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Breeding Loquat

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ABSTRACT

Loquat (*Eriobotrya japonica* Lindl., Rosaceae, Maloideae) is a subtropical, evergreen fruit tree ($2n = 34$) indigenous to China, that blooms in the fall and early winter, and ripens in the spring. The association of fruit ripening with a traditional Spring Festival in South China makes it one of the most popular fruits of that area but loquat is now being grown in various other countries and is being exported from Spain. Most loquat cultivars are derived from clonal selection from open-pollination but at present various hybridization programs

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have been carried out for both scions and rootstocks. Seedless loquat has been achieved by application of gibberellic acid to triploids that occur infrequently by nonreduction in open-pollinated diploids. Advances in genomics offer possibilities for marker-assisted selection (MAS), and the transfer of useful genes into loquat from other species within the Rosaceae.

KEYWORDS: *Eriobotrya japonica*; Rosaceae; seedlessness; selection; synteny

I. INTRODUCTION

Loquat (*Eriobotrya japonica*) is a subtropical evergreen fruit tree that blooms in the fall and early winter and ripens in the spring. The tree is cold hardy to −10°C but fruits are damaged at −3°C. Production is particularly well suited for Mediterranean climates. Despite its Latin binomial loquat is indigenous to Southern China and its English name derived from the Chinese *luju* although it is better known there as *pipa*. Its various names include *bibassier* or *neflier du Japon* (French), *níspero japonés* (Spanish), *Japonische mispel* (German), *nepola Giaponese* (Italian), emeixa do Japao (Portugal), and Japanese plum or medlar (United States).

Loquat has been cultivated in China for over 2000 years and many species occur there in the wild state (Lin et al. 1999). Loquat was introduced to Japan in ancient times and described as early as 1189. Although long a favorite fruit in Japan, crop area has recently declined since cultivation is very labor-intensive. It was first described in the West by the German traveler and physician Englebert Kaempfer who observed it in Japan and named it *Amoenite Exotic* (1712), and more fully described by Thunberg (1784) who named it *Misfiles japonica* or Japanese medlar. In 1784, the loquat was introduced from Guangdong, China into the National Garden at Paris and reached the Royal Botanical Gardens at Kew, England in 1787. The genus was subsequently changed to *Eriobotrya* by John Lindley in 1812, in reference to the woolly clustered panicles. It was introduced to Florida from Europe and to California from Japan between 1867 and 1870. Chinese immigrants carried the loquat to Hawaii. Loquat was distributed around the Mediterranean to various countries including Algeria, Cyprus, Egypt, Greece, Israel, Italy, Spain, Tunisia, and Turkey. Cultivation spread to India, Nepal, Pakistan, and Southeast Asia including Korea, Laos, and Vietnam and reached Australia, New Zealand, Madagascar, and South Africa. It is now grown in Armenia, Azerbaijan, and Georgia and in the Americas including Argentina, Brazil, Chile, Ecuador, Guatemala, Mexico, and Venezuela.

Loquat fruits are round, ellipsoid, to obovate with diameter ranging from 2 to 5 cm and average weight from 30 to 70 g but larger fruit is possible. The thin peel and flesh is white or orange. Soluble solids content vary from 7% to 20%. The seeds, usually about three to five per fruit, are relatively large, each about 1.2–3.6 g and may be obtrusive when the fruit is consumed fresh. The fruit can be process in various forms including juice and wine. The flesh is about 70% of seeded fruit by weight and is aromatic, juicy, delicately flavored, and often considered delicious. However, loquat has some serious drawbacks including the high number of large seed, poor storage life, and susceptibility to bruising. Leaves and fruits have been traditionally considered to have high medical value and there is evidence of pharmaceutically active compounds (Lin et al. 2007).

Loquat is extremely popular in China because it is the first tree fruit of the season ripening around the popular Spring Festival. Production in China reached 512,000 tonnes (t) from 133,000 ha in 2010 and is increasing. Spain, the leading world exporter, produces 40,000 t from about 3,000 ha. Significant producers include Turkey, India, Japan, and Pakistan with small production in Brazil, Chile, Guatemala, Greece, Israel, Italy, Morocco, and Portugal. Although loquat is a minor crop in most of the world, there is considerable room for growth of the industry.

Breeding has long been identified as a priority for expansion of the loquat industry. Most major cultivars of loquat are derived from chance seedlings and there are many selections in the various provinces of China. Breeding programs based on hybridization have been initiated in several countries. The objective of this chapter is to review the current state of loquat genetics and breeding.

II. GERMPLASM

A. Genus *Eriobotrya*

The number of loquat species had been under dispute and the opinions of authors in different countries vary. As described in the literature and collected as specimens in herbariums in China, there are about 32 species or variants (Lin et al. 2004; Yang et al. 2005); most originated in Southern China with the rest from Southeastern Asia (Table 5.1). From 2005 to 2011, S. Lin and his team searched for these species or variants from China and Southeast Asia and found most of them. Only three species were not found such as *E. bengalensis* f. *contract* Vidal, *E. hookeriana* Decne, and *E. philippinensis* Vidal.

Species and variants in China could be divided into three groups (Fig. 5.1) based on stamen and style number and leaf size (Yang et al. 2007). The first group has about 15 stamens, two to four styles, and small leaves (width < 2 cm) and consists of three species: *E. seguinii* Gard, *E. henryi* Nakai, and *E. angustissima* Hook. f. The second group has 20 stamens, five styles, and large leaves (width >5 cm) and consists of three species: *E. japonica* Lindl., *E. malipoensis* Kuan, and *E. elliptica* Lindl. and two additional variants of this species: var. *petelottii* Vidal and f. *petiolata* Hook. The third group has 20–30 stamens, 2–4 styles and medium sized leaves, and is divided into four subgroups based on their

Table 5.1. Loquat species and varieties as listed in diverse literature and/or indicated on specimens in Chinese herbaria.

Eriobotryo species	Representative area
E. angustissimo Hook. f	Laos, Southern Vietnam
E. bengalensis Hook. f.	South-western China
f. *angustifolia* Vidal	Yunnan, China
f. *contract* Vidal	Vietnam
f. *intermedia* Vidal	Yunnan, China
E. cavaleriei Rehd.	Guangdong, China
E. deflexa Nakai	Taiwan, China
var. *buisanensis* Nakai	Taiwan, China
var. *koshunensis* Nakai	Taiwan, China
E. elliptica Lindl.	Tibet, China
var. *petelottii* Vidal	Vietnam
f. *petiolata* Hook.	Tibet, China
E. fragrans Champ.	Guangdong, China
var. *furfuracea.* Vidal	Vietnam
E. henryi Nakai	Yunnan, China
E. hookeriana Decne	Tibet, China
E. japonica Lindl.	Southern China
E. kwangsiensis Chun	Guangxi, China
E. latifolia Hook.	Moalmayne
E. macrocarpa Kurz	Burma
E. malipoensis Kuan	Yunnan, China
E. obovata W.W. Smith	Yunnan, China
E. philippinensis Vidal	Philippines
E. poilanei Vidal	Vietnam
E. prinoides Rehd. & Wils.	Yunnan, China
var. *laotica* Vidal	Laos
var. *dadunensis* H.Z. Zhang	Sichuan, China
E. salwinensis Hand.-Mazz.	Yunnan, China
E. seguinii Card	Guangxi, China
E. serrata Vidal	Yunnan, China
E. stipularis Craib	Cambodia
E. tengyuehensis W.W. Smith	Yunnan, China

distribution as shown in Fig. 5.1. Subgroup 1 is distributed around Southern China and South Asia and Southeast Asia. Subgroup 2 is distributed along valley of the Pearl River. Subgroup 3 is distributed along Salvin River, and Subgroup 4 is divided along the Mekong River.

Among these species, only *E. japonica* is cultivated for its fruits. *E. deflexa* and *E. prinoides* have been used as rootstock, but they are less widely used than *Photinia serrulata* Lindl. in China and *Cydonia, Malus, Pyrus*, and *Pyracantha* in Mediterranean regions. Although more than 95% of loquat are grafted on common loquat (*E. japonica*)

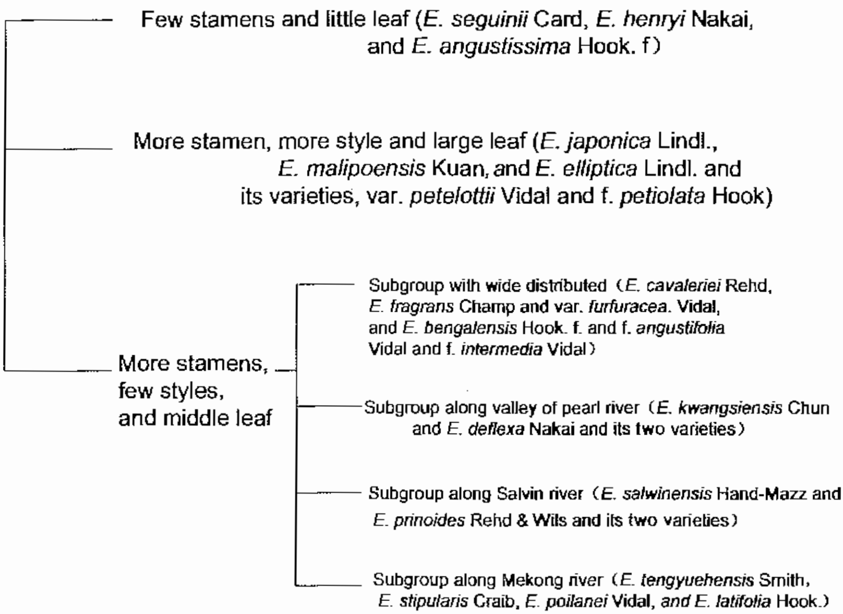

Fig. 5.1. Loquat species in China.

seedlings, other species of *Eriobotrya* could be used as rootstock and 10 species are under evaluation in China. *E. cavaleriei* Rehd. has been used to make wine and *E. prinoides* Rehd. & Wils. has been used in Chinese medicine with additional species under evaluation for this purpose.

B. Species *E. japonica*

There are more than 500 cultivars in China, most of which are seedling selections that are no longer in production although they are all conserved in the National Fruit Germplasm Repository iu Fuzhou. There are 83 cultivars (or selections) in Zhejiang, more than 100 in Fujian, 57 in Jiangsu, 31 in Anhui, 18 in Guangdong, and 9 in Sichuan. However, less than 50 cultivars are widely cultivated around China. Germplasm banks are also located in Spain (ca. 100 cultivars), and Japan (<100 cultivars). Spanish commercial production depends on only four cultivars: 'Magdal,' 'Algerie,' 'Golden Nugget (from United States)', and 'Tanaka (from Japan),' with the majority of production from 'Algerie'. Three cultivars, 'Mogi', 'Tanaka', and 'Nakasakiwase', account for 95% of the total loquat commercial area in Japan. The characteristics of the major cultivars grown worldwide are presented in Table 5.2.

Table 5.2. Major loquat cultivars in the main producing areas of the world.

Country	Area	Name of cultivar	Origin	Outstanding characteristics
Brazil		Nectar de Cristal	Obtained by open pollination of Togoshi (Japan), 1970s	Yellowish-red flesh, high yield, fruit uniformity
		Parmogi	Obtained by open pollination of Mogi (Japan), 1970s	Yellowish-red flesh, high yield, pleasant taste
		Precoce de Itaquera	Selected from Japanese seedling	Yellowish-red flesh, very productive
China	Anhui	Guangrong	Selected from seedling of Dahongpao	Yellowish-red flesh, vigorous growth, stable yield, quite large fruit, good keeping quality
	Fujian	Changhong No. 3	Selected from a natural hybrid seedling of Changhong, 1990	Yellowish-red flesh, elongate-obovate fruits, 50 g, ripening in mid-April; high stable yield
		Guifei	Selected from seedling of whitish cultivar	Whitish flesh, fruit 52–68 g, Brix 13.8–15.2°
		Jiefangzhong	Dazhong seedling, selected 1950	Yellowish-red flesh, large fruits, average 70 g with some as large as 172 g; high yield
		Zaozhong No. 6	Jiefangzhong × Moriowase, 1992	Yellowish-red flesh, ripening in the beginning of April, average 53 g, attractive, good quality
	Jiangxi	Duhe	Introduced from unknown cultivar, 1958	Yellowish-white flesh, single seed, high yield, single seed, medium eating quality
	Sichuan	Dawuxing	Seedling	Yellowish-red flesh, large fruits with star-like calyx-lobe
		Longquan No. 1	Seedling	Yellowish-red flesh, high yield
	Zhejiang	Dahongpao	Old seedling cultivar	Whitish flesh, vigorish growth, stable yield
		Loyangqing	Selected from Dahongpao, 1980s	Yellowish-red flesh, strong disease resistance, high stable yield, good keeping quality

(continued)

Table 5.2. (*Continued*)

Country	Area	Name of cultivar	Origin	Outstanding characteristics
		Ninghaibai	Selected from whitish cultivar, 2004	Whitish flesh, fruit 52 g, Brix 13–16°, good eating quality
Egypt		Golden Ziad	Seedling of Premier	Yellowish-red flesh, high yield, early season
		Moamora Golden Yellow	Seedling of Premier	Yellowish-red flesh, high yield
India		Pale yellow		White flesh, fruit large
		Safeda		Flesh cream-colored, early to midseason
		Thames Pride		Yellowish-red flesh, bears heavily, early season, juicy, canned commercially
Israel		Akko 13	Japanese origin	Yellowish-red flesh, early season (March), juicy, agreeable flavor, good keeping quality, requires cross-pollination
		Zerifin	Seedling	Yellowish-red flesh, bears regularly and abundantly, excellent quality, stores well
Italy	Sicily	Marchetto (or Marturana)	Seedling from Ficarazzi, near Palermo	White–orange flesh, fruit 70–80 g, Brix 15°, easy peeling, does not ship will, adapted to local sales
		Nespolone di Trabia	Seedling from Trabia, near Palermo	Orange flesh, subacid, fruit 60–80 g, Brix 14.5°, ripening in May–June, very good eating quality, ships well
		Nespola Rossa	Seedling from Ficarazzi, near Palermo	Pink flesh, fruit 40–50 g, Brix 9.3°, harvested end of April, good pollinator for Nespolone di Trabia and Sanfilipparo, flowering Oct.–Dec.
		Sanfilipparo (or Gigante)	Derived from Nespolone di Trabia	Orange flesh, characteristics very similar to Nespolone di Trabia, but easily adaptable to different environments and climates; ships well
		Virticchiara	Seedling from Bocca di Falco, near Palermo	White-pink flesh, fruit 45–50 g, Brix 9.3°, sweet, very early ripening (April)

Japan	Honshu; Shikoku	Tanaka	Seed brought to Tokyo from Nagosaki 1888	Yellowish-red flesh, harvest in May, 60–76 g, good keeping quality
	Kyushu	Nakasaki-wase	Mogi × Hondawase, 1976	Yellowish-red flesh, prone to cold damage, very early ripening, excellent quality
		Suzukaze	Kusunoki × Mogi, 1974, Released in 1999	Yellowish-red flesh, fruit 55 g, Brix 12.7°, vigorous
		Yougyoku	Mogi × Morimoto, 1973, Released in 1999	Yellowish-red flesh, fruit 60 g, Brix 12.1°, sensitive to loquat canker
Spain	Alicante	Algerie	Seedling from Algeria	Yellowish-red flesh, 95% of Spanish production
	Andalusia	Golden Nugget	A clone of Tanaka obtained in California, 1888–1890	Yellowish-red flesh, 80% of total in Andalusia, good keeping, ships well
Turkey		Akko 13	Japanese origin	Yellowish-red flesh, juicy, sweet, agreeable flavor, dark orange good keeping
		Golden Nugget	A clone of Tanaka obtained in California, 1888–1890	Yellowish-red flesh, juicy, sweet flesh, apricot-like flavor, good keeping, ships well
		Hafif Cukurgobek	Seedling selection in Turkey	Yellowish-red flesh, sweet, juicy; pleasant flavor, good keeping
		Takaka	Japanese seedling	
United States	California	Advance	Seedling, selected 1897	Yellowish-red flesh, good pollinator
		Champagne	Seedling introduced, 1908	Yellowish-red flesh, juicy, excellent flavor, good for preserving
	Florida	Fletcher	Parentage unknown, 1957	Yellowish-red flesh, good keeping quality and flavor
		Wolfe	Seedling of advance, released 1965	Pale-yellow flesh, excellent flavor, stable yield, resistant to bruising

Cultivars in China can be divided into three groups: whitish flesh, large fruit with orange flesh, and medium-to-small fruit with orange flesh. Most cultivars in Japan belong to the medium-to-small fruit with orange flesh group but several belong to the other two groups. 'Shiro Mogi' is in the whitish-flesh group, and 'Tanaka' is in the group with large fruit size and orange flesh. In China, 70% of widely planted cultivars have orange flesh with the remainder having whitish flesh. Whitish-flesh cultivars, such as 'Zaozhong' and 'Baiyu', are the leading ones in Jiangsu Province. Among orange cultivars, there are different ecological types in various zones formed during the long course of their cultivation and acclimatization. Ecotypes in China have been divided into two cultivar groups: the north subtropical cultivar group (NSCG) and the south subtropical cultivar group (SSCG) (Ding et al. 1995). NSCG are primarily distributed in the mid- and north subtropical area, roughly in the provinces in the basin of the Yangtze River, located in the range of 27°–33°N where average annual temperature is 15°–18°C, with an absolute low temperature of −5° to −12°C, and 800–1500 mm of annual rainfall and snows and frost can occur. NSCG cultivars are characterized by strong cold-resistance. Fruits are mostly late ripening, with orange flesh, and small but with high quality. Representative cultivars are 'Luoyangqing' in Zhejiang, 'Zhaozhong' in Jiangsu, and 'Guangrong' in Anhui. These cultivars have been successfully introduced to the south subtropical zones and margins of the tropical zones and their fruits ripen as early as April. SSCG cultivars are grown in the south subtropical zone and margins of the tropical zone, approximately 19°–27°N, with only a few days of frost and snow or temperatures lower than 0°C, and with more than 1,500 mm of annual rainfall. The SSCG cultivars have poor cold-resistance but are high yielding and early, while their fruits are large but generally less flavorfull than NSCG fruits. Representative cultivars are 'Dawuxing' in Sichuan and 'Jiefangzhong' in Fujian. Flowers and fruits will be injured by cold if they are grown in the north subtropical zones. Attempts to introduce 'Jiefangzhong' to Zhejiang and Jiangsu have been carried out since the 1970s, but failed (Ding et al. 1995). Several Spanish cultivars with large fruit and orange flesh such as 'Marc' and 'Pelluches' are also included in the SSCG group.

C. Rootstock

Seedlings of *E. japonica* are the most widely used rootstocks and usually produce large trees (Hartmann and Kester 1975; Morton, 1987). In Japan, seedlings of domestic cultivars, such as 'Mogi' and 'Champagne', are usually used as rootstocks (Nesumi 2006) but clonal quince rootstocks are used in some modern orchards. In Mediterranean countries, loquat seedlings are also usually used as rootstocks since they are well adapted to calcareous soils, which are abundant in this region. In Spain, seedling loquats are the most common rootstocks.

There are reports of other rosaceous species being evaluated as rootstocks for loquat in various countries. These include hawthorn (*Cratagous scabrifolia* Rehd.), apple (*Malus* × *domestica* Borkh.), firethorn (*Pyracantha fortuneana* Roem.), medlar (*Mespilus vulgaris* Rchb.), pear (*Pyrus communis* L.), Chinese photinia (*Photinia serrulata* Lindl.), and quince (*Cydonia oblonga* Mill.).

Quince and pyracantha rootstocks may cause extreme scion dwarfing. Dwarfing on quince rootstocks has encouraged expansion of loquat cultivation in Israel since 1960. Quince rootstocks selections (A, C, BA29) produce smaller, more compact trees, a shorter juvenile period, and larger fruits with high sugar content and good color. However, quince rootstocks are very sensitive to calcareous soils and show graft union incompatibility with many loquat cultivars (Llácer et al. 2003; Polat and Kaska 1992; Polat 2007a). The growing of dwarf trees greatly reduces the labor of pruning, flower- and fruit-thinning, fruit bagging, and harvest. However, loquat on quince suffers from zinc deficiency, and some trees break off at the graft union (Blumenfield 1995). Quince rootstock, which tolerates heavier and wetter soils, is widely used in Egypt (Morton 1987). In Spain, under experimental conditions, quince conferred resistance to saline conditions due to their ability to reduce the transport of Na and Cl to the shoots (García-Legaz et al. 2005; Manuel et al. 2008). Chinese photinia was sometimes used as rootstocks for loquat cultivars in the Suzhou district of Jiangsu province, China, but this genus induces shallow-rooting and late-bearing.

Most of the 32 species of *Eriobotrya* have seemingly never been used as rootstocks for loquat in commercial plantings. Until recently, there had been only one report of another species of *Eriobotrya* beyond *E. japonica* used as rootstocks, that is, Taiwan loquat (*E. deflexa*) (Lin et al. 1999) but a number of candidate species could be used as rootstocks. The potential of six wild species of *Eriobotrya* as rootstock for domesticated loquat cultivars was evaluated in China (Zhang et al. 2010). When the domestic loquat, 'Zaozhong No. 6', was grafted onto different wild loquat species seedlings, differences in compatibility were observed and graft compatibility was lower for all combinations in comparison to 'Zaozhong No. 6' grafted onto domestic loquat seedlings. Delayed incompatibility was observed when wild Henry loquat (*E. henryi*) was used as a rootstock. Differences in soluble solid contents due to the use of different rootstocks were observed, and there was a significant negative correlation between graft compatibility and this difference. Rootstock affected anatomical changes in graft unions. Wild rootstocks influenced scion growth, fruit bearing, and fruit quality. The wild Fragrant loquat (*E. fragrans*) appeared to have value as a rootstock as it produces greater cold resistance and improved scion growth.

III. REPRODUCTIVE PHYSIOLOGY

A. Flowering

Loquat flower-buds differentiate from terminal shoot buds. Flowers initiated from May to August. Muramatsu et al. (1963) working in Japan found that flower-bud initiation can be affected by the time and duration of shading treatments with the crucial period for physiological differentiation 1 month after fruit harvest. Lin observed that the flower-bud physiological differentiation of 'Dahongpao' was initiated in late May, with two differentiated peaks, one is in mid-late June and the other is in mid-late July.

Flower development of loquat has been reviewed by Li (1982) and Lin (1992), and Rodriguez et al. (2007). In Ningbo City, Zhejiang province of China (29°N), the main shoot of inflorescence panicles differentiate in the beginning of August; the spring lateral shoot and the summer main shoot in the middle or the end of August, sepals and petals in the beginning of September, and stamens and pistils in the middle or end of September. Sperm and egg nuclei develop in October, and anthesis occurs in November. The summer lateral shoot begins to differentiate flower buds in September, 1 month later than the spring main shoot, but anthesis takes place in November. Until late October, the process of differentiation in these four kinds of shoots (spring main and lateral shoots and summer main and lateral shoots) is similar with pistil primorida reaching 1.5 mm (Chen 1958). The stigma of loquat is closed, with some internal transfer cells for nutrition transportation.

B. Blossoming

The main panicle typically usually bears 70–100 flowers on 5–10 branched, secondary axes. Depending on panicle size, flower number can range from 30 to 260. One month after the panicle appears, inflorescences begin to bloom and flowers remain from late November to early-mid January in China, and 1 month earlier in the Mediterranean basin countries. In most cultivars, the solitary floret at the top of main panicle axis blooms first, followed by the secondary axes in the middle of the panicle, and then the basal axes. If the panicle bends downward or droops, progression of flower opening is from the center of the bend to both sides. The opening sequence of the secondary-axes inflorescence is as follows: first, one floret at the base blooms, then the other at the apex or near the apex; and finally all the rest bloom in an acropetal direction. Solitary flower wither after 8–14 days.

Generally speaking, loquat blooms from late fall to mid-winter; thus low temperatures in early winter affects blossoming and fruit set. Loquat is floriferous between 11 and 14°C and flowering duration is prolonged below 10°C. Temperatures below −6°C causes severe damage to blossoms and result in seriously damage to trees (Wang 2003). Under ordinary climatic conditions in China, the best period of bloom and fruit set is from mid-November to mid-late December (Wu et al. 1991). Sustained low but nondamaging temperatures may induce cold resistance of the flower bud but slows the development.

C. Pollen Biology

1. Morphology. Yang et al. (2009c) observed pollen morphology of six species of *Eriobotrya* with scanning electron microscopy (SEM). Pollen is of the N3P4C5 model, prolate with three equally distributed colposates, triate-foveolate with many stripes, and a tectum punctured exine with distinct differences among the six species. Guo et al. (2010) found significantly different morphologies among 15 triploids derived from 'Longquan' loquat.

2. Germination. Loquat pollen begins to germinate when the temperature is above 10°C, and germination can reach 70% at the optimum temperature of 20°C. Germination is very low above 35°C, and no pollen germinates below 5°C. Exposure to suitable temperatures for a few hours is sufficient to induce good pollen germination. In the northern edge of the loquat cultivation zone in China, though the weather is cold during the blossom period; however, pollination, fertilization, and fruit-setting are not influenced because the noon temperature is above 15°C (Huang 2000). Both plant growth regulators (GA, IAA, and NAA) and plant nutrients (B, Mn, and Ca) promote pollen germination of loquat (Ding et al. 1991).

3. Storage. In cryopreservation studies of loquat pollen, 30% water content was adequate for survival (Wang et al. 2004). Thawing temperatures did not significantly affect pollen vitality after storage in liquid nitrogen (−196°C).

D. Pollination and Fertilization

1. Environmental Factors. Loquat is cultivated in the northern edge of the subtropical zone, so that low winter temperature is the main factor affecting pollination and fertilization (Zhang et al. 2005). Loquat cannot set fruit properly below 10°C. At blooming and frnit-setting stages, uusuitable weather such as coutinuous chilly temperature with rain, cold temperatures below −3°C, and lack of sunshine causes pollination and fertilizatiou failure and low fruit set (Li and Tang 2006). Liang et al. (2011a) found that the mixed solution of boron, sucrose, calcium, and diethyl aminoethylhexanoate (DA-6) significautly promoted *in vitro* pollen tube elongation in 'Zaozhong No. 6' and 'Jiefangzhong'.

2. Pollen Compatibility. Pollen compatibility studies have been long neglected because the amount of flowers and fruits in loquat are usually sufficient to meet growers' need, and are usually excessive, requiring thinning for optimal commercial quality. It was long assumed that all loquat cultivars are cross- and selfcompatible. However, Xia (1993) discovered that cross-pollination between different loquat cultivars set better than selfing. It is now clear that selfcompatibility is not a common trait in loquat. Selfincompatibility is an evolutionary strategy used by flowering plants to prevent selffertilization and promote outcrossiug (De Nettancourt 1977). However, the release of flower-visiting insects, such as bees (*Apis dorsata* and *A. mellifera*), during the flowering period can increase fruit set (Mann and Sagar 1987). Loquat specifically shares with the rest of the Rosaceae species a gametophytic selfincompatibility (GSI) system based on S-RNases (Igic and Kohn 2001). This system exhibits high polymorphism at the selfincompatibility locus. Several alleles have been identified in loquat and intercompatibility groups have been established (Carrera et al. 2009; Cisbert et al. 2009b). The high number of alleles fouud at the *S*-locus makes it particularly iuteresting for genetic diversity studies and it is essential for correct orchard planning and suitable design of breeding programs. As with other members of the Rosaceae, interplanting cultivars with the same *S*-alleles would be unsuitable for cross-polliuation.

E. Embryology

1. Micro- and Macrosporogenesis and Embryogenesis. Microsporogenesis and the development of the male gametophyte was iuvestigated by Li and Ding (1984) and Li et al. (1986). After one periclinal division, one or two lines of primary sporogenous cells are formed in the sporogoninms and then divide into many microspore mother cells. The meiosis of the microspore mother cell is simultaneous and prodnce androspore tetrads which developed into mature pollen grains each with two cells.

The macrospores appear when the flower buds are diamond-shaped. The layer cells nnder the placental intine epidermis increase rapidly and gradually expand to be ovular primordia, which change the diamond-shaped flower buds into round-ones. The sporogoninm under the top nucellus epidermis has large nuclei and dense cytoplasm. After periclinal division, the sporogoninm divide into a parietal cell and a sporogenous cell. The sporogenous cell develops into the embryo sac mother cell. After periclinal and anticlinal divisions, the parietal cell divides to form poly-layered periphery nucellar tissue and pushes the embryo sac mother cell to the deep nucellar tissue forming the crassinucellate ovule. The embryo sac mother cell produces four macrospores after meiosis; the macrospore in the chalazal end divides three times continuously and forms an 8-nucleate embryo sac. The other three macrospores degenerate and are eliminated. The megasporocyte divides into two unclei that move to different poles to form a binncleate embryo sac that divide again to form a 4-nucleate embryo sac. After the third division, four nuclei are produced in both the micropylar and chalazal ends. The Polygonium-type embryo sac has eight nuclei immersed in the same cytoplasm and contains an egg apparatus (egg cell and two synergids), three antipodal, and a central cell containing two polar nuclei (Lin 1992).

The proembryo development of loquat is of the Cruciferae (ouagrad) type (Zheng and Liang 1989). The zygote laterally splits to an embryoid with a small roof-cell at the chalazal end and a large basal cell at the micropylar end. The basal cell splits laterally again and forms an upside down T-shaped proembryo with four cells. After two or three longitudinal and lateral divisions, the basal cell develops into a suspensor with four to eight cells. Endosperm development of loquat belongs to the nuclear type. As the embryo develops to the globular stage, wall formation commences in the micropylar end of the embryo sac. At the heart stage, the endosperm around the proembryo in the micropylar end moves gradually to the chalazal end, followed by disintegration until elimination. When the typical dicotyledonous embryo evolves, the endosperm and nucellar cap is absorbed completely (Lin 1985).

2. Seedlessness. GA_3 applied before bloom can induce seedless fruits as a result of endosperm abortion or embryo degradation (Goubran and El-Zeftawai 1986). Endosperm abortion causes the lack of nutrition for embryo development, so the zygotic embryo divides abnormally and finally disintegrates. The ovule when deprived at the initial phase forms abortive seed forms (Deng et al. 2009).

3. Seed Morphology and Physiology. The mature loquat seed is oval-shaped and slightly flat, 2.3×1.4 cm in size. Each seed weighs 1.5–2.1 g and the weight ratio of cotyledous to embryo and hypocotyls is greater than 400. There is great diversity in seed characters within loquat germplasm. Number of seed per fruit may vary from one to eight, generally speaking three or four. Among 128 loquat germplasm accessions tested, most had two to four fully developed seeds and a few abortive seeds per fruit under favorable pollination conditions (Jiang et al. 2009). Seeds are triquetrous, beige, with a few spots and a small indehiscent sheath.

Loquat seed is recalcitrant and loses germinating ability rapidly. Seed moisture content and temperature are the two most important factors for seed storage (Ellis et al. 1991). Storage at 15°C with 51% relative humidity prolonged storage life (Chen et al. 1998a,b). At 10°–15°C, germination rate was 95% after 330 days.

Water imbibition in fresh loquat seed is restricted by the seed coat resulting in a low germination rate. Moisture content of loquat seed can reach 58.7% in the cotyledon and 73.2% in the embryonic axis (Chen et al. 1998a). Seed vigor decreases rapidly if too much water is lost and will be completely lost when the moisture content is reduced to 35.5% in cotyledons (or 28.0% in the embryonic axis. Germination is optimal at 25°C and is inhibited below 10°C or above 32°C. Germination can be increased by light desiccation (3%–5%) or GA_3 (0.1–0.5 mmol L^{-1}) but is inhibited by abscisic acid. Seed germination can be increased by stratification at 4°C for 30 days (Polat 1997).

F. Fruit

Loquat fruit, as other pomes, derives from the receptacle beneath the ovary and is considered a pseudocarp containing a fleshy edible receptacle in addition to the ripened ovary. There are two peaks and two troughs during fruit development. Fruit development can be divided into four stages: fruitlet development, seed development, fruit enlargement, and fruit ripening. During the whole process, the vertical diameter of the fruit grows faster than the transverse diameter, resulting in an elipsoid fruit shape in longitudinal section (Jia 2009). Loquat fruitlets freeze below −3°C (Fan et al., 2010).

Generally, the flesh of seeded loquat is thin and has many large seeds, with flesh recovery as low as 65%–70%, which adversely affects its commercial value. Therefore, seedless loquat would have great economic significance. Hu and Lin (2010) had suggested the induction of the seedless loquat by treatment with gibberellic acid (GA_3) and *N*-(2-chloro-4-pyridyl)-*N*-phenylureia (CPPU) treatment before fertilization at the flower-bud stage. The laboratory of G. Liang has used triploidy and GA application to develop seedless loquat (see Section V. E).

IV. BREEDING OBJECTIVES

Breeding objectives in loquat must include consumer requirements for appearance and quality as well as grower acceptability. Objectives for scion cultivars include low seed number or seedlessness, attractiveness, various colored skin and flesh, fruit size, increased quality especially high soluble solids, cold resistance, disease resistance, season of ripening, and productivity.

In the Southern subtropical areas of China, there is a demand for cultivars that ripen earlier than 'Zaozhong No. 6' which is harvested in February or March. In the Northern subtropical areas (Southern Shaanzi, Southern Gansu, and Central Jiangsu), late ripening (July to August) is desirable. Loquat is soft and susceptible to mechanical damage, which leads to difficulty in storage and transportation. However, it is possible to prolong the shelf life of loquat for up to 6 months. Desirable tree characters inclnde short shoots to facilitate crop thinning and fruit bagging to rednce labor costs, which have donbled over the last decade in China.

Breeding objectives in the Mediterranean countries are focnsed on increasing the diversity of cultivars. In the case of Spain, 90 % of the production relies on 'Algerie' and its bud mutations and 'Magdal'. New cultivars earlier that 'Algerie' with better fruit quality and tolerauce to scab are sought. In other Mediterranean conntries, the industry is based on clones that do not meet the international commercial standards of quality, and new cultivars with better fruit quality and yield are the main objectives.

Breeding of loquat rootstocks has been neglected for many years. Rootstocks inflnence scion growth, fruit bearing, and fruit quality. In addition to providing adaptability to soil conditions the major objectives for rootstocks include dwarfness and graft compatibility. Breeding objectives in Spain are focused on production of dwarfing rootstocks, tolerance to the calcareous soils, and tolerance to soilborne diseases such as *Phytophthora* and *Armillaria*.

The breeding of clonal rootstocks that would be vegetatively propagated has not been considered in China becanse of the problem of shallow rooting. Seedling rootstocks have tap roots that prevent orchards being blown down by typhoons and strong winds. However, the use of clonal rootstocks would reduce orchard variability. Thus, loquat rootstocks with strong root systems are an important breeding aim.

V. BREEDING METHODS

A. Genetic Studies

Genetic studies of loquat carried out in China have been summarized by Zheng (2007). Fruit characters included juvenility, size, pericarp and flesh color, maturity, and soluble solids and plant characters included resistance to leaf spot caused by *Fabraea maculata*. Early fruiting was shown to be quantitatively inherited. Bearing age ranges from 3 to 9 years averaging about 5. In general, selections from early parents bear fruit earlier than from late parents. There is evidence that early fruiting is maternally inherited. Heritability of fruit shape, weight, and quality was 96%, 80.8%, and 90.0%, respectively. Soluble solids inheritance is similar to that of fruit quality. No differences have been found in reciprocal crosses. Fruit maturity is quantitatively inherited.

Inheritance of pericarp and flesh color was complex suggesting incomplete dominance of one or several genes. Flesh color did not segregate if parents were white or yellow-white. Crosses involving gold flesh color showed segregation for white, gold, light salmon, and deep salmon. Salmon color was dominant to gold.

Seedlings from crosses involving only selections susceptible to leaf spot were all susceptible. Reciprocal crosses of resistant and susceptible cultivars showed evidence of maternal inheritance suggesting the use of resistant seed parents in crosses.

B. Selection

Till the 1980s, loquat planting in China was based mainly on seed propagation and the use of grafted selections was unpopular. Seed propagation from natural hybrids is associated with abundant genetic variation and provides abundant material for clonal selection. Both white-fleshed and orange-fleshed cultivars have been selected (Zheng 2007).

White flesh in loquat is appreciated for the tender, juicy, thick flesh, and sweet fine flavor. China has the most abundant white-fleshed germplasm. Cultivars selected from various parts of the country include 'Baili', 'Baiyu', 'Guanyu', 'Keyansuobaisha', 'Libai', 'Ninghaibai', 'Ruantiaobaisha', 'Wugongbai', 'Xinbai No. 1', 'Xinbai No. 3', and 'Xinbai

No. 8'. The orange-fleshed loquat selections include 'Auhuidahongpao', 'Bahong', 'Changhon No. 3', 'Dawuxing', 'Donghuzao', 'Hongdenglong', 'Jiefangzhong', 'Longmen No. 1', 'Luoyangqin', 'Luzhou No. 6', 'Puxinben', 'Puxnan1', 'Taicheng No. 4', and 'Yangmeizhou No. 4'.

Selection of rootstocks is underway in China. The Fruit Research Institute of the Fujian Academy of Agricultural Science has selected dwarfing rootstocks from selections of *E. japonica*, such as 'Daduhe' and 'Min'ai No. 1'.

C. Hybridization and Mass Selection

Hybridization between selected elite cultivars that carried the traits of interest followed by selection, characterization, and testing of seedlings is the prominent breeding method in loquat. Breeding programs are being carried out in China (Zheng 2007; Shih 2007), Japan (Terai 2002), and Spain (Gishert et al. 2007). At present new cultivars still primarily come from selection made by growers, since dedicated breeding efforts are recently initiated and the loquat has a fairly long juvenility period. Most cultivars currently grown come from commercial fields or from evaluation of broader germplasm collections. Cultivar surveys have been carried out in all countries where the crop is present: China (Zheng 2007), Mediterranean countries (Llácer et al. 2003), Pakistán (Hussain et al. 2007), and Turkey (Karadeniz and Şenyurt 2007; Polat 2007b).

D. Mutagenesis

Jiang et al. (2007) demonstrated that loquat showed a strong sensitivity to gamma irradiation, with high mutation rate. The chemical mutagens, most useful, belong to the class of alkylating agents including ethyl methyl sulfate. Colchicine has been widely used to increase ploidy. 'Shiro Mogi' loquat was obtained from induced mutation of irradiated seed of 'Mogi' (Lin 1998, Prederi 2001). Compared to 'Mogi', 'Shiro Mogi' has erect stems and greater vigor with larger fruit. The blossom period is nearly 10 days later so that cold weather can be avoided. Harvest of 'Shiro Mogi' is later than 'Mogi' and fruit is juicy with a fine, tender texture, with 13%–14% total soluble solids. The fruit shape was rounder and the whitish flesh was thicker with less seeds than 'Mogi'.

E. Breeding Seedless Loquat

Seedlessness in fruits is a commercially valuable trait in a number of traditional fruit crops (Table 5.3). This trait is especially desirable in

Table 5.3. Seedless fruit crops and mechanisms.

Crop	Seedless mechanism	References
Apple (& pear)	Parthenocarpy, genetic sterility (apetelous), triploidy	Chan and Crain (1967)
Atemoya	Triploidy, GA application	George and Paull (2008)
Banana	Parthenocarpy, genetic sterility reinforced by triploidy	Simmonds (1976)
Citrus	Triploidy, irradiation induced sterility	Ollitrault et al. (2008)
Cucumber	Parthenocarpy, genetic sterility, growth regulator induced parthenocarpy	Paris and Maynard (2008)
Fig	Parthenocarpy, lack of pollinator	Condit (1947)
Grape	Parthenocarpy or stenospermocarpy (early embryo abortion)	Ledbetter and Ramming (1989)
Loquat	Triploidy, growth regulators (GA)	Lin et al. (1999); Liang et al. (2011a)
Pineapple	Parthenocarpy, genetic sterility, selfincompatibility, elimination of pollinators (humming birds)	Collins (1960)
Tomato	Growth-regulator induced parthenocarpy	Gorguet et al. (2008)
Watermelon	Triploidy	Kihara (1951)

loquat because it has large seeds as in *Prunus* and multiple seeds, usually between 4 and 6, as in *Malus* (Lin et al. 1999; Janick 2011) and seed weight is a significant portion (15%–20%) of total fruit weight.

1. Induction of Seedlessness. Seedlessness is often associated with parthenocarpic fruit development achieved without fertilization (vegetative) although in some cases stimulus by pollination is required (aitonomic). Apparent seedlessness (pseudoparthenocarpy) is associated with early embryo abortion (stenospermocarpy) and is common in grapes (Ledbetter and Ramming 1989) where traces of seed are observable.

There are a number of strategies to induce seedlessness. These include: (1) exploiting unbalanced gametes in triploidy (apple, atemoya, banana, citrus, loquat, and watermelon); (2) sterility either genetic or induced by irradiation (apple, cucumber, and citrus); (3) elimination of pollination in self-incompatible parthenocarpic plants (fig and pineapple); and (4) application of growth regulators, usnally gibberellic acid (atemoya and loquat) or auxin (tomato).

The exploitation of triploidy to induce seedlessness is a promising breeding technique in those crops where the triploid condition is a suitable ploidy level as found in the Rosaceae. For example, 10% of apple and pear cultivars are triploid although the frequency of occurrence is less that 1%. In many triploids such as watermelon, pollination by diploids is still required to stimulate fruit development.

2. Production of Seedless Triploids in Loquat. There are four potential routes to the induction of triploids.

Meiotic Nonreduction (NR). NR in mega- or microsporogenesis of diploids ($2n = 34$) produces diploid instead of haploid gametes either from a failure of division II in meiosis or fusion of sporocytes in division II (Fig. 5.2). NR gametes occur with a frequency of <1% in rosaceous species. In loquat, the frequency of nonreduced gametes was estimated as 0.28% from an analysis of meiosis in pollen mother cells (Yan et al. 2011).

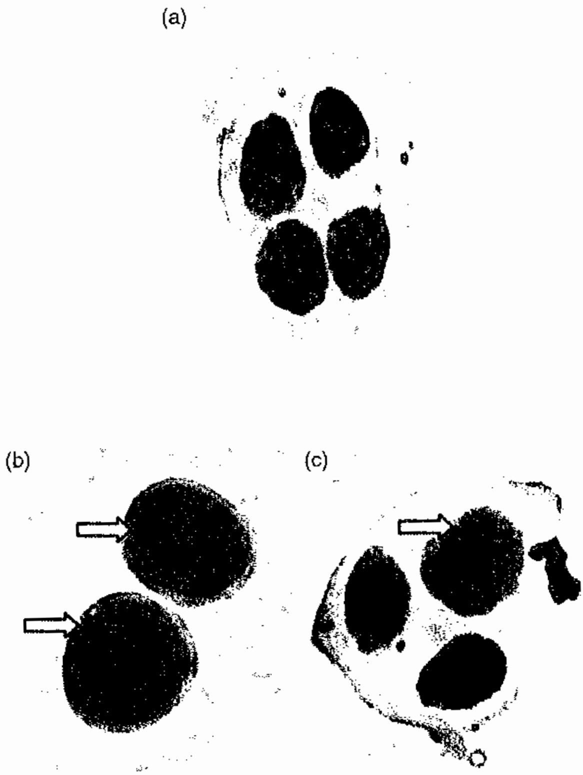

Fig. 5.2. "Tetrad stage" of diploid meiosis in microsporogenesis: (a) normal tetrad; (b) dyad due to failure of division II; (c) triad due to incomplete division II. Arrows show evidence of nonreduced microspores. *Source*: Yan et al. (2011).

Table 5.4. Frequency of ploidy levels in seedlings of diploid × diploid crosses in apple and loquat.

Crop	No. $2n \times 2n$ seedlings	Distribution of seedlings % (no.)				
		Diploid	Triploid	Tetraploid	Pentaploid	Mixaploid
Apple	6,825	99.63	0.28 (19)	0.09 (6)		
Loquat	44,828	99.31	0.50 (225)	0.11 (50)	0.02 (10)	0.06 (26)

Sources: Einset (1959), Guo et al. (2007), Liang et al. (2011b).

The snbseqnent fnsion of diploid and haploid gametes in diploid × diploid crosses leads to triploidy, often referred to as natural triploidy. In apple, triploid seedlings from $2n \times 2n$ crosses occur with a frequency of 0.28% (Einset 1959) as compared to about 0.50% in loquat (Guo et al. 2007; Liang et al. 2011b) as shown in Table 5.4. In loquat, the frequency of triploidy varied with the seed parent from 0.18% to 1.62% (Gno et al. 2007). A total of 366 triploids in loquat have been identified by an analysis of 99,272 seed (G.L. Liang, unpnblished).

Genomic *in situ* hybridization (GISH) of naturally occnrring triploids using genomic DNA of the seed parent as the probe demonstrated that unreduced gametes resulted from NR in the seed parent with the haploid male gamete derived from either the seed parent or a different clone as a resnlt of cross-pollination. (Liang et al. 2011c). However, karyotypic analysis of chromosomes of diploids and their related triploids (Fig. 5.3) indicate that some triploids may result from NR in microsporogenes based on an analysis of satellited chromosomes (Liang et al. 2011d).

Triploidy in loquat was determined by chromosome counts. Seeds extracted from frnits of individual cultivars, are disinfected and

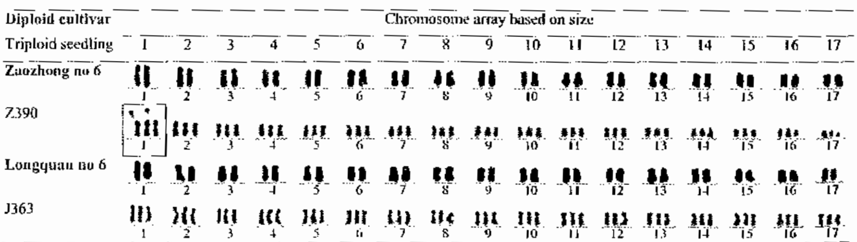

Fig. 5.3. Karyogram of two diploids and their related triploid seedlings. Satellite chromosomes are boxed. The two satellilted chromosome 1s in triploid Z390 must have been derived from nonrednction in a gamete of the diploid pollen parent containing one or two satellited chromosomes. *Source*: Liang et al. (2011d).

germinated in a peat mixture in the dark. Root tips were subjected to wall degradation by the hypotonic treatment method, pretreated with 0.002 M 8-hydroxyquinoline (a reagent to suppress spindle fiber formation); Carnoy's fixative (3 methanol:1 glacial acetic acid), enzymolysis (3% cellulose +3% pectinase), hypotonic treatment, fixation, smearing, flame drying, 5% Giemsa staining, microscopic examination, and photomicrography. Skilled technicians are able to evaluate 300 seedlings per day (Guo et al. 2007). Flow cytometry methods have been used in citrus for ploidy determination (Ollitrault et al. 2008) and may be useful in high-throughput assessment of ploidy in loquat seedlings.

Diploid × Tetraploid Crosses. If tetraploids are fertile, 100% of $4n \times 2n$ crosses are expected to be triploid, The production of natural tetraploids sometimes occurs from spontaneous somatic doubling but tetraploids can be readily induced by colchicine treatment in loquat (Kihara 1981). Triploids have also been achieved in loquat from $2n \times 4n$ crosses, where the pollen parent is tetraploid (Huang 1984, 1989). Blasco et al. (2011) applied different concentration and duration of colchicine to ungerminated seeds, apical buds, and whole seedlings, and obtained different rates of survival and ploidy level. Tetraploid plants were obtained in all treatments but the best treatment varied according to the plant material used. Since cytochimeras are a problem, in the Rosaceae, the tetraploid sectors must include LII of the meristem (Pratt 1983) to give rise to diploid gametes. The reliance on genetically prepotent tetraploids is a limitation of this system.

Culture of Triploid Nucellar Tissue. Since the endosperm of loquat is triploid, culture of endosperm tissue could lead to triploid clones (Chen and Lin 1991). Triploid plants from endosperm culture have been achieved in loquat (Liu et al. 1999).

Somatic Hybridization of $2n + n$ Cells. This technique involving protoplast fusion has been achieved in citrus. Haploid plants in citrus are produced, with very low efficiency, by pollination using irradiated pollen (Ollitrault et al. 2008) and can then be used to create haploid protoplasts. Protoplast isolation and culture for embryos has been achieved in loquat (Lin et al. 1989; Lin 1985, 1991, 1995) but protoplast fusion has not been reported. Development of haploids in loquat has been explored using microspore culture (Blasco et al. 2011; Padoan et al. 2011). Microspores from the late uninucleate to early binucleate pollen stages are most suitable for somatic embryogenesis. Callus production was 31.5% with 'San Filiparo' loquat in a medium supplemented with 1 mg L^{-1} of NAA and Zeatin.

3. Morphology and Pomology of Seedless Triploid Loquat. Triploid loquats set fruits in 2003 and 2004 but were aborted at a young stage without exogenous growth regulators. However the addition of GA during the flowering period resulted in fruit formation and it was later determined that GA at 100 ppm was the appropriate concentration (G. Liang, pers. commun.). By 2011, 86 triploids obtained from diploid cultivars had fruited and many selections had been propagated.

As compared to the diploid parents, triploid plants grow exuberantly producing large trees with few branches, dark green leaves with long dense villi, and notched leaf edges (Liang et al. 2011c).The circumference of trunks, diameter of annual branches, length, width and length × width of leaves of triploids were 1.7, 1.4, 1.6, 2.0, and 3.3 times greater than the diploid seed parent, respectively (Table 5.5). The number of branches and leaf index in triploids were 45% and 81% of diploids. Size of flowers and flower parts were significantly greater in triploids than diploids. Pollen germination of diploids averaged about 88% while triploid pollen germination ranged from 0% to 6.7%. Of 34 triploids examined, pollen germination was observed only in 19 plants, averaging 2.3%. (G. Liang, pers. commun.).

The seedless fruit show great variability is size and shape. Among 45 triploids analyzed, six had larger fruits and are considered elite or super strains (Table 5.6, Fig. 5.4). These triploids had thicker flesh with a flesh

Table 5.5. Comparison of plant morphology of diploid loquats and their related triploid seedlings.

Cultivar	Trunk circum. (cm)	No. of branches	Annual branch diam. (cm)	Leaf: Length (cm)	Leaf: Width (cm)	Leaf: Length × width (cm^2)	Leaf: Leaf index
Dawuxing (2x)	25.5b[z]	7.2a	5.3b	27.5b	7.3b	201b	3.8a
3x seedling	50.0a	3.6b	7.1a	45.8a	15.6a	718a	2.9b
Longquan No.1 (2x)	29.7b	6.0a	6.5b	22.2b	7.0b	154b	3.2b
3x seedling	50.0a	2.4b	8.5a	36.3a	13.4a	488a	2.7a
Jinfeng (2x)	24.9b	7.0a	6.4	25.6b	7.2b	186b	3.5a
3x seedling	45.0a	3.0b	7.5	43.2a	15.6a	676a	2.8b
Zaohong No.3 (2x)	36.0b	7.0a	5.8b	25.7b	7.7b	197b	3.4a
3x seedling	49.0a	4.0b	7.4a	38.7a	14.1a	546a	2.8b

[z]Mean separation of 2x and related 3x means at 5% level.
Source: Liang et al. (2011e).

Table 5.6. Characteristics of fruit in diploid loquats and their related triploids.

Cultivars	Ploidy	Fruit: Shape[z]	Fruit: Flesh color[y]	Fruit: No. of seeds	Fruit: Weight (g)	Fruit: Length (mm)	Fruit: Width (mm)	Shape index	Edible portion (%)	TSS (%)	Composition (g 100 ml⁻¹): Sugar	Composition (g 100 ml⁻¹): Acid	Composition (g 100 ml⁻¹): Vit. C
Raotiaobaisha	2x	R	W	4–6	25.2 (34)[x]	37.8 (40)[x]	38.4 (40)[x]	0.97	62.0	14.3	8.97	0.47	1.70
H324	3x	LO	OY	0	50.3 (68)	56.2 (70)	40.1 (48)	1.40	86.0	12.5	8.16	0.36	1.82
Jinfeng	2x	O	OY	4–6	61.1 (133)	58.2 (86)	46.9 (68)	0.97	65.0	12.0	8.65	0.68	1.85
D425	3x	LO	OY	0	79.3 (103)	78.5 (94)	52.7 (61)	1.49	84.9	12.0	6.83	0.48	1.70
D327	3x	LO	OY	0	78.1 (85)	73.8 (90)	41.3 (50)	1.78	85.6	11.8	6.66	0.60	1.70
Dawuxing	2x	O	OY	4–6	58.7 (96)	62.5 (77)	45.1 (59)	0.97	65.0	12.8	8.65	0.68	1.85
A322	3x	LO	OY	0	65.8 (85)	73.0 (76)	50.0 (53)	1.56	83.5	11.5	6.53	0.61	1.88
A313	3x	LO	OY	0	62.2 (83)	70.0 (79)	49.0 (43)	1.49	82.5	11.7	6.83	0.48	1.70
A35	3x	LO	OY	0	63.1 (85)	73.0 (78)	49.0 (44)	1.78	85.2	11.5	6.66	0.60	1.70

[z]LO, long ovoid; O, ovoid; R, roundish.
[y]OY, orange yellow; W, white.
[x]Maximum.
Source: Liang et al. (2011b).

Fig. 5.4. Seedless loquat: (a) of H324 (white-fleshed); (b) D425 (orange flesh) with diploid 'Jinfeng' at far right. *Source*: Liang et al. (2011b).

recovery averaging 84% as compared to 64% for the diploid seed parents. Fruit index (L/W) tended to be greater in triploids iudicating that increase in length of triploid fruits was greater than width. In addition, triploids tended to have a sunken calyx. The average fruit weight of triploids averaged 48.9% higher than corresponding diploids. Flesh color ranged from white to orange reflecting color in diploid parents. Sugar content and acid were slightly lower than corresponding diploids but fruit quality of triploids reached levels required by the industry (Table 5.4). Elite strains are currently under testing in various locations in China.

The morphological characteristics and fruit quality of these super strains indicate that this breeding technique holds enormous promise for creating commercial seedless loquat. However there are a number of issues that need to be solved as follows:

1. Is pollination required for fruit set in triploids? If so, diploid pollinators would be required in solid blocks of triploids since only $(1/2)^{17}$ of pollen of triploids is expected to be haploid or diploid assuming random chromosome segregation of the 17 extra chromosomes in Division I of meiosis.
2. For seedless loquat to be successful, a range of types must be generated including a range of ripening time and various internal and external quality features (color and shape) to meet market expectations. This will require selection from literally hundreds of triploids. It is suggested that many thousands of seeds from the best diploid cultivars be grown out to identify potential triploid cultivars resulting from unreduced gametes.
3. Flow cytometry which is more efficient than chromosome counts should be adapted as a technique to evaluate triploidy,
4. The optimum levels of GA application will have to be determined for each triploid.

F. Molecular Breeding

1. Molecular Markers. Molecular markers in loquat have been used mainly for assessing genetic diversity relationships among *Eriobotrya* species and related genera using PCR-derived dominant and codominant markers.

Dominant Markers. The first type of markers used in loquat were random amplified polymorphic DNA (RAPDs) (Williams et al. 1990). These markers were used for genotyping of plant material and genetic diversity studies in loquat collections. Since they are dominant, this type of markers gives less genetic information, but they can be used when there is a lack of DNA sequence information. The first RAPD marker studies were applied to Mediterranean germplasm collections. Vilanova et al. (2001) studied cultivars with common origin, mostly local selections from Spain, and although the diversity found was low, as expected, the markers allowed the identification of cultivar homonyms and synonyms, and identification of derived natural mutations. Later, a larger collection of loquat genotypes was characterized using these markers by Badenes et al. (2004a). Although the accessions had a close origin, they were mostly local cultivars from the Mediterranean basin countries, the markers provides a high rate of cultivar identification. RAPD markers have also been used to elucidate the identity of cultivars in Chinese collections (He et al. 2007; Yang et al. 2009a, b). In all these studies, RAPD markers were useful for identification of loquat genotypes, however, the lack of reproducibility among laboratories made them less convenient for genotyping collections, since the data are hardly exchangeable. They were replaced later by amplified fragment length polymorphism (AFLPs) (Vos et al. 1995). These markers consisted of selective amplification of restriction fragments from a total digested genomic DNA, combining a restriction enzyme analysis with ligation with previous amplified adapters. In this way the PCR amplification was not based on random priming but was selective, and the reproducibility of the technique was acceptable, allowing comparison of genotyping experiments among laboratories. Yang et al. (2009c) used these markers for establishing relationships among *Eriobotrya* species. Wu et al. (2011) analyzed the genetic diversity in 34 cultivated loquat germplasm accessions using AFLP. A total of 1,091 AFLP bands were generated with eight informative primer combinations, of which 993 (91.0%) were polymorphic bands. A mean of 124.1 polymorphic bands was detected for each AFLP primer combination.

Codominant Markers. Microsatellites, simple sequence repeat or SSR markers (Tautz and Schlötterer 1994) have also been used for loquat diversity stndies. Badenes et al. (2004b) used SSRs cloned from *Malus* species to study a set of loquat accessions, found a good degree of transferability between *Malus* and *Eriobotrya* genera. This transferability was confirmed by Soriano et al. (2005), who found a high percentage of transferability of the SSR markers developed from *Malus* (Gianfranceschi et al. 1998) to *Eriobotrya*, confirming the usefnlness of microsatellite markers from apple species as a suitable tool for loquat cultivar identification and genetic studies.

However, microsatellites optimized from the same species may be of greater value in assessing diversity. Gishert et al. (2009a) developed and characterized the first 21 polymorphic microsatellite loci from a CT/AG-enriched loquat genomic library. The observed heterozygosity ranged between 0.20 and 1.00, and expected heterozygosity rauged between 0.17 and 0.81. Three markers were multilocus and eight loci departed significantly from Hardy–Weinberg equilibrium, and so were excluded from further evaluations The remaining markers were nsed by Gisbert et al. (2009h) for stndying the genetic relationships among 83 loquat accessions from different countries belonging to the European loquat germplasm collection held at the Instituto Valenciano de Investigaciones Agrarias (IVIA). A total of nine SSRs from *Malus* and

Eriobotrya revealed 53 informative alleles and S-RNases consensus primers detected 11 selfincompatibility putative alleles. The combined data allowed an unambiguously separation of 80 of the 83 accessions studied.

Another type of marker used for genotyping loquat is the internal transcribed spacer (ITS) from ribosomal DNA (Li et al. 2007). Following the study of Zhai et al. (2008), ITSs from loquat rDNA were isolated by Yang et al. (2011). The anthors characterized the sequences and found 130 variable points suitable for classification of *Eriobotrya* species.

Intersimple sequence repeats (ISSR) (Zietkiewicz et al. 1994) combined with RAPD markers has been used to establish the genetic relationships among Daduhe loquat (*E. prinoides* var. *dadunensis*), oakleaf loquat (*E. prinoides*) and common loquat (*E. japonica*) by Luo et al. (2011). ISSR markers proved very usefnl for studying genetic relationship among loquat germplasm from China (Xie et al. 2007)

2. Marker-Assisted Selection (MAS). Development of molecnlar markers linked to the traits of interest has the advantage of allowing selection of desirable genotypes as seedlings, reducing the number of plants evaluated. The lack of genetic studies has made MAS of limited value in loquat breeding to date. However, the selfcompatibility trait in *Eriobotrya*, is a gametophytic mechanism mediated by RNAs, is common to other rosaceous species, and homology of conserved regions have been used for development of markers for the selfincompatibility trait in loquat. S allele fragments were determined by amplification of partial degenerated primers designed from conserved regions of S allele sequences of *Malus* × *domestica* and *Pyrus* spp. (Raspé and Kohn 2002). Differences among the alleles amplified in accessions allows determination of the S alleles, to identify the compatible allele Sc and establish the groups of intercompatibility in loquat (Gisbert et al. 2009b). Differences in length of S alleles amplified by primers derived from conserved regions of RNAs gene on five selfincompatible loquat cultivars were determined by Carrera et al. (2009). Both studies provided a genomic PCR-based approach useful for identifying S-RNase alleles in loquat, which allow prediction of selfcompatibility of genotypes and intercompatible groups of cultivars for planning of crosses in breeding.

3. Genetic Linkage Maps. Construction of molecular marker linkage maps, is an important tool to expedite plant breeding. Such maps are of greatest value to breeding when markers are identified which are tightly linked to traits expanding the use of marker-assisted selection (MAS), which is described above. It is to then possible to use MAS for traits in which the associated gene is unknown, and where multiple loci (quantitative trait loci, QTLs) contribute to important traits. Linkage mapping, which allows quantitative character dissection, combined with candidate genes approaches, are new genomic tools for molecular breeding (Yamamoto et al. 2004).

Gisbert et al. (2009c) reported the construction of the first genetic maps of two loquat cultivars based on AFLP and microsatellite markers from *Malus*, *Eriobotrya*, *Pyrus*, and *Prunus* (Fig. 5.5). An F_1 population

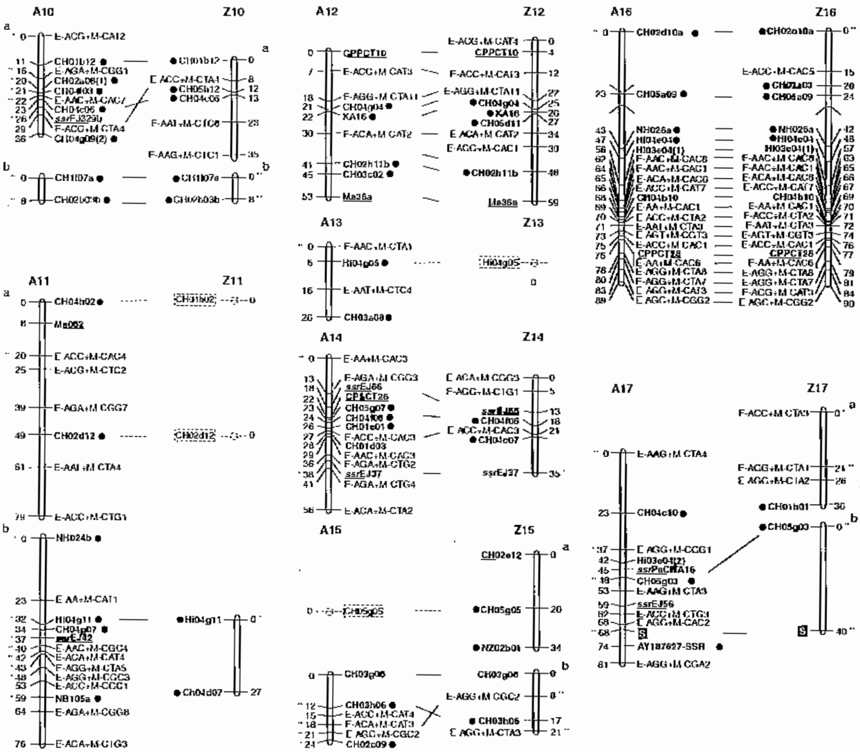

Fig. 5.5. Molecular genetic maps of 'Algerie' and 'Zhaozhong-6'. The map includes AFLP and SSR markers. The codominant markers came from *E. japonica* (16), *P. persica* (9), *Malus* × *domestica* (3), *P. armeniaca* (1), and *P. salicina* (1). Seventeen linkage groups were obtained, which agrees with the single haploid chromosome number of Pomoideae. The map of 'Algerie' contains 177 loci (83 SSRs y 94 AFLPs) expanding 900 cM. The map of 'Zaozhong-6' contains 146 loci (64 SSRs y 82 AFLPs), expanding 870 cM. In bold the codominant markers used. SSR markers from loquat underlined. The arrows indicated the markers establishing synteny between linkage groups. Solid circles indicate anchor markers with other *Malus* and *Pyrus* maps. *Source*: Adapted from Gisbert et al. (2009c).

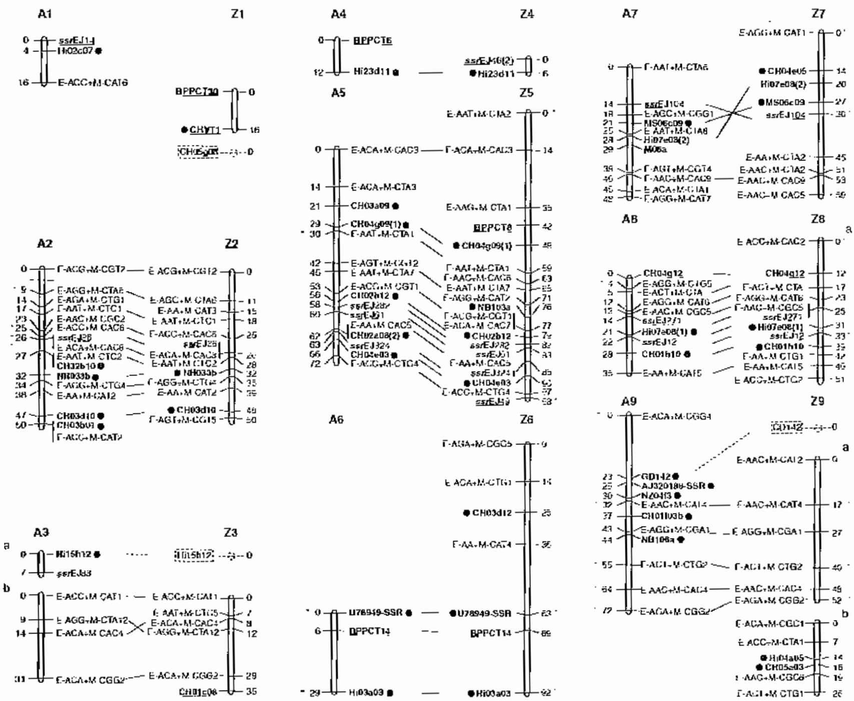

Fig. 5.5. (*Continued*)

consisting of 81 individuals, derived from the cross between 'Algerie' and 'Zaozhong-6', was used to construct both maps. A total of 111 scorable simple sequence repeat (SSR) loci were identified by testing of 440 SSR primer pairs in the analyzed progeny. The SSR transferability to *Eriobotrya* was found to be 74% from apple, 58% from pear and 49% from *Prunus* spp. In addition, 183 polymorphic AFLP were used to establish the maps. The 'Algerie' map was organized in 17 linkage groups covering a distance of 900 cM and comprising 177 loci (83 SSRs and 94 AFLPs) with an average marker distance of 5.1 cM. The 'Zaozhong-6' map covered 870 cM comprising 146 loci (64 SSRs and 82 AFLPs) with an average marker distance of 5.9 cM. The selfincompatibility trait (S-locus) was evaluated as an additionalcodominant marker, by nsing the primers described above, and permitting assessment of all alleles present. The segregation of the S-locns marker was consistent with a half-compatible reaction in which one S-allele was shared by both parents (1:1). Selfincompatibility trait was mapped at a LOD score of 6.0 at the distal part of LG17, agrees with maps from species from Maloideae subfamily. The bigh degree of synteny (retention of gene order on linkage groups) observed between *E. japonica* and other *Maloideae* species supports the potential nse of the biotecbnology resources from *Malus* to loquat, such as markers, sequence information (Velasco et al. 2010), and candidate genes.

4. Transformation. Loquat transformation has not been acbieved. However, regeneration protocols are in progress from leaves, stems, and roots (Blasco et al. 2011)

VI. FUTURE PROGRESS

Loquat is an ancient rosaceous fruit of China with records indicating that has been grown for over 2000 years (Lin 2008). The fruit is still widely acclaimed and appreciated in China and plantings are still increasing. The fruit has possibilities of becoming an important crop in subtropical and Mediterranean climates. Loqnat is an intrigning species that blooms in antnmn and early winter and is one of the first gifts of spring. The delicious and attractive fruit is nonclimacteric and has the potential of long storage life and can be consnmed both fresh and processed. Its main disadvantage is the many large seeds and the tender flesh. It is clear that many of the problems of this crop could be improved by breeding efforts. A survey of cnltivars that have been selected from open pollinated seed indicate many with high productivity, disease resistance, large size, various flesh color, high Brix, rich flavors, attractive appearance, various ripening dates, and long keeping quality.

Most efforts at crop improvement have been achieved by evaluation and selection of existing cultivars that have been selected aronnd the world but it is clear that vigorous programs of hybridization and recurrent selection should be able to produce superior clones that are adapted to specific areas. Efforts are underway in China (Zheng 2007), Japan (Lin 1998), Spain (Gisbert et al. 2007), and Turkey (Tepe et al. 2011). At the present time seedlessness has been achieved in China by selection of natnrally occurring triploid clones which require applications of gibberellic acid for fruit set. A number of seedless triploids have been produced and are currently under test to determine which selections have a chance at commercial success. However, more efforts are needed to exploit an even wider selection of germplasm.

The production of loquat would be greatly improved by the use of dwarfing rootstocks (Janick 2011). Dwarfing in loquat has been achieved with quince rootstocks but there are compatibility problems. Evalnation efforts are underway to determine if dwarfing rootstocks could be obtained within diverse *Eriobotrya* species (Zhang et al. 2010)

Advances in genomics and synteny in the Rosaceae offer exciting possibilities for loquat improvement. (Hummer and Janick 2009; Badenes et al. 2009). The development of saturated gene maps with codominant and transferable markers suggest that MAS could be used advantageously in loquat. Many of the genes responsible for desirable quality traits within *Prunes*, *Malus*, *Pyrus*, *Cydonia*, and *Fragaria* may be relevant to loquat. These include genetic dwarfing, enhanced fruit quality, and disease resistance. Furthermore, nse of transformation to incorporate critical genes from other fruit in the Rosaceae might minimize consumer resistance, which is greater with transgenesis using more distant species. Joint conventional and molecular breeding efforts involving scientists involved with loquat throughout the world would be the best strategy to achieve the desired goals of genetic improvement.

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