Litchi Breeding and Plant Management in Taiwan

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Abstract

Litchi (*Litchi chinensis* Sonn.) was introduced from Fujian Province of China into Taiwan by immigrants nearly 300 years ago. It is an economically important horticultural crop. Now, the total harvested area under litchi cultivation is 11000 ha, roughly, and the main variety "Hak Ip" accounts for over 70% of all area. The main constraint in the litchi industry is the short production season lead to the imbalance between supply and demand in market, due to "Hak Ip" planting too much. The seven novel varieties released from Taiwan Agricultural research institute have different fruit maturity seasons and good fruit quality. Based on the policy of the "right cultivar for the right land," cultivating them in proper ecological regions will effectively diversify/extend the production period and match market requirements. As for plant management, by means of studying the biology of flower and fruit development, researcher and grower have developed several special cultural technologies to apply for the commercial production of litchi. The current status of breeding, biology of flower and fruit development, and cultural research in Taiwan are discussed in this review.

Keywords

Litchi • Cultivation • Agricultural research • Plant management • Commercial production

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2.1 Introduction

Litchi or lychee (*Litchi chinensis* Sonn.) was a subtropical evergreen fruit tree, which originated in the area between latitudes 23° N and 27° N; it is more accurate to say that its source was in the region between southern China, northern Vietnam, and Myanmar (Menzel 2002a, b) and belongs to the Sapindaceae family. The earliest record of litchi cultivation in China began in 111 BC, during the Han dynasty (Huang 2002). Now it spreads between latitudes 31° N (the south Sichuan Province) and 18° N (the south of Hainan Province), whereas the commercial production zone lies between latitudes 19° and 24° N (Guangdong, Guangxi, Fujian, and Hainan Province) in China (Wu 1998). Litchi was introduced into Burma (Myanmar) by the end of the seventeenth century and reached India and Thailand about 100 years later. Litchi reached Madagascar and Mauritius around 1870 and was introduced in Hawaii in 1873 by a Chinese trader. It arrived in Florida, from India, between 1870 and 1880 and was introduced in California in 1897. Litchi was probably introduced into Israel around between 1930 and 1940 (Mitra 2002). Litchi might be reached in Australia around in the 1940s, though it was not as a commercial crop until the 1970s (Menzel 2002a, b). Since the twentieth century, litchi had become a worldwide fruit tree; it had about 800,000 ha orchard in 2005, and China, Vietnam, India, Thailand, Madagascar, Taiwan, South Africa, Mauritius, and Australia are now major lychee-producing countries in the world (Young 1970). Although the annual yield of 2005 was about 2.5 million tons, fruit storage and shelf life were very short, resulting in only 50–60 thousand tons of fruit for international trade (Chang and Chang 2015).

Economical litchi production is limited by its strictly environment requirement and biannual bearing behavior. The litchi crop is best in regions with frost-free winters and summers with high rainfall and humidity. It needs cool and dry weather in autumn to winter for flower bud formation, warm and enough water supply but not too much rainfall during late winter to early spring for flowering, and warm to hot and humid from spring to summer for fruit growth. So, the economical production area is limited in humid subtropical area, especial between altitude 18°–28° N and 6°–29° S.

Compared to other fruit trees, litchi breeding is a little bit complicated and difficult, because any of the following difficulties need to be overcome, which include very long juvenile stage, unique and strict environmental condition for flower bud formation, abundant but few efficacy florets, and low fruit set rate no matter by using hand or open pollination, very short storage lives of pollen and seed, chilling damage sensitive during flowering and fruit developing stage, and low propagation efficiency. So, the breeding achievements of litchi are still lower than most of the other fruit crops.

2.2 Industry Status in Taiwan

According to the history, the litchi was introduced from Fujian Province of China into Taiwan by immigrants nearly 300 years ago (Chang 1961; Huang 1966); however, mass cultivation did not begin until the 1950s. In 1964, the producing area was only 695 ha. But after that it increased very quickly and gradually become an economically important fruit crop. In 1988, it reached a peak with 14,682 ha. However, due to the first major cultivar, "Hak Ip," planting too much caused the short production season and resulted in the imbalances in market supply and demand. Following 1988, litchi production in Taiwan declined (Chang et al. 2005). According to Taiwan Agricultural Year Book in 2015, the total harvested area under litchi cultivation was only 11,187 ha, with a total production of 70,537 tons; the average yield was 6.3 tons/ha. Litchi ranks only behind citrus, mango (*Mangifera indica* L.), and pineapple *(Ananas comosus)* in the fruit crop yield in Taiwan (Taiwan Agricultural Statistic Year Book 2015).

Although more than 30 cultivars have been reported, only three of these are cultivated on a large economic scale (Chang et al. 2009a, b). The cultivation status of the three cultivars and their general characteristics in Taiwan are described as follows:

"Hak Ip" ("Haak Yip," "Hei ye," "Black Leaf," "O-ia" (Chang and Cheng 2002)) is the most popular cultivar among fruit growers, accounting for over 70% of all area under litchi cultivation (Chang et al. 2012). The fruit weighs around 22 g, with uneven heart fruit shape. It has medium-sized seed and 70% flesh recovery (Teng et al. 2004). "Hak Ip" adapts to a wide range of geological conditions and has abundant yield results in very popular for grower. Its growing areas are located in central and southern Taiwan (Chang et al. 2009a, b).

"Yu Her Pau," also called "Fei Zi Xiao" in China and "Fay Zee Siu" in Australia (Menzel et al. 2005), is an early-maturing cultivar with a superior quality of fruit. The fruit weighs around 22–40 g with round to oval fruit shape. It has 50% shriveled seed (chicken-tongue seed) and 73% flesh recovery on average (Teng et al. 2004). It accounts for about 25% of the land under litchi cultivation and almost located in southern Taiwan (Chang et al. 2012). Productivity of this cultivar is unstable, although its fruit gets high price.

"73-S-20," a branch line of "No Mai Tsz," was found by Yen et al. (1984) in Nantou, central Taiwan. There is another variety named "No Mai Tsz" in Taiwan. Both varieties have similar fruit appearance and taste, except that "73-S-20" has sharper protuberances, firmer flesh, a higher proportion of shriveled seeds (upmost 90%), and higher flesh recovery (70–80%). After agricultural administration office promoted for many years, "No Mai Tsz" has been almost replaced by "73-S-20." In Taiwan, now, growers call "No Mai Tsz" which in fact means "73-S-20." Although this cultivar is considered to be with the best fruit quality among all of the litchi cultivars commercially cultivated (Yen 1995) and has good sale and better price, it accounts for less 1.5% of litchi production area in Taiwan, and most of its growing areas are located in central–northern Taiwan (Chang et al. 2009a, b). The reasons include the following: it bears irregularly, fruit shows a significant variation in shriveled seed ratio, and it is prone to fruit cracking (Chang et al. 2009a, b). It has been assumed that "73-S-20" is unlikely to be genetically identical to the original China cv. "No Mai Tsz" (Yen 1995). But it most likely is "Guiwei" in China when you judge it from the fruit appearance and characteristics data.

Other cultivars, such as the "Sah Keng," "Kwai Mi," "Sam Yee Hong," "Nansi Early," and Tainung series, occupy for less than 4% of all area under litchi cultivation (Chang et al. 2012).

In the early 1980s, the problem of the short production season led to the imbalance between supply and demand in market, due to too much "Hak Ip" planting which was apparent in Taiwan. The Taiwan Agricultural research institute (TARI) started the litchi program in 1982 in attempts to breed new varieties with diversified fruit maturing and excellent qualities to solve the problem. The responsible units were Chiayi Agricultural Experiment Station (CAES) and Fengshan Tropical Horticultural Experiment Station (FTHES), two branches of TARI (Yen 1995).

2.3 Breeding Objective and Strategies

2.3.1 Breeding Objectives

An ideal litchi cultivar for modern requirement includes regular high yields, large fruit size (individual weighing over 25 g), higher aril percentage or higher fresh recovery, bright red skin color, good fruit flavor and texture, good storage life, resistance to physiological disorders and pest, desirable tree structure, and wider adaptability to diverse ecological conditions (Ray 2002). However, to breed an ideal litchi cultivar is not necessary. If we take it as the breeding objective, it is difficult to conduct the program even in primary selection owing to too high selection pressure. The breeding goal is to solve the industry's problems. In Taiwan, the most serious problem is the short production season leading to imbalance between supply and demand for fresh fruit. Early or late fruit maturity with regular high yields to extend the production is the most important consideration (Chang et al. 2009a, b). Other important considerations depend on the characteristic of fruit maturity season. Generally, the standards of fruit qualities to require for late-maturity variety are higher than early-maturing variety. For early-maturing variety, the fruit qualities only need to accord with the consumer's demand. But as to late-maturing varieties, the standards of the fruit qualities and storage life need higher than the main commercial variety, "Hak Yip."

2.3.2 Breeding Strategies

There are different breeding strategies applied for litchi to improved traits, such as conventional breeding, genetic engineering, in vitro mutagenesis, and molecular-assisted breeding (Sarin et al. 2009). However, conventional breeding is still the main approach in Taiwan (Chang et al. 2013). The molecular technologies are only used to assist litchi breeder to identify the genetic relationship among varieties, currently (Lee et al. 2007; Wu 1998; Kumar et al. 2006).

2.4 Germplasm Resources

There are more than 45 varieties at Chiayi Agricultural Experimental Station and Fengshan Tropical Horticultural Experimental Branch, TARI, in Taiwan. Except for local collections, many of them are introduced from China and Hawaii.

2.4.1 Parental Choice

According to the breeding objectives, selecting the appropriate parent is the first step to ensure that breeding success. Factors to consider parental choice include the following three categories.

2.4.1.1 The Utilization of Varieties with Special Characteristics

In Taiwan, the varieties which relatively commonly used as parents include the following categories (Chang et al. 2013):

Giant fruit size: Ziniangxi, Dadingxiang (China variety), and Chakrapad (Thai-land variety)
Seedless: Hainan Wuheli and Guangxi Wuheli (China variety)
Chicken-tongue seed: Yu Her Pau, No Mai Tsz (73-S-20, Taiwan variety), and Dadingxiang
Extreme Harvest Season: San Yue Hong, Kwai Mi, and Khom (Thailand variety)
Abundant yield: Sah Keng (Taiwan variety), Hak Yip, and Kwai Mi

2.4.2 The Utilization of Varieties with Special Characteristics

To get more genetic diversity of offspring is one of the concerns of the breeder. Means of using difference in morphology and/or DNA markers to compare parents are efficient ways to maximize the probability of selecting those parents with different gene set. In West Bengal, the cultivars were grouped into four clusters on the basis of six fruit characteristics. Crossing between cultivars of cluster I with cluster IV is expected to give maximum extent of heterosis (Dwivedi and Mitra 1996). Kumar et al. (2006) reported the genetic relatedness among Indian litchi cultivars by random amplified polymorphic DNA (RAPD) markers. Twenty-five sample accessions were classified into five groups. In Taiwan, Yen et al. (1984) evaluated 20 cultivars based on 11 fruit characteristics and tree growth vigor and divided them into three groups, named “Hon Li” group, “Hak Ip” group, and “Kwai Mi” group. The cultivars such as “Yu Her Pau” and “Chung Yuang Hong” belong to “Hon Li” group that have oblong to ovoid fruit with distinct protuberance and early maturity.

The cultivars that belonged to “Hak Ip” group have cordate and dark red fruit with smoother protuberance and middle maturity. “Hak Ip,” “Sah Keng,” and “San Yueh Hong” belong to this group. The cultivars that belonged to “Kwai Mi” group have globular and colorfully red fruit with a specific fragrance in aril. “Kwai Mi,” “Hawai Li,” and “No Mai Tsz” belong to this group. Lee et al. (2007) and Kumar et al. (2006) used RAPD markers to identify the genetic relationship among native cultivars in India and China, respectively. The results show that they could be divided into two major groups. Among them, “Yu Her Pau” and “Sun Yueh Hong” belong to one group, and “Hak Ip,” “Sah Keng,” “No Mai Tsz,” “Kwai Mi,” and “Hawai Li” belong to the other. Wang and Chang (2010) used RAPD markers to identify the genetic relationship among 14 cultivars and find that they could be divided into two major groups. Among them, “Tainug No. 7 (Early Big),” “Tainug No. 1 (Tusey Yuh),” “Tainug No. 5 (Ruby),” “Yu Her Pau,” “Hak Ip,” and “Sah Keng” belong to one group; their fruits mature early to middle. “Tainug No. 3 (Rose Red),” “Tainug No. 4 (Lucky Lychee),” “No Mai Tsz,” “Kwai Mi,” and “Hawai Li” belong to the other; their fruits mature late. By using inter-simple sequence repeat (ISSR) markers, the same samples were identified to show that they could be divided into three major groups. “Tainug No. 7 (Early Big),” “Tainug No. 1 (Tusey Yuh),” “Yu Her Pau,” and “Tainug No. 4 (Lucky Lychee)” belong to one group; their fruits mature early. “Tainug No. 3 (Rose Red),” “Tainug No. 5 (Ruby),” “Hak Ip,” and “Sah Keng” belong to another group; their fruits mature middle. “No Mai Tsz,” “Kwai Mi,” and “Hwai Li” belong to the other; their fruits mature late. Both ways showed that the genetic relationship between “Yu Her Pau” and “Hak Ip” was closer than “No Mai Tsz.” The genetic relationship between “Hak Ip” and “Sah Keng” was very close, so as to between “No Mai Tsz” and “Kwai Mi.” It also supported the description that “Tainug No. 7 (Early Big),” “Tainug No. 3 (Rose Red),” and “Tainug No. 5 (Ruby)” are the offspring of “Yu Her Pau,” “No Mai Tsz,” and “Sah Keng,” respectively.

2.4.3 Assess the Genetic Dynamics of Offspring

After the breeding work lasted for some time, the breeder should assess the performance of offspring from different mother sources. Using the data to choose the parents and adjust the quantity of seeds for planting will contribute to save the cost, labor, and land and increase breeding efficiency. Yen et al. (1984) accorded to the results of controlled hybridization indicated that “Kang Wei,” “Hwai Li,” “Sah Keng,” and “Hak Ip” were the best mother plants. Chang et al. (2011) compared the results of 32 controlled hybridized compositions and got the results that “Sah Keng” and “Kwai Mi” used as mother parents could get more seeds than other varieties. In this article they also described the results of assessing 324 open pollination seedlings from 12 mother plants and indicated the following points:

1. “Sah Keng,” “Kaohsiung early,” and “Kang Wei” used as mother plants contributed to shorter juvenile period of offspring.

2. The offspring of “Sah Keng” showed divergence toward early maturity season. Some of the second offspring even had maturity season 1 week earlier than the mother plants and 3 weeks earlier than “Sah Keng.”
3. The contributions of mother plants to fruit size of offspring were not significantly correlated. The offspring of mother plants which had small fruit size produced small fruit. However, mother plants which had large fruit size might not have offspring with large fruit.
4. Flesh recovery of offspring was not positively correlated with that of mother plants. However, total soluble solids in fruits of offspring were rather consistent with those in mother plants.

2.4.4 Seedlings Selection from Open Pollination

A multi-variety gene pool orchard composed of 14 varieties with nine replications (Yen et al. 1984), by using “Sah keng,” “Sun Yueh Hong,” “No Mai Tsz,” “Kwai Mi,” and “Hawai Li” as stocks, 35 varieties as scions making up poly-cross compositions (Chang et al. 2013), was established to increase the hybridization ratio under natural conditions in Chiayi Agricultural Experiment Station (CAES), TARI. In the past 34 years, a total of more than 7000 seedlings were planted for evaluation and selection.

2.4.5 Controlled Hybridization

The hybridization technique used in Taiwan was as follows

The inflorescence of litchi is determinate and composed of several panicles. Each panicle of litchi produces three types of flowers as type I, type II, and type III, named by Mustard (1960). Type II is defined as functional female, type I and type III as functional males, but type III with more hermaphrodite feature than type I (Galan et al. 1989). The researchers prefer the terminology based on their sexual functionality (male 1, female, and male 2). Usually, M1, F, and M2 open in sequence on the same panicle (Robbertse et al. 1995). But many variations have been found. Sometimes M1 and F and F and M2 are partially overlapped (Galan Sauco and Menini 1989). In order to avoid pollution of mother plants by M1, emasculation is necessary. Only about 25–30 F flowers expected to open next day remain on the panicle. The remaining flowers are removed. The panicle is then bagged to protect it from unwanted pollens. One day before pollination, the chosen male flowers were taken and placed in petri dishes, under exposure of a bulb, making the anther to split as the pollen source. Pollination was conducted 2–3 days after F flower anthesis, and only 15–20 flowers are pollinated (Yen et al. 1984). The remaining F flowers were removed and the panicle was bagged again.

2.5 Breeding Procedure

The litchi breeding procedure in Taiwan shows as Fig. 2.1. The focal points need to consider are as follows.

2.5.1 Seedlings Management

In order to reduce the cost and save the land use, handling the seedlings with high population was practiced. The spacing of 3 m × 1 m was adopted in CAES now, and the seedlings were zoned to plant according to the growth vigorous. Top grafting and the ways of chemical applying to shorten the juvenile phase were used (Chang et al. 2013).

2.5.2 Primary Selection

To access the fruit characteristics of the seedlings is the most important work of the primary selection phase. Five selected criteria were laid down in this phase in CAES. They include single fruit weighing over 20 g, flesh recovery over 70%, total soluble solids over 16∘Brix, harvest season different from "Hak Ip," and special flavor. Only the data for 2 years in succession met three of these five criteria to be chosen as a superior line and move on to the next phase of the trial. When the fruits

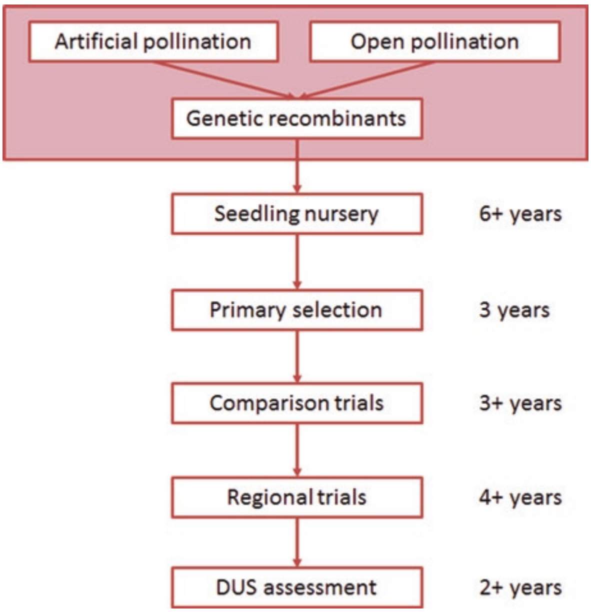

Fig. 2.1 The breeding procedure in Taiwan

of each seedling are near maturity, they are harvested twice, space for 1 week or 10 days, to access harvest season more precisely (Chang et al. 2013).

2.5.3 Comparison Trials

Usually, we use "Hak Ip" as the check variety. Similar sizes of air layers were used to avoid the stock factors which affect the performance. At least five plants were needed for each test clone. Yield, percentage of chicken-tongue seeds, and harvest season were the three key items to access in this phase (Chang et al. 2013). The pollen parent effects on seed size have been reported in litchi literature (Sun et al. 2010; Chu et al. 2015). At primary selection phase, owing to high-density planting leading to different seedling sources that pollinated each other very easily, it was hard to evaluate the percentage of chicken-tongue seeds. Fruit maturity season is easily influenced by climate factors. It usually changes slightly year by year. But our real purpose is to understand the gap of maturity season between the test line and "Hak Ip" at same producing area (Chang et al. 2013).

2.5.4 Regional Trials

Regional test as the name suggests is to assess the adaptability of the clones to regard as reference in the future promotion. Usually, we take the main local variety as the check. For example, if the trial was taken in southern Taiwan, we chose "Yu Her Pau" as the check. However, if it was done in central Taiwan, "Hak Ip" was chosen, and sometimes "No Mai Tsz" was chosen as the reference. In principle, similar sizes of air layers were used. However, top grafting was used to esteem the opinions of the cooperative growers. At least five plants were needed for each test clone. But if the cooperative grower was willing to provide more land, the quantity is increased (Chang et al. 2013).

2.5.5 Other Related Trials

The investigations of plant diseases, insect pests, and fruit storage are the other important data for breeders to understand the characteristics of test clones in further. The investigated kinds of diseases and pests we usually used were downy blight, litchi sour rot, litchi anthracnose, and brown root rot and litchi borer. In the fruit storage trials, the controlled temperatures generally we used were 5 °C, 10 °C, and 15 °C [16].

2.5.6 Apply and Get "the Plant Variety Right"

In Taiwan, the law of "the plant variety and plant seed act" was promulgated in 2004. The breeder would apply "the plant variety right" to acquire the protection by government. The report of distinctness, uniformity, and stability (DUS) assessment is requested in the qualification of the course. Generally, there is no problem in uniformity and stability assessment. Because vegetative propagation is used for the commercial production of litchi nursery, normally, the performance of the plant characters of the materials is very uniform and stable. However, in distinctness assessment, the chosen of check variety needs to pay attention specially. Check variety must be chosen in the morphology closest to the application variety. On the real affair, the parent, mother parent, especially, is chosen as the check variety. However, when the parents are not commercial varieties, the closet commercial variety in morphology always is requested as another check variety.

2.5.7 Achievements

Seven novel litchi varieties, namely, “Tainung series,” have been released by the Taiwan Agricultural Research institute (TARI), four varieties from Chiayi Agricultural Experiment Station (CAES) and three varieties from Fengshan Tropical Horticultural Experiment Station (FTHES), up to now. Their characteristics are laid as follows:

1. “Tainung No.1” (Tsuey yuh) is a hybrid resulted from “Hak Ip” × “Yu Her Pau” and released in 2004 from CAES. The commercial name “Tsuey yuh” litchi meant the litchi has green peel at suitable harvesting time. “Tainung No.1” has fruit weighting around 15.7–27.7 g with total soluble solids of 17.6–20.2° Brix. “Tainung No.1” has high chicken tongue seed percentage (51.9–100%) with seed weighting around 0.4–1.3 g. These characteristics results have higher flesh recovery (80.5–86.3%) than other commercial varieties in Taiwan (Fig. 2.2). The harvest period was from middle May to early June. It was 7–10 days earlier than that of “Hak Yip.” The yield is nearly 12 tons/ha, assessed by 5-year-old trees at planting density 400 plants/ha (Chang et al. 2005).
2. “Tainung No. 2” (Wuang Lee) is a hybrid resulted from “Sah Keng” × “Yu Her Pau” and released in 2007 from FTHES. It is an early variety. The harvest season is around the early of May in southern Taiwan, which is the same as “Nansi Early” (named Souey Tung in other countries) and about 10 days earlier than “Yu Her Pau.” The fruit quality is excellent with fruit weighting 21.4 g and 18.5。Brix total soluble solids on average. It bears the fruits that have 100% of small seeds with average weight of 1.2 g. This characteristic result has 77.0% flesh recovery on average (Teng and Liu 2007a, b).
3. “Tainung No. 3” (Rose Red) has been released in 2006 from CAES. It is an open-pollinated seedling. The seed was gotten from the multi-variety gene pool litchi orchard of CAES in 1986. Its parents most likely are “No Mai Tsz” and “Kwai Mi,” when RAPD markers used to identify the genetic relationship (Wang and Chang 2010). The name “Rose Red” refers to the litchi fruit with rose

Fig. 2.2 "Tainung No.1" (Tsuey yuh) produces abundant yield, and fruit has high chicken tongue seed percentage and shows green pee at suitable harvesting time (Adapted from Chang, Jer-Way)

fragrance of aril and red rose peel in color (Fig. 2.3). The fruit qualities of "Tainung No.3" are excellent. The fruit weight, seed weight, aril percentage, total soluble solids, and titratable acidity are 23–29 g, 1.0–3.2 g, 67–75%, 17.4–20.2 。Brix, and 0.12–0.17%, respectively. The flesh is firm. The peel segments are swelling, and the protuberances are protruding (Chang et al. 2009a, b). Tainung No. 3 is also named "zipper" lychee (Fig. 2.4) by consumer, because the suture of the fruit is obvious and easily peeled only by means of using the thumb of both hands, squeezing, and stripping the peel from the both side of suture. By using this way to enjoy the aril, consumers don't need to worry the juice making the hands wet. For it is so convenient and clean for consumer, it is the most welcomed and expensive litchi in the market of Taiwan, currently. The harvest period is from late June to early July. It is 7–27 days later than that of "Hak Ip." The "Rose Red" lychee has good storage life. When the fruits were wrapped with plastic film and stored at 4 °C, the storage life was estimated 30 days (Wang, Yi-Tien, personal communication). "Tainung No.3" is an exciting candidate for litchi growers to replace "Hak Ip" and to exploit the economic potential of litchi production in Taiwan.

Fig. 2.3 "Tainung No. 3" (Rose Red) has red and rose-colored peel and rose fragrance (Adapted from Chang, Jer-Way)

4. "Tainung No.4" (Lucky) was selected from the open-pollinated offspring of "Chakrpad" in 1993 and has been released in 2008 from the FTHES. It produces abundant yield. "Tainung No.4" is a late variety. The harvest season is around the early of July, which is the same as "Kwai Mi" (the latest variety in Taiwan) and about 20–28 days later than that of "Hak Ip." "Tainung No.4" is known for giant fruit size weighing around 41.5 g with oval fruit shape, purple red peel color, 70.2% flesh recovery, and 17.8 total soluble solids on average (Teng and Liu 2007a, b).
5. "Tainung No.5" (Ruby) is selected from the open-pollinated offspring of "Fay Zee Siu" (not the same as "Fay Zee Siu" or "Feizixiao in other countries) in 1989 and has been released in 2008 from the CAES. "Tainung No.5" produces abundant yield (more than 12t/ha of 5-year-old trees). It has the averaged fruit weight of around 18–20 g, with long cordate shape, bright red peel color, protruding protuberance, and unobvious suture. In addition, there are two attractive characteristics when it is compared with other main commercially available litchi cultivars: (1) it bears the fruit that have 50–80% of shrivelled seeds, and (2) its cropping is regular (Fig. 2.5). Fruits were harvested from late June to early July, about 7–14 days later than "Hak Ip" (Chang et al. 2010). It needs a longer cool period to induce flower than "Hak Ip" (Chang et al. un-published data).

Fig. 2.4 "Tainung No. 3" (Rose Red) also named "zipper litchi" (Adapted from Yan, Hong-Ren)

6. Tainung No. 6 ("Colorful") was selected from "Khom" open-pollinated offspring in 1999 and has been released from the FTHES in 2011. It is well known for its very early-maturing trait. The harvest season is around the middle of April, which is earlier than "San Yueh Hong" (also named "Sum Yee Hong" in other countries which is the earliest variety in Taiwan even in China) and about 30 days earlier than "Yu Her Pau." The fruit is large weighing around 27.9 g with elliptic fruit shape and blight red peel color (Teng and Chen 2011).
7. Tainung No. 7 ("Early Big") has been released in 2010 from CAES. It was selected from "Sah Keng" open-pollinated offspring and its father plant most likely is "Yu Her Pau" with RAPD and ISSR markers to identify the genetic relationship (Wang and Chang 2010). Tainung No. 7 is known for its early-maturing trait and large fruit size compared with other commercial varieties. The harvest of Tainung No. 7 is the same as "Nansi Early" (named "Souey Tung" in other countries) and about 10 days earlier than "Yu Her Pau" and 17–21 days earlier than "Hak Ip" or "Sah Keng" grown in the same region; in addition, the averaged fruit weight is generally more than 30 g. Tainung No. 7 has red peel, elliptic or cordate fruit shape, and mild–sweet and juicy aril (Fig. 2.6) (Chang et al. 2014a, b). With these special fruit characteristics and higher regular yield than "Nansi Early" and "Sanyuehong," Tainung No. 7 has become a popular variety for orchard renewal among growers.

2.6 Biotechnology

The traditional selection methods cost long time because of the long juvenile phase (Chang et al. 2001). Tissue culture and molecular tools were applied to accelerate the selection and propagation procedures. In the section we will briefly introduce the aspects and methods of litchi genetic improvement.

Fig. 2.5 "Tainung No.5 (Ruby)" produces abundant yield, and the fruit has long cordate shape, bright-red peel color, and high chicken tongue seed percentage (Adapted from Chang, Jer-Way)

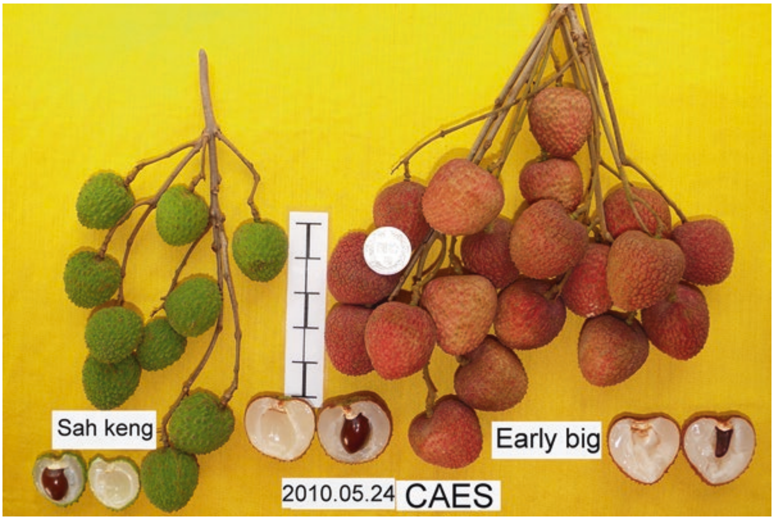

Fig. 2.6 Tainung No. 7 ("Early Big") is known for its early-maturing trait and large fruit size compared with other commercial varieties. The harvest of Tainung No. 7 is 17–21 days earlier than "Sah Keng" grown in the same region (Adapted from Chang, Jer-Way)

For the outcrossing crops, the increase in the genetic diversity of parent population is a concern for breeders. This can be achieved by germplasm introduction, mutation, and genetic modification/editing. On the other hand, the commercial names of litchi are confusing between countries during the dispersal in the past hundred years. These individuals may share morphological similarity, while some cultivated lines or varieties were derived from the "ancient" cultivars. Collection of the divergent germplasms is difficult to achieve with the limited resources (space, labor, and budget) in reality. The effective ways to manage the parent population or germplasm conservation will benefit long-term crop improvement.

The molecular tools provide reliable approaches to identify the litchi lines. Degani et al. (1995a) used the leaf isozyme banding patterns to identify the litchi cultivars; for example, in Taiwan, the "Yu Her Pau" has identical isozymic polymorphisms to "Fay Zee Siu," and the unnamed line #3 from Taiwan is highly similar to "Haak Yip (Hak Ip)" from Hawaii. This study also supports the description that the land-race "Sah Keng" in Taiwan is the offspring of "Hak Ip" (Menzel and Simpson 1990). These genetic relationships were confirmed using the inter-simple sequence repeat (ISSR) markers (Degani et al. 2003). Other nucleotide markers like simple sequence repeat (SSR), random amplified polymorphic DNA (RFLP), and single-nucleotide polymorphism (SNP) have been developed to be utilized in litchi (Vos et al. 2009; Madhou et al. 2013; Liu et al. 2015; Long et al. 2015). Identification and grouping of the litchi lines by informative markers increases the efficiency of germplasm introduction and conservation, parent selection, and hybrid detection for the conventional hybridization procedures.

Interspecies hybridization is one of the new techniques that results in greater genetic variations with the Sapindaceae family. Longan (*Dimocarpus longan*) is one of the good candidates with better pest and disease resistance and later harvest season compared to litchi. The hybridization between litchi and other S. fruit has been done in Australia and China (McConchie et al. 1994; Zhao et al. 2008).

In China, the majority of litchi varieties or lines were selected from seedling, whereas others were derived from somatic mutation which can carry out different traits on cool temperature requirement, fruit appearance and its quality, and the harvest season (Sun et al. 2010). Mutation tended to create novel cultivars, especially when it is targeted to reduce seed size or extend the production season (Jain 2000). Gamma-ray and X-ray irradiations have been reported to be applied on litchi budwood in the breeding procedures in South Africa. For gamma-ray treatment, approximate 20 Gy irradiation was suggested for selecting the survival, although the LD50 was estimated at 36 Gy (Vos et al. 2009).

Tissue culture methods provide other options on creating novel lines, for example, the haploid plant acquiring via anther culture (Fu and Tang 1983), which was established to create doubled haploid pure lines (Fu 1990). The tissue culture practice also generates genetic variation in conjunction with the crop breeding procedures (Jain 2001). Several in vitro methods of litchi micropropagation have been established in the past. For example, anthers, leaves, and zygotic embryos from immature fruit have been used as the litchi explant resources on in vitro regeneration and somatic embryogenesis (Liao and Ma 1998; Puchooa 2004; Das et al. 2016).

These methods can be further used to reduce the length of time for breeding and create transgenic individuals, as *Agrobacterium* mediated transformation has been succeeded in litchi (Das and Rahman 2012).

2.7 Plant Management

To induce flowering and to improve fruit set are always important issues, no matter for breeder or producer. Researchers in Taiwan have to pay much effort to study the biology of flower and fruit development and have developed several cultural techniques to apply for the commercial production of litchi.

2.7.1 Growth Rhythm

The growth rhythm of litchi tree is a continuous processing, and the vegetative or reproductive types of next new flush are dependent on the environment. Although litchi is considered no dormancy stage as temperature fruit trees does, a vegetative dormancy between the last shoot growth and the next new flush growth exists within each growth section in the growth rhythm of litchi. When the environment is suitable for bud burst, the apical bud starts to expand and to develop new leaves. The number of shoot flushes in each year depends on the cultivar and the environmental condition. The period of vegetative dormancy between each flush is an important stage, and the morphology of next growth was decided during this vegetative dormancy. The switch of this period is dependent on the temperature. When the weather is warmer, the duration between two vegetative flushes is shorter and the next growth of flush will become the shoot. However, when the temperature is lower than the critical temperature of the chilling requirement for inflorescence initiation, the cessation period is going to be longer, and the buds will have high potential to transfer to an inflorescence bud. The several alternative growths of vegetative shoots and one reproductive growth complete the litchi growth rhythm.

2.7.2 Flower Bud Formation

Poor litchi flowering is a worldwide problem (Menzel 1983, 1984; Galan 1989), especially in regions where the weather during the induction period is too warm (Groff 1943; Young 1970; Menzel 1983). Cool and dry weather from late autumn to winter is the basely environmental condition for litchi flower bud formation, but Nakata and Suehisa (1969), Huang and Weng (1978), and Teng (1988) depicted that even under flower bud formation condition, immature flush, with the leaves still purple red to light green, could not change from vegetative to reproductive statute. Huang and Weng (1978) and Chang et al. (2014a, b) found that in Taiwan climate condition, late flushes should fully mature before November, or most of them would form vegetative flush in next year spring. So, dry weather in autumn is a benefit for shoot to cease growth and to move to the vegetative dormancy stage and then increased the flowering intensity at next season. In Israel, got similar results from the study on “Mauritius” and “Floridian” litchi.

Chang et al. (1997) and Chang (1999) used 2-year potted “No Mai Tsz“ litchi air layers to conduct a serious experiments in order to define the condition needed for inducing flower bud formation. In experiment 1, the durations of 15 weeks for temperature treatments and 6–9 weeks for water stress treatments were conducted in phytotron. At 25/20 °C (day/night) temperature, irrigation promoted vegetative growth, plants under water stress treatments unable flushing. Even after the water supply resumed, no panicles were produced. At 20 °C/15 °C (day/night), only a few panicles formed in the irrigation treatment, and over 70% the plants in both treatments showed vegetative dormancy. Over 80% shoots of both irrigation and water stress treatments under 15 °C/13 °C (day/night) for 7 weeks induced the development of visible panicles from terminal buds. In experiment 2, by comparing the cyclical water stress (irrigation when soil water tension attained −70 to −80 centibars during October to early December then resumed the water supply until the next spring season) with continuing irrigation treatments under nature environment, the results showed that only very few terminal buds developed panicles in cyclic water stress treatment and all plants in irrigation treatment grew flush without any flowers. In experiment 3, three sets of plants with all shoot growth ceased were treated to define the influence of slight water stress on flower formation. Two sets of the plants treated water stress for 31 days and 58 days under natural environment, then moved to the controlled growth chamber maintained at 20/12 °C, and resumed irrigation daily, respectively. The other one is irrigated daily at the same artificial situation as the other two treatments from the beginning of the experiment. The plants of water stress treatments were controlled at a constant pre-dawn leaf water potential of −1.2 to −1.4 MPa which is monitored by measuring leaf stomata resistance daily. The results showed that the “58-day water stress” treatment got nearly 70% shoots flowering which significantly higher than “31-day water stress” and “continuing irrigated” treatments, which got 9% and 43%, respectively. However, the “31-day water stress” had the highest percentage of shoots with growth ceased, over 80%, compared with “58-day water stress” and “continuing irrigated” treatments, which got 21% and 43%, respectively. These experiments indicated that water stress reduced vegetative growth but couldn’t induce “No Mai Tsz“ litchi flowering if the enough cool temperature requirement were not met or it might be the water condition in the root zone that will influence the degree of cool temperature requirement for inducing flowering. Both Chaikiattiyos et al. (1994) and Menzel (1983) got similar result in Australia. It meant that cool temperature was essential for flower bud formation. Menzel et al. (1989) mention that water stress appears to act by synchronizing vegetative dormancy in the branches before exposure to low temperatures, as to the degree of cool temperature required for inducing flowering that depends on the varieties. Basically, below 20 °C may meet the minimum requirements, while below 15 °C may get much higher flowering intensity (Chang et al. 1997;

Menzel and Simpson 1988, 1995). In Taiwan, according to the results of a temperature model of panicle formation which established by Chen et al. (2016b) to predict panicle burst date, "Yu Her Pau" may just need below 23 °C.

Roots play very important role in litchi flower bud formation. Under low-ambient-temperature condition, "Yu Her Pau" litchi could not develop panicle if root temperature is higher than 25 °C (Teng 1988). Menzel et al. (1989) also found that a number of panicles were lesser when day shoot temperatures and root temperature exceeded 20 °C. They also considered that low starch reserves, especially in the twigs, branches, and trunk, may be related with the phenomenon of poor flowering at high temperatures.

2.8 Cultural Practices Affected to Flower Bud Formation

2.8.1 Nitrogen Fertilizer Dosage and Application Season

Too much nitrogen fertilizer and too late nitrogen fertilizer application always result in too much and too late vegetative growth, especially under soil water content which is still enough for vegetative growth. In such condition, last flush of the year could not mature enough to accept the cool and water stress stimulate for flower bud formation (Menzel and Simpson 1987b). While Chang et al. (Chang and Cheng 2002) also depicted that low N dosage tree had more panicle.

2.8.2 Girdling, Cincturing, and Strangulation

Phloem interruption techniques are commonly used in fruit tree to stimulate flower bud formation, promote fruit set, and increase fruit size. In Taiwan, girdling was used to overcome biannual bearing. Girdling in late autumn or early winter showed high efficacy in promoting flower bud formation (Teng 1996; Cheng et al. 2005). Girdling or strangulation treatment that increased carbohydrate, soluble sugar, and K content of leaves was considered to be associated with the phenomenon (Teng 1996; Chen et al. 2011). In Australia, Menzel and Paxton (1986) found that cincturing "Bengal" litchi increased flowering on a branch with dormant vegetative growth and with early or late flush, but the effect was much stronger on the former. However, flowering was not improved with a branch with mid-flush. Menzel and Simpson (1987a) found that promotion effect of cincturing was better in trees which next year will be off season than those trees which next year will be on season. The authors also concluded that adequately fertilizing and completing a significant vegetative flush after harvest should be necessary before cincturing treatment.

2.8.3 Late Flush and Panicle Pruning and Panicle Length Control

2.8.3.1 Late Shoot Pruning

Cool weather in autumn to winter is needed for flower bud formation of litchi. The matured leaves are necessary for litchi flower induction. The time for most shoots that flowered in subsequent spring was called “the putative marginal time for flowering (pFMT)” (Chang et al. 2015). Late flush in late autumn to early winter should thin off to make sure the shoots reach “pFMT” and to stimulate the axillary bud, which just below thinning place develop panicle. Huang and Weng (1978) think late flush, which did not fully mature in November, induce 60–100% shoot to produce panicle in the spring of next year. Used same method in “Yu Her Pau” to overcome biannual bearing problem.

2.8.3.2 Panicle Thinning

During winter season, thinning off whole panicle could induce new panicle. It was widely used in Taiwan to avoid winter low-temperature damage (Chen et al. 2016a, b). Another kind of thinning off panicle is removing some parts of inflorescence. This thinning method could decrease the nutrition loss during flowering in order to promote the fruit setting. Usually pruning off 1/3–2/3 parts of whole inflorescence controls the size of inflorescence; for example, around 20 cm inflorescence retained in “Yu Her Pau” litchi (Chen et al. 2013).

2.8.4 Plant Growth Regulators

Paclobutrazol (PP333, α-tert-butyl-β-(4-chlorobenzyl)-1H-1,2,4-triazole-1-ethanol) was used to replace hand removal of leaflets on panicles and promote the normal development of panicles, even during a warm spring (Liang and Yu 1991) (39% 2-chloroethylphosphonic acid or 39% ethephon), and naphthaleneacetic acid (NAA) was used to induce late flush panicle development (Huang and Weng 1978; Nakata and Suehisa 1969; Teng 1996).

Although paclobutrazol is an inhibitor of GA3 synthesis and to substitute the effect of water stress, Chen et al. (2014) found that the application of GA3 could increase the ratio of leafy inflorescence and replace the manual inflorescence and late flush pruning in “Yu Her Pau” litchi.

2.8.5 Irrigation

Autumnal water stress significantly increased flowering intensity and yield in “Mauritius” and “Floridian” litchi. Six weeks of autumnal water stress, terminated by winter rains, were sufficient to effect these changes. But after panicle emergence, litchi trees should be irrigated at full rates for panicle development, flowering, and fruit set (Batten et al. 1994; Menzel et al. 1995).

2.9 Floret Development

Litchi panicles have three types of Floret, two male flowers, which defined as M-1 and M-2, respectively, and one female (pseudohermaphrodite female) flower.

One litchi female (pseudohermaphrodite) flower has two ovaries. Generally, each ovary has one embryo sac. Normally only one embryo sac sets fruit or does not set at all. This resulted in the low fruit-setting rate of litchi. The rates of fruit set for certain cultivars were very low in Taiwan, especially the early and small seed cultivar "Yu Her Pau." Normally, the ratio of female flowers on the inflorescence of litchis has been considered to be an important factor which affected fruit set. However, the ratio of female flowers on the inflorescence varied by varieties (Menzel 1984) and influenced by water conditions (Menzel and Simpson 1992), ambient temperature (Chang 1999; Menzel and Simpson 1992; Shih 2000), and root temperature (Shih 2000). There are many factors also considered with the low productivity in litchi, which includes the separation of blooming time of male and female flower (Menzel 1984; Mustard 1960; Robbertse et al. 1995; Stern et al. 1997a, b), short pollen acceptable duration of stigma (McConchie and Batten 1989; Menzel 1984), and the nutrition shortage of the tree.

Moreover, Mustard (1960) found some abnormal development of litchi embryo sacs. Stern et al. (1996) and Shih and Chen (2000) also found that some low-productivity varieties had a high rate of abnormal embryo sac of female flower. Stern et al. found that some embryo sacs of the "Mauritius" are still undeveloped at anthesis. Shih and Chen (2000) pointed out the well-developed embryo sac rate of "Yu Her Pau" attained the maximum level at three to five days after anthesis, but most of its stigma lost their acceptability for pollen. Shih and Chen (2000) also found that the two embryo sacs of the female flower of "Yu Her Pau" do not develop equally (Fig. 2.7) and had high aborted rate. Therefore, the rate of well-developed embryo sac during the stage of stigma which are still acceptable for pollen became an important factor affecting the rate of fruit set. Stern et al. claimed that the abnormal embryo sacs of litchi can be as high as 53–97% for 2-day-old female

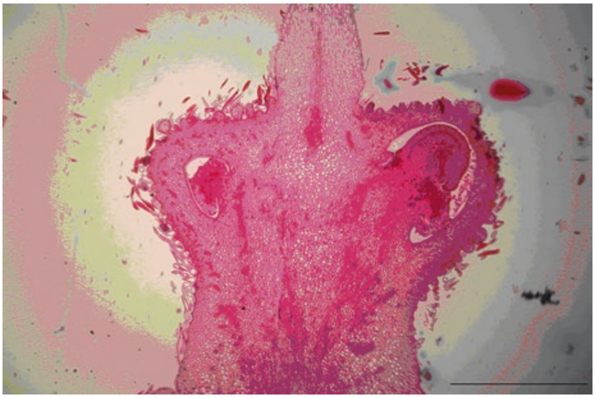

Fig. 2.7 The embryo sac of "Yu Her Pau" litchi pseudohermaphrodite flower. Normally, litchi pseudohermaphrodite flowers have two embryo sacs, but their developments are asynchronized. Bar =150 μm (Adapted from Chen, Iou-Zen)

flower (2 days after anthesis). However, according to the result of Shih and Chen (2000), part of the embryo sac in 2-day-old female flowers is still not well developed. Therefore, the rate for abnormal embryo sac observed by Stern et al. might be overestimated, just because they are different varieties.

2.10 Pollination and Pollen Storage

There were many kinds of pollinators for litchi flowers, but honeybee species were the most important and efficient group, especially *Apis dorsata* F, *A. mellifera* L, *A. cerana* F., and *A. florea* F. Generally, for the outset of flight activity in honeybee species, the ecological conditions should meet 15.5–18.5 °C temperature, 600–1700 Ix light intensity, and 9–20 mW/cm2 solar radiation at least (Abrol 2006). Stern and Gazit (1996) found that pollen density on bees collected from "Mauritius" inflorescences was very low during the M1 phase and increased to very high values during the M2 phase. These results indicate that for "Mauritius" the M1 may not be as an effective pollen source. Besides, according to the in vitro germination tests, M2 pollen from "Mauritius," "Floridian," "No Mai Chee," "Wai Chee," and "Early Large Red" had a much higher germination rate than M1 pollen from those same varieties. In all the five varieties investigated, the adequate germination rates for M2 were found at 35, 30, and 25 °C, but the optimal incubation temperature for in vitro pollen germination was 30 °C. Consistently and significantly higher final fruit set was gotten after hand pollination with M2 pollen, relative to M1 pollen (Stern and Gazit 1998). This experiment indicated that hot and warm regimes during flower development had pronounced detrimental effect on pollen viability compared to a cool regime and pollen should be collected from M2 flower.

Pollen parent will influence the selective abscission of lychee fruitlets. In Israel, trees adjacent to the "Mauritius" pollenizer got 36% higher yield than "Floridian" itself (Degani et al. 1995b). Pollen storage has also been one of the most important works in crop breeding. In China, Wang et al. (2015) found the use of an air-blowing electric dryer at 35 °C for 6 hours and pollen cryo-stored (−86 °C) for one year showed significantly higher germination rates than those stored under the other conditions. However, in a real application that storage of pollen at 4 °C was suitable for field pollinations in the blooming season, the pollen germination rate was still good for up to two months.

2.11 Fruit Set and Growth

Chang et al. (2015) studied fruit growth of "Early Big" litchi. "Early Big" litchi fruit showed a sigmoid growth pattern on the basis of fresh and dry weight. Seed began to develop about week 5 after full female bloom when the embryo was visible. Generally, in week 4 AFFB, 95% small fruit would drop.

In "73-S-20" litchi trees require a minimum number of three flushes for adequate fruit production (Chang and Lin 2008). Batten et al. (1994) found that fruit shedding was significantly less in irrigated trees. The reasons may be water deficits at anthesis, reduced ratio of daytime stomatal conductance, and CO2 assimilation [Batten et al. 1994, Menzel and Simpson 1990).

Plant growth regulators, especially auxin, have been used to maintain high fruit set rate. Under good pollination condition, Teng (1988) applied 2,4,5-TP (2-(2,4,5-Trichlorophenoxy)propionic acid prior to blooming of pistillate flower and just after floret blooming shedding once, respectively, which significantly increased fruit set. Stern et al. found similar results after immersion in 2,4,5-TP at the stage when fruitlets at the ca. 2 g. Bhat et al. (1997) depicted that naphthalene acetic acid (NAA) and 2,4-dichorophenoxy acetic acid (2,4-D) could significantly control fruit crack and increase fruit set and NAA was better than 2,4-D. Compared to 2,4,5-TP, Stern and Gazit (1997) mention that 3,5,6-TPA (3,5,6-trichloro-2-pyrid yl-oxyacetic acid) was better than 2,3,5-TP when sprayed at the stage of initial fruit set. They recommended 3,5,6-TPA could be able to fully replace 2,4,5-TP if 2,4,5-TP is banned. Finally, Stern et al. (2000) found that at the young fruitlet stage, application of 2,3,5-TP followed by 3,5,6-TPA a week later got the best results more than did either substance alone. In India, using polyamine putrescine at the beginning of female bloom increased the yield of "Mauritius" which have been made sure after 6 years of being studied (Sanyal and Mitra 2000).

2.12 Seed Storage

Litchi seed was relatively high in moisture and dies quickly (4–7 days) upon dehydration in open conditions (Fu et al. 1990). One hundred percent germination was obtained if seeds contained 28.5% moisture (wet weight basis), and the physiological of the seed was at the end of the ninth week after anthesis. Seed with 20% moisture was the critical level moisture content for germination, and when kept for 1 week under ambient conditions (29–33 °C), the moisture content fell below 19% that means that the seeds completely have lost their germinability. In practical application, seeds should be planted as soon as possible once the fruit is harvested. However, if we need to store seeds, seeds stored in sealed polyethylene bags or retained seeds with fruits treated with benomyl (0.05%) and wax emulsion (6%) and sealed in polythene bags were good choices; the former method showed 50.7% germination after 10 days, and the latter could maintain 42% viability for up to 24 days (Ray and Sharma 1987).

2.13 Vegetative Propagation

Traditionally, litchi propagated by air layer method in spring to summer season. Top working was commonly used to change cultivar of mature tree (Fig. 2.8). Actually, graft method had been tested, but not popular. Chen et al. found that some scion and rootstock combination had graft incompatibility, and graft compatibility could be well judged by graft joint performance and leaf color. By comparing the compatible

Fig. 2.8 Top working of litchi tree (Adapted from Chen, Iou-Zen)

with the incompatible ones, the former showed dark green leaf and smooth graft joint, while the incompatible ones had yellow leaf, at 6 months after grafting. When the graft union formation was observed, the compatible combinations had higher superoxide dismutase (SOD), peroxidase (POD), and polyphenol oxidase (PPO) activities than that in the incompatible ones.

2.14 Conclusions

Litchi industry in Taiwan suffers from certain constraints such as the short production season, which is due to too much "Hak Ip" planting, short storage time of fruit, the poor/irregular production of fruit, and fruit physiological disorders in "Yu Her Pau" and "No Mai Tsz" ("73-S-20"). These constraints led to the imbalance between supply and demand in market. The seven novel varieties released from TARI have different fruit maturity seasons and good fruit quality. Based on the policy of the "right cultivar for the right land," cultivating them in proper ecological regions will effectively diversify/extend the production period and match market requirements. As for plant management, by means of studying the biology of flower and fruit development, researchers and growers have developed several special cultural technologies to apply for the commercial production of litchi, such as girdling, strangulating, manual removing or ethrel applying to get rid of young foliage and to promote flower initiation, and panicle thinning and plant growth regulators applying to improve fruit set and growth besides. However, there are still some breeding and cultural technology efforts that need to work to the future of litchi industry in Taiwan, which includes the following:

1. More extremely early or late fruit maturing varieties, especially the latter. The novel varieties released up to now still not later than the commercial variety "Kwai Mi."
2. Big fruit with high and stable percentage of shriveled seed but not at the cost of sacrificing productivity.

3. Varieties with particular used such as for fast frozen, dried, canning, juice and wine processing, or with high anthocyanin content in the peel. Furthermore, the traditional selection methods spent too much time and labor cost because of the long juvenile phase and huge seedling handling work. Tissue culture and molecular tools were applied to accelerate the selection and propagation procedures in future.
4. Cultural practices with low labor cost to fit the force trend of lacking and aging of the grower.

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