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Underutilised Crop Genomes

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The Mangosteen Genome

7

Mohd Razik Midin and Hoe-Han Goh

Abstract

Mangosteen is one of the most popular tropical fruits in Southeast Asia. It is called 'The Queen of Tropical Fruits' as its thick sepals collectively resemble a crown. Mangosteen fruits contain white and juicy edible pulp with a sweet flavour and pleasant aroma. They are rich in beneficial phytochemicals such as xanthones, which make mangosteen a potential medicinal plant. Traditionally, mangosteen has been used to treat fever, diarrhoea, and wounds. In recent studies, researchers found that mangosteen has anti-cancer and anti-diabetic properties. However, mangosteen is still an underutilised crop due to its slow growth rate with a long juvenile period that usually takes eight to ten years to bear fruit. It is also an obligative apomict with asexual reproduction, hence producing clones of progenies with low genetic variations. Therefore, the breeding programme of mangosteen is challenging with a very low success rate. Furthermore, genetic information on mangosteen accessions in different countries is limited to unravel its lineage and parental history. Other constraints in mangosteen improvement include low viability of recalcitrant seeds and the lack of a rapid propagation method. Efforts have been made to understand this crop through functional genomic studies. Recent genomic studies of mangosteen, including genome sequencing, genome survey, genome size estimation, and cytogenetic analysis, are highlighted in this chapter.

7.1 The Genus *Garcinia* L.

The genus *Garcinia* L. belongs to the family Guttiferae (Clusiaceae) and was characterised by the Swiss botanist, Linnaeus. Linnaeus named the genus after the French naturalist Laurent Garcin (1683–1757) in honour of his botanical contributions in the eighteenth century, and it was Garcin who provided the detailed description of *Garcinia* fruits (Corner 1952). It is a pantropical genus with 400 species distributed in the Southeast Asian region (Maheshwari 1964; Whitmore 1973). Out of 400 *Garcinia* species estimated worldwide, 49 species were discovered in Peninsular Malaysia (Whitmore 1973; Nazre et al. 2007).

Garcinia species comprises small- to medium-sized dioecious trees or erect shrubs with hard timber and abundant gummy latex

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(Ridley 1967; Whitmore 1973; Richards 1990a). Morphologically, male flowers have many stamens (with or without a pistillode) and female flowers have a large hypogynous ovary with a sessile, plate-like stigma (with or without staminodes) inserted on a variously shaped receptacle (Richards 1990a; Osman and Milan 2006). According to Richards (1990b), most *Garcinia* species are thought to be facultative agamosperms, except possibly *G. scortechinii* King and *G. mangostana* L.

Garcinia is an economically important genus in Southeast Asia. Most of the species produce edible fruit (Yapwatannapun et al. 2002). Their fruits can be used as flavourings, for instance, *G. atroviridis* Griff. *ex* T. Anderson, *G. gummi-gutta* (L.) Roxb. (syn. *G. cambogia*) (Gaertn.) Dessr., and *G. planchonii* Pierre are used as ingredients in dishes in Peninsular Malaysia, India, and Indochina (Cox 1976). Numerous species of *Garcinia* are also used for purposes other than the edible fruits. Several species are important in domestic uses such as landscaping and the furniture industry (Osman and Milan 2006; Nazre et al. 2007). *Garcinia* species also contain phytochemicals that have pharmaceutical and therapeutic value (Hemshekhar et al. 2011). Several *Garcinia* species have antioxidant, anti-tumour, and antiviral properties with potential for the treatment of cancer and HIV (Ampofo and Waterman 1986; Rukachaisirikul et al. 2003; Nabandith et al. 2004; Pedraza-Chaverri et al. 2008; Aizat et al. 2019).

According to Lim (2012), *Garcinia* species originate from the Malay Archipelago and several are considered important Asian species of *Garcinia* including *G. dulcis* Griffith ex. T. Anderson, *G. tinctoria*, *G. atroviridis* (asam gelugor), *G. celebica* syn. *G. hombroniana* (seashore mangosteen), *G. indica* Choisy (kokum), *G. prainiana*, and *G. mangostana* (mangosteen) (Osman and Milan 2006). The most famous *Garcinia* species is *G. mangostana* as it possesses white and juicy edible pulp with a sweet flavour and pleasant aroma (Jung et al. 2006).

7.2 Botanical Description of Mangosteen

Mangosteen is a slow-growing tree that is believed to be native in Malaysia and known as a cultivated species (Verheij 1991). It was recognised and grouped by Linnaeus (1753) in the family Guttiferae (Clusiaceae). Locally, it is called as 'manggis'. It is a desirable species and sometimes referred to as 'The Queen of Tropical Fruits'.

The following description relies on information provided by Corner (1952), Richards (1990b), and Verheij (1991). Mangosteen is a medium height evergreen tree with a straight trunk. All parts of the plant contain yellow latex. Its leaves are shining and coriaceous, dark green, rarely yellow-green, dull pale green, or yellow-green beneath. Its flowers are red in colour and solitary, paired, or rarely 3 at apices of branchlets. The ovary is broadly ellipsoid to globose, sessile, and 4–8 celled. Stigma is sessile and smooth. The fruit is a depressed-globose-shaped berry with thick pericarp, dark purple colour with fleshy sweet arils (Richards 1990b; Osman and Milan 2006). Figure 7.1 shows the tree, leaves, and fruits of two varieties of mangosteen in Malaysia, the common mangosteen 'manggis' and mesta.

7.2.1 Mangosteen as an Apomictic Species

Mangosteen reproduces through apomixis. Apomixis is defined as asexual plant reproduction via seeds from the maternal tissues of the ovule that results in the production of genetically uniform progeny. The term apomixis is synonymous with the term agamospermous (Richards 1997). It can be divided into two main types based on the way of apomictic embryo development: (1) sporophytic apomixis, also known as adventitious embryony and (2) gametophytic apomixis (Koltunow and Grossniklaus 2003; Bicknell and Koltunow 2004; Brukhin 2017; Šarhanová et al. 2017). To date, 250 species from 57 families of

Fig. 7.1 Commercial mangosteen in Malaysia of the **a** common variety and **b** Mesta variety located in Pahang, Malaysia (i) Tree (ii), immature, and (iii) ripe fruits. Photos are taken by Mohd Razik Midin.

flowering plants have been categorised as sporophytic apomicts including several economical plant species (Naumova 1992; Naumova et al. 2001; Brukhin 2017).

Apomixis has been discovered previously in paleotropical *Garcinia* species as reported by several researchers (Sprecher 1919; Gustafsson 1946; Horn I940; Grant 1971). According to Richards (1990b), most *Garcinia* species are facultatively apomictic. In Malaysia, he found that at least ten *Garcinia* species are facultative apomicts except for two species, *G. scortechinii* and mangosteen whereby males are absent (Richards 1990a). The current opinion suggests that mangosteen is exclusively female as it exhibited obligate apomictic and adventitious embryony in which embryo produced via sporophytic mechanism from ovular tissues (Lim 1984; Richards 1990a, b). However, several researchers previously reported the presence of male trees of mangosteen such as Burkill (1935) and Idris and Rukayah (1987). In Peninsular Malaysia (and Southeast Asia in general), the rarity of males in mangosteen may be due to the local planters' activity in chopping them down as they bear no fruit (Nazre 2014). The only male

tree found by Idris and Rukayah (1987) at Ulu Kundor Village of Linggi District in Negeri Sembilan had been chopped down by villagers because they believe it was not worth keeping.

7.2.2 Genetic Variation of Mangosteen

Although mangosteen has been considered as an obligate apomictic species (Richards 1990b), genetic variation has been reported among its cultivars in molecular studies (Ramage et al. 2004; Mansyah et al. 2010; Sobir et al. 2011; Matra et al. 2016). Sobir et al. (2011) studied the genetic variability of mangosteen in Indonesia through field evaluation and molecular analysis (using RAPD, AFLP, and ISSR markers) to select superior trees with desirable traits. Mansyah et al. (1999) reported that mangosteen trees in West Sumatra exhibit wide variability in leaf length, fruit weight, and rind thickness. Mansyah et al. (2010) also found that mangosteen in Tembilahan, Sumatera Island, shows a flattened fruit shape, very short peduncles, and an elliptic stigma lobe. Several mangosteen varieties were released by Bogor Agricultural University, Indonesia, for cultivation including Wanayasa, Puspahiang, and Malinau. Julu, a variety produced in the Philippines, possessed larger fruits than other varieties with large seeds and more acidic pulp (Horn 1940). Cytologically, mangosteen is considered a polyploid species with apparently non-uniform chromosome number ($2n$ = 76, 96, 88–90, 110–120) as reported by Krishnawary and Raman (1949), Tixier (1960), Ha (1978), Richards (1990b), Sarasmiryati (2008), and Midin et al. (2018). If chromosome count data are correct, the chromosome instability may contribute to morphological variation observed between groups (Ramage et al. 2004; Nazre 2014). Instead of chromosome instability, environmental differences and geographic adaptation may cause morphological variation.

In 1991–1993, the Malaysian Agricultural Research and Development Institute (MARDI) started a project to collect and study the genetic diversity of mangosteen germplasm in Malaysia (Osman and Milan 2006). In this study, MARDI found that there are some distinct variations in fruit characteristics such as fruit size and shape. The Malaysian Department of Agriculture has also identified several accessions that show variations in fruit size and shape, seed number, shelf-life, fruiting precocity, and external coloration. Recently, two types of mangosteen are being cultivated in Malaysia, one with normal globose fruits (common mangosteen) and another with ovoid-shape fruits known as Mesta (Osman and Milan 2006; Raziah et al. 2007). Mesta (Fig. 7.2b) is the more popular commercial Malaysian seedless variety with smaller fruit size and less juice than the common mangosteen. The pulp texture is also more solid than the common variety.

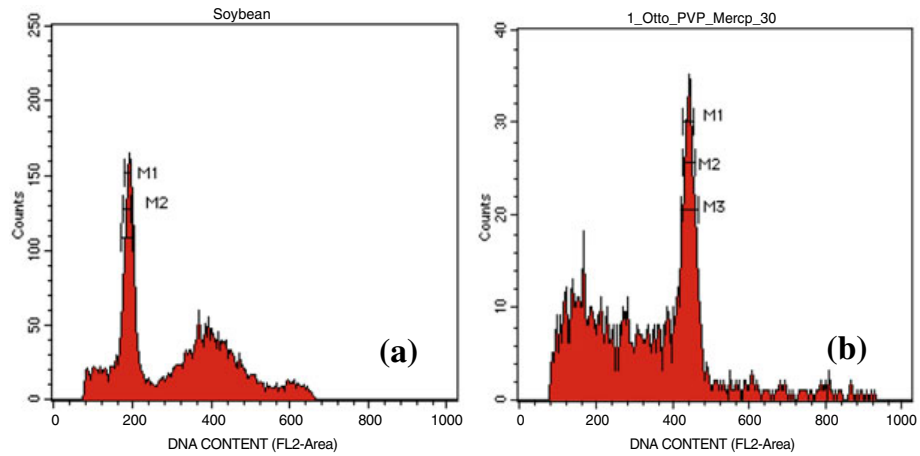

Fig. 7.2 Fluorescent intensity histogram peaks **a** *Glycine max* cv. Polanka and **b** mangosteen. (Reproduced from Midin et al. 2018 with permission)

7.3 Origin and Distribution of Mangosteen

7.3.1 Origin of Mangosteen

Up to now, the origin of mangosteen is still debatable. Mangosteen is thought to be closely related to two *Garcinia* species which are *G. celebica* (syn. *G. hombroniana*) and *G. malaccensis*. These two species are indigenous to Malaysia although the distribution of *G. celebica* extends to Nicobar Island. Richards (1990b) was the first who proposed that mangosteen is a hybrid between two species, *G. celebica* (syn. *G. hombroniana*) and *G. malaccensis.* However, as pointed by Nazre (2014), *G. malaccensis* from Pasoh Forest Reserve studied by Richards (1990b) was a misidentified *G. penangiana* Pierre. The misidentified *G. penangiana* was also used by Abdullah et al. (2012) in their phylogenetic analysis. They claimed that *G. opaca* and *G. penangiana* are the closest relatives to mangosteen. Due to this misidentification, Nazre (2014) conducted morphological analysis on the three *Garcinia* species including *G. penangiana*, *G. malaccensis,* and *G. mangostana* as listed in Table 7.1. Morphological characters of *G. celebica* as described by Richards (1990b) are also listed.

Other phylogenetic studies were also conducted by Yapwattanaphun et al. (2004), Nazre et al. (2007), Abdullah et al. (2012), Sulassih et al. (2013), and Nazre (2014). Yapwattanaphun et al. (2004) found that *G. celebica* was more distant from mangosteen as compared to *G. malaccensis* in their phylogenetic study. Nazre et al. (2007) reported that mangosteen was most closely related to *G. penangiana* among the cultivated *Garcinia* of Peninsular Malaysia based on ITS (internal transcribed spacer) sequences, but later Nazre (2014) found that mangosteen was clustered together with *G. malaccensis* rather than *G. penangiana*. Based on morphology and ISSR markers, Sulassih et al. (2013) claimed that the possible ancestors of mangosteen were *G. malaccensis* and *G. celebica*. In the most recent study based on morphology and ITS sequences, Nazre (2014) proposed two theories on the origin of mangosteen. First, mangosteen is a hybrid of different varieties of *G. malaccensis*, and second, that it may be a product of multiple, superior selections from different populations of female trees of *G. malaccensis*. Table 7.2 summarises the hypotheses on the origin of mangosteen and reveals that four *Garcinia* species including *G. celebica, G. malaccensis*, *G. opaca*, and *G. penangiana* are closely related to mangosteen.

7.3.2 Closely Related Species of Mangosteen

Since the origin of mangosteen remains uncertain, many studies related to close species of *Garcinia* provide hints on the parent species of mangosteen. Four candidate species are described below based on the consensus from the literature which showed the closest relationship with *G. mangostana*.

Table 7.1 Morphological characters of *G. celebica, G. malaccensis, G. mangostana*, and *G. penangiana*

Character	*G. celebica*	*G. malaccensis*	*G. mangostana*	*G. penangiana*
Latex	White	Yellow	Yellow	White
Petal colour	Cream	Pinkish red	Pinkish red	No information
Stigma surface	Smooth	Corrugated	Rather smooth surface	Nodule-like surface
Fruit shape	Globose	Ovoid, ellipsoid, globose	Ovoid, ellipsoid, globose	Ovoid, globose
Fruit colour (ripe)	Red	Yellowish red. reddish pink, purple-black	Purple-black	Reddish pink
Fruit flavour	Astringent	Sweet–sour	Sweet–sour	Sour

Table 7.2 List of hypotheses on the origin of mangosteen

No	Hypothesis	References
1	Mangosteen might be a hybrid between *G. malaccensis* (misidentified *G. penangiana*) and *G. celebica*	Richards (1990b)
2	Mangosteen more closely related to *G. malaccensis* than to *G. celebica*	Yapwattanaphun et al. (2004)
3	Mangosteen was closely related to *G. penangiana* than to *G. celebica*	Nazre et al. (2007)
4	The closest relative to mangosteen is *G. opaca* and *G. malaccensis* (misidentified *G. penangiana*)	Abdullah et al. (2012)
5	The possible ancestors of mangosteen are *G. malaccensis* and *G. celebica*	Sulassih et al. (2013)
6	a. Mangosteen is a hybrid of different varieties of *G. malaccensis* b. Mangosteen may be a product of multiple, superior selections from different populations of female trees of *G. malaccensis*	Nazre (2014)

7.3.2.1 *G. celebica* L. (Syn. *G. hombroniana* Pierre)

The scientific name of seashore mangosteen as *G. hombroniana* was first established by Pierre (Nazre 2010). Seashore mangosteen has been recorded in Southeast Asia (between Singapore and Malacca in Peninsular Malaysia), Nicobar Island, South Thailand, and Peninsular Malaysia (Maheshwari 1964; Whitmore 1973; Richards 1990b; John et al. 2008; Nazre 2010). *G. celebica* is known as 'Beruas', 'Manggis hutan', and 'Minjok' in Malaysia (Osman & Milan 2006; Nazre 2010). In India, it is called as 'Puli mangosteen' while in Thailand, it is called 'Waaa' (Osman and Milan 2006; John et al. 2008).

Taxonomically, Pierre grouped *G. hombroniana* with two Linnaeus species, *G. cornea* and *G. celebica* in the same section based on their morphological characters (male flowers and fruits) and geographical distribution (Nazre 2010). However, morphological evidence based on the literature and herbarium specimen suggested that *G. hombroniana* in Thailand, Peninsular Malaysia, and Borneo, and *G. cornea* and *G. celebica* in Indonesia actually refer to the same species (Nazre 2010). Due to this, the valid taxonomic name of seashore mangosteen that should be used is *G. celebica* as it was published earlier by Linnaeus (1754) than *G. cornea* (1772) and *G. hombroniana* (1882–1885) (Nazre 2010).

Garcinia celebica is planted for its timber that can be used for fencing and its fruit for flavouring in local dishes (Richards 1990b; Jamila et al. 2017). For traditional medicine, its roots and leaves have been used to treat skin infections and women after childbirth (Burkill 1935). Previous phytochemical investigations on *G. celebica* found that its extract such as xanthones, flavonoids, and triterpenes (garchombronanes) exhibits anticholinesterase, lipoprotein antioxidant, and antiplatelet (Jamila et al. 2015, 2017). This species can be used as a rootstock for the improvement of slow-growing *Garcinia* species (Yacob and Tindall 1995; Hammer 2001; John et al. 2008).

7.3.2.2 *G. malaccensis* Hook. F.

Garcinia malaccensis is a wild species in Peninsular Malaysia, Sumatera, and Brunei. Locally, it is known as 'Manggis burung' (Lim 2012). It is confined to the lowland forest in Peninsular Malaysia. As is the case for *G. celebica*, *G. malaccensis* is dioecious and a facultative agamosperm (Richards 1990a). It is considered one of the closest related species to mangosteen. Morphologically, it seems to have been confused with mangosteen (Richards 1990b; Yapwattanaphun et al. 2004; Abdullah et al. 2012; Nazre 2014). It also produces fruits as delicious as mangosteen that tastes acidic sweet. However, no cultivated tree could be found today. A study conducted by Jabit et al. (2009) revealed that the extracts from its leaves exhibited moderate activity and selectivity towards non-small-cell lung cancer cells, whereas its stem extracts exhibited inhibition against nitric oxide production. However, its

timber has no commercial value as it splits after drying (Lim 2012).

7.3.2.3 *G. penangiana* Pierre

Garcinia penangiana is a widely distributed species in Peninsular Malaysia (Nazre et al. 2007). Locally, it is known as 'Kandis Burung'. Morphologically, its bark is dark brown colour (Nazre 2014). Its leaves are bright reddish colour when dry. The intramarginal vein is absent on the leaves of *G. penangiana* which produce whitish latex. Its fruit shape is ovoid or globose with fewer stamen bundles that are widely spaced and display a nodule-like surface. Its colour is reddish pink and tastes sour. Traditionally, it is used to treat skin diseases and fever (Jabit et al. 2009). The previous study conducted by Jabit et al. (2009) revealed that its methanol extract is potent with selective cytotoxic activity against breast cancer. Its xanthone compounds, such as penangianaxanthone, cudratricusxanthone, macluraxanthone, and gerontoxanthone, showed strong cytotoxic activity towards tumour cell lines (Jabit et al. 2007).

7.3.2.4 *G. opaca* King

Morphologically, *G. opaca* is a small tree or shrub. It is a widely distributed lowland and hill forest species and endemic to Peninsular Malaysia (Kochummen 1997). *G. opaca* is facultatively apomictic which can propagate via apomixis and also through sexual reproduction (Abdullah et al. 2012). The fruit of the former is flask-shaped with a thin wall, while the latter is globose-shaped with a thick wall. Its fruits are eaten by local people, and decoction of leaves is used to improve blood circulation (Jabit et al. 2009). The bark of *G. opaca* was identified to possess cytotoxic activity (Mori et al. 2014). The compounds extracted from its bark such as terpenoid and opaciniol showed moderate cytotoxicity against tumour cell lines (Jabit et al. 2009; Mori et al. 2014). Its xanthone compounds strongly inhibit platelet-activating factor receptor binding (Jantan et al. 2001).

7.3.3 Geographic Distribution of Mangosteen

Mangosteen is naturally distributed as well as cultivated throughout Southeast Asia. Recently, it has also been introduced and cultivated in other countries including Australia, Cuba, Dominica, Ecuador, Gabon, Ghana, Guatemala, Honduras, India, Jamaica, Liberia, Myanmar, Philippines, Puerto Rico, Singapore, Sri Lanka, Tanzania, and Vietnam (Cruz 2001; Murthy et al. 2018). Four countries including Malaysia, Indonesia, Philippines, and Thailand are considered as the major producers of mangosteen (Osman and Milan 2006; Rozhan et al. 2011). About 85% of the total production of these four countries is produced by Thailand. The producing countries of mangosteen show a variety of climates. Regarding this, the production seasons in these countries demonstrate some distinct differences. Generally, Malaysia, Indonesia, Philippines, Thailand, and Vietnam have similar production seasons with fruits available from May to January. However, their production seasons contrast to those of Australia, which has its production seasons from November to April.

7.3.4 Mangosteen Export from Malaysia

Previously, the cultivation of mangosteen has never been targeted for commercial purposes. In the Malaysian Third National Agriculture Policy (1998–2010), mangosteen was identified as a flagship of the Malaysian fruits for export. Due to its potential, mangosteen has been imported by several countries including China, Singapore, and Thailand. In 2017, 1254 tonnes of mangosteen was exported from Malaysia (Department of Agriculture 2018). Based on the market potential and world demand, the Malaysian Ministry of Agriculture (MOA) implemented a mangosteen planting programme (Rozhan et al. 2011).

7.4 Conservation of Mangosteen Germplasm

Various approaches of conservation including *in situ* and *ex situ* programmes have been implemented to conserve genetic resources of mangosteen (Murthy et al. 2018). Large-scale collection and conservation of mangosteen from Southeast Asian countries such as Myanmar, Laos, Thailand, Cambodia, Vietnam, Malaysia, Brunei, Singapore, Philippines, Indonesia, and Papua New Guinea have been conducted by International Plant Genetic Resources Institute (IPGRI) (Coronel 1995). As mangosteen produces recalcitrant seeds, *in situ* conservation methods are prominent. Mangosteen seeds are high in moisture content, possess no dormancy, exhibit low seed viability, and short lived, without a differentiated embryo, endosperm, or embryonic axis (Normah et al. 1992; Malik et al. 2005). Due to this, seed storage is hampered (Murthy et al. 2018). For ex situ conservation, in vitro conservation is preferred to maintain the explants such as shoots, meristems, embryo, or plantlet in a sterile environment. This condition will assist in the production of recalcitrant seeds. Research institutes involved in germplasm collection and research on mangosteen in Southeast Asia countries include the Forest Research Institute of Malaysia (Malaysia), National Biological Institute, Bogor Agricultural University (Indonesia), Horticultural Research Station, Chanthaburi (Thailand), and Institute of Plant Breeding, UPLB College of Agriculture and Food Sciences, University of the Philippines Los Baños (Philippines) (Murthy et al. 2018).

7.5 Why Mangosteen is Underutilised?

Mangosteen has a long juvenile period which usually takes 8–10 years to bear fruit (Horn 1940). The slow growth is caused by poor root system (no root hairs, poor branching, easily broken, and disturbed by adverse environments resulting in very small contact surfaces between roots and soil), poor nutrient and water uptake, low photosynthetic rate, low cell division rate in the apical meristem, and long shoot dormancy period (Cox 1976; Wieble et al. 1992; Ramlan et al. 1992; Poerwanto et al. 1995; Poerwanto 2002). To address its slow growth, grafting technique has been employed (Fairchild 1915; Galang 1955; Ochse et al. 1961; Poerwanto 2002). As well as slow growth rate, the strict climatic requirements, short viability of seeds, lack of rapid propagation methods, delayed precocity of trees, limited research manpower, and budget in producer countries can be other constraints for mangosteen improvement and thus make it underutilised (Osman and Milan 2006; Murthy et al. 2018). Further, the breeding programme of mangosteen is very slow and potentially has a very low success rate as it has a long juvenile period. Nonetheless, a breeding programme is necessary to improve the development and plant growth of mangosteen by shortening its juvenile period as well as increasing the yield and fruit quality. The information of mangosteen accessions in different countries is still limited to unravel the genetic background of species (Osman and Milan 2006). Genetic diversity information of mangosteen will provide clues of hybridisation and occurrence of important mutations.

7.6 Benefits of Mangosteen

Mangosteen has considerable economic potential in several Southeast Asia countries for the local and export markets. Its designation as 'The queen of tropical fruit' is because of its fruits with thick sepals that collectively resemble a crown aside its popularity due to the white and juicy edible pulp with a sweet flavour and pleasant aroma (Jung et al. 2006). Various components of mangosteen including stem, rind, leaves, and fruits have been used for many purposes. For instance, its rind contains tannins that can be utilised to tan leather and to dye fabric black (MacMillan 1956; Coronel 1983; Nakasone and Paull 1998).

Besides, mangosteen trees provide timber for making furniture and are used in carpentry (Nakasone and Paull 1998; Yapwattanaphun

et al. 2002). Its fruits are also commercialised as a functional food or drink, with the addition of other minor components such as vitamins, which exhibits general health boost and even promoted as an anti-diabetic supplement (Udani et al. 2009; Xie et al. 2015). Mangosteen has been also used in traditional medicine. Different parts of mangosteen such as fruit hulls, barks, and roots have been utilised for hundreds of years in Southeast Asia as traditional medicine. Its rind has been used to cure diarrhoea, dysentery, skin infection, and respiratory disorder (Burkill 1966; Yaacob and Tindall 1995; Ohizumi, 1999).

Further, its leaves and roots are used for the cure of wounds and medicine for menstruation (Burkill 1966). Phytochemical studies conducted on mangosteen found that its extracts, such as xanthones, have antioxidant, anti-tumour, anti-allergic, anti-inflammatory, anti-bacterial, anti-diabetic, and anti-viral activities (Mahabusarakam et al. 1983; Yapwattanaphun et al. 2002; Pedraza-Chaverri et al. 2008; Obolskiy et al. 2009; Aizat et al. 2019; Ansori et al. 2020). For anti-cancer, α-mangostin, the largest constituent of xanthone in mangosteen pericarp extract, is applied in various cancer types such as gastric, cervical, colorectal, hepatocellular, and breast cancers (Ying et al. 2017; Mohamed et al. 2017; Muchtaridi et al. 2018). Mangosteen extract is also described to have anti-diabetic properties such as garcinone E and mangostanaxanthones III (Abdallah et al. 2017; Liang et al. 2018; Aizat et al. 2019). Several mangosteen extracts such as isogarcinol and γ-mangostin can be used for liver protection (Liu et al. 2018; Wang et al. 2018).

7.7 Genomics Study of Mangosteen

Determining the entire DNA sequence of an organism is known as genome sequencing. This process involves sequencing the chromosomal DNA, mitochondrial DNA, and for plants also the chloroplast DNA. To date, only a few studies concerning the mangosteen genome and chromosomal characterisation have been reported despite being one of the important fruits throughout Southeast Asia (Murugan et al. 2014). Studies conducted on mangosteen were mostly on tissue culture, seed characterisation, and morphology. A recent study reported the transcriptome-wide gene expression changes with transcriptional reprogramming during mangosteen seed germination (Goh et al. 2019). Several studies related to the mangosteen genome and organelle sequences have been reported (Abu Bakar et al. 2016; Midin et al. 2017; Jo et al. 2017; Wee et al. 2022a, Wee et al. 2022b). Data obtained from these works are necessary for genome size study, chromosome characterisation, and future genome sequencing project. The genome size and chromosome number data of mangosteen are important to study the genetic variability of mangosteen. The information will also contribute to other research areas including taxonomy and evolutionary studies. The correct information on the chromosome number and genome size of mangosteen will help on-going efforts to assemble and annotate mangosteen genome in future.

7.7.1 Genome Sequencing of Mangosteen

Several studies on the mangosteen genome have been reported. Abu Bakar et al. (2016) conducted the first genome sequencing on the common variety of mangosteen in Malaysia to study its genome composition as well as attempted draft genome assembly by using Illumina HiSeq 2000 sequencer platform. They have predicted the best k-mer length (41 bp) for assembly through KmerGenie (Chikhi and Medvedev 2014) and SGA Preqc (Simpson 2014). De novo assembly was then conducted by using Minia assembler v2.0.3 followed by scaffolding using SSPACE. The assembled genome draft was evaluated using CEGMA pipeline. Table 7.3 shows the sequencing and assembly statistics obtained in the study.

Genome sequence analysis has also been performed on another popular variety of mangosteen, ‘Mesta’ as reported by Abu Bakar et al. (2017) using Illumina HiSeq 2000 and Midin

Table 7.3 Statistics of mangosteen sequencing and assembly (Adapted from Abu Bakar et al. 2016 with permission)

Attributes	Values
Raw reads	
Total number	505,856,290
Total bases (bp)	51,091,485,290
Filtered reads	
Total number	418,812,062
Total bases (bp)	42,300,018,262
N (%)	0.0089
GC (%)	38.14
Q20 (%)	99.19
Q30	95.43
Minia assembly	
K-mer	41
Number of contigs	281,494
Total contig size (bp)	272,873,894
N50 (bp)	1,006
Contig size range (bp)	83–14,015
SSPACE scaffolding	
Number of scaffolds	284,879
Scaffold size	279,483,966
N50 (bp)	1,022

et al. (2017) with single-molecule real-time (SMRT) sequencing on a PacBio RS II platform. These data allow comparative analysis of genome composition between the two varieties of mangosteen in Malaysia, which are invaluable for crop improvement due to the lack of mangosteen molecular genetics information. The data can be utilised in the genome assembly and provides sequence information on the GC content, as well as genome size estimation of mangosteen as reported in Midin et al. (2018) (see also Sect. 7.2).

Jo et al. (2017) reported the first complete plastome sequence of mangosteen. The size of the mangosteen plastome is 158,179 bp in length. It contains a large single copy of 86,458 bp and a small single copy of 17,703 bp. Both are separated by two inverted repeats of 27,009 bp. Recently, there is a new report on the plastomes (156,580 bp) of mangosteen from the Malaysia varieties Mesta and Manggis (Wee et al. 2022a), which suggested a different origin to that of Thailand variety as reported by Jo et al. (2017). Furthermore, the mitogenome of the Mesta variety (371,235 bp) has also been described for the first time from the Garcinia genus and Clusiaceae family (Wee et al. 2022b). These complete plastome and mitogenome sequences are useful for the phylogenetic and evolutionary studies of Clusiaceae.

7.7.2 Genome Size of Mangosteen

Genome size is the total amount of DNA in the nucleus of an organism that is measured either in picograms (pg; i.e., 1×10^{-9} g) or megabase pairs (Mbp, with 1 pg = 978 Mbp) (Dolezel et al. 2003; Pellicer and Leitch 2013). Genome size is an important characteristic of eukaryotes that correlates with the chromosome number (Bennett and Leitch 2005). It gives important information for ecological and evolutionary studies, plant breeding, understand of somaclonal

variation in tissue culture, and the development of genome sequencing project (Rival et al. 1997; Srisawat et al. 2005; Kron et al. 2007; Ochatt et al. 2011; Leitch and Leitch 2012; Cardoso et al. 2012). Various methods have been employed to estimate the genome size of plant species such as Feulgen densitometry, chemical extraction, pulse-field gel electrophoresis, reassociation kinetics, genome sequencing, and flow cytometry (Bennett and Leitch, 2011). Midin et al. (2018) measured the genome size of mangosteen by using two approaches, namely flow cytometry and k-mer analysis. The correct information on the genome size of mangosteen will help on-going efforts to assemble and annotate mangosteen genome.

Flow cytometry (FCM) is a powerful tool used to determine the genome size of an organism. However, optimising nuclei preparation before FCM analysis is quite challenging for some plant species especially those containing high amounts of secondary metabolites. The presence of secondary metabolites compounds such as phenolic may cause stoichiometric errors during sample preparation, especially in woody plants such as mangosteen (Loureiro et al. 2006; Mallón et al. 2009; Midin et al. 2018). They will decrease the fluorescence and increase the CV level, hence reducing the quality of nuclei suspension as well as causing errors during the FCM analysis (Noirot et al. 2003; Bennet et al. 2008; Obae and West 2010). To minimise this problem, the selection of lysis buffer, plant materials, and DNA fluorochrome play important roles. Three types of lysis buffer namely LBO1, Tris-$MgCl_2$, and Otto were used for mangosteen sample preparation by Midin et al. (2018). As a result, they found the most suitable lysis buffer for mangosteen sample preparation was Otto buffer supplemented with reducing agents (mercaptoethanol and PVP-40) and propidium iodide (PI), a DNA intercalator.

The quality of DNA peak histogram from FCM analysis was evaluated based on the coefficient of variation (CV) value as well as the amount of debris produced in the background. The different types of lysis buffer generate different level of resistance to negative effects of phenolic compounds (Loureiro et al. 2006, 2007; Vrána et al. 2014). The addition of reducing agents such as mercaptoethanol and PVP-40 in Otto buffer counteracted the interference of phenolic compound with DNA staining, thus decreased the CV value (Price 2000; Yokoya et al. 2000; Noirot et al. 2003; Loureiro et al. 2006). Young leaves of mangosteen used in FCM analysis also can reduce CV value as they may contain less secondary metabolite components (Jedrzejczyk and Sliwinska 2010). Meanwhile, the amount of plant material and chopping intensity can also be decreased to reduce the effect of secondary metabolites (Loureiro et al. 2006; Doležel et al. 2007).

For genome size estimation of mangosteen, Midin et al. (2018) utilised *Glycine max* cv Polanka (soybean) as an external reference standard for two reasons: (1) It has a well-established genome size of 2.5 pg (Doleželˇ and Bartos 2005) and (2) its leaves have a soft structure easy to process during sample preparation (Madon et al. 2008; Midin et al. 2013). In the genome size estimation, the selection of a reference standard is important (Johnston et al. 1999). The genome size of an ideal reference standard should be known and not too close or too distant to the target species to avoid the risk of non-linearity and offset errors (Vindelov et al. 1983; Bagwell et al. 1989; Doleželˇ et al. 1992; Johnston et al. 1999; Bennet et al. 2003). Too close a genome size might cause DNA peak of sample and standard to overlap. The external standard method was selected following Hendrix and Stewart (2005) to avoid the overlapping of DNA peak. Figure 7.2 shows the histogram of DNA peak in reference standard, *Glycine max* cv. Polanka compared to mangosteen.

The 2C peak of *Glycine max* cv. Polanka was not overlapping with the 2C peak of mangosteen which are located on the channel 180–200 and 420–440, respectively. Midin et al. (2018) used five biological replications for the genome size determination of mangosteen (Table 7.4). Based on the FCM result, the genome size of mangosteen was found to be 2C = 6.00 pg. However, Matra et al. (2014) previously reported that the genome size of common mangosteen was

Table 7.4 Genome size estimation of mangosteen using *Glycine* max cv. Polanka (2C = 2.5 pg) as external reference standard (Adapted from Midin et al. 2018 with permission)

Replicate	Genome size (pg)
1	6.04
2	6.09
3	6.08
4	5.70
5	6.10
Mean ± SD	6.00 ± 0.17

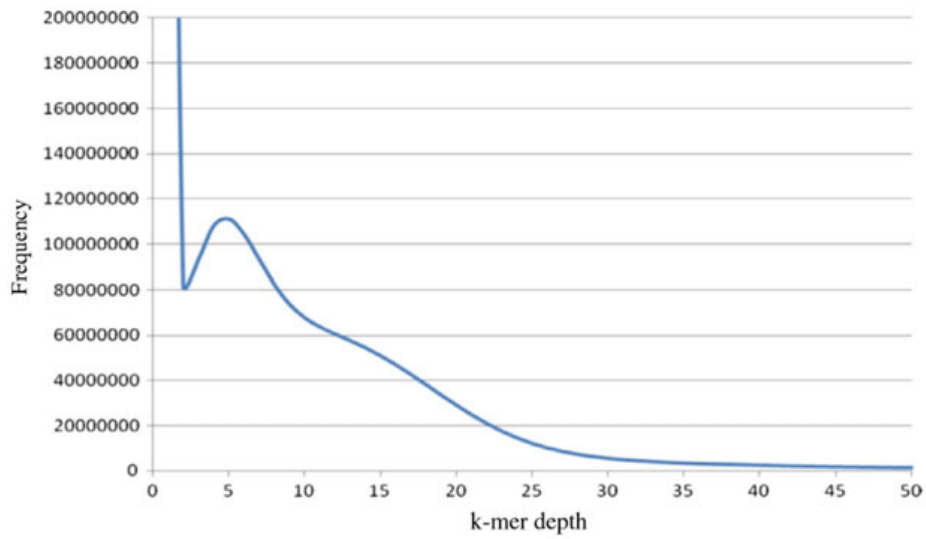

Fig. 7.3 Distribution of k-mer 41 depth in whole-genome Illumina reads of a common mangosteen variety (Reproduced from Midin et al. 2018 with permission)

2C = 7.42 pg. The difference may be due to the different DNA fluorochrome used during the FCM analysis. Matra et al. (2014) utilised DAPI while in this study, PI was used. DAPI intercalates preferentially on a specific region of DNA, which was AT-selective (Doležĕl et al. 1992).

To resolve this discrepancy, Midin et al. (2018) incorporated an in silico method of k-mer analysis to confirm the genome size of mangosteen. Figure 7.3 presents the genome size estimation for mangosteen using k-mer analysis using a k-mer value of 41.

The total number of k-mer predicted by Jellyfish version 1.1.11 1 (Marçais and Kingsford 2011) was 29,604,414,280, and the peak value of k-mer frequency distribution was 5. The genome size of mangosteen was estimated at 5.92 Gbp. The genome size was then converted according to the following relationship: 1 pg DNA = 9.78×10^8 (Doležel and Bartos 2005). The calculation gave approximately a genome size of 6.05 pg for mangosteen. Together, Midin et al. (2018) concluded that the genome size of mangosteen to be between 6.00 and 6.05 pg via FCM and k-mer analysis. The correct genome size is necessary for informing genome sequencing projects (Bennett et al. 2000). As the project scale and cost depend on the genome size, it is necessary to have an accurate knowledge on genome size (Doležel and Greilhuber 2010; Cardoso et al 2012).

7.7.3 Cytogenetics of Mangosteen

Information on chromosome number is also essential for a genome project. Chromosome count analysis is always difficult for species with many chromosomes (Mallón et al. 2009). It was not easy to count the chromosome number of *Garcinia* species due to high number of chromosomes (Robson and Adams 1968). Chromosome numbers for several *Garcinia* species have been reported including *G. celebica* L. [$2n$ = 48 (Tixier

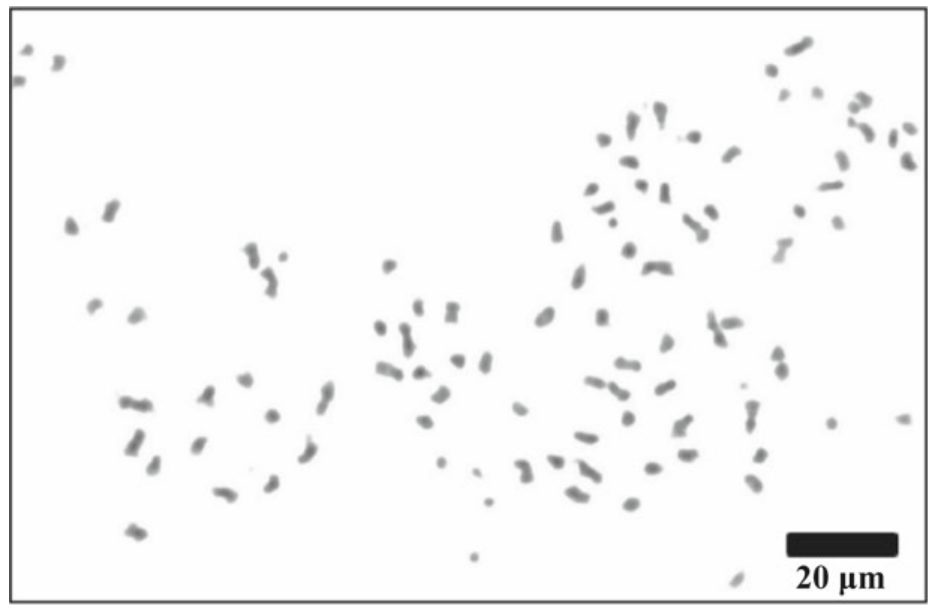

Fig. 7.4 A mitotic metaphase spread of mangosteen root

Table 7.5 Previous findings of mangosteen chromosome number

Chromosome number (2*n*)	Reference
76	Krishnawary and Raman (1949)
88–90	Richards (1990b)
90	Sarasmiryati (2008)
96	Tixier (1960)
110–120	Ha (1978)

1960)], *G. hanburyi* Hook. f. [$2n = 44$ (Tixier 1953)], and *G. indica* Choisy [$2n = 48$ (Thombre 1964); $2n = 54$ (Anerao et al. 2013)]. The chromosome number of mangosteen has also been reported by previous researchers (Table 7.4).

In the most recent study, Midin et al. (2018) revealed that mangosteen has $2n = 74–110$ chromosomes by evaluating more than twenty metaphase chromosome spreads (e.g., Fig. 7.4) which agrees with findings obtained by previous researchers (Table 7.5).

Their results therefore suggest the occurrence of numerical chromosome variation in mangosteen genome. This variation could contribute to the phenotypic differences among mangosteen variety and explain the occurrence of phenotypic variations, such as fruit morphology between the common and mesta varieties of mangosteen.

This variation might be due to the presence of variable number of B chromosomes (Bs) (Sarasmiryati 2008). The B chromosomes are also known as supernumerary chromosomes and defined as additional dispensable components of the genome which exhibit a characteristic non-Mendelian and irregular pattern of inheritance (Datta et al. 2016). These chromosomes are classically understood as a sea of repetitive DNA sequences that are poor in genes and maintained by a parasitic-driven mechanism during cell division (Valente et al. 2017). These chromosomes contribute to the occurrence of numerical chromosome variation (Roberto 2005). The variable number of Bs may have caused the difficulty in determining the chromosome number. Besides the presence of Bs, other factors including genome mutation and the occurrence of polyploidy and aneuploidy also cause the numerical chromosomes variation.

Natural mutation in mangosteen has been reported previously by Ray (2002) and Sobir et al. (2011). This phenomenon can impair chromosome segregation, which could also cause

aneuploidy (Zuzana 2012). According to Huettel et al. (2008) and Birchler (2013), aneuploidy refers to unbalanced changes in chromosome number from the basic chromosomal complement that characterises each species. These changes in chromosome numbers are determined in relation to the somatic chromosome number of the species (Dar et al. 2017). Recent findings revealed the occurrence of aneuploidy in polyploid species (Ganem et al. 2007; Chester et al. 2012; Zhang et al. 2013; De Storme and Mason 2014; Wu et al. 2018). Polyploidy may induce aneuploidy by increasing the chromosome number and complexity of their pairing and segregation during meiosis and mitosis (Comai 2005). This explains the occurrence of numerical chromosome variation in mangosteen as itself is determined as a polyploidy species by Richards (1990b) and Matra et al. (2016). Matra et al. (2016) revealed the evidence of tetraploidy in mangosteen. This was proved via microsatellite analysis where they found that mangosteen has more than two alleles per locus. A maximum of four alleles per locus was found in mangosteen from five populations which indicated tetraploidy. The combination of findings obtained by Matra et al. (2016) and Midin et al. (2018) concluded that mangosteen is an apomictic species with a polyploid genome. Previous studies have linked the occurrence of polyploidy in apomictic species (Galdeano et al. 2016). Plant species with tetraploid genome are often associated with apomicts (Quarin et al. 2001; Bicknell and Koltunow, 2004). However, not all polyploids are apomicts. The formation of apomictic species requires polyploidy genome as diploid or aneuploid gametes are necessary for the transmission of genes that cause apomixis (Comai 2005), which possibly explains the occurrence of apomixis in mangosteen.

7.8 Conclusion

Crop improvement of mangosteen requires special approaches due to its long juvenile period as well as a slow growth rate. To date, a rapid propagation method of mangosteen is still lacking, which is confounded by recalcitrant apomictic mangosteen seeds. The breeding programme of mangosteen is time-consuming and laborious with a very low success rate. As mangosteen has a long juvenile phase, hence it is difficult to perform progeny analysis. Another strategy to improve this crop is to identify genetic variation in mangosteen through molecular analysis. Mutational breeding has also been conducted on mangosteen to increase genetic variation. By using this approach, gamma-ray radiation was applied to mangosteen seeds. As genetic variation increase, superior trees with desirable traits can be selected. To date, genome-scale studies of mangosteen are limited, with a few recent reports on attempts of genome sequencing but a draft genome assembly is still lacking. Nevertheless, these sequence data provide essential information on mangosteen genome size and complexity. Ascertained number of chromosomes, genome size, and cytogenetics of mangosteen will help on-going efforts to assemble and annotate mangosteen genome. A reference mangosteen genome is important to provide a blueprint of molecular genetics information for crop improvement, through studies of genetic diversity and genomics-assisted selection.

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