Manoj Kumar · Vivek Kumar
Ram Prasad · Ajit Varma *Editors*

The Lychee Biotechnology

Springer

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Preface

Litchi (*Litchi chinensis* Sonn.) is an important fruit crop commercially grown in some states with tremendous export potential and plays a significant role in their economy. There has been an ever-increasing demand for litchi in domestic and export market. Owing to specific climatic requirement, successful litchi cultivation has been restricted in certain areas, but now with the development of improved cultivation technologies, it is spreading to many other parts of India. Litchi is an evergreen subtropical fruit, known for its delicious, juicy aril and refreshing taste. Fruits are consumed fresh or processed into value-added products. The pulp, canned aril and dried fruits ('litchi nuts') are exported. The aril of dried litchi is eaten like raisins. The Chinese use dried aril in their tea as a sweetener. Litchi fruits are also spiced or pickled or made into sauce, preserves or wine. Litchi seeds are used as anodyne in neuralgic disorders and bronchitis.

'The acquisition of this book by researchers will undoubtedly provide them with great enthusiasm and a clear insight into the development of future research'. *Experimental biotechnology* is comprised of the following: allelochemicals, breeding strategies, canopy architectural engineering, pest and disease management, bioactive compounds, genetic transformation, molecular marker, mycorrhizae, tissue culture, aetiology, endophytes, etc.

Editors have the deep-rooted thrust on the litchi system which acquires the zest of the proposed book that will provide the great enthusiasm and a clear insight to the contents of the book and its beneficiaries.

For growing the litchi/lychee, it is hopeless because it is definitely too advanced for the average grower; moreover, cultivators, scientists and students need information on growing litchi, and therefore, objectives of the book meet all the requisite inputs.

In this book, editors compiled researches carried out by potential contributors in the form of documented assortment with elaborate description that relate with the 'role of biotechnology in litchi improvement and sustainability'.

Chapter 1. This chapter provides a wide understanding on fruit set, development, maturation and health benefit property which will be helpful to increase yield, produce high-quality fruit and increase the consumption of litchi at commercial level.

Chapter 2. 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. The current status of breeding, biology of flower and fruit development and cultural research in Taiwan are discussed in this review.

Chapter 3. This chapter focuses upon contemporary information on biotechnological advances made in lychee by overcoming the problems encountered during in vitro propagation, generation of disease-resistant cultivars and enhancement of shelf life.

Chapter 4. Widening of the genetic base of native cultivars using different molecular markers and introduction of genetic engineering to produce promising hybrids with large fruit, resistance to pericarp browning and long life span are highly discussed with reference to biotechnological tools. Authors have attempted to overview the combined research and development for the improvement of fruit quality and postharvest storage using various conventional as well as biotechnological tools.

Chapter 5. Propagation of lychee from seeds is difficult and not practicable because of longer juvenile period and non-viable, abortive and genetically diverse nature of the seedlings. However, the techniques such as cell, tissue and organ culture (micropropagation) can overcome the difficulties of lychee propagation. In a nutshell, lychee is an important commercial fruit crop, and there is a need to develop technical research so as to sustain and enhance its yield, postharvest management, medicinal value and marketing. This chapter comprises of botanical description, cultivation, medicinal uses, micropropagation and trading of *Litchi chinensis*.

Chapter 6. This chapter explains cracking problems on the litchi pericarp skin which acts as point of entry for the invasion of postharvest microbial pathogens during cold storage and transport. Though browning triggered by withering does not harshly influence the corporeal attributes of lychees, involuntary injury and postharvest deterioration could lead to deadly effects on sensory attributes of lychee aril. Pericarp skin browning and postharvest deterioration during storing and transport are presently measured by adopting SO_2 fumigation in numerous lychee-growing and lychee-exporting countries. However, SO_2 fumigation leaves unwanted remains, changes fruit taste and results in health issues for customers and workers.

Chapter 7. In this chapter, authors have deliberately discussed phytochemical composition and important bioactivities of litchi and its different parts emphasizing the mechanism of action underlying bioactive properties.

Chapter 8. This chapter discusses the necessity to develop fruit crop varieties that are resilient to abiotic stresses to ensure nutritional and financial security to a large population of the world. With the development of new biotechnological tools such as genomics, transcriptomics, microarray and next-generation sequencing, a plant scientist can investigate molecular, physiological and biochemical regulatory pathways activated *in planta* to cope with various abiotic stresses and use this information for genetic improvement of crop as well as the formation of new-generation GMOs. Various abiotic stresses interfere with lychee growth and development and affect its productivity as well as provide a detailed update on recent researches

which contributes to a better understanding of stress regulatory mechanism to combat abiotic stresses in lychee.

Chapter 9. The respiratory burst is associated with larger production of reactive oxygen species (ROS), responsible for accelerating the fruit senescence. Postharvest cold storage prolongs litchi shelf life, but storage of lychee at ambient condition after pre-cold storage has not been proved considerably effective. Comprehensive genomic, transcriptomic and metabolomic analyses help in revealing the molecular background of postharvest senescence of lychee.

Chapter 10. As lychee biotechnology has huge potential to offer societal issues at farming level which must be discussed at industrial and academia level, patents can be given to farmers (stakeholders) for their novel approaches in harvesting the products which could be enhanced with high-throughput technology. The country's patent law and the scopes of patentable claims for lychee plants/products that can popularize lychee in the international market have been discussed with international standards.

Chapter 11. In vitro plant regeneration has been harnessed to give an impetus to the production of litchi, but litchi is a recalcitrant plant and restrictions in explant collection slow the progress in this regard. Genetic transformation along with omics approach and biotechnology tools may immensely help in the development of desired cultivars of litchi. Authors have discussed the challenges and possibilities of genetic manipulation of litchi.

Chapter 12. A research protocol has a comprehensive discussion with comprehensive illustrations. It addresses the technical inputs for reproducible and efficient method of in vitro regeneration of elite litchi trees appropriate for clonal propagation. The protocol has been referred as advantageous to the horticulturists and the industry for recalcitrant trees that can be developed as true to the parental type.

Chapter 13. Phytochemical investigation revealed that the major chemical constituents of litchi are flavonoids, sterols, triterpenes, phenolics and other bioactive compounds. Crude extracts and pure compounds isolated from *L. chinensis* exhibited significant anti-oxidant, anti-cancer, anti-inflammatory, antimicrobial, antiviral, anti-diabetic, antiobesity, hepato-protective and immunomodulatory activities. It is now being used in many cultures for the treatment of cough, flatulence, stomach ulcers, diabetes, obesity, testicular swelling, hernia-like conditions and epigastric and neuralgic pains. From the toxicological perspective, litchi fruit juice and extracts have been proved to be safe at a dose.

Chapter 14. The application of biotechnological tools for in vitro regeneration, micropropagation and genetic engineering in litchi species has been practised with success, especially in the last decade as, by using genetic engineering, the addition of introducing a desired gene in a single step is possible in litchi. This chapter reviews some of the basic aspects and advancements made in litchi propagation and genetic transformation techniques for further improvement.

Chapter 15. Two major approaches used for conservation of plant genetic resources are in situ and ex situ. Both approaches are important and complementary to each other for sustainable agriculture. It is challenging to conserve litchi germplasm through seed, field maintenance and in vitro storage because of its

recalcitrant nature and owing to various biotic and abiotic factors. Of all the various strategies of ex situ conservation of litchi, cryopreservation of litchi germplasm using its embryonic axis or pollens is a promising option for conservation of germplasm.

Chapter 16. The major flavanols in litchi fruit pericarp (LFP) are reported to be procyanidin B4, procyanidin B2 and epicatechin, while cyanidin-3-rutinoside, cyanidin-3-glucoside, quercetin-3-rutinoside and quercetin-3-glucoside are recognized as main anthocyanins. Furthermore, some genes are responsible for anthocyanin accumulation in LFP. Litchi flavonoids exhibit good potential anti-oxidant activity. Additionally, LFP extract displays a dose- and time-dependent inhibitory effect on human breast cancer, which could be attributed, in part, to its inhibition of proliferation and induction of apoptosis in cancer cells through upregulation and downregulation of multiple genes. It is suggested that flavonoids from LFP play an important role as potential components for functional foods and anti-breast cancer drugs.

Chapter 17. Litchi cultivation is highly specific to its climatic requirements as different temperature and humidity conditions are required for flowering and fruit development. Soil factors (edaphic) are quite common for the cultivation of litchi which restricts the spread of litchi genepool. Heterozygosity is another natural instinct which is unavoidable at generic growth of litchi progeny and eventually discourages the true-to-type concept at generation level. Several research articles have been published on the known limiting factors in terms of asexual and sexual growth and conditions.

Chapter 18. Genetic transformation in plants is synergistic to conventional plant breeding technologies. By using this, the breeders can introduce novel genes irrespective of species barrier and can create phenotypes with desired characters. Over the last decade, some remarkable achievements have been made in the field of development of efficient transformation methods in field crops. Also in litchi genetic engineering, a technique can be used to introduce new traits into popular genotypes, which can result into new cultivars with desirable traits. In this chapter, authors review the transformation methods which are being used or can be used for genetic improvement in litchi.

Chapter 19. Litchi cultivation is still based on conventional approaches, viz. grafting, air layering, etc., which have wearing and tearing. In current scenario, litchi biotechnology is still in scarcity which needs to be enhanced with modern approaches. Here author proposes the potential ways (micropropagation, germplasm culture, anther culture, etc.) to propagate litchi trees with modern tissue culture approaches.

In the preparation of this book, it has been the authors' aim to keep in mind not only the requirements of researchers and students in this specialized domain but also the needs of plant biotechnologists.

Editors are grateful to Springer Nature for publishing *The Lychee Biotechnology* with their customary excellence. Special thanks are due to Dr. Mamta Kapila and Ms. Raman Shukla, without whose constant efforts the book could not be published. Finally, the editors wish to thank the technical staff team of Springer for their promptness and their helpful action.

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Manoj Kumar
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About the Editors

Manoj Kumar, Ph.D.
Editor, The Lychee Biotechnology

Dr. Manoj Kumar is a scientist with sanguine behavior who is adoring about research and development, with a commitment to lifelong learning. He is determined on high-quality science that contributes broadly to both increasing intellectual knowledge of plant development and to increasing the ecological niche. He has a high level of professional desire and intellectual hunt, and the potential to fulfill the dream of his high-impact publications and the future recognition of these by academic peers.

Dr. Kumar has pursued doctorate in plant biotechnology specialized in lychee genetics and tissue culture. He has been awarded prestigious DBT-PDF from the Indian Institute of Science, Bangalore, and NRF-PDF from the University of Pretoria. His starting career includes tree genetics and forest molecular genetics which have been expanded with his current approaches at plant-microbe interaction level.

Dr. Manoj Kumar is a researcher of plant biotechnology in the Division of Microbial Technology at the Amity University Uttar Pradesh, India. Dr. Kumar has been involved in tree genetic improvement using the modern approach at functional analysis level. He has developed user-friendly approaches for regeneration and genetic transformation of recalcitrant tree species like litchi, eucalyptus, populous, etc. in which the functional aim is to adapt crop plants in order to increase productivity and adaptability on such Indian soils, with consequent improvement of sustainability in both developed and developing countries. Dr. Kumar has set an intellectual aim to understand the metabolic fate of microbial-mediated precursors in whole plant physiology and genetics through processes occurring at the level of metabolism, particularly through processes such as rhizosphere communication under in situ and in vitro plants. This aim is being addressed by combining functional genetics and metagenomics approaches with a broad-based understanding of plant-microbe healthy interaction.

Vivek Kumar, Ph.D.
Editor, The Lychee Biotechnology

Dr. Vivek Kumar is a scientist involved in teaching, research and guidance, with a pledge to enduring knowledge. Dr. Kumar is working in the Division of Microbial Technology at Amity University, Uttar Pradesh, Noida, India. He is serving in the editorial board of reputed international journals, viz. *EnvironmentAsia*, *International Journal of Biological and Chemical Sciences*, *Journal of Advanced Botany and Zoology* and *Journal of Ecobiotechnology*. He is also reviewer of *Journal of Hazardous Materials*, *Science International*, *Acta Physiologiae Plantarum*, *International Research Journal of Plant Sciences*, *International Journal of Microbiology*, *African Journal of Microbiology Research*, *Journal of Microbiology and Antimicrobials*, *Environmental Science and Pollution Research* and *Rhizosphere*. He has published 61 research papers, 19 book chapters, six review articles and two books. Dr. Kumar has also served as microbiologist for 8 years in the Department of Soil and Water Research, Public Authority of Agricultural Affairs and Fish Resources, Kuwait.

Dr. Kumar's research areas are plant-microbe interactions, environmental microbiology and bioremediation. He has been credited with first-time reporting and identification of pink rot inflorescence disease of date palm in Kuwait caused by *Serratia marcescens.* He has been awarded 'Young Scientist Award' for the year 2002 in 'Agricultural Microbiology' by the Association of Microbiologists of India (AMI).

Dr. Kumar is establishing an 'unearthing and deliverance system', where a balance is being strived between development of drought- and salinity-resistant microbiome for better crop production in rain-fed and saline areas. In the bioremediation research programme, isolation and characterization of autochthonous microbiome from textile dye effluent and soil performed very well in remediation of dyes under laboratory conditions. Selected microbiome will be further employed in bioremediation of textile dyes at larger level.

Ram Prasad, Ph.D.
Editor, The Lychee Biotechnology

Dr. Ram Prasad is assistant professor at the Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, India. Dr. Prasad has completed his Ph.D. from the Department of Microbiology, Chaudhary Charan Singh University, Meerut, UP, India, in collaboration with the School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi, India. Dr. Prasad received his M.Sc. in life sciences at JNU and also qualified CSIR-NET, ASRB-NET and GATE. His research interest includes plant-microbe interactions, sustainable agriculture and microbial nanobiotechnology. Dr. Prasad has 93 publications to his credit, including research papers and book chapters and five patents issued or pending, and edited or

authored several books. Dr. Prasad has 11 years of teaching experience, and he has been awarded the Young Scientist Award (2007) and Prof. J.S. Datta Munshi Gold Medal (2009) by the International Society for Ecological Communications, the FSAB fellowship (2010) by the Society for Applied Biotechnology, the Outstanding Scientist Award (2015) in the field of microbiology by Venus International Foundation and the American Cancer Society UICC International Fellowship for Beginning Investigators (USA, 2014). In 2014–2015, Dr. Prasad served as visiting assistant professor in the Department of Mechanical Engineering at Johns Hopkins University, USA.

Prof. Dr. Ajit Varma
Editor, The Lychee Biotechnology

Professor Ajit Varma is distinguished scientist and professor of eminence at Amity Institute of Microbial Technology (Amity University, Uttar Pradesh). He has been leading an international research group of microbial technology in collaboration with several prestigious institutions worldwide. He is also holding several other responsibilities in Amity University, like vice chairman of Amity Science, Technology and Innovation Foundation and chairman of the Faculty Research Council at university level. He has pursued his doctorate from Allahabad University in 1964 and then started his academic and scientific journey from the Indian Agricultural Research Institute, New Delhi, and then retired as an eminent professor from prestigious Jawaharlal Nehru University in 2004. Since then, his leading role incepted in Amity University to harness the Amity Research at international level. Professor Varma has numerous national and international research and academic awards in his credit and headed several councils in plant-microbial world. He has visited several countries as a visiting scientist, professor and academician for his world novel discovery *Piriformospora indica* – a magic fungus which has been popularized as *ROOTONIC*. Apart from the above-mentioned facts, Professor Varma has achieved the academic height based on the following mentioned accreditations:

Awards and recognitions:

- Commonwealth Fellowship (Australia)
- National Research Council (Canada)
- Alexander von Humboldt Foundation (Germany)
- National Science Foundation (USA)
- Indo-Czechoslovakia Exchange Programme (Prague)
- DAAD Fellowship (Germany)
- Deutsches BMFT Programme, Georg-August-Universität Göttingen (Germany)
- RAISA Fellowship for Innovative Research in Biotechnology (Italy)
- Swiss Federal Research Fellowship (Switzerland)
- BP Koirala Award (Nepal)

- DFG-INSA Fellowship (Indo-Germany)
- FAMI Award 2011 (India)
- Honorary Diploma, UMF, Cluj-Napoca, Romania (2011)
- Lifetime Achievement Award, Bombay University (2011)
- Special felicitation for outstanding research in the field of microbiology, JNU (2012)

  Number of Ph.D. degrees awarded: 56
  Number of D.Sc. degrees awarded: 1

Litchi Fruit Set, Development, and Maturation

1

Hui-Cong Wang, Biao Lai, and Xu-Ming Huang

Abstract

In broad terms, fruit development can be divided into three stages: set, growth, and maturation. The fruit set of litchi are established soon after fertilization except for the parthenocarpic cultivars, which grow fruits without fertilization. In structure, the fruit of litchi is a drupe with an edible aril enclosing a single seed surrounded by a pericarp. Some cultivars produce a proportion of aborted seeds and thus have a higher flesh recovery than others, while a few rare strains produce seedless fruit. The aril (flesh) of litchi is white, semitranslucent, and juicy with sweet taste and fragrant flavor. The tuberculate skin or pericarp is green, yellow-red, or red, depending on the cultivar. Fruit set, development, and maturation of litchi are the crucial period for yield and quality formation. Understanding the fruit set, development, maturation, and the health benefit property will be helpful to increase yield and produce high-quality fruit and the consumption of litchi.

Keywords

Fruit set • Fruit size • Maturation • Sugar accumulation • Anthocyanin biosynthesis • Health benefit compounds

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1.1 Embryology, Histology, and Organogenesis

The ovary of litchi generally has two loculi, rarely has three or more, one that grows and the other one atrophies. Sometimes, normal well-developed twin or triple fruit set on one pedicel. The surface of the ovary has protuberance that gives the fruit rough surface (Fig. 1.1). A normal ovule is composed of funiculus, obturator, two integuments, and an embryo sac containing egg apparatus (Fig. 1.1). Litchi fruit development takes between 70 and 100 days after anthesis (DAA), depending on the cultivar and location (Subhadrabandhu and Stern 2005).

According to Lü et al. (1985), double fertilization takes place 2–3 days after pollination, followed by division of the nucleus of the primary endosperm. In normal-seeded cultivar Heiye, the embryo reached the globular stage after 20 DAA, the heart stage with a rudimentary cotyledon after 30 days, and the torpedo stage after 40 days. The liquid endosperm was absorbed by the developing cotyledon after 50 days. In aborted-seeded cultivar Nuomici, embryo development slowed after 30 days and aborted after 40–50 days, at torpedo stage (Qiu et al. 1994; Xiang et al. 2001). The volume of the liquid endosperm began to decrease after 25 days and it disappeared after 40 days.

A microscopic study showed that the cell division in the ovary wall of "Huaizhi" was very active before anthesis, but relatively quiescent during bloom (Li 2001). A second wave of cell division occurred in the pericarp at 14 days after anthesis (DAA). Cell division ceased in various parts of the pericarp at different times: at 19 days for inner mesocarp, day 32 for the outer mesocarp, and day 47 for the endocarp and epicarp. Difference in fruit size among cultivars was related to variations in the number of pericarp cells rather than to their final cell size (Li et al. 2002).

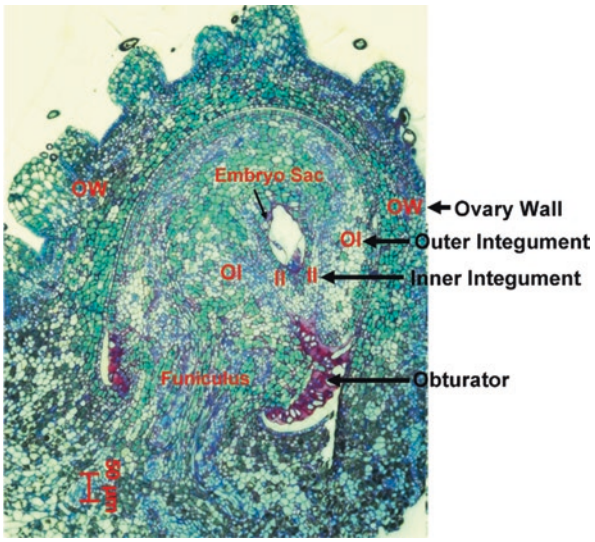

Fig. 1.1 Longitudinal section of a 3-day-old "Huaizhi" female flower. *OW* ovary wall, *OI* outer integument, *II* inner integument

There is much divergence of opinion about the origin of the litchi aril. Huang et al. (1983) obtained valid microscopic evidence that shows that the primordium was not derived from the obturator, but from a site immediately above it on the funicle. However, Ye et al. (1992) suggest that the primordium originated from the outer integument rather than from the funicle. Aril development begins around 21–35 DAA and the growth stage lasts about 49–56 DAA.

1.2 Type of Fruit

There are three types of fruit: normal, aborted, and seedless (Fig. 1.2). Seedless and aborted-seeded fruit are preferred by consumers, since they have a high flesh recovery. Normal-seeded fruit have a dark-brown seed containing a viable embryo when mature and a fully developed aril. Aborted-seeded fruit has a well-developed aril that fills the whole space provided by the preformed pericarp. The seed is small and shriveled, with an empty cavity and dead or rudimentary stunted embryo. According to Huang (2005), shriveled seeds in litchi are called "chicken tongues"; however, botanically this phenomenon is called "stenospermocarpy," which means "fruit with slim or narrow seeds." Seedless fruit, botanically termed "parthenocarpic," does not have a seed because the ovary is not fertilized.

Normal-seeded cultivars, such as "Heiye," "Huaizhi," and "Dazao" ("Maritius"), have a low incidence of aborted seeds, while aborted-seeded cultivars, such as "Nuomici" and "Lühebao," have very low incidence normal seeds. Other cultivars, such as "Guiwei" and "Lanzhu," have a variable proportion of aborted seeds. Lü et al. (1985) term this kind of cultivars as "partly aborted-seeded cultivar." Seed abortion can occur after unfavorable weather (Stern and Gazit 1996). This helps to explain why the proportion of aborted seeds in some cultivars, such as "Guiwei," varies from year to year in the same orchard. However, the seed abortion rate of "Guiwei" also varies for individual plants in the same orchard or even panicles in

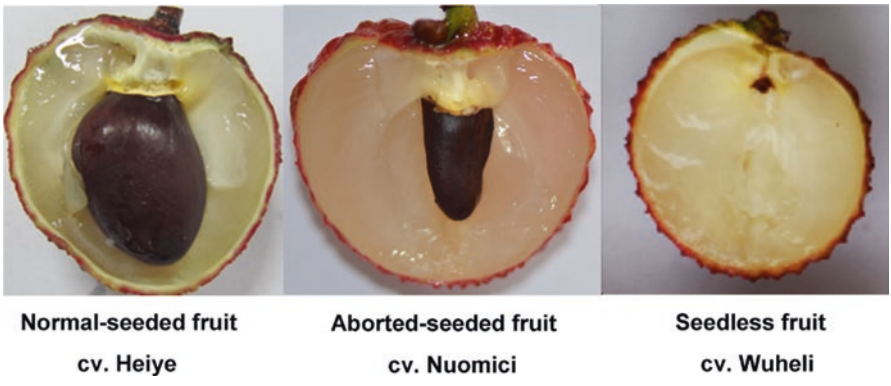

Fig. 1.2 Three different types of litchi fruit (The picture of cultivar Heiye cited from Wang et al. 2015)

Fig. 1.3 "Guiwei" fruit with different seed size samplings from the same tree

Table 1.1 The effects of different pollen sources on seed and fruit features of litchi cv. Guiwei

Pollen source	Seed diameter (cm)		Abortion rate (%)	Pericarp thickness (cm)	Aril thickness (cm)	Recovery rate (%)
	Vertical	Transverse				
Feizixiao	1.64b	1.18b	83.3a	0.14a	1.00a	80.4a
Nuomici	1.73b	1.24b	87.5a	0.12b	1.04a	78.6b
Guiwei	1.65b	1.28b	95.4a	0.15a	1.05a	82.8a
Hongxiuqiu	1.87a	1.30b	50.0b	0.13b	0.99b	75.6b
Huaizhi	1.98a	1.29b	37.5c	0.13b	1.04a	80.1a
Xuehuaizi	2.06a	1.56a	0.0c	0.13b	1.00a	52.2c
Heiye	1.71b	1.30b	66.6b	0.14a	1.01a	80.4c
Chenzi	1.82a	1.32b	63.2b	0.13b	0.95b	75.2b
Jiangjunli	2.02a	1.56a	28.6c	0.15a	0.93b	78.1b
Shangshuhuai	1.93a	1.39a	30.0c	0.16a	0.93	76.5b

From Qiu et al. (2006)
Note: Column followed by different letters is significantly different at $p < 0.05$

the same tree. Figure 1.3 shows that "Guiwei" produce fruits with different seed size in the same tree. Stern et al. (1993) found that seeds from self-pollinated flowers more likely to abort than seeds from cross-pollination. Pollen sources have a direct influence on seed features (xenia) and the surrounding tissues (metaxenia) of litchi (Qiu et al. 2006). Fruit pollinated with the pollens of "Xuhuaizi," "Huaizhi," "Jiangjunli," and "Shangshuhuai" displayed significant lower abortion rate than "Guiwei" self-pollination and pollens of "Feizixiao," "Nuomici," and "Heiye" (Table 1.1). Pollen source significantly affects the fruit set, seed weight, and shriveling of litchi (Degani et al. 1995; Chu et al. 2015). These results help to explain

Table 1.2 The fruit weight and seed development of different lines of "Wuheli" (unpublished data)

Lines	Fruit weight (g)	Seed weight (g)	Big seed rate (%)	Aborted seed rate (%)	Seedless rate (%)
"A4"	22.34 ± 0.61	0.15 ± 0.03	1.4	24.8	73.8
"Nandao"	24.99 ± 0.43	1.61 ± 0.10	39.1	23.2	37.7

Note: Data presents as means ± Se or means (n = 150 for fruit and seed weight, n = 300 for seed development)

why the abortion rate varied among trees, orchards, and years for different planting varieties in orchards and different weather might affect the chance of self-pollination.

"Wuheli" is the only commercial litchi cultivar that produces seedless fruit. This cultivar produces normal-seeded, aborted-seeded, and seedless fruits. The seedless rate differed among production years and lines. Embryo sac sterility was the reason for failure to bear seeds normally (Liu et al. 1999; Feng et al. 2010). Delay and a relatively higher level of abnormal embryo sacs occur under 23/17 °C (day/night) as compared to 33/27 °C in "Feizixiao." This might explained the variation in seedless rates among production years in the same orchard (Shi and Chen 2000). However, genetic factors might play priority role in determining the type of embryo that develops. "A4 seedless" and "Nandao seedless" are two popular "Wuheli" lines of this cultivar with a seedless rate at around 75% for the former and 40% for the latter (Table 1.2). And the seedless rate remains relative consistent in fruits from different orchards and production years.

1.3 Fruit Abscission

Initial set depends on success of fertilization in normal and aborted seed cultivars. Fruit abscission is a normal event during fruit development. Normal-seeded cultivars have two periods of fruit abscission (Joubert 1986), while aborted-seeded cultivars have three to four waves of fruit abscission (Yuan and Huang 1988; Qiu et al. 1998). The first wave of abscission occurred 1 week after full bloom (AFB) and was associated with a lack of fertilization. Low viability of the embryo sac and/or pollen resulted in severe initial fruit drop. The proportion of 2-day-old fertile flowers abscising ranged from 3 to 27% in 11 orchards in Israel (Stern and Gazit 1996). In extreme cases, all the fruits may be lost, leading to bare panicles after prolonged rained or overcast weather. Wave II occurred around 3 weeks AFB, before the liquid endosperm was full. A third wave occurred 6–7 weeks AFB, when the embryo grew rapidly. Wave IV was specific to cultivars with aborted-seeded cultivars and occurred 2–3 weeks before harvest. The final fruit set is around 5% or less (Stern and Gazit 2003), depending on cultivars, weather, and the status of tree nutrients.

Litchis are self-compatible, since orchards based on single cultivars can yield heavily. However, some researches found that self-pollination in cultivar "Floridian" but not "Mauritius" showed lower fruit retention and produced smaller seeds than

outcrossing (Stern et al. 1993; Degani et al. 1995). The lower fruit retention rate might derive from the poor seed development for some litchi cultivars such as "Guiwei" displayed distinct xenia phenomenon. Higher seed abortion rate and lower fruit retention were noticed in self-pollinate "Guiwei" as compared with pollinated the pollen of "Xuehuaizhi," "Huaizhi," and "Baila" (unpublished data). Excessive midterm fruit drop can substantially reduce final yield and is associated with poor vitality of the liquid endosperm and the failure of the embryo.

Competition for nutrients occurs between flower and fruits within panicles. Summer flush or the active growth of roots also causes excessive fruit drop. Some large panicle cultivars such as "Feizixiao" have plentiful second round of male flower, and the blooming of second round male flower might deplete assimilates. Flowering use stored carbohydrate reserves (Yuan et al. 2009). The lower fruit retention rate in "Feizixiao" is related to the excessive consumption of carbohydrate reserves by flowering, leaving little for fruit set (Jiang et al. 2012). Overcast weather or rain, which frequently occurs during fruit development in South China, reduces photosynthesis and fruit set (Yuan and Huang 1992). Chilling during flowering and large inflorescence resulted in lower fruit set (Chen et al. 2013). Emasculation is a common practice in South China to increase fruit set of "Feizixiao" (Wu et al. 2000; Chang and Lin 2003). Chen et al. (2014) applied 100 mg L^{-1} GA_3 to panicle significantly reduced the length of inflorescence and increased fruit set.

Summer vegetative flush can sometimes be detrimental, causing fruit drop before rapid fruit growth (Huang 2005). An additional early peak of root growth in young trees of "Nuomici," which is a shy bearer, coinciding with an early summer leaf flush, caused a heavy fruit drop (Huang 2002). Girdling prior to or during bloom enhanced fruit set by inhibiting root growth and thus eliminated root-fruit competition (Fig. 1.4). The extent of midterm drop can be mediated by competition among sinks. In "Nuomici," trees with summer flushes on 50% of terminal shoots lost 59% of their fruit during the period between 22 May and 5 June compared to trees with summer flushes of 7% of terminal shoots, which lost 43% of the fruits (Huang 2005). The effect of summer flushes on fruit retention usually occurs only within individual twigs or shoots. Hieke et al. (2002), who are working with "Tai So" and several other cultivars, found that pruning of one side of a tree to induce summer flushed did not affect fruit growth on the unpruned side.

Recently microarray, next-generation sequencing technology, and global transcriptome analyses have been widely used to investigate the molecular regulatory networks on fruit abscission. Carbohydrate stress by girdling plus defoliation resulted in 100% fruit drop of litchi and increased the transcript level of two IAA-responsive genes (*LcAUX/IAA1* and *LcSAUR1*), one cell wall degrading enzyme gene (*LcPG1*), and one ethylene biosynthetic gene (*Lc-ACO1*), in contrast to the decreasing accumulation of auxin response factor (*LcARF1*) mRNA (Kuang et al. 2012; Peng et al. 2013). Differentially expressed candidate genes involved in fruit abscission induced either by carbohydrate stress (2771 unigenes) or ethephon (2730 unigenes) in litchi were identified by Li et al. (2015a, b).

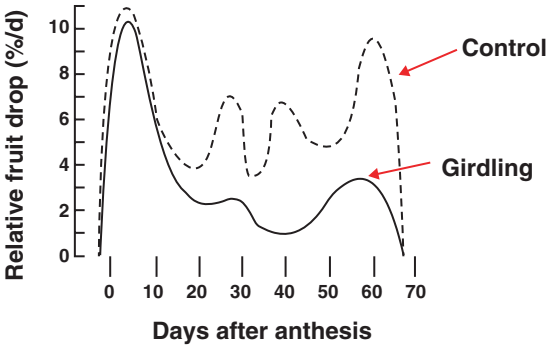

Fig. 1.4 The effects of girdling on the fruit drop of "Nuomici" (From Huang 2002)

1.4 Fruit Size

Genetic factors contribute the most toward fruit size. Fruit weight of a litchi fruit may vary from less than 10 g for some cultivars like "Hexiachuan" to over 50 g in cv. "Erdanli" (Wu 1998). Huang and Xu (1983) proposed a "ball skin versus bladder effect" to conceptualize the restraints exerted by a preformed fruit skin to the expanding aril. The weight of the aril and the whole fruit were found to correlate with the pericarp weight, irrespective of the fruit having normal or aborted seed (Huang and Qiu 1987). These findings implied that a large pericarp is a prerequisite for a large fruit in litchi.

Li et al. (2010) published an overview of factors related to fruit size in litchi. His research group carried out a serial studies about litchi fruit size. According to Li et al. (2003a), litchi fruit growth could be reasonably divided into two stages (Stage I and Stage II). Stage I, which constitutes about two thirds of the whole fruit growth period (0–53 DAA), mainly involved pericarp and seed coat growth. Stage II mainly involved embryo and aril growth (53–88 DAA). Using large-fruited "Erdanli" and small-fruited "Huaizhi" litchis as materials for comparison, Li et al. (2002) found that the cell number in the pericarp of "Erdanli" was significantly greater than that of "Huaizhi" with no significant difference in cell size between them, suggesting that difference in fruit size is primarily a result of difference in cell number rather than cell size. Xia et al. (2012) confirmed this pattern using large-fruited "Siliangguo" and small-fruited "Chenzi" litchis as materials. Cell division in the pericarp of litchi was found to occur mainly during the periods prior to and after anthesis. Thus it was assumed that factors affecting cell division before and after anthesis could impact on the final fruit size.

Environmental factors during the period of fruit development might play important roles in fruit size. A study comparing the fruit from the early bloom and from the late bloom on the same panicle in "Feizixiao" showed that the fruit size from the early bloom was over 1.5 times larger than that from the late bloom (Li et al. 2003b). The total growth degree days (GDD) for fruit development were almost the same between the two types of fruit, but fruits from early bloom usually have longer Stage I for relative lower fruit development temperature as compared with late bloom fruits. Longer length of Stage I, a phase mainly involved in pericarp growth, resulted

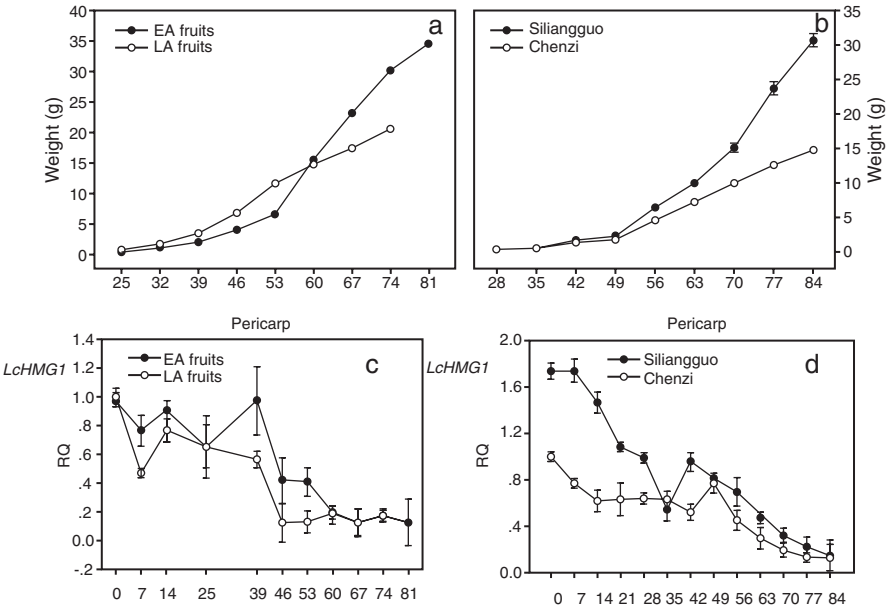

Fig. 1.5 Fruit development of early bloom and late bloom "Feizixiao" fruits (**a**) and large and small fruits of different genotypes (**b**) and expression analysis of *LcHMG1* (c and d) during fruit development in the pericarp of different types (From Xia et al. 2012)

in larger weights of pericarp and harvested fruit. *LcHMG*, a gene encoding an enzyme catalyzing the first committed step in the mevalonic acid pathway for the biosynthesis of isoprenoids, was suggested to be involved in early cell division and fruit size determination in litchi (Fig. 1.5, Xia et al. 2012).

Water stress during rapid fruit growth decreases the size of harvested fruit in most fruits. Nonirrigated trees during fruit growth produced 10% smaller fruit than regularly irrigated trees (Batten et al. 1994; Menzel et al. 1995). Li and Huang (1994) compared the fruit growth from trees of "wet" treatment to those from "drought" treatment. The lower fresh weight percentage of pericarp during Stage I of fruit growth and the higher fresh weight percentage of the aril during Stage II of the fruit growth were shown in "drought" treatment compared with "wet" treatment. It implies that water stress during the whole fruit development has more influence on the pericarp development than that on the aril growth.

Phytohormones are considered to be involved in most phases of fruit growth. A high ZRs/ABA ratio favors fruit growth in litchi (Li et al. 2005; Li and Zhou 2015). "Erdanli" had higher concentration of zeatin ribosides (ZRs) than "Huaizhi" at three out of six sampling periods during fruit development and lower concentrations of abscisic acid (ABA) from 40 DAA. In "Feizixiao," the fruit from early bloom was found to have a higher concentration of ZRs than the fruit from late bloom in all of the sampling dates and lower concentrations of ABA on three out of five sampling dates. A synthetic auxin, 3,5,6-TPA, applied when the fruit attained a size of about

2 g to increase the fruit set and fruit weight in litchi production (Stern et al. 2000; Goncalves et al. 2014).

Girdling or bark ring incision was commonly used to enhance flowering and fruit set in litchi production. However, the negative effect of girdling on fruit growth should not be ignored. Hieke et al. (2002) pointed out that girdling on small branches reduced the average fruit weight by 11.7% (21.8 g vs. 24.7 g), and girdling done on big branches had no effect on fruit size (18.5 g vs. 18.3 g). Li et al. (2004) demonstrated that the continuous two rings of bark incisions made on a fruiting shoot every 3 weeks from 30 days after anthesis significantly decreased the fruit size of "Nuomici" litchi by 15.2% in 2000 and by 23.3% in 2001.

1.5 Aril Sugar Accumulation

1.5.1 Sugar Compositions

Sucrose, glucose, and fructose have been identified in litchi fruit in different ratios between different litchi cultivars (Paull et al. 1984; Wang et al. 2006). Both the amount and types of sugars in fresh fruit directly influence its quality and flavor. Investigations were conducted into sugar contents and compositions in the arils of 42 litchi cultivars (Fig. 1.6) (Yang et al. 2013). In consistent with the previous reports (Wang et al. 2006), a significant difference in sugar contents and hexose/

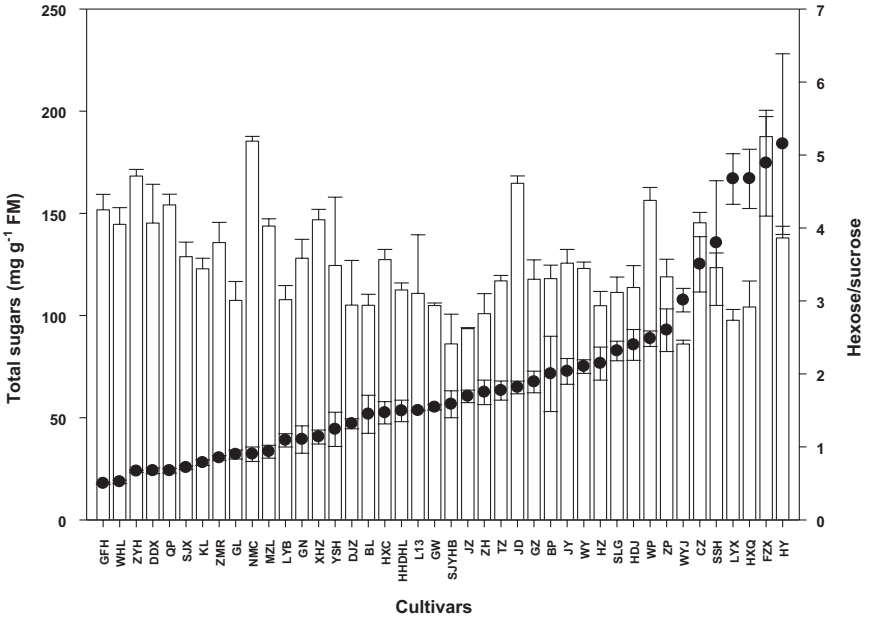

Fig. 1.6 Total soluble sugars and hexose/sucrose ratios in the arils of 42 cultivars at maturity. The *vertical bars* represent the standard error of three replicates (From Yang et al. 2013)

sucrose ratio were observed. Base on hexose/sucrose ratio, litchi cultivars were grouped into three types: the sucrose-prevalent type (hexose/sucrose < 1), the intermediate type (1 < hexose/sucrose < 2), and the hexose-prevalent type (hexose/sucrose > 2). According to Yang et al. (2013), the sugar composition in the litchi aril depends mainly on the sucrose cleavage enzymes acid invertase (AI) and sucrose synthase (SS) rather than on the sucrose synthetic enzyme sucrose phosphate synthase (SPS). The activities and expression levels of soluble acid invertase (SAI) and SS displayed highly significant positive correlations with hexose/sucrose ratios among the 15 cultivars tested. (Fig. 1.7, Yang et al. 2013).

1.5.2 Post-phloem Transport Pathways in Parenchyma Cells of Litchi Fruit

It is well established that phloem unloading and metabolism of imported sugars in sink cells play a key role in the partitioning of photo-assimilates and that post-phloem transport of sucrose into terminal sink organs can take symplastic and/or apoplastic routes depending on the type of organ and developmental state (Patrick 1997). In sinks which accumulate high concentrations of soluble sugars, the apoplasmic step is largely associated (Patrick 1997). The apoplastic route depends on carrier-mediated electrogenic transporters. Unlike other fruit species, the aril of litchi is an organ without vascular tissue with the seed stalk or funicle serves as the connection between the vascular pedicel and the aril (Fig. 1.8).

Wang et al. (2015) investigated the post-phloem unloading pathway in the aril of litchi. In litchi fruit, phloem transport ended in the funicle, and the spongy parenchyma funicle cells were the first receiver cells of the photo-assimilates (Fig.1.8). An assay of carboxyfluorescein (CF) dye infiltration demonstrated symplastic connection between vascular tissue and funicle parenchyma cells. And furthermore, the frequency of plasmodesmata was counted in funicle parenchyma cells (Fig. 1.9). These data indicated that a symplasmic post-phloem transportation operated in the funicle of litchi. However, the aril of litchi fruit is apparently symplasmically separated from the funicle reflecting by the dye of CF confined to the parenchyma cells of funicle tissue connecting the aril.

1.5.3 The Mechanism of Sugar Accumulation

The amount of sugars in fresh fruit is one of the most important quality traits. The aril of litchi accumulates 15 to 20% sugars of the fresh mass, and the total amount of sugars accumulated varies among cultivars (Wang et al. 2006; Yang et al. 2013). In studies of fruit species including citrus (Komatsu et al. 1999, 2002), peach (Vizzotto et al. 1996), tomato (Ngugen-Quoc and Foyer 2001), and banana (Choudhury et al. 2009), sucrose metabolism enzymes mainly invertase (EC 3.2.1.26), SS (EC 2.4.1.13), and SPS (EC 2.4.1.14) have been investigated in relation to sugar accumulation. Although significant activities of invertase, SS, and SPS

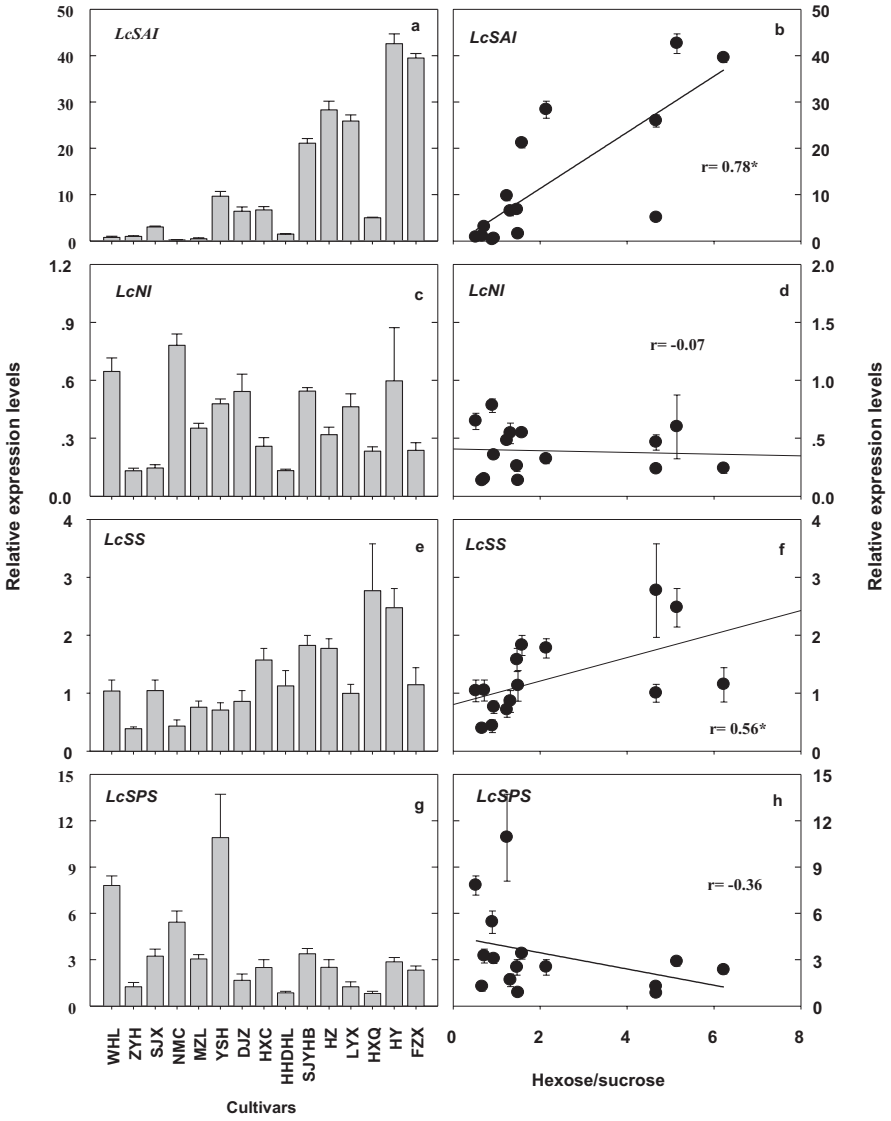

Fig. 1.7 Activities of CWAI, SAI, NI, SS, and SPS (**a**, **c**, **e**, **g**, and **i**) and their correlations with the hexose/sucrose ratio (**b**, **d**, **f**, **h**, and **j**) in the mature arils of 15 cultivars. The *vertical bars* represent the standard error of three replicates. Correlation coefficient r with "*" indicates significant correlation at $P < 0.05$ (From Yang et al. 2013)

were detected and distinct gene transcriptions of these enzymes were observed in litchi aril, sugar accumulation was inconsistent with either the activity or expression patterns of sucrose metabolism enzymes (Yang et al. 2013). These results suggest that these sucrose metabolism enzymes are not necessarily related to sugar accumulation.

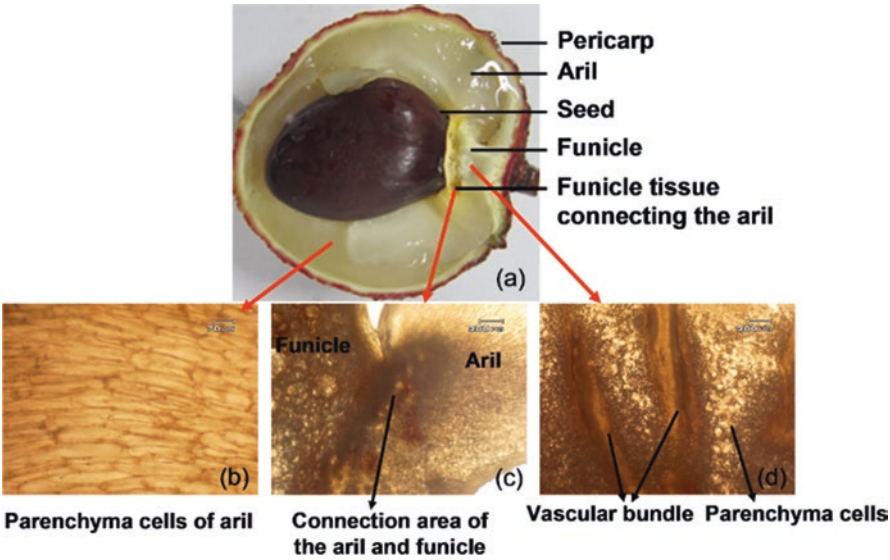

Fig. 1.8 Structure of litchi fruit. (**a**) Longitudinal section of a mature litchi fruit, showing various fruit structures. (**b**) A longitudinal section of the aril with no vascular bundle. (**c**) A longitudinal section of the funicle adjoining the aril with no vascular bundle. (**d**) A longitudinal section of the funicle consisting of the vascular bundles and spongy tissues (From Wang et al. 2015)

Figure 1.10 demonstrates the sugar accumulation strategy summarized base on the data of Yang et al. (2014) and Wang et al. (2015). In the funicle, sugars mainly sucrose unload from phloem via plasmodesmata. The osmotic pressure was kept low due to the conversion of soluble sugars into less osmotically active polymorphic forms such as starch and pentasaccharide. This facilitated the symplastic solution flow from the phloem by increasing the sugar concentration difference. Sucrose cleavages into hexoses by SS and/or invertase facilitate its unloading and utilization (Ruan et al. 2010). Hexose-prevalent cultivars, such as Feizixiao and Heiye, displayed significantly higher activities of cell wall acid invertase and soluble acid invertase (SAI), which result in a higher starch and soluble sugars than those of sucrose-prevalent cultivars Nuomici and Wuheli. However, the higher sugar levels in the funicle do not necessary mean higher sugar levels in the aril. Cultivar Nuomici accumulated higher sugar levels in the aril but lower sugar levels in the funicle as compared with cultivar Heiye. As mention earlier, the aril of litchi is apparently symplasmically separated from the funicle. In addition, much higher concentrations of sugars were measured in the aril than in the funicle.

The abovementioned results provided evidences for an apoplasmic post-phloem transportation in the aril of litchi. Thus, energy-driven transporters and energy metabolism might play crucial roles in determining sugar accumulation in the aril of litchi. Both ATPase inhibitor (EB, eosin B) and sucrose transporter inhibitor (PCMBS, *p*-chloromercuribenzene sulfonate) inhibited sugar uptake into the aril (Fig. 1.11). And furthermore, Wang et al. (2015) found that the sugar accumulation in the aril of litchi was highly correlated with the expression of a putative aril

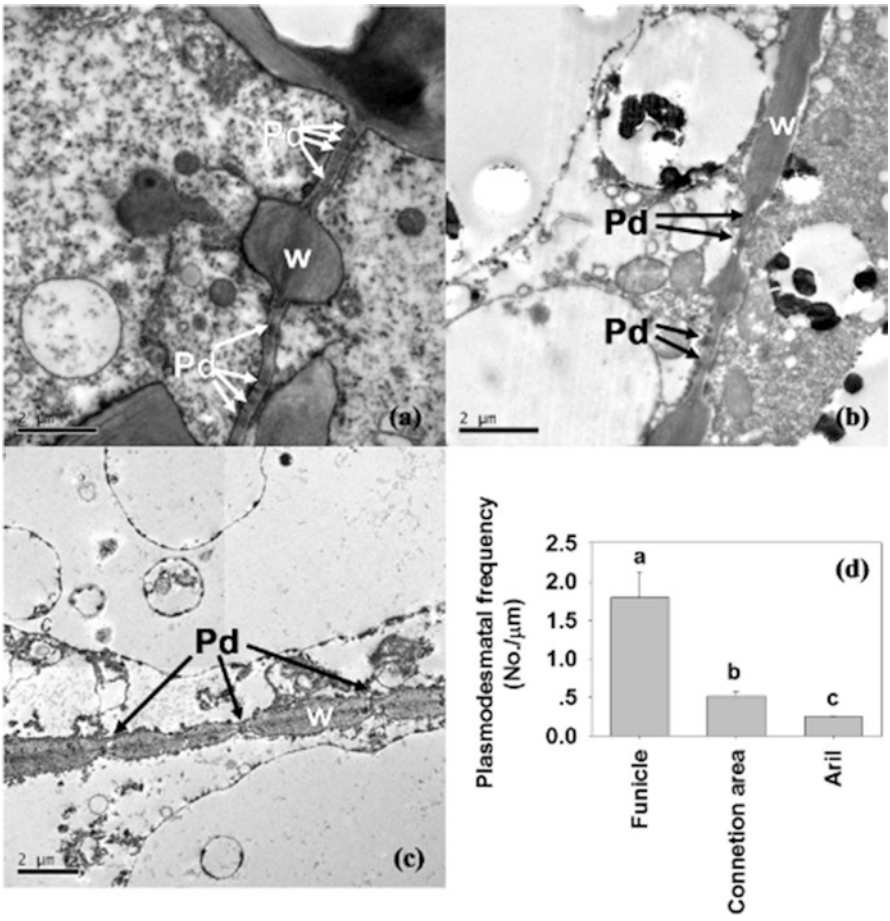

Fig. 1.9 The ultrastructure of the funicle (**a**), the funicle tissue adjoining the aril (**b**) and the aril (**c**), and the plasmodesmatal frequencies between parenchyma cells of these tissues in cultivar Feizixiao. The *vertical bars* represent the standard error of five replicates (From Wang et al. 2015)

vacuolar membrane sucrose transporter gene (*LcSUT4*). Taking together, these data suggest that apoplasmic transport is critical for sugar accumulation in litchi aril and that *LcSUT4* is involved in this step.

1.6 Pericarp Pigmentation

1.6.1 Fruit Color

Pericarp pigmentation is the result of chlorophyll degradation and anthocyanin accumulation coinciding with the onset of litchi maturation. Anthocyanins and chlorophylls are present mainly in the outer cell layers of the pericarp, particularly

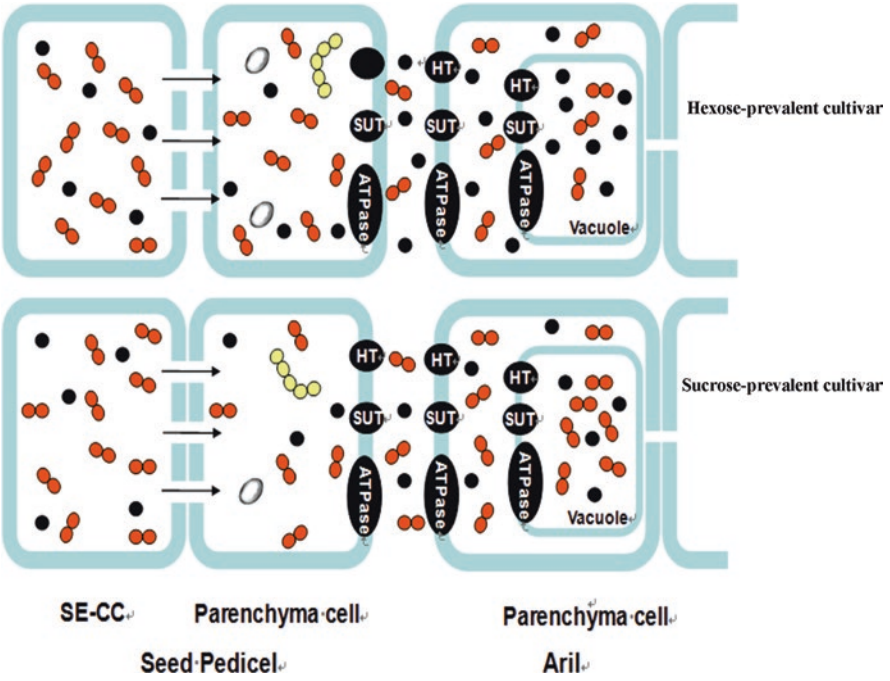

Fig. 1.10 Sugar accumulation strategy in the aril of hexose-prevalent and sucrose-prevalent cultivars. ● represents hexose, ●● represents sucrose, ●●●●● represents pentasaccharide, ⬭ represents starch, HT represents hexose transporter, and SUT represents sucrose transporter

between the protuberances (Underhill and Critchley 1994). With increasing anthocyanin and decreasing chlorophyll levels, anthocyanin-accumulating fruit often displays a range of intermediary colors from green to red, then blue, and, finally, purple to black. Litchis were basically divided into three fruit coloration types according to the color appearance and concentrations and distribution of anthocyanins and chlorophylls: (a) the non-red ones that accumulate no or extremely low anthocyanins, such as "Quixingqingpitian" and "Xingqiumili"; (b) the unevenly red cultivars such as "Feizixiao" and "Sanyuehong," which accumulate some anthocyanins while retaining relatively high levels of chlorophylls; and (c) the evenly red cultivars that accumulate significant amounts of anthocyanins with decreased chlorophylls such as "Meiguili" and "Baila," which display a serial color progressing from pink to dark red (Fig. 1.12, Wei et al. 2011).

1.6.2 Anthocyanin Biosynthesis

The expressions of anthocyanins result in red pigmentation on the pericarp of litchi fruit (Lee and Wicker 1991; Zhang et al. 2004; Wei et al. 2011). Red pigments start to accumulate in litchi fruit pericarp at very late developmental stage about 62 days

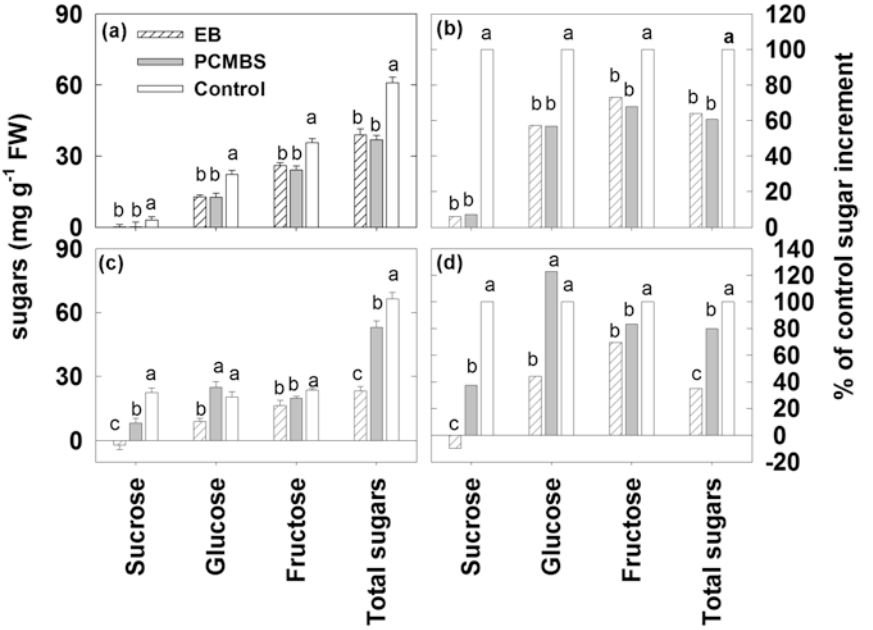

Fig. 1.11 Effects of EB and PCMBS on the sugar accumulation in the aril of cultivar Feizixiao and Nuomici. (**a**) Sugar increment in the aril of FZX. (**b**) Sugar increment in the aril of NMC. (**c**) % control sugar increments in the aril of FZX. (**d**) % control sugar increments in the aril of NMC. The *vertical bars* represent the standard error of eight replicates (From Wang et al. 2015)

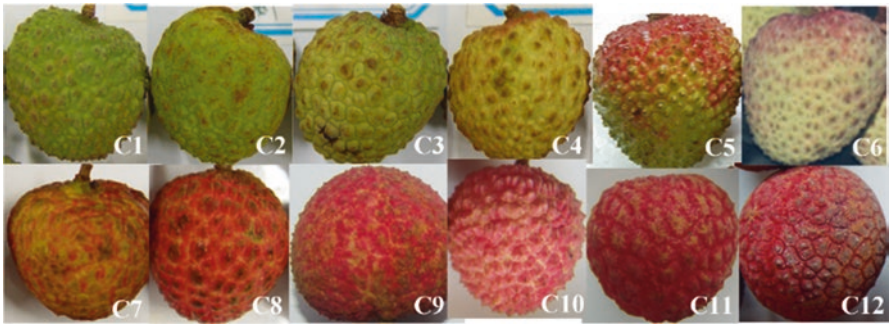

Fig. 1.12 Appearance of 12 litchi cultivars. C1, "Kuixingqingpitian"; C2, "Xinqiumili"; C3, "Yamulong"; C4, "Yongxing No. 2"; C5, "Feizixiao"; C6, "Sanyuehong"; C7, "Meiguili"; C8, "Baila"; C9, "Baitangying"; C10, "Guiwei"; C11, "Nuomici"; C12, "Guinuo" (From Wei et al. 2011)

after anthesis, and the fruit will be fully red and ripen in another 10 days (Lai et al. 2014). Cyanidin-3-glucoside and cyanidin-3-rutinoside were the major anthocyanins in the red pericarp of litchi (Lee and Wicker 1991; Wei et al. 2011; Li et al. 2016a). Anthocyanin contents in the pericarp of litchi were variable among cultivars and subjected to the influence of agronomic factors and fruit maturity, while anthocyanin profile was primarily determined by genetic factors (Li et al. 2016a, b).