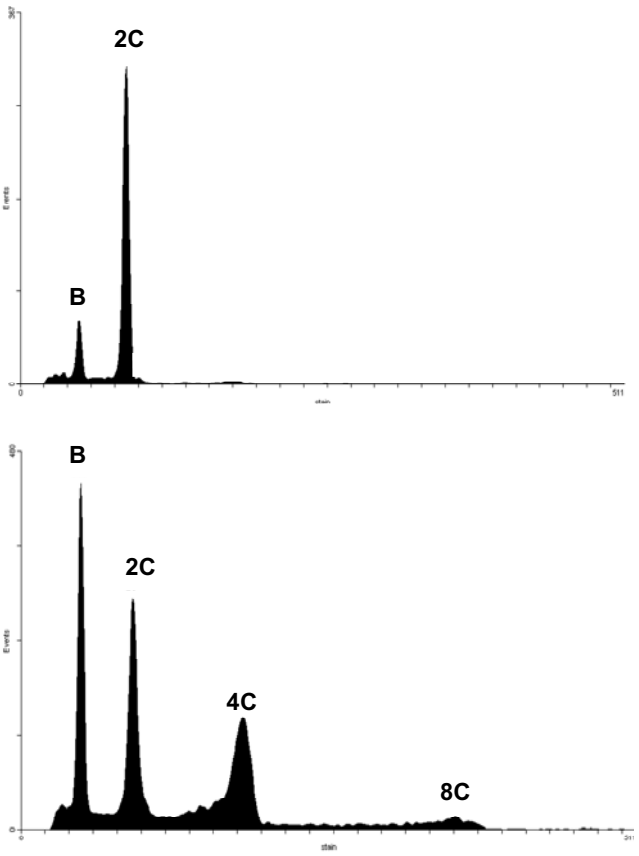

Fig. 1 Ploidy changes found in *in vitro*-grown papaya following DAPI staining. Upper histogram: control *in vitro* papaya leaves. Lower histogram: Callus induced by 1 mg/l 2,4-D resulting in endopolyploidy, as high as 8C. B = control, barley (*Hordeum vulgare*). (JA Teixeira da Silva, unpublished results).

TGAC-3′) primer could be used to identify the sex type of papaya plants. This primer produced two polymorphic bands: one of ~365 base pairs (bp) from hermaphrodite bulk DNA and the other of ~360 bp from the male bulk DNA. Neither band was detected for females. Only recently new diagnostic tools have been made available to early detection of this virus (Tavares *et al.* 2004; Araújo *et al*. 2007).

More recently, Ming *et al*. (2007) proposed that two sex determination genes control the sex determination pathway in trioecious papaya: one, a feminizing or stamen suppressor gene, causes stamen abortion before or at flower inception while the other, a masculinizing or carpel suppressor gene, causes carpel abortion at a later flower developmental stage.

A detailed description of cultivars and the origin of cultivar names can be found in Morton (1987).

THE PLANT AND FRUIT: STRUCTURE, USES AND MEDICINAL PROPERTIES

Papaya fruits are borne by both female and hermaphrodite trees, but their shapes differ. Fruits from female trees are round whereas fruits from hermaphrodite trees are elongated. The fruit is a berry that can range from 5 cm in diameter and 50 g in weight to 50 cm or longer, weighing 10 kg or more (Storey 1969). Papaya fruits are covered with a smooth thin green skin that turns to yellow or red when ripe. The flesh is succulent, varying in texture and colour ranging from yellow to orange to red.

Papaya is a major fruit crop worldwide that is primarily consumed as fresh fruit. Papaya fruits consist mostly of water and carbohydrate, low in calories and rich in natural vitamins and minerals, particularly in vitamins A and C, ascorbic acid and potassium (Chan and Tang 1979; **Table 3**). One hundred g of papaya contains: 55 calories, 0.61 g pro-

Table 3 Nutrient content of ripe papaya.

Constituent	Appropriate value	Constituent	Appropriate value	Constituent	Appropriate value
Water	89 %	Calcium	24 mg	Sodium	3 mg
Calories	39 kcal	Iron	0.1 mg	Niacin	0.34 mg
Protein	0.61 g	Phosphorous	5 mg	Pantothenic acid	0.22 mg
Fat	0.14 g	Potassium	257 mg	Vitamin A	1094 IU
Carbohydrate	9.8 g	Magnesium	10 g	Vitamin E	0.73 mg

Source: USDA Nutrient Database for Standard Reference, Release 18 (2005).

tein, 9.8 g carbohydrates, 1.8 g dietary fiber, 89% water, 283 IU vitamin A, 62 mg vitamin C, 38 mg folate and 257 mg potassium (IFAS 1998). As a result, papaya is consumed as jams, pickles, and desserts. Unripe fruit is frequently used in Thai and Vietnamese cooking, cooked as a vegetable, fermented into sauerkraut, or candied (Sankat and Maharaj 1997). In addition, fruit and seed extracts have pronounced bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella flexneri* (Emeruwa 1982). Flath and Forrey (1977) identified 106 volatile components in papaya. Fermentation with brewer's yeast and distillation yielded 4% alcohol, of which 91.8% was ethanol, 4.8% methanol, 2.2% *n*-propanol, and 1.2% an unknown (non-alcohol) (Sharma and Ogbeide 1982). Chinoy *et al*. (1994) showed extracts of papaya seeds could be used as a contraceptive in rats, specifically two principal compounds, MCP I and ECP I (the code names of the major purified compounds of methanol and ethyl acetate subfractions of the benzene chromatographic fraction of the chloroform extract of the seeds of *C. papaya*, respectively; Lohiya *et al*. 2005, 2006; N. K. Lohiya pers. comm.) demonstrated that the methanol sub-fraction or MSF of the seeds of *C. papaya*, a putative male contraceptive, could be safely used in rats as a male anti-fertility agent.

Papaya plants are also produced for papain and chymopapain, two industrially important proteolytic enzymes found in the milky white latex exuded by fruits. In general, female fruits tend to exude more papain than hermaphrodite fruits (Madrigal *et al*. 1980). The latex serves as an excellent meat tenderizer, for treatments of gangrenous wounds or burns (Starley 1999; Hewitt *et al* 2000), and is used in cosmetic products (Singh and Sirohi 1977; Knight 1980), the light industry and food processing. Papaya latex is often used as a cheap and affordable substitute for protease in high school DNA extraction experiments (Teixeira da Silva, unpublished results). Green fruits are generally better sources, containing more papain than ripe fruits. Benzyl isothiocyanate and the corresponding glucosinolate (benzyl glucosinolate, glucotropaeolin) can be found in papaya. Some of the highland papayas, whose center of origin lies in Ecuador, have latex of unripe fruit has activity 15-fold higher than *C. papaya* (Scheldeman *et al*. 2002).

Nakamura *et al*. (2007) separated papaya seed and edible pulp and then quantified the amounts of benzyl isothiocyanate and glucosinolate in both. The papaya seed (with myrosinase inactivation) contained >1 mmol of benzyl glucosinolate in 100 g of fresh weight which is equivalent to quantities found in Karami daikon (the hottest Japanese white radish) and cress.

Papaya milk latex shows anti-bacterial properties, inhibits fungal growth, especially that of *Candida albicans* (Giordani and Siepai 1991), and thus would be useful in the treatment of skin eczema caused by this fungus. Emeruwa (1982) reported that extracts from fruits showed effective anti-microbial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* sp. and *Shigella* sp. The Dutch and Malays use leaves and young fruit extracts to eradicate intestinal worms and to treat boils (Burkill 1966) while young shoots and male flowers are consumed as a vegetable dish in the Malay Peninsula. In Mauritius, the smoke from dried papaya leaves relieves asthma attacks. In Australia it is believed in some quarters that several cancer diseases can improve after drinking papaya leaf extract.

Papaya is used in tropical folk medicine. According to Reed (1976), papaya latex is very much useful for curing dyspepsia and is externally applied to burns and scalds. Okeniyi *et al*. (2007) showed that the fruit and seeds have antihelminthic and anti-amoebic activities. Packages of dried, pulverized leaves are sold by "health food" stores for making tea, despite the fact that the leaf decoction is administered as a purgative for horses in Ghana and in the Ivory Coast it is a treatment for genito-urinary ailments. The dried leaf infusion is taken for stomach troubles in Ghana and it is used as a purgative. In India, unripe and semi- ripe papaya fruits are ingested or applied on the uterus to cause abortion. Recently a study with rats at different stages of gestation showed that the consumption of unripe and semi-ripe papaya fruits could be unsafe during pregnancy given the high levels of latex in the fruits at these stages of maturity. But consumption of ripe fruits during prenancy causes no risk (Adebiyi *et al*. 2002). In addition, allergies to papaya fruit, latex, papain and papaya flower pollen exist among sensitive individuals (Blanco *et al*. 1998). IgE-mediated reactions induced by the ingestion of papaya and papain have been reported (Mansfield *et al*. 1985; Sagona *et al*. 1985; Castillo *et al*. 1996). Moreover, occupational IgE-mediated asthma induced by the inhalation of papain has been described (Tarlo *et al*. 1978; Baur and Fruhmann 1979; Novey *et al*. 1979; Baur *et al*. 1982). Externally the latex is an irritant, dermatogenic, and vescicant. Internally it causes severe gastritis. The acrid fresh latex can cause severe conjunctivitis and vesication. Anaphylaxis is reported in about 1% of cases of chymopapain injections.

Although described as a tree, the papaya plant is a large herb or soft-wood tree (1.8 to 6 meters). Generally papaya wood has very little application. It has long been used in the manufacture of rope but it was recenty shown that papaya bark can be used as a new biosorbent of heavy metals and has potential application to the treatment of waste water. Saeed *et al*. (2006) demonstrated that 97.8, 94.9 and 66.8% of 10 mg/L copper (II), cadmium (II) and zinc (II) solutions, respectively were removed with 5 g/L papaya wood during a shake flask contact time of 60 minutes.

CHEMISTRY, PHYTOCHEMISTRY AND BIOCHEMISTRY

C. papaya contains many biologically active compounds. Two important compounds are chymopapain and papain which are widely known as being useful for digestive disorders and disturbances of the gastrointestinal tract. Huet *et al*. (2006) showed that papaya-derived papain, caricain, chymopapain, and glycine endopeptidase can survive acidic pH conditions and pepsin degradation. However, at low pH, a conformational transition that instantaneously converts their native forms into molten globules that are quite unstable and rapidly degraded by pepsin. Thus, they may need to be protected against both acid denaturation and proteolysis for them to be effective in the gut after oral administration for the control of gastrointestinal nematodes.

Apart from papain and chymopapain, *C. papaya* contains many biologically active compounds. *C. papaya* lipase, or CPL, a hydrolase, is tightly bonded to the water-insoluble fraction of crude papain and is thus considered as a "naturally immobilized" biocatalyst. Domínguez de María *et al*. (2006) reviewed several applications of CPL: (i) fats and oils modification, derived from the sn-3 selectivity of CPL as well as from its preference for short-chain fatty

acids; (ii) esterification and inter-esterification reactions in organic media, accepting a wide range of acids and alcohols as substrates; and (iii) more recently, the asymmetric resolution of different non-steroidal anti-inflammatory drugs (NSAIDs), 2-(chlorophenoxy)propionic acids, and non-natural amino acids.

The papaya Kunitz-type trypsin inhibitor, a 24-kDa glycoprotein, when purified, stoichiometrically inhibits bovine trypsin in a 1:1 molar ratio (Azarkan *et al*. 2006). A novel α-amylase inhibitor from *C. papaya* seeds was recently shown to be effective against cowpea weevil (*Callosobruchus maculatus*) (Farias *et al*. 2007).

A comprehensive list of the compounds found in various parts of the papaya plant can be accessed from the USDA Phytochemical and Ethnobotanical Databases. Of note, levels of the compounds vary in the fruit, latex, leaves, and roots. Furthermore, plant parts from male and female trees have been found to differ in the amounts of the compounds produced. For example, phenolic compounds tend to be higher in male plants than female plants. Cultivars also vary in the quantity of the compounds.

POST-HARVEST MANAGEMENT OF PAPAYA

Geographic distribution and nomenclature

Postharvest losses in papaya of approximately 40-100% have been reported in developing countries (Coursey 1983). The losses are mainly due to decay, physiological disorders, and mechanical injury, the result of improper harvesting and handling practices.

Because of its thin skin, papaya is damaged very easily by handling and this can lead to infection by fungi such as *Colletotricum gloeosporioides* (Palhano *et al*. 2004), the causal agent of anthracnose and the main post-harvest disease. *Rhizopus* rot, stem-end rot and gray mold rot also affect papaya fruits during storage and transportation. Numerous physiological disorders are associated with mineral deficiencies. For example, fruits with low flesh calcium at harvest ripen at twice the normal rate. Maturity at harvest is a very important determinant of storage-life and final fruit quality; harvesting fruits at improper maturity can lead to uneven ripening and over ripe fruits (Ceponis and Butterfield 1973). A number of non-pathological disorders also

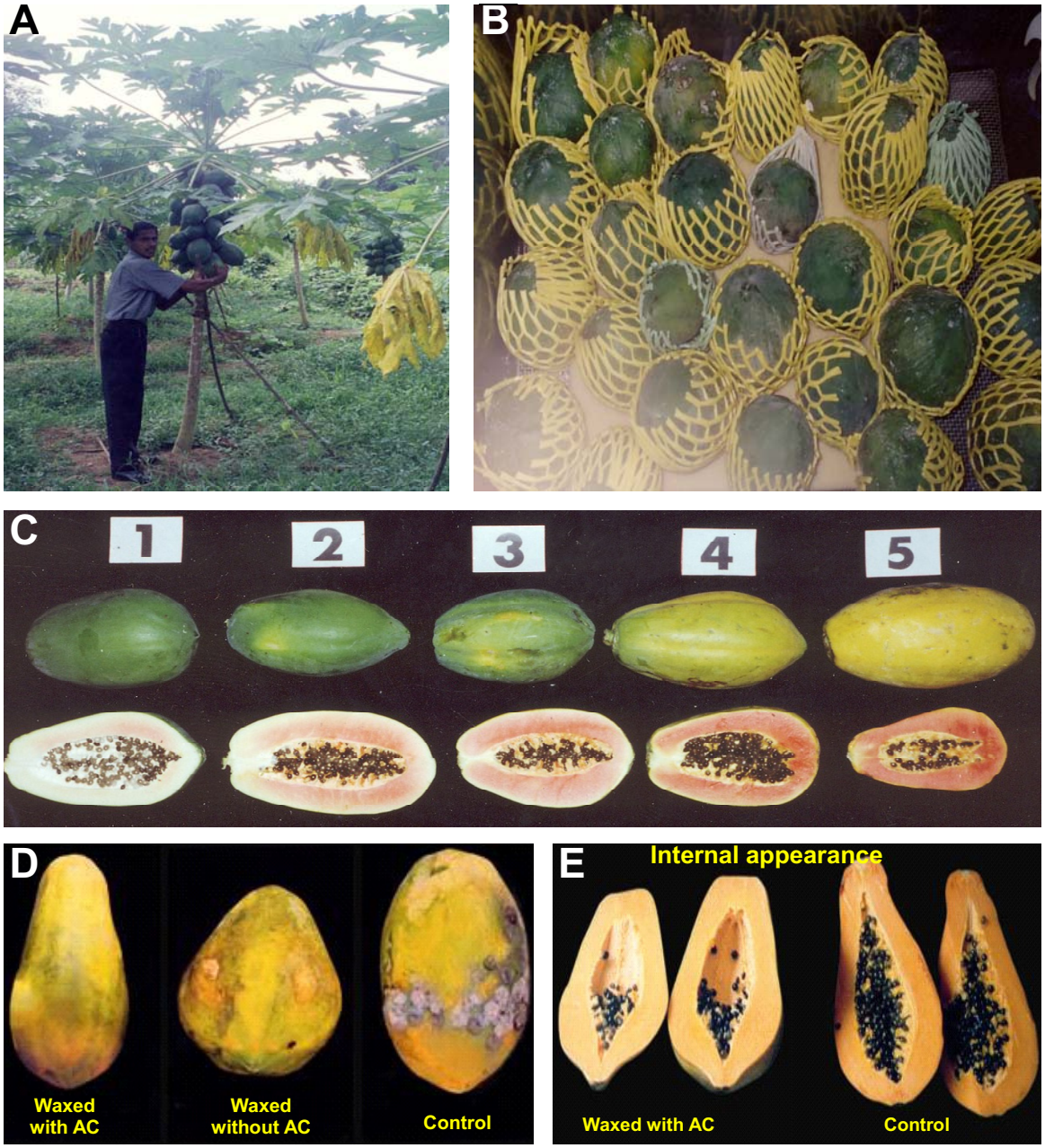

Fig. 2 Postharvest aspects of papaya. (**A**) Harvesting papaya. (**B**) Papaya fruits are sleeved with plastic netting to prevent mechanical injury due to aberration during transportation. (**C**) Harvesting maturity indices for papaya. Papaya for export must be harvested at the mature green stage (= No. 2). (**D, E**) Quality retention of papaya fruits after 14 days in cold storage. These fruits were waxed with a paraffin wax-based formulation. AC = ammonium carbonate.

contribute to quality loss, e.g., the soft fruit symptom is caused as a result of mechanical impact injury during ripening. Common mechanical injuries in papaya include sunken damage due to abrasion damage, scaring and bruising.

Postharvest defects are catogorised as; decay and mold, sunken areas on skin, discolouration, overripe, soft, scarring of the skin, bruising of flesh, brown spot on the skin, and shriveled appearance at cargo inspection.

Harvesting, handling, heat treatment, storage and ripening

With increased consumer awareness of papaya and the expansion in production and exports, papaya fruit ripening and handling research has become more important over the last decade. Major research issues are on quality retention and postharvest storage life since extreme or fluctuating temperature treatments and mechanical damage combined with improper harvesting and handling practices can result in fruit with poor appearance, flavor and nutritional value (Proulx *et al*. 2005).

Papayas are hand harvested (**Fig. 2A**) at the colour break stage or when they have started to ripen as judged by the appearance of skin yellowing. Fruits are collected in smooth surfaced plastic crates or in clean collection bags and thereafter transferred into large lug collection bins (ca. 25 L). Fruits are sorted at the field according to colour stages and defects. They are subsequently washed in packing sheds and, in some, countries subjected to vapour heat treatment (Paull and Armstrong 1994) or double dip hot water treatment to kill insects and their larvae (42°C x 30 min followed by 20 min or more at 49°C) (Nishijima 1995).

The vapour heat treatment raises the temperature of the fruit center to about 47.5°C over a period of 6-8 hours. After this treatment, fruits are cooled to 30°C in water. Hot water treatment or hot water treatment with fungicides is usually adopted to control decay (Couey and Farias 1979; Couey *et al*. 1984). Exposure of papaya fruit to high temperatures results in the disruption of softening. The pattern of ripening related events such as the change in skin colour, climacteric respiration, ethylene production, 1-aminocyclopropane-1-carboxylic acid (AAC) content, net ethylene forming enzyme (EFE) activity and internal carotenoid synthesis are also altered by the high temperature treatments. Paull (1995) suggested that the response of papaya to heat treatments depends on maturity, growing season and temperature changes. Chemical treatments can cause fruit damage and reduce the external fruit quality.

C. papaya β-galactosidase/galactanase (β-galactoside galactohydrolase; EC 3.2.1.23) isoforms, β-gal I, II and III are as softening enzymes during ripening that hydrolyze pectins while still structurally attached to unripe fruit cell wall (Lazan *et al*. 2004). In assessing flesh firmness in ripening papaya fruit, Manrique and Lajolo (2004) found that cellulose residue exhibited decreasing quantities of galacturonic acid and non-glucose monosaccharides during ripening indicating that the association between polysaccharides from matrix and microfibrilar phases may be involved in the softening process while Almora *et al*. (2004) claimed that butanol, 3-methylbutanol, benzyl alcohol and α-terpineol showed maximum concentrations in the third maturation stage, in correspondence with fruit ripeness.

If the pre-sorting was not done previously the fruits are sorted by weight and colour at this stage. Fruit is generally packed by hand and individually sleeved (**Fig. 2B**). Quality indices for papayas have been defined by the market. The export specifications adopted for papaya in India are given in **Table 4**.

Storage temperature depends on the type of papaya cultivar. The storage temperature usually ranges between 10-13.5°C. According to Chen and Paull (1986), papaya harvested at colour break stage can be stored in cold storage at 7°C for 14 days and will ripen normally when transferred to room temperature. Storage below 10°C is known to cause chilling injury (Maharajh and Shankat 1990). Symptoms of chilling injury occur in mature green fruits or in 60% yellow fruits as skin scald, hard lumps in the pulp around the vascular bundles, water soaking of flesh and high susceptibility to decay.

Papaya is a climacteric fruit and exhibits a characteristic rise in ethylene production during ripening which is accompanied by softening, change in colour (**Fig. 2C**), and the development of a strong and characteristic in aroma. The main compounds produced by the fruit are esters and alcohols. The most abundant esters are ethyl acetate, and ethyl butanoate, methyl butanonate, and butyl acetate comprising 88% of the volatiles in fully ripe fruit. Butanol is the most abundant alcohol. Among the volatiles produced, ethyl butanoate, ethyl acetate, ethyl hexaonate and ethyl 2-methylbutanoate are reported to be most potent odour compounds (Balbontìn *et al*. 2007). An increase in the abundance of alcohol has also been observed in fruits after 1-MCP (1-methyl cyclopropane) treatment (Lurie *et al*. 2002). Ethylene treated papayas ripened faster and more uniformly in terms of de-greening, softening and flesh colour development. To induce ripening in papaya, fruits must be stored between 18°C and 25°C and treated with ethylene gas at 100 ppm (0.01%) for 24 h. Under this condition, fruit will take 3-4 days to develop full yellow skin (Ann and Paull 1990). Severe weight loss and external abnormalities become more prominent at temperatures higher than 27°C. Delaying the process of fruit ripening helps to control the release of ripe fruit to the market. Treatment of fruit with 1-MCP (0.3 μL/L) for 16 h at 20°C inhibits the increase in ethylene production and the ripening process (Balbontìn *et al*. 2007). Although 1-MCP treatment reduces the production of esters in papaya, a large increment of alcohol was reported by Balbontìn *et al*. (2007). The increase in alcohol abundance has also been observed in other fruits after 1-MCP treatment (Lurie *et al*. 2002; reviewed in Lurie 2007). According to Manenoi *et al*. (2007), papayas treated with 1-MCP (100 nL/L) at colour break stage are firmer but show a rubbery texture at the ripe stage whereas fruit treated with 1-MCP at more than 25% skin yellow ripened normally. Ethephone (2-chloroethyl) phosphoric acid generates ethylene and is used commercially as a ripening agent. However, treatment of papayas with Ethephone was not successful in reducing the effect of 1-MCP on fruit firmness at the ripening stage. Papayas ('Solo') at the 20% yellow stage kept in sealed polyethylene bags for 5 days at 22°C, were significantly more firm and showed slower skin colour develop-ment than 1-MCP treated fruits (Manenoi *et al*. 2006). Moya-León *et al*. (2004) showed that treatment with 1-MCP could offset the increase in ethylene during the climacteric phase of mountain papaya (*V. pubescens*) and thus increase shelf-life.

Genes involved in papaya fruit ripening were recently identified. Devitt *et al*. (2006) generated a total of 1171 expressed sequence tags (ESTs) from randomly selected clones of two independent cDNA libraries derived from yellow and red-fleshed papaya fruit varieties. The most abundant sequences encoded: chitinase, ACC oxidase, catalase and methionine synthase. DNA sequence comparisons revealed ESTs with high similarities to genes associated with fruit softening, aroma and colour biosynthesis. Putative cell wall hydrolases, cell membrane hydrolases, and ethylene synthesis and regulation sequences were identified with predicted roles in fruit softening. Expressed papaya genes associated with fruit aroma included isoprenoid biosynthesis and shikimic acid pathway genes and proteins associated with acyl lipid catabolism. Putative fruit colour genes were identified based on similarities with carotenoid and chlorophyll biosynthesis genes from other plant species. Devitt *et al*. (2007) identified candidate genes that are differentially expressed during papaya fruit ripening in 'Tainung' (red-fleshed) and 1B (yellow-fleshed) hybrid varieties. In all, 1022 ESTs were searched, identifying seven putative carotenoid and aroma biosynthesis genes. Colour complementation identified papaya cDNA clones with significant homology to three carotenoid pathway genes and gene expression analysis of these genes in two colour-contrasted papaya

Table 4 Specific requirements, storage conditions for the export market.

Fruit colour	Greenish yellow skin colour. Hermaphrodite fruit must be pear shaped and female fruit uniformly round, all fruits must be fresh, free of shriveling, discolouration and exhibiting non-uniform ripening.
Packing	Packed according the fruit weight, based on fruit counts. Following weight count is used for 4 Kg net weight carton for both female and hermaphrodite fruit. Small: 12-15 count (260-330 g); Medium: 8-12 count (360-500 g); Large: 4-8 count (570-1000 g).
External appearance	Absence of latex stains or surface debris, absence of wounds during harvesting postharvest handling procedures, absence of insect bites, scars or spray damage, Fruit skin colour should not exceed greenish yellow.
Storage and ripening	To attain the maximum marketing period fruits must be stored at 10-12°C. Temperatures below this range can cause chilling injury. To develop ripening in papaya, fruits must be stored at 18-25°C and treated with ethylene gas at 100 ppm (0.01%) for 24 h.

Source: Punjab National Bank-Krishi 2007, http://www.pnbkrishi.com/papayatech.htm

cultivars identified cultivar-specific differences in patterns of mRNA accumulation during fruit development. Differential expression of the two carotene desaturase encoding genes, phytoene desaturase and ζ-carotene desaturase and a gene encoding the carotene desaturase co-factor 4-hydroxy phenylpyruvate dioxygenase were identified and may be associated with colour phenotype differences in papaya. In an earlier report, Chen *et al*. (2003) proposed the association of the ACC oxidase gene *AP-ACO1* with maturation while *CP-ACO2* is late-stage associated, occurring during organ senescence, such as fruit ripening and leaf senescence. Related to fruit colour, Saraswathi *et al*. (2007) could discriminate between the red and yellow types of dioecious papaya using RAPD primer OPC-05; similarly primer OPK-13 distinguished the indigenous dioecious from exotic gynodioecious forms.

Other post-harvest treatments

The major postharvest disease anthracnose (*Colletotrichum gloeosporioides*) can be controlled by prochloraz or propiconazole during storage and transportation (Sepiah 1993). Dembele *et al*. (2005) investigated the association of fruit maturity, presence and attack of rots, and the accumulation of fungicide residues in papaya fruits. Of the fungicides tested, thiabendazole-treated fruits did not rot 21 days after treatment. Moreover, low levels of the fungicide were detected on treated fruits; they were reported lower than those defined in the EU's guideline.

Hot water dip treatment in combination with fungicides improves the efficiency in controlling anthracnose. However, hot water dip treatments can affect the ripening process (Paull 1990) and the use of fungicides for extended periods may cause the emergence of fungicide-resistant strains of the fungus. As a result and given the health conscious consumers demand for "fungicide treatment free fruits", the development of non-hazardous methods for controlling postharvest disease is ongoing.

Gamma irradiation was proposed as a promising treatment since the low doses conferred anti-microbial as well as insecticidal effects on fruit flies (Chitarra and Chitarra 1990). Gamma irradiation was found to be effective on all stages of the life cycle of fruit flies (Moy and Wong 1996). Several studies have since investigated the effects of the stage of fruit maturity at the time of irradiation and report an association between the efficiency of gamma radiation and maturity stage in delaying the ripening process (Pimentel and Walder 2004). Papaya can tolerate up to 1 kGy before surface scald occurs (Paull 1996) and fruit irradiated at 0.5-1 kGy retained the fruit firmness for 2 days longer than non-irradiated control fruits (Zhao *et al*. 1996). Moreover, a major advantage of the method is that gamma irradiation is a physical treatment that does not leave residues on the fruit and can help to reduce the postharvest use of fungicides. Cia *et al*. (2007) reported that doses of 0.75 and 1 kGy could exhibit direct and indirect effects on *C. gloeosporioides*. The physico-chemical characteristics of the fruit were apparently modified resulting in firmer fruits (than the controls) and this made colonisation by the fungus more difficult. Zaho *et al*. (1990) showed that irradiation at 0.5-1 kGy at 25-30% yellow stage reduced the polymerization of pectic substances causing firm texture at full ripe stage and about 2 days longer than the non-irradiated fruits. But it was concluded that irradiation had no direct effect on firmness of papayas and acted by altering the ripening induced synthesis of cell wall enzymes, mainly pectin methyl esterase. However, the greatest obstacle in the use of irradiation for postharvest treatment is the high cost and prejudice by consumers against irradiated foods (Gomaz *et al*. 1999).

A range of materials are being investigated as alternatives to chemicals for the control of postharvest diseases of papaya during storage. The GRAS (Generally Regarded As Safe) compounds such as ammonium carbonate (3%) in paraffin wax-based formulation effectively reduced the incidence of anthracnose by 70% and treated fruits retained the overall quality during storage (Sivakumar *et al*. 2002). Furthermore, combined application of the biocontrol agent *Candida oleophila* with sodium bicarbonate-incorporated wax coating also resulted in significant and commercially acceptable control of anthracnose (Gamagae *et al*. 2003, 2004). A yeast isolate CEN63, *Cryptococcus magnus*, was found effective in controlling anthracnose in papaya (de Capdeville *et al*. 2007a, 2007b). Chitosan at 2% and 3% showed a fungicidal effect against *C. gloeosporioides* (Bautista-Baños *et al*. 2003). However, chitosan (1%) in combination with ammonium carbonate (3%) significantly reduced the incidence of anthracnose and the recovery of *C. gloeosporioides* from naturally-infected fruit compared to the untreated fruit. Treated fruits were of acceptable eating quality (Sivakumar *et al*. 2005). Similar findings were made by Hewajulige *et al*. (2007). The mode of action of the carbonate salts on the fungi appears to be by collapse and shrinkage of hyphae and inhibition of sporulation because of a reduction in cellular turgor pressure (Aharoni *et al*. 1997). Bautista-Baños *et al*. (2003) also reported that chitosan had a protective effect rather than a therapeutic effect on papaya fruit, since chitosan was more effective when applied before *C. gloeosporioides* inoculation than when applied after inoculation with the fungus.

Of note, chitosan treatment of papaya increased the internal CO_2 concentrations, delayed ripening and colour development, resulted in retained high fruit firmness and caused less weight loss (Sivakumar *et al*. 2005). Palhano *et al*. (2004) suggested the combined use of essential oil (lemon grass, *Citrus citratus*) and high hydrostatic pressure could limit fungal infection in harvested papaya fruit.

Research is also focused on using chitosan on harvested papaya to prevent fruit-to-fruit transmission of causal agent of anthracnose. Although *C. gloeosporioides* enters the papaya fruit by direct penetration in the field (Chau and Alvarez 1983), postharvest anthracnose is primarily a result of latent infections. During transportation and storage, *C. gloeosporioides* can spread rapidly from infected to healthy fruit by direct contact (Chau and Alvarez 1983). Therefore, the presence of a chitosan coating with ammonium carbonate on the fruit surface should prevent fruit-to-fruit disease transmission by acting as a physical barrier. This technology could be adopted to protect freshly harvested papaya, especially during sea shipments, at least to destinations within 14 days from the harvest site (Sivakumar *et al*. 2005). However, further research is needed to evaluate the effect of pre-harvest application of chitosan for the effective control of anthracnose.

Papaya fruits cv. 'Sunrise' exposed to methyl jasmonate vapours (10^{-4} or 10^{-5} M) for 16 h at 20°C inhibited fungal decay, reduced chilling injury and loss of firmness during

storage for 14-32 days at 10°C. A shelf life of 4 days at 20°C was obtained. The postharvest quality of papaya was retained significantly by combining the methyl jasmonate (10^{-5} M) treatments and a modified atmosphere (MA) created by low-density polyethylene film. According to González-Aguilar *et al*. (2003), the MA created (3-6 kPa O_2 and 6-9 Pa CO_2) inside the package did not induce off-flavour development during storage at 10°C. It was further confirmed by Yahia and Paull (1997) that the gas composition of 3-6 kPa O_2 and 6-9 Pa CO_2 within the package during papaya storage at 10°C, is within the range of concentrations that does not adversely affect postharvest fruit quality. 'Sunrise Solo' papaya was stored at 10°C for 31 days under a controlled atmosphere containing 8% CO_2 and 3% O_2 and thereafter for 5 days at 25°C at the retail market (Cenci *et al*. 1997). However, further research is needed to optimize atmosphere container shipment conditions and determine suitable gas composition for different export varieties. In Malaysia, a biotechnology-derived papaya has been developed for resistance to PRSV and improved postharvest qualities. The improved variety is under field evaluation (Abu Bakar *et al*. 2005).

Postharvest loss assessment also involves changes in weight. Paull and Chen (1989) reported that weight losses greater than 8% considerably diminish the postharvest quality of papaya. However, the use of polymeric film wraps and waxing of papaya (Paull and Chen 1989) or chitosan coating (Sivakumar *et al*. 2005) were shown to successfully reduce water loss and shriveling of fruits (**Fig. 2D**). Mosca and Durigan (1995) tested coating of 'Sunrise Solo' Line 72/12 with stretchable PVC (Polyvinyl Chloride), packaged in plastic sacks or immersed in wax (Sta Fresh™), diluted 3:7 with Benomyl (500 mg/L). The fruits were kept under environmental conditions (29.5°C, 68.3% RH) or under refrigeration (12°C, 85-90% RH). The authors concluded that the treatment with wax and wax plus Benomyl under environmental conditions did not influence fruit conservation, while plastic bags with partial vacuum and refrigeration increased their useful lifetime for up to 19 days.

Chauhan *et al*. (2006) described the synergistic effects of calcium infiltration, mild acidification to pH 4.5 and presence of MAs on the keeping quality and maintenance of optimum texture of pre-cut papaya (*C. papaya*) slices. Kakaew *et al*. (2007) applied 0.5% calcium chloride to shredded green papaya 'Kaek Dum' at 25 or 40°C. After treatment, shreds were stored at 4°C for 10 days and weight loss, surface color (lightness and hue value), firmness, respiration rate and sensory evaluation were determined every 2 days in storage. Application of a calcium dip at both temperatures resulted in a decrease of respiration rate throughout storage and heat treatments with calcium chloride or distilled water improved surface color, firmness and reduced weight loss of shredded green papaya. The results indicate that calcium chloride could maintain the quality and prolong shelf-life of shredded papaya, especially at higher dipping temperature. Members of the same group of researchers (Srilaong and Chansamrankul 2007) found that, when using the same cultivar, active MAP (MA packaging in a polyethylene bag with heated seal) and passive MAP (packaging in a nylon laminated polyethylene bag flushed with 2.5 and 5% O_2) were more effective than the control in maintaining better firmness and colour (Hue value).

Karakurt and Huber (2003, 2007) used mRNA differential display reverse transcription polymerase chain reaction (RT-PCR) to isolate genes expressed in fresh cut and intact papaya fruit. Fourteen differentially expressed cDNAs ranging from 154 to 777 bp were cloned and sequenced. High identities were found between the clones and genes previously reported as signaling pathway genes, membrane proteins, cell-wall enzymes, proteases, ethylene biosynthetic enzymes, and enzymes involved in plant defense responses. It was concluded the expression of proteins involved in membrane degradation, free radical generation, and enzymes involved in global stress responses were induced during the fresh-cut process.

CONVENTIONAL PROPAGATION: SEEDS, SEEDLINGS AND SYNSEEDS

Even though scion grafting (Sookmark and Tai 1975) and rooting of cuttings (Allan 1964; Allan and MacMillan 1991) are possible, these methods are not routinely used for commercial papaya propagation. Propagation of papaya is mostly through seeds. Farmers generally collect fruits of good quality from their orchards and the extract seeds for subsequent plantings. Numerous black seeds are enclosed in a gelatinous sarcotesta (or aril) and are attached to the wall of the ovary in five rows (Purseglove 1968). Seeds germinate in 3-5 weeks, but this can be reduced to 2-3 weeks if the sarcotesta is removed. The seeds are, therefore, washed to remove gelatinous material and are allowed to air dry. Attention is always given to damping-off diseases. Once seeedlings have attained a height of 15-20 cm, they are ready to be transplanted to the field. Fertilizer application and irrigation may be required depending on the location of the orchard and the variety. But Marler and Discekici (1997) found that it was not necessary to modify fertilizer treatments when 'Red Lady' papaya plants were grown on a hillside, however, a change in the irrigation schedule was required for the development of good root systems. Marler *et al*. (1994) also found that sufficient substrate aeration was important for effective plant physiology and growth.

The use of seeds for papaya production has both positive and negative facets. Numerous seeds are available from one papaya fruit, but seed germination can be slow and sporadic (Perez *et al*. 1980). Reyes *et al*. (1980) and Yahiro and Yoshitaka (1982) isolated "germination inhibitors" in the sarcotesta and inner seed coat but not in the embryo and endosperm. Moreover, heterogeneity caused by cross-pollination can be a disadvantage. Seeds derived from open-pollinated flowers can produce plants with considerable variation in sex types (a mix of male, female and hermaphroditic plants) which is highly undesirable when this results in variation in fruit quality and type.

Much research over the years has focused on understanding the factors contributing to seed germination and emergence in papaya. Furutani and Nagao (1987) found that, after removing the sarcotesta, that the application of 1.8 mM GA_3 or 1.0 M KNO_3 resulted in a higher percentage germination (44% and 56%, respectively) at 35°C than at 25°C (33% and 49%, respectively); SE = ± 3.2-3.3. Furthermore, the number of days to 50% seedling emergence was reduced from 19 to 15 days, and from 17 to 14 days when the temperature was increased from 25°C to 35°C, when 1.8 mM GA_3 or 1.0 M KNO_3 were applied, respectively; SE = ± 0.9-1.0. In three independent studies, seed germination was improved by removal of the sarcotesta (Gherardi and Valio 1976; Perez *et al*. 1980; Reyes *et al*. 1980) or by soaking in GA_3 (Yahiro and Oryoji 1980; Nagao and Furutani 1986). Soaking in KNO_3 (Perez *et al*. 1980) or GA_3 (Yahiro and Oryoji 1980; Nagao and Furutani 1986), or sowing seeds at elevated temperatures (Yahiro 1979) improved the uniformity and percentage of seedling emergence.

Bhattacharya and Khuspe (2001) did extensive tests on the differences between seed germination *in vitro* and *in vivo* in 10 cultivars, and their main findings were: (a) there are large differences due to cultivar, with 'Honeydew' showing the smallest difference (6.3%) and 'Disco' the largest (68%), (b) direct germination in soil resulted in an average of 40.2% germination (range = 3–71%), while soaking for 24 h in 200 ppm GA_3 increased to an average of 56.5% (range = 12–79%), a finding also reported by Sen and Gunthi (1977), Nagao and Furutani (1986) and Tseng (1991); (c) TDZ applied at 1 µM, NAA at 5 µM or BAP at 1 µM showed the highest percentage seed germination (values from all 10 cultivars were pooled), amounting to 92%, 80% and 82%, respectively; (d) light hastens the germination process, as does exposure to 30°C; high concentrations of BAP, and (e) all concentrations of 2,4-D and 2,4,5-T resulted in explant callusing.

Recently, somatic embryogenesis, encapsulation, and plant regeneration were achieved with papaya cultivars. Castillo *et al*. (1998a) produced uniformly-sized somatic embryos in a high-frequency liquid production system consisting of MS + 10 µM 2,4-D, 50 mg/L *myo*-inositol, and 3% sucrose. These somatic embryos, 2.0 mm in diameter, when encapsulated in a 2.5% sodium alginate solution in ½MS for only a 10 min exposure to $CaCl_2$ resulted in uniform encapsulated synseeds with a high frequency (77.5%) of germination (Castillo *et al*. 1998b). Ying *et al*. (1999) also used liquid culture for the induction of somatic embryos. Saha *et al*. (2004) first induced somatic embryos in nine cultivars, and synseeds created from these somatic embryos using 4.6% sodium alginate in a 100 mM $CaCl_2$ solution for the formation of tougher, or more gelatinous beads, respectively. Synseeds were subsequently placed onto MS or MS + 0.2 mg/L BAP + 0.02 mg/L NAA. In order to avoid bacterial and fungal contamination associated with planting of synseeds directly in the greenhouse, a second layer was added (i.e. double-layered synseeds in several combinations) following treatment with 150 mg/L Rose Bengal, a bactericide or Bavistine (100 mg/L), and a general fungicide. Less contamination (30%) was found in synseeds which had a fungicide/bactericide treatment than in control synseeds simply sown in the greenhouse. Germination percentages ranged from 8-58%, but depended on the plant growth regulator present and on the cultivar.

Seedling germination was considerably improved (95-100% depending on the treatment) in 'Solo' according to a simple but effective method devised by Teixeira da Silva and Giang (unpublished results) following *in vitro* culture, and by Teixeira da Silva (unpublished results) when placed under different LEDs.

MICROPROPAGATION

Efficient micropropagation of papaya has become crucial for the multiplication of specific sex types of papaya and in the application of genetic transformation technologies. Significant progress has been achieved using organogenesis and somatic embryogenesis.

Shoot tip, axillary bud and single node culture

Papaya is most commonly propagated by shoot tip or axillary bud (explants around 20 mm in length) culture. This is the most reliable method used for the micropropagation of this fruit tree to date. Prior to the collection of shoot tip or axillary bud explants, the mother plant should be tested for the presence of pathogens, in particular viruses and bacteria. Virus indexing should be conducted prior to the establishment of cultures and smaller explants are in general recommended. Bacterial indexing is also essential, since up to fourteen bacterial isolates can be found in surface-sterilized shoot tips. In one study with shoots of *C. papaya* 'Surya', six Gram-negative genera, two Gram-positive genera (Thomas *et al*. 2007a, 2007b) were identified. Chan and Teo (1993b) used the following method to surface sterilize explants for *in vitro* culture and obtained a 77-84% successful regeneration rate. The steps involved; a wash in detergent, then a 30 minute rinse with running tap water; excised apical and axillary buds were placed in 95% ethanol for 15 sec, the surface sterilized for 20 min in 20% chloride (commercial bleach); three rinses with sterile distilled water; immersion in an antibiotic solution containing 100 mg/L chloramphenicol, 100 mg/L streptomycin with continuous agitation on an orbital shaker for 24 h; three rinses with sterile distilled water; incubation in 4% sucrose solution between Whatman № 1 filter paper sheets for 48 h; sterilization for 5 min in 5% chloride (commercial bleach); three rinses with sterile distilled water; plate explants on solid plant growth regulators (PGR)-free MS medium. Although this method is effective, it is tedious and time consuming and the use of antibiotics is now be discouraged.

Mehdi and Hogan (1976) also established papaya plantlets from shoot tips, and Yie and Liaw (1977) established plantlets from internode sections from seedlings of an unspecified age. While numerous researchers (Litz and Conover 1977, 1978a; Rajeevan and Pandey 1983, 1986; Mosella and Iligaray 1985; Drew and Smith 1986; Drew 1988; Mondal *et al*. 1990; Reuveni *et al*. 1990; Kataoka and Inoue 1991; Drew 1992; Chan and Teo 1993a; Chan 1996; Castillo *et al*. 1997; Lai *et al*. 1998, 2000) established plantlets from both shoot tips and axillary buds. Ashmore *et al*. (2001) obtained micro-cuttings from cryopreserved shoot meristems. Agnihotri *et al*. (2004) could establish male and female plants through shoot tip culture, but noted much callusing at the base of micro-cuttings. Despite this, they could successfully root shoots in a 4-stage process in which the first step involved a 24 h 10 mg/L IBA-pulse. Mondal *et al*. (1990) used gibberellins to restore apical dominance following growth on a cytokinin medium, which tends to induce bushiness *in vitro*. Azimi *et al*. (2005) could successfully cryopreserve shoot tips and seeds of papaya when the following procedures were followed: Shoot tips were incubated for 1-6 days before vitrification with an optimum treatment time of 1-4 days; Duration of exposure to vitrification solution varied and 70% recovery was obtained from the shoot tips which had been exposed to 100% PVS2 for 20 min at 0°C; Treatments for <20 min or >40 min resulted in no regeneration after liquid nitrogen treatment. *C. papaya* cv. 'Washington' pollen stored at -196°C for eight years was still effective in pollination and brought about fruit set and seed development to the extent of 80-86% (Shashikumar *et al*. 2007).

The accumulation of ethylene in papaya cultures tends to cause an increase in senescence: 3.5-fold higher when the ethylene concentration is 50 ppm as compared to controls, according to Magdalita *et al*. (1997), who used nodal culture. These authors reduced ethylene accumulation and senescence by adding a loose cap of aluminum foil and thus increased aeration. Other strategies employed by Magdalita *et al*. (1997) to reduce ethylene accumulation and senescence involved the use of larger culture vessels and the inclusion of the ethylene-suppressant aminoethoxyvinylglycine (AVG) at 1.2 µM, or the ethylene-antagonist, silver thiosulphate (STS) at 0.3 mM. The use of AVG and STS increased nodal culture growth by 283% and 289%, respectively, while leaf area production was increased by 350% and 211%, respectively. Even though nodal culture is an easy technique, involving the sprouting of axillary buds from a node, on suitable medium, this technique is not commonly used.

Lai *et al*. (1998) showed that by aerating shoot buds two weeks after no aeration gave a 41% increase in the number of shoots ≥ 0.5 cm, a 42% increase in leaf expansion and a 17% increase in leaf numbers compared to unaerated cultures. In independent experiments, Teixeira da Silva (unpublished data) showed how the use of aeration (using a Vitron™ vessel or Milliseal®) and 3000 ppm CO_2 photoautotrophic micropropagation (**Fig. 3**) could increase the general physiology of papaya *in vitro*-regenerated plants, including fresh leaf weight and number, and SPAD, i.e. measure of chlorophyll content while the manipulation of the light quality could allow for the formation of "mini"-papaya plantlets through the used of blue LEDs (light emitting diodes; **Fig. 3**). Lai *et al*. in 2000 went further by adding the ethylene biosysntheis precursor, ACC and the inhibitors, AVG and $CoCl_2$, to medium in sealed containers. Shoot number was enhanced 75% with the addition of 2 µM ACC and 23% and 49% by the addition of 0.5 µM AVG and 5 µM $CoCl_2$, respectively.

Geneve *et al*. (2007) showed that pawpaw cultures typically produce many shoot-bud clusters that do not readily elongate and that shoot-bud cultures that had been maintained on a BA (8.9 µM) + NAA (2.3 µM) medium for over five years showed evidence of cytokinin habituation. Single shoot-buds (1.5 cm) moved to a media with or without PGRs continued to initiate new shoots at a similar rate (~ 5 to 8 shoots per culture).

Fig. 3 Papaya 'Solo' *in vitro*. (**A**) Photoautotrophic micropropagation in a Vitron™ (i.e. whole vessel allows for air exchange) or using Milliseal® (allowing localized aeration; **B**) and 3000 ppm CO_2. Manipulation of growth and size of seed-derived plantlets under 100% blue (**C**) or 100% red (**D**) light emitting diodes. (**E**) Control plants growing under fluorescent lamps at 40 µmol/m^2/s. (**C-E**) Medium: Hyponex 3 g/l, 3% (w/v) sucrose in 1 L culture bottles. (All figures by JA Teixeira da Silva, unpublished results).

Organogenesis, anther and ovule culture, and regeneration from protoplasts

There is only a single study that reports on the successful regeneration of plants directly from petioles in papaya (Hossain *et al*. 1993). Litz *et al*. (1983) reported papaya regeneration by organogenesis from cotyledons of axenically-grown *C. papaya* seedlings. Litz and Conover (1978a) cultured anthers to obtain haploid plants with a chromosome number of 2n=9. But their initial conversion rate was low (1 in every 1000 anthers cultured) and only improved slightly to 0.4% in later attempts (Litz and Conover 1979). Tsay and Su (1985) improved the conversion rate (0.7%) when anthers were cultured on simple medium. Rimberia *et al*. (2005) induced somatic embryos from anthers in a liquid-to-solid 2-phase step using 0.1 mg/L BA and 0.1 mg/L NAA. The maximum embryo induction rate increased to 4% when anthers were treated with water for 1 day or MS medium with sucrose for 3 or 5 days. These authors then used sex-diagnostic PCR to confirm that the plants were female. Initial studies were done by Sondur *et al*. (1996), Somsri *et al*. (1998), Lemos *et al*. (2002) and Urasaki *et al*. (2002a, 2002b) in which DNA markers (almost exclusively RAPDs) were used to establish sex-specific bands. Rimberia *et al*. (2007) showed tremendous variability in the morphology and fruiting characters of 26 anther-derived triploid dwarf cv. 'Wonder blight' while Gangopadhyay *et al*. (2007) used both RAPDs and ISSR to determine the sex of plants.

Ovule culture is limited, and almost exclusively conducted for the production of somatic embryos, as discussed below.

Even though Litz and Conover (1978b, 1979) and Litz (1984) proposed the use of protoplasts as a means of producing virus-free papaya plants, they were unsuccessful in attempts to regenerate plantlets from protoplast-derived calli. It was Chen *et al*. (1991) and Chen and Chen (1992) (summarized by Chen 1994) who successfully isolated protoplasts from highly regenerable suspension cultures from interspecific crosses of *C. papaya* × *C. cauliflora* zygotic embryos. These protoplast-derived somatic embryos proliferated rapidly and some formed plantlets.

Callus induction and somatic embryogenesis

Not all callus tissue induced in papaya is embryogenic, with clusters of meristematic points. Once callus with embryogenic potential has been formed or isolated, it can be maintained effectively using cell suspension cultures. Since somaclonal variation and the possible production of off-types is a constant worry, somatic embryogenesis is not a commonly used method for the micropropagation of papaya, even though several positive results have been obtained. It remains nonetheless an important method for genetic transformation, as described later in the review.

de Bruijne *et al*. (1974) first induced somatic embryos from papaya callus using seedling petiole segments but no plants were regenerated. In contrast, Yie and Liaw (1977) when using the internode stem of seedlings, first induced callus on MS containing 5.0 µM NAA and 0.5 µM kinetin, then somatic embryos on MS containing 0-0.25 µM IAA and 5.0-10 µM kinetin, and subsequently regenerated plantlets. Arora and Singh (1978a) advanced this finding by also inducing roots *in vitro* from shoots derived from somatic embryos (Arora and Singh 1978b). The authors showed that auxin was critical for the initiation and subsequent growth of callus and that out of the 3 auxins tested, NAA was most effective, followed by 2,4-D and IAA. Addition of 1.0 mg/L NAA was sufficient for good callus growth, occasionally assisted by the addition of GA_3 up to 1.0 mg/L. These authors claimed that the milky latex inhibited the establishment of *in vitro* cultures from mature tissues of both male and female plants, although this problem was not encountered by Litz and Conover (1980) who induced somatic embryos from the peduncles of adult *C. stipulata* plants. *C. stipulata* is not important as a fruit crop, but is important germplasm since it is resistant to PRSV. Litz and Conover (1982) furthered their own findings by inducing callus from ovules, and somatic embryos that subsequently formed germinated in 10-20% of the cultured ovules both solid and liquid White's medium supplemented with 60 g/L sucrose, 400 mg/L glutamine, 20% (v/v) filter-sterilized coconut milk and 8 g/L agar. Mehdi and Hogan (1979) could regenerate somatic embryos on MS medium containing coconut water (CW), IAA, IBA, NAA and kinetin, although the concentrations were not specified. Chen *et al*. (1987) regenerated somatic embryos in three months from 'Sunrise Solo' seedling root explants cultured on ½MS containing 5.4 µM NAA, 2.3 µM kinetin and 2.6 µM GA_3, and finally 100 plants per explant. Litz *et al*. (1983) induced callus from the midrib (0.3-2.0 mg/L BA with 0.5-3.0 mg/L NAA) and lamina (0.6-3.0 mg/L BA with 1.2-5.0 mg/L NAA) of cotyledons of axenically-grown *C. papaya* seedlings when cultured on MS basal medium. Lin and Yang (2001) also generated somatic embryos from adventitious roots within four months. Fitch (1993) and Fitch *et al*. (1998) induced somatic embryos in 'Kamiya Solo' from an initial callus phase when

hypocotyl sections were cultured on ½MS with modified MS vitamins, 2.3 to 112.5 mM 2,4-D, 400 mg/L glutamine, and 6% sucrose. Cônsoli *et al*. (1995) also claimed success with the use of hypocotyls, epicotyls and leaves, although no details of the medium were defined, nor was the cultivar used mentioned. Similar generalizations were made by Neupane *et al*. (1998) when using 'Sunrise Solo', 'Kapoho Solo' and 'Sunset Solo'. Yamamoto and Tabata (1989) also induced hypocotyl somatic embryos using 0.1-1.0 µM 2,4-D. One-cm long explants of an unspecified age were cultured on Linsmaier and Skoog (1965) medium containing 10 µM 2,4-D (Yamamoto *et al*. 1986). Pale yellow, friable embryogenic calli were produced but plantlets were not regenerated since the focus of their studies was on laticifer development in papaya somatic embryos. Monmarson *et al*. (1995) produced embryogenic calli from the integuments of immature seeds at a high-frequency. Bhattacharya and Khuspe (2000) induced somatic embryos in 'Honey Dew' and 'CO_2' following the culture of immature zygotic embryos on MS + 3 mg/L 2,4,5-T in the dark for 3-6 weeks. Maturation of embryos was achieved in medium supplemented with ABA at 0.1 mg/L or on PGR-free medium (71% in 'Honey Dew' and 59% in 'CO_2'). Romyanon *et al*. (2007) found that somatic embryos cultured in half-strength liquid MS medium containing 22.5 µM 2,4-D and 2.5 µM ABA yielded higher cell mass (dry-weight basis) than parallel treatments with other combinations of PGRs.

Ovules are an excellent source of regenerable papaya cultures via somatic embryogenesis. 'Ovular' somatic embryos are mainly derived from nucellar tissue (Litz and Conover 1981, 1982, 1983), but also from highly embryogenic zygotes produced in interspecific crosses between papaya and *C. cauliflora* (Moore and Litz 1984; Manshardt and Wenslaff 1989). Litz and Conover (1981) also reported that occasionally cultured ovules from self-pollinated papayas also became embryogenic, although the zygotic or maternal origin was not specified. Gonsalves *et al*. (1998), based on earlier work by Fitch *et al*. (1990) induced somatic embryogenesis in 'Sunrise Solo' immature zygotic embryos. Davis and Ying (2004) induced somatic embryos from immature seeds, placed aseptically on Fitch's liquid medium, ½MS and vitamins, 50 mg/L *myo*-inositol, 6% sucrose, 10 mg/L 2,4-D and 400 mg/L glutamine; two months thereafter, they were transferred to a similar medium, the difference being the inclusion of 2% sucrose, 0.1 mg/L BAP and 0.01 mg/L NAA. Magdalita *et al*. (2002) were able to induce 7730 somatic embryos from a initial culture of 11,900 zygotic embryos of 'Solo' (i.e. 65% conversion) on de Fossard medium with 0.25 µM each of BAP and NAA and 10.0 µM GA_3.

Fitch (1993) found that an increase in osmoticum up to 7% sucrose resulted in a simultaneous increase in the percentage somatic embryogenesis of 'Kapoho Solo' hypocotyls. Similar findings were reported by Litz (1986).

Genotype also played a role in the success of somatic embryogenesis, with 'Kapoho' > 'Sunset' > 'Sunrise' > 'Waimanalo' (Fitch 1993). Fitch and Manshardt (1990) had previously found, however that the order was 'Waimanalo' > 'Sunrise' > 'Kapoho' > 'Sunset', although this order varied depending on the medium constituents and concentration of phytohormones added. For example 'Waimalo' showed the lowest (57%) embryogenic yield compared to 'Sunset' (93%) when 5 mg/L 2, 4-D was included in the medium. In their study, CW, BA, TDZ, 2,4-D or picloram could induce somatic embryos, singly, or in combination. Litz and Conover (1982, 1983) also found 20% (v/v) CW to be efficient on either MS or White's medium for the induction of somatic embryos.

Jordan *et al*. (1982) could induce somatic embryogenesis in 'mountain papaya' or *C. candamarcensis* (i.e. *C. pubescens*) using hypocotyl calluses induced from greenhouse-grown seedlings on a medium containing 5-25 µM NAA and 5 µM kinetin.

Micropropagation and scaling-up

Manshardt and Drew (1998) were able to commercially produce and grow 14,000 elite female clones generated from micro-cuttings of nodes of apically dominant plants, using a method established earlier by Drew (1992). Lai *et al*. (1998) could mass produce plants when papaya plantlets were repeatedly subcultured on MS medium supplemented with 0.88 µM BA and 0.1 µM NAA, and this method is currently used to mass produce papaya in Taiwan. Similar propagation medium (MSNB) for multiple shoot formation was devised by Yang and Ye (1996) in which shoots were induced from petioles on MS supplemented with Gamborg's B5 vitamins (Gamborg *et al*. 1968), 0.8 µM BA and 0.1 µM NAA. Castillo *et al*. (1997) claimed the importance of an equal concentration (100 µM) of $FeNa_2EDTA$ and FeNa EDDHA (Sequestrene®) in producing the highest shoot proliferation. Chan and Teo (1994) used a 10-week solid proliferation medium followed by a 10-week liquid proliferation medium to mass produce shoots (116 plants per explant). The proliferation medium consisted of MS + 0.1 mg/L BA + 500 mg/L casein hydrolysate + 0.38 mg/L riboflavin. Suksa-Ard *et al*. (1998) showed how elongation of shoot masses, initiated on an MS medium with BA, could be achieved with the application of 2.5 µM 2-iP on medium containing 3% sucrose and 12 g/L agar.

Drew (1992) found that 1% fructose resulted in better plant production, especially over repeated sub-cultures, than when 2% sucrose was used.

Rooting and acclimatization

Mass propagation by *ex vitro* rooting was attempted by Reuveni and Schlesinger (1990) and Kataoka and Inoue (1992) but stringent rooting conditions, seasonal factors, and explant type affected rooting success (Kataoka and Inoue 1992; Teo and Chan 1994), and thus its application to a mass micropropagation unit.

Many papaya tissue culture scientists believe that the addition of an auxin to the medium is an essential prerequisite for successful rooting of *in vitro* shoots. Miller and Drew (1990) determined the minimum size for a shoot tip to root is 5 mm, while Drew *et al*. (1993) claimed a short, 3-day exposure to 10 µM IBA is sufficient to induce roots and that a longer exposure period inhibits root formation. These authors also rooted papaya shoots on NAA- or CPA-supplemented medium. Earlier studies by Drew (1987) showed that when riboflavin was added to the medium, it synergistically acted to promote rooting. When Teo and Chan (1994) embedded micro-cuttings on a full or half strength medium (MS) + 2.2 µM BA + 29.5-49.2 µM IBA, thick, stumpy roots formed with basal callusing. To avoid callusing, the same authors suggested a dip in a low agar concentration medium with 12.3 µM IBA. Surprisingly, only a 68% success rate was achieved, as opposed to 90% when micro-cuttings were dipped in 11.1 µM IBA and then grown *ex vitro* in vermiculite.

Although early reports of acclimatization claimed poor field performance and survival of papaya (de Winnaar 1988), later reports claim a 100% acclimatization success (Drew 1988; Manshardt and Drew 1998). Thick, short, stumpy roots and yellowing of leaves have frequently been reported on agar-supplemented medium (Drew 1987; Kataoka and Inoue 1987; Drew and Miller 1989; Drew *et al*. 1993; Teo and Chan 1994; Yu *et al*. 2000). Suksa-Ard *et al*. (1998) showed how the choice of medium substrate affected the *in vitro* rooting percentage and demonstrated high rooting rates in starch (96%), then agar (76%), and rockwool (76%). Lower rates were observed with vermiculite (56%) and gellan gum (8%).

It would appear that the physical and chemical nature of the rooting substrate affects the rooting capacity in papaya considerably (Kataoka 1994). Yu *et al*. (2000) established a more efficient protocol in which papaya shoots were cultured for one week in darkness on MS + 2.5 µM IBA fol-

lowed by two weeks in aerated flasks on ½MS, and plantlets acclimatized in vermiculite. Resulting survival rates were 94.5% from aerated vermiculite, 87.8% from non-aerated vermiculite, 42.2% from aerated agar, and 35.6% from non-aerated agar. Drew (1988) claimed that a 1:1:1 ratio of peat: perlite: vermiculite provided sufficient aeration to avoid bacterial and fungal diseases. Agnihotri *et al*. (2004), following a rather complex 4-step rooting process (basically transferring from an IBA-supplemented medium to an IAA-supplemented medium) culminating in a final *in vitro* rooting step on sterilized Soilrite, demonstrated an 80% survival upon transfer *ex vitro*, with plantlets reaching the fruiting stage in only 6 months.

Field performance trials of tissue-cultured papaya were conducted by Pandey and Singh (1988) in India, by Drew and Vogler (1993) in Australia, and by Chan and Teo (1994) in Malaysia. In the latter two trials, the juvenile period was shortened as compared to control *ex vitro* propagated plants, with either earlier flowering, or flowering at a significantly reduced height.

GENETIC TRANSFORMATION

Although, *in vitro* techniques namely somatic embryogenesis and somaclonal variation are useful tools for genetic manipulation, genetic transformation can be used and has been used in papaya to alter superior cultivars for a specific trait. Stable transformation of papaya has been achieved through the use of various DNA transfer technologies since the initial report of Pang and Sanford (1988). Pang and Sanford (1988) obtained transgenic callus with the *neomycin phosphotransferase* type II (*npt*II) marker gene from oncogenic *Agrobacterium tumefaciens*-mediated transformation of 'Sunrise Solo' and 'Kapoho Solo' papaya leaf discs, stems and petioles. But they could not regenerate plantlets from these calli. Since then several efficient transformation and selection protocols have been developed and have resulted in transgenic plants expressing new traits including herbicide tolerance, increased the shelf-life of fruits, virus-resistance, and aluminium tolerance. According to do Carmo and Souza Jr. (2003), most studies (>55%) used *Agrobacterium*-mediated trans-formation systems, 80% of those by *A. tumefaciens* (GV311, LBA4404, A136, C58-Z707) and the remaining 20% used *A. rhizogenes* (LBA9402, A_4T, 8196). In addition, many transgenic papaya studies have utilized *npt*II as the marker gene of choice although Cabrera-Ponce *et al*. (1995) used the *bar* gene, which codes for phosphinothricin acetyl transferase and allows for the breakdown of phosphinothricin, or PPT, a herbicide. Given that antibiotic and herbicide resistance genes in widely grown transgenic crops may pose a risk, real or perceived, of transfer to weedy relatives or microorganisms, an alternative selection technology using phospho-mannose isomerase (PMI) was developed (Bolsen *et al*. 1999) and has been tried with papaya (Souza Jr. *et al*. 2001; Zhu *et al*. 2005). PMI converts mannose (Man) to mannose-6-phosphate. The results from these two groups were different, probably due to the different papaya cultivars used. Souza Jr. *et al*. (2001) used 'Sunrise Solo', while Zhu *et al*. (2005) used 'Kapoho Solo'. Zhu *et al*. demonstrated that embryogenic papaya calli have little or no PMI activity and cannot use Man as a carbon source. However, calli transformed with the *pmi* gene showed PMI activity and were able to use Man as efficiently as sucrose. The green fluorescent protein (GFP) from jellyfish (*Aequorea victoria*) is also becoming a popular alternative reporter gene in plant transformation. Zhu *et al*. (2004a) successfully transformed the papaya variety 'Kapoho Solo' with the GFP gene via microprojectile bombardment of embryogenic callus. A reduction in selection time (3-4 weeks as compared to the average 3 months experienced when using a geneticin [G418] selection-based medium) was demonstrated, a 5- to 8-fold increase in the number of transformants (compared to antibiotic-based selection), and a 15- to 24-fold increase in transformation throughput.

Fitch *et al*. (1993) were the first to successfully transform and regenerate transgenic papaya plants. Transgenic papaya plants were regenerated from microprojectile bombarded immature *in vitro* 'Sunrise Solo' and 'Kapoho Solo' papaya zygotic embryos, hypocotyl sections, or somatic embryos derived from both embryos or hypocotyls that were cultured on medium containing 2,4-D (Fitch and Manshardt 1990). The transgenes included *npt*II, β-glucuronidase (GUS) and coat protein (*cp*) of a mild strain of PRSV (PRSV HA 5-1). The latter gene codes for the viral capsid protein used for packaging the viral RNA, assisting the movement of the virus *in planta* and interaction with insect vectors. The objective of the study was to develop resistance to PRSV. By the late 1990s, the first transgenic line designated as line 55-1 was used to develop PRSV-resistant transgenic cultivars 'Rainbow' and 'SunUp'. In 1998, two PRSV resistant papaya cultivars, 'SunUp' and 'Rainbow', were released to growers in Hawaii (Fitch *et al*. 1992; Manshardt 1998). The transgenic papayas have offered durable resistance to PRSV and have controlled the virus in Hawaii (Ferreira *et al*. 2002). According to figures out of the USDA's statistical service, 'Rainbow' makes up 47% of the Big Island's 779 papaya hectares. 'Rainbow' is a yellow-flesh F_1 hybrid of a cross between the transgenic cultivar 'SunUp' and nontransgenic cv. 'Kapoho Solo' (Manshardt 1998; Gonsalves 2002) which is the preferred non-transgenic cultivar in Hawaii. 'SunUp' is homozygous for the single *cp* gene insert of the mild strain PRSV HA 5-1 (Manshardt 1998) and was derived from the red-flesh transgenic papaya line 55-1 (Fitch *et al*. 1992).

Initial greenhouse studies of transgenic line 55-1, hemizygous for the *cp*, showed that although the plants were resistant to Hawaiian virus isolates, they were susceptible to PRSV isolates from 11 geographical regions, including Bahamas, Florida, Mexico, Jamaica, Brazil, and Thailand (Tennant *et al*. 1994). Later work showed that the resistance of line 55-1 is RNA-mediated and dependent on the dosage of the *cp* gene, *cp* sequence homology of the challenge virus, and plant development stage (Tennant *et al*. 2001). Even though 'Rainbow' (RB), a transgenic papaya cultivar hemizygous for PRSV *cp* gene, exhibited early plant susceptibility (>70% of plants infected) to mechanical inoculation with crude preparations of PRSV isolates from Hawaii, RB plants become highly resistant by approximately 9 weeks after seeding in the greenhouse (2.5% of plants infected) and by 13 weeks in the field (<16% of plants infected) (Gaskill *et al*. 2002). In contrast 'SunUp', a transgenic papaya cultivar homozygous for the *cp* gene, exhibited complete resistance against all isolates of PRSV from Hawaii, but is susceptible to isolates from outside of Hawaii. Among 18 virus isolates collected in Taiwan, four (5-19, CY4, TD2, and DL1) were able to breakdown the transgenic resistance of papaya lines carrying the *cp* gene of PRSV and caused symptoms on non-transformed papaya plants different from those induced by the strain YK (Chen *et al*. 2002); the DL1 isolate was further identified as *Papaya leaf distortion mosaic virus*. Resistance against PRSV through a *cp* gene of mild PRSV was also shown to be transmitted to non-transgenic 'Solo' plants through conventional crossing between a female transgenic R_0 and a non-transgenic plant (Tennant *et al*. 1995). Mode-rate genetic resistance to PRSV in papaya germplasm has been used in other conventional breeding programs in Florida, Jamaica and Hawaii to create PRV-tolerant cultivars (Manshardt *et al*. 1995; Turner *et al*. 2004). Thus, the hemizygous 'Rainbow' is resistant to Hawaiian isolates, but susceptible to isolates from outside of Hawaii whereas homozygous 'SunUp' is resistant to isolates from outside of Hawaii, with the exception of the Thailand isolate. The resistance of another Hawaiian transgenic line, line 63-1, was recently tested against PRSV from various locations (Tennant *et al*. 2005). Line 63-1 originated from the same transformation experiment that resulted in line 55-1 from which the transgenic commercial cultivars, 'Rainbow' and 'SunUp', were derived. ELISA and PCR tests provided evidence that there are at least two segregating *cp* loci

in line 63-1. Souza Jr. *et al*. (2005) further demonstrated that line 63-1 has two sites of transgene insertion (designated locus S and locus L) and that both the *cp* and the *npt*II genes are present in both loci. Unlike line 55-1, a significant percentage of inoculated transgenic plants were susceptible to some isolates from Hawaii and others were resistant to Hawaiian and non-Hawaiian isolates. Line 63-1, therefore, presents Hawaii with PRSV-resistant transgenic germplasm that could be used as a source of transgenes for resistance to PRSV isolates within and outside of Hawaii. Souza *et al*. (2005) also provided evidence that the number of resistant plants in a 63-1-derived population is directly correlated with the number of plants with multiple transgene copies (Souza *et al*. 2005).

Other countries, Brazil, Jamaica, Venezuela, Thailand, Australia (Lines *et al*. 2002), Taiwan (Bau *et al*. 2003), and recently with Bangladesh and the east African countries of Tanzania, Uganda, and Kenya, have since used the technology and the *cp* gene from their region to develop their own transgenic varieties. The transgenic papayas are at various stages of development and evaluation. For example, translatable and untranslatable versions of the *cp* gene of PRSV collected in the State of Bahia, Brazil, were engineered for expression in papaya varieties, 'Sunrise Solo' and 'Sunset Solo' (Souza *et al*. 2005). The genes were transferred to somatic embryo cultures derived from immature zygotic embryos via microprojectile bombardment. Fifty four transgenic lines, 26 containing translatable and 28 containing untranslatable gene versions, were regenerated. Greenhouse evaluation of the resistance of the regenerated transgenic plants was conducted with PRSV from Brazil, Hawaii and Thailand. The plants showed mono-, double- and even triple-resistance against the viruses from the three countries. However, the transgenic papayas have been subjected to very limited field evaluation in Brazil. Fermin *et al*. (2004) used *Agrobacterium* to transform local Venezuelan varieties of papaya with the *cp* gene from two PRSV isolates, El Vigía (VE) and Lagunillas (LA), Merida. They found that transgenic plants were effectively protected against both homologous (VE and LA) and heterologous isolates from Hawaii and Thailand. Field evaluations were initiated but activists destroyed all transgenic plants before useful data was collected (Fermin *et al*. 2004). In Jamaica, the transgenic papayas were developed by microprojectile bombardment of somatic embryogenic materials (Cai *et al*. 1999). Transgenic papayas, containing translatable (CP_T) or nontranslatable coat protein (CP_{NT}) gene constructs, were evaluated over two generations for field resistance to PRSV in a commercial papaya growing area in Jamaica (Tennant *et al*. 2005). Trees with acceptable horticultural characteristics exhibited a range in resistance phenotypes. Reactions of R_0 CP_T transgenic lines ranged from asymptomatic, mild or severe leaf and fruit symptoms, or all three phenotypes in one line or between different lines. Trees of most CP_{NT} lines exhibited severe responses to infection and some also showed mild reactions. R_1 offspring showed phenotypes previously observed with parental R_0 trees, however, phenotypes not previously observed or a lower incidence of the phenotype was also obtained. It was concluded that the transgenic lines appear to possess virus disease resistance against PRSV that can be manipulated in subsequent generations for the development of a product with acceptable commercial performance. However, local deregulation efforts have stalled research and the development of a transgenic product.

In Thailand, the transgenic papaya has been field trialed extensively. Three lines were selected for their horticultural characteristics and resistance. These lines, derived from 'Khaknuan' papaya variety, yielded fruit 70 times that of the nontransgenic Khaknuan'. Safety assessments have shown no impact on the surrounding ecology and there were no differences in the nutritional composition of the transgenic fruit compared to the nontransgenic fruit (Sakuanrungsirikul *et al*. 2005).

While the processes for deregulating the transgenic papaya are well under way, public acceptance of genetically modified products appears to be keeping the project from reaching the ultimate goal of deregulation and commercialization of the transgenic papayas.

The efficacy of other genes in the control of PRSV is being investigated. In other studies with transgenic lines against PRSV in Hawaii, lines containing a nontranslatable *cp* version of the mild strain of PRSV conferred varying degrees of resistance (Cai *et al*. 1999; Gonsalves 1998). Twenty-two lines of 77, conferred complete resistance against the homologous isolate and 23 lines showed 47% resistance. When inoculated with PRSV isolates from other regions of Hawaii, moderate levels ranging from 11 to 26% were obtained. Chen *et al*. (2001) reported the first successful PRSV-resistant 'Tai-nong-2' papaya through replicase-mediated resistance, i.e. using the RP or viral replicase gene with *A. tumefaciens* as vector. The RP fragment that was used showed a 82.8%, 91.83% and 95.07% sequence similarity to the sequences of PRSV strains HA5-1 from Hawaii (Quemada *et al*. 1990), Sm from mainland China (Liu *et al*. 1994) and YK from Taiwan (Wang *et al*. 1994), respectively.

Since the early reports on the transformation of papaya, a number of laboratories have modified the protocols and reported success with different explants, *Agrobacterium* species or strains, and selection systems. Cabrera-Ponce *et al*. (1995) established a particle bombardment protocol for 'Maradol' zygotic embryos and embryogenic callus derived from immature zygotic embryos. Ye *et al*. (1991), Fitch *et al*. (1993; in 'Kapoho Solo') and Yang *et al*. (1996) obtained transgenic 'Sunrise Solo' papaya after transformation of somatic embryos or the petioles of *in vitro* propagated multishoots, respectively, using *Agrobacterium*. Using cross sections of papaya petioles, Yang *et al*. (1996) introduced the *npt*II and *uid*A genes, used as a selection marker and reporter gene, respectively, into 'Sunrise Solo' papaya following *A. tumefaciens*-mediated transformation. Cabrera-Ponce *et al*. (1996) used *A. rhizogenes*. Cheng *et al*. (1996) inserted the PRSV YK *cp* gene using *A. tumefaciens*. Cabrera-Ponce *et al*. (1996) could induce hairy roots in Yellow-large hermaphrodite type *C. papaya* after infection with *A. rhizogenes*, and then induced somatic embryos from the hairy roots. Cheng *et al*. (1996) found the inclusion of carborundum to be important in the effective *Agrobacterium*-mediated transformation of 'Tainung №2' papaya embryogenic tissues with the *cp* gene of PRSV, which tended to reduce, or eliminate the high frequency of abnormalities, and reduce the regeneration time after transformation experienced by Yang *et al*. (1996). Carbenicillin and cefotaxime, two antibiotics used to suppress *Agrobacterium* growth, were shown to stimulate the number of somatic embryos at 125 mg/L for the former and 250 mg/L for the latter (Yu *et al*. 2001). Yu *et al*. (2003) found that *npt*II-transformed papaya root explants (using three PRSV-*cp* transgenic lines, 16-0-1, 17-0-5 and 18-0-9; Bau *et al*. 2003) were strongly inhibited by kanamycin, and authors recommended the use of geneticin at 12.5-25 mg/L.

Of note, varying transformation rates have been described for papaya. A low transformation efficiency (0.42% and 0.6%) was reported by Fitch *et al*. (1990, 1992, 1994) in 'Sunrise Solo' and 'Kapoho Solo' and by Fitch *et al*. (1993) in 'Kapoho Solo' for microprojectile bombardment or *A. tumefaciens*-mediated transformation, respectively. A 9% transformation efficiency, defined as number of transgenic plants obtained per number of immature zygotic embryo excised, was claimed by Souza Jr. *et al*. (2005a) and do Carmo and Souza Jr. (2003), after producing 54 'Sunrise Solo' and 'Sunset Solo' transgenic plants. Cheng *et al*. (1996) reported 15.9% transformation efficiency in 'Tainung №2', 41% by Mahon *et al*. (1996) in Queensland papaya line (OE), while Cabrera-Ponce *et al*. (1996) in yellow-large hermaphrodite type and Cai *et al*. (1999) in 'Sunrise Solo' both reported 100% efficiency. Transformation efficiencies are however difficult to compare, since the regeneration and transformation protocols vary (Cai *et al*. 1999).

Other transformation studies have focused on improving papaya germplasm and developing transgenic papaya

with resistance against *Phytophthora*, spider mites, and aluminum toxicity. Fitch *et al*. (2002) developed a novel clonal propagation system to replace multiple seedlings in which, following growth and yield trials, the clonally propagated plants bore fruit earlier, lower on the trunk, and could be harvested without extra equipment for a longer period than could seedlings. These authors further developed new PRSV-resistant cultivars by direct genetic transformation and also introgressed the PRSV-resistance gene from 'Rainbow' F2 progeny into the soil-adapted, *Phytophthora*-tolerant cultivar 'Kamiya'.

Zhu *et al*. (2004a) successfully transformed 'Kapoho Solo' with GFP and the stilbene synthase gene, *Vst*1, from *Vitis vinifera*. Increased resistance to *P. palmivora,* the main cause of root, stem and fruit rot diseases, was demonstrated. In another study, the *Dahlia merckii* defensin gene, *DmAMP*1, was used in the transformation of papaya (Zhu *et al*. 2007). Bioassays with extracts of total leaf proteins and leaf discs from transgenic papaya revealed inhibited the growth of *Phytophthora*. Similarly, transgenic plants in the greenhouse exhibited increased resistance against *P. palmivora* following inoculation. A reduction in the growth of *P. palmivora* at infection sites was observed. Murad *et al*. (2007) reviewed the use of defensins in transgenic plants.

Tolerance to carmine spider mites (*Tetranychus cinnabarinus* Boisd.) has been recently introduced in transgenic papaya varieties. McCafferty *et al*. (2006) reported on the development of papaya plants transgenic for the tobacco hornworm (*Manduca sexta*) chitinase protein for improved tolerance to spider mites. Transfer of the gene to embryogenic calli derived from the hypocotyls of the papaya cultivar 'Kapoho Solo' was done by microprojectile bombardment. Subsequent insect bioassays showed that plants expressing the chitinase gene had significantly lower populations of spider mites. Tolerance was also observed under field conditions and exposure to natural mite populations.

The technology of genetic engineering has not only been applied to developing resistance to biotic factors but also abiotic environmental factors that result in poor crop productivity and soil fertility. Poor crop productivity and soil fertility in acid soils are mainly due to aluminum toxicity. Aluminum has a clear toxic effect on roots by disturbing plant metabolism and decreasing mineral nutrition and water absorption. The potential role of organic acid release, for example citric acid, in Al-tolerance was originally proposed in the early 1990s. Citric acid chelates Al^{3+}. The strategy of producing transgenic plants with an increased capacity to secrete citric aicd was appealing since papaya production in the tropics is affected by acid soils. A citrate synthase gene (*CSb*) from *Pseudomonas aeruginosa* was cloned and biolistics used to successfully transform papaya (de la Fuente *et al*. 1997). Transgenic plants that could root and grow on aluminium concentrations up to 300 mM were regenerated. Non-transformed controls did not root on 50 mM Al^{3+} or less.

Projects aimed at improving the postharvest qualities of papaya by increasing the shelf-life of the fruit have been initiated and possibly have potential in reducing one of the industry's principal problems in fruit exportation. The strategy adopted to delay fruit ripening in papaya involved the suppression or inhibition of the key enzyme, ACC synthase (*ACS 1* and *ACS 2*) in ethylene production during the ripening process (Neupane *et al*. 1998) or ACC oxidase genes (*CP-ACO1* and *CP-ACO2*; Burns *et al*. 2007; Sew *et al*. 2007). Field evaluation of transgenic papayas was reported by Muda *et al*. (2003). Research is also being conducted on manipuating fruit softening. The gene of fruit cell wall enzyme β-galactosidase has been cloned and used to transform papaya (Umi *et al*. 2005).

More recently, the use of transgenic papaya as an antigen-delivery system for subunit vaccines has been explored. Transgenic papayas that carry the epitopes KETc1, KETc12, and GK-1, three promising candidates for designing a vaccine against *Taenia solium* cysticercosis, were developed (Hernández *et al*. 2007). Cysticercosis the most common parasitic infection of the central nervous system world-wide is caused by the pork tapeworm, *Taenia solium*. Infection occurs when tapeworm larvae enter the body and form cysticerci (cysts). Nineteen different transgenic papaya clones expressing synthetic peptides were found to confer resistance against cysticercosis. Complete protection against cysticercosis was induced with the soluble extract of the clones that expressed higher levels of transcripts of up to 90% of immunized mice. The results indicate that transgenic papayas may be a new antigen-delivery system for subunit vaccines (Hernández *et al*. 2007). The results are significant as there is an urgent need for affordable and reliable vaccines in developing countires. Costs associated with the production, maintenance and delivery of traditional vaccines are often very high resulting in limited distribution of vaccines in these countries. Theoretically, the expression of recombinant proteins in transgenic plants offers inexpensive vaccines that could be produced directly "on site".

GENETICS AND GENOMICS

Papaya offers several advantages for genetic and evolutionary studies including a small genome of 372 megabases, a short generation time of 9-15 months, numerous flower types, a unique evolutionary process in female flowers, an intriguing system of sex determination and an established transformation system (Storey 1941; Arumuganathan and Earle 1991). Genetic and genomic research was enhanced with three major accomplishments: a) Transgenic improvement by the transfer the PRSV *cp* gene and the successful development and release of transgenic varieties to save Hawaii's papaya Industry from collapse because of susceptibility to *Papaya ringspot virus* disease (Fitch *et al*. 1992; Gonsalves 1998); b) sex-linked DNA markers, where four sequence-characterized amplified region (SCAR) markers tightly liked to sex forms were developed (Parasnis *et al*. 2000; Deputy *et al*. 2002; Urasaki *et al*. 2002a, 2002b) as a means to sex the plant prior to flowering. All known sex-linked vegetative characters are too far from the sex determining locus to be of practical use; c) The genetic linkage map first reported by Hofmeyr (1939) consisted of only three morphological markers: sex form, flower color and stem color. But in 1996, Sondur *et al*. developed a second map based on 62 RAPD markers and mapped the sex determination gene on linkage group-1.

Sex determination in papaya has been a frequent subject of genetic analyses (Hofmeyr 1938; Storey 1938; Hofmeyr 1967; Storey 1976) because it is directly related to efficient commercial fruit production. Genetic analysis of papaya sex determination was carried out by crossing individuals of different sex types (Storey 1941). Storey (1953) hypothesized that papaya sex is determined by three alleles, *M*, *H* and *f*, at a single locus *Sex1*. The alleles *M* and *H* were assumed to be dominant over the *f* allele. Thus, the male, hermaphrodite and female sexes are determined by the *sex1* locus genotypes, *Mf*, *Hf* and *ff*, respectively, whereas, homozygotes of dominant alleles (MM and HH) as well as a heterozygote (MH) were assumed lethal. The cytological traits to identify heteromorphic chromosomes were not successful by Hofmeyr (1939).

Some molecular markers tightly linked to the sex of dioecious plants have been reported. In papaya, RAPD and microsatellite markers linked to sex have been reported (Sondur *et al*. 1996). In a southern hybridization study using the oligo-nucleotide $(GATA)_4$ as a probe, Parasnis *et al*. (1999) identified sex-linked DNA fragments. However, a papaya DNA marker both tightly linked to sex and easy to score has not been available. Studies by Urasaki *et al*. (2002a, 2002b) reported a RAPD marker specific to male and hermaphrodite plants of papaya. They showed that the marker, PSDM (papaya sex determination marker, 450 bp fragment), SCARs can be used to determine the sex of papaya plants at an early developmental stage (Giovanni and Victor 2007). The conversion of RAPD to SCAR marker allowed rapid sex identification in papaya. The result

suggested that PSDM, occurring only in the male and hermaphrodite genomes, might be located in the chromosome region that is specific to different sexes indicated the possibility of chromosomal differentiation between sexes. However, sex was correctly predicted with SCAR marker in each of 49 papaya plants tested for sex. These DNA sex markers are now used in selection of desired sex types at the seedling stage for more efficient papaya production (Deputy *et al*. 2002).

The papaya sex locus has been genetically mapped to linkage group 1 (LG 1; Sondur *et al*. 1996). Two studies were undertaken by Liu *et al*. (2004) and Yu *et al*. (2007); using fluorescence *in situ* hybridization mapping of Y(h)-specific bacterial artificial chromosomes and showed that papaya contains a primitive Y chromosome, with a male-specific region that accounts for only about 10% of the chromosome. In the former study, they found severe recombination suppression and DNA sequence degeneration which provided direct evidence for the origin of sex chromosomes from autosomes. Those authors found that hermaphrodite and male papaya plants share identical DNA sequences in most parts of the male-specific (MSY) region. These two sex types appeared to share a halotype for the MSY region that differs from that of females and is recently derived from a common ancestral chromosome. Their studies suggested that hermaphrodites with an MSY region are already genetically different from the ancestral hermaphrodites.

In comparison to other crop species, the genetic mapping of papaya lagged behind that of many other plant species, due partly to the low level of polymorphism among existing germplasm (Sharon *et al*. 1992; Stiles *et al*. 1993; Kim *et al*. 2002).To develop a high-density genetic map of papaya and to characterize its sex-locus, Ma *et al*. (2004) constructed 54 F_2 plants derived from cultivars 'Kapoho' and 'SunUp' with 1501 markers, including 1498 amplified fragment length polymorphism (AFLP) markers, the PRSV *cp* marker, morphological sex type and fruit flesh color. They mapped those markers into 12 linkage groups with a recombination frequency of 0.25. The study revealed severe suppression of recombination around the sex determination locus with a total of 225 markers cosegregating with sex types. Therefore, the high-density genetic map was recommended for the cloning of specific genes of interest such as the sex determination gene and for the integration of genetic and physical maps of papaya.

CONCLUDING REMARKS

Papaya continues to increase in importance as a fruit crop. Several of the limitations in tissue culture can now be overcome using some of the novel techniques in *in vitro* culture outlined in this review. Genetic transformation is now well established and improved virus detection techniques and integrated pest control programs should allow the modern breeder to find novel solutions to any remaining problems in the progation and production of papaya. One such example is the heavy use of pesticides in papaya culture (Hernández-Hernández *et al*. 2007). A transgenic approach could be used to introduce traits to provide resistance to important pests. However, despite all these possibilities, several factors (cultural, governmental, ecological, and environmental) need to be considered when introducing transgenic papaya varieties into new cultivation areas (http://www.greenpeace.org/international/news/the-scent-of-ge-papaya). Despite the great advances in transgenic papayas, certain markets, e.g. the Japanese market, continue to insist on the importation of non-transgenic'Kapoho Solo' and 'Sunrise' papaya fruits through a joint Identity Preservation Program between Japanese exporters in Hawaii and the Hawaii Department of Agriculture (Mochida 2007). Some excellent, local updates on papaya research and marketing can be appreciated in *Acta Horticulturae* **740**.

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