

# Chapter 3

## Breeding Guava (*Psidium guajava* L.)

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### 3.1 World Production

Guava tree (*Psidium guajava* L.) has its origin in the American tropics and is today distributed throughout the tropical and subtropical areas of the world (Kwee and Chong 1990; Gonzaga Neto and Soares 1994; Medina 1988). It belongs to the Myrtaceae family, comprising a large number of fruit yielding species (Pereira and Nachtigal 2002; Ray 2002; Kwee and Chong 1990; Subramanyam et al. 1992). The guava fruit is important for fresh consumption and for substantial industrial preservation (Maia et al. 1988). The fruit contains vitamin A and B, and are exceptionally rich in vitamin C (ascorbic acid), superior to that present in the citric juices.

According to Ellshoff et al. (1995) *P. guajava* was first named by Linnaeus in 1753. As Ruehle (1964) stated, initial references to the guava tree are from the Spanish chronicler Oviedo, from the period between 1514 and 1557, when he was in Haiti. On that occasion, Oviedo referred to the guava tree as guayabo and made considerations about the vegetative behaviour of the plants found in some areas of West Indies. Oviedo (1959) made this insightful statement about guavas: 'Fruits have many seeds that are bothersome only to those who eat the fruit for the first time. Foods with such a heavenly taste and smell just might be considered sinful'. It is believed, on the other hand, that the Spaniards transported the guava tree of the Pacific to the Philippines and India, from where it passed to the Malay Archipelago, to Hawaii and to South Africa (Soubihe Sobrinho 1951). However, there is enough scientific evidence of guava having a pre-historic anthropogenic distribution all over the Antilles (Newsom 1993).

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The species is widely cultivated for its fruit and has become naturalised in tropical and subtropical areas worldwide. The guava has been cultivated and distributed by man, birds and other animals for so long that its place of origin is uncertain, but it is believed to be an area extending from southern Mexico into or through Central America (Morton 1987). It is common throughout all warm areas of tropical America and in the West Indies (since 1526), the Bahamas, Bermuda and Southern Florida where it was reportedly introduced in 1847 and was common over more than half the State by 1886. Although the guava plant was domesticated more than 2,000 years ago, it was not until 1526 when the first commercial cultivation of guava was reported in the Caribbean islands. Later it was spread by explorers into the Philippines and India (Yadav 2006). Early Spanish and Portuguese colonisers were quick to carry it from the New World to the East Indies and Guam. It was soon adopted as a crop in Asia and in the warm parts of Africa. Egyptians have grown it for a long time and it may have travelled from Egypt to Palestine. It is occasionally seen in Algeria and on the Mediterranean coast of France.

The world production of guava is increasing. Figures presented by FAO (Table 3.1) shows an increase of more than 10% in the last 5 years.

The status in Brazil, one of the major guava producers, gives a good idea on how growers are responding to market demand. Although the total area and the production increased 45% from 1999 to 2004, in Northeast Brazil, where guava is produced under irrigation and intensive technology, it increased by 90%. In this particular case, improved varieties played a very important role, as newly released varieties, such as 'Paluma', were used to establish new orchards. In India, the largest world producer, guava is produced in more than 150,900 ha (Fruits 2006) yielding over 1.6 million tons, but productivity is as low as 10–12 t/ha, due to poor management and post-harvest losses (Khushk and Lashari 2006).

**Table 3.1** World production of Guava by country (1,000 t)

Country	2000	2001	2002	2003	2004
Total	3646.2	3792.2	3952.9	3984.8	4035.5
India	1710.5	1631.5	1715.5	1700.0	1700.0
Pakistan	494.5	525.5	550.0	580.0	600.0
Mexico	254.2	263.4	283.3	299.2	317.0
Brazil	117.6	281.1	300.0	300.0	300.0
Egypt	216.8	228.8	243.9	231.2	230.0
Thailand	170.1	154.4	160.0	160.0	160.0
Colombia	130.6	149.6	145.0	145.7	154.7
Indonesia	137.6	138.1	138.1	138.1	138.1
Venezuela	120.0	120.0	120.0	120.0	120.0
Sudan	96.3	100.0	100.0	100.0	100.0
Bangladesh	48.0	49.0	49.9	50.9	51.8
Vietnam	38.5	37.8	34.0	35.0	35.0
Malaysia	11.7	13.0	13.1	24.8	28.9
Others	100.0	100.0	100.0	100.0	100.0

Source: FAO

Mexico is one of the major guava producers in the world with an increasing crop that surpassed 23,000 ha in 2003 (SIAP 2003) (Table 3.2). Others are Pakistan, Taiwan (6,644 ha as of 1999 and yielding 20 to 35 t/ha), Thailand, Colombia and Indonesia. Other tropical countries plant guava on parcels that vary along the years, such as Cuba and Venezuela (Table 3.2). Guava is also planted in smaller scale in other countries: Malaysia 1,641 ha in 2001 (16,861 t), Australia and South Africa (Table 3.2).

According to the South African Guava Producers Association, there are two main production areas: Limpopo/Mpumalanga (in the North of South Africa) producing around 10,000–15,000 tons of guava (puree or juice) and Western Cape (in southern South Africa) producing around 25,000 tons of guava mainly as fresh fruit. Area under guava in this region was 440 ha in 2002 and 500 ha in 2005. In Vietnam, guava is planted mainly in the Mekong delta region on 2,000 ha. Often, many farmers inter-plant guava with other fruit trees like king orange and pummelo.

In the USA, Florida and Hawaii have a very limited area devoted to guava. Even so, acreage has declined on the islands, down from a total of 125 farms that grew guava on 376 ha (including new orchards) in 1992, amounting to \$1,896,000.

United States is a major importer of guava products (paste, puree, jams) and there was an increase in the import from 2002 to 2004. United States imports mainly from Brazil, Dominican Republic, Ecuador and Mexico (Table 3.3). Although international trade in tropical fruits continues to be dominated by pineapples, significant growth in both the volume and value of exchange of other tropical fruits has developed in recent years, particularly mango and, to a lesser extent, avocados, carambola, guava, lychee, mangosteen, passion fruit and rambuttan (FAO 2002). Most of the recent growth in the tropical fruit trade is based on expanded crop areas specifically intended for export.

**Table 3.2** Guava: World area (A, hectares) and production (P, tones)

Country		2000	2001	2002	2003	2004	2005
Mexico	A	20,619	20,441	22,763	16,089	16,184	n.a.
Colombia	A	n.a.	n.a.	n.a.	n.a.	n.a.	16,124
Cuba	A	4,609	5,253	6,019	7,267	7,991	7,312
	P	17,092	23,206	28,454	40,052	52,670	47,878
South Africa	A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	P	21,911	25,179	26,637	22,879	17,645	n.a.
USA (Hawaii)	A	275	247	222	214	202	260
	P	7,212	6,940	4,400	3,039	3,674	3,674
Brazil	A	14,354	14,387	16,066	17,776	18,826	16,399
	P	n.a.	281,102	321,127	328,747	408,283	345,533
Pakistan	A	60,200	63,400	64,300	62,700	61,600	63,471
	P	494,500	525,500	538,500	531,600	549,500	571,800
India	A	150,000	150,000	190,000	n.a.	n.a.	151,000
	P	1,710,000	1,630,000	1,680,000	1,700,000	1,700,000	1,710,000

Sources: IBGE (Brazil); SIEAP/SAGARPA (Mexico) (SIAP, 2003); Statistics, Ministry of Agriculture, Cuba; South Africa Department of Agriculture (adapted); USDA – National Agricultural Statistics Service; Plan Frutícola Nacional (Colombia)

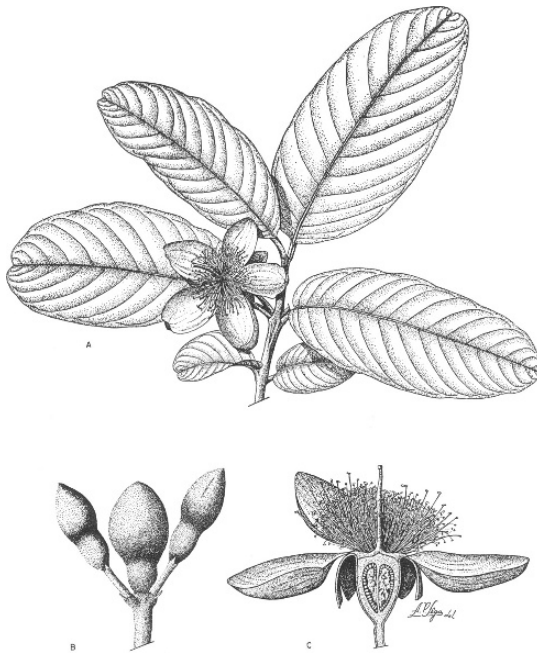
**Table 3.3** U.S. guava imports: quantity, by country, 2004

Country	Quantity			
	Guava – paste, puree	Guava – prep. or preserved	Guava – jams	Guava, Mango and Mangosteen – dried
	Metric tons			
Brazil	1,836	353	264	0
Colombia	419	77	2	84
Costa Rica	0	97	379	38
Dominican Republic	624	1,367	63	0
Ecuador	248	1,591	3	0
Fiji	17	0	0	0
France	3	8	0	1
India	533	267	0	11
Malaysia	0	259	0	2
Mexico	495	660	0	700
Netherlands	0	0	0	0
Philippines	141	0	2	2,872
Singapore	0	0	0	6
South Africa	33	262	0	19
Thailand	1	355	0	1,387
Venezuela	0	0	0	0
Others	3	100	1	146
Total	4,352	5,397	713	5,266

Source: U.S. Department of Commerce

### 3.2 Botanical Aspects

In guava, flowers are white, hermaphrodite, solitary or in 2–3 flowered cymes, emerging in the axils of the leaves (Fig. 3.1). According to Soubiê Sobrinho (1951), however, only the flowers located between the middle and the base of the branch have larger probability of producing fruits. More than three flowers can appear besides the usual two or three floral buttons, but it was observed that not always all produce fruits. The calyx is entire in the bud, splitting into 4–6 sepal lobes 1–1.5 cm long, reflexed, pubescent and persistent. The corolla consists of 4–5 petals that are white, obovate, concave reflexed and 1–2 cm long. The stamens are numerous and inserted in rows on a disc, 1–2 cm long with white filaments and yellow anthers that dehisce longitudinally (Kwee and Chong 1990). The stigma is capitate, greenish yellow, 1.5–2 cm long with a filiform style arising from a 4–5 locular ovary. The guava flower has a superior calyx with 5 lobes and the corolla of 6–10 petals arranged in 1 and 2 whorls (Subramanyam and Iyer 1993). The androecium consists of 160–400 thin filaments carrying bilobed anthers closely packed together. The gynoecium consists of an inferior ovary, syncarpous with axile placentation and subulate terminal style. The style is smooth and red at the summit. It is larger than filaments, but bent over stamens in bud stage. The stigma is exerted above the stamens, thus self-pollination without the help of external agency is rather uncommon.



**Fig. 3.1** A – frontal view of a guava flower in current year shoot; the five petals and the numerous stamens can be seen. B – guava inflorescence (cyme) with three buttons and a dichasium structure. C – flower in longitudinal section view with numerous ovules inside the ovary, persistent sepals, stigma with a slightly conic shape, large number of stamen and the petals typically boat-shaped. (from Soubihe Sobrinho 1951)

About 30 days are required from flower bud differentiation to complete the development up to calyx cracking stage (Subramanyam and Iyer 1993). The flower bud, when fully developed, has two distinct parts, namely, proximal ovoid adnate and distal free part that is ovoid or round and slightly pointed at apex. The cracking of the calyx occurs nearly 24 hours before flower opening. Sehgal and Singh (1967), however, observed that the calyx splits anywhere between 13 and 26 hours before flower opening.

It is observed that the fruit originating from the central floral button almost always presents faster development. This characteristic is of great importance because it can signal the correct way to practice the thinning of fruits. This is valuable in the evaluation of germplasm accessions. Observation and knowledge of such traits can guide a programme of genetic improvement, especially those pursuing derivation of varieties through recombination breeding. Guava prefers cross pollination that can vary from 25.7 to 41.3%, with an average of 35.6% (Soubihe Sobrinho and Gurgel 1962). Singh and Sehgal (1968) made a contradictory observation that guava is self-pollinated. Medina (1988) observed 62–82% open pollination. Domestic bee, *Apis mellifera*, is the main pollinating agent. While studying the natural fruit set, variations were registered from 22 to 75%, verified in cultivar Lucknow-49 (Soubihe Sobrinho 1951; Medina 1988).

According to Subramanyam et al. (1992), it takes 45–51 days to complete development of flower bud from 2 mm to full bloom during winter. Flower development in *P. quadrangularis*, *P. molle*, *P. cattleianum* and *P. friedrichsthalianum* take 36–45 days. Flowering in all the cultivars of *P. guajava* occurs three times a year as in *P. quadrangularis*. In *P. friedrichsthalianum* few flowers are observed between two flushes. Fruit set varies between 40 and 85.7% in *P. cattleianum*. Among the varieties of *P. guajava*, it ranges between 30.4 (cv. Pear Shaped) and 80% (cv. Allahabad Safeda and Apple Colour).

One of the most critical botanical characteristics of guavas is that the flowers are always borne on newly emerging vegetative terminals irrespective of the time of the year (Shigeura and Bullock 1976). Consequently, blossom bud formation and subsequent fruit set can be very erratic during the year or between years, depending on the rainfall pattern and the availability of fertiliser and water. However, trees can possibly be exploited by cultural manipulation to flower and fruit when desired. Under temperate conditions, there is only one fruit season a year with little choice. Irrespective of the time of the year in the tropics, new vegetative growth on guavas can be induced in several ways. The easiest and most common is by pruning the branches in a manner so that the apical dominance of the pruned branch will not be disrupted. This is done by taking out other branches by cutting at the junction to the main branch. The duration of flowering in each of the two peak flowering seasons is 35 to 45 days in India (Ray 2002). The fruit bud differentiation is practically continuous throughout the year except during winter.

### 3.3 Genetic Resources

The guava tree belongs to the Myrtaceae family, comprising more than 70 genera and 2,800 species. The Myrtaceae family also includes other agriculturally important plants that yield economic products such as aromatic spices (clove, cinnamon, allspice), aromatic oils (eucalyptus), ornamental plants (myrtle, Callistemon) and a number of fruits (roseapple, Surinam cherry, Java plum, wax jambu, feijoa and many others) (Kwee and Chong 1990). The genus *Psidium* presents about 150 species, among which stand out *P. Guajava* L. (guava,  $2n = 22$ ), *P. cattleianum* Sabine (sweet, beach or crown araçá) and *P. guineense* Swartz or *P. araçá* Raddali (true or acid araçá) (Pereira 1995).

Description of *Psidium* species is scattered throughout a number of papers and authors, but from the breeders perspective, the essential knowledge is presented by Soubihe Sobrinho (1951), Kwee and Chong (1990), Subramanyam and Iyer (1993), Gonzaga Neto (1999), Ray (2002) and Pereira and Nachtigal (2002). Also Ellshoff et al. (1995) presented an annotated bibliography on *Psidium*, while treating guava as a forest weed in Hawaii.

*Psidium* was described as a member of the pimentoid sub-tribe of the Myrtaceae having a C-shaped or uncinat embryo, hard or bony seeds and the calyx splitting between the lobes at anthesis (McVaugh 1968). Except for a few species that have become widespread through cultivation, most species of the genus occur as native

plants. The greatest diversity of species is from central and southeastern Brazil, but there are also a good number (about 15) from northern South America, quite a few from the West Indies, a handful from continental North America and a scattering of peripheral species from the Andes and southern Brazil. Numerous species described from central and southeastern Brazil has not been adequately studied since his time. Contrary to what other authors have accepted as a distinguishing characteristic, McVaugh found no sharp distinction in *Psidium* between groups of species with closed buds and those with open buds.

As a basis for hybridisation and improvement of cultivated *P. guajava*, Seth (1963) established comparative studies of the floral morphology, floral biology, incompatibility, cytology, embryology and seed development of *P. guajava*, *P. guineense*, *P. chinense*, *P. molle* and *P. cattleianum* var. *lucidum*. Floral biology of all the species was reported to be very similar to one another. *P. cattleianum* differed slightly in several ways from the other species. However, the author stated that maturation of floral buds and fruits was most rapid in this species; anthers dehisced after anthesis rather than before as in the other species; pollen had the lowest viability; optimum temperature for pollen germination was lower (25 °C as opposed to 30 °C for the other species); pollen longevity was shortest, the stigma becoming receptive the day the flower opens, remaining so for up to 72 hrs. (in other species, the stigma became receptive the day after anthesis and remained so up to 32 hrs.); when *P. cattleianum* var. *lucidum* and *P. guajava* were crossed, the fruits were seedless; and *P. cattleianum* var. *lucidum* was reported to be octaploid (as compared with *P. molle*, which was tetraploid and the other above-listed species, which were diploids of  $2n = 22$ ).

Seedless varieties were reportedly common in *P. guajava* as stated by Seth (1959). The seedless trait is related to many factors, of which self-incompatibility and chromosomal abnormalities were considered to be the major ones. The variety 'Seedless' was diploid with  $n = 22$ . Both embryo sac and pollen grains were found to be functional, but the percentage of viable pollen grains was low. The meiotic division was highly abnormal showing monovalent and bivalent laggards as well as bridging of chromosomes, indicating the hybrid origin of the variety and explaining the low pollen grain fertility.

The chromosome number of *P. guajava* was reported as  $2n = 22$  (Soubihe Sobrinho 1951; Kumar and Ranade 1952), but the level of ploidy was verified. Kumar and Ranade (1952) mentioned a seedless variety found to have a somatic complement of 33 chromosomes, which appeared to be the first record of triploidy.

In this matter, Hirano (1967) reported the following results obtained in his study for chromosome counts: *P. cattleianum* ( $2n = 77$ ; based on two specimens); *P. cattleianum* var. *lucidum* ( $2n = 66$ ; based on one specimen); *P. cujavillus* ( $2n = 44$ ); *P. guineense* ( $2n = 44$ ); *P. friedrichsthalianum* ( $2n = 44, 66$ ); *P. polycarpum* ( $2n = 22$ ); *P. guajava* several cultivars ( $2n = 21, 22, 24, 25, 33$ ). Hirano (1967) also observed that pollen of *P. cattleianum* var. *lucidum* could not be germinated; *P. cattleianum* and *P. cattleianum* var. *lucidum* could be crossed in both directions; *P. guajava* could not be crossed with *P. cattleianum* or with *P. cattleianum* var. *lucidum*. *P. cattleianum* and *P. cattleianum* var. *lucidum* could not be crossed with *P. guineense*, *P. cujavillus* or *P. friedrichsthalianum*.

Polyploidy within *P. guajava* was reportedly uncommon but the genus *Psidium* itself is represented by di-, tetra-, hexa- and octoploid species (Hirano and Nakasone 1969<sup>a</sup>). For *P. guajava* and *P. polycarpum*  $2n = 22$ ; for *P. guineense* and *P. cujavillus*  $2n = 44$ ; for *P. friedrichsthalianum*  $2n = 66$ . Although reports of  $2n = 88$  have been made for the chromosome number of *P. cattleianum* and its botanical form *P. cattleianum* f. *lucidum*, two plants of *P. cattleianum* were found to be heptaploid with  $2n = 77$  and three plants of *P. cattleianum* f. *lucidum* were hexaploid with  $2n = 66$  in this study. *P. guineense* and *P. cujavillus* were introduced into Hawaii as such, but similarities in chromosomes as well as in vegetative characters between these species casts some doubt as to their identities. *P. cattleianum*, *P. cattleianum* f. *lucidum*, *P. guajava*, *P. guineense*, *P. cujavillus* and *P. friedrichsthalianum* were subjects of pollen germination and crossing studies by Hirano and Nakasone (1969b). Pollen of *P. guajava* (cultivars used had  $n = 22$  and  $n = 33$ ) generally had high germination rates, which were higher than those for species with higher chromosome numbers. *P. cattleianum* and *P. cattleianum* f. *lucidum*, with reports of  $n = 88$ , were sometimes found to be heptaploid (*P. cattleianum*,  $2n = 77$ ) and hexaploid (*P. cattleianum* f. *lucidum*,  $2n = 66$ ). Pollen of *P. cattleianum* showed 32% germination, while that of *P. cattleianum* f. *lucidum* failed to germinate. Pollen tubes of *P. cattleianum* were shorter than those of other species.

### 3.3.1 Cultivars

Although selective breeding of guava cultivars started almost a century ago, the easiness of plant propagation through seeds hindered preserving improved cultivars without significant changes of their attributes. Only after establishment of good cloning methods such as rooting herbaceous cuttings of guava, cultivars started to be well preserved and maintaining the original characteristics.

Many breeding programmes in the world have released improved guava cultivars (Table 3.4) but the most common way of getting new varieties is through growers' actions, such as identification of outstanding plants in their orchards and their propagation. This is possible because of the great diversity in open pollinated plants used to form orchards. There are probably more than 400 guava cultivars around the world, but only a few dozen are responsible for the majority of plantings. As stated by Subramanyam and Iyer (1993) and Pathak and Ojah (1993), the description and nomenclature of guava varieties is often confusing. Usually new selections are named according to shape of the fruit, skin colour, flesh colour and after the place of origin.

Allahabad Safeda: Fruits are big in size, round, smooth skin, white flesh, soft, firm, light yellow and, on ripening, develops very sweet taste, pleasant flavour and a few seeds. It is the most popular variety in India and other countries.

Beaumont: Selected from a seedling population derived from fruits found in Halemanu, Oahu, Hawaii. Medium to large, roundish fruits weighing up to 240 g.



**Table 3.4** Commercial cultivars of guava in the world

Country	Cultivars
Australia	Allahabad Safeda; Beaumont; Lucknow-49; Ka Hua Kula
Bangladesh	Swarupkathi; Mukundapuri; Kanchannagar; Kazi
Brazil	Paluma; Rica; Pedro Sato; Kumagai; Sassaoka; Ogawa; Yamamoto; XXI Century
Colombia	Puerto Rico; Rojo Africano; Extranjero; Trujillo
Costa Rica	Tai-kuo-bar
Cuba	Enana Roja Cubana; EEA 1-23
Egypt	Bassateen El Sabahia; Bassateen Edfina; Allahabad Safeda
India	<i>White fleshed</i> : Allahabad Safeda; Apple Colour; Lucknow-42; Lucknow-49; Safeda; Karela; Seedless; <i>Red Fleshed</i> : Lalit; Hybrid Red Supreme; Red-fleshed; Benarasi; Sardar; Chittidar; Harijha; Arka Mridula; Arka Amulya
Malaysia	Kampuchea (Vietnam, GU8); Hong Kong Pink; Jambu Kapri Putih; Maha 65; Bentong Seedless (Malaysian S.); Taiwan Pear
Mexico	Media China; Regional de Calvillo; China; la Labor; Acaponeta; Coyame
Puerto Rico	Corozal Mixta; Corriente; Seedling 57-6-79
South Africa	Fan Retief; Frank Malherbe
Taiwan	Tai-kuo-bar
Thailand	Glom Sali; Glom Toon Klau; Khao Boon Soom
Vietnam	Xa ly nghe; Ruot hong da lang; Xa ly don
USA (Hawaii)	Beaumont; Pink Acid; Ka Hua Kula

Pink flesh, mildly acid and seedy. Excellent for processing. Somewhat susceptible to fruit rots. Tree vigorous, wide spreading and very productive.

Lalit: Recently released, fruits are medium sized (185 g) with attractive saffron-yellow colour and red blush. Its flesh is firm and pink with good blend of sugar and acid. It gives 24% higher yield than the popular variety 'Allahabad Safeda'.

Xa ly nghe: Pear-shape, 260 g, rough skin, flesh thickness 1.5–1.6 cm, sour-sweet taste, few to moderate seediness and white.

Ruot hong da lang: Pear-shape, 400 g, smooth skin, flesh thickness 1.3–1.4 cm, acidish sweet taste, few to moderate seediness and pink.

Xa ly don: Spheroid, 270 g, roughish skin, flesh thickness 1.4–1.5 cm, sourish sweet taste, few to moderate seediness and white.

Paluma: Seedling from open-pollinated Rubi-Supreme (UNESP, Brazil). Highly productive plants (more than 50 t.ha<sup>-1</sup>), vigorous, good tolerance to rust (*Puccinia psidii* Wint.). Fruits are large (over 200 g, even in not-thinned plants), pyriform, smooth surface, yellow colour in ripe fruits, pulp of an intense dark red, firm, thick (1.3–2.0 cm), nice flavour due to high sugar content ( $\pm 10^\circ$ Brix) and few seeds. Most-planted cultivar in Brazil.

Rica: Seedling from open-pollinated Supreme (UNESP, Brazil), vigorous and highly productive plants (more than 50 t.ha<sup>-1</sup>), oval to pear-shaped with an average weight (100–250 g), green-yellowish peel, slightly rough, red pulp, thick and firm, very pleasant flavour (11 °Brix) and low acidity. Few and small seeds.

Pedro Sato: Cultivar selected by growers from open-pollinated orchards, probably from 'Red Ogawa N° 1', in Rio de Janeiro, Brazil. Vigorous plants with

relatively good yields, fruits slightly oval, good appearance (150 to 280 g), sometimes reaching 400 g in thinned branches, very rough peel, pink pulp, thick and firm, pleasant flavour and few seeds. At present, it is the table cultivar with rough peel most planted in São Paulo (Brazil).

**Sassaoka:** Originated from a seedling of Common Red, in Valinhos (Brazil), large fruits (weight superior to 300 g when in thinned plants), rounded, light-pink, thick and firm pulp and few seeds.

**XXI Century:** Recently released (2003), it was obtained from a controlled cross between Supreme-2 and Paluma (UNESP, Brazil) presenting a very productive plant with a short cycle (130 days from bloom to harvest), large fruits (average 200 g) with thick pulp (160 mm), rosy-red, great flavour and with little and small seeds (1.3 g/100 seeds).

**Tai-kuo-bar:** Introduced from Taiwan, it is a table guava with large and roundish fruits, weighing 400 to 800 g in average, white pulp, juicy and crunchy and for fresh consumption.

India, the world's largest guava producer, relies on well established and very effective breeding programmes. For instance (Guava Technical 2006), at Fruit Research Station, Sangareddy, Andhra Pradesh, 2 hybrids, Safed Jam and Kohir Safeda were selected from reciprocal crosses involving Allahabad Safeda and Khoir, were released. These hybrids have been recommended for semi-arid tropical areas and have also been found suitable for juice. Subramanyam and Iyer (1993) reported that at Horticultural Research Station, Saharanpur, efforts to obtain varieties (Singh 1953) having good fruit quality and yield resulted in a superior selection, Sol, having good fruit shape, few seeds, sweet taste and high yield (Singh 1959). At Central Institute of Horticulture for Northern Plains, Lucknow, a large germplasm was introduced and evaluated for morphological characteristics, fruit quality and yield. Evaluation of 20 varieties indicated that Lucknow-49 was the best (Chadha et al. 1981).

In Brazil, pioneer work was carried out by Soubihe Sobrinho at the IAC (Agronomic Institute of Campinas) who established the basis for all subsequent breeding work involving flower biology, rate of natural crossing and other fundamentals (Soubihe Sobrinho 1951; Soubihe Sobrinho and Gurgel 1962). The first Brazilian variety may have been IAC-4 most likely resulting from a cross between a seedless and a seeded variety with round and small fruits (100–160 g).

### 3.4 Breeding Objectives

Nakasone and Paull (1998) indicated that the fact of being a fruit with a lot of seeds makes guava suitable for controlled hybridisation. The same authors affirm that resulting progenies of open pollination can be appropriate for development programmes of cultivars. The selection criteria are:

I – Fruits: (a) large size (200–340 g) with few seeds and thick pulp; (b) white pulp for table and dark rose for industry; (c) flavour and aroma characteristics of the fruit;

(d) content of total soluble solids superior to 10%; (e) acidity from 1.25 to 1.50% in those destined for processing and from 0.2 to 0.6 for table; (f) content of vitamin C equal or larger to 300 g.kg<sup>-1</sup>; (g) minimum number of stone cells (probably the ones that make a stir of hardness in the pulp); (h) good post-harvest quality; and (i) resistance to diseases and insects that damage the fruits.

II – Plants: (a) vigorous trees, with a crown widely opened and low development in height; (b) resistance to pests and diseases; (c) high production; and (d) dwarfing rootstocks.

Gonzaga Neto (1999) reported that the guava breeding programme, at Empresa Brasileira de Pesquisa Agropecuária Semi-Árido ([www.cpatsa.embrapa.br](http://www.cpatsa.embrapa.br)) in Brazil, includes the following objectives: (a) collect, introduce, characterise and select guava genotypes with defined and appropriate characteristics to production; (b) select genotypes with higher productive potential and with resistance mechanisms to pests and diseases; (c) establish important botanical descriptors for the guava tree, seeking to eliminate redundancies in data collection; (d) to maintain collection of guava tree genotypes in strategic areas of development; and (e) to select and diffuse guava tree genotypes, seeking the formation of commercial orchards and supply of elite material for other improvement programmes and nurseries.

Kwee and Chong (1990) reported that, in general, the attributes of a good commercial cultivar are:

- Good size – greater than 7 cm in diameter
- Consistent high yield – about 40–60 t/ha/year
- Pleasant flavour and aroma
- Sweet to mildly acid
- Smooth – textured and palatable, with little stone cells
- Thick flesh with a small seed core or seedless
- Deep pink flesh (rich in vitamin A)
- Soluble solids around 9–12%
- Resistant to pests and diseases.

Pereira and Nachtigal (2002) presented an extensive list of guava breeding objectives carried on at UNESP/Jaboticabal (Brazil)

Fruit, external aspect:

1.1.1 Medium weight superior to 100 grams in no-thinned plants

- Oval shape, with short neck
  - Halos of medium and/or small size
  - Green-yellowish or yellow peel when ripe
  - Resistant to transport and good keeping quality
- Fruit, internal aspect:

1.1.2 Pulp colour rosy or red

- Ratio pulp/total weight superior to 70% and pericarp thickness superior to 100 mm
- Absence or few stains in the pericarp and absence of stone cells
- Few seeds and seeds of small size

**Table 3.5** Comparison between the characteristics of the fruits of two guava varieties and the desirable characteristics listed in the literature

Characteristic	1	2	'Rica' (3)	'Paluma' (3)
Fruit diameter (cm)	7.62	8.4–8.9	6.5–8.0	8.0–10.0
Cavity diameter (cm)	3.81			
Fruit weight (g)	196–280	224–672	100–160	140–250
Seeds (%)	1–2			4.96
Pulp colour	Dark pink	Dark pink	Red	Intense red
Soluble solids (%)	9–12	9–12	10.9	
pH		3.3–3.5	3.72	
Vitamin C (mg/100g fruit)	≥ 300	≥ 300		
Stone cells	Few			
Purée ratio (%)		90		93.76

(1) Hamilton and Seagrave-Smith (1954); (2) Boyle et al. (1957); (3) Pereira (1984)

#### Fruit organoleptic traits and contents:

Total soluble solids (SST) superior to 10° Brix and with ratio SST/TA superior to 11.7

Vitamin C content around 100 mg of ascorbic acid per 100 g of pulp

Pleasant flavour and aroma, remaining in the industrialized products.

Plants:

Productive with a minimum yield of 30 t.ha<sup>-1</sup>

Resistant or tolerant to rust (*Puccinia psidii* Wint.)

Low and open crown

Table 3.5 shows desirable characteristics in guava fruits, according to Hamilton and Seagrave-Smith (1954) and Boyle, Seagrave-Smith, Sakata and Sherman (1957), side by side with the characteristics of the fruits of two varieties, Rica and Paluma, released some 20 years ago (Pereira 1984). Pereira (1984) selected, in segregating populations from seeds of open pollination, the genotypes that gave origin to cultivars Rica and Paluma, respectively derived of the varieties Supreme and Rubi Supreme.

Reddy et al. (2006) suggested the future line of work for widening the genetic base for effective breeding through inter-varietal hybridisation involving less seeded triploid varieties with those of high yielding, better keeping quality and less seed content. He stated that emphasis should also be given to breed scion and rootstock separately for abiotic/biotic stress situations.

### 3.5 Breeding Techniques

Most of the improvement programmes are based on controlled artificial pollination, using crossings among plants that present characteristics of interest for obtaining new cultivars. Plants crossed may be of the same species, in the inter-varietal crosses or from different species, constituting the inter-specific crosses. The accomplishment of controlled crossings depends largely on the adequacy of technique employed for pollen collection. São José and Pereira (1987) observed that flower

emasculation, removal of anthers, sepals and petals, when the calyx ruptures, prevents selfing. The pollen grains are viable from the phase of developed floral button to the phase of totally open floral button (blossom) and it is advisable to accomplish pollination immediately after the emasculation.

According to Pereira and Nachtigal (2002), the first step of the work is the selection of the parents, in order to make possible the combination of favourable characters in the descendants to be selected. Once the crossings are programmed, during the blossom period, collection and conservation of pollen from male parents is to be provided. During the first hours of the morning, recently opened flowers are collected from the male parent, displayed into cardboard boxes and dried in the shade for 1–2 hours. Stamens are separated and dried for 3–4 hours at 35°C. Material is passed through 16-mesh sieve and the collected pollen is kept on glass vessels at temperatures not higher than 25°C. Soon afterwards, during the rupture of the sepals, the emasculation of female parents is done followed by pollination. Pollination must be repeated on the two subsequent days to assure success. Usually, to obtain 200 seeds, it is necessary to pollinate about 20 flowers that can have 2–5 fruits.

Soon after pollination, fruits are labelled and protected with water repellent paper bags. Harvest should be accomplished when fruits reach the stage of maturity because the seeds turn physiologically ripe before completely ripe. The seeds should be dried in shade, treated with fungicide and conserved in paper bags. Sowing can be made in 3 liter plastic bags, with two or three seeds per unit. If all seeds happen to germinate, only one plant must be allowed in each bag; the others should be carefully transplanted to other containers. During plant development, special care should be taken in management and identification.

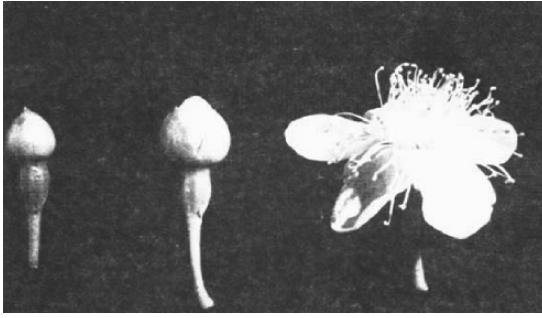
When the flower buttons reach their maximum development, the sepals begin breaking up in several points, signalling the beginning of anthesis. The following day, at around 6 a.m., for approximately an hour, the gradual opening of almost all the buttons begins. (Soubihe Sobrinho 1951). The hour at the beginning is variable and depends on diurnal temperature. Ray (2002) stated that anthesis starts at 4.00 a.m. and continues till 10.00 a.m., the peak opening occurring between 5.00 and 7.00 a.m.

According to Soubihe Sobrinho (1951), the first insect to visit the guava tree is the bee (*Apis mellifera* L.). During anthesis, bees fly over the tree, butting against the petals in order to remove that obstacle in search of pollen. It is said in 'search of pollen' because exams done on some flower buttons did not reveal the existence of nectar glands. It can be said that bees assist in opening the flower, though anthesis happens in a lesser time than necessary for it to usually take place.

The flowers are immediately visited and pollinated by that insect. As diurnal temperature increases, other insects may appear.

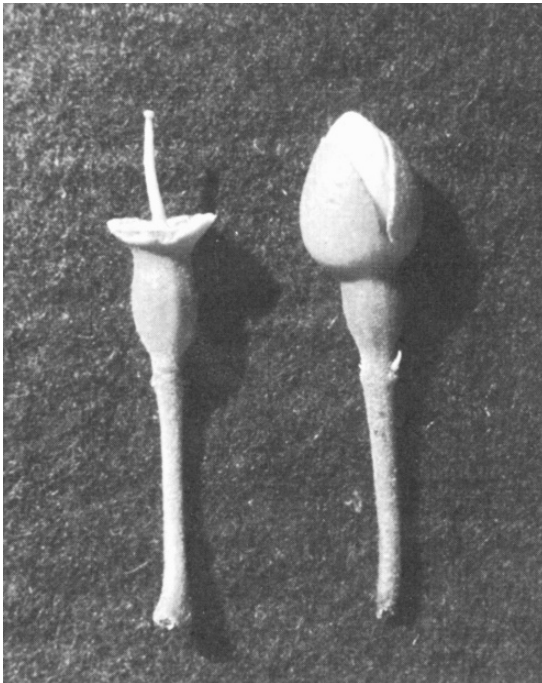
Dehiscence of anthers and receptivity of the stigma occur just after the opening of the flower. As stated by Souza (1998), pollen viability was 99.59% and frequency of diads and triads was 0.25%.

The flower buds that open the next morning show a cracking of the calyx on the previous day, nearly 24 hours in advance. São José and Pereira (1987) verified



**Fig. 3.2** Guava flower: developed button; button showing calyx rupture; open flower (São José and Pereira 1987)

the most adequate stage of flower development for pollen grain collect and the most efficient and safe pollination technique in controlled crosses of guava aiming to obtain specific knowledge for genetic breeding research work. They tested the efficiency of pollen obtained from closed flowers with calyx rupture and previously packed open flowers (Figs. 3.2 and 3.3), associated with pollination just after emasculation or 24 hours after emasculation. The authors concluded that emasculation of



**Fig. 3.3** Guava flower: emasculated button; button showing calyx rupture (São José and Pereira 1987)

guava flowers with calyx rupture and total elimination of anthers, sepals and petals prevents any possibility of self-pollination; pollen grains showed to be viable at the three tested stages of flower development; pollination is more effective when it is done 24 hours after emasculation when results are evaluated through fruit set percentage and average seed number.

Though anthesis starts at 4.00 a.m. and continues till 10.00 a.m., the peak opening occurs between 5.00 and 7.00 a.m. The dehiscence of anthers starts 15–20 minutes after the opening. In majority of the cultivars, peak dehiscence time is 6.00–8.00 a.m. In *P. Friedrichsthalianum*, peak dehiscence occurs between 7.00 and 9.00 a.m., while in *P. cujavillus* and *P. cattleianum* it is between 9.00 and 11.00 a.m. Viability of the freshly collected pollens varies from 42 to 95% depending upon the varieties. Seedless cultivars, in general, have less than 50% pollen viability whereas the seeded varieties like Chittidar or Allahabad Safeda show over 90% pollen viability at the time of dehiscence (Ray 2002).

Pollen grains of *P. guajava* L., *P. guineense* Swartz, *P. molle* Bertol, *P. chinese* Lodd and *P. cattleianum* Sabine var. *lucidum* remain viable for 1 day under field conditions but are viable for 90–135 days at low temperature (0–4.5 °C) and low relative humidity (0–25%). Pollen grains of commercial cultivars like ‘Chittidar’ could be stored for about 5 months at 0 °C with 25% R.H. The stigma becomes receptive within 2–3 hours after opening and remains so up to 48 hours thereafter. The maximum fruit set occurs when the stigmas are pollinated within 2 hours after anthesis. Singh and Sehgal (1968) have reported that the receptivity commences even 2 days before anthesis and lasts up to 4 days after anthesis.

However, Singh and Sehgal (1968) obtained maximum germination of pollen grains on stigma when pollination done just 2 hours after anthesis. In wild species of *Psidium*, the best period of stigmatal receptivity is within an hour of opening of the flowers. For making crosses in a hybridisation programme, flowers are emasculated at least one hour before anthesis and bagged. It is pollinated within 2 hours after emasculation with freshly collected or stored pollens and rebagged. Bags are removed only after 5–6 days of pollination. In the beginning of the rainy season, in case irrigation is provided, the new hybrids should be taken to the field (6 × 4 m), because the most effective evaluations are accomplished after the initial phase of plant development.

### 3.6 Breeding Progress

Historically guavas have been grown from seed and plantings that are quite variable due to insect pollination of flowers. Seedlings segregate a lot and this has been the basis of the variation used for selection all over the world by breeders as well as growers. Schrader et al. (1954) reported considerable variability among guava seedlings, leading to real possibilities in the selection of quite different genotypes. In conventional improvement, Soubihe Sobrinho (1951), at IAC, developed a scheme for the improvement of guava and determined the fruit set percentage (22%),

indicating predominance of selfing in the species. Later, the same author determined the cross-pollination rate in guava tree (Soubihe Sobrinho and Gurgel 1962), reporting a rate of crossing from 25.7 to 41.3% with an average of 35.6%, the same standard value presented by Nakasone and Paull (1998).

Considerable variability is present whenever a seedling guava population is obtained. Du Preez and Welgemoed (1990) observing plantings of guava seedlings at the CSFRI, Nelspruit, verified production of trees with fruit that varied widely in physical and chemical characters. This variation was used as a means of selecting cultivars to improve stability of an industry based only on one cultivar, namely Fan Retief. From an evaluation of 8,000 trees, 5 selections were made over a 3 year period. Differences were found among seedlings in fruit size, shape, flesh thickness, flesh colour, soluble solids, acidity and ascorbic acid. With the exception of ascorbic acid, all other characteristics were better in the selections than in Fan Retief. The variability observed in these fruit traits indicates that they would be responsive to further, more controlled selection and breeding.

Dinesh and Yadav (1998) provided the analysis of half-sib progenies derived from the variety Apple Colour and verified that the genotypic variability was smaller than the phenotypic for all the studied characteristics. They reported that the level of genetic variability was low and the heritability showed to be moderately high for all of them. Physiochemical characters of the fruits, such as shape, texture, pulp ratio, peel and pulp colour and contents of sugars, acids and volatile compounds plays a significant role in the selection process. Martinez Jr (1992) and Carvalho (1996) verified that pulp colour, soluble solids content and flavour of the fruits were the attributes that most contributed to the rejection of undesirable plants in the programmes of genetic improvement of the guava tree at UNESP (Brazil). Schrader (1955), in his research towards improvement of guava in Brazil, has found genotypes producing fruits of up to 475 g and others with vitamin C indexes of up to 560 mg of ascorbic acid/100 g of fruits. He also considered fruit shape and colour, pulp texture and seed amount.

In some areas of Brazil, use of cultivars with production that does not coincide with the normal pick of the harvest (precocious or late) can provide a crop with a better price in the fresh fruit market (Gonzaga Neto et al. 1991<sup>a</sup>; Gonzaga Neto et al. 1991b; Gerhardt et al. 1995). However, in the State of São Paulo and in other areas, with innovative cultural practices (pruning and irrigation), guava production is extended practically throughout the year.

### ***3.6.1 Breeding for Disease Resistance***

A guava tree is attacked by several pests and diseases that harm mainly the fruits (Campacci and Chiba 1983). Rust, caused by *Puccinia psidii* Wint., is one of the most serious diseases of the guava tree, limiting cultivation due to damage to the fruits, spoiling them for consumption as well as for processing (Campacci and Chiba 1983; Figueiredo et al. 1984). Till date, the only feasible control measure is through weekly sprayings with fungicides, which raises production costs.



Wan and Leu (1999) crossed and selfed 12 varieties and lines in 32 combinations and 9,434 resulting seedlings were inoculated artificially with spores of *Myxosporium psidii*, agent of guava tree wilt in Taiwan. One year later, almost half (46.85%) had died due to the disease, a proportion that increased to 98% after 6 years and 192 remained healthy. The commercial variety, Peipa, seems to be the best resistance source, having the largest survival rate (11.69%) obtained among the descendants of the crossing Peipa × R1 (this last one, a lineage). Out of the surviving clones, 57 were selected by their fruit quality, having derived from 8 crossings and one selfing.

Ribeiro and Pommer (2004) studied half-sib progenies resulting from 22,950 seeds from fruits originated through open pollination of 306 accessions. Seedlings were grouped into different number of accessions as: (a) 35 primary selections of white guava, obtained in the breeding programme IAC (identification: White LxPy); (b) 64 primary selections of red guava, obtained in that same programme (identification: Red LxPy); (c) 118 commercial varieties (some with 2 up to 6 accessions) as Supreme, Indiana, Weber, FAO, Australian, Patillo, Paluma, Rica, Ruby Supreme, IAC-4 and others; (d) 55 advanced selections of IAC programme (with acronym MAS), of Conceição de Almeida, BA (with acronym EEFT) and others named Sigla (II to XIII) and; (e) 34 accessions not clearly identified or without identification. Selection was applied in the initial stages of the seedlings and after artificial inoculation with the fungus. The heritability for rust resistance was estimated in a broad sense being  $h^2 = 0.275$ . The results of the evaluations in the half-sib progenies showed variation in the proportion of plants without symptoms: 25% in Group 1 (IAC selections of white guava); 28% in Group 2 (IAC selections of red guava); 44% in Groups 3 (commercial varieties) and 5 (miscellany); and 64% of plants without symptoms in Group 4 (advanced selections of Monte Alegre do Sul and of Conceição do Almeida). The analysis of variance showed that the plants of Group 4 differed from others in that aspect (t test,  $p > 0,05$ ) evidencing the selection pressure made in that sense. After 2 years, 105 individual plants were selected with absolutely no symptoms of the disease and are under selection for other traits, such as yield, fruit characteristics, colour and flavour.

An attempt to overrun a problem with Guava Wilt Disease (GWD) in South Africa was done by Du Preez (2006). Through the use of tissue culture she submitted 30,000 seedlings to a fungal filtrate from the fungus after removing its toxin, using this as a selection agent in the tissue culture, and selected 10 that survived the disease. Those that survived were multiplied in tissue culture and transplanted into pot trials. Out of those 10 selections, she selected 3 rootstocks that seemed to do well in the pot trials. TS-G-1 and TS-G-2 are almost resistant. TS-G-3 was tolerant. Fungus was found in the rootstock, but it did not kill the rootstock. No disease symptoms were observed. None of the rootstocks have ideal fruit quality, but according to the author they do compare favourably with the cultivar that was being used. At the moment, new plantings are being made with the rootstock plants being used as cultivars without grafting. The breeding and selection processes are ever continuing using the same methods in the hope of getting better cultivars that are resistant.

Cell-free filtrates derived from *Penicillium vermoeseni* were used by Vos et al. (1998) to screen 30,000 guava seedlings in vitro. Ten promising selections were made and cloned in tissue culture. Three of the selections exhibited 100% tolerance or resistance to GWD. The major advantage of using this technique to screen for resistance is that the juvenile growth phase of the plants could be maintained. This facilitated the use of nodal and split-nodal cuttings from tissue culture derived ramets instead of the slow, conventional propagation techniques such as air-layering and hardwood cuttings. As a result, 25% of the trees lost to GWD in South Africa have been replaced by trees with tolerant rootstocks within a research period of 5 years.

Studying GWD in South Africa, Schoeman and Vos (1998) verified that six months after inoculation with the fungus, all the Fan Retief plants in the non-grafted experiment inoculated in the stem or in the roots were dead. Except for one plant of selection TS-G2, inoculated in the stem, none of the plants of the other selections showed any symptoms. In the grafted trial, 100% of the Fan Retief plants grafted onto Fan Retief were dead six months after inoculation, while only one plant grafted onto selection TS-G3 showed symptoms. In the field trial, three Fan Retief plants were dead three years after planting while none of the plants of the other selections showed any symptoms. These results indicate that these selections are more resistant to GWD than the commercial Fan Retief cultivar. Selection TS-G3 appears to be tolerant to GWD since the Fan Retief scion was affected by the fungus. Selections TS-G1 and TS-G2 have been used as rootstocks for Fan Retief in commercial plantings in South Africa.

### 3.6.2 *Inheritance in Guava*

Compared to other organisms, guava is not an appropriate plant for studies on inheritance. The fact of being a perennial tree, demanding huge areas and labour to cultivate, presenting high heterozygosity and demanding large populations for this type of study ends in very little information on inheritance in guava.

Heritability in the broad sense includes all types of gene action such as dominance, additive and epistasis (Ray 2002). Considerable research effort has gone into estimating the heritability pattern in guava. It has been observed that commercially important traits, such as yield, fruit size, certain types of disease resistance and quality characteristics (Vit. C, acidity, pectin, etc.) are often in the low-heritability category. None of these characters are determined solely by major genes, although basic genes, subject to the modifying effects of polygenes, have been identified for some quality characters like skin colour and acidity. Obovoid shape of the fruit is dominant over round (oblate) and pyriform.

Continuing its original studies on guava breeding in Brazil, Soubiê Sobrinho and Gurgel (1962) observed that red is dominant to white pulp colour. Later on, Subramanyam and Iyer (1992) showed that red colour of pulp is dominant to white and that this character is governed monogenically. A linkage was found between

flesh colour and seed size. It was also observed that the attractive pulp colour and high yields of 'Beaumont' can be transferred to other white sweet cultivars.

Seth (1960) reported varietal cross incompatibility since neither fruit nor seed set was obtained when crosses were made between Behat Coconut X Lucknow-49, S1 X Behat Coconut, Behat Coconut X Apple Colour and Apple Colour X S1. Triploidy and some other genetic factors have been reported to be responsible for female sterility. The variation observed in triploids was possibly due to their independent origin from a different diploid variety. At Coimbatore, in the triploid fruits, a black mass of degenerated ovules was observed in the centre due to less stimulation by placenta during fertilisation. There is a need to further study qualitative and quantitative inheritance in order to assist the guava breeder in interpreting phenotypic values in terms of potential genetic gain.

Dinesh and Yadav (1998) studied the F1 progenies of four crosses among 'Apple Colour' and three other guava varieties. They found that genotypic variance was less than phenotypic variance for all the five characters analysed (fruit weight, length, volume, width and TSS). The coefficient of variation also followed the same trend, implying greater manifestation of these characters. The low genotypic coefficient of variation indicated low degree of genetic variability present in half-sib progenies. The higher phenotypic coefficients of variation imply the greater manifestation of these characters. The coefficients of variation indicated only the variability in different characters and did not indicate the heritable portion. The heritability in narrow sense was observed to be moderately high in fruit length (44.45%) and TSS (42.88%). Heritability was least in fruit width (31.68%). Thus, selection can be practiced to improve the yield characters since these traits are controlled by additive effects. The fruit weight had positive correlation with fruit volume, fruit length and width. However, negative correlation was observed with TSS. This character was negatively correlated with other four characters. The genotypic correlation was higher than phenotypic correlation for all the characters except TSS. This can be attributed to the relative stability of the genotypes. This happens not only when genes governing the traits are similar but environmental factors pertaining to it also have similar effects. Coheritability estimates were moderately high for most of the pairs of characters. The TSS goes down with the selection of big-sized fruits. However, selection of medium-sized fruits would not bring down the TSS.

The proportion of genetic and environmental variances for fruit weight (FW), flesh thickness (FLT), flesh weight (FLW), fruit firmness (FF), seed cavity weight (SCW), total soluble solids (TSS), titratable acidity (TA), juice acidity (pH) and ascorbic acid (AA) in guava were estimated with eight genotypes, four trees per genotype and five fruits per tree for two seasons by Thaipong and Boonprakob (2005) (Table 3.6). Eight clones were randomly selected from the collection of breeding materials. These consisted of six white flesh dessert types ('Klom Salee', 'Yensong', 'Pan Seethong', 'Khao Um-porn', 'Pan Yuk' and 'Nasuan'), one red flesh dessert type ('Philippines') and one pink flesh processing type ('Pijit 12-102'). A high proportion of genotypic variance was found with FW, FLT, FLW, SCW and AA indicating that genetic improvement for these traits through breeding and selection was achievable. Seasonal variance was high for pH, while among fruits within

**Table 3.6** Estimated variance values of the fruit characteristics of eight clones

Variance (%)						
Trait	Genotypic	Seasonal	Genotype by season	Trees within genotype	Tree by season	Fruits w. tree
FW	64.5	3.0	2.3	2.4	3.2	24.6
FLT	61.8	0.0	3.2	0.0	4.8	30.2
FLW	65.1	1.6	2.0	1.8	3.9	25.6
FF	4.2	22.5	15.2	0.0	7.4	50.7
SCW	43.6	10.7	7.0	6.3	0.0	32.3
TSS	21.2	26.9	7.4	0.2	2.7	41.6
TA	33.4	20.6	2.9	0.0	2.9	41.2
pH	6.3	61.0	5.3	4.2	4.2	19.0
AA	46.8	10.8	17.0	3.9	0.0	21.5

FW, fruit weight; FLT, flesh thickness; FLW, flesh weight; FF, fruit firmness; SCW, seed cavity weight; TSS, total soluble solids; TA, titratable acidity; pH, juice acidity; AA, ascorbic acid.

tree variance was greatest for FF, TA and TSS. The traits that were high in either season were more difficult to improve genetically.

### 3.6.3 Ploidy and Breeding in Guava

In a guava orchard with 1,600 plants in Brazil, Soubihe Sobrinho, Pompeu, and Gurgel (1961) have found six that differ greatly from the others in growing habit, leaf structure and low fruitfulness. The fruits presented apple-shape with no distinction from external and internal pulp that formed a mass with few seeds (25 on average). They observed that cells of these plants presented 44 chromosomes instead of 22 confirming them as tetraploid plants.

Subramanyam and Iyer (1993) in their review mentioned that cytological studies made on structure and behaviour of chromosomes in different varieties of *P. guajava* indicated that the meiosis was normal with the formation of 11 bivalents at diakinesis and normal distribution of the chromosomes at later stages. The diploid chromosome number of *P. friedrichsthalianum* was also  $2n = 22$ . A natural triploid was reported in the genus with somatic chromosome number to be  $2n = 33$  as also reported in a seedless variety of *P. guajava*, suggesting that triploidy is the cause of seedlessness in guava.

Ray (2002) reported that in guava, majority of the commercial varieties are diploids ( $2n = 22$ ) while the seedless variety is triploid and shy bearing in nature. To evolve a variety with less seeds and better yield potential, crosses were made between a triploid (Seedless) and diploid (Allahabad Safeda) at IARI, New Delhi. Out of 73 F1 hybrid seedlings, 26 were diploids ( $2n$ ), 9 trisomics ( $2n + 1$ ), 5 double trisomic ( $2n + 1 + 1$ ) and 14 tetrasomics ( $2n + 2$ ). Distinct variation in tree growth habit, and leaf and fruit characters were observed.

The breeding behaviour of aneuploids of guava (*P. guajava* L.) such as trisomic, tetrasomic and higher aneuploids has been studied by Mohammed and Majumder (1974). Reciprocal crosses between aneuploids and diploids indicated less than 100% crossability. The aneuploids when used as male parents crossed

less frequently than as female parents and certain aneuploids crossed more readily than others. Differences were observed in fruit size, fruit weight and seed number in the reciprocal crosses. The extra chromosome was found to be transmitted through both the egg cell and the pollen. However, the frequency of transmission was greater through the egg cell than the pollen. As high as 26% transmission of extra chromosomes were obtained through the egg cell. There was no clear-cut difference between trisomics and higher aneuploids with regard to the frequency of transmission of extra chromosomes.

Sharma and Majumdar (Anonymous 2006) identified a promising aneuploid rootstock for guava and demonstrated its potentials. The results on growth have highlighted the dwarfing effect of the rootstock on cultivar Allahabad Safeda. The tree size reduced significantly and it also showed higher yield potential, with an estimated yield of 28.33 tons of fruits per hectare. The trees were found to be tolerant to guava wilt. It produced fruits of better quality in terms of flesh thickness, vitamin C content, softness of seeds (due to light seed weight) and sweetness compared to Allahabad Safeda on its own roots. The trees grew to a height of 3–4 m, and were ideal for high density planting at a spacing of 3 m by 3 m. The short-statured plants had short internodes and small cup-shaped and lanceolate leaves. The dwarf rootstock is a tetrasomic guava developed by crossing a diploid (Allahabad Safeda) with a triploid (Seedless) variety. It has a wider adaptability, dwarfness and field tolerance to guava wilt.

### 3.7 Molecular Markers in Breeding

In agriculture, biotechnology has become a routine tool in cell and tissue culture to achieve rapid propagation of plant species; in diagnostics, for detecting plant pests and diseases based on the use of monoclonal antibodies and nucleic acid probe; and in genetic engineering of plant species, to introduce new traits and in aiding conventional plant breeding programmes using molecular markers (FAO 2003).

Morphological data have traditionally been used for variability evaluation. In order to supplement and refine the morphology-based descriptions, enzyme markers were used in a first approach to assess genetic variability (Belaj et al. 2003). Subsequently, DNA-based markers provided a new option for genetic studies and showed significant advantages as compared to morphological and biochemical markers (Sunil 1999; FAO 2003).

DNA markers are becoming increasingly important in a wide range of tasks: construction of genetic linkage maps; comparative mapping analysis; tagging economically important genes; marker-assisted selection and map-based cloning. They also provide genetic information in key areas of germplasm conservation both *ex situ* and *in situ* (Karp et al. 1997).

There is a great potential for the application of molecular markers to tropical, subtropical and indeed all perennial fruit crops. For instance, in fruit trees, this activity could be complicated by factors such as self-incompatibility, apomixes,

dioecy, seedlessness, embryo maturity, heterozygosis and long juvenile periods. Consequently, conventional breeding and assessment based on morphological markers could be a difficult and slow process (Moore and Durham 1992).

Although biotechnology is becoming increasingly important in agriculture, the fact that over 50% of the agricultural productivity in the world has been achieved through traditional plant breeding should not be ignored. Although DNA marker technology cannot replace plant breeding, it will certainly facilitate this activity by providing new tools to ease the many problems faced by breeders (Sunil 1999).

### ***3.7.1 Types of Molecular Markers***

Molecular markers that reveal polymorphisms at the protein level are known as biochemical markers, whereas DNA markers do it at the DNA level. The former are proteins produced as a result of gene expression that can be separated by electrophoresis to identify the alleles. The most commonly used are isozymes that are variant forms of the same enzyme (Vodenicharova 1989). Protein markers reveal differences in the gene sequence and function as co-dominant markers.

Depending upon how the polymorphism is revealed, DNA-based markers can be classified into two categories: hybridisation-based polymorphisms and PCR-based polymorphisms. Some authors have considered a third category that combine both.. DNA markers can be both dominant and co-dominant (Valadez and Khal 2000).

In guava, different PCR-based techniques have been used to verify DNA quality, to establish fingerprint of individual accessions, to assess the genetic diversity, to construct a genetic linkage map and for tagging economically important genes for marker-assisted selection. These are Random Amplified Polymorphic DNA (RAPD) (Williams et al. 1990), Amplified Fragment Length Polymorphism (AFLP) (Vos et al. 1995), Inverse Sequence-Tagged Repeat (ISTR) (Rohde 1996) and Simple Sequence Repeat (SSR) (Litt and Luty 1989; Tautz 1989; Weber and May 1989).

### ***3.7.2 Applications of Molecular Markers for Guava Breeding***

#### **3.7.2.1 DNA Isolation and Purification Protocols**

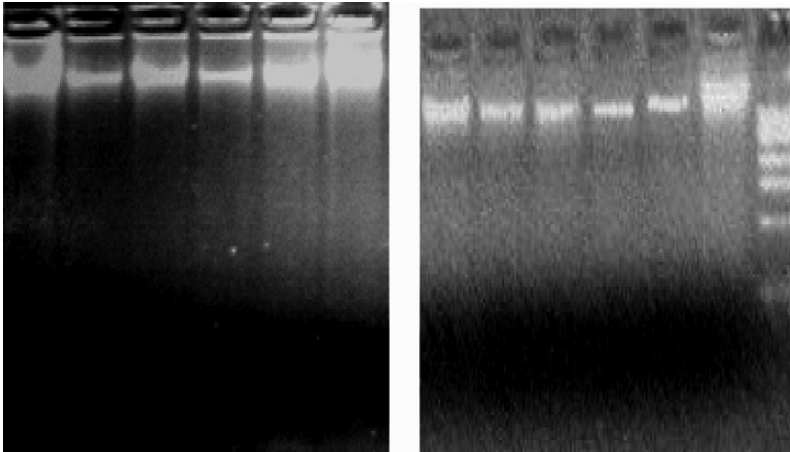
The different applications of molecular markers in agriculture have allowed breeders to increasingly utilize it in breeding programmes. Nevertheless, to ensure a routine use of Molecular Biology, it is a pre-requirement to have protocols that would enable fast DNA isolation and purification. Several methods that make possible direct amplification of plant genomic DNA from leaf, seed and root tissue have been reported (Rehman et al. 2001). Some of these techniques, while rapid, have inherent problems of contaminants, such as polysaccharides, polyphenols and other secondary metabolites, which can inhibit the amplification reaction (Sharma et al. 2000).

When DNA was first isolated from a plant species, problems invariably occurred due to the presence of the contaminants mentioned above. With cell rupture, polyphenols and polysaccharides can make contact with nuclei and other organelles. In their oxidized forms, polyphenols bind to DNA covalently, giving it a brown colour and making it useless for most research applications (Rogstad et al. 2001). Polysaccharides are detected in DNA solution by their viscous, glue-like texture, which difficult the pipetting and also makes DNA no amplifiable by inhibition of the Taq polymerase activity and unrestrictable for endonuclease digestion (Sharma et al. 2002).

For tropical fruit trees, these problems have already been reported (Guillermout and Marechal-Drovart 1992). In guava, leaves are far sensitive to oxidation, resulting in polyphenol presence. A more serious problem is the extremely high content of polysaccharides that co-precipitate with DNA throughout the standard purification (Ramírez et al. 2004). Several protocols have been performed to obtain good DNA quality and concentration (Prakash et al. 2002; Hernández et al. 2003; Rueda et al. 2003). Also, a variation (Ramírez et al. 2004) of the CTAB method described by Doyle and Doyle (1990) and further purification using NucleoSpin Extract Method (Macherey-Nagel 2002; Fig. 3.4) have been used, providing very good results for molecular applications in this crop.

### 3.7.2.2 SSR Development from *P. guajava* L.

(GA)<sub>n</sub> and (GT)<sub>n</sub> micro-satellite-enriched library was developed to improve the type of molecular markers available for genetic studies and further marker-assisted selection in guava as well as its close related species (Risterucci et al. 2005). To



**Fig. 3.4** DNA quality and concentration extracted by a modification (Ramírez et al. 2004) of the CTAB method described by Doyle and Doyle (1990) followed by an additional purification with NucleoSpin Extract Method (Macherey-Nagel 2002). Left: Before NucleoSpin purification; Right: After NucleoSpin purification. M: 1 kb DNA ladder marker

determine the usefulness of the primers designed, DNA samples of guava from diverse origins (Cameroon, Colombia, Cuba, Florida, Hawaii and Martinique) and also from *P. acutangulum* D.C., *P. cattleianum* Sabine var. *lucidum* and *P. friedrichsthalianum* (O. Berg.) Nied. were utilized for PCR amplification. All the SSR primers have been successfully amplified in *P. guajava* L. For the rest of *Psidium* species, except for four loci, the amplification revealed reliable SSR patterns. This library appears to be the first reported for guava and can be used for genotype identification, pedigree analysis, germplasm diversity and mapping studies. Furthermore, it is a potentially useful molecular resource for genetics investigations in the genus *Psidium* (Risterucci et al. 2005). The primer combinations mPgCIR05, mPgCIR07, mPgCIR09, mPgCIR10, mPgCIR11, mPgCIR15, mPgCIR16 and mPgCIR19 revealed clear polymorphism in guava accessions from Cuban germplasm (Rodríguez et al. 2007).

### 3.7.2.3 DNA Markers for Guava Fingerprinting

The use of molecular markers to establish fingerprint of individual accessions have been suggested for several crops (Sunil 1999). In addition, the International Union for the Protection of New Varieties of Plants (UPOV) is pushing for a distinct, uniform and stable (DUS) testing, the introduction of new test methods and to overcome the legal implications of such changes for plant variety protection (Donini et al. 2000).

In this regard, one approach was the use of four isoenzymatic systems ( $\alpha$ -esterase,  $\beta$ -esterase, acid phosphatase and peroxidase) to distinguish intra- and inter-specific variation on *Psidium* spp. However, a clear genotyping was not observed (Albany et al. 1998). Isozymes markers sometimes exhibit an insufficient polymorphism. In addition, spatial-temporal and environment variation could also occur (Dettori and Palombi 2000). Then, their use for identification purposes is restraint to a local germplasm as isoenzyme profiles are not transferable.

With the advent of PCR-based marker system, RADP, AFLP and micro-satellites (SSR) techniques have been the common choice for variety identification in fruit trees (Tessier et al. 1999; Dettori and Palombi 2000; Aranzana et al. 2001; Belaj et al. 2003), but to date, AFLP and micro-satellites are the prevalent option for variety profiling and, hence, identification (Donini et al. 2000). In addition to this, a remarkable degree of polymorphism detected through retrotransposon sequences has also been reported (Inverse Sequence-Tagged Repeat [ISTR]) (Ramírez et al. 2002; Capote et al. 2003).

The selection of a given marker is a balance between the level of polymorphism it can detect (information content) and its capability to identify multiple polymorphisms (Powell et al. 1996). Tessier et al. (1999) defined the D parametre (discriminating power), which evaluates primer efficiency for varieties identification (i.e. the probability that two randomly chosen individuals have different patterns). The D parameter can be used to compare different type of markers even if only the allele frequencies are known.



Despite the fact that a comparative study related with the use of molecular markers for guava fingerprinting is still to be developed, the utility of AFLP, ISTR and SSR for genotype identification have been corroborated (Hernández et al. 2003; Rodríguez et al. 2003; Valdés-Infante et al. 2003; Rodríguez et al. 2007). Besides, RAPD analysis has proven good results (Prakash et al. 2002; Rueda et al. 2003), although their reproducibility across different laboratories remain under discussion (Donini et al. 2000).

#### 3.7.2.4 Genetic Diversity Analysis for Guava Germplasm

A pre-requirement for improving the overall plant characteristics is the knowledge of the structure of the germplasm collection that in turn will lead to a systematic sampling for breeding and conservation purposes. DNA markers have been used to quantify the genetic diversity and determine phylogenetic relationships (Sunil 1999).

Understanding and management of the natural variation present within the domestic cultivars and wild relatives of a plant species is essential for the establishment of an efficient programme aiming at crop improvement. Taking advantage of natural variation is very important for several reasons: genetic uniformity is undesirable because it tends to make the crop vulnerable to epidemics and environmental disasters resulting in yield loss. Many wild relatives contain genes that confer resistance to biotic stresses such as pests and diseases, as well as tolerance to abiotic stresses such as drought, cold and salinity. When such traits are incorporated into economically important varieties, large yield losses can be prevented (Sunil 1999).

Comparisons of molecular markers for measuring genetic diversity have been carried out in several plant species (Belaj et al. 2003), but to our knowledge, such studies have not been so far reported in guava. However, different molecular markers have been used individually to assess the genetic diversity.

Some studies on Myrtaceae have revealed the utility of isozyme to assess the structure and diversity in *Eucalyptus* spp; *Eugenia dysenterica* D. C. and camucamu (*Myrciaria dubia*) (Kunth) McVaugh populations (Turner et al. 2000; Pires de Campos et al. 2001; Teixeira et al. 2004). Although biochemical markers have not been broadly exploited in guava, they can be a potential tool for variability estimation in this species.

Rueda et al. (2003) found a relatively high level of genetic diversity in Corpoica Palmira germplasm (Colombia); while Prakash et al. (2002) detected from low to moderate variability in India germplasm, both using the same molecular marker (RAPD). On the other hand, Valdés-Infante et al. (2003) detected a low level of diversity using AFLP, although micro-satellites revealed a moderate heterozygosity level in the same Cuban guava germplasm (Rodríguez et al. 2007). This difference might result from the inheritance of each molecular marker and the genomic region explored. The codominant nature of SSRs markers allows the detection of a high number of alleles per locus and contributes to detect higher levels of expected heterozygosity than AFLPs. However, this also depends on the species under study (Belaj et al. 2003).

Genetic diversity can be associated with geographical origin of different genotypes within species. In addition, climatic differences in the same region can lead to ecotypes and therefore to new variability sources (Zizumbo-Villauea et al. 2005). Using RAPD markers in guava germplasm collections, some authors have identified genotypes coming from diverse foreign regions (Prakash et al. 2002; Rueda et al. 2003). Nevertheless, overall interpretation of the genetic relationships among guava accessions with AFLP (Valdés-Infante et al. 2003) and SSR (Rodríguez et al. 2007) in Cuba indicates the absence of separate clusters representing local and foreign germplasm. This reflects the selection of guava lines from open pollination rather than from controlled crosses. Micro-satellites also detected a high number of alleles shared for the majority of guava genotypes in this germplasm. This suggests that most of the plant material analysed shares a common genetic ancestry; this comes from the fact that relatively few accessions were used for breeding programmes and many hybrids derived from them were conserved in the germplasm bank. On the other hand, some individual and combined rare alleles were detected in such accessions. This information provide ground for parental selection in guava breeding programmes and conservation strategies.

The correspondence among the results derived from individual data sets is by far the most important issue to be considered when combining different data sets. Several studies in recent years have analysed correlation among genetic distance-similarity matrices derived from the application of various DNA-based marker systems. However, very few analyses have attempted to compare results originated from individual versus combined data, regarding the genetic diversity assessment and collection management (Mohammadi and Prasanna 2003).

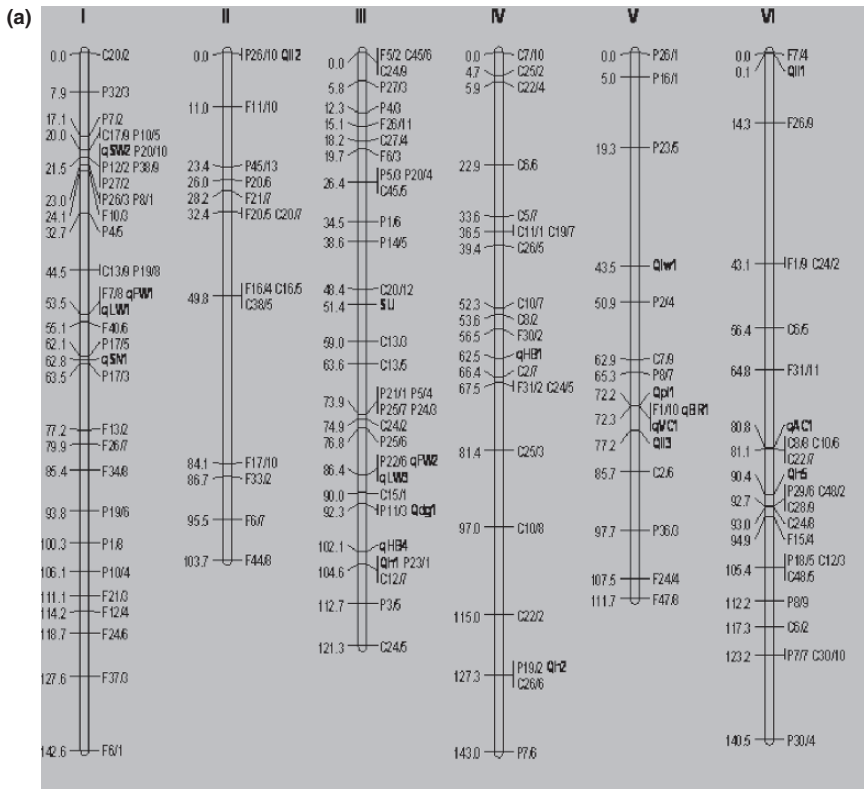
The generation of a higher number of polymorphic markers is not necessarily correlated with the resolution power (Capote et al. 2003). Although AFLP detected lower diversity than SSR in the guava collection from Cuba, the second could not identify between two highly related genotypes 'N6' and 'Ibarra', using the same primer number (Rodríguez et al. 2007). This corroborates the necessity of an integrated study to prevent wrong deductions related to misclassification, duplicates detection and variability estimation.

Also, a comprehensive analysis of data set of distinct nature (morphological, biochemical and DNA markers) to ascertain whether such combination provides a better understanding of genetic diversity is highly scarce (Mohammadi and Prasanna 2003). The use of different methods to evaluate genetic diversity may reveal dissimilar patterns of variation. Phenotypic differences are not necessarily correlated with the number of underlying gene mutations and differences in phenotypic characters are not necessarily reflections of different genetic events (Persson 2001). Besides, morphological traits are often influenced by environmental conditions. On the other hand, DNA markers can cover coding as well as non-coding regions of the genome. For that reason, classical methods for evaluating genetic variation have been complemented by molecular techniques (Persson 2001). The low correlation coefficient detected by Rodríguez et al. (2004) from the comparison of similarity matrixes between morph-agronomical and AFLP analysis in guava germplasm corroborates this assertion.

### 3.7.2.5 Construction of Guava Genetic Linkage Map

In order to efficiently use the countless polymorphisms as genetic markers, knowledge of their individual genomic locations is necessary and this information can be obtained by constructing a genetic linkage map. Thus, a genetic linkage map graphically represents the arrangement of the innumerable loci, which includes morphological and isozyme as well as DNA markers along with the chromosome (Sunil 1999).

For a future implementation of marker-assisted selection to improve the efficiency of guava breeding programme in Cuba, three mapping populations were produced under controlled-pollination conditions with three individual trees of the cultivar ‘Enana Roja Cubana’ as the female parent and pollen from cultivars ‘N6’ (mapping population 1 = MP1), ‘Suprema Roja’ (MP2) and ‘Belic L-207’ (MP3).



**Fig. 3.5 a and b:** Integrated molecular linkage map for guava mapping population MP1 (Rodríguez et al. 2007). Single linkage groups are indicated in roman numbers with distances on the left given in cM. Mapped AFLP markers are listed by their origin as to the parent (P: parent 1, ‘Enana Roja Cubana’; F: parent 2, ‘N6’; C: marker common to both parents) and the AFLP primer combinations (e.g. P17/3 is the parent 1-specific AFLP fragment #3 produced by primer combination 17). The mapped QTLs are indicated in bold

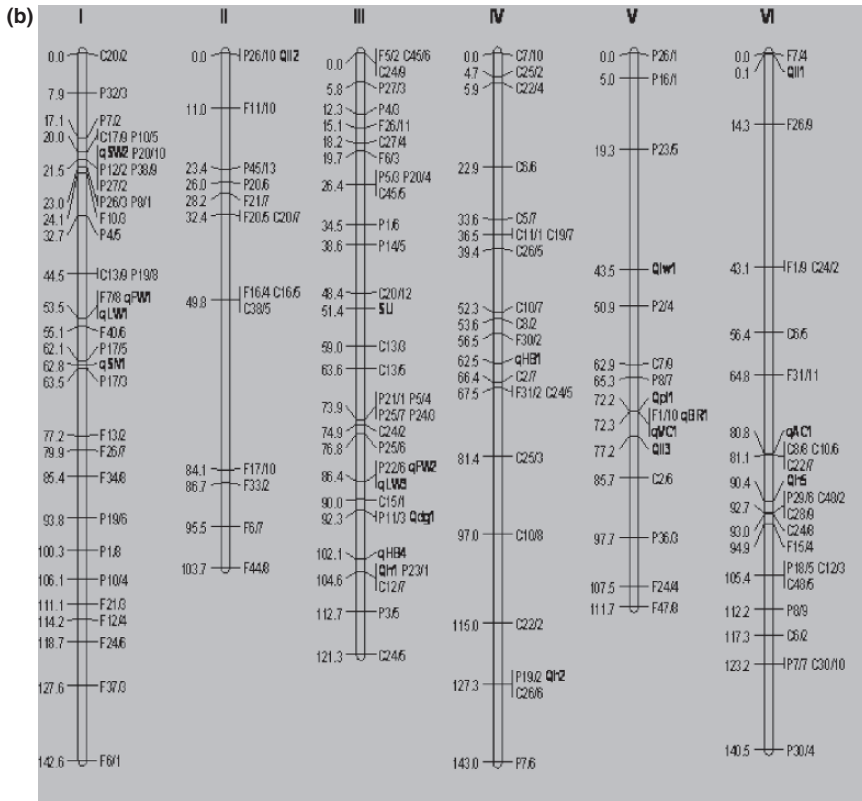


Fig. 3.5 (continued)

The MP1 was used for the initial identification of co-segregant markers in the progeny by AFLP technique.

Based on these results, the first genetic linkage map was established by Valdés-Infante et al. (2003) with a total of 167 markers mapped onto 11 linkage groups that presumably represent the 11 chromosomes of the haploid guava genome. These efforts were further extended by increasing the number of AFLP primer combinations and mapping additional markers onto the linkage map. The high number of common markers enabled the fusion of the two individual parental maps into an integrated linkage map for the two guava genotypes with a total of 220 markers mapped up to date (Fig. 3.5). The individual linkage groups contain from 11 to 30 markers each, vary in length between 104 and 150 cm, and result in a total map length of 1,379 cm (Rodríguez et al. 2007).

Although the initial investigations about genetic linkage maps have been developed on cereals species, this technique can represent an important and efficient tool for fruit trees breeding programme; due to long juvenile periods that delay the evaluation and characterisation of fruit and overall plant for many years (Moore and Durham, 1992; Kijas et al. 1997).

### 3.7.2.6 Marker-Assisted Selection in Guava

A direct application of genetic linkage maps has been in tagging genes of economic importance with molecular markers (Mohan et al. 1997). In general, the likelihood of identifying a marker linked to a gene is inversely proportional to the distance between the marker and the gene. Several important traits such as yield, fruit quality and maturity, and resistance to several biotic and abiotic stresses are controlled by a relatively large number of loci, each of which makes a small positive or negative contribution to the final phenotypic value of the trait. Such loci are termed ‘quantitative trait loci’ (QTLs) and those traits that show a continuous variation in phenotype are termed ‘polygenic traits’ because the final phenotypic expression is determined by the genetic variation at a large number of loci, modified by environmental effects (Sunil 1999).

By using molecular markers, chromosomal positions can be assigned to individual QTLs in order to establish the types and magnitude of gene effects of individual QTLs and also to determine which parent possesses the positive allele at each QTL (Sunil 1999).

Morphoagronomic characters such as leaf length, leaf width, petiole length, height and the growth rates for height and trunk diameter were recorded at regular intervals on the established guava mapping population (MP1; see 3.7.2.5 topic). Fifteen QTL loci, which originated predominantly from ‘N6’ (male parent of MP1; tall genotype in contrast to the female dwarf ‘Enana Roja cubana’) could be mapped in total (Valdés-Infante et al. 2003).

Subsequently, further characters related to fruit quality (fruit width and weight, seed number and seed weight, total soluble solids, acidity, vitamin C content and pulp thickness) were also assessed. In total, 21 QTLs were identified for these traits and mapped onto different linkage groups of the integrated linkage map (Fig. 3.5). Additional efforts along these lines will form the basis for marker-assisted selection (MAS) in guava breeding programme (Rodríguez et al. 2007).

### 3.7.3 New Trends

The results presented here are a further step (1) to compare the discriminating capacity and informativeness of the different molecular markers for genotype identification and genetic diversity analyses; (2) to determine the genetic similarity estimates and genetic relationships among genotypes as well as to compare the patterns of variability between morph-agronomic and molecular markers; (3) to characterise wild relatives looking for germplasm diversity and resistance to biotic and abiotic stresses; (4) to increase the marker density of the guava molecular linkage map; (5) to identify co-dominant DNA marker such as micro-satellites for an alignment of individual maps into a guava reference map; (6) to identify markers that co-segregate with important breeding traits; (7) to detect resistance gene-like sequences (RGLs) as potential candidates for resistance genes to map these RGLs

onto the guava map and record a putative segregation of tolerance in the mapping population to pest and diseases.

### 3.8 New Varieties: Present and Future

Research institutions from countries where guava is a cash crop, such as India, Brazil, Mexico and others devote substantial efforts to produce and release new varieties. The example of India, where guava improvement work for the first time was initiated during 1907 at Ganeshkhind Fruit Experimental Station, must be followed by others. Subramanyam and Iyer (1993) listed a number of promising hybrids from different Research Stations in India, resulting from a survey of many authors and their own work.

At Narendra Dev University of Agriculture and Technology, Faizabad, out of the 23 strains collected as a result of survey in guava growing region, 3 seedlings of Allahabad Safeda (AS1, AS2 and AS3) and 2 of Faizabad Selection (FS1 and FS2) were found to be promising with respect to fruit quality and yield. From plantations around Navalur, a village in Karnataka, 16 high performing seedlings were selected from the variety Navalur, which is hardy, drought tolerant and canker resistant, based on fruit quality, yield and plant characters. Twelve strains were collected from Aurangabad and Bhir districts of Marathwada, out of which ABO 3 and BHR 3 and 5 were observed to be superior. At IIHR, Bangalore, from 200 open pollinated seedlings of variety Allahabad Safeda, one seedling selection, Selection-8, was found to be promising. Plants are dwarf and give higher yields.

At IIHR, Bangalore, by hybridisation among Allahabad Safeda, RedFlesh, Chitidar, Apple Colour, Lucknow-49 and Benaras, 600 F1 progenies were raised. Two selections, Hybrid I and Hybrid 16-1 were found to be promising. Hybrid-I: hybrid between Seedless and Allahabad Safeda, giving heavy yield. Fruit size is medium, pulp is white with few soft seeds. Fruit quality in terms of sugar content and TSS is excellent. Keeping quality is good. Hybrid 16-1: from a cross between Apple Colour and Allahabad Safeda, showing plants semi-vigorous plants giving a moderate yield. The fruit skin has a very attractive bright red colour. The flesh is firm, white with very high TSS and good keeping quality. The seeds are few and soft.

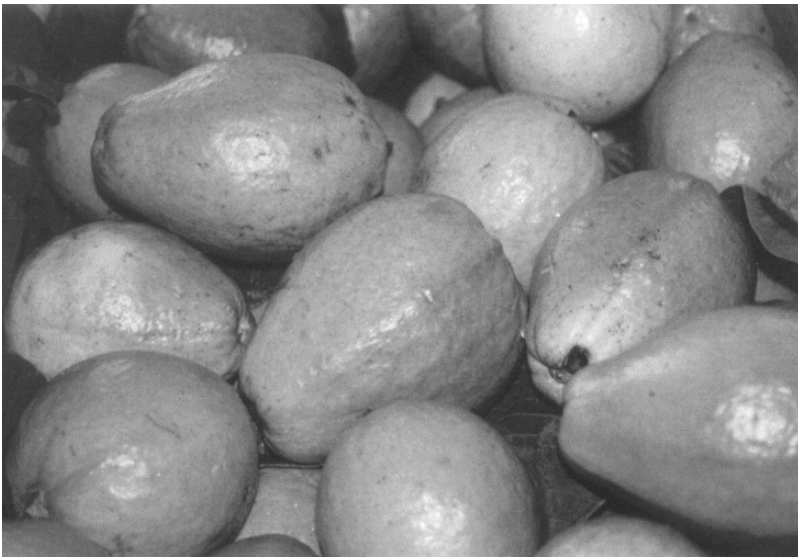
At Horticultural Experiment and Training Centre, Basti, inter-varietal hybridisation was undertaken to obtain a variety with higher vitamin C and attractive skin and flesh colour utilizing the cultivars Allahabad Safeda, Seedless, Lucknow-49, Patilla, Apple Colour, Kothrud and Red Flesh. Fifty-five F1 hybrid seedlings were obtained by crossing which are being evaluated. At Fruit Research Station, Sangareddy (AP), inter-varietal hybridisation resulted in the isolation of two superior hybrids, Safed Jam and Kohir Safeda, which were released for commercial cultivation, particularly in semi-arid tropical areas of Telangana and Rayalseema. Safed Jam: a hybrid between Allahabad Safeda and Kohir, is similar to Allahabad Safeda in growth habit and fruit quality. The fruits are bigger in size with good quality and few soft seeds.

Kohir Safeda: it is a heavy yielding cross of selected line of Kohir x Allahabad Safeda. Tree is vigorous, the fruits are larger with few soft seeds and white flesh.

Ray (2002) described 8 guava cultivars recently developed through selection, such as Allahabad Surkha, Lalit and Bangalore local and other 12 superior guava hybrids developed at different fruit research centres in India, such as Safed Jam and Kohir Safeda. One hybrid 'Arka Amulya' has already been released and two (Hybrid 16-1 and Hybrid 31-1) are likely to be released shortly on account of their better characteristics.

Pereira and Nachtigal (2002) in Brazil, at UNESP, Jaboticabal, started in 1976 a selection programme of new cultivars, through the introduction and selection of seedlings originated from open pollinated American, Indian and Brazilian varieties of different provenance and local selections, leading to the obtaining of the cultivars Rica and Paluma (Fig. 3.6). Since 1985, in that same institution, the second phase of genetic improvement of the guava tree is under development, with the goal of obtaining plants with favourable agronomic attributes and fruits that can be destined to industrialisation as well as for consumption as fresh fruit. After a long evaluation and selection period, the programme achieved selections with potential as new options for the guava growers. The following crossings were accomplished:

- 8501 – Rica × EEF-3
- 8502 – Supreme-2 × Paluma
- 8503 – Rica × Patillo 5
- 8504 – Paluma × Rica.



**Fig. 3.6** Paluma, the guava cultivar most planted in Brazil  
(Source: TodaFruta ([www.todafruta.com.br](http://www.todafruta.com.br)))

The following genotypes were selected:

8501-01 – It presents productive and vigorous plants and normal maturation period; the fruits are of medium size (122 g without thinning), ovoid, with neck of reduced size, thickness of pulp of 118 mm and around 74%; the pulp is of rosy colour, with soluble solids of 8.4° Brix, relationship TSS/TA 18 and vitamin C content of 134.25 mg ascorbic acid.100 g of pulp-1. The main characteristic of this selection is low susceptibility to the psyllidium (insect, Psyllidae) attack.

8502-01 – Productive plants, with ramifications predominantly horizontal, medium vigour and precocious maturation (about 130 days from blossom to the maturation of the fruits); it presents fruits of big size (193 g without thinning), with a ratio of firm pulp of 76%, thick pulp (137.5 mm), ovoid, with neck of reduced size; the pulp is of rosy colour, intense and brilliant, with total soluble solids content close to 10° Brix, relationship TSS/TA close to 20 and vitamin C content around 100 mg of ascorbic acid.100 g of pulp-1; presents few seeds and reduced size.

8503-08 – Presents productive and vigorous plants, and with precocious maturation period; the fruits are of medium size (127 g without thinning), ovoid, with neck of reduced size, thickness of pulp of 113 mm and ratio of firm pulp around 72%; the



**Fig. 3.7** Século XXI (XXI Century), a new guava cultivar in Brazil  
(Source: TodaFruta ([www.todafruta.com.br](http://www.todafruta.com.br)))



pulp is of rosy colour, with soluble solids content of 8.8°Brix, relationship TSS/TA 17 and vitamin C content of 101.45 mg ascorbic acid/100 g of pulp-l. The main characteristic of this selection is the production of fruits lacking a strong pungent odor, even when ripe, characterizing most guava cultivars.

In Brazil, Pereira, Carvalho and Nachtigal (2003) released the XXI Century (Fig. 3.7) guava cultivar obtained from 219 plants originated from several crossings after 10 years of evaluation. It was from the cross Supreme-2 × Paluma and its main characteristics are a very productive plant with a short cycle (130 days from bloom to harvest), big fruits (236 g in average) with thick pulp, rosy-red, great flavour and with little and small seeds.

At least in Brazil, it is quite evident that increases in the area planted with guava and the high yields obtained in the main producing regions are due to the availability of improved varieties, such as Paluma.

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