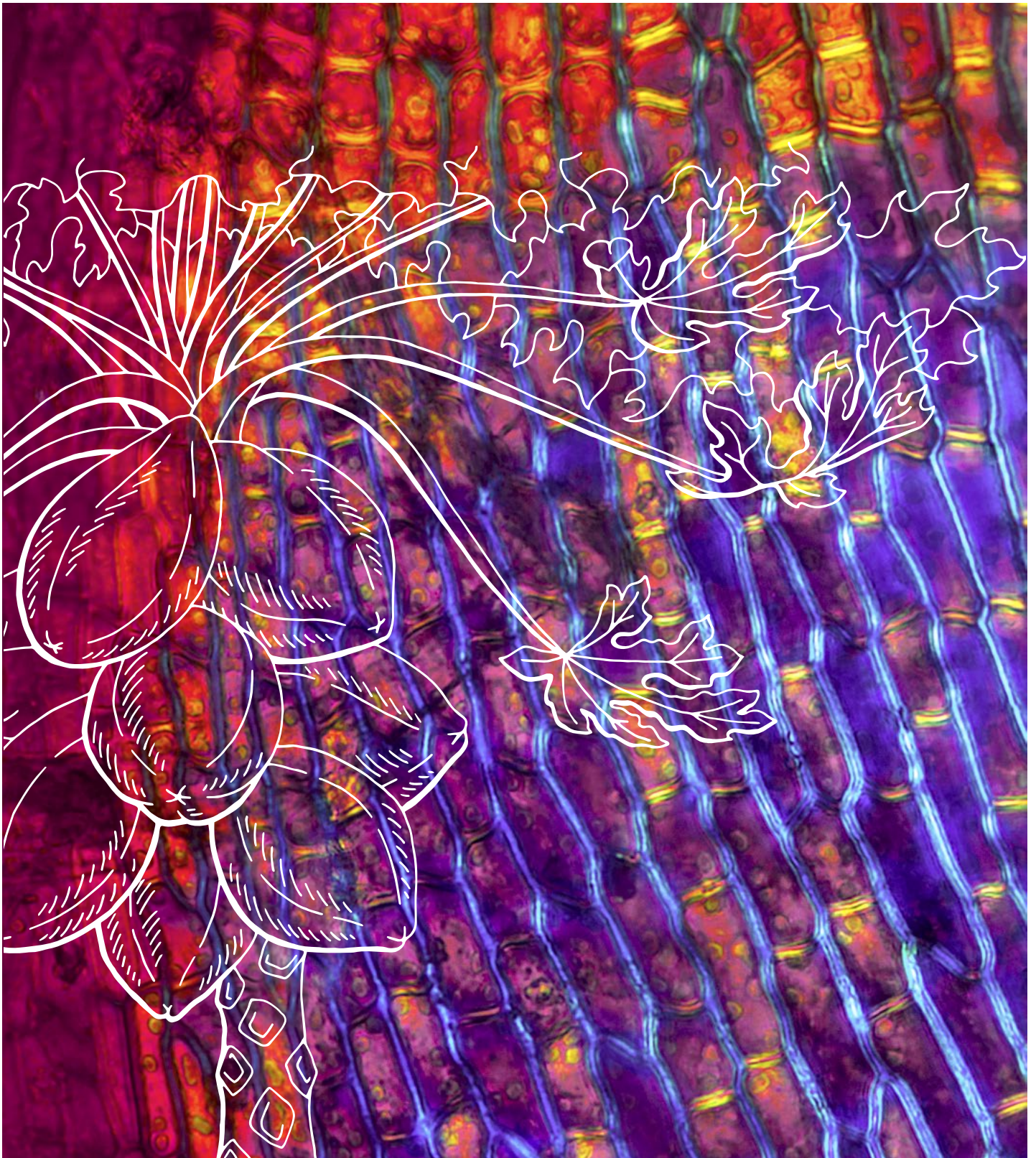


# BIOLOGY OF PAPAYA

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*(Carica papaya L.)*







## Biology of Papaya (*Carica papaya* L.)

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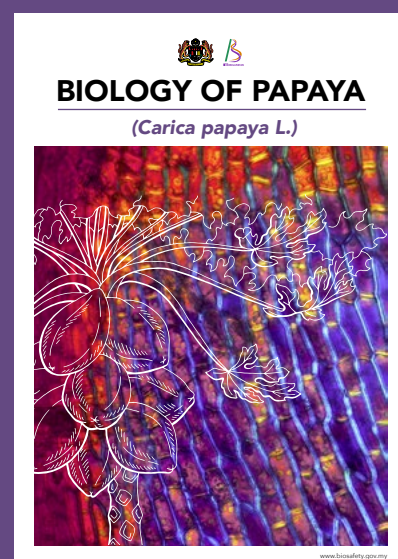
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### About the cover image



A polarizing micrograph of papaya leaf image with illustration of papaya tree.

BIOLOGY OF  
**PAPAYA**

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## PREAMBLE



Malaysia has been involved in the development of genetically modified (GM) papayas since the 90's and its progress accelerated after the Malaysian Agricultural Research and Development Institute (MARDI) joined the Papaya Biotechnology Network of South East Asia coordinated by the International Services for Acquisition of Agri-biotech Applications (ISAAA) in 1998. The focus of the Network was on development of papayas with resistance to papaya ringspot virus disease and papayas with delayed ripening, the two major constraints to the papaya industry at that time. In Malaysia, the current research is on development of GM papayas with resistance to Bacterial Die Back (BDB), the most devastating disease that currently holds back investment in papaya cultivation. Several lines for delayed ripening papaya are ready for field testing and ultimately commercialization. However, all GM materials must undergo preliminary contained and later restricted field trials to establish the

risk assessment with findings of no significant impact (FONSI) before they can be released for more extensive open field trials and commercial planting. The Hawaiians for example, have spent many years in the deregulatory process before they could release their world's first GM papaya 'Rainbow'. The documentation of the Biology of Papaya particularly in the Malaysian context is important for the risk assessment in the later release of GM papayas. This document covers the origin, distribution and trade, the ecology the plant requires for optimum growth and development, botany, genetics, propagation and cultural practices. Important considerations in Findings of No Significant Impact (FONSI), relating to toxins, allergens, weediness and potential for gene transfer are also presented. This is an important reference for researchers and regulatory bodies for the release of GM papayas in the Malaysian environment.



## I. INTRODUCTION



**P**apaya (*Carica papaya* L.) is a popular fruit native to tropical America. It is usually grown for its small to large melon-like fruit. It is a herbaceous short-lived perennial, bearing fruits continuously at the leaf axils spirally arranged along the single erect trunk. Papaya trees can normally live for 5-10 years, but in commercial plantings they are replanted every 2-3 years because the trees become too tall for economic harvesting. The papaya is also called papaw, pawpaw, papayer (French), melonenbaum (German), lechosa (Spanish), mamao, mamoeiro (Portuguese), mugua (Chinese) and betik (Malaysian, Indonesian).

The papaya is popular as a backyard tree in many developing countries but increasingly becoming more important in commercial plantings for domestic markets and in countries like Mexico and Malaysia, for export. The advantage in papaya cultivation is the rapid return to investment due to its early maturation, intensive cultivation and high yield. Most papayas in the tropics can be harvested 8 or 9 months after sowing and yields can range from 60-100 t/ha/year for improved varieties. The ripe fruit has a delicate aroma and sweetness and have high contents of vitamins A and C. One medium-sized papaya exceeds the Dietary Reference Intakes (DRI) of 3000 IU for vitamin A and 90 mg for vitamin C established by the US Food and Nutrition Board (OECD, 2005).

There is great diversity in the size, shape and quality of the fruit. In unselected germplasm or backyard trees, fruits are usually very large and not very palatable, but for varieties such as 'Solo' and 'Eksotika' specifically selected for export or up-markets, they are usually small for convenience in packaging and have much better taste and storage attributes.

Papaya is usually eaten fully ripe when the flesh is soft and succulent. However, it can also be eaten raw, sliced into thin strips and eaten as vegetable, or processed into various products such as candy, pickle or puree. The 'Eksotika' papayas imported by China, are served as a delicacy in high-end restaurants. The half-cut fruit with seed scooped out are filled with 'sharks-fin' or 'birds-nest' and steamed before serving. The latex from unripe fruit and leaves contains a proteolytic enzyme papain, which can be used for tenderizing meat, chill-proofing beer, tanning leather and for making chewing gum. In pharmaceuticals, papain is used for suppression of inflammation, treatment of gangrenous wounds and for various digestive ailments. As a proteolytic enzyme, it has exfoliating property that removes the dead surface cells of the skin, giving it a rejuvenated feeling. It is therefore popularly used in soaps, creams, shampoos and lotions in the cosmetic industry.

## 2. ORIGIN, DISTRIBUTION AND TRADE

### 2.1. Origin and distribution

The cultivated species of *Carica papaya* L. has not been found wild in nature. Its origin is rather uncertain, but there is some agreement among botanists that it originated in the lowlands of Central America between southern Mexico and Nicaragua (Storey, 1969a). It is believed to have originated from hybridization between two Mexican species. Early distribution over wide geographical regions was enhanced by abundance of seeds in the fruit and their long viability. Following the discovery of the New World, the papaya was taken by early maritime explorers along tropical trade routes and reached Panama as early as 1535, Puerto Rico by 1540 and Cuba soon thereafter (Storey, 1969a). By 1611, it was grown in India and by 1800 was widely distributed in the South Pacific, Malacca and Philippines. Don Francisco Marin, a Spanish explorer and horticulturist, is credited with the introduction of papaya into Hawaii from the Marquesas Islands during the early 1800's. Papaya is now grown in all tropical countries and in many subtropical regions of the world.

Major commercial production of papaya is found primarily between 23° N and S latitudes. Man has extended cultivation into regions as far as 32° N and S. At these latitudes, papayas may be best grown in well-protected areas at sea level or under greenhouses as seen in the Canary Islands and Okinawa. In Hawaii, at 19 to 22°N, papaya is grown at sea level and up to 300 m elevation (Nakasone and Paull, 1998).

### 2.2. Cultivation and trade

The area of papaya cultivation in Malaysia is only 1,579 ha (Anon., 2013) with a production of 31,748 mt and a net value of RM 62 million in 2013. The contribution of East Malaysia (Sabah, Sarawak and Labuan) to area of production was only 18% (Table 1). The papaya production in Malaysia was insignificant compared to the global production of 11.22 million mt in 2010 (Anon., 2012) (Table 2). Of this, 42% was produced by India (4.7 million mt), followed by 17.6% from Brazil (1.87 million mt), and about 6% each from Indonesia, Nigeria and Mexico. The most important papaya exporter in 2011 was Mexico leading with 40.9% of the world export, followed by Brazil (11.2%), Belize (11.2%) and Malaysia (9.4%) (Table 3). It was surprising that Malaysia with just a fraction of the world's production, can achieve the fourth position in world exports. In fact, during its heydays in 2005, Malaysia exported 42,008 mt and was the second most important exporter in the world. The major market for Malaysian papayas was China (including Hong Kong) and the rapid decline in export was due to quarantine restrictions that require pre-entry fruit disinfestation and also due to the drop in production because of diseases. The Netherlands and Belgium do not produce papayas but are mentioned as significant exporters because they act as transient ports for the redistribution of papayas to other cities in the European Union.

**TABLE 1** Production of papayas in Peninsular Malaysia, Sabah and Sarawak (2013)

States	Hectareage (Ha)	Harvested Area (Ha)	Production (Mt)	Value of Production (RM)
Johor	853.5	789.1	16,108.9	31,412,265
Kedah	135.9	105.7	1,267.8	2,472,215
Kelantan	68.7	64.4	1,405.0	2,739,742
Melaka	51.2	43.1	484.0	943,810
Negeri Sembilan	38.2	37.0	802.0	1,563,884
Pahang	186.6	173.9	949.8	1,852,067
Perak	167.6	157.2	4,561.6	8,895,206
Perlis	5.8	5.8	104.4	203,580
Pulau Pinang	50.8	48.8	794.3	1,548,944

States	Hectareage (Ha)	Harvested Area (Ha)	Production (Mt)	Value of Production (RM)
Selangor	16.5	13.4	55.0	107,273
Terengganu	4.6	2.5	23.0	44,909
<b>Peninsular Malaysia</b>	<b>1,579.4</b>	<b>1,441.0</b>	<b>26,555.8</b>	<b>51,783,895</b>
Sabah	170.4	158.5	3,505.1	6,834,945
Sarawak	182.0	129.3	1,653.3	3,223,935
W.P. Labuan	2.2	2.2	34.1	66,495
<b>MALAYSIA</b>	<b>1,934.0</b>	<b>1,731.0</b>	<b>31,748.3</b>	<b>61,909,270</b>

Source: Fruit Crops Statistic Malaysia (2013)

**TABLE 2** Global papaya production, 2006-2012 (mt)

Countries	2006	2008	2010	2012	2012 (%)
India	2,482,100	3,629,000	4,713,800	5,160,390	41.6
Brazil	1,897,640	1,890,290	1,871,300	1,517,696	12.2
Indonesia	643,451	717,899	695,214	906,312	7.3
Nigeria	759,000	688,782	703,800	775,000	6.2
Mexico	798,589	638,237	616,215	712,917	5.7
Ethiopia	259,174	250,000	232,400	38,694	0.3
Congo	217,900	221,800	225,772	230,000	1.9
Colombia	164,606	207,698	263,178	163,939	1.3
Thailand	134,443	201,099	211,594	215,000	1.8
Guatemala	113,277	190,000	200,000	206,500	1.7
Other	1,432,796	1,413,564	1,489,758	2,485,118	20.0
<b>TOTAL</b>	<b>8,902,976</b>	<b>10,048,369</b>	<b>11,223,031</b>	<b>12,411,566</b>	<b>100.00</b>

Source: FAOSTAT (2015)

**TABLE 3** Global papaya exports, 2007-2011 (mt)

Countries	2007	2009	2011	2011 (%)
Mexico	101,306	134,960	104,797	40.6
Brazil	32,267	27,554	28,823	11.2
Belize	33,341	27,152	30,839	11.9
Malaysia	26,938	24,301	22,207	8.6
India	10,880	17,573	18,657	7.2
United States	9,604	8,090	11,946	4.6
Netherlands	8,625	8,023	6,313	2.4
Guatemala	6,680	7,375	11,537	4.4
Ecuador	5,486	5,370	9,270	3.6
Belgium	527	2,496	532	0.2
Others	40,103	16,790	13,369	5.3
<b>TOTAL</b>	<b>275,757</b>	<b>279,684</b>	<b>258,290</b>	<b>100.0</b>

Source: FAOSTAT (2015)

## 3. ECOLOGY

### 3.1. Soil

Papayas are not very fastidious to soil types but they must be well-drained as even short periods of flooding (> 8 hours) can lead to root rot and death of plants. A porous loam or sandy loam soil with pH between 5.0 and 7.0 is ideal. At pH levels below 5.0, plant growth is poor and field mortality high and lime applications are needed to increase growth and yield.

Papaya can be grown successfully in certain marginal soils, such as peat, sandy soils (tin-tailings) and acid sulphate soils, if their inherent shortcomings are taken care of. For peat and acid sulphate soils, high rates of lime application (6 - 8 t/ha) are essential for successful cultivation. On peat, micronutrients like boron, zinc and copper should also be applied regularly to ensure production of quality fruits. Water table in peat and acid sulphate areas is usually high and the area requires drainage before papaya, which is very sensitive to flooding, can be grown. Yields of more than 100 t/ha for some selected hybrids have been obtained from peat soils (Chan and Raveendranathan, 2003). However one major constraint encountered on peat is the poor root anchorage and trees will dislodge if they are not staked or supported. It is also not possible to employ heavy farm equipment on the soft peat ground.

Cultivation of papaya on sandy soils requires incorporation of large amounts of organic matter such as chicken dung and Palm Oil Mill Effluent (POME) (> 30 t/ha), heavy chemical fertilization (8 kg/plant/year) coupled with irrigation facilities. Susceptibilities to boron deficiency and nematode infestation are two major problems encountered on sandy soils.

Papaya has been ranked from extremely sensitive to moderately tolerant to salinity. Germination and early seedling growth appear to be the most sensitive stages. It is probably moderately salt sensitive at other growth stages.

Another marginal soil for papaya cultivation is very heavy clay with a shallow impervious hardpan that makes the soil prone to water-logging. For the first crop, shallow beds covered with plastic mulch to prevent compaction worked quite well (Chan and Baharuddin, 2010a). Under this system, the trees grew and yielded well only for the first year. After this, the root system was confined and unable to penetrate sideways of the plastic mulch or underneath the hardpan and the vigour of the trees was abruptly halted. The confined root system was unable to anchor the trees and dislodging and loss of production was common during storms. In the subsequent plantings, the subsoil should be broken up using bulldozer drawn tines or in the case of deep hardpans, excavators may be used (Chan and Baharuddin, 2010a). Papayas cultivated on marginal soils have a relatively shorter economic life span and the texture and sweetness of the fruits may be slightly inferior to that obtained from the usual loamy soils.

### 3.2. Climate

#### 3.2.1. Rainfall

Papaya is a continuous cropper and fruiting may be disrupted even with short periods of drought lasting a week or so. It grows and yields well in the humid tropics where there is a uniform annual precipitation of about 1200 mm. However, supplementary irrigation is still needed as perfect uniform rainfall distribution does not occur in tropical areas with monsoon-type climates of well-defined wet and dry seasons. Irrigation can increase yield by 20% over rainfed papayas in Malaysia and this increase more than compensates for the cost of the irrigation system (Chan *et al.*, 1991).

During the drought season, water stress causes flowers to go into a sterile phase resulting in non-productive 'skips' in fruit production. During the wet season, the consistently high moisture level promotes the production of hermaphrodite flowers with reduced number of stamens (Awada, 1961). Such flowers develop into misshapen or 'cat-faced' (carpelloid) fruit that are unmarketable. Drought also leads to the rapid shedding of older leaves and the fruit stay unripe on the trees longer. With the sudden onset of rain, several fruits may be harvested from the same tree as they simultaneously turn 'breaker-colour'. Prolonged wet spells especially in poorly drained areas, are detrimental to

papayas. Within five days of continuous rainfall, chlorosis of the lower leaves is evident and in severe cases, the leaves lose their turgidity and hang bunched around the trunk but do not abscise. Recovery from non-lethal flooding is slow due possibly to the low regeneration of new roots in fruiting trees. In Queensland, Australia, a dieback disorder of papaya was linked to excessive rainfall followed by a hot dry period and exacerbated by heavy, poorly drained soils (Glennie and Chapman, 1976).

### 3.2.2. Temperature

The optimum temperature for papaya growth is between 21 °C to 33 °C. Papaya is extremely sensitive to frost and if temperature falls below 12 to 14 °C for several hours at night, growth and production is severely affected (Nakasone and Paull, 1998). Dioecious cultivars are more stable towards variation in temperatures, as female trees at <20 °C do not exhibit the sex changes shown by the more sensitive hermaphrodite cultivars. Cool temperatures causes a reduction in stamen number in hermaphrodite flowers, a condition commonly known as 'carpellody'. Hermaphroditic cultivars like Solo grown under minimum temperature less than 17 °C can have 100% carpelloid flowers. At higher temperatures (>35 °C), there is a tendency of hermaphrodites to form functional male flowers with poorly developed and non-functional female parts. Reversal to this sterile stage leads to skips in fruiting. This tendency varies with cultivars and as well as within a cultivar. Net photosynthetic rate also rapidly declines above 30 °C. High temperature following excessive moisture was believed to cause the dieback disorder of papaya (Glennie and Chapman, 1976).

Temperature during the growing season significantly influences fruit development and maturation and may stretch it from the normal 120 to 150 days especially in subtropical areas. Flowers and fruit usually abort in winter and if the fruit set in late fall, it can take up to 90 days longer to mature. Such fruits are unattractive, with pale flesh colour and low total soluble solids. Final fruit size is determined in the first 4 to 6 weeks of fruit development and temperature plays a dominant role in the process especially in subtropical areas (Nakasone and Paull, 1998).

### 3.2.3. Radiation

Papaya is a sun-loving plant. When shaded, the plants are etiolated, have smaller leaves, lower stomatal density, increased internode and petiole length and decreased chlorophyll content. Partial stomatal closure and opening occurs rapidly with cloud related changes in irradiance, thereby maximizing plant water use efficiency (Clemente and Marler, 1996).

No photoperiodic effects on tree growth, production or sex expression were reported (Lange, 1961). Allan *et al.*, (1987) however, reported that short daylengths coupled with cool temperature caused reversion of usually sterile staminate flowers from male trees to fertile, elongate type hermaphrodite flowers.

### 3.2.4. Wind

Papaya trees are delicate and require protection from strong winds. The root system is well-developed but relatively shallow and the tree can be uprooted by winds of 64 km/hr, especially if the soil is softened by rain (Nakasone and Paull, 1998). Even though trees may withstand uprooting, considerable shattering of the large leaves occurs, resulting in reduced photosynthesis. This leads to flower and young fruit abscission and reduction in total soluble solids of the maturing fruit. Abrasions of the fruit caused by swaying petioles and leaves also scar the fruit and reduce its marketability. Recovery from wind damage can take from 4 to 8 weeks.

## 4. BOTANY

### 4.1. Taxonomy and species

Papaya belongs to the family Caricaceae under the genus *Carica*. Until recently, this family consisted of three other genera, i.e. *Cyclimorpha*, *Jacaratia* and *Jarilla* and *Carica* itself was made up of 21 species (Badillo, 1993). However, the family Caricaceae is now re-classified as having six genera (with the addition of *Vasconcellea* and *Horovitzia*) and *Carica* now has only one species i.e. *C. papaya* which represents the cultivated papaya (Badillo, 2000). *Vasconcellea* whose species were formerly assigned to the genus *Carica*, includes several species with edible fruits and have great importance for breeding and genetic studies. The species with edible fruits are *V. cundinamarcensis* (chamburo), *V. pubescens* (ababai), *V. stipulata* (siglalon or jigacho), *V. monoica* (col de monte), *V. goudotiana* (papayuelo), *V. quercifolia* and *V. cauliflora* (bonete or mountain pawpaw). Most of the fruits from the *Vasconcellea* are small with thin, dry pulp and are usually cooked and flavoured before eating. *V. pentagona* (babaco) is of subtropical origin found in the highlands of Ecuador (Oosten, 1986) and commercially cultivated for its large, parthenocarpic fruit in New Zealand (Little, 1982).

Some *Vasconcellea* species have resistance to diseases which *C. papaya* is susceptible to, e.g. *V. cauliflora* and *V. quercifolia* are resistant to distortion ringspot virus, and some attempts have been made to transfer resistance to *C. papaya* by intergeneric hybridization. Successful interspecific hybrids have been reported among *Vasconcellea* species but *C. papaya* was not cross compatible with any of them (Mekako and Nakasone, 1975). However, hybrids of *C. papaya* with *V. cauliflora* (Manshardt and Wenslaff 1989a) and with *V. pubescens*, *V. quercifolia* and *V. stipulata* (Manshardt and Wenslaff, 1989b) were obtained using embryo rescue techniques to overcome post-zygotic barriers to hybridization. More recently, Drew *et al.*, (2005) reported success in interspecific hybridization between *C. papaya* and *V. quercifolia* and a few interspecific hybrids resistant to papaya ringspot virus have been selected. These backcrossed fairly easily to *C. papaya* and further backcrosses would be expected to develop a resistant genotype with the economic traits of *C. papaya*.

### 4.2. Mating system

*C. papaya* is a polygamous species. In nature, it is dioecious with male and female trees in the population, but possibly due to Man's interference and deliberate selection against non-productive male trees, gynodioecious populations with female and hermaphrodite trees also exist (Storey, 1969a).

For dioecious populations, several workers (Prest, 1955; Storey, 1969a) agreed that wind is the main agent of pollen dispersal. The long pendulous male inflorescence which readily sheds pollen in the breeze, lends support to this belief. However, Allan (1963) reported that very little papaya pollen was airborne and suggested that honeybees were responsible, although papaya flowers were not the priority sites for visits. Free (1975) reported that papaya flowers were often visited by many Skipper butterflies (*Perichares philetus philetus*) during dusk in Jamaica, and suggested their use as pollinators if the need arose. Garret (1995) showed that pollination by wind and honeybees was rare in Queensland, Australia; the main pollinator being hawkmoth (*Hyles* sp.). Some native vegetation in Queensland and cultivation of grape and sweetpotato help to attract these pollinators and increase fruit production in papaya. Dioecious papaya varieties are enforced cross-pollinators because of physical separation of the androecium and gynoecium.

In gynodioecious populations, the role of wind as the pollinating agent is diminished. This is because the stamens are packed inside the corolla tube and seldom protrude prominently out of the flower. Many gynodioecious varieties such as 'Sunrise Solo', 'Kapoho Solo' and 'Eksotika' are self-pollinated and are, therefore, purelines. The hermaphrodite flowers are mostly cleistogamous, i.e. anthers dehisce and release the pollen to effect self-pollination prior to anthesis of the flower (Rodriguez-Pastor *et al.*, 1990). Such varieties are enforced self-pollinators and seed gathered from these hermaphrodite fruit will usually breed true to type. Self-pollination in papaya does not appear to result in any loss of vigour (Hamilton, 1954).

Parthenocarpic seedless fruit may sometimes form without fertilization of flower. This usually

happens to isolated female trees without any nearby pollen source. Parthenocarpy is usually not a criterion pursued in breeding programmes because seeds are quite easy to remove in papaya. Moreover, parthenocarpic fruit are small and although seedless, the tiny undeveloped embryos are bitter and still need to be scraped off before the fruit is eaten.

Papaya pollen production and viability varies according to variety and season. In the production of the F<sub>1</sub> hybrid 'Eksotika II', two inbred parents Line 19 and Line 20 were used, with the latter as male parent because of consistently higher pollen production (Chan, 1993). Variation in pollen production was observed with decreased quantities during winter and early spring (Garret, 1995). Pollen from freshly anthesized stamens have 90% viability during summer but drops to as low as 4.5% in winter when temperatures below 10 °C severely affect viability because of degeneration of pollen mother cells (Allan, 1963). Under ideal storage conditions, pollen remains viable for 5-6 years. In contrast to pollen production and viability, stigma receptivity remains high throughout the year. Both female and hermaphrodite flowers pollinated with viable pollen will successfully set fruit even in winter.

In studies of pollen movement using GUS transgene marker, Manshardt *et al.*, (2005) have shown that papaya trees located 0.4 km downwind, did not get pollinated by pollen from an acre of GUS-marked 'Rainbow' papaya. The Papaya Biotechnology Network of Southeast Asia (ISAANA) has also proposed a separation of 400 m as safe distance for preventing gene transfer from transgenic to non-transgenic papayas (Hautea *et al.*, 1999). Singh (1990) recommended 2-3 km isolation distance to preclude foreign pollen from contaminating the production of foundation seed for papaya.

## 4.3. Morphology

### 4.3.1. Stem

The stem is soft-wooded, usually single and erect and sometimes branched if the terminal shoot is injured. The stem is hollow, and marked by prominent half-moon shaped leaf scars on the surface. A 2-year old tree in an orchard will have a trunk diameter of 20-25 cm at the base and about 5-10 cm near the crown of leaves. The height would be about 4-5 m.

### 4.3.2. Leaves

The leaves are clustered at the apex of the stem. They consist of large, palmate laminae, 40-60 cm in width, normally with 7-9 lobes and held by long, hollow, pale green or purple tinged petioles. Usually 15 mature leaves are present; the older, lower leaves shrivel and sometimes remain attached for a while to the trunk. Under Malaysian conditions, varieties like 'Sunrise Solo' and 'Subang 6' produce about three leaves per week (Chan and Toh, 1984).

### 4.3.3. Fruit

The papaya fruit is a fleshy berry, variable in weight from 200 g to well over 10 kg. Fruit shape is a sex-linked character and ranges from spherical to ovoid from female flowers to long, cylindrical or pyriform (pear-shaped) from hermaphrodite flowers. The skin of the fruit is thin and usually green when immature, turning to yellow or orange when ripe. The immature fruit when bruised, exudes a white sticky latex that contains a proteolytic enzyme papain. The flesh is succulent, usually yellow or reddish orange in colour. The fruit has a central ovarian cavity that is lined with the placenta carrying numerous black seeds. The ovarian cavity is larger in female fruit than in hermaphrodite fruits. The shape of the cavity at the transverse cut, ranges from star-shaped with 5-7 furrows to smooth circular-wall.

### 4.3.4. Seed

The papaya seed consists of a small laterally flattened embryo with ovoid cotyledons surrounded by fleshy endosperm, and a seed coat made up of a dark brown, hard, muricate endotesta and a translucent sarcotesta which contains a thin mucilaginous fluid. This fluid contains growth inhibitors that prevent germination while the seed are still in the fruit. A well-pollinated fruit produces about 800-1,000 seed attached to the interior wall (placenta) of the ovarian cavity.

### 4.3.5. Floral biology

#### 4.3.5.1. Flower types and sexes

Storey (1941) classified papaya flowers into five basic types:

<b>Type I:</b>	Pistillate or female flower devoid of stamens, with a distinct ovoid ovary terminating in a five-lobed stigma (Figure 2a)
<b>Type II:</b>	Hermaphrodite (pentandria) flower having five functional stamens and a globose five-furrowed ovary (Figure 2b)
<b>Type III:</b>	Hermaphrodite (carpellid) flower having six to nine functional stamens and an irregularly-ridged ovary (Figure 2c)
<b>Type IV:</b>	Hermaphrodite (elongata) flower having ten functional stamens and an elongate, smooth ovary (Figure 2d)
<b>Type IV+:</b>	Hermaphrodite (barren) flower having ten functional stamens but the pistil aborts, becomes vestigial and lacks a stigma (Figure 2e)
<b>Type V:</b>	Staminate flower having ten functional stamens only. The ovary is completely absent and flowers are bunched in an inflorescence (Figure 2f)

Although five basic floral types are listed, certain male and hermaphrodite trees undergo sex reversal and morphological changes to varying degrees under the influence of climatic and environmental changes (Storey, 1958).

#### 4.3.5.2. Derivation of floral types

The evolution and derivation of the pistillate (Type I) and staminate (Type V) flowers started basically from a common ancestor, i.e. the elongata hermaphrodite flower (Type IV) (Storey, 1969b). From the elongata flower, two phylogenetic lines diverged intraspecifically, each terminating with the derivation of a unisexual form.

The staminate flower was derived along classical lines, i.e. with the phylogenetic loss of the gynoecium without appreciable disturbance to other floral organs. Transitional forms of hermaphrodite flowers leading to complete maleness showed reduction in ovary size, numbers of stigmatic rays, dorsal vascular bundles, carpels and placenta (Nakasone and Lamoureux, 1982).

Derivation of the pistillate flower, on the other hand, represents a departure from the classical theory. It arose not through the loss of stamens from the hermaphrodite (elongata) type but by the incorporation or fusion of the stamens to the ovary tissues. The sequence leading to the derivation of the pistillate flower began in the upper whorl of five stamens of the elongata flower with the stamens fusing with the ovary, thus leading to the formation of the intermediate pentandria (Type II) flower. This is the start of the final steps to the formation of the pistillate flower. After further fusion of the remaining five stamens to the ovary was complete, the pistillate flower is the result. The process of fusion of the stamens to the ovary is called carpelody of stamens. Between the elongata flower and the pistillate flower, many intermediate, carpellid forms therefore exist, depending on the number of stamens that are fused. Such flowers develop into misshapen or 'cat-faced' fruits which are not marketable.

With regard to the female or pistillate flower, its morphological structure is strongly fixed genetically in the female tree. Therefore, unlike hermaphrodite trees where sex reversal is commonplace, the pistillate tree is virtually unknown to undergo any change of sex (Storey, 1969b).

Somsri *et al.*, (1998) also hypothesized that sex in papaya is controlled by a single locus with three alleles, i.e.  $M_1$  (staminate),  $M_2$  (hermaphrodite) and  $m$  (pistillate). Staminate ( $M_1m$ ) and hermaphrodite plants ( $M_2m$ ) are heterozygous whereas pistillate plants ( $mm$ ) are homozygous recessive. Combinations of dominants, namely  $M_1M_1$ ,  $M_1M_2$ , or  $M_2M_2$  are lethal, leading to post-zygotic abortion of such ovules; the lethal effect of these homozygous dominant sex-determining alleles is further evidence of the degeneration of the Y chromosome. This hypothesis predicts that viable males can only be  $M_1m$  (dioecious) and viable hermaphrodite papaya plant can only be  $M_2m$  (gynodioecious). A cross of two hermaphrodite flowers normally yields a 2: 1 (hermaphrodite:pistillate) ratio.



Ming *et al.*, (2007) proposed that two sex determination genes were involved in the sex determination pathways in trioecious papaya. One is a feminizing or stamen suppressor gene, which causes stamen abortion before or at flower inception while the other, a masculinizing or carpel suppressor gene, which causes carpel abortion at a later flower development stage.

There have been reports of molecular markers tightly linked to the sex of dioecious plants (Somsri *et al.*, 1998; Somsri and Bussabakornkul, 2007). Urasaki *et al.*, (2002a; 2002b) that reported a Random Amplified Polymorphic DNA (RAPD) marker specific to male and bisexual papaya plants. Giovanni and Victor (2007) converted RAPD to Sequence Characterized Amplified Regions (SCAR) marker to allow rapid sex determination. In the Philippines, two pairs of primers have been used successfully to predict the sex types of papaya in the seedling stage. These primers are:

T1-F 3'TGCTCTTGATATGCTCTCTG 5'	T1-R 3'TACCTTCGCTCACCTCTGCA 5'
W11-F 3' CTGATGCGTGTGTGGCTCAT 5'	w11-R " CTGATGCGTGATCATCTACT 5'

#### 4.3.5.3. Environmental influences on sex expression

Although the basic sex types in papaya are genetically determined, certain male and hermaphrodite trees have been known to undergo sex reversal under the influence of environmental changes (Storey, 1958).

*Temperature.* Cool temperatures like those experienced during winter months of subtropics promote femaleness in hermaphrodite trees. The flowers revert from the Type IV hermaphrodite which has ten stamens to a Type III or carpelloid form with 6-9 stamens or the Type II (pentandria) having 5 stamens. This reduction in the number of stamens is brought about by fusion of the stamens to the ovary wall. At high elevations i.e. cooler temperature, Solo papayas have a greater number of Type II and Type III fruits which are not marketable (Awada, 1958). At the other extreme, warm temperatures tend to promote the production of Type IV+ (barren) hermaphrodite flowers resulting in sterility of the trees. When such conditions persist over a period of time, a production gap (sterility skip) is evident along the trunk. Allan *et al.*, (1987) reported that male trees also showed reversion to femaleness under cool temperatures. Night temperatures of 12 °C and short daylengths caused reversion of sterile staminate (Type V) flowers to fertile, elongate type hermaphrodite (Type IV) flowers.

*Moisture and Nitrogen.* High soil moisture and nitrogen levels that promote vigorous plant growth encourage the expression of femaleness. Hermaphrodite trees stressed by drought produced more sterile Type IV+ flowers, while consistently high moisture levels promoted the production of hermaphrodite flowers with reduced number of stamens (Type II and Type III) (Awada, 1961). Increased nitrogen application from 0.1 to 2 lb/tree at every 6-week interval brought about a 58% increase in fruit rejects arising from carpelloid fruits (Awada *et al.*, 1979). Moisture and nitrogen levels affect the well-being and vigour of trees, indirectly bringing about reversal in sex. Vigorous growing trees during the first year of bearing had higher incidence of carpelloid than subsequent harvests (Chan, 1984).

## 5. PROPAGATION

### 5.1. Seed (sexual reproduction)

Papaya is almost entirely propagated from seed in commercial cultivation. Sound seed usually germinate after two weeks in the polybags and are ready for transplanting at the 8-12 leaf stage after another six weeks. The cost of production for each seedling is estimated to be RM 0.35 (about USD 0.1) (Chan *et al.*, 1991), and this works out to about 3.5% of the total production costs for papaya (estimated at RM10/tree over two years).

The seeding rate for papaya is very low. This is because dry papaya seed are relatively light, weighing about 14.5 g per 1,000 seeds. Further, the density of the crop at 2,000 trees/ha is relatively low compared with cereals and horticultural crops like vegetables. For establishing a hectare of papaya, about 3,000 seed or only 50 g are required (Chan, 1994). This would be increased to 75-100 g if multiple planting per point is practised.

It is important to maintain the purity of the varieties during seed production so that the desirable qualities of the fruit are not lost. The majority of gynodioecious varieties are self-pollinated purelines, and even in cross-pollinated gynodioecious or dioecious varieties, seed may be reproduced with good genetic purity if care is taken. The growers themselves can produce their own seed without having to buy them from nurseries. However, for the  $F_1$  hybrid like 'Eksotika II', its seed are difficult to reproduce because they need two inbred parents ('Line 19' and 'Line 20') to be crossed for production of the hybrid seed (Chan, 1993). Seed production procedures therefore are different for different mating systems of the varieties.

#### 5.1.1. Seed production procedures

##### 5.1.1.1. Self-pollinated varieties

The 'Solo' group consists of self-compatible gynodioecious varieties whose hermaphrodite flowers can set fruit when pollinated with their own pollen. The anthers are located very close to the stigma and as the anther sacs burst, they release the pollen before the flower itself opens to ensure self-pollination.

Self-pollinated seed are reproduced with good purity by covering each hermaphrodite flower with a wax paper bag (7 cm x 10 cm) before the flower opens. This is important because if the flower had already opened, there may be a chance that pollen from other varieties may have caused fertilization, and the seed that develop later are not pure. The wax paper bags will also keep away excessive moisture from the flowers for good fruit set.

The paper bags can be secured over the hermaphrodite flowers with paper clips or staples. When the anther sacs burst and self-pollination occurs in the flower, the fruit will develop inside the paper bag. It is not necessary to remove the bag to expose the developing fruit because when the fruit reaches a certain size, it will burst out of the bag. It usually takes about 135-150 days from bagging of flowers to fruit ripening and seed harvest.

##### 5.1.1.2. Cross-pollinated varieties

Some gynodioecious varieties like 'Sekaki' is considered cross-pollinated because the fruit does not set well or produce very little seed when self-pollinated. The hermaphrodite flowers are bagged in the same way as described above to reproduce seed of such varieties with good purity. Next, pollen is collected from hermaphrodite flowers in the population. It is important to collect pollen from many trees so that the highly heterozygous genetic makeup and vigour of the variety is maintained. Pollination with pollen from too few trees will gradually result in narrowing of the gene pool due to genetic drift, and subsequently loss of varietal identity. Pollination is carried out when the hermaphrodite flower is freshly opened. The bag that covers the flower is removed temporarily and the pollen mixture introduced onto the stigma with a light brush. There is no necessity for emasculating the flower prior to pollination. The bag is replaced immediately after pollination. This same procedure applies for production of seed from dioecious varieties.

##### 5.1.1.3. $F_1$ hybrid varieties

$F_1$  hybrid seed like 'Eksotika II' are produced by hybridisation of the inbred parents 'Line 19' and 'Line 20'. Where there are no reciprocal differences in the cross, the male parent is the one that produces abundant

pollen and the female should be very fruitful. In 'Eksotika II', 'Line 19' is used as the maternal parent because it does not produce pollen well due to high carpellody of stamens and sterility (Chan, 1993). Production of hybrid seed requires more effort because of the need to emasculate the hermaphrodite flowers prior to pollination. However, it is easier in papaya because of the large number of seed that can be obtained from a single pollination. Prior to pollination, the selected unopened hermaphrodite flower is emasculated before the anther sacs dehisce. The corolla tube of the bud is gently opened to expose the stamens attached to the petal beneath the stigmatic lobes. A pair of fine forceps is used to remove the ten anthers. After emasculation, another pair of fine forceps is used to pick a freshly dehisced anther from the pollen donor parent and to gently rub it onto the stigma. The flower is covered with a wax paper bag immediately after the operation.

Hybridisation using female flowers will obviate emasculation and the seed production is also four times higher compared to using hermaphrodites (Chan and Mak, 1993b). However, seed developed from female fruit have a higher percentage of undesirable female trees and for this reason alone, the higher cost in using hermaphrodites for hybrid seed production in papaya is justified.

### 5.1.2. Seed treatment and storage

Papaya seed can be harvested when the fruit reaches 'colour breaker' stage. The seed should not be harvested too late because a high percentage has been known to germinate within the fruit in some varieties.

Papaya seed are non-recalcitrant and can be dried to moisture levels of 9-12% for long-term storage (Teng and Hor, 1976). Seed freshly harvested from fruit have very low and variable germination. This is because the sarcotesta (Arumugum and Shanmugavelu, 1975) and the seed itself (Yahiro and Hayashi, 1982) contain growth inhibitors that prevent wasteful germination while the seed are still in the fruit. Certain treatments must be done to the seed prior to storage or planting, so that they can store longer, as well as germinate with good viability and uniformity. Removal of the sarcotesta promotes germination considerably even in fresh, undried seed, but germination is further enhanced by seed drying and cool temperature storage at 15°C (Yahiro, 1979). The requirement of cool temperature to break dormancy in papaya is similar to vernalisation for temperate seeds; although the temperature for vernalisation is lower (5-10°C). Yahiro and Hayashi (1982) reported that storage of papaya fruit for 30-50 days under 15°C greatly reduced the activity of growth inhibitors in the seed, resulting in improved rate and uniformity of germination.

In the tropics, the practical methods for drying of papaya seed are either under the sun or air-drying in the shade. Chacko and Singh (1972), using the Washington variety, reported that there was no difference in germination using either method. Chan and Tan (1990), however, found significant interaction between four genotypes and drying treatments (sun or shade, with or without sarcotesta) for seed germination. Sun- or shade-drying, with or without sarcotesta, gave good germination for three of the varieties, but for 'Eksotika', sun-drying with the sarcotesta intact gave very poor results. Papaya seed without sarcotesta, well-dried and stored at a temperature of 5°C can retain good germination of 60-70% even after five years of storage (Chan, 1991).

## 5.2. Vegetative (asexual) propagation

Vegetative propagation is the norm in perpetuation of perennial fruit trees and many horticultural crops, particularly ornamentals. The main advantage in propagation by vegetative means is that it allows fixing of the maternal genotype and faithfully reproducing it from one generation to another. Several methods of vegetative propagation are available for papaya. Although these are still not widely used in commercial plantings, there are good prospects and potential for their adoption and utilisation in the near future.

Allan (1964) was the first to report success in propagating papaya by cuttings. Large, leafy, lateral shoots that developed after winter were initially used as cuttings for rooting under intermittent mist. In subtropical countries, the cool winter checks growth and temporarily overcomes apical dominance, resulting in the proliferation of lateral shoots. Availability of cuttings became less season-dependent when they were induced from vigorous one- to two-year-old trees by topping off the shoot terminus to remove apical dominance. The method for induction and proliferation of suitably sized lateral shoots for cuttings was improved further with the application of cytokinin and gibberellic acid mixtures (Allan, 1993). The ideal size of cuttings would be 50-150 mm long and 8-12 mm diameter with 4-5 leaves. These are harvested,

trimmed to leave 3-4 small leaves and treated with fungicide and a basal dip in Indolebutyric acid (IBA) to encourage rooting before they are planted in intermittent mist beds with bottom temperature of 30°C. The cuttings will root in about three weeks. Papaya cuttings have been tried in Malaysia by MARDI and Department of Agriculture, Sarawak but the success is very low (Sim, *pers. comm*).

In field performance, Fitch *et al.*, (2002) reported that clonally propagated 'Rainbow' transgenic papaya flowered 1-3 months earlier and 30 cm lower compared with seedlings. Clonal plants yielded significantly higher than seedlings, and the difference was more marked in less favourable environments.

Papaya can also be propagated by grafting. Airi *et al.*, (1986) successfully cleft-grafted scion shoots from cultivars Co-1 and Honey Dew onto uniformly established seedlings. Chong *et al.*, (2005) also used cleft grafting to establish clonal hermaphrodite 'Eksotika' plants and reported an initial success of 80% although this was reduced later due to infection of soft-rot fungi. Patch and T-budding can also be used, but the success rate was poorer than with cleft grafting. In Malaysia, some papaya growers have used field grafting to replace female trees of the 'Eksotika' cultivar in the orchard (Cheah *et al.*, 1993). As soon as the sex of the trees can be determined, the female trees are side-cleft grafted with scion shoots (basal diameter 2-3 cm) harvested from hermaphrodite 'Eksotika' trees. When the union is established in about 2-3 weeks, the female tree is cut back to about 60 cm from the ground to allow the scion to take over. The time in bearing and yield of these in-field grafted hermaphrodites are not significantly different from seed-propagated trees. This practice is economically justifiable because of the much better price paid for hermaphrodite 'Eksotika' fruit.

### 5.3. In vitro propagation

Early successes of *in vitro* propagation of papaya were reported by Mehdi and Hogan (1976) and Yie and Liaw (1977). However, seedling tissues were used as primary explants, and as such the application is limited because the sex of the plants, which is of prime importance, could not be ascertained. Later, Litz and Conover (1978) reported successful regeneration of papaya plantlets by culturing apices of mature, field-grown papaya plants in modified Murashige and Skoog media. This success stimulated more *in vitro* research on papaya because of the prospects for mass propagation, predictable plant sex and greater uniformity of the crop.

In Peninsular Malaysia, *in vitro* propagation research was carried out using primarily the 'Eksotika' papaya (Nathan and Tan, 1989; Chan and Teo, 2002). Field trials of *in vitro* plantlets indicated that they propagated true to sex without somaclonal reversion. Therefore, the problems of sex segregation and variation of fruit shape which seed propagation faces do not arise in micro-propagated plants. Besides greater uniformity, the other benefits are earlier bearing, lower bearing height and improved yield (Drew, 1988; Nathan and Tan, 1989; Chan and Teo, 2002). In Sarawak, micropropagation of papaya using the *in vitro* culture technique was initiated in 2006 with dual objectives of multiplying true-to-type planting material and accelerating the breeding programme in which the selected hybrids can be multiplied *bona-fide* (Sim *et al.*, 2010). A papaya micropropagation protocol using axillary bud as the explant was established (Sim *et al.*, 2009; Rosmah *et al.*, 2010). Field trials of micropropagated 'Eksotika' plants showed that they were true-to-type in fruit size and yield similar to those derived from certified seeds (Jong, 2014).

Despite all the clear advantages, the use of *in vitro* propagated papaya in commercial plantings appears to be an exception rather than the rule. The most likely reason may be related to economics. Demand for such planting materials may not be high enough to justify the economies of scale for the large capital investment. Under limited demand, some tissue culture laboratories in Malaysia have been known to sell *in vitro* propagated plantlets for USD 0.40-0.50 each. This is about five to six times the price of raising a plant from seed. Survival of micro-propagated plants *ex vitro*, reported to be as low as 30% (Fitch *et al.*, 2002), may also pose a problem.

Besides the prospects of using *in vitro* as a method for rapid mass propagation of papaya, it has also been used in embryo rescue to obtain plants from otherwise incompatible intergeneric crosses between *C. papaya* and other *Carica* (now *Vasconcellea*) species (Manshardt and Wenslaff, 1989b, rapid disease resistance screening (Sharma and Skidmore 1988), anther culture for generating haploid papaya lines (Tsay and Su, 1985) and transformation and regeneration of genetically modified papaya for transferring resistance to papaya ringspot virus disease (Pang and Sanford, 1988).

## 6. GENETICS AND VARIETIES

The chromosome number of the papaya is  $2n = 18$ . There are no known polyploid varieties. Genetics and inheritance (heritability) studies are important in understanding how traits are passed from parents to progenies. High heritability indicates major gene control of traits and progenies are likely to resemble much like the parents in that particular trait as opposed to low heritable traits which are controlled by multiple genes whose expression is often influenced by environmental conditions.

### 6.1. Genetics and inheritance of traits

#### 6.1.1. Flower types and sex expression

Sex of papaya is determined by monogenic inheritance involving three alleles (Hofmeyr, 1938). The alleles are  $M$  for male,  $M^H$  for hermaphrodite and  $m$  for female. All homozygous dominants i.e.  $MM$ ,  $MM^H$  and  $M^H M^H$  are lethal to the zygotes. Therefore male genotypes ( $Mm$ ) and hermaphrodite ( $M^H m$ ) are enforced heterozygotes while the female genotype ( $mm$ ) is a double recessive.

Table 4 shows the sex segregation obtained from eight possible combinations. For gynodioecious varieties such as 'Sunrise Solo' and 'Eksotika', it is desirable to have a high proportion of hermaphrodite trees in the orchard because the pyriform hermaphrodite fruits fetch higher prices. In this case, selfing hermaphrodite flowers or hybridisation of hermaphrodite flowers with hermaphrodite pollen should be used in the production of seed. Seed derived from these cross combinations will have twice the number of hermaphrodites compared with females.

**TABLE 4** Pollination combinations and sex segregation in papaya

Pollination combination	Segregation ratios			
	Female (mm)	Herma-phrodite ( $M^H m$ )	Male (Mm)	Non-viable zygotes ( $MM, M^H M, M^H M^H$ )
1. $mm \times Mm$	1		1	
2. $mm \times M^H m$	1	1		
3. $Mm$ (selfed)	1		2	1
4. $Mm \times Mm$	1		2	1
5. $M^H m$ (selfed)	1	2		1
6. $M^H m \times M^H m$	1	2		1
7. $M^H m \times Mm$	1	1	1	1
8. $Mm \times M^H m$	1	1	1	1

For dioecious varieties, the preferred combination for seed production is  $mm \times Mm$ , i.e. to use pollen from male flowers for crossing female flowers. A ratio of 1:1 male to female will be obtained. This high proportion of males in the seed far exceeds the amount that is required for pollination. Normally for dioecious varieties, a higher density is planted in the field with subsequent culling of male trees to about 10% when the sexes can be identified (Agnew, 1968).

It is possible to self or cross males (combinations 3 and 4) only if there is reversal of sex from a staminate flower to a form that has a functional ovary. This reverted 'sexually ambivalent male' (SAM) has been used for development of inbred lines in breeding of dioecious papaya (Aquilizan, 1987). Night temperatures of  $12^\circ\text{C}$  and short daylengths can also bring about the 'SAM' condition (Allan et al., 1987).

Crosses between females and hermaphrodites may be used for hybrid seed production because it obviates emasculation when female flowers are used as the maternal parent. The disadvantage, of course, is the high proportion of females in the seed. With regard to the other crosses, i.e. males with hermaphrodite flowers, these produce variable, trioecious populations but are not commonly used either for commercial seed production or in breeding.

There are no reliable practical methods for determining sex at the seedling stage. Molecular tools (i.e. micro-satellites and PCR- based techniques) can be used to predict sex in papaya at the seedling stage but these cannot be economically applied on a commercial scale.

The practical method is to determine sex at flowering stage, usually 4-6 months after planting. Multi-point planting of three or more seedlings per hill followed by culling, once sex is determined, is a common practice to achieve high hermaphrodite stands in orchards.

The Taiwan Seed Improvement and Propagation Station has recently announced the successful development of a new papaya  $F_1$  hybrid 'TSS no. 7' with the unique characteristic that the seed are 100% hermaphrodite. It is not known whether this was based on successful development of the  $M^H M^H$  homozygote which in natural state is deleterious (Storey, 1969). The Taiwan Seed Improvement and Propagation Station has welcomed seed producers to apply for a non-exclusive license for the production of 'TSS no.7' (Chiu, 2015).

The East West Seed also reported a mutant allele EWSMHP, which confers production of highly hermaphroditic progenies upon selfing of its hermaphrodite plants and also production of highly hermaphroditic  $F_1$  progenies when crossed with normal female and normal hermaphrodite papaya plants. Unlike the 'TSS no.7' this mutant gene can reproduce nearly 100% hermaphrodites, the remaining may be females. The application for patent of this invention has been filed (Anon. 2014) under US201402368A1.

### 6.1.2. Fruit size and shape

There is a wide variation in fruit size in papaya, ranging from about 5 cm in diameter and 50 g weight to over 50 cm in length and 10 kg or more in weight. Size preferences vary among countries and markets. Export markets in USA, Europe and China prefer the small fruits of the 'Solo' and 'Eksotika' types while the domestic market in Malaysia prefers the medium fruit of the 'Sekaki' variety. Overly large fruits have lost out in popularity because of the inconvenience in handling and generally poorer eating quality. Fruit weight is a quantitative character determined genetically by multiple alleles. The weights of hybrid fruit commonly lie at or near the geometric mean of the parents. However in certain crosses, heterosis in fruit weight over the better parent had been obtained (Chan, 2001), indicating the presence of over dominant gene action.

Fruit shape in papaya is a sex-linked character. The female flower has a globose ovary that develops into round or ovoid fruit. In contrast, the elongata or hermaphrodite flower has a slender, tapering ovary and this subsequently develops into a fruit that is elongated and cylindrical or pyriform in shape, depending on the variety of papaya.

Fruit shape preference varies among countries. In Australia, for example, the round female fruit from dioecious varieties are preferred, while for the majority of other papaya-growing countries like Hawaii, Brazil, India and the Southeast Asian countries including Malaysia, the gynodioecious varieties bearing elongated hermaphrodite fruit are more popular. In Malaysia, the hermaphrodite, pyriform fruit of the 'Eksotika' variety fetches a premium price which is more than twice that of the round female fruit, although there is no difference in the eating quality and total soluble solids content of fruit between the two sexes (Chan, 1986).

### 6.1.3. Precocity and plant stature

Precocity or earliness of bearing and height at initial fruiting are important factors in papaya production. Precocity or earliness to fruiting is a factor of the number of nodes produced before the first flowering node while the number of nodes to flowering and the internode length determine height to first fruit (Nakasone and Storey, 1955; Lim and Siti Hawa, 2005). Additive gene effects govern these characteristics with the hybrid having the geometric mean between the two parents (Nakasone and Storey, 1955). Subhadrabandhu and Nontaswari (1997) reported that height of first flower and number of nodes to fruiting are controlled both by additive and non-additive genes. All the hybrids produced from the tall bearing parent 'Eksotika' have mean values that are skewed more towards that parent. Lim and Siti Hawa (2005) studied the earliness in flowering and dwarfism of accessions in the MARDI

germplasm and concluded that internode length have strong correlation with plant height and the number of nodes to first flower was correlated to earliness in flowering (precocity).

#### 6.1.4. Carpellody of stamens

Carpellody of stamens is the development of misshapen or 'cat-faced' fruits due to fusion of the stamens to the ovary tissues in hermaphrodite flowers. Carpelloid fruit are not usually marketable. High heritability ( $h^2 = 82\%$ ) was obtained for carpellody, but effective phenotypic selection may be interfered by the 'change-in-rate' type of interaction between genotype and plant age (Chan, 1984). Selection against this trait must be done early to coincide with the period of rapid growth and development when expression of carpellody is highest. Increased expression of carpellody may also be brought about by rapid growth due to cool temperature (Awada, 1958), high soil moisture (Awada, 1961) and high nitrogen application (Awada and Ikeda, 1957).

#### 6.1.5. Flavour and total soluble solids

Flavour and total soluble solids are important traits that influence the eating quality of papaya. Flavour and odour can range from pleasantly aromatic as in the 'Solo' and 'Eksotika' varieties to undesirably musky as in the 'Maradol' variety. Muskiness has been reported to be due to the homozygous recessive allele of a single gene that could easily be bred out of a line (Storey, 1969a). Total soluble solids content is usually associated with sweetness, and may range from 8° Brix or less in nondescript cultivars to about 16° Brix or more in the 'Solo' and 'Eksotika' varieties. This trait is governed by quantitative genes with additive effects, and hybrids are expected to have intermediate values between the parents (Subhadrabandhu and Nontaswari, 1997).

#### 6.1.6. Flesh colour

Papaya flesh colour ranges from pale yellow to deep red with varying intermediate shades. In Malaysia, the preference is definitely for red-fleshed varieties. Flesh colour is governed by a single gene with yellow ( $R$ ) being dominant over red ( $r$ ). Varying intermediate shades of pink may be attributed to modifier gene effects influenced by environment. All red-fleshed ( $rr$ ) varieties like 'Sunrise Solo' and 'Eksotika' will breed true for flesh colour but progenies of heterozygous ( $Rr$ ) yellow  $F_1$  hybrids like 'Rainbow' will segregate in this trait.

#### 6.1.7. Fruit skin colour

The skin colour of papaya fruit is usually green when immature, changing to yellow or reddish-orange when fully ripened. The 'Morib' is an interesting local selection that has attractive yellow fruit skin even at the immature stage. The tree is dwarfed and has yellowish-green foliage. A single gene governs fruit skin colour with green ( $G$ ) being dominant and yellow is expressed in a double recessive ( $gg$ ). This attractive trait has been transferred to the 'Sunrise Solo' after hybridisation with the Morib, followed by selection for promising yellow-skinned progenies in subsequent inbred generations. The  $F_1$  between 'Sunrise Solo' and Morib was an all green-fruit population, while the  $F_2$  generated a typical 3:1 segregation of green: yellow progenies. An inbred line called 'Niensee' that has most of the 'Solo' characteristics but with yellow fruit skin was subsequently selected (Chan, 2009). The constraint in commercialization of this attractive variety lies in the difficulty in identifying the appropriate maturity index of the fruit. The 'colour breaker' stage normally used for fruit harvesting is quite impossible to determine against an all yellow background.

#### 6.1.8. Disease resistance

##### 6.1.8.1. Papaya Bacterial Dieback

This disease is the most devastating and had caused the decline of the papaya industry in Malaysia (Chan and Baharuddin, 2010b). The disease is caused by the *Erwinia* bacteria and the species previously identified as *papayae* and *agglomerans* and now called *mallotivora* (Noriha *et.al.*, 2011). It is an airborne disease and infected leaves and shoots rapidly rots and die back. Currently, there are no effective chemicals or cultural practice to stop this disease. Tolerant dioecious accessions have been found in Venezuelan and Guadeloupean germplasm while all commercial cultivars are highly susceptible. The level of tolerance is variable and inherited in an additive manner subject to strain virulence, but genotypic ranking for tolerance remains the same. Tolerance is transmitted in a co-

dominant way involving a few genes, with  $F_1$  hybrids intermediate in reaction between susceptible and tolerant parents (Ollitrault *et al.*, 2005). Chan (2011) made crosses of the highly resistant 'Glimmer' variety with the susceptible 'Frangi' and found the  $F_1$  population was highly resistant suggesting that single or few additive dominant genes governed resistance. There is good prospect of overcoming this disease through conventional resistance breeding and selection.

#### 6.1.8.2. Papaya Ringspot Virus Disease

The papaya ringspot virus (PRSV) is considered the single most devastating disease of papaya in the world. In Malaysia the PRSV was first detected in Johor in 1991 and although it is a gazetted disease, it is no longer a threat as it was long overshadowed by the bacterial dieback disease. The ringspot virus belongs to the potyvirus group, which is readily transmitted by aphid vectors in a non-persistent manner. Control is made even more difficult because of the existence of alternate hosts in Cucurbitaceae. The most prominent tell-tale symptom is the dark green concentric rings on the fruit, hence the name for the disease. Infected trees are necrotic and eventually die and fruit from diseased trees are low in sugar. Some tolerant varieties have been reported such as 'Cariflora' (Conover *et al.*, 1986), 'Tainung 5' (Lin *et al.*, 1989) and 'Sinta' (Villegas *et al.*, 1996). Chan (2004a) developed four lines with good tolerance to PRSV from a single seed descent programme involving the 'Eksotika' and the resistance donor 'Tainung 5'. Drew *et al.*, (2005) successfully obtained intergeneric hybrids between *Carica papaya* and PRSV resistant wild relative *Vasconcellea quercifolia* and then backcrossed to *C. papaya* to get the  $BC_1$  population. There was variability in development of PRSV symptoms in the  $BC_1$ , but one fertile resistant plant was selected that holds promise for development of a commercial variety. In genetic modification, 'Rainbow' is the world's first commercial transgenic papaya developed with coat-protein mediated virus resistance by the University of Hawaii (Gonsalves, 1998). 'Rainbow' is not cultivated anywhere else outside Hawaii because of several reasons; respect for intellectual property, government policies preventing introduction and testing of GM varieties and most importantly the resistance of 'Rainbow' has been shown to be specific to the virus strains in Hawaii only. A number of countries like those in the Papaya Biotechnology Network of Southeast Asia have started their own programmes to develop papaya varieties with PRSV resistance. All are transforming with their own local strain of virus because the resistance of transgenic papayas like 'Rainbow' seemed to indicate strain specificity (Tennant *et al.*, 1994). The danger in using the coat protein of only one specific strain like the PRSV-YK in Taiwan for transformation is the breakdown of resistance by other PRSV strains or new unrelated viruses like the papaya leaf distortion mosaic virus (Chen *et al.*, 2002).

Estimation of heritability of tolerance to PRSV by parent-offspring regression conducted on 35 papaya lines (Chang and Guo, 2002) indicated  $h^2 = 61\%$  on disease index. Yield and total soluble solids were positively correlated to the degree of tolerance to PRSV.

#### 6.1.8.3. Fruit freckle

Fruit freckle is widespread in the 'Solo' papayas including 'Eksotika' and is considered a disease of unknown cause in Hawaii (Hine *et al.*, 1965). The symptoms appear as superficial water-soaked spots of variable sizes, more apparent on the exposed surface of the maturing fruit on the tree and apparently associated with the lenticels of the fruit skin. The freckles do not affect the eating quality of the fruit, but the cosmetic appeal of infected fruit may be considerably reduced. Freckle is genetically controlled, as shown by Chan and Toh (1988) when three backcrossed sib lines, 'Line 7', 'Line 19' and 'Line 20' showed distinct differences in response to this disorder over two seasons. Wet season caused higher severity, not in the increase in number of freckle spots, but in enlargement of freckle size. 'Line 20' was the most susceptible, 'Line 7' intermediate while 'Line 19' was the most resistant. The progeny of 'Line 19' x 'Line 20' showed very good tolerance shifting more towards the resistant parent than the mid-value of both parents, suggesting partial dominance of the resistant genes (Chan and Toh, 1988).

## 6.2. Varieties

Storey (1969a) reported that the only *bona fide* varieties of papaya in existence were 'Solo' and 'Bush' of Hawaii and 'Hortus Gold' of South Africa. Since then, however, numerous distinct, true-breeding varieties have been developed from many parts of the world. Many are from systematic breeding programmes, while others are from judicious selection efforts by growers. Recently, biotechnology



tools have been used to develop transgenic varieties. Papaya varieties can be self-pollinated (in which case they are purelines), or cross-pollinated. In general, gynodioecious varieties (having hermaphrodite and female trees) are self or cross-pollinated, while the dioecious varieties (having male and female trees) are enforced cross-pollinators.

### 6.2.1. Gynodioecious varieties

#### 6.2.1.1. 'Solo' types

Gynodioecious varieties are also known as 'bisexual' or 'hermaphrodite' varieties where no male trees are present. The most popular hermaphrodite varieties in the world today are those from the 'Solo' family, so called because of the small fruit size suited for one serving. Within the 'Solo' are many lines such as 'Line 5', 'Line 8', 'Line 10', 'Kapoho', 'Waimanalo' and 'Sunrise' (Yee *et al.*, 1974), 'Wilder' and 'Higgins' (Nakasone *et al.*, 1974), 'Sunset' and 'Kamiya' (Fitch, 2002). 'Solo' is typically small-fruited around 500g, pyriform shaped with a distinct 'neck' for hermaphrodite fruits and usually yellow to yellow orange flesh (except for Sunrise and Sunset which are orange-red). The flavour is distinct and pleasant with usually high TSS of 13-16%. 'Line 5', 'Line 8' and 'Line 10' were the earliest recommendations by the University of Hawaii and these have been phased out or restricted to domestic markets because the fruit softens quickly and has poor keeping quality. 'Wilder' and 'Higgins' too did not make commercial impact because of high occurrence of carpellody. Trials in Malaysia recorded occurrence of 40% and 75% respectively for these two varieties although Nakasone *et al.*, (1974) have indicated less than 10% when grown in Hawaii. Below are descriptions of the 'Solo' types of commercial importance.

'Waimanalo' ('Waimanalo Solo X-77'). Waimanalo was selected from crosses between 'Betty' from Florida and 'Line 5' and 'Line 8' Solo strains (Yee *et al.*, 1974) and released by the Hawaii Agricultural Experiment Station in 1968, superseding the early 'Solo' lines. It bears fruit much lower to the ground than other Solo types. The fruit is medium-sized, weighs 600-700g, roundish with a short neck with smooth, shiny 'freckle-free' skin. The flesh is orange-yellow, thick, and fairly firm with TSS around 14-17%. It is very tolerant to *Phytophthora* root rot as well as yield decline due to repeated cropping in the same area (Nakasone and Aragaki, 1973).

'Sunrise' and 'Sunset Solo'. These two are the only 'Solo' types that have red flesh. 'Sunrise' (formerly 'HAES 63-22') is the most popular of the 'Solo' and is grown worldwide because of its wide adaptability. The fruit is small, weighs around 400-500 g, elongate with a distinct neck with smooth and usually 'freckled' skin. The flesh is orange-red, fairly soft with an exquisite pleasant fragrance and high TSS around 15-17%. Sunrise was used as a recurrent parent in a backcross breeding programme to improve Malaysian cultivars (Chan, 1987). 'Sunset' is of the same parentage as 'Sunrise' and is similar in all respects except for smaller fruit size of about 350-450 g. 'Sunset' was the target variety used in transformation for cp-mediated virus resistance resulting in selection of the first papaya transgenic 'SunUp' (Gonsalves, 1998).

'Kapoho'. This is the major cultivar grown in Hawaii before the industry was crippled by PRSV in the mid 1990's. Many hectares are now superseded by the resistant genetically modified 'Rainbow' especially in the 'hotspot areas' (Mochida, 2005). However, 'Kapoho' continues to be the dominant variety grown in Hawaii for export especially to Japan which does not accept GM papayas. The 'Kapoho' fruit is small, weighs 400-550 g and pyriform. The flesh is orange-yellow, thick, and very firm with TSS around 14-16% and strong flavour. It has very good shelf life and withstands well during shipping. However it is sensitive to drought which can drastically reduce fruit to unmarketable sizes. In Hawaii, it is grown primarily in the eastern part of the island where annual rainfall is more than 2500 mm (Yee *et al.*, 1974). 'Kapoho' was used to cross with the transgenic 'SunUp' to produce the commercial PRSV resistant 'Rainbow'.

'Kamiya'. 'Kamiya' is a selection from Waimanalo. The tree is quite dwarf and high yielding. It is a large-fruited variety with distinct, blocky shape and a very short neck. Its flesh is thick, deep orange yellow, firm and juicy and has a flavour reminiscent of coconut or mango (Fitch *et al.*, 2002).

'Eksotika', 'Eksotika II'. 'Eksotika' and 'Eksotika II' are the flagship varieties for export in Malaysia (Chan, 2004b). 'Eksotika' has similar features as 'Sunrise Solo' because 'Sunrise' was used as a recurrent parent in the backcross programme that developed this variety (Chan, 1987). 'Eksotika' is a good bearer, yielding about 50-60 tonnes/ha/year. The fruit is medium size (600-800 g) with orange-red flesh (Figure 3). It has the Solo pleasant aroma and high sugar content of 12-14° Brix but does not keep well because of its soft texture. It is also quite susceptible to fruit freckles and malformed top disease (Chan and Mak, 1993a). 'Eksotika II' is a F<sub>1</sub> hybrid between the 'Line 19' and 'Line 20' (Chan, 1993). Compared with its predecessor, Eksotika II has higher yield due to slightly larger fruit (600 - 1000 g), firmer flesh for longer storage and less prone to fruit 'freckles'.

#### 6.2.1.2. Large-fruited types

There are several gynodioecious papaya varieties that are large-fruited and bear little resemblance to the 'Solo' types. These are usually grown and consumed locally because the large fruit is inconvenient to handle and pack for export. The varieties in the ASEAN region are 'Batu Arang', 'Subang 6' and 'Sitiawan' from Malaysia, 'Kaegdum' ('Khaek Dam'), 'Kaegnuan', 'Koko' and 'Sainampeung' from Thailand, 'Cavite Special' from the Philippines, 'Dampit', 'Jingga' and 'Paris' from Indonesia (Chan et al., 1994). The other large-fruited varieties popular in other parts of the world are 'Coorg Honey Dew', 'Maradol', 'Red Lady #786' and the 'Tainung' series 1-3. They generally have elongate or cylindrical fruit, ranging from 1 kg to 6 kg in the case of 'Cavite Special'. 'Khaek Dam' is Thailand's best-known variety. It is vigorous, bears red-fleshed fruit about 1.2 kg with 10.6% TSS content (Subhadrabandhu and Nontaswatsri, 1997). 'Coorg Honey Dew' is a popular selection from 'Honey Dew' made by the Indian Institute of Horticultural Research. The fruit is long, weighs 2-3.5 kg with yellow flesh and a large cavity and keeps fairly well (Morton, 1987). 'Maradol' originates from Cuba, but now grows widely in Mexico and South America. It is a short-stature variety that bears fruit very close to the ground. Fruit weighs 1.5 kg, attractive with firm red flesh with 10-11 % TSS content. It has a characteristic strong flavour described by some as 'musky' and the fruit is quite susceptible to anthracnose when grown in the humid tropics. 'Red Lady #786' is a red fleshed fruit averaging 1.5-2 kg and quite tolerant to papaya ringspot and ripe-rot diseases such as anthracnose. 'Tainung' series 1-3 are popular varieties in Taiwan and to some extent in Caribbean, South America and South Africa. 'Tainung 1' and '2' hermaphrodite fruits are elongate with a pointed end, weigh around 1.1 kg and are red fleshed. These two varieties were introduced into South Africa in the early 1990's and because of their superior quality and consistency of fruit, have replaced most of the dioecious varieties there (Louw, 2004). 'Tainung 3' fruit is more pear-shaped, larger (1.3 kg) and orange fleshed. 'Red Lady' and 'Tainung' series 1-3 are F<sub>1</sub> hybrids developed by the Known You seed company in Taiwan and listed in several international seed catalogues.

More recently, the Philippines introduced a new hybrid 'Sinta' with moderate tolerance to PRSV (Villegas et al., 1996). 'Sinta' is a F<sub>1</sub> hybrid between 'Py-5' and 'Py-3', bears fruit of 1.2-1.3 kg with yellow orange firm flesh and with 11.9° Brix TSS. Its yield is 35-60 kg/tree in one fruiting cycle. In PRSV hotspots, 'Sinta' is recommended as an annual crop; otherwise it can keep fruiting for at least three years. In Malaysia, the recent introduction is 'Sekaki' meaning the 'foot-long' papaya in Malay and it has replaced to a large extent, the older varieties like 'Subang 6'. 'Sekaki' sometimes called the 'Hong Kong' papaya, is a prolific bearer (60 tonnes/ha/year) with medium sized cylindrical fruit of 1.5-2 kg (Figure 4). The tree is quite dwarf, bears fruit low to the ground and is resistant to malformed top disease. 'Sekaki' fruit is attractive with smooth, even-coloured and freckle-free skin. The flesh is red, firm but sugar content is not high at 10° Brix or less (Chan 2001). 'Sekaki' has a small export market, mainly to Singapore and Hong Kong, but most of the fruit are sold locally.

In general, the large-fruited gynodioecious varieties do not have the quality, flavour and sweetness of the 'Solo' types. Some, as in 'Kaegnuan', the immature fruit is consumed as a vegetable in Thailand.

#### 6.2.1.3. Transgenic papayas

'Rainbow' is the world's first commercial transgenic papaya developed with coat-protein mediated virus resistance by the University of Hawaii (Gonsalves, 1998). It is an F<sub>1</sub> hybrid made from a cross between the transgenic 'SunUp' and the non-transgenic commercial cultivar 'Kapoho'. Transgenic varieties of 'Kapoho' and 'Kamiya' have also been developed by introduction of the coat-protein transgene from 'Rainbow' through conventional hybridization and backcrossing. The 'Poamoho Gold' is

a backcross-1 hybrid between 'Kapoho' and 'Rainbow F2', while the 'Laie Gold' is a F<sub>1</sub> hybrid between 'Kamiya' and 'Rainbow F<sub>2</sub>' (Fitch *et al.*, 2002).

In the near future, many transgenic papaya varieties will be developed in Malaysia, Thailand, Indonesia, Philippines and Vietnam. These countries come under the ISAAA-coordinated Papaya Biotechnology Network of Southeast Asia formed in 1998, and have been actively working on the development of ringspot virus (PRSV) resistance and delayed fruit ripening (Hautea *et al.*, 1999). Working in close collaboration with Monsanto and University of Hawaii in the PRSV resistance project, and with Syngenta (Zeneca) and University of Nottingham in the delayed fruit ripening project (Abu Bakar *et al.*, 2000), several countries in the network notably Thailand, Vietnam and Malaysia, already have transgenic lines in field trials. In Thailand, Phironrit *et al.*, (2005) developed a coat protein mediated PRSV resistant line KN1165 and it went as far as a limited field trial. However, further work was curbed due to public dissent. In Malaysia, Rogayah *et al.*, (2013) reported success in genetic modification in the expression of delayed ripening of Eksotika papaya using the ACO2 gene construct. Transformed lines showed down regulation of ACO2 gene resulting in up to 15-day delayed ripening characteristic compared to only 4 days for non-transformed lines. Potential lines are ready for open field trials.

### 6.2.2. Dioecious varieties

Dioecious varieties with male and female flowers on separate trees are enforced cross-pollinators. Dioecious commercial varieties are not common and restricted only to some countries like Australia and South Africa. The disadvantage of dioecious varieties is that 50% of the population is unproductive male trees. In commercial cultivation of dioecious varieties, a high initial stand of seedlings is first established with subsequent culling of male trees to 5-10% for pollination purpose. The advantage of dioecious varieties is that fruit size, shape and appearance is more uniform and stable because female flowers do not undergo sex reversal as in hermaphrodites. 'Hortus Gold', 'Honey Gold', 'Sunnybank', 'Hybrid No. 5', 'Cariflora', 'Co1' and 'Co2' are the known commercial dioecious varieties.

'Hortus Gold' is a South African cultivar released in the early 1950's. It is early maturing with round-oval, golden-yellow fruit weighing 0.9-1.36 kg (Morton, 1987). 'Honey Gold' is a selection made from 'Hortus Gold' and has many similar features but with improved sugar content and disease resistance (Morton, 1987). It is a late season bearer and its fruit fetch better prices at the tail end fruiting of other varieties. Commercial orchards of these two varieties are propagated by cuttings (Allan, 1993). 'Sunnybank' and 'Hybrid No. 5' were popular cultivars in Queensland, Australia (Agnew, 1968). 'Sunnybank' strain S7 was selected at the Redlands Horticultural Research Station while 'Hybrid No. 5' is a F<sub>1</sub> hybrid between 'Bettina 100A' and 'Peterson 170'. These Queensland varieties bear round to ovoid fruit, about 1-2 kg in weight, with attractive clear smooth skin that colours uniformly on full ripening. The flesh is yellow and usually soft with 11-12% TSS. 'Cariflora' is fairly dwarf and bears small rounded fruit (0.5-0.8 kg) with a slightly pointed end. The fruit has yellow flesh, soft texture and low TSS (9.5-10.8%). It is tolerant to papaya ringspot virus and is recommended for cultivation in diseased areas in Florida and the Caribbean (Conover *et al.*, 1986). It was also used as a parent for improving PRSV tolerance of the 'Eksotika' papaya in Malaysia (Chan, 2004a). 'Coimbatore 1 and 2' or 'Co1' and 'Co2' were developed at the Tamil Nadu Agricultural University. The dwarf trees bear fruits low to the ground. Fruits are medium sized, 1.5-2.5 kg, with yellow, sweet flesh. (Morton, 1987). 'Co2' is also recommended for papain extraction.

## 7. CULTURAL PRACTICES

### 7.1. Nursery

The nursery structure should be covered with a plastic roof with open sides. The optimum shade for raising papaya seedlings in nursery is 30%. At higher shade levels the seedlings produced are thin and etiolated while in the open sun, seedlings tend to be heat-damaged, short and stunted.

For small holdings, the seed is sown into 10 cm x 15 cm perforated polythene bags and placed in rows on the ground usually covered with plastic sheet for weed control. Commercial papaya nurseries use the 'Trays in Pool' or 'TIPS' system (Jayalaxmi *et al.*, 2008). This consists of moulded plastic "Ebb and Flow" table top pools (e.g. STAL & PLAST) of dimension 1.68 m x 2.8 m and plastic seedling trays (e.g. Humibox) with dimensions 55 cm x 28 cm x 11 cm (depth), each with 50 cells. Thirty seedling trays should fit in each table top pool. It is estimated that 1 ha of nursery space with 252 table tops, should have a capacity to produce 378,000 seedlings in 6-8 weeks. The seedling trays are irrigated by continuous flooding of the table tops and soluble fertilizers can be supplied simultaneously by in and out flow pipings.

For conventional nursery practices using polybags, the soil mixture is 25% sand, 25% organic manure (cow dung or chicken dung) and 50% top soil mixed by volume (Chan *et al.*, 1991). The relatively high proportion of sand is required for good drainage. However, this mixture is inadequate for commercial nurseries using the 'TIPS' system as the media is often too wet under the continuous flooding. The recommended medium for this system is peat moss (e.g. Lata Gold) with perlite, vermiculite, compost and carbon from burnt rice husk in the ratio of 40: 20: 20: 10: 10. Fifty grams of effective microbes (e.g. 'Mycoplex' (Novozyme) are also added into each tray of 50 cells (Jayalaxmi *et al.*, 2008).

Before sowing, seeds should be immersed in clean water the night before to leach out the inhibitors and also to soften the testa for quicker germination. Normally only one seed is sown into each bag or cell. The seeds should be sown about 1 cm deep. Seeds buried too deep will not germinate well while if sown too shallow, they will be easily exposed during rain or watering. It is necessary to sow 10% extra seeds in trays to replace non-germinating seeds in polybags. Transplanting seedlings from the trays to replace non-viable seeds in the polybags can start as soon as the hypocotyl appears.

The seeds germinate 10 to 15 days after sowing. Plant replacements for non-viable seeds in polybags should start after four weeks of sowing when it is certain that the seeds are not viable. The soil in the polybags must be kept moist but should not have standing water. Plants should be watered just once a day in the morning.

It is good practice to put snail pellets around the periphery of the polybags to keep away these pests that damage the emerging shoots. Disease and pest problems may be quite serious at the nursery stage. *Cladosporium* and thrips for example, cause severe stunting and malformation of young leaves. A mixture of Benomyl fungicide and Pyrethroid insecticide at fortnightly intervals should be used as protection. The seedlings should be given a foliar nutrient spray at the six-leaf stage. It is extremely important that no systemic herbicides like Glyphosate be sprayed around the nursery area because they can cause very extensive damage to the young plants. The best way to keep weeds down in the nursery is to line the floor space with black polythene sheets or granite dust and stones.

After about six to eight weeks in the nursery, the seedlings should reach the eighth to twelfth-leaf stage and this is the most suitable age for field planting. When planting is delayed further, the seedlings will appear spindly and etiolated, and fruit production in the field will be delayed and fruits will be borne higher up the tree than normal. When seedlings are too old for planting, they may be cut back to 10 cm from the ground. They will re-shoot and may be planted a month later.

### 7.2. Planting

#### 7.2.1. Land preparation

All vegetation should be cleared before planting papaya because the crop is very sensitive to competition. Assuming an average soil condition, one pass of disc plough followed by rototiller would be sufficient. Otherwise the area is disc ploughed with two runs and disc harrowed once to obtain a finer tilth. If the terrain is fairly steep, the area should not be rotovated because with such a practice, the tilth will be

too fine and erosion may be a problem. With a disc harrow, the earth will remain in fairly large clumps that will reduce the risk of erosion as well as delay compaction of the soil. An additional pass of raking or disc harrow would be desirable after the planting operation to remove any compacted wheel tract. On heavy or compacted soils, sub-soiling or ripping down to 50 cm is recommended to facilitate roots to penetrate deeply. Sub-soiling provides better drainage if done parallel to the contour lines.

It is a good practice to lime the soils before growing papaya because the crop does well under pH of 6.0 to 6.5 and most tropical soils are acidic and well below this optimum range. Well-limed papaya areas result in earlier crops as well as higher yields.

The best time to add the lime (calcium carbonate or ground magnesium limestone) to the soil is after the two runs of the disc plough. About three to four tons per hectare are required and this is broadcasted uniformly either by tractor or more tediously by hand. After the broadcast, the lime is worked into the soil by single run of the disc harrow.

After incorporating the lime into the soil, it is recommended to blanket spray the ploughed area with a pre-emergent herbicide (e.g. s-metalachlor (Dual G)). This will ensure that the area remains free of weeds for the first 6-8 weeks of growth.

Lining should be done along the contour lines and each row is spaced 2.7 m apart. Within row, holes are dug about 1.8 m apart. With this spacing, about 2 000 plants/ha can be obtained. The size of the hole depends on the nature of the soil. If it is friable, a 10 cm diameter hole will be sufficient but increased to 30 cm if the soil is compact or has hard lateritic concretions. Holes should not be dug with a mechanically operated soil augur as it will compact the side wall and will not allow lateral seepage of water.

### 7.2.2. Transplanting

The ideal stage for transplanting seedlings from the nursery to the field is at the eighth to twelfth-leaf stage. This is the stage when it will suffer the least transplanting shock because of its optimal plant size and minimal root injury during removal of the polybags.

Just prior to transplanting, about 200 g of T.S.P. (Triple Superphosphate) with 45%  $P_2O_5$ , should be added to the hole and mixed well with the soil. This is preferred over the cheaper Christmas Island Rock Phosphate (CIRP) because the nutrient is more readily available to the young seedlings. As mentioned, most Malaysian soils are acidic and low in phosphate and this early booster will be of considerable advantage to the initial development of the roots and shoots.

The actual process of planting is simple. The surrounding soil of the hole is filled back with loose earth and mixed with the T.S.P added earlier. A small depression, large enough to fit in the size of the polybag is made. The polybag is carefully torn with minimal damage to the earth ball containing the roots and the ball of earth is lowered into the hole. In the case of 'TIPS' system, the seedlings can be pulled out the cell with the intact root system and planted. The soil is pushed around the collar of the plant but the normal practice of tamping the soil with the feet is discouraged because this rapidly compacts the surrounding soil. The best depth of planting is when the soil level covers about 2 cm of the collar of the seedling. If it is shallower than this, the plant may run the risk of exposure of the surface roots later due to erosion while deeper planting may result in collar rot.

The double and triple-point planting systems basically involve the planting of two or three seedlings to a point, spaced about 30 cm apart. After three months, culling of seedlings to maximize hermaphrodite trees in the field should be carried out (see 7.2.3). Polyethylene mulch over the beds reduces moisture losses, fertilizer leaching and effectively controls weeds. Organic mulches of grasses, wood shavings, rice hulls, oil palm empty bunches and other kinds of material are also beneficial.

### 7.2.3. Culling of seedlings

Three months after transplanting, the seedlings should be flowering and the sex can be determined by the shape of the flowers. The female flowers are more rounded and stamens are absent as compared with the elongated hermaphrodite flower with a constricted corolla tube and the presence of stamens. Female trees are culled by pulling them out of the ground. In the case when both plants in the same planting point are hermaphrodites, one is trimmed of nearly all the leaves and carefully transplanted to holes where both happen to be females. If the farmer is skilled, grafting of hermaphrodite scion on female trees can be done in the field. By culling and grafting, the field should have nearly 100% hermaphrodite stand.

### 7.3. Fertilization

Papaya is a fast and continuously growing tree that provides fruits all the year round. Therefore, it needs an abundant supply of nutrients at regular intervals to sustain good growth and production. In general, an adequate supply of nitrogen and phosphorus should be provided during the early stages to ensure optimum foliage, trunk and root development. However, at the fruiting stage, the level of potassium should be raised considerably because it is very important for improving the quality of fruits. Flesh colour will be richer, taste sweeter and texture firmer when adequate potassium is supplied. On the other hand, the level of phosphorus should be reduced at the fruiting stage because high levels will reduce fruit size. The level of nitrogen remains somewhat unchanged through the juvenile and fruiting stages because foliage is continuously produced.

Diversity of soil types, climatic conditions and practices makes it necessary to develop recommendations for specific areas, based on soil and foliar analyses. In Malaysia 50 - 100 g/tree of 15:15:15 formulation is given monthly till trees are four-months old, after which 180 g/tree of 12:12:17:2 formulation is given monthly (Chan *et al*, 1991). On top of this, Processed Organic Manure [processed chicken manure, Palm Oil Mill Effluent (POME) or fish and bone meal] should be supplemented at 2 kg per tree every 4 months, in the first year and 3 kg per tree in the second year. The organic manure helps to improve the soil structure of the heavy clay and creates a micro flora and fauna in the root zone suited for good crop growth. It is important not to use raw manure from poultry or livestock. Use of raw manure is forbidden under Good Agricultural Practices (GAP), and will also increase risks of introduction of serious plant disease to the farm, including bacterial dieback.

Papaya is sensitive to boron deficiency especially when cultivated on sandy soil. Plants suffering from this deficiency exhibit fairly characteristic symptoms. In the nursery the first noticeable symptom is slight yellowing and downward curling of the leaf tips with very slight necrosis of the leaf tips and margins. The leaves are brittle in texture and claw-like. These symptoms are observed first on the young leaves.

However, deficiency symptoms during the fruiting phase are detected on the fruits rather than the leaves, which in many instances appear normal. In the early stages, secretion of milky latex often occurs on the fruit surface, which subsequently turns brown in colour. At the later stage, the fruit surface becomes rough or 'bumpy' giving the fruit an overall distorted or malformed appearance (Wang and Ko, 1975). This malformation is the earliest symptom and the most sensitive indicator of boron deficiency in papaya.

Treatment of boron deficiency includes soil and foliar application of boron. For soil application, 5-10 g borax per tree is applied as side dressing along the dripline. The borax must be evenly spread as uneven application could cause boron toxicity. For foliar sprays a thorough wetting of the leaves with a 0.25% borax solution is recommended. Generally three sprays at fortnightly intervals are required to rectify the problem. Fruits that are already showing deficiency symptoms will not respond to remedial measures but new flushes of fruits will be normal. Chan and Raveendranathan (1984) found that varieties have differential sensitivity in expression of boron deficiency symptoms. Maradol was found to be very sensitive while the Solo was very tolerant. Maradol showed the 'bumpy' fruit symptom even at soil boron levels that are adequate for other varieties. This can be exploited by random planting of Maradol trees in the orchard as indicators for early boron deficiency in the field.

### 7.4. Irrigation and drainage

Water is required for papaya during the early stages of growth and during periods of prolonged drought. Lack of moisture generally retards plant growth, shifts sex expression to male and causes abortion of flowers and fruitlets leading to sterile phases or fruiting skips.

Generally the adoption of irrigation facilities in papaya cultivation results in an increase of both the number and size of marketable fruits. While supplementary irrigation would suffice for regions with uniform rainfall, scheduled irrigation is quite essential in drought prone areas. A monthly minimum rainfall of 100 mm is needed without supplementary irrigation for mediocre production. Irrigation should replace at least that lost by a pan evaporation and 1.25 pan evaporation is required for maximum yield of mature tree. Young trees may only need about 0.3 to 0.5 pan evaporation. Good production occurs with 60 to 90 liters per tree per week immediately after planting or during the wet season and 120 to 240 liters per tree per week during the dry period.

Irrigation may be by flooding between the row space by furrows running along both sides of rows of trees or via micro-sprinklers, jets, or drip emitters. Irrigation intervals of around 10 to 15 days may be necessary to sustain production unless this interval is broken by rainfall. Overhead sprinklers are not recommended because they constantly wet leaves and trunks, predisposing them to diseases and they also introduce ground water deposits onto leaves and fruits. Aerial water droplets during irrigation will also hinder movement of insect pollinators and compact the soil. The drip system is very popular and the in-line dripper hoses (e.g. Netafim Typhoon 25) with drippers spaced 30 cm apart and dispensing 2.75 L/h are most suitable. For jets and micro-sprinklers, a single one with output of 30 L hr<sup>-1</sup> will meet the requirements.

Delivery of fertilizers in a drip system (fertigation) can be achieved quite easily and cheaply. It requires a pressure tank and the inlet and outlet hoses attached to it. Soluble fertilizers are mixed into the pressure tank and the solution is forced out of the tank by the pressure of the flowing water in the pipes. A valve controlling the outflow calibrates the correct amount of fertilizer coming out of the tank and an electrical conductivity (EC) meter measures the amount in the solution. Fertigation using soluble fertilizer 15: 5: 40 during fruiting stage at a rate of 1-2 g per tree daily is recommended.

Good drainage in the farm is a very important consideration because papaya is very sensitive to water logging. Roots will rot after 24 hours of submergence. The affected tree remains stunted and the lower leaves wilt, turn yellow and defoliate, leaving a few leaves at the apex. After the water has subsided, new adventitious roots may grow from what is left of the healthy roots, but plant recovery is extremely slow and the yield is uneconomical. The trees often dislodge because of the loss in anchorage. It is important to ensure that the site chosen has good soil texture with adequate drainage to avoid this problem. In places where hardpans occur at a shallow depth, breaking them with deep tines or excavators may be necessary.

## 7.5. Replanting

When the crop reaches 30 months, it will be due for replanting. Under certain circumstances, the crop may need to be replanted earlier, for example in very heavy soils where soil compaction may reduce the economic life of the trees. Also when the field shows a strong build-up of diseases such as bacteria dieback, an early replanting is recommended. A tractor equipped with a front grader-blade is used to push down the trees. As much of the plant residues including the roots should be collected and used later for compost-making. When fields show high build-up of diseases, the land should be fallowed or planted with another crop that does not share the same diseases such as a 2:1 papaya to pineapple crop rotation system.

A large amount of biomass can be expected when the field is cleared for replanting. The trunks, leaves and immature fruit are good sources of material for the production of compost. The plant residues are collected, chipped into small pieces and mixed with a N-source such as chicken manure. Kept in the compost dry yard and turned periodically to prevent build-up of heat, the compost will be ready for use in about 60 days.

## 8. PESTS AND DISEASES

### 8.1. Pests

#### 8.1.1. Mites

Several species of mites are known to infest papaya, but the common ones found in the tropics are the red spider mite (*Tetranychus cinnabarinus*) and broad mite (*Hemitarsonemus latus*). They cause little damage when the numbers are low, but if allowed to build up to high levels they will cause severe cosmetic injury to the fruit cuticle. The mite population increases dramatically during the dry and cool spells and they can be located on the undersurface of emerging leaves and petioles. They are hardly visible to the naked eye but the presence of spider mites is easily detected by their telltale webs on the underside of leaves.

In severe infestations, the leaves are shed prematurely and the shoot terminals suffer die back. The young leaves are often distorted and brittle in texture. They also cause extensive scarring (netted corky appearance) on the surface of the fruits when they feed on the epidermal tissues.

Monthly sprays of Kelthane at 0.1% when infestation is apparent during the drier months give effective control. In Hawaii, 2.7 kg of 95% wettable sulphur in 380 liter of water is recommended for mite control. Since the mites are located on the underside of leaves and hidden in emerging shoots, the spray should cover these areas as well. Beneficial organisms such as coccinellid beetles, carabids or predator mites appear to reduce mite populations. Frequent use of pesticides such as pyrethroids (e.g. Karate) that harm these organisms can cause 'flare up' of the mite populations.

#### 8.1.2. Thrips

Thrips (*Thrips parvispinus*) are very small insects, hardly visible to the naked eye. They are common on papaya flowers during the blooming season. On very large healthy well-established trees, they do not appear to be very serious pests. However, during the early stages of plant growth, particularly in the nursery stages and on newly transplanted seedlings in the field, they hide among the shoot terminals and feed on the supple young leaves and shoot. By doing so, they provide the infection site for the invasion by the saprophytic fungus *Cladosporium oxysporum*. The close association of the thrips and the fungus is thus responsible for the outbreak of the bunchy/malformed disease in papaya. Young fruitlets damaged by thrips feeding will mature with unsightly scabs on the skin.

Control of the disease and the thrips can be achieved by weekly spraying of a fungicide e.g. Benlate, Bavistin or Octave alternated with a contact fungicide e.g. Dithane M-45, Anthracol or Coprantol or other Cu based fungicides. Suitable insecticides e.g. decamethrin or methamidophos should be incorporated in the sprays to control thrips infestation at the early stages of plant growth, but once flowering has occurred, it should be used judiciously as thrips are believed to play an important role in pollination. Unpollinated or under-pollinated fruits tend to be small and may drop prematurely.

#### 8.1.3. Fruit Flies

Fruit Flies (*Bactrocera* spp.) are a potentially serious problem in papaya. Their presence influences many of the agronomic practices, including harvest timing and post-harvest treatments, and also affects the export of the fruit to many countries due to quarantine concerns. Although there are numerous species of fruit flies in Malaysia, only *Bactrocera papayae* is an important concern, due to its habit of ovipositing in fruits at a very early stage (usually from index 3). During the mature green stage, the flies are not attracted to the fruits because they contain the papain latex, a proteolytic enzyme that will destroy the eggs. When fruits are picked according to the recommended stage 2, this problem is minimal as egg-lay is a problem only when fruit have 25% or more skin yellowing (Seo et al., 1982). Fruit fly population management is most effectively dealt with as part of an area-wide pest management system covering all the farms in the area. However it can also be implemented at a smaller scale although with less effective results. Generally, the minimum area for effective management of fruit flies is about 20 to 40 ha (depending on field shape and local environment). Two methods are used to reduce fruit fly populations.



a) *Protein Bait Application Technique*- Protein is an important diet for adult flies and when these are laced with suitable insecticides (such as Success or Regent), will kill adult flies effectively and reduce their populations.

b). *Fruit mimic and male annihilation techniques*- Yellow painted bottles act as fruit mimic and they are coated with a glue (e.g. Neopeace) to trap the flies. Methyl eugenol, (a parapheromone that attracts and entraps male flies) is put inside the bottles.

As a result of papaya being a host to fruit fly, Sanitation and Phyto-Sanitation (SPS) and strict quarantine restrictions have effectively restricted export of Malaysian papayas to the United States of America (USA), Australia, Japan and China. Papaya fruits have to be treated with Hot Water Dip for the China market and Vapour Heat Treatment for Japan, Australia and USA markets.

#### 8.1.4. Scale insects

There are three species of scale insects known to infest papaya in Malaysia but only two, i.e. the palm scale (*Aspidiotus orientalis*) and the oriental scale (*Aonidiella orientalis*) are economically important. *Aspidiotus orientalis* is considered the most destructive. Both the nymph and adult feed on the leaf, stem and fruit. If left unchecked, the papaya loses its vigour and eventually results in trunk collapse. The shoots will not develop while fruits will be generally smaller. *Aonidiella orientalis* is only found on the fruit and seldom on other parts of the plant. It does not spoil flesh quality but the cosmetic appeal of the fruit will be lost. Even when the scales are removed during fruit washing, 'green island' marks remain in places where they have fed (Figure 5).

Termicide (Chlorpyrifos) is the most effective chemical for control, and is applied as a basal spray application to the collar and lower trunk of the tree. The chemical is systemically absorbed into the plants' vascular system and is translocated into the upper trunk area and foliage and to a lesser extent into the fruits. This chemical is very phytotoxic and caution is advised in its use. In less severe incidences, scale insects can be effectively managed using white oil (95 ml/4.5 L) and dimethoate (5 - 8 ml/4.5 L).

#### 8.1.5. Nematodes

This pest, common only on sandy soils, will not be expected to be important in orchards where the soil is made up of a large proportion of clay. They damage the root systems and cause stunting of the plants.

Two groups of nematodes are important locally i.e., the root-knot nematode (*Meloidogyne incognita*) and reniform nematode (*Rotylenchulus reniformis*). Treatment for nematode infestation is difficult and expensive. It is best that sandy soils especially those with a history of nematode infestation, be avoided for cultivation. Such areas require fumigation with Nematicur or Basamid before planting. When trees are infected in the field, it may not be economical to save them. Removing infested trees with their root systems with good sanitation practices is recommended.

## 8.2. Diseases

### 8.2.1. Bacterial dieback (BDB)

The papaya industry in Malaysia today is practically destroyed by the bacterial dieback (BDB) disease earlier thought to be caused by *Erwinia papayae* (Maktar et al., 2008) but recently changed to *Erwinia mallotivora* (Noriha et al., 2011). First detected in the southern state of Johor in 2003, it had rapidly spread countrywide since and today farmers are reluctant to invest in this crop because of the high risks. Known also by other names like Bacterial Crown Rot and Bacterial Canker, this disease has been reported in most of the West Indies, Virgin Islands to Venezuela, Java, Taiwan and the Mariana Islands (Ollitrault et al., 2005). It is surprisingly deemed unimportant and had gone unreported in many papaya-growing countries such as Brazil, India, Mexico and USA (Hawaii). In Malaysia, the impact of the disease had been devastating. Maktar et al., (2008) reported that the disease affected about 800 ha resulting in the destruction of one million trees and a loss of 200,000 mt equivalent to USD 58 million. The 200 ha papaya farm at Lanchang owned by the Malaysian Agri Food Corporation was completely obliterated by this disease in 2006.

It is now a national gazetted disease and under the purview of the Ministry of Agriculture, Malaysia.

A typical early symptom is the collapse of the petiole near the lamina, giving it a 'flag leaf' appearance (Figure 6). This later leads to complete rotting and disintegration of the crown (Figure 7). Circular dark lesions also develop on green shoots (especially at the attachment of the leaf petiole) as well as on fruit. The spread of the disease is extremely rapid, especially if the source of inoculum is not removed immediately. It is not soil-borne but believed to be spread by wind, and entry into the host is usually through injury points such as wind-shattered leaves or insect feeding.

Management of bacterial dieback is through an 'exclusion programme' in new, uninfected fields that includes the restriction of visitors and traffic, and the treatment of vehicle tyres and shoes with sanitizing agents. When the field is already infected, the 'containment programme' is activated. This programme requires constant surveillance and culling of infected trees when the symptoms are severe, such as death of the crown. Where trees show early symptoms such as the 'flag-leaf', the diseased petiole may be cut back near to the trunk and the tree may be saved. Another method is to cut the tree down, leaving a 1 m stump for re-shooting. The new shoot may be disease-free and continue to bear fruit normally. When severely diseased trees are removed, the empty points may be re-planted immediately with new seedlings, and these usually thrive without recurrence of the disease.

Breeding for resistance appears to be the best option. The variety 'Glimmer' has been reported to be resistant and has been used for hybridization to produce tolerant hybrids (Chan, 2011).

### 8.2.2. Papaya Ringspot Virus

Papaya ringspot virus or PRSV in short, had severely restricted the papaya industry in Thailand, Philippines and Taiwan and had also been recorded as the major papaya disease in India, Hawaii, the Caribbean, Malaysia and Vietnam. In Taiwan, papaya is restricted to annual rather than perennial production and cultivation is often carried out in large net-houses to keep out the disease-carrying vectors. In Malaysia, the disease was first detected in 1991 and although gazetted, it has not been treated as a significant threat, perhaps being overshadowed by the more devastating bacterial dieback disease.

The most prominent tell-tale symptom appears on the fruit as dark green concentric rings (Figure 8) hence the name for the disease. The symptoms appear as elongated green water-soaked streaks on the younger portion of the stem which is still green and supple and also on the petioles. Infected plants also show chlorosis in the young leaves, and the upper portion of the crown appears prominently yellowed. The symptoms on older leaves are typical mosaic with dark green necrotic spots or patterns on the underside of the leaves. Infected trees may not be killed immediately but are gradually debilitated with very low fruit production. Fruits are also low in sugar content with poor flavour.

The virus is transmitted by several species of aphids in a non-persistent manner. The aphids acquire the inoculum from an infected plant after only a short probe of a few seconds. They do not stay on one tree, but are quickly attracted to another; hence the mode of spread is extremely rapid. The situation is made worse because the virus has an alternative host in Cucurbits such as the widely grown watermelon, pumpkin and squash.

There is no effective treatment for the virus but culling infected trees and desiccating the remains quickly with herbicide can curb its spread. Transportation of seedlings and fruits out of the infected areas and inter-state movement should be prohibited. Isolation of infected areas and formation of buffer zones have been quite successful in preventing the spread of disease in Malaysia and Hawaii. Cross-protection using a mild strain inoculated into the plants confers some immunization, enabling plants to be 82% more productive than unprotected trees in field trials (Yeh *et al.*, 1988). Breeding and selection have resulted in development of tolerant varieties such as Cariflora (Conover *et al.*, 1986), Tainung No. 5 (Lin *et al.*, 1989), Sinta (Villegas *et al.*, 1996) and Eksotika-derived breeding lines (Chan, 2004a). Genetically modified variety Rainbow transformed with the virus coat protein gene has shown excellent resistance to the disease in Hawaii (Gonsalves, 1998).

### 8.2.3. *Phytophthora*

*Phytophthora* commonly causes collar and root-rot and sometimes stem canker and fruit rot in papaya. Papaya is predisposed to the disease in areas with high water table, poor aeration and persistent high rainfall. *Phytophthora* with *Pythium* also cause seedling 'damping-off', a serious disease during nursery stage.

Infected plants show a soft rot round the trunk near the soil line. The root system may sometimes be totally damaged and dislodging of the tree is common. In less severe cases, the trees are stunted and show wilting symptoms and premature defoliation. Even when treated with Ridomil or Aliette (systemic fungicides normally effective against *Phytophthora*), the trees may not fully recover and do not bear economically as the unaffected healthy trees. It is best that infected trees are quickly removed before the disease spreads further.

The first crop of papaya on a new area will not face much problems but continuous cropping will increase the chances of its occurrence. In Hawaii, it is also known as the 'replanting problem' because newly planted seedlings seem to be quickly infected with the disease in areas that have been cropped many times with papaya. The reason for this is that regular cropping increases the amount of papaya residues in the soil and this increases the inoculum level. Therefore, a useful practice in papaya cultivation is to keep the area under good sanitation and fallow with an alternate crop to give at least a three-year break. Ripe papaya fruits, root and trunk residues, which are rich food sources for this pathogen, should not be left on the ground but should be collected and buried or burned. Breeding and selection in Hawaii had resulted in the development of Waimanalo with high tolerance to *Phytophthora* root rot (Nakasone and Aragaki, 1973).

#### 8.2.4. Bunchy/Malformed Top

Bunchy or malformed top is a relatively new disease of papaya, being reported in Malaysia only in late 1985. Thrips were originally thought to be responsible for the malady but subsequent investigations showed it to be a disease complex involving both thrips and a normally saprophytic fungus *Cladosporium oxysporum* (Lim, 1989).

Severely infected plants remain stunted and are slow to recover. If infection occurs before maturity, almost no yield can be obtained. Symptoms are typified by the appearance of new flushes of leaves that are malformed with leaf spots and 'shot-holes' (ranging from 1 to 3 mm wide). Short, light-yellow streaks (due to feeding by thrips) are usually interspersed with the spots.

The young unfolding leaves are most susceptible to infection, appearing as translucent spots or streaks. As the leaves enlarge, the spots and malformation become more evident. A grey mould which is the *Cladosporium* fungus is usually found on severely blighted undeveloped leaves. New infection does not occur on already matured leaves. The Eksotika and Solo varieties are exceptionally susceptible at juvenile stage while local large-fruited varieties such as Subang are quite tolerant (Chan and Mak, 1993a).

Effective control can be obtained by weekly spraying of a systemic fungicide e.g. Benlate, Bavistin or Octave alternated with a contact fungicide e.g. Dithane M 45, Antracol, Coprantol or other Cu based fungicides. Insecticides e.g. decamethrin or methamidophos to control the thrips can be mixed with the fungicides.

#### 8.2.4. Anthracnose/ Brown blotch

This disease caused by a fungi *Colletotricum gloeosporioides*, often infects papaya fruits at the ripening stages. At times, the fungus is also found on leaves and petioles causing leaf spotting and lesions. When the infected leaves fall to the ground prematurely, they become an important source of inoculum for infection of the fruits later on.

At the early stage, the infection is manifested as tiny dark spots on the skin of the fruit and if left untreated, form larger depressed lesions when the fruits start to ripen. It was also referred to as 'brown blotch' (Figure 9) and the 'Frangi' variety is especially susceptible (Chan and Baharuddin, 2010b). In more severe cases, especially when the fruits turn full colour, the lesions may coalesce with each other and the pinkish spores of the fungi are evident in the depressed lesions. The infected fruit becomes soft, dark coloured and unattractive.

Dithane M-45 at 0.2% concentration alternating with Amistar sprayed on the leaves and fruits every 10 days when infestation is apparent will give effective control. During the wet spells, Tenac sticker should be applied as well to reduce wash-off of the fungicide. Folicur (Tebuconazole 25.9%) at 1ml product/ L used as a dip will considerably reduce the incidence of this disease during storage, transportation and export.

## 8.4. Weed

One of the major problems in the cultivation of papaya is weed management. After clearing and disc harrowing the area, it is necessary to blanket spray the ploughed area with pre-emergent herbicides such as Diuron or s-metalachlor (Dual G). This allows the land to be relatively free of weeds for about six to eight weeks. The minimum usage of herbicides during the early establishment of the crop is very important because the soft, supple trunks of the seedlings are very susceptible to damage by herbicide drift.

At the third month after transplanting, weeds would appear in patches in the field and these should be attended to immediately. Spraying should be done in the mornings when there is little wind (speeds of less than 1 m per sec.) to avoid herbicide drift damage. The stem of the papaya remains green for about four or five months after transplanting and direct contact with herbicides at this stage can cause necrosis, stem rot and lodging of plants.

To reduce further any chances of herbicide drift damage during the critical green stem stage, mulching of individual plants is strongly recommended. With mulching immediately around the trunk, not much herbicide use is required around the plant and the occasional weed can be safely spot-sprayed or hoed out.

The rounds of chemical weeding may be carried out every six weeks or so but during wet seasons, the rounds may have to be done sooner. Monthly sprays may have to be done during this time because of rapid establishment of the weeds. Despite its higher cost, the herbicides Basta (glufosinate) and Fusilade (fluazifop) are recommended for use during the early growth period, as they are comparatively safer to use on papayas. However when the trees are more than six months old, these can be substituted with other cheaper herbicides like paraquat.

Keeping the orchard free of weeds is an accepted practice in papaya growing. In farms located on slopes with high risks of erosion, inter-row strips of grass are recommended. Blanket legume covers are not suitable because papaya has very weak and shallow root systems and it cannot compete with the aggressive cover crops. Keeping the orchard weed-free is a lot of work during the early stages of growth but the burden is considerably lessened once the trees achieve full crop coverage when many of the common weed species are shaded out. Under these circumstances, spot-spray of occasional patches of weeds need only be done at two-month intervals or so.







Young papaya seedlings are very sensitive to systemic herbicides like 2, 4-D Amine and Glyphosate (Roundup). Young leaves damaged by these systemic weedicides become distorted, slender and claw-like with curling at the foliage tips. The stem will be pitted or necrotic caused by the spray droplets. In more severe cases, defoliation and shoot die back occurs. Most plants generally recover from mild drift damage in about a month, although flower abortion during that period will invariably affect yield to a large extent.

## 9. PRE HARVEST HANDLING

### 9.1. Fruit maturity

Papaya trees are expected to flower 3 to 4 months after transplanting and the fruits will mature about 5 months later. This means that the maturation age of trees of most papayas is about 8 to 9 months. The optimum stage of maturity is 17 to 20 weeks from flower anthesis. Harvesting at the proper stage of maturity is very essential so that quality and optimum storage life are ensured.

The ripening of the Eksotika papaya is divided into 6 stages of skin colour as follows:

Colour index 1:		full green
Colour index 2:		green with trace of yellow
Colour index 3:		more green than yellow
Colour index 4:		more yellow than green
Colour index 5:		yellow with trace of green
Colour index 6:		full yellow

### 9.2. Harvesting

Fruits should be harvested at colour indices 2 and 3. At these stages, there is also little damage due to anthracnose or fruit flies. Fruits at full green index 1 are not suitable for table consumption because they do not ripen well or ripen with poor quality. Fruits harvested at colour indices 4 and 5 are too mature and do not allow adequate time for transportation and marketing.

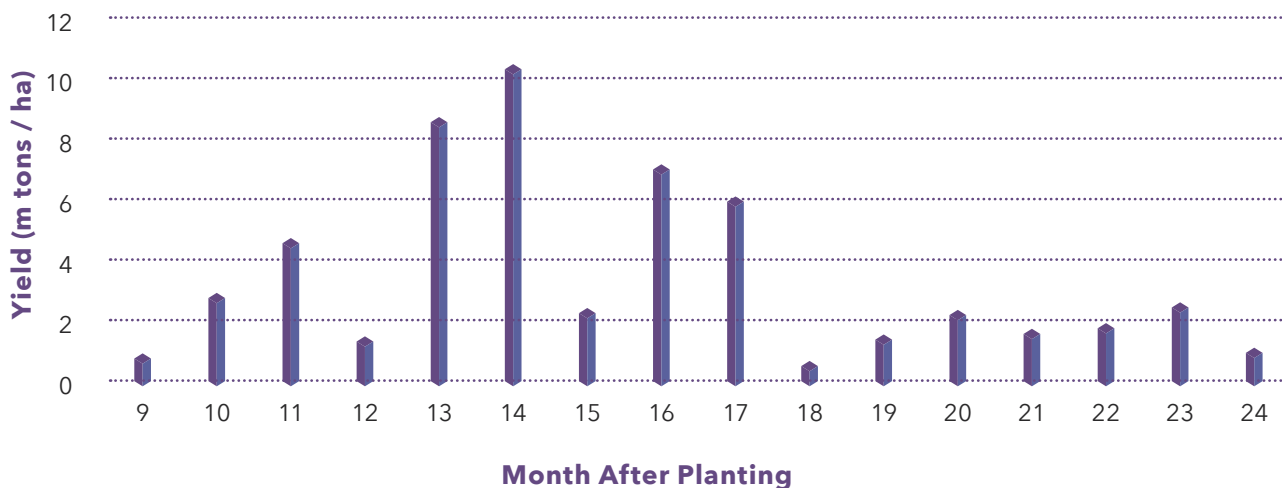
The first fruits are borne about 3 to 4 feet from the ground, so the harvesting in the first year can be conveniently done by hand. In the second year, the harvesting process becomes more tedious because the trees will bear fruits beyond the reach of the harvester. In such circumstances, a long stick with a small wire basket lined with sponge attached to the end will facilitate the harvester. At the end of the stick, a nylon net secured around a ring would be useful to catch the fruit as it detaches from the tree.

The normal practice of harvesting papaya is to use a stackable plastic basket for collecting harvested fruits. It must be emphasized, however, that the basket must be lined with cloth or plastic sheets so that bruising of the fruits is minimal in order to sell them for a premium price.

### 9.3. Seasonality in fruit production

Although papaya is generally known to be a non-seasonal fruit type that produces fruits throughout the year, it shows distinctive production peaks and non-productive phases when its whole cropping cycle is considered.

The general pattern of fruit production over a period of 24 months on a commercial farm is shown in Figure 1. The peaks of production generally come in at every three monthly intervals beginning with the first peak at the eight to ninth month period. The best production phase is expected to be around the 13 to 14 month period, when the yield of the plants is highest. The peaks after this progressively become less marked when the plants reach the latter part of their crop cycle around 18 to 24 months.



**Figure 1. Fruit production trends of Eksotika papaya**

The production trends do show variation, depending on the rainfall and management practices. As fruit production shows fluctuation, sometimes for a month or more, there will be few fruits for harvesting. This being the case, fruit harvesting should, where possible be organized on contract basis.

Finally, the peaks of the harvesting do not appear to be staggered even with the staggering of the planting dates of several papaya plots. The reason for this is that the papaya plant is very sensitive to weather changes and flower production is most active during the rainy periods. Therefore, all plants, irrespective of their ages, will respond accordingly and show similarity in production pattern at the same location. This fruiting pattern characterized by periods of heavy and poor fruiting (resulting from variable weather conditions) causes instability in the supply of fruits even though papaya is considered as a non-seasonal fruit.

## 10. POST HARVEST HANDLING

### 10.1. In-Field Collection and Transport

Papaya has tender skin and can be easily bruised or cut by rough handling. Therefore, careful handling is required right from the harvesting time and throughout the process chain to obtain high quality fruits. Handling during the first mile, i.e. transportation activities from the field to the processing center, is one major episode contributing to quality degradation. Damages occurred in the field will carry over and become diseased through packing and storage processes. Therefore it is important that efforts be made to minimize the movement of fruits during transport.

Harvested fruits should be collected in plastic crates that are padded with rubber foam to minimize damage to the fruits during in-field transportation. Bubble wrap sheet can be used to inter-layer the fruits in the crates to minimize the fruits from rubbing against each other. The crates are quite sturdy and can be stacked up to 4 layers which economize on transport space.

The picked fruits should be shaded from strong sunlight to reduce the absorption of field heat. Special care need be taken during transportation using lorries, to avoid shocks on bumps and pot holes on farm roads.

It is good practice to have pre-cooling facilities (15°-20°C) to ensure that the temperature of harvested fruits is quickly brought down. This is to prevent accelerated ripening and subsequent vulnerability to storage diseases.

### 10.2. Pre-sorting and Washing

At the packing house, the fruits need again be sorted according to size, shape (sex), colour index, deformation (carpellidoidy) and damages due to pest, disease, mechanical injury, etc. For Eksoitika fruits weighing less than 250 g or over 1 kg are considered out of the acceptable range, but these can still be sold in specialty markets. Damaged, deformed, overripe and round (female) fruits are considered rejects.

Sorting is very important especially if the fruits are meant for supermarkets or for export. For export, only fruits with colour index 2 or 3 are accepted for packing.

Fruits with too much scars are usually sold in local markets. After sorting, the fruit stalks are trimmed off. The fruits are then washed in clean water to remove latex, dirt, insects and foreign matters.

### 10.3. Sanitizing

Sanitizing treatments are carried out in order to kill microorganisms on the surface of the fruits. These include both potential human pathogens (e.g. colliforms) as well as the causal agents of post-harvest disease (e.g. anthracnose or soft rots).

The sorted fruits are normally dipped for 2 minutes in sodium hypochlorite solution ('chlorox') at concentration 150-200 ppm free chlorine at ambient temperatures, 24°-26°C and the solution is most effective when maintained at pH 6.0 - 7.0. The sanitization efficacy is due to the oxidising power of the chemicals added to water, in this case, the concentration of hypochlorous acid. An easier way to measure this is by using oxidising-reduction potential (ORP) meter measured in mV. Between 700-1000 mV of ORP at pH 6.0-7.0 is required for good sanitizing. Mixing 1 L of chlorox (5.25%) in 200 L water is sufficient to sanitize 1 mt of fruits.

If water contains high amount of dissolved iron (1-3 ppm), it should first be sufficiently aerated to precipitate the iron oxides and then have them filtered out or simply allowed to sediment in the storage tank. Adding acetic acid to lower the pH of the alkaline Chlorox mixture is recommended.

## 10.4. Chemical dip

Fruits for immediate consumption need not be treated with fungicides. However, fruits that are to be stored or exported should be treated with Folicur (Tebuconazole 25.9%) at 1ml product/ L of clean water by dipping for 20-25 seconds.

It is recommended not to leave the fruits in Folicur solution for too long (not more than 1 or 2 minutes), or necrosis of the cuticle will occur.

Dipping the fruits in the fungicide helps to reduce the severity of postharvest diseases such as anthracnose (*Colletotrichum*) for a period of 10 to 14 days. Folicur will also suppress stem end rots caused by *Botryodiplodia* or *Phomopsis*. After the dip, the fruits are force-air dried.

## 10.5. Ripening Induction

All papaya varieties respond very well to ripening induction which helps the fruit develop a uniform, attractive full yellow skin. It also softens the texture of the flesh which may otherwise be still too firm at full yellow index 6. Ripening treatment can be done by using ethylene gas. This will be carried out in a ripening room and only after the fruits have been packed into boxes or crates. Maintaining the relative humidity at about 90% during induction will produce fresh, turgid and an attractive sheen on the skin.

Facilities for ethylene ripening are (i) air-tight cool room of minimum size of 50 m<sup>3</sup> that has cooling fans with temperature control of 20°-26°C, (ii) room with relative humidity (RH) of 90%, (iii) ethylene generator (e.g. Easy Ripe of Catalytic Generators, Inc.), (iv) supply of ripening liquid (e.g. Ethy-Gen II) which is made up of 90% ethanol, and (v) ethylene meter. Safety is important as ethylene is explosive when the concentration exceeds 27,000 ppm. The amount of ripening liquid has to be calculated based on the room size. As a general guide, a room of 50 m<sup>3</sup> will need 200 ml of the Ethy-Gen II to produce 100-150 ppm ethylene gas by the generator. Ethylene treatment of 24 h at 22°-23°C is sufficient to produce good ripening quality.

Only fruits that have been packed in boxes or crates can be ripened in a ripening room. The airflow through the boxes and crates is important to produce uniform ripening. Most fruit can be ripened from index 2 to index 5 in 24 h but may vary depending on the ethylene concentrations and the temperature applied. The usual practice is to apply 100-150 ppm of ethylene at 22°-23°C. To extend the shelf life, low temperatures at 12C° and high RH are maintained for fruit that have ripened to index 4.

## 10.6. Sorting, Grading and Standards

The grading of small-fruited Eksotika papayas for the export market has been clearly spelt out in Malaysian Standard (MS 1145:2003). During sorting and grading, many non-pathological defective fruits may be found and these should be discarded early (Table 5).

**TABLE 5** Quality grading system of Eksotika papaya

Grades	Requirements	Tolerance
Premium	The fruit shall be characteristic of the variety, uniform in size and shape, free from blemishes with the exception of very slight superficial irregularities in the skin. There shall be no freckled fruits.	Fruit which do not meet these requirements shall not exceed 5% by count but shall conform to the next lower grade.
1	The fruit shall be characteristic of the variety, fairly uniform in size and shape and free from blemishes.	Fruit which do not meet these requirements shall not exceed 10% by count but shall conform to the next lower grade.
2	The fruit shall be characteristic of the variety, fairly uniform in size and shape and reasonably free from blemishes.	Fruit which do not meet these requirements shall not exceed 10% by count.



Size	Weight (g)
Extra large	> 851 - 1,000
Large	651 - 850
Medium	451 - 650
Small	351 - 450
X-Small	280 - 350

For most export markets, the pyriform-shaped hermaphrodite fruits are the preferred choice. Medium sized fruits of 451-650 g (Table 6) are normally exported to Singapore, China and the Middle East and Europe. All fruit sizes are marketed locally. At present, Malaysian papayas may not be exported to Japan, Australia, New Zealand and the United States of America because these countries have very strict quarantine regulations which require postharvest disinfestation treatments.

## 10.7. Packaging and Labeling

Papaya is a fragile fruit. For long distance transport and export, papaya fruits must be packaged in suitable container and maintained in appropriate temperature and humidity. This is to ensure that the shelf life and quality of the fruits are maintained when reaching the consumers. Being a climacteric fruit, papaya is sensitive to ethylene. For this reason, precise sorting is crucial at packing time into boxes in that a more ripe fruit, which releases more ethylene, would speed up the ripening of other fruits in the package. Smaller size fruits normally would ripen faster. Higher temperature ripens the fruit even faster. Dehydration causes the skin to wrinkle, whereas high humidity in the package encourages diseases. Therefore the packages used must fulfill the following requirements:

- ✓ Sufficiently rigid to protect the fruits from external forces, such as shock and vibrations;
- ✓ Keeps the fruits separate to avoid bruises by wrapping with styrofoam sleeve or newsprint to cover the whole fruit, folded at the bottom but remained open at the top;
- ✓ Allows good flow of air and vapour through the boxes, even when stacked multi-layered
- ✓ Protects from external heat and excessive loss of moisture;
- ✓ The packaging material is not easily weakened by moisture and temperature change;
- ✓ The materials that make the packaging containers do not release chemicals that affect the shelf life of the fruits, like ethylene;
- ✓ The packaging containers are light, easy to handle and can be placed on pallets;
- ✓ The packaging containers must be labeled according to the specifications required by the market regulatory authorities, local and importing countries.

Most papaya packers pack the fruits in open stackable plastic crates for domestic market or corrugated fibre board boxes for export. The plastic crates, can hold 15 kg containing about 25 fruits. For corrugated fibre board boxes, Malaysian papayas are packed in 2 box designs, i.e., the open top 5 kg box that holds 6-12 fruits and the telescopic 3.5 kg box holding 6-8 fruits. The latter packaging is sturdier and normally used for export by air or sea. Before packing the fruits into boxes or crates, each fruit is wrapped in wood-free paper to protect them from bruising during transportation. Occasionally, styrofoam sleeves are used for the export fruits.

After packing the fruits in boxes, they are placed on pallets (Euro type: 100 cm x 120 cm). The boxes can stack up to 10 layers in cold trucks for local market and 12 layers in reefer containers for sea export. Each pallet should have fruits within the same harvest date that is important for shelf life management. Ready-stacked pallets must be protected from insects, birds and rodents by using nylon netting.

## 10.8. Cold chain storage

Temperature plays a very important factor in storage of papaya fruits, as ripening is hastened by high temperatures that increase the ethylene release. Diseases like anthracnose and soft rots would appear faster as the ripening accelerates. All these will shorten the shelf life resulting in poor quality for marketing. Postharvest diseases like anthracnose, stem end rot and other rots caused by fungi are common if recommended temperature during storage is not followed.

Fruits with colour indices 2 and 3 can ripen without chemical induction at ambient conditions (22°-30°C) at 8 to 12 days, while fruits at colour indices 4 and 5 can keep for 4 to 6 days respectively. Papayas at index 2 can be stored up to 2 weeks at 12°C before it turns to index 4-5. Temperature at 10°-12°C is preferred to keep the fruits green up to 3 weeks. Lower temperature and longer storage period can result in skin browning, dehydration, lowering of sweetness, bitter taste and appearance of lumpy tissue.

Risk of postharvest disease will increase if storage exceeds 10 days at ambient conditions. The lumpy tissue can be avoided if the fruits are ripened first to index 4 before extending the chill storage. Long chill storage of fruits treated first with ethylene will get too soft at index 5. Storage of papayas at index 2 at less than 10°C may cause the appearance of greenish blotches, which is a chill injury aggravated by the wetting of the fruit skin due to moisture condensation. This minor form of skin injury can diminish if the fruit skin is kept dry and the blotchiness will disappear when the fruit fully ripens to index 6. Papayas at colour index 2, must not be stored for more than 3 weeks at temperature lower than 12°C to avoid serious chill injury.

Freezing temperatures should never be used as the fruit will stop to ripen. Severe chill injury shows bronzing of the fruit skin as well as whitish hard tissues in the vascular bundles at the periphery of the fruit cavity. For exports to distant markets, sea-reefer is the economical means of transport and the long storage at low temperatures invariably will result in some form of chill injury that depreciates market value. But some of the injuries such as green blotches are only unsightly on the skin and do not affect the eating quality.

## 11. BIOCHEMISTRY

### 11.1. Chemicals in plant parts and products of papaya

The papaya plant and its latex have medicinal and industrial uses. Minerals and chemical compounds, many of them biologically active, are found in various parts of the papaya plant. Some of the more important ones compiled from published papers (Duke, 2007, cited in OGTR, 2008, Aravind, *et al.*, 2013 and Boshra and Tajul, 2013) are listed in Table 7. A comprehensive list can be retrieved from Dr. Duke's Phytochemical and Ethnobotanical Databases ([www.ars-grin.gov/cgi-bin/duke/farmac2.pl](http://www.ars-grin.gov/cgi-bin/duke/farmac2.pl)).

**TABLE 7** Chemicals found in various parts of *Carica papaya*

Plant part/product	Chemicals
<b>Fruit</b>	Protein, fat, fibre, carbohydrates, calcium, iron, magnesium, potassium, manganese, zinc, vitamin C, thiamine, riboflavin, niacin, $\beta$ -carotene, amino acids, citric, butanoic acid, carpaine, <i>cis</i> - and <i>trans</i> - linalool oxide, $\alpha$ -phellandrene, $\alpha$ -terpinine, methyl-thiocyanate  In unripe fruit: papain, chymopapain, malic acid
<b>Leaf</b>	Calcium, iron, magnesium, potassium, manganese, zinc Carpaine, pseudocarpaine, dehydrocarpaine, choline, carposide, vitamin C and E, flavonols, tannins, nicotine, prunasin (cyanogenic glycoside)
<b>Shoot</b>	Myricetin, kaemferol
<b>Bark</b>	$\beta$ -sitosterol, glucose, fructose, sucrose, xylitol
<b>Root</b>	Carposide and enzyme myrosin
<b>Seed</b>	Fatty acids, crude protein, crude fibre, papaya oil, carpaine, benzyl-isothiocyanate, benzylglucosinolate, glucotropacolin, benzyl-thiourea, hentriacontane, $\beta$ -sitostrol, enzyme myrosin, caricin, carpasamine, linoleic acid, oleic acid, palmitic acid
<b>Latex</b>	Papain, chemopapain, glutamine, cyclotransferase, chymopapains A, B and C, peptidase A and B, lysozymes, benzy-lglucosinolate, caoutchouc, chymopapain A and B, malic acid
<b>Juice</b>	N-butyric acids, n-hexanoic and n-octanoic acids, lipids, myristic, planets, stars, linolec, linolenic and <i>cis</i> -vaccenic and oleic acid

Papaya is a laticiferous plant that has specialised cells called laticifers dispersed throughout most of its tissues and are capable of secreting a white milky substance known as 'latex' when damaged. The latex of papaya plants is rich in enzymes and is used widely for protein digestion functions in the food and pharmaceutical industries. Commercially, papaya latex is harvested from fully grown but unripe fruit the skin of which contains numerous laticifers. Ripe papaya fruit contains no latex (Villegas, 1997, cited in OGTR, 2008). The main constituent in papaya enzymes is cysteine proteinase which may constitute as much as 80% of the enzyme fraction in papaya latex (El Moussaoui *et al.*, 2001, cited in OGTR, 2008). The most well studied proteinases from papaya are papain, chymopapain, caricain and glycyl endopeptidase. Other enzymes known from papaya latex include glycosyl hydrolases such as  $\beta$ -1,3-glucanases, chitinases and lysozymes, protease inhibitors such as cystatin, glutaminyl cyclotransferases and lipases (El Moussaoui *et al.*, 2001, cited in OGTR, 2008).

## 11.2. Allergens

Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies known as Immunoglobulin E (IgE), or sensitisation of specific tissue bound lymphocytes (Taylor and Lehrer, 1996 cited in OGTR, 2008; FAO/WHO 2000). Allergy has a well-defined etiology (i.e. biochemical cause) that is quite different from toxicity.

Many studies have shown that plant parts, latex and root extracts of papaya have inherent toxicological or allergenic properties, many of which apparently relate to the complex, largely uncharacterised, chemical composition of papaya latex. Sensitised individuals may develop allergic symptoms when exposed to papaya latex.

Papain, a product of papaya latex, is widely used in both the food manufacturing and pharmaceutical industries. Sensitisation to papain among workers in these industries is well known (Baur *et al.*, 1988, cited in OGTR, 2008; Iliev and Elsner, 1997, cited in OGTR, 2008). Immunoglobulin E (IgE) antibodies against all four of the major papaya cysteine proteinases in latex, i.e. papain, chymopapain, caricain and glycyI endopeptidase have been identified in people who show an allergic response to a pharmaceutical product derived from papaya latex (Dando *et al.*, 1995, cited in OGTR, 2008). The presence of these antibodies demonstrates that all four cysteine proteinases are allergenic. In another study of the cysteine proteinases, it was found that papaya latex also contains other enzymes as minor constituents: a class-II and a class-III chitinase, an inhibitor of serine proteinases, and a glutaminyl cyclotransferase. The presence of a  $\beta$ -1,3-glucanase and of a cystatin is also suspected, but they have not been isolated yet (OGTR, 2008).

Externally, papaya latex is an irritant, dermatogenic and vesicant. Papaya fruit pickers manually harvesting fruit had been advised to wear gloves and protective clothing, as latex oozing from the fruit stalk may cause skin irritation (Morton, 1987). Papaya latex may induce asthma and it may also have allergenic properties when ingested. Iliev and Elsner (1997, cited in OGTR, 2008) reported an allergic reaction, manifesting as a skin rash developed after the use of throat lozenges containing papaya extract. Diaz-Perales *et al.*, (1999, cited in OGTR, 2008) noted that papaya fruits contain class I (allergenic) chitinases that correspond to proteins detected with a pool of sera from patients with latex-fruit allergy. Latex-allergic patients may develop oropharyngeal itching (the oropharynx is the part of the pharynx at the back of the mouth) and angioedema (rapid swelling of the skin, mucosa and submucosal tissues, particularly around the mouth/throat and hands; sometimes followed by itchiness) if they eat papaya (De Clerck *et al.*, 2007, cited in OGTR, 2008). An extreme allergic reaction to skin contact with unprocessed papaya fruit has also been reported (Ezeoke, 1985, cited in OGTR, 2008).

Papaya pollen is able to induce respiratory IgE-mediated allergy (Blanco *et al.*, 1998, cited in OGTR, 2008) and one IgE-reactive, 100k Da protein component with esterase activity has been identified. The pollen can contribute significantly to the aeropollen and aeroallergen load in areas where papaya plants occur (Chakraborty *et al.*, 2007, cited in OGTR, 2008).

## 11.3. Toxins

Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot, 2000, cited in OGTR, 2008). Although the whole of papaya plant is useful in various medicinal properties, some of the active compounds like carpine and papain are toxic. In nature, carpine is present in traces in papaya seeds. However, it is used in large quantity to lower the pulse rate and depress the nervous system (Eyo *et al.*, 2013). There is a compound present in crushed papaya seed that is believed to have activity against helminthic intestinal parasites. Benzyl isothiocyanate (BITC - derived from benzylglucosinolate), has been shown to have an effect on vascular contraction using a canine carotid artery *in vitro* model (Wilson *et al.*, 2002, cited in OGTR, 2008). Other studies have suggested possible purgative effects of papaya root extracts (Akah *et al.*, 1997, cited in OGTR, 2008) and antihypertensive activity of papaya fruit extracts (Eno *et al.*, 2000, cited in OGTR, 2008). The presence of cyanogenic compounds in papaya has also been reported (Seigler *et al.*, 2002, cited in OGTR, 2008). Papaya seed extract was found to be toxic to larvae of *Aedes aegypti* (Nunes *et al.*, 2013). Powder prepared from seeds of papaya (Ayotunde *et al.*, 2010) and aqueous extract of papaya seeds (Eyo *et al.*, 2013) had

been found toxic to fingerlings of sharptooth catfish. Generally plants that produce glucosinolates do not produce a cyanogenic glycoside and *vice versa*. However, papaya appears to be a rare example of a species in which a glucosinolate (benzylglucosinolate) and a cyanogenic glycoside (prunasin) co-occur (Olafsdottir *et al.*, 2002, cited in OGTR, 2008). Excessive consumption of plant parts that contain cyanogenic glycosides (leaves and roots, in the case of papaya) may induce adverse reactions, due to the formation of cyanide in the digestive system of the consumer, although none have been recorded for papaya (OGTR, 2008).

#### 11.4. Other effects of phytochemicals from papaya

Excessive consumption of papaya may cause carotenemia, the yellowing of soles and palms, which is otherwise harmless. However, a very large dose would need to be consumed to have this effect; papaya contains about 6% of the level of beta carotene found in carrots (the most common cause of carotenemia).

Carpine and papain also have antifertility properties particularly of the seeds. Thus may be used in birth control. A complete loss of fertility has been reported in male rabbits, rats and monkeys fed an extract of papaya seeds (Lohiya *et al.*, 2002 cited in Ayotunde *et al.*, 2010), suggesting that ingestion of papaya seeds may adversely affect the fertility of human males or other male mammals.

In India and parts of south-east Asia and Indonesia, consumption of papaya fruit is widely believed to be harmful during pregnancy, since papaya is believed to have abortifacient properties (induces miscarriage during pregnancy) or teratogenic properties (causes malformations of the foetus) (Adebiyi *et al.*, 2002, cited in OGTR, 2008). Conversely, a papaya fruit extract is used for prevention of miscarriage by traditional African healers (Eno *et al.*, 2000, cited in OGTR, 2008). Although a number of early studies, largely conducted in India, suggested that unripe papaya fruit, latex extracts or papaya seeds have deleterious effects on pregnancy in laboratory animals (Schmidt, 1995, cited in OGTR, 2008), more recent analysis suggests that ripe papaya fruit or purified papain do not cause malformations of rat foetuses (OGTR, 2008).

Ingestion of unprocessed ripe papaya fruit has no impact on the number of viable foetuses or foetal weight in rats (Adebiyi *et al.*, 2002, cited in OGTR, 2008). Likewise, purified papain derived from latex of unripe papaya did not impact adversely on prenatal development when administered orally to pregnant rats (Schmidt, 1995, cited in OGTR, 2008). However, *in vitro*, crude latex derived from unripe papaya fruit stimulates contractions in non-pregnant rat uterus (Adebiyi *et al.*, 2002, cited in OGTR, 2008).

#### 11.5 Health benefits, uses and nutritive values of papaya

Apart from medicinal and industrial uses, papaya is famous for its nutritive value and has many health benefits. It is a natural source of vitamins and minerals that are essential for the normal functioning of the body. The vitamin A and C content of one medium papaya fruit (approx. 350 g edible portion) exceeds the Dietary Reference Intakes established by the US Food and Nutrition Board for adult minimum daily requirements (OECD 2005). The vitamin C content is much higher than in either tomatoes or oranges (Benson and Poffley 1998, cited in OGTR, 2008). Papaya is also rich in vitamin B (folate and pantothenic acid).

Papaya is primarily a fresh-market fruit. It is used in drinks, smoothie, milkshake, jams, candy and as crystallized fruit. The green fruit can be used in pickle and salad. The leaves, flowers and roots can be cooked as vegetable. Latex derived from the raw fruit is used as a meat tenderizer and is also used in the manufacturing of several cosmetic, skin, and beauty products, as well as certain chewing gums. They are available for consumption throughout the year. The whole fruit, including other parts of the papaya tree, are beneficial to health in several ways. An enzyme called papain is present in the fruit which helps in the digestion of proteins. Papaya in raw form contains this enzyme, which is used in the preparation of dietary supplements and chewing gums. Papaya is rich in antioxidant nutrients such as carotenes and flavonoids. It is also a good source of fiber and minerals such as magnesium. Together, these nutrients help to improve cardiovascular health and protect against colon cancer. The key nutritional elements of the papaya fruit are listed in Table 8.

Papaya Fruit Nutritional Values (Per 100g edible portion only)\*  
(Source: OGTR, 2008)

<b>Water</b>	89.3g	88.83g
<b>Energy</b>	123kJ / 29kcal	163kJ/39kcal
<b>Protein</b>	0.4g	0.61g
<b>Fat</b>	0.1g	0.14g
<b>Carbohydrate (total)</b>	6.9g	9.81g
<b>Carbohydrate (sugar)</b>	6.9g	5.9g
<b>Dietary fibre</b>	2.3g	1.8g
<b>Cholesterol</b>	Nil	Nil
<b>Sodium</b>	7mg	3mg
<b>Potassium</b>	140mg	257mg
<b>Calcium</b>	28mg	24mg
<b>Magnesium</b>	14mg	10mg
<b>Iron</b>	0.5mg	0.1mg
<b>Zinc</b>	0.3mg	0.07mg
<b>Beta-carotene</b>	910ug	276ug
<b>Thiamin</b>	0.03mg	0.027mg
<b>Riboflavin</b>	0.03mg	0.032mg
<b>Niacin</b>	0.3mg	0.338mg
<b>Vitamin C</b>	60mg (171% of RDI)	61.8mg
<b>Vitamin A Eq</b>	150µg	N/A

\* The nutritional data taken from the Papaya Australia (2007) website and the USDA National Nutrient Database for Standard Reference (2006)

## 12. DEVELOPMENT OF GENETICALLY MODIFIED PAPAYAS

### 12.1. Resistance to papaya ringspot virus

Papaya ringspot virus (PRSV) disease is serious worldwide causing substantial losses in papaya fruit production. First identified on Oahu Island in Hawaii in 1945, it had become a threat to the industry in the 1950s (Bury, 2014). To escape the PRSV disease, papaya cultivation was moved from Oahu to the then virus free Hawaii Island (also known as the Big Island) where it stayed free from PRSV for 30 years until the early 1990s when PRSV was detected in the Puna district of Hawaii Island (Bury, 2014). It was widespread by 1995. The PRSV disease is also detected in many papaya growing countries and regions. In China, PRSV was first reported in 1959 in Guangdong province (Bury, 2014). In Taiwan, PRSV first detected in 1975 and by 1980 had spread across the entire island. PRSV was first reported in the Philippines in 1982, in Thailand in 1990 and a year later in Vietnam and Malaysia as well.

In Hawaii, using the concept of pathogen-derived resistance, the coat protein gene of a mild mutant of a PRSV strain from Hawaii, HA55-1 was used in biolistic transformation of embryogenic cultures of the yellow-pulped papaya cultivar Kapoho, the red-orange pulped cultivar, Sunrise and its sib, Sunset cultivar (Fitch *et al.*, 1992). The R<sub>0</sub> line 55-1 of cv. Sunset and the R<sub>1</sub> lines derived from outcrossing with non-transgenic lines were found highly resistant to Hawaiian PRSV-P isolates in the glasshouse and in the field (Fitch *et al.*, 1992; Lius *et al.*, 1997). Transgenic line 55-1 of cv. Sunset was inbred to homozygosity for the single copy coat protein gene and named 'SunUp'. In Hawaii, the dominant cultivar has been the yellow-pulped 'Kapoho'. The GM (genetically modified) papaya 'SunUp' however, has red-orange pulp. No suitable transgenic lines from the yellow-pulped 'Kapoho' was obtained. In order to make a virus resistant yellow-pulped papaya available to growers, the GM 'SunUp' papaya was crossed with the PRSV susceptible non-GM 'Kapoho'. This resulted in a F<sub>1</sub> hybrid which is yellow-pulped and resistant to PRSV (Manshardt, 1998). This F<sub>1</sub> hybrid is called 'Rainbow' (Manshardt, 1998).

In 1995, a large field trial of GM papayas 'Rainbow' and 'SunUp' was laid down in a severely infected farm located in Puna where the virus was already widespread (Ferreira *et al.*, 2002). A section of the trial included small replicated blocks of 'Rainbow', 'SunUp', a GM Sunset line 63-1 and a non-GM 'Sunset'. Adjacent to these replicated blocks was a large solid block of 'Rainbow' that was surrounded by border rows of virus-susceptible 'Sunrise'. PRSV inoculum source existed in a nearby-infected block of non-GM 'Sunrise' as well as selected 'Sunrise' plants in the border rows that were mechanically inoculated with PRSV. The results showed that all non-GM plants became infected within 11 months of starting the field trial while none of the GM test plants became infected. This resistance was still evident up to termination of the trials in early 1998 (Gonsalves, 2004).

Although PRSV resistant papayas had been successfully developed in Hawaii, these resistant R<sub>1</sub> lines showed varying degrees of susceptibility when challenged with PRSV-P isolates from different geographic regions (Tennant *et al.*, 1994; Yeh and Gonsalves, 1994). These lines developed in Hawaii would likely be ineffective in controlling PRSV-P of other geographical regions. In view of this, development of GM papaya resistant to the PRSV isolates specific to each of the countries were implemented notably in Australia, Bangladesh, Brazil, China, Jamaica and Venezuela. Coordinated by ISAAA, the Papaya Biotechnology Network of Southeast Asia was established in March 1998 for collaborative efforts of partners to develop GM papaya for PRSV resistance and GM papaya with delayed-ripening trait. Five countries, namely, Indonesia, Malaysia, Philippines, Thailand and Vietnam were members of this Network. The collaboration also involved University of Nottingham and two private sector companies, Monsanto and Syngenta as the technology providers.

Inspired by the success in Hawaii, researchers from South China Agricultural University, in Guangdong province initiated a similar research project. Instead of expressing the PRSV coat protein, the Chinese researchers chose to work on the viral replicase gene. GM papaya 'Huanong 1' resistant to PRSV was successfully developed. The Chinese Biosafety Committee approved it for cultivation and commercialization in 2006 after numerous field tests. Since 2007, China has also been growing its own domestically developed PRSV resistant GM papayas. In 2012, the cultivated area devoted to GM papayas was 6275 ha which accounted for more than 60% of the total cultivated land area used for papayas in China (Bury, 2014).

In Australia, Lines *et al.*, (2002) reported the development of two Australian papaya cultivars that are immune to infection with PRSV. In Taiwan, researchers at the 'National Chung Hsing University' and the Taiwanese agricultural research institute published in 2003 the development of a transgenic papaya resistant to Taiwanese PRSV. The Taiwanese GM papaya is not just resistant to PRSV viruses from Taiwan but also to PRSV from Hawaii, Thailand and Mexico. However, the Taiwanese GM papaya has not been cultivated commercially. During the early 1990s, the Jamaican papaya industry was seriously affected by PRSV infections. In collaboration with Cornell University, a GM papaya was developed based on coat proteins from the Jamaican variant of PRSV. The GM papaya was ready for field testing in 1998 but because the necessary legal framework for commercialization was absent, no further steps have been made. Efforts in Brazil and Venezuela had also resulted in the development of GM papaya resistant to PRSV that is suitable for their countries (Cai *et al.*, 1999; Tennant *et al.*, 2002). However, no information regarding the deregulation process is available.

In Thailand, a collaborative research between the Department of Agriculture and Cornell University was initiated in 1995. Two Thai cultivars were transformed by microprojectile bombardment using a non-translatable coat protein gene of PRSV from 'Khon Kaen'. Three R<sub>3</sub> lines derived from Khaknuan papaya showed excellent resistance to PRSV (97% to 100%) and one R3 line initially derived from Khakdam papaya showed 100% resistance. Safety assessments of these transgenic papayas found no impact on the surrounding ecology. No natural crossing between transgenic and non-modified papaya was observed beyond a distance of 10 m from the plots. Analysis of the nutritional composition found no differences in the nutrient levels in comparison with the non-modified counterparts (Sakuanrungrisirikul *et al.*, 2005). Unfortunately, the development of PRSV resistant GM papaya in Thailand was terminated at the confined field test stage due to a countrywide moratorium on all field testing of transgenic crops.

In the Philippines, a ceremonial planting of genetically modified papaya was held in February 2009 at the University of the Philippines, Los Baños-Institute of Plant Breeding (UPLB-IPB) experimental field to mark the start of the limited field release of the GM papaya resistant to the papaya ringspot virus disease. However, no further information on the field release is available.

In Vietnam, twenty-five transgenic lines had been generated using three different constructs and were evaluated under greenhouse conditions (Tecson-Mendoza *et al.*, 2008).

## 12.2. Increasing shelf-life

Papaya fruit is highly perishable and this is one of major problems in fruit exportation. In general, papayas have a short shelf life of 4 to 5 days stored at room temperature of 25°C-28°C and up to three weeks at lower temperature of 10°C to 12°C (Paull *et al.*, 1997). However, when stored at 15°C to 20°C, the quality, thus the marketability of papaya fruit are affected because the flesh turns soft and the fruit shrivels (Proux *et al.*, 2005). Increasing the shelf-life by delaying the ripening of the fruit will have potential in reducing this problem. The strategy for delaying ripening of papaya fruit is to suppress or inhibit the enzyme ACC (1-aminocyclopropane-1-carboxylic acid) synthase or ACC (1-aminocyclopropane-1-carboxylic acid) oxidase involved in ethylene production process during ripening of papaya fruit.

In Malaysia, using the antisense technology, GM papayas with delayed-ripening traits were being developed by MARDI (The Malaysia Agricultural Research and Development Institute) to stretch the shelf life and maintain the quality of its fruits during shipment (Abu Bakar *et al.*, 2001). Somatic embryogenic calli of papaya cultivar Eksotika was transformed with the antisense 1-aminocyclopropane-1-carboxylic acid oxidase 2 gene (ACO2) construct. Several potential transgenic 'Eksotika' papaya lines with delayed-ripening characteristic have been produced and a confined field trial to evaluate the lines had been conducted. According to Rogayah *et al.*, (2013) no significant difference in the physical stature of the GM and non-GM papaya plants was found. Physiological evaluations of the fruits of GM papaya showed a 15-day delay in ripening compared with four days of the non-GM seed-derived papaya fruits. The total soluble solid (TSS) of the transgenic fruits was comparable to that of the non-transformed seed-derived fruits with similar 11-15°Brix, implying the transgenes did not affect the TSS content. The transgenic fruits remained firm for an additional 4 to 8 days at room temperature (25 ± 2°C) after achieving the full maturity index (index 6), whereas the non-transformed seed-derived fruits lost their firmness after 2 days.



In Australia, the Botany Department of the University of Queensland had generated 100 transgenic papaya trees. In 2003, OGTR (the Office of Gene Technology Regulator) approved the application from the University for the limited and controlled release of the eight GM papaya lines into the environment to evaluate the modified fruit ripening characteristics. The fruits from transgenic trees exhibited increased shelf life of up to two weeks from colour break stage. Similar result was also obtained in the Philippines.

### 12.3. Resistance to Bacterial Dieback Disease

Bacterial Dieback Disease (BDB) caused by *Erwinia mallotivora* had become another major disease of papaya. It was first recorded in Malaysia in 2003 and has since obliterated most of the papaya farms in the country. All commercial cultivars are susceptible and management practices by farmers have not been proven successful so far in managing this disease. Effort has been initiated recently in MARDI to develop BDB resistant papaya through biotechnological approach.

### 12.4. Deregulation and Commercialization of GM papayas

Efforts to move the transgenic papaya line 55-1 through the deregulation processes of the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) and the US Environmental Protection Agency (EPA), as well as the consultation process with US Food and Drug Administration (FDA), were started in late 1995 (Gonsalves, 2004). APHIS was concerned with the effects the produced coat protein could have on other plant viruses and the presence of small RNA fragments as a result of the RNA silencing mechanism. Based on the fact that the PRSV coat proteins are naturally present in all plants infected with PRSV, no new situation was being created, so the concerns were refuted (Bury, 2014). A third concern of APHIS was that the new trait could probably be transferred to wild papaya varieties, giving them a selective advantage and causing them to become invasive plants. As there are no wild papaya varieties in Hawaii and even in areas where there are no PRSV infections papaya is not considered invasive, green light was given by APHIS (Bury, 2014). As far as EPA is concerned, the coat protein is considered to be a pesticide due to the resistance to plant viruses that it provides. This meant that the coat protein would need to be subjected to the same risk analyses as a pesticide. However, the coat proteins are present in all PRSV-infected papayas and despite their spots, are still consumed without causing any health problems. Furthermore, the coat protein's concentration is much lower in the GM papaya than it is in infected non-GM papayas. There was no evidence suggesting that coat proteins from any plant virus could produce allergic reactions or have any other negative effects on human health (Bury, 2014). Therefore, approval was given by EPA. The FDA was interested in how much the GM papayas differed from non-GM papayas with regards to nutritional value (Bury, 2014). After finding that there is no difference in the nutritional value of GM compared with that of non-GM papaya, the approval from FDA was obtained in September, 1997. Finally, all licenses that were needed to commercialize the transgenic papaya were obtained by April 1998.

Between the two GM papayas i.e. 'SunUp' and 'Rainbow', farmers in Puna favoured the yellow-pulped 'Rainbow'. Thus 'Rainbow' became the most popular GM papaya cultivated in Hawaii. First harvesting of 'Rainbow' was in 1999 (Gonsalves, 2004). GM papaya was also approved for consumption in the U.S. In 2003, Canada gave the green light to the import of Hawaiian GM papayas for consumption. Canada, and Japan, which had historically been a major consumer of papaya from Hawaii, was slow in accepting the 'Rainbow'. However, after evaluation of all the food safety and environmental factors, approval for importation of GM papaya into Japan was granted in December 2011 (Gonsalves, 2004).

The introduction of the virus resistant papayas was a widespread success in Hawaii. In 2009, the GM papaya 'Rainbow' had a share of 76% of the total papaya harvested land. For the non-GMO papaya variety 'Kapoho' its share declined from 49% in 2002 to 9% in 2009. While GM papayas have solved the PRSV disease problem, pollen drift from GM papaya has lately becoming a concern in the papaya industry in Hawaii. Papaya pollen could disseminate through wind, insect, animal or human. Between 2004 and 2010, assays for the GUS ( $\beta$ -glucuronidase) transgene in embryos were done to

study transgenic-pollen drift in commercial non-GM 'Kapoho' plantings and in replicated field plots (Gonsalves *et al.*, 2012). Pollen drift averaged 1.3% of tested embryos in field plots where individual hermaphrodite 'Kapoho' trees were adjacent to two or four 'Rainbow' trees. In contrast, 67.4% of tested embryos were GUS positive in similarly located female 'Kapoho' trees. This showed that while the hermaphrodite papaya trees are self-pollinated before the stigma is exposed to pollen from the open, the female papaya trees can be open-pollinated. It is possible (especially in the female fruit) for the pulp to be non-GM but any number of seeds inside could be GM seeds. Farmers who produce non-GM papaya were advised to grow only hermaphrodite non-GM papaya plants and to bag flowers before anthesis to avoid contamination of seeds resulting from pollination from GM papaya plants.

GM papaya contamination would cause the loss of lucrative export of non-GM papaya and organic-papaya markets. It also would lead to expensive testing and roguing to the non-GM papaya growers. In March, 2014, America's first anti-GM farming law went into effect on Hawaii Island. This has banned the cultivation of other GM crops and limited cultivation of GM papaya in the Island.

Although GM papaya has been approved for consumption in the U.S., Canada, China and Japan, cultivation of GM papaya is only allowed in the U.S. and China. To-date, three GM events i.e. 55-1, 63-1 and x17-2 all modified with *cp* (coat protein) gene of PRSV were approved for cultivation in the U.S. Only one event, Huanong No.1 which was modified with the *replicase* gene of PRSV was approved for cultivation in China. Importing, marketing and consumption of GM papaya is approved in Canada and Japan both for one event, i.e. 55-1. Importing and marketing of GM papayas are not permitted in the European Union and other countries including those which had been involved in development of GM papaya and even conducted confined field tests on GM papaya.

## 13. MAJOR ENVIRONMENTAL CONCERNS OF GM PAPAYAS

### 13.1. Weediness

Weediness refers to the situation in which a cultivated plant becomes established as a weed in other fields or as an invasive species in other habitats. The term 'weed' here refers to an invasive plant that causes harm to the health of human and animals and the environment. Invasiveness refers to a high ability to spread (disperse, expand population) and persist (establish, survive and reproduce). As invasiveness may or may not cause harm, weed status of plant considers both invasiveness and adverse impacts (Conner *et al.*, 2003).

Australian weed scientists had developed two weed risk assessment approaches: (1) a pre-border screen for proposed novel plant introduction and (2) a post-border approach used to assess plants already present in the environment. The post-border weed risk assessment system (PBWRA) is based on scoring answers to a list of questions related to either harm or invasiveness of a plant (Standard Australia 2006, cited in Keese *et al.*, 2014). The PBWRA has been adopted by Food and Agriculture Organization of the United Nations and various Australian government departments, agencies and research bodies including the Office of Gene Technology Regulator (OGTR).

Based largely on the questions listed under PBWRA, the possibility of papaya becoming a weed can be assessed as below:

The attributes which indicate papaya could become a weed in the unmanaged natural environment are:

- Papaya is an introduced species and can naturalise where it is grown.
- Papaya plant can grow in tropical and sub-tropical regions under a wide range of climatic and soil conditions.
- The plant may persist after cultivation. In Malaysia, left over old plants or reshooting of plants that were cut about one foot (30cm) above ground were seen in previous planting sites and abandoned farm sites or old house sites.
- Papaya has the features of pioneer species i.e. fast growing, short life cycle, capable of produce a lot of viable seeds.
- Papaya seed has dormancy.
- Papaya plant flowers and bear fruit all year round.
- It is self- as well as cross- pollinated and does not require a specialist pollinator.
- Papaya fruits are consumed by a wide range of vertebrates including birds, bats, squirrels, rats etc. This could help in spreading the seeds.
- Genetic diversity within commercial cultivars exists and this provides a wide range of adaptability and allows cultivation under a relatively wide range of conditions.

However, there are features which do not favour the establishment of the plant as a serious weed. These are:

- Optimal growth of the plant is restricted by requirements for warm temperature, adequate moisture and good nutrition.
- The plant does not have any significant asexual reproduction mechanism. Vegetative propagation is not possible naturally.
- Trees yield well for two years, after which production declines.
- Due to the highly complex capability in sexual expression sex ratios for obtaining optimal fruit (and seed) set would be unlikely to occur without human intervention.
- Papaya plant is well controlled by herbicides.
- Papaya has limited taxonomic affinity to other weedy species. *Vasconcellea pubescens* (formerly *Carica pubescens*; 'mountain papaya'), a small shrub is the only relative of papaya that has been recorded as a weed (Randall, 2002). *Vasconcellea pubescens* is not found in Malaysia and no other species of Caricaceae occurs in Malaysia.

Papaya has naturalised in many tropical and sub-tropical countries (Randall, 2002). It has been variously described as an incidental escapee from cultivated sites, an opportunist, a pioneer species or sometimes even regarded as an invasive or potentially invasive species but on balance, it is not considered to be a significant weed in any region of the world (OECD, 2005). According to Groves *et al.*, (2003, cited in OGTR, 2008) papaya is naturalised and may be a minor problem but not considered important enough to warrant control at any location. In agricultural ecosystems, papaya is also not considered a problem. Furthermore, papayas are sensitive to most herbicides - it can be eliminated using herbicides like paraquat, glyphosate and triclopyr (OECD, 2005).

## 13.2. Gene Transfer

Gene transfer refers to the exchange of genetic material between different organisms. There are two types of gene transfer, the vertical and the horizontal gene transfers. They are different phenomena and have different implications for the safety of genetically modified crops.

### 13.2.1. Vertical gene transfer

Crossing of two plants sexually and passing their genes on to following generations is an example of vertical gene transfer. Gene transfer via pollen between plants of the same or related species takes place in the wild. For papaya, gene transfer could happen in nature when viable pollen from one papaya plant pollinates the receptive stigma of: (1) another cultivated papaya plant, (2) a naturalised papaya plant or (3) a cross-compatible wild relative of papaya for example, species of *Vasconcellea*.

#### 13.2.1.1. Intraspecific crossing

For the dioecious papaya plants, wind is the main agent of pollen dispersal. For the gynodioecious papaya plants, insects (including thrips and bees) are the most likely pollen vectors in Malaysia. Viable seeds and potentially fertile progeny would be produced when pollen is transferred between papaya plants, irrespective of whether the transfer occurred to cultivated or naturalised papayas.

Various organisations and agencies have specified isolation distances for growing papaya; these are cited in OECD (2005). The Hawaiian Identity Preservation Protocol for non-GM papaya seed production specifies a minimum of 400 m isolation from other varieties; the Papaya Biotechnology Network of Southeast Asia proposed that non-GM papaya should be separated by 400 m from GM papaya; USDA-APHIS approved an isolation distance of 500 m for GM papaya field trials. Manshardt (2002) reported that a series of experiments conducted in Puna, Hawaii in 1997 indicated that when commercial fields of hermaphrodite plants are separated by more than 400 m, cross-pollination will be a rare event (Manshardt *et al.*, 2007). It was noted, that the type of flowers that are present on a papaya plant would influence the level of cross-pollination that will occur. Female plants were found to have a higher frequency than the hermaphrodite plants. Further experiments conducted in Oahu, Hawaii in 2003 confirmed that transgene dispersal through pollen drift between hermaphrodite papayas is an inefficient process (Manshardt *et al.*, 2007).

If crossing were to take place between a GM and a non-GM papaya plant, the latter could acquire a novel gene. The safety assessment of GM papaya would include assessing both the possibility of out-crossing of the GM papaya to other non-GM papaya plants including its close relatives and evaluating the potential ecological consequences if it were to occur. It is necessary to assess if the transgene could confer a trait that could enable the plant to gain an unfair advantage in the wild.

#### 13.2.1.2. Interspecific and intergeneric crossing

Species in the genus *Vasconcellea* (formerly, *Carica*) are the closest relatives of papaya (*Carica papaya*) and are most likely to hybridize and exchange genes with *Carica papaya*. These species, however, do not occur in Malaysia, thus eliminating the likelihood of genes transferring naturally to wild papaya species.

Wild papayas (*Vasconcellea* spp.) possess a number of desirable traits including resistance to pathogens, cold tolerance and higher sugar content of fruit (Drew *et al.*, 1998). Breeders have sought

to introduce these traits into *Carica papaya* using traditional plant breeding techniques. Difficulties in producing hybrids between *Carica papaya* and *Vasconcellea* spp. underscore the negligible risk of gene transfer from *Carica papaya* to wild papayas in Australia. For instance, several investigations (Manshardt and Wenslaff, 1989a;1989b; Drew *et al.*, 1998) have indicated that following pollination of *Carica papaya* with pollen from wild papayas (and vice versa), pollen grains germinated on the stigma successfully and pollen tubes grew through the style and penetrated the ovules, thereby facilitating fertilisation. Subsequently, however, abortion of these ovules, or endosperm failure, prevented further development of hybrid embryos or production of viable mature seed. Using embryo rescue and micropropagation techniques, some intergeneric hybrids have been generated (Manshardt and Wenslaff, 1989a; 1989b). However, these F<sub>1</sub> plants were sterile and produced no F<sub>2</sub> progeny. Drew *et al.*, (1998) obtained limited fertile hybrids of *Carica papaya* and *Vasconcellea quercifolia*. Large numbers of F<sub>1</sub>s were obtained following embryo rescue. These F<sub>1</sub>s were backcrossed to *Carica papaya* and produced one male BC<sub>1</sub> that was fertile and tolerant to papaya ringspot virus. This work is ongoing.

However, the inability of papaya to hybridise naturally with its closest relatives and the infertility of such hybrids when they are formed artificially, illustrates the reproductive isolation of *Carica papaya* from other plant groups (Manshardt and Wenslaff, 1989a) and indicates that the likelihood of gene transfer between *Carica papaya* and other plant species in the wild is negligible.

### 13.2.2. Horizontal gene transfer

This refers to the transmission and/or absorption of genetic material not involved with sexual reproduction and independent of acknowledged species boundaries. Depending on a variety of conditions, horizontal gene transfer (e.g. from a plant to a soil bacterium) may be possible, but very seldom occurs under natural conditions. In the safety assessment of genetically modified plants, both the possibility and the probability of the foreign gene being transmitted by horizontal gene transfer have to be considered. For example, the antibiotic resistance genes used as marker genes might be transferred from a transgenic plant to soil or intestinal bacteria. Regulators need to ask if horizontal gene transfer is likely, and if it were to occur, what the consequences could be.

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Figure 2(a - f) Flower types of *Carica papaya* (reproduced from Chan, Y.K. (2008))

Figure 2a Type I Pistillate



Figure 2b Type II Pentandria



Figure 2c Type III Carpelloid



Figure 2d Type IV Elongata



Figure 2e Type IV+ Barren elongata



Figure 2f Type V Staminate





Figure 3. Eksotika, a small fruited Malaysian variety



Figure 4. 'Sekaki' a large fruited Malaysian variety



Figure 5. 'Green island' injury caused by scales



Figure 6. 'Flag-leaf' early symptom of Bacterial Dieback Disease



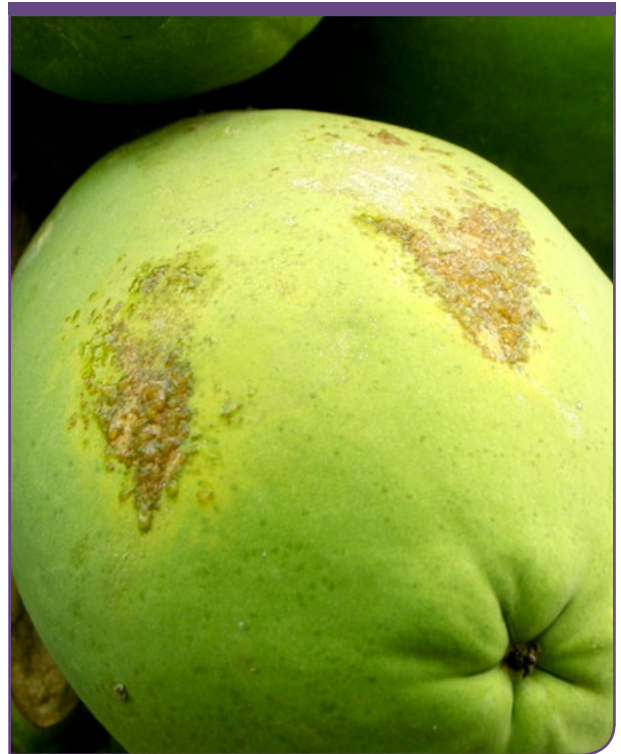
Figure 7. Rotting of terminal shoot in late symptom of Bacterial Dieback Disease



Figure 8. Papaya Ringspot Virus symptom on fruit



Figure 9. Anthracnose 'Brown Blotch' on maturing fruit














## Biology of Papaya (*Carica papaya* L.)

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