Systemic Information for Future Perspectives in Litchi Crop Improvement

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Abstract

Litchi (*Litchi chinensis* Sonn.) is a subtropical evergreen fruit crop of the Sapindaceae family. Over the years, significant growth in production and productivity coupled with the fast-expanding market of litchi has been recorded at both the national and international level. Most commercial cultivars in litchi have been clonally selected under Chinese or Indian conditions and have been adapted to a limited climatic condition. Disease infestation in litchi has been a long-term debate in the horticulture and agriculture sector. Keeping the damage factor as a prime concern, clonal selection as the basis of cultivar selection must rely on limited characteristics such as systemic susceptibility in terms of fruit size, quality, and period of maturity, which narrows down the diversity, focusing on only a few commercial traits. Hence, creation of variability within the litchi gene pool is of paramount importance to yield desirable characters such as precocity, dwarfness, regularity of bearing, wider adaptability, and resistance and avoidance of pests and disorders. The heterozygosity of litchi produces a wide extent of variability, which serves as the baseline for new selections through harnessing precocious genes and exploiting natural hybrid vigour and other genetic manipulations. Different strategic efforts on a breeding programme need to be undertaken on a large scale with considerations of a comprehensive survey of various genotypes and trait inheritance patterns, raising a large population of open pollinated seeds with known parentage, mutation breeding because of obvious difficulties with traditional litchi breeding and the lack of pure lines. This context provides the basic information for further improvement and genetic enhancement of the breeding programme in litchi disease resistance.

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8.1 Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical evergreen fruit crop of the Sapindaceae family (Leenhouts 1978). Its climatic requirements are highly specific, and thus its commercial cultivation is restricted to only a few subtropical countries in the world. The major litchi-producing countries are China, India, Taiwan, Thailand and Vietnam. A relatively small amount of litchi is produced in the United States, Mexico and Central and South America (Menzel and Waite 2005). World production of litchi is estimated to be around 2.11 million tons, with more than 95% of the area and production shared by Asia. India and China account for 91% of the world production, but it is mainly marketed locally. Over the years, significant growth in production and productivity of litchi have been recorded coupled with a fast-expanding market at both the national and international level (Singh et al. 2012).

There has been a substantial increase in the area and production of litchi in India during the past 50 years. The area has increased from 9400 ha (1949–1950) to 84,950 ha (2014–2015). The contribution of litchi to the total area under fruit cultivation has increased from 0.75% to 1.70%. Increase in area during 1991–1992 to 2014–2015 (22 years) has been more than 80%, whereas production increase during the same period is to the tune of more than 150% (NHB 2015). Productivity also recorded an increase of about 50% during the same period. Evidently, production and productivity of litchi are constantly increasing in the country. The fresh fruit market dominates the trade, followed by dried and canned fruit. The main importers are the European Union, the United States, Hong Kong, Singapore, Japan and Canada, and China, Taiwan, Thailand, Madagascar, South Africa, Australia and Mexico are the main exporting countries.

Globally, the litchi industry is beset with myriads of problems. Low success rate of establishment, lesser fruiting span, low and irregular yields because of poor flowering and fruit set, fruit cracking, browning and rotting of fruits, fruit borers and mites, poor shelf life, and lack of suitable varieties with early and late maturity and good-quality fruits are some of the factors encountered.

8.2 Origin and Distribution

The *Litchi* genus contains three subspecies: *Litchi chinensis* ssp. *chinensis*, *L. chinensis* ssp. *philippinensis*, and *L. chinensis* ssp. *javensis* (Leenhouts 1978). The subspecies *philippinensis* is found growing wild in the Philippines from sea level to 500 m altitude (Sotto 2002). The fruits are distinguished from cultivated litchi by their long and oval shape, with long thorny protuberances and inedible flesh that partially covers the seed (Menzel 1991). Fruits of the subspecies *javensis* from the Malay Peninsula and Indonesia produce an aril thinner than that of the cultivated litchi. Neither of these two subspecies is grown commercially. The subspecies *chinensis*, a litchi of commerce, originated in Southern China and Northern Vietnam from wild populations of these regions (Wu 1998b; Hai and Dung 2002). In China, enormous diversity has been observed in the moist forests of Hainan from low elevations up to 1000 m, below 500 m in hilly areas of western Guangdong and eastern Guangxi, and in valley or hilly regions of southern Yunnan below 1000 m (Wu 1998a). Litchi dominates most of these forest compositions and often grows in mixed stands with *Vatica astrotricha* (green plum), *Hopea hainanensis*, *Heritiera parvifolia* of the Chinese parasol family, *Coelodepas hainanensis*, *Polyathia laui*, and *Diospyros hainanensis*, which belongs to the Ebenaceae family. The small area of wild litchis have also been found growing in the natural rainforest of Vietnam at low elevation in the Ba Vì mountains and in the forests of Tam Dao (Vinh Phuc Province) and Tuyên Hóa of Quang Binh Province (Hai and Dung 2002). Generally, in Hainan the wild trees look similar to cultivated litchi and produce edible fruit, but the flesh is relatively thin and sour. Fruits are variable and can be characterized into three groups based on their shape and arrangements of skin segments and protuberances: group 1 possesses sharp and pointed protuberances, group 2 has protruded but obtuse skin segments, and group 3 has flat skin segments. The wild types evolved in two directions, with skin segments becoming protruded and long, as in 'Dazao' ('Tai So') and 'Guiwei' ('Kwai May'), or flattened, as in 'Sanyuehong' ('Sum Yee Hong'), 'Shuidong' ('Souey Tung'), 'Nuomici' ('No Mai Chee'), and 'Huaizhi' ('Wai Chee') (Wu 1998a). In Yunnan, a population of wild litchis was identified in which the trees required less cool weather to initiate flowering. These trees mature earlier and produce a crop in warmer climates than can the traditional subtropical ecotypes. Flowers from these wild specimens have sepals with brownish pubescence, which gave the species the name 'brown-hair litchi' or 'Hemaoli.'

Litchi, a luscious fruit crop, originated from the Kwantung and Fukien Provinces of Southern China (Tao 1955). According to de Candolle (1909), 'Chinese authors living at Peking knew about litchi only late in the third century of our era.' Although controversial, the first citation for this fruit in the literature probably can be traced back to as early as 1766 BC. However, a clear reference has been mentioned in the literature of the Han dynasty (140–86 BC). Possibly the first complete book on litchi in English (Groff 1921) was a monograph published by Ts'a' Hsiang (1059 AD).

Until the tenth century, litchi was propagated through seedlings. Later, vegetative propagation through air layering or marcotting became widely accepted among the growers, being first practised in the fourth century AD. By the sixteenth century, grafting was also recorded (Anonymous 1978). However, their detailed application was first documented in the Registers by Xu Bo in 1579 and by Deng Qingcai in 1628, respectively.

Until the late seventeenth century, litchi cultivation was restricted to Southern China and Northern Vietnam (Tindall 1994; Hai and Dung 2002). It then spread to other regions through the route suggested by Galán Saúco and Menini (1989). Apparently, it reached Burma and Eastern India in the late seventeenth century (Hayes 1957). Subsequently, by the end of the eighteenth century, it made its way to Bengal, whence it diversified to other parts of India on a commercial scale and later to Nepal (Budathoki 2002) and Bangladesh (Abu Baker Siddiqui 2002). The litchi industry has grown significantly in these countries. Litchi was first introduced into Thailand from China 300 years ago by merchants who carried the fruit with them. Some plants adapted successfully to the tropical conditions of the central region of the country. Promising seedlings were selected and accordingly named by local growers as lowland litchi or tropical litchi, as the trees do not require a long period of cold to initiate flowers (Subhadrabandhu 1990). In Chiang Mai, litchi was planted around 1890, through air layers brought by emigrants who migrated from Yunnan through Laos or Myanmar (Boonrat 1984). These are truly subtropical types, requiring a longer period of low temperature for flowering. Many of them still retain their Chinese names, such as 'O-Hia,' 'Hong Huay,' and 'Kim Cheng'; however, the Thai spellings and pronunciations are different (Subhadrabandhu and Yapwattanaphun 2001a). Litchi reached the Philippines from China before 1916 but failed to flower and set fruit at low altitudes until 1931 (Sotto 2002). Later, in 1931, trees were introduced from other sources that bear fruit and gave hope for litchi growing in the more elevated areas. The crop was introduced in Australia through Chinese migrants attracted by gold rushes around 1854 (Menzel et al. 1988) and reached Southern Africa about 50 years earlier.

In Madagascar the arrival of litchi dates back to 1802 and the 1940s, with many old and relatively few new plantations still in existence at a commercial level (Jahiel and Abraham 2001). Evidence revealed that the first litchi trees were imported into South Africa from Mauritius in 1876. However, trees had already been observed in Natal in 1875, suggesting there must have been earlier imports (Oosthuizen 1991). From Natal, trees traveled to the Transvaal Lowveld as well as to other suitable frost-free areas. The species arrived in Florida in the 1880s, but commercial cultivation started in the 1940s. 'Brewster' ('Chenzi') is the main cultivar of the industry in the region, but currently it is replaced by 'Mauritius' ('Dazao,' 'Tai So') (Knight 2001). The first trees, known as 'Afong,' which were transported to Hawaii in 1873 by Chinese merchants, later were identified as being similar to 'Dazao' (Nakasone and Paull 1998a). In Israel litchi was introduced in the 1930s from different parts of South Africa ('Mauritius'), California ('Floridian'), and India ('Bengal'); however, a commercial industry developed only after the 1980s (Goren et al. 2001).

At a later event, litchi was introduced to several other countries. In Hawaii, it was probably successfully introduced in 1873. Its cultivation in the West Indies and Natal, South Africa, dates back to 1775 and 1869 (Marloth 1947), respectively. Commercial cultivation of litchi in Queensland (Australia) was of late occurrence (Batten and Lahav 1994), although it was introduced as early as 1854. In the United States, it reached from Saharanpur (India) to Florida in 1883, to California in 1897, and subsequently again to Florida from Fukien Province of China in 1906 (Singh and Singh 1954), where it was named 'Brewster' litchi. Early in the nineteenth century, it also reached England and France but was unsuccessful (Pandey and Sharma 1989).

8.3 Classification of Litchi

The majority of species in the Sapindaceae are either trees or shrubs native to Asia, although there are a few species in South America, Africa, and Australia (Bailey 1949; Leenhouts 1971, 1978, 1986). The family derived its name from the soapberry, *Sapindus saponaria*, whose fruit is used as a soap substitute in the tropics (Nakasone and Paull 1998b). Litchi relatives from Southeast Asia include rambutan, *Nephelium lappaceum*, and pulasan, *Nephelium mutabile*. These species differ from litchi in having long hairs or spinterns instead of protuberances. Litchi and longan are long-lived evergreen trees producing new leaves, flowers and fruit on terminal shoots. The inflorescences produce hundreds of functionally male and female flowers, which bear 5 to 80 fruits at harvest. Other tropical species of local significance of the subfamily Sapindoideae include tuan, dawa, or Fiji longan, *Pometia pinnata*, from Southeast Asia and the Pacific (tribe Nephelieae), mamoncillo, *Melicoccus bijugatus*, from the Caribbean (tribe Cupanieae); ackee, *Blighia sapida*, from West Africa (tribe Sapindaceae); and guarana, *Paullinia cupana*, from the Amazon basin (tribe Paullinieae) (Yeap 1987; Menzel et al. 1993). The sapindaceous trees were first described by Cambessedes in 1828; however, detailed systematic study was published only after the start of the twentieth century.

Classification of litchi by Radlkofer (1932) was based on a wide range of evidence including the presence or absence of a terminal leaflet, the number of ovules per carpel, the structure of the fruit, the presence or absence of an aril, and pollen morphology. Although several revisions on the classification of the Sapindaceae have been made, the scheme of Radlkofer is essentially accepted with only minor modification. According to plant characteristics, pollen morphology, and geography, the Sapindaceae are grouped into two subfamilies: Dodonaeoideae (Austral distribution) and Sapindoideae. The latter can be separated into three main groups centred around Sapindeae (pantropical) or Cupanieae (pantropical) and a third group separating into Thiouinieae and Paullinieae, both predominantly American (Leenhouts 1971, 1978, 1986). The majority of the cultivated species in the Sapindaceae belong to Litchi, Nephelium, Dimocarpus, and Blighia, with their horticultural classification based largely on fruit characteristics (Tindall 1994). Minor attention has been paid to leaf and flower structures. Subsequently, Leenhouts (1971, 1978, 1986) and Choo and Ketsa (1991) reviewed the taxonomy of litchi.

According to Leenhouts (1978), there are three subspecies of *Litchi chinensis* based on the thickness of the twigs, arrangement of the flowers, number of stamens, and fruit characteristics. *Litchi chinensis* subspecies (subsp.) *chinensis* is the commercial litchi that grows wild in Southern China, Northern Vietnam, and Cambodia (Groff 1921). The tree possesses slender twigs and bears flowers in lax cymules.

The flowers usually have six stamens. The fruit is smooth or with protuberances up to 2 mm high (Groff 1921; Singh and Singh 1954; Menzel and Simpson 1990; Tindall 1994). The two other subspecies are not commercialized (Menzel et al. 1993). *Litchi chinensis* subsp. *philippinensis* is rarely cultivated although it is common and known in the Philippines and Papua New Guinea as alupag, arupag, or mamata. The taxonomic classification of *Litchi* is described as follows:

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Sapindaceae – Soapberry family
Genus	*Litchi* Sonn.
Species	*Litchi chinensis* Sonn.

8.4 Flower Biology

Litchi requires a period of cool weather (15°–20°C) for successful flower initiation. The flowering period in litchi varies with the genotype and environmental condition (Zhang 1997; Chen and Huang 2001; Ghosh 2001; Singh et al. 2012). Generally, the flower bud differentiation in litchi starts in December and is completed by the end of January (Singh et al. 2012). The flower panicle emerges from January to the end of February. Before floral bud initiation, the twig produces vegetative growth that subsequently throws a reproductive bud in the form of a racemose panicle (Menzel et al. 2000; Subhadrabandhu and Yapwattanaphun 2001; Stern and Gazit 2003; Menzel and Waite 2005).

Litchi bears a determinate panicle either terminally or axillarily composed of several branches produced on the current season's wood. The panicles are normally produced terminally in clusters of 10 to 20. The terminal panicles are more robust and productive compared to lateral panicles (Shukla and Bajpai 1974; Scholefield 1982; Naphrom et al. 2001; Singh et al. 2012). Litchi produces mixed panicles with the lowest buds producing leaves only, the middle buds producing floral buds in the axils of the leaves, and the topmost buds producing mostly floral branches (Menzel and Waite 2005). The number and percentage of different types of flowers in litchi vary with cultivar, environmental conditions, tree and panicle within a tree (Menzel and Waite 2005; Singh et al. 2012). The proportion of functional female flowers varies between 10% and 60%, depending on tree age. Each panicle produces several small, white, greenish or yellowish flowers (Zheng et al. 2001a, b; Batten and McConchie 1995; Menzel and Waite 2005; Singh et al. 2012).

8.4.1 Flower

The flower is terminal or auxiliary, arising from the axil of the first or second uppermost leaf of the new shoot. It bears tiny, greenish white or yellowish flowers and is branched racemosely. The inflorescence varies from 20 to 60 cm in length and from 15 to 30 cm in width. Litchi flowers measure 3–6 mm wide when fully open and the rest, on pedicels, measure approximately 1.5 mm. Flowers are apetalous and possess a cup-shaped calyx with four or five short, serrated sepals (Liu 1954; Costés 1988; Goren et al. 1998). Three types of flowers open in succession on the same panicle, and they vary in terms of degree of sexual development pertaining to length and functionality of the stamens and development and functionality of the pistil (Menzel and Waite 2005; Singh et al. 2012).

8.4.1.1 Male or Staminate Flowers (Type I or M1)

These flowers possess a structure which contains a pink, pubescent protuberance and very rudimentary pistil lacking both stigma and style (Joubert 1985; Costés 1988; Robbertse et al. 1995). The pistil is surrounded by 4 to 12 stamens (usually 8) with hairy filaments. The filament varies from 5 to 6 mm in length (Fig. 8.1a–c). In some flowers, the filaments are two to three times as long than the anther, whereas in some the filaments are equal to the length of the anthers (Liu 1954; Mustard et al. 1953). Type I is defined as the nonfunctional male, which opens for 10 days. Stamens are long with thin creamy white filaments which are deflexed and inserted into an angular glabrous nectary disc. Anthers are small, two-celled, elliptical, emarginated at apex, fixed and longitudinally dehiscent; initially pale white, they become brownish at maturity (Das and Choudhury 1958; Joubert 1985; Stern and Gazit 1996). During anthesis, the pollen sacs mature and the anther dehisces longitudinally and successively. Pollen grains are small and triangular, having nipple-like angles with three germinating pores. The ovary is very small, reddish white and abortive. The flowers possess nectar discs at their base, although they are not well developed (Menzel and Waite 2005; Singh et al. 2012).

8.4.1.2 Hermaphrodite Female Flower (Type II or F)

These flowers resemble the hermaphrodite male flower with a short filament except that the lobes of the stigma open down the vertical cleft (Fig. 8.1d–f). Called type II or F flowers, they appear and remain functional only for 2 days. These flowers have a small but well-developed pistil attached to a short peduncle (Joubert 1985; Costés 1988; Robbertse et al. 1995). The ovary has two to four carpels, each containing an ovule. The surface of the ovary is pubescent with protuberances persisting throughout the fruit development period. The ovary is long and composed of a short style with a bilobed, white and sticky stigma. The nectar disc at the base of the ovary produces abundant secretions, attracting insects. Generally, only one of the ovary lobes develops into a fruit; the rest abort. Occasionally, however, two lobes may develop, producing two fruits each containing a seed, which remain embedded at their bases (Das and Choudhury 1958; Joubert 1985; Stern and Gazit 1996). The pistil is usually surrounded by five to eight stamens with very short filaments (less

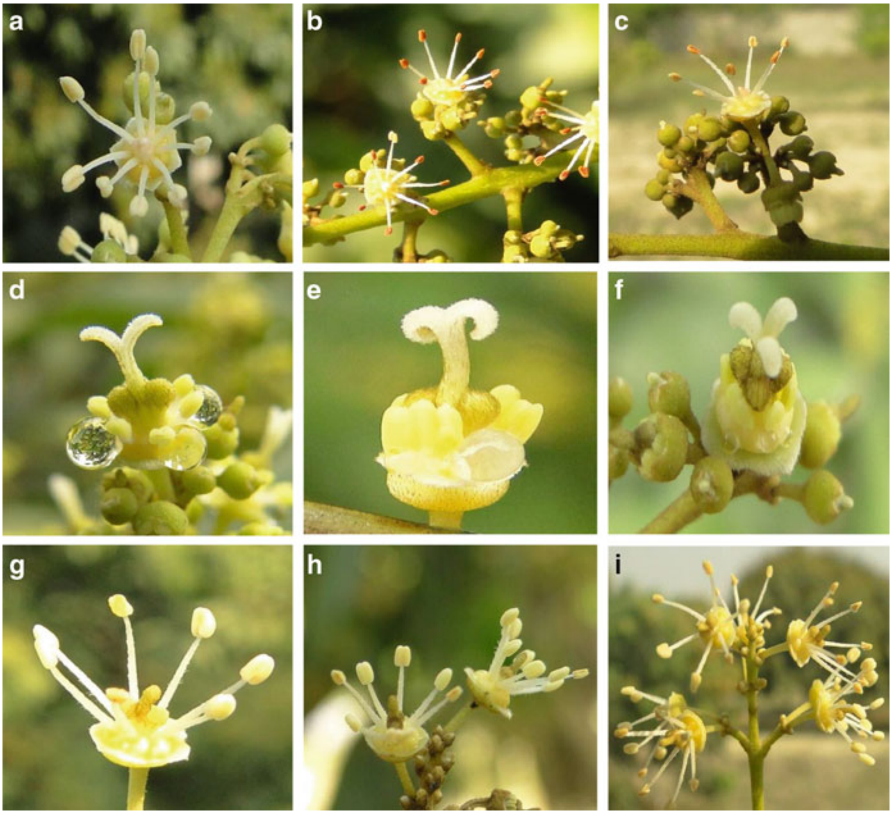

Fig. 8.1 Pattern of flowering in litchi. **a–c** Male flower (M_1) in full bloom with ripe pollen and unopened females. **d–f** Fully opened hermaphrodite female flowers (F) with well-developed stigma, style and rudimentary stamens. **g–i** Fully developed hermaphrodite male flowers (M_2) with rudimentary stigma and degenerated ovary in the centre of the stamens

than 1.5 mm long). The anthers do not normally dehisce and contain little viable pollen, thereby ensuring cross-pollination (Menzel and Waite 2005; Singh et al. 2012).

8.4.1.3 Hermaphrodite Male Flower (Type III or M2)

Type III is a functional male flower with more hermaphrodite features than the type I flower. Both stamens and pistil are present, but the lobe of the stigma fails to open to permit pollination. The filaments vary in length. Stamens arise in two forms, of which those with long filaments are fertile. The pistil consists of a short undivided and nonfunctional style. Type III flowers have an ovary with certain resemblances to both the aforementioned types (Fig. 8.1g–i). Anthesis occurs for 7 to 10 days. The flower is surrounded by six to ten stamens which are similar to those of type I flowers, possessing a rudimentary style, stigma and a nectary disc (Liu 1954; Costés 1988; Goren et al. 1998). Anthesis of the three flower forms usually occurs in succession on the same tree, usually on different panicles or branches. However, the first flowers to appear on any tree may not necessarily be type I. There seems to be a tendency in young trees for type II flowers to open first (Costés 1988; Menzel and Waite 2005; Singh et al. 2012).

The duration of flowering (anthesis to pollination) in litchi is between 20 and 45 days, depending on the cultivar, season and location. Anthesis occurs during both the day and night, with peak opening in the early hours of the day (0600). Temperatures below 8 °C suppress flower opening, but this occurs in the rainy and dry seasons. Under very dry conditions, the young flowers may dehydrate and fail to develop. High night temperatures (approximately 21 °C) reduce the duration of female flower anthesis (Mustard et al. 1953; Menzel and Waite 2005; Singh et al. 2012).

Anthers dehisce 1 day after anthesis and continue for up to 3 days, occurring continuously and more frequently between 0800 and 1000, with no apparent environmental, cultural or genetic effect. The pollen of type III flowers is more viable than that of type I flowers, which are, in turn, more viable than that of type II flowers. The stigma becomes receptive as soon as the lobes separate and remains receptive up to 3 days after anthesis. Maximum receptivity (75%) occurs 1 day after anthesis. When the stigmas cease receptiveness, they become dry and brown and lose their glossy appearance. Abundant and maximum viable pollens are produced by M2 flowers. Estimated pollen viability ranges from 4% to 40% at the time of pollen release and decreases rapidly thereafter (Wang and Qiu 1997; Goren et al. 1998; Menzel and Waite 2005; Singh et al. 2012).

8.4.1.4 Sex Ratio

Sex ratio in litchi varies with cultivar and the environment. The proportion of F, M1, and M2 flowers ranges from 1.00:0.16:0.32 (‘Mountain Litchi’) to 1.00:2.30:3.90 (‘Brewster’) litchi in Florida (Mustard et al. 1953) and from 1.00:0.16:0.90 (‘Calcuttia Late’) to 1.00:1.60:2.40 (‘Dehradun’) in India (Chadha and Rajpoot 1969). In ‘Mauritius’ the ratio varies from 1.00:1.10:1.10 in Israel (Stern et al. 1993) to 1.00:1.20:1.50 in Reunion (Costes 1988). Chaturvedi (1965) observed 32% female flowers in ‘Early Large Red’ in India; the proportion of female flowers in different cultivars at several parts of Queensland, Australia, varied from 16% to 43%, with the extent of variation as much as observed within cultivars (Menzel and Simpson 1992). ‘Fay Zee Siu’ in China produced a high proportion of male flowers in long inflorescences, whereas a high proportion of female flowers was noted in short inflorescences (Wang and Qiu 1997). Pruning the inflorescence 6 weeks before anthesis increased the female:male ratio and initial fruit set (Wu et al. 2001). Sex ratio is also affected by temperature. When five cultivars of age 2 years were kept at 15 °/10 °, 20 °/15 °, 25 °/20 °, or 30 °/25 °C, the highest proportion of female flowers (72%) was noted from the lowest temperature regime. At high temperature (25 °/20 ° or 30 °/25 °C), ‘Bengal,’ ‘Souey Tung’ and ‘Wai Chee’ fail to set female flowers (Menzel and Simpson 1991; Menzel and Waite 2005; Singh et al. 2012).

8.5 Pollination

Litchi is a highly cross-pollinated crop. Although anthesis of functional male and female flowers coincides, the presence of sterile pollen grains necessitates cross-pollination (Dhaliwal et al. 1977; Du Toit 1994; King et al. 1989; Butcher 1957; Pandey and Yadava 1970). The nectar produced by flowers attracts pollinators such as honey bees, flies, ants and wasps, facilitating cross-pollination (Stern and Gazit 1996). A nectary disc occurs on every flower as a large fleshy crenulate gland within the calyx to which the stamens and pistils are inserted. Nectar, secreted only in the morning, is highly attractive to honey bees and flies (Das and Choudhury 1958; Chadha and Rajpoot 1969; Wang and Qiu Wang and Qiu 1997). At the time of flowering, installation of honey bee boxes in a litchi orchard increases the yield up to 30–40%. Pollinators forage primarily between 0600 and 1200, although foraging continues later in the day at much lower levels (Stern and Gazit 1996).

Honey bees are the major pollinators in litchi (Fig. 8.2). Other pollinators include screwworm, ants, wasps, coleopterans, hemipterans, homopterans, and lepidopterans. Pollinator activity is affected by extremes of temperature, cloud cover, heavy rain and strong winds, and in the presence of insecticides. The presence of 10 to 12 standard bee colonies is sufficient to ensure good pollination and fruit setting in a 1-ha area (Menzel and Waite 2005; Singh et al. 2012).

8.6 Fruit

Botanically, litchi fruit is pendant in a loose cluster (up to 0.6 m long) of several dozen fruits. Fruits are round, ovoid, heart shaped or even kidney shaped and vary in size depending on the cultivar but can measure up to 5 cm in length and up to 4 cm in diameter. The fruit possesses shoulder, suture, apex and skin segments as observed externally (Deng et al. 1999; Huang et al. 2005a). Different parts of the fruits are briefly described in following paragraphs (Menzel and Waite 2005; Singh et al. 2012).

8.6.1 Epicarp

The rind of the fruit (pericarp) is thin, tough, hard, brittle and frangible. At maturity, its colour changes from green to bright red. The intensity of redness varies among cultivars, and fruits may even be yellow or green depending upon cultivars. The skin possesses prominent protuberances but is less marked than in related species such as rambutan and pulusan. When overmature, the skin assumes a dirty brown colour and loses its extensibility (Huang et al. 2005b); this occurs rapidly, even when the fruit is still perfectly edible (Menzel and Waite 2005; Singh et al. 2012).

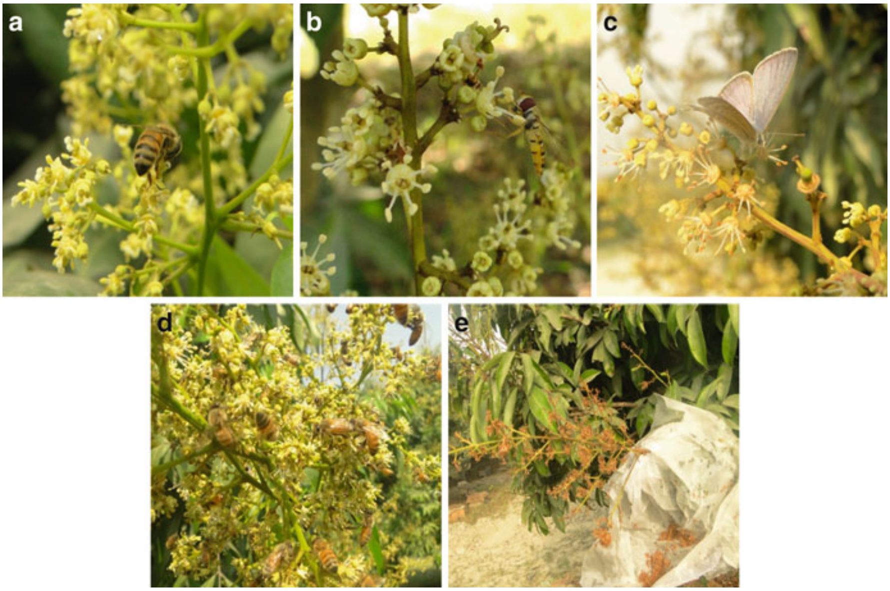

Fig. 8.2 **a–d** Pollinators collecting nectar from the calyx gland of litchi flowers. **e** Flower drop in bagged and unpollinated litchi panicles

8.6.2 Mesocarp

The mesocarp tissue has a loose consistency without clear demarcation between the epicarp and the mesocarp. In the initial stages, this tissue is composed of ordinary parenchymatous cells which become separated with time as large intercellular spaces develop. Botanically, the edible part of the fruit is known as the aril. In a developing fruit, the aril grows continuously as an outgrowth of the outer cells of the seed coat (outer integument); the rate of outgrowth varies greatly among cultivars (Huang 2005). The aril is translucent, white, slightly acid, juicy and sweet, with a faint and pleasant aroma. Many cultivars of litchi can be distinguished by their flavour and aroma (Menzel and Waite 2005; Singh et al. 2012).

8.6.3 Endocarp

Two distinct tissues have been noted in the endocarp. Lying next to the aril is a dual layer of rectangular cells which are considerably thickened and lignified; just above it is the tissue composed of thick, elongated parenchymatous cells with tapering walls (Huang 2005; Menzel and Waite 2005; Singh et al. 2012).

8.6.4 Seed

The fruit contains a single dark brown seed 6 to 12 mm wide and 10 to 23 mm long. Some cultivars have a high proportion of aborted seeds (chicken-tongued seeds) which are shrivelled and nonviable (Huang 2005). The seed is surrounded by a creamy and pulpy edible aril. It is cylindrical, compressed piano convex or concavo convex, exalbuminous and chocolate in colour (Menzel and Waite 2005; Singh et al. 2012).

8.7 Leaf

Litchi produces compound leaves with 7 to 12 lamellae, alternate, coppery red or red to pale green, creamy when young and bright and dark green when matured. Leaf shape can be lanceolate, oblong lanceolate or even elliptical, measuring 7.5–20 cm in length and 2.5–6 cm in width (petiole and rachis), smooth but coriaceous in texture with rounded base. Eight to ten pairs of leaflets may be arranged along the rachis directly or opposite to each other, with petiole length ranging between 3 and 25 mm. Leaflets are glossy with short internodal length, dark green adaxially and dull and waxy on the abaxial surface. Low-vigour varieties have small leaves with shorter internodal length. In some genotypes, young flushes tend to produce attractive reddish-bronze-coloured leaves (Menzel and Waite 2005; Singh et al. 2012).

8.8 Cytogenetics

There is a dearth of information on the aspects related to the cytogenetics of litchi (Liu 1954; Chapman 1984) and this has not drawn much attention among litchi researchers. The species is probably a natural hybrid involving more than one wild progenitor. Variable haploid chromosome numbers of 14, 15, 16, and rarely 17, have been reported in litchi, thus suggesting its polyphyletic origin (Chapman 1984; Sarin et al. 2009).

8.9 Germplasm Collection and Conservation

Historically, litchi cultivar breeding relied primarily on seedling selection. To date, more than 300 cultivars have been planted in the National Litchi Germplasm repository located in Guangzhou, China, established in 1998, which is the largest litchi germplasm gene bank in the world (Wu et al. 2007). Globally, standardized protocols are being followed for cryopreserved tissues and excised embryos, recalcitrant seeds, and vegetative propagated plants of litchi for long-term storage at −196 °C. Besides seed banks, in vitro gene banks have also been established for germplasm conservation.

Selection of improved cultivars in litchi has been practised for thousands of years in Asia. Fay Zee Siu, Bah Lup, Lanzhu, Baitang-ying, Haak Yip, Kwai May (Red), No May Chee and Wai Chee, the main cultivars evolved in China, form the basis of the litchi industry in many other countries; for example, Tai So and Wai Chee in Thailand; Tai So, Kwai May Pink and Wai Chee in Australia; and local seedling selections of Chinese origin in Vietnam, India, Nepal, Bangladesh and Southern Thailand. Breeding effort in the past 50 to 60 years led to the development of popular seedless or small-seeded Dongguan Seedless and Hexiachuan and late maturity Maguili in Guangdong (China), SahKeng in Taiwan (China), Kom and Chacapat in Thailand, UPLB Red in the Philippines and Salathiel in Australia. There is a great potential for improving productivity through breeding new selections, with emphasis on traditional breeding rather than on biotechnology Bose (2001). According to the Chinese, litchi has more cultivars than any other fruit Bose (2001). Bah Lup, Baitang-ying, Haak Yip, Fay Zee Siu, Kwai May, No Mai Chee and Wai Chee are the important cultivars in Guangdong with Wai Chee accounting for more than 80% of the area in Guangxi. However, in Fujian, Lanzhu is the predominant cultivar. No Mai Chee and Kwai May with high proportion of chicken-tongued or aborted seeds and Fay Zee Siu having good fruit size (24–32 g) with excellent eating quality Bose (2001). Haak Yip dominates in the Taiwan Province of China and accounts for more than 50% of the area. Other important cultivars include Sum Yee Hong, Chong Yun Hong, No Mai Chee and SahKeng Bose (2001).

In Vietnam, 80% of the area is occupied by Vaithieu Bose (2001). Tai So (Hong Huay) is the main cultivar in Northern Thailand, besides Wai Chee, O-Hia (Baidum) and Chacapat (Chakrapad). Kom, Luk Lai, SampaoKaow, KalakeBaiYaow and Red China are ecotypes developed for Bangkok regions but of low fruit quality. In India, most cultivars are of seedlings origin introduced from China. Selections of more than 30 cultivars are grown in this region, but only Shahi (Muzaffarpur), China, Calcuttia, Bedana, Late Bedana and Longia, possessing large fruits of excellent quality, are commercially important. In West Bengal, Bombai, Shahi and Rose Scented yield up to 40 kg/tree compared to 15–25 kg/tree in many other cultivars Bose (2001). In Nepal, Majfpuri, Raja Saheb, Dehraduni, China and Calcuttia are the established cultivars probably introduced from India, whereas commercial production in the hilly tract is primarily based on seedling populations. Bombai, Muzaffarpuri, Bedana and China Number Three are the popular cultivars of Bangladesh. In the hilly areas of the Philippines, Mauritius and a local selection from China, Sinco, dominates production, and an introduction from Thailand, UPLB Red, is planted in the lowlands. In Australia, Kwai May Pink accounts for more than 50% of the area, besides Tai So, Souey Tung, Fay Zee Siu, Salathiel and Wai Chee Bose (2001).

Litchi has a narrow genetic base. However, ecological variations do exist in litchi, bringing the total of cultivars to more than 60, although commercially only a few varieties such as Shahi, China, and Bedana are popular. The National Active Germplasm Site at NRCL maintains more than 50 such variants, 20 clonal variants, and a large number of seedling populations arising from various parental combinations. The crop improvement programme in litchi started back in the 1950s at Sabour and Ranchi, resulting in promising cultivars such as Swarna Roopa, Sabour Bedana, and Sabour Priya. The main target of crop improvement in litchi is the integration of such characters as larger fruit size, smaller seed, high pulp ratio and acceptable pulp quality. In clonally propagated species, genetic characterization of clones held in gene banks is important for proper documentation and assessment of variability in the collection, thereby enhancing the effectiveness of gene conservation efforts. The pulp ratio of the existing varieties is moderate with associated problems related to irregularity in bearing, incidence of fruit cracking, and susceptibility to pest and disorder, thus affecting the overall yield potential. Development of improved cultivars coupled with efficient cultural practices is vital for improving crop production in a region. Improved varieties of litchi have been identified and evaluated for their potential from ICAR-NRC on Litchi, Muzaffarpur. The striking features of these varieties have been described.

Shahi Shahi is the most popular cultivar, grown in North Bihar, Jharkhand, Uttarakhand and Uttar Pradesh. Shahi fruits are known for excellent aroma and quality with 65–70% pulp recovery. Fruits are globose, heart or obtuse in shape; pulp is greyish white, soft, moderately juicy and sweet [19–21° B total soluble solids (TSS)]; seeds are large but 6–8% small shrunken seeds are produced. Besides high-quality fruit, Shahi possesses a rosy flavour and heavy fruit weight (22–26 g), earliest in maturity (20–30 May); this is the first variety to reach the market. Tree size is large with high-yield potential (140–150 kg^{-1} tree) and fruit is moderately prone to cracking (Fig. 8.3a).

China This variety has a very high yield potential and is tolerant to heat waves and fluctuations in soil moisture. It is medium to late in maturity (30 May–10 June), with comparatively compact trees and high-yield potential (150–160 kg^{-1} tree), but prone to alternate bearing. Fruits of China are large (22–25 g), oblong in shape and tyrant rose in colour with dark tubercles at maturity. The aril is creamy white, soft, juicy and sweet (18–19° B); seeds are large and less prone to cracking with good flesh recovery (60–67%). The maturity period of this variety coincides with pre-monsoon showers (Fig. 8.3b).

Swarna Roopa A late-maturing, cracking-resistant cultivar selected at Ranchi has attractive red-coloured fruits with small seeds and high (65–70%) aril content; fruits are medium in size weighing 15–17 g and have a high pulp content. The pulp contains high TSS and low acidity. The cultivar is suitable for extended harvest as it matures after China and is prized for its attractive fruit colour.

Purbi Purbi is mostly grown for table purposes in the eastern part of Bihar. Fruits are medium large, oblong conical in shape, and ripen at the end of May or first week of June. At maturity red tubercles appear on the pinkish-brown background. The average yield is 100–120 kg/tree.

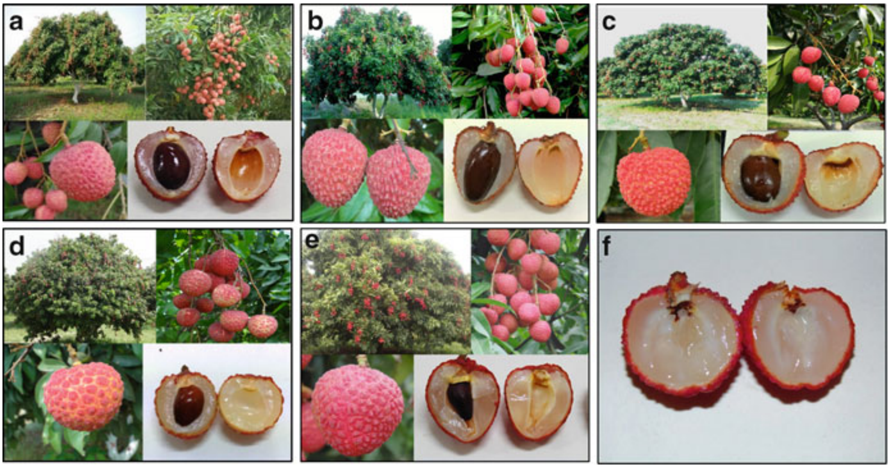

Fig. 8.3 Important litchi varieties of ICAR-NRC on litchi, Muzaffarpur: Shahi (**a**), China (**b**), GandakiLalima (**c**), Gandaki Yogita (**d**), and Gandaki Sampada (**e**). (**f**) Seedlessness in litchi resulting from embryo abortion

Gandaki Sampada A late-maturing strain ripens during mid-June. Fruits are large in size, conical in shape and vermilion to carmine in colour at maturity with dark blackish-brown tubercles (Fig. 8.3e). Pulp is creamy white, soft and juicy, with large fruit size (35–42 g), cracking resistance, pleasant aroma and good yield potential (120–140 kg^{-1} tree). It has a very high percentage of shrivelled and small-seeded fruits, resulting in 80–86% pulp recovery. This variety has potential for export purpose.

Gandaki Yoita This is a dwarf plant, comparatively compact, and tolerant of heat waves and fluctuations in soil moisture. The fruits are free from fruit borers and are of very late maturity (5–15 June). Fruits are round in shape, tyrant rose in colour with dark tubercles and flexible seeds at maturity. The aril is creamy white, soft, juicy and sweet; fruit size ranges from 15 to 19 g with small seeds (Fig. 8.3d). Pulp recovery is 70–75%, possessing melting texture with pleasant aroma and a good blend of sugar and acid. This variety has good yield potential (70–80 kg^{-1} tree) and can be recommended for high-density planting as a speciality variety.

Gandaki Lalima Trees are moderately vigorous, attaining an average height of 10–12 m and almost uniform spread. It is the most nutrient-efficient strain having dark green leaves and the capacity to withstand climatic aberrations. Fruits are conical, bright marigold-orange red in colour with sharp tubercles. A late-maturing cultivar ripens in the second week of June; a heavy yielder has an average yield of 130–140 kg per tree and the fruit weighs between 28 and 32 g. The fruit pulp is creamy white in colour, sweet (18–19° B TSS), soft and juicy with agreeable flavour. Seeds are moderate in size and pulp recovery is above 60% (Fig. 8.3c).

This variety can offer a better alternative for commercial exploitation for export of litchi from India.

8.10 Breeding for Litchi Improvement

8.10.1 Breeding Objectives

Litchi, being a cross-pollinated crop, has a high degree of heterozygosity which does not produce true-to-type plants through sexual means. Most commercial cultivars clonally selected under Chinese or Indian conditions have been adapted to limited climatic conditions (Pandey and Sharma 1989). Clonal selections as the basis of cultivar selection rely on limited characters such as fruit size, quality, and period of maturity. Creation of variability within the litchi gene pool is of paramount importance for desirable characters such as precocity, dwarfness, regularity of bearing, wider adaptability in tree characteristics and resistance to physiological disorders. Cross pollination results gametic variations which serve as the baseline for new selections through genetic manipulation; this stresses the need for initiating a planned breeding programme and raising plants from seeds (Sarin et al. 2009; Singh et al. 2012). The literature reveals that less attention has been paid to breeding work for raising new varieties; although programmes were initiated and conducted sporadically at Hawaii (Storey et al. 1953) and Florida, USA (Knight 2001), Queensland, Australia (Cull 1977), and Saharanpur, India (Lal and Nirwan 1980). As a result, important cultivars such as 'Groff' and 'Brewster' were developed. A strategic effort for a breeding programme on a large scale is needed with consideration of a comprehensive survey of various genotypes and trait inheritance patterns because of the obvious difficulties in litchi breeding (Sarin et al. 2009; Menzel and Waite 2005; Singh et al. 2012).

8.10.2 Inheritance Pattern or Linkage

Studies on the trait inheritance pattern in litchi have been very limited, complicating our understanding of the segregation pattern of genetic traits (Galen Sauco 1989). Differentiating cultivars through genetic markers revealed that most cultivars shared similar parentage. Variability among litchi cultivars is still unknown because breeding for new cultivars is done by growers and is based on a small number of parents (Kumar et al. 2006). However, high heritability and high genetic advances were recorded for fresh seed weight, fruit weight, fruit volume and fresh and dry pulp weight. Fruit and seed weight had strong positive correlations with total sugars, ascorbic acid, protein and tryptophan content but a significant negative correlation with acidity and phenol content (Singh et al. 1987). A negative partial correlation between embryo and aril and a direct repressive effect of the former on the latter were confirmed. These results may be taken into account when breeding varieties resistant to cracking. In nature, the extent of outcrossing in litchi varies from 65% to 87% depending on the proximity of the pollen source. Inbreeding depression occurs on selfing with respect to fruit and seed weight (Stern et al. 1993).

Understanding the genetic diversity within a collection is of prime importance in facilitating germplasm utilization (Strauss et al. 1998). To identify genetic materials containing desirable traits, a systematic evaluation of genetic diversity is required to understand the genetic structure and relationships among accessions and their corresponding collecting site environment (Steiner and Greene 1996). Comparison of parents using different DNA markers is one of the approaches by which breeders can increase the probability of selecting parents with different gene sets. Cultivars with high percentages of aborted seeds (chicken tongue) are highly sought after in litchi. Even within the same cultivar, some trees consistently produce higher percentages of chicken-tongued seeds, and this stresses the needs to determine whether this phenomenon is attributable to environmental conditions or to genetic variation (Madhou et al. 2010).

8.10.3 Breeding Strategies

Up to the present, the majority of litchi breeding work has been attempted through conventional methods with minor attention paid to modern biotechnological tools. Reducing the breeding period in evolving new improved cultivars is very desirable from a breeder's view. Breeding methods combined with novel technologies including genetic engineering, in vitro mutagenesis and molecular assisted breeding would assist litchi breeders to develop new cultivars in a cost-effective manner.

8.10.4 Conventional Breeding

Seeds are the main source of variability. However, the lengthy juvenile period associated with seedling populations hinders the breeding progress. Breeding programmes have been undertaken in several countries within the past two decades. One was initiated during 1992–1993 at the Institute for Tropical & Subtropical Plants (ITSP), South Africa, for the purpose of cultivar selection for South African conditions (Froneman and Oosthuizen 1995). In China, breeding programmes and selection of cultivars with longer duration of bearing period and good-quality fruits have been initiated (Huang et al. 2005a). A seedless litchi variety was produced by conventional breeding in Hainan, China (Sarin et al. 2009; Menzel and Waite 2005; Singh et al. 2012).

Reciprocal controlled crosses among several existing cultivars have been reported in Australia (Dixon et al. 2005). Most seedlings bear fruit within 3 years under subtropical and tropical conditions, and one promising genotype producing 32 kg of fruits with an individual fruit weight of 35–45 g was identified. According to Miao et al. (1998), four new litchi selections were recommended for cultivation in Hainan Province of China. Of these, Aili is a dwarf selection producing an average fruit weight of 24.8 g, and Ziangxi has large fruits (39.1–59.5 g) with acceptable eating quality. Apart from yield and quality characters, there is still a great scope for evolving cultivars that are less prone to cracking and browning as these are directly related to fruit quality. Extension of bearing period, irregular bearing, poor shelf life and disease resistance are the other significant areas to be addressed in future programmes on litchi breeding (Sarin et al. 2009; Menzel and Waite 2005; Singh et al. 2012).

8.10.4.1 Crossing Techniques

Litchi requires cross-pollination, carried out primarily by bees. Although self-sterility has been reported in litchi, bagging of individual panicles is necessary to avoid outcrossing. Male flower development of litchi was defined in three stages. In the first stage, the anther extended just beyond the receptacle, and the filaments were not visible. In the second stage, the filaments extended by half. In the third stage, the filaments extended further before the anthers dehisced. The maximum number of mature pollen grains was found in the third stage before anther cracking (Wang et al. 2015); however, exclusive collection was inefficient because anther development in the same male flower was asynchronous. Therefore, anthers between stage 2 and stage 3 can be collected to ensure sufficient pollen for a crossing programme. For effective crossing, flowers should be collected at the second stage (half-extended filaments light yellow in colour), and at the third stage, bags are removed and fresh male flowers are collected when the anthers turn light yellow in colour. The pollen can be collected either directly from freshly dehisced anthers, if used immediately, or from indehiscent mature anthers when it will be stored for a day or two. Anthers are first separated from flowers by passing them over a screen, which allow them dehisce overnight in petri plates at room temperature. Moist cotton is placed inside the petri plates for obtaining fresh, quality and abundant pollens used for crossing. Pollen obtained in this way can be used directly in crosses and remains viable for 3–5 days at room temperature. It can remain for several weeks if refrigerated under low relative humidity. For long-term storage, litchi pollen can be stored at −86 °C for 2 years. Litchi pollen stored at 4°C remained viable after 52 days, which may meet the demand for artificial supplementary pollination in production, but the demand for artificial cross-breeding is not completely met (Wang et al. 2015).

8.10.4.2 Emasculation and Pollination

To emasculate flowers, scissors are used to remove excess flowers; only 10–15 flowers per panicle are retained for effective crossing, and flowers can be pollinated on the same or the next day. By dipping a small brush into vials or petri plates, pollens are placed on the stigma. Holding the dehisced anthers by their filaments using forceps can also be adopted to transfer pollen. After pollination, flowers are bagged. Successful fertilization occurs after a period of several days.

8.10.4.3 Technique/Procedure of Artificial Hybridization/Artificial Crossing

M_2 pollen has a higher germination rate over a wide range of temperatures. Hand pollination with M_2 pollen produces a satisfactory fruit set compared to M_1 pollen. Hot (32/27 °C) and warm (27/22 °C) temperature regimes during flower development had a pronounced effect on pollen viability compared with cool regimes (22/17 °C) (Stern and Gazit 1998). The pollen germination percentage varied greatly among cultivars and was also significantly affected by weather conditions before the opening of staminate flowers, especially during the anther development stage (Xian et al. 1994).

Fruit and leaf characters are widely used to identify litchi hybrids, although it is known that phenotypic traits can be influenced by environmental conditions and are not reliable indicators (Anuntalabhochai et al. 2002). Hence, along with morphological characterization, molecular studies are essential to confirm the identity of litchi cultivars for optimum germplasm management and establishment of appropriate breeding programmes.

8.10.4.4 Germplasm Evaluation and Clonal Selection

Because many of the existing cultivars originated from a relatively limited ancestral stock, the introduction of new germplasm from wild forms and varieties into the genetic composition of existing cultivars is of paramount importance for achieving the breeding objectives. The important genetic resources of litchi identified for different characters are listed in Table 8.1.

At present, most cultivars grown are of Chinese origin, and the genetic base of the commercial cultivars is relatively small. the majority of the cultivars arose through clonal propagation of high-performing parents. Wild forms or types of the three known litchi subspecies have been widely collected but have been minimally used for integration in breeding programmes, and genetic enhancements are largely based on collections of commercial cultivars selected from open-pollinated Chinese seedlings.

8.10.4.5 Hybridization

Selection for high-yielding and good-quality litchi has been attempted over a long period, but the breeding of new hybrids has not yet attained a significant impact. Recent breeding efforts at Sabour (India) led to the development of two hybrids, namely, Sabour Madhu and Sabour Priya (Table 8.2). The growth of the hybrid seedlings is slow, and only 4% of the total population reached maiden flowering at the age of 14 years (Thakur 1992). Thus, in addition to a short period of seed viability, the late-bearing habit of the seedlings poses serious problems for hybridization work. Besides, these hybrids showed erratic flowering which made it difficult to obtain pollen for breeding purpose.

8.10.4.6 Distant Hybridization

Hybridization was also attempted between litchi and longan, and seedling progenies were variable with small fruit size, which appeared to be a dominant

Table 8.1 Special characteristics of some of the litchi cultivars

Special traits	Source of traits
Small seed	Bedana, Nuomici, Lingshan Xiangli, Hainan Xiaodingxiang, Guangxi Zhangluoli
Crisp and sweet flavour with low tannin	Lingshan Xiangxi
Early maturity	Shahi, Shanyuedong Red, Early Bedana, Dehra Rose
Large fruit	Edanli (60–70 g), Vnsdasu (40–45 g)
High yields	Heiye (Black Leaf), Baitang-ying (White Super Poppy)
High temperature tolerance	Shanyehong: better flower bud differentiation at 20 °C as compared to 12 °C to common varieties
Good for canning purpose	Heiye, Xuangxi
Drought tolerance	Tianyan (Sweet Stone)
Good on-tree storage	Huaizhi: fully ripe fruits can be left on trees for fresh picking
Late maturity	Xuehuazi: ripening during early/mid-July, with high-yielding characteristics; Fijian Xiafanli (Fujian Xiafan litchi): maturing during late July or early August, enabling extended supply period in combination with early and intermediate maturity types; Longia, Kaselia, Yogda

Table 8.2 Litchi cultivars developed through hybridization

Number	Hybrids	Description
1	Sabour Madhu	This hybrid resulted from Purbi × Bedana. It has a higher number of fruits (24) per panicle and ripens 8 days later than another late-maturing cultivar, Kasba. It has higher TSS and aril percentage than Purbi. Fruit shape resembles Purbi.
2	Sabour Priya	This is a product of Purbi × Bedana. It has better fruit quality than Purbi in terms of higher aril percentage and TSS content. The fruit shape is intermediate between both parents. The fruit weight is higher than the better parent (Purbi).

character. Attempts on diallele crosses between *Nephelium lappaceum*, *N. rambutanakee*, *Dimocarpus longan*, and *Litchi chinensis* were made, and only intergeneric crosses between longan and litchi were successful. The pollen of both litchi and longan germinated on stigma of rambutan but was arrested in the embryo sac. However, there appears to be no breeding barrier between cultivars or species within a genus except when seedless fruits are commonly produced (Fig. 8.3f).

McConchie et al. (1994) attempted reciprocal crosses between commercial cultivars of litchi (Bengal and Kwai May Pink) and longan (McLeans Ridges and Duan Yu) and found that hybrid progeny developed only when litchi was used as the female parent. Morphologically the hybrid plants were similar to litchi, but the leaves were smaller. Three types of seeds developed in litchi following pollination with longan pollen: (1) normal seeds with well-developed testa and embryo, (2) seeds with aborted embryo but normal testa development, and (3) seedless, wherein the ovule development is arrested.

8.10.5 Characterization of Litchi Based on Morphological Traits

Litchi cultivars were classified into four categories according to fruit shape: long oval, heart shaped, short heart shaped and round (Li and Fang 1956). Guangdong litchi cultivars were grouped into seven types in 'The Flora of Guangdong Litchi' (Guangdong Academy of Agricultural Sciences 1978). These types include Guiwei, Xiaozhi, Jinfeng, Sanyuehong, Heiye, Nuomici and Huaizhi, based on the shape of tortoise shell-like cracking segments and pericarp with or without sharp protuberances as well as the shape of leaf, inflorescence and fruit, maturity time and quality of fruit. Later, in 1986, litchi cultivars were separated into three types and seven groups according to a four-rank classification criterion considering pericarp, fruit shape and other morphological characteristics. These classification principles were adopted in the subsequent 'The Flora of Chinese Fruit Tree, the Volume of Litchi' (Wu 1998b); Wu et al. (2016) classified litchi cultivars into three types based on the smoothness or roughness of the pericarp. Morphological characteristics such as tree height, canopy spread, tree shape, foliage texture and colour, leaf length, width, shape and orientation, intermodal distance, number of leaflets per leaf, number of leaves per flush, flush colour, panicle length, number of anthers and carpels per flower, filament and style size and fruit colour and size have been used to quantify genetic diversity in litchi (Khurshid et al. 2004).

8.10.6 Characterization of Litchi Based on Isozyme Analysis

Isozyme markers were effective in differentiating litchi accessions. Ambiguities resulting from synonyms and homonyms and sexual propagation of accessions have been resolved to some extent by comparing isozyme fingerprints of different accessions. However, an authentic collection and evaluation of native Chinese cultivars would help in establishing the identity of the present litchi collection. Liu et al. (1989) and Zhou et al. (2001) analysed the PER isozyme of 24 Guangxi litchi cultivars and 35 Guangdong litchi cultivars and produced almost identical results (Wu et al. 2007). Liu et al. (1989) also reported the stability of the litchi PER isozyme pattern as unaffected by the age of leaves. Aradhya et al. (1995) reported genetic diversity among 49 litchi (*Litchi chinensis* Sonn.) accessions using eight enzyme systems encoding 12 loci and revealed moderate to high levels of genetic variability. Comparison of isozyme fingerprints revealed different isozyme patterns in 'Nomai tsz', 'Kwai mi' and 'Hak ip,' believed to be synonyms, whereas some others with different names displayed identical patterns. Wu et al. (2016) reported the usefulness of morphological traits of leaf and branching to discriminate 146 Chinese litchi germplasms.

8.10.7 Characterization of Litchi Based on Molecular Markers

Molecular studies on genetic diversity of litchi are limited. The presence of variable litchi cultivars in China and India provides a good basis for development of new cultivars (Groff 1921). Recent development in molecular tools further enhances our efficiency in understanding the genomic variability and the diversity between and within different species of litchi.

Initially, studies on genetic diversity in litchi have been largely based on morphological characters. Development of molecular markers in the past few decades yielded accurate and precise information on the extent of genetic diversity in litchi. The most commonly used DNA marker is random amplification of polymorphic DNA (RAPD) (Ding et al. 2000; Chen et al. 2004, 2005; Liu and Mei 2005; Wang et al. 2006; Bajpai et al. 2016), followed by amplified fragment length polymorphism (AFLP) (Yi et al. 2003; Peng et al. 2006) and simple sequence repeat (SSR) (Li and Zheng 2004). However, in most of the tested litchi cultivars, contradictory results were obtained from different markers, and even those obtained from the same DNA marker are not totally identical. Results of RAPD and AFLP analysis indicated a low level of genetic diversity within litchi collections; several workers characterized litchi cultivars using molecular markers and reported the occurrence of synonyms (Ding et al. 2000; Yi et al. 2003; Chen et al. 2004) such as 'Nongmei No. 9' and 'Qiongshan No. 27' (Chen et al. 2004), 'Dazao' and 'Zaohong,' 'Baiye' and 'Guahong,' 'Feizixiao' and 'Zhimali,' 'Ziniangxi' and 'Zengchengdaguoli' (Liu and Mei 2005) and 'Fengshuang' and 'Tunchangfengshuang' (Wang et al. 2006). However, Ding et al. (2000) reported that 'Dazao' and 'Zaohong' are two entirely different cultivars. Viruel and Hormaza (2004) obtained 12 microsatellites enriched in CT repeats from a genomic library of the litchi cultivar 'Mauritius.' Assessment of genetic diversity of litchi at Kalyani, West Bengal (India), revealed the segregation of cultivars into two clusters which can give heterotic hybrids when intercluster cultivars are crossed (Dwivedi and Mitra 1995, 1996). Recently, Liu and Mei (2005) reported an average of 15.8% polymorphic and 0.10% monomorphic RAPD markers from a population comprising 60 litchi cultivars, one longan cultivar, and one tentative intergeneric hybrid of litchi and longan. Two accessions (LH80 and LH109) were genetically distinct as revealed by RAPD and AFLP markers. In Thai litchi cultivars, the percentages of polymorphic markers for RAPD and AFLP were 34.6% and 36.3%, respectively (Tongpamnak et al. 2002), and each marker system was able to differentiate all accessions. RAPD markers have been widely used to study the genetic relatedness among litchi cultivars (Kumar 2006); Ding et al. (2000) investigated the segregation patterns of RAPD markers in an F_1 population of litchi from a cross between 'Wuye' and 'Luhebao.' Bajpai et al. (2016) reported genetic diversity of 20 litchi cultivars from the Indian peninsula, and phylogenetic analysis based on RAPD and microsatellites revealed clustering of the cultivars into four major groups, although within a very narrow range (0.63–0.90) of similarity, viz. Seedless (i.e., Bedana), Mandarji, Shahi and China groups. Simple sequence repeats (SSRs) markers previously developed for litchi have also been evaluated for polymorphism in different populations (Ekuae et al. 2009). The Power Core programme based on 30 EST-SSRs and a combined dataset of the EST-SSRs and 16 phenotypic traits has been used to construct two core collections from 96 accessions (Sun et al. 2012). Madhou et al. (2013) conducted molecular characterization study of litchi accessions from Mauritius and Reunion and compared them with Spanish litchi cultivars. Liu et al. (2015) obtained the potential of single nucleotide polymorphisms (SNP) for the identification of 96 representative litchi accessions, and their genetic relationships in China were evaluated using 155 SNPs that were evenly spaced across the litchi genome. Ninety SNPs with minor allele frequencies above 0.05 and a good genotyping success rate were used for further analysis. A relatively high level of genetic variation was observed among litchi accessions, as quantified by the expected heterozygosity (He =0.305).

8.10.8 Mutation Breeding

So far, induced mutations have not been in vogue in litchi improvement. It was reported that seedlessness in the litchi cultivars Lanzhu and Luhebao might have resulted from mutation, gene interaction and long-term artificial selection and is not the result of variation in chromosome number or structure (Lu et al. 1987). Qinzhou red litchi, a new variety of litchi, was derived from spontaneous mutation of the cultivar Black Leaf (Peng et al. 2001).

8.10.9 Transgenic Approach

Green fluorescent protein (GFP) gene expression in leaf tissues of litchi after transformation is using *Agrobacterium* (Puchooa 2004a). In vitro, grown leaf tissues were used for transformation. After 4 weeks in culture, expression of GFP was apparent when the regenerated callus and the leaves were observed under a fluorescence microscope fitted with a blue exciter filter, a blue dichroic mirror and a barrier filter. Although no transformed litchi plantlets were regenerated, screening for GFP gene expression may prove useful to improve transformation efficiency and to facilitate the detection of transformed litchi plants. Ouyang et al. (1985) reported T-DNA transfer and tumour formation induced by *Agrobacterium tumefaciens* on litchi. Therefore, genetic transformation of litchi using *Agrobacterium* could be exploited in the future.

8.11 Constraints in Litchi Breeding

The development of improved cultivars in litchi is a lengthy and cumbersome process, and trees when fruited only produce less than 1% of the seedlings worthy of selection. Breeding for a new hybrid cultivar through conventional methods would require about 40 years in fruit trees such as litchi (Zheng et al. 2001). In addition to poor seed viability and wide variability in seedling progenies, erratic flowering poses a hindrance for pollen collection during the breeding process. Although crosses between litchi and longan are successful, the use of longan as parents in a hybridization programme is limited by its biennial bearing tendency. In crosses involving plants that have a tendency to produce chicken-tongued seeded fruits as the female parent, many of the most valuable progeny are lost before the harvest (Puchooa 2004b). Only a few cultivars have been bred through conventional methods because of the long juvenile period, the apparent lack of genetic variability in the existing germplasm, and the great expenditure that is required in terms of land, time and money (Litz et al. 2005). Thus, the improvement of litchi appears to be confined mainly to selections of improved chance seedlings or genotypes. Future efforts in plant breeding need to emphasize identification of potential parents followed by their reciprocal crossing.

8.12 Future Prospectives

Despite constraints and the long time frame needed for conventional breeding, opportunities do exist to develop new cultivars with improved traits that can improve productivity and fruit quality against the endogenous and exogenous attack of pathogens, mites and insects. New ideotypes through appropriate blending of traditional breeding and biotechnological tools can be used in the future for disease management in the litchi system. Recent and future development in high-throughput MAS can substantially improve the efficiency of the conventional litchi breeding to attain the resistance in susceptible litchi crops. Application of molecular markers in the creation of a genetic map and other pre-selection techniques has the potential to substantially reduce selection time frame in litchi breeding programmes. Identification and cloning of genes of horticultural interest from litchi and its wild relatives will enhance the rate of conservation of plant survival from germinated transgenic somatic embryos and would certainly revolutionize the genetic improvement of litchi cultivars. The advantage of mutation induction in obtaining the hidden genetic variation and improvement of vegetatively propagated plants when one or few characters of an outstanding cultivar are to be modified can also be exploited to a great extent. Biotechnological developments and statistical analysis of current breeding populations would immensely improve our understanding of the genetic traits and their inheritance in litchi which, in turn, make it easier for the breeders to select parents and design a systematic breeding programme with more specific breeding goals than has been pursued in the past. However, there is still significant constraint in controlled hybridization that needs to be addressed.

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