

Breeding the papaya—*Carica papaya*

The modern papaya is the result of plant breeding under cultivation. Chan Ying Kwok is well-known for the breeding and development of papaya in Malaysia. In November 2014 he gave a seminar in UTAR on the challenges facing the papaya industry, in particular, the devastating bacterial die-back disease caused by *Erwinia mallotivora*.

Francis Ng interviews Chan Ying Kwok

The first reference to papaya in Malaysia was by the Dutch traveller Linshoten who noted in 1610 that the ‘papaios’ then being grown in Malacca had been introduced recently from the Philippines. He wrote: “There is also a fruit that came out of the Spanish Indies, brought from beyond the Philippines or Luzon to Malacca, and from thence to India, and is very like a melon ... and will not grow but always two together, that is male and female ... and when they are divided and set apart one from the other, they yield no fruit at all” (from Popenoe, 1920). From Linshoten, we know that the papaya has been cultivated in Malacca (and Malaysia) from the early 1600s, for about 400 years.

The early Spanish explorers in tropical America found the papaya as a cultivated plant. Truly wild papayas were not detected until recently when wild populations were found in the Caribbean coastal lowlands of Southern Mexico and Northern Honduras (OECD). The wild plants are either male or female, and the female plants produce bitter-tasting fruits the size of golf balls, filled with small seeds.



Fruiting hermaphrodite tree of the Sekaki variety



Male tree



Male inflorescence

Female plants bear female flowers on short stalks and each flower contains a fully developed pistil¹ but no stamens². In contrast, male plants bear male flowers on long, branching, many-flowered inflorescences, each flower containing ten stamens but no pistil or a rudimentary non-functional pistil. The pollen is produced in sticky clumps and is carried to the female flowers by insects. However, it is difficult to spot the insects, and pollination may happen at night when the flowers are open and strongly fragrant. The fragrance of the flowers and the stickiness of the pollen both rule out pollination by wind.

**In cultivation,
hermaphrodite (bisexual)
trees have replaced males
and females**

Most papaya trees are nowadays hermaphrodite (bisexual), with flowers bearing a functional pistil together with five functional stamens. The anthers burst open and pollen is scattered on the stigma of the pistil even before the flower opens (cleistogamy), and fertile fruits are produced. Hence, in cultivation, papaya male and female forms are no longer needed for propagation.

¹ The pistil is the female reproductive organ, consisting of an ovary that contains ovules and a pollen-receiving structure called the stigma. After fertilisation the ovary grows into a fruit and the ovules become seeds.

² The stamen is the male reproductive organ, consisting of a pouch-like structure called the anther in which pollen grains develop. Pollen grains are transferred to the stigma in the process of pollination, to fertilize the ovules.



Fruits of Sekaki variety : L—female; R—hermaphrodite. "Sekaki" as a fruit name should only be applied to the hermaphrodite.

Hermaphrodite trees were not mentioned by Linshoten, who noted that the trees were separately male and female, and the female alone

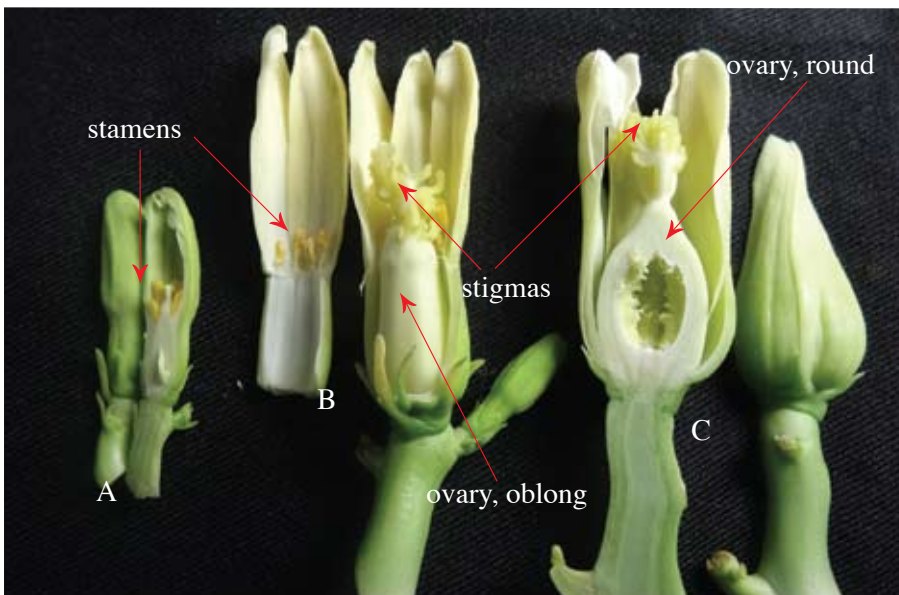
does not produce fruits. When, where and how did hermaphrodite trees come into existence?

In Indonesia, Ochse noted in 1931 that hermaphrodite flowers occurred among male flowers on male trees and produced small abnormal fruits but he did not mention hermaphrodite trees. Burkill in 1936 noted that the papaya trees in the Malay Peninsula were male and female, and remarked that hermaphrodite trees were rare and sterile.

Burkill's observation that hermaphrodites were sterile has never been confirmed. In Molesworth-Allen's excellent account of Malayan fruits in 1967, male and female plants were the rule and hermaphrodite flowers were mentioned in passing as intermediate between male and female. Probably she was referring to

hermaphrodite flowers that occur occasionally on male trees. She did not mention hermaphrodite trees. Hence it may be concluded that hermaphrodite trees were either absent or unnoticed in Malaysia up to 1967.

It was in Hawaii, at the Hawaii Experimental Station, that hermaphrodite plants were noticed and targeted by breeders. The breeders were working on the Solo variety that, according to Purseglove,



Dissected flowers of Sekaki : A - male flower from a hermaphrodite tree
B - hermaphrodite flower
C - female flower



Ungraded Sekaki fruits labelled as "Hong Kong"

had been introduced from Barbados. This work was mentioned by Popenoe in 1920. Popenoe observed that unisexual plants are difficult to improve by breeding because the males and females are separate and the males do not show the characters that are inherent in them and which will appear in the fruits of their progeny. With hermaphrodites, one can select an individual of known qualities and this may be used as the sole parent stock or may be combined with another parent of known qualities. Hence hermaphrodites would make better targets for breeding than males and females. The "Solo" name became the name of the hermaphrodite fruit developed by breeding in Hawaii.

To explain the range of non-Solo hermaphrodites now present in Malaysia and China, we think hermaphrodites must have been independently and informally noticed and propagated by local growers in Malaysia and China in the late 1960s or early 1970s. The hermaphrodite trees must have been derived from male trees because male trees are known to occasionally bear hermaphrodite flowers. In Brazil, only male and

female plants were known until the 1970s when hermaphrodite plants arrived with the introduction of the Solo variety from Hawaii and the Formosa variety from China.

For any given variety, the fruits from the hermaphrodite plant are different in shape and size from the fruits of the female plant. In the Solo variety, the hermaphrodite fruits are pear-shaped while female fruits are rounded, with five bulging

lobes. With selection efforts concentrated on the hermaphrodite for the reasons given by Popenoe, the pear-shaped hermaphrodite fruits of the Solo group, including Eksotika, have become quite uniform in shape and size and such uniformity facilitates handling and packing for export. The hermaphrodite Solo has become the export standard and the female fruit is discarded.

When Eksotika was first made available by MARDI in 1987, the local farmers refused to cull the female plants, so the fruits they produced were highly variable, with small rounded female fruits of various sizes, mostly undersized, mixed up with the larger and more uniform pear-shaped hermaphrodite fruits. It took over 20 years for growers and consumers to get used to papayas of uniform size and shape and farmers are now beginning to practice culling of female trees and to produce uniform hermaphrodite fruits.

The foot-long oblong-shaped Sekaki fruit is hermaphrodite and it is so popular in Hong Kong that it is now also known as the Hong Kong papaya although it was developed in Malaysia. The female fruit is shorter and rounder.



Eksotika packed for export market



Sekaki packed for the local market

- female x male → 1 female: 1 male;
- hermaphrodite x male → 1 hermaphrodite, 1 female and 1 male.

Since hermaphrodites reproduce by selfing and produce progeny in the ratio of 1 female: 2 hermaphrodites, selection and propagation centred on hermaphrodites would result in twice as many hermaphrodite plants as females, and males would not appear. In commercial plantations producing fruits for export, the female plants would be culled as soon as they are identifiable by their flowers, hence in such plantations, males and females would rarely occur.

Eksotika and Solo are stable pure lines. Seeds produced from the hermaphrodite fruits would be *bona-fide* since the hermaphrodite flowers are self-pollinated. The same applies to Sekaki. Seeds taken from fruits in an orchard that are totally hermaphrodite will be true to type unless stray pollen gets in from outside. To make sure, the flowers could be bagged to exclude stray pollen.

In 1968, Purseglove described the results of crossing between the three different sex forms and the resulting sex of the progenies:

- hermaphrodite x hermaphrodite (as in selfing of hermaphrodites) → 1 female: 2 hermaphrodites;

Eksotika 2 is quite different because it is a F1 hybrid produced by crossing two MARDI³ pure lines (Line 19 and Line 20). Seeds taken from the Eksotika 2 fruit will not propagate true to type, hence Eksotika 2 seeds have to be produced by MARDI by continual crossing of the parental lines.

³ MARDI: The Malaysian Agricultural Research and Development Institute, established in 1969.

Papaya seeds are abundantly produced. A medium-sized hermaphrodite fruit weighing 1kg may contain over 600 seeds. Female fruits bear fewer seeds—their pollen source is external and less assured. Each seed is enclosed in a gelatinous coat (sarcotesta). The seeds can be spread out to air-dry on an absorbent towel. The sarcotesta will dry up and will not inhibit germination as sometimes claimed. Dried seeds, stored under cool conditions, will remain viable in storage, reportedly for two to three years. Upon planting, the seeds germinate in about two weeks, to grow into single-stemmed trees, each bearing a rosette of large, deeply lobed leaves at the apex. The trees are planted about 3 m apart and begin to bear flowers in 3-4 months. Three seedlings may be planted per planting site to allow for selection of one hermaphrodite plant and removal of the others as soon as their sex can be identified by their flowers. The first fruits may be harvested in 7 to 9 months.

It is the duty of tropical horticulture to encourage the dissemination of the better forms and further to improve them by means of breeding.

Wilson Popenoe, 1920

Fruits are produced at the rate of one per leaf-axil. Under the constantly warm and moist conditions in Malaysia, a healthy young tree can produce 13 – 15 leaves (and fruits) per month every month of the year (Ng, 1979). A fruit is harvested as soon as a streak of yellow appears on the skin. After harvesting, the fruit ripens in about 5 days. Large fruited varieties like Sekaki have fruits as heavy as 3 kg compared to Solo at about 500 gm and Eksotika at about 600 gm. Huge fruits weighing over 10 kg have been recorded in some old varieties.

The length of time it takes for a fruit to develop

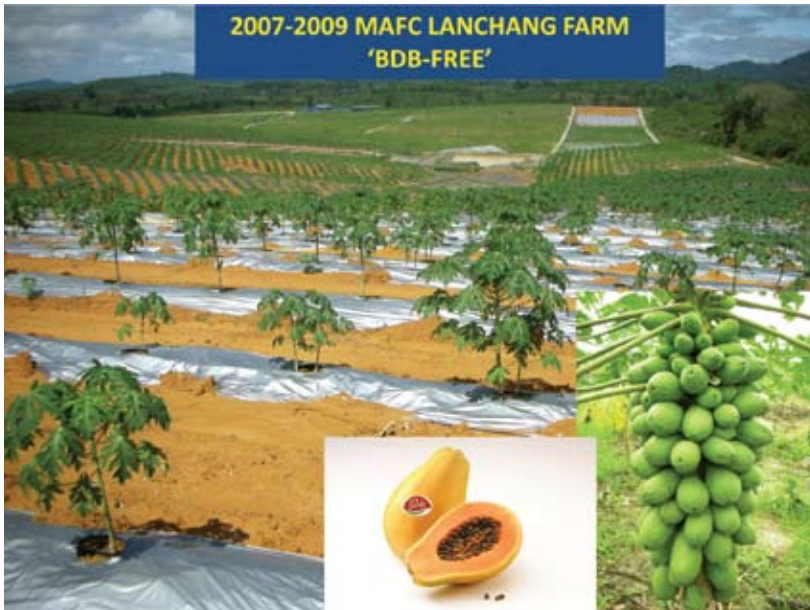
and mature is about 4-5 months for small-fruited varieties. Large-fruited varieties may take longer.

A tree may grow to 10 m tall but is usually cut down when it is too tall for the fruits to be harvested. Instead of replanting, the tree may be cut to about 30 cm (1 ft) above the ground and allowed to produce new shoots (coppice shoots), from which one shoot is selected to continue production. The ability of a tree to coppice declines with age and after one or two coppice cycles, the trees lose their vigour and have to be removed to make way for new plantings.

All parts of the tree exude white latex when cut. The latex is a source of the enzyme papain, which has many uses in the food, pharmaceutical and other industries. In Malaysia, fresh leaves, ground up with water to form a paste, or the sap

extract, have been fed to patients as a treatment for dengue-fever.

Before the establishment of MARDI in 1969, there was no official papaya breeding programme. Growers propagated papayas from seeds, and over time, different parts of the country became identified with different kinds of papayas. For example, those in the district of Sitiawan produced very large, long fruits while those in Subang produced medium-sized fruits. The variety known as Sekaki (meaning ‘one foot’) was a one-foot-long variety that was first grown in Coldstream Village near Bidor.



Malaysian AgriFood Corporation (MAFC) in Lanchang, Pahang

Bacterial die-back disease

Bacterial die-back disease has previously been reported in the Caribbean region, in Venezuela and elsewhere. In Malaysia, it was first detected in the southern state of Johor in 2003. In 2009, it was spotted in the 200 ha papaya farm of the Malaysian AgriFood Corporation (MAFC) in Lanchang, Pahang, which was then the biggest commercial producer of papaya in Malaysia. The disease eventually got out of control and decimated the plantation. The disease went on to devastate papaya plantations

1972–1975: MARDI picked ‘Subang 6’ to promote as fresh fruit and ‘Sitiawan’ for canning.

1972–1987: Chan crossed ‘Subang 6’ with ‘Sunrise Solo’ from Hawaii. The progeny was backcrossed to ‘Sunrise Solo’ and one of the results was the ‘MARDI Backcross Solo’ that was later released under the name of ‘Eksotika’.

1985–1991: Eksotika was found to be susceptible to fruit freckles caused by the combined action of the fungus *Cladosporium* and a small insect of the genus *Thrips*. However, one of backcross lines related to Eksotika, known as Line 19, was resistant. Eksotika was crossed with Line 19 and out of this cross came Eksotika 2 with improved fruit appearance, storage life, and yield. This was the first commercial F1 hybrid papaya in the world.

throughout Peninsular Malaysia .

The first noticeable symptom of bacterial dieback is when individual leaves begin to wither and decay. The weakened leaf blade then hangs limply on its stalk like a flag in still air. The infection spreads to other leaves and eventually the whole crown decays. The disease spreads by dispersal of the bacteria by wind or insects, entering the plants through wounds made by wind damage or by insects. The disease is not soil-borne because if a diseased plant is removed, the empty spot can be replanted with another papaya plant. It may be possible to control the spread of the disease by removal of infected plants or leaves, done carefully so that healthy surrounding plants are not damaged and infected in the process.



Subang (left) compared with the Sunrise Solo (right) in the backcross breeding programme



Papayas cleaned for export



Ekstotika in a supermarket



Malaysian papaya at Marks & Spencer



Bacterial dieback disease symptoms

Another possibility is to select plants for resistance to the disease, 19 different accessions⁴ have been screened and one accession has been found to have remarkable resistance. Further research and development of resistance may enable the industry to recover its former glory. However there is a possibility, as with all severe contagious diseases, for the disease to die out by itself after it runs out of susceptible hosts.

⁴ An accession is a plant-type that is assumed to be genetically different from others because it is collected from a distinct location where it would have been subjected to local selection pressures; a germplasm collection or 'genebank' is a collection of many different accessions maintained for the different genes they contain.

What are F1 and F2?

A breeding programme begins with two plants selected to be parents, one as the pollen provider (male) and the other to receive the pollen and bear the fruit (female). The aim is to bring together desirable features from the two parents.

The parental plants used to start a breeding programme are plants with characteristics that are relatively fixed and known to the breeder. Such plants are developed by inbreeding of selected plants for several generations to create 'pure lines' with known characteristics. Using pure lines as parents would give greater control and precision to breeding. In papaya, Chan crossed his Lines 19 and 20 to produce Eksotika 2. Only by crossing Lines 19 with 20 can one produce Eksotika 2.

The offspring from a cross are called the F1 progeny. 'F' alludes to the Latin word *filius* for 'son'. F1 plants tend to display a blend of the characteristics of the two parents.

When F1 plants are inbred or selfed, the next generation is called the F2 generation. In the F2 generation, the genes responsible for the characteristics would segregate into different combinations. If the F1 generation does not produce what the breeder wants, the F2 generation will provide more opportunities for selection. Many breeders prefer to concentrate on the F1 because a desirable F1 provides good commercial control— whoever controls the parental lines controls the production of the desired F1.

Questions

When you began crossing Solo with Subang, what combination of characters were you trying to obtain? What was the reason for backcrossing with Solo? Is Subang still being grown?

The main aim was to get the eating qualities of Solo with the local adaptability of Subang, hence after the first cross, the progeny was backcrossed to Solo to build up the Solo genetic background with the benefits of Subang vigour and adaptation. Subang is now gone since the village lost its agriculture due to development.

The first new breeds produced at MARDI were Eksotika and Eksotika 2. In your seminar you referred to 'Glimmer' as a new variety resistant to bacterial die-back and also to 'Frangi'



Chan Ying Kwok

developed in MAFC and marketed as 'Paiola'. Paiola became a big hit in the European market. What is origin of Frangi/Paiola?

Frangi is a F1 hybrid developed in 2006 resulting

from a cross between the LSGC1 and LSGC2 pure line parents. They are all proprietary of the MAFC.

Some papaya names have been registered under the Malaysian Plant Variety Protection Act. What are the implications of registering a name?

Registering a variety under MPVP Act will give the breeder/ proprietor the rights over the variety. Other growers of a registered variety may have to pay royalty or face legal actions.

You have mentioned that 19 accessions of papaya were tested for resistance to bacterial die-back disease. These accessions would have come from a germplasm bank. How are these banks maintained, bearing in mind that papaya trees have a short life span and would require constant renewal by replanting from seeds; also gene banks can be affected by disease.

The 19 accessions were kept at MAFC and MARDI. MARDI has a large germplasm collection, about 75 accessions. In the old days, accessions were obtained quite easily through exchange with other research institutions, but now everyone is quite reluctant to share because of intellectual property issues. Depending on their breeding systems, accessions are kept pure through controlled pollination.

The variation in papayas in the market has narrowed almost completely to Eksotika and Sekaki. It has been argued that the best security for germplasm is to encourage farmers to develop different varieties as in the past instead of all growing the same few varieties. What is your view on this?

Fully agree that diversity is the best defence against adversity. But this is more easily said

than done as the established commercial varieties translate to revenue and diverse varieties have too much variation for effective marketing and sales.

Are Sabah and Sarawak free of bacterial dieback disease? Who identified the disease and its mechanism of infection? How could the disease have reached Malaysia from overseas, considering that it has to travel within a diseased plant?

I've seen the disease in Tenom, Sabah some 15 years ago, but Sarawak has not reported the disease, but that does not mean it is not there. Maktar *et al.* at UTM identified it as *Erwinia papayae* in 2008 and Noriha *et al.* from Biotech MARDI in 2011 established it as *Erwinia mallotivora*. It was suspected that it came at the time when there was active import of dragon fruit cuttings and dragon fruit shares a similar *Erwinia* dieback.

As a plant breeder you have had to breed for fruit quality, resistance to disease, productivity and so on. So how should a plant-breeder be trained? How did you get started as a plant breeder?

In my agricultural degree training days at University of Malaya back in late 60's, we could stream in the final year, and plant breeding was the option I picked, although it wasn't my first choice. Poultry breeding was, but I was too allergic to the feed dust and had to abandon that for a project on tomato breeding. There were good teachers—Prof. Keith Graham and Dr. Yap Thoo Chai were inspirational. The basic breeding principles learnt from the basic degree was enough to get started for the research at MARDI—there were also good mentors, Dr.

Hamilton from U. Hawaii deserves special mention and also Dr. Ooi Swee Chai. The basic training was one thing—the rest comes from the day-to-day research, reading and writing. And most importantly, breeding and selection can be more an art than science. Selecting Eksotika is a case in point. No breeder would have picked this miserable tree for the next cycle of selection if based on the data sheet. Somehow, the vibes when one passed it said that it deserved a second chance, and it did not disappoint.

Many graduates now begin with a degree in biotechnology. How do they fit into agriculture and into plant breeding?

Biotechnology is a great tool in agriculture and breeding, going into the molecular scale of things. However, a biotechnologist is more competent if he or she is trained as well in the basic principles of agriculture and breeding. At

least one knows how to grow those plants that come out of the lab rather than depending on the agriculturists as is happening now.

The papaya has changed a lot, from the small bitter fruits of the wild form to the sweet large fruits of the current varieties, and from female fruits to hermaphrodite fruits. In what way could it be further improved?

The papayas in this country have undergone a step-wise improvement programme since the 70's beginning with taste, yield and uniformity improvement, followed by resistance breeding. For now and the future, considerations should be given to breeding for industrial and pharmaceutical uses of papaya—papain, anti-oxidants, vitamins etc. At the summit in the step-wise programme is the improvement of designer traits like convenience in serving the papaya, perhaps a peeling papaya?

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