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

The Lychee Biotechnology

Springer

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Editors
Manoj Kumar
Amity Institute of Microbial Technology
Amity University
Noida, Uttar Pradesh, India

Vivek Kumar
Amity Institute of Microbial Technology
Amity University
Noida, Uttar Pradesh, India

Ram Prasad
Amity Institute of Microbial Technology
Amity University
Noida, Uttar Pradesh, India

Ajit Varma
Amity Institute of Microbial Technology
Amity University
Noida, Uttar Pradesh, India

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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.

Noida, Uttar Pradesh, India

Manoj Kumar
Vivek Kumar
Ram Prasad
Ajit Varma

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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

H.-C. Wang (✉) • B. Lai
Guangdong Litchi Engineering Research Center, College of Horticulture, South China Agricultural University, Guangzhou 510642, People's Republic of China

Department of Life Sciences and Technology, Yangtze Normal University, Fuling 408100, People's Republic of China
e-mail: wanghc1972@263.net

X.-M. Huang
Guangdong Litchi Engineering Research Center, College of Horticulture, South China Agricultural University, Guangzhou 510642, People's Republic of China

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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).

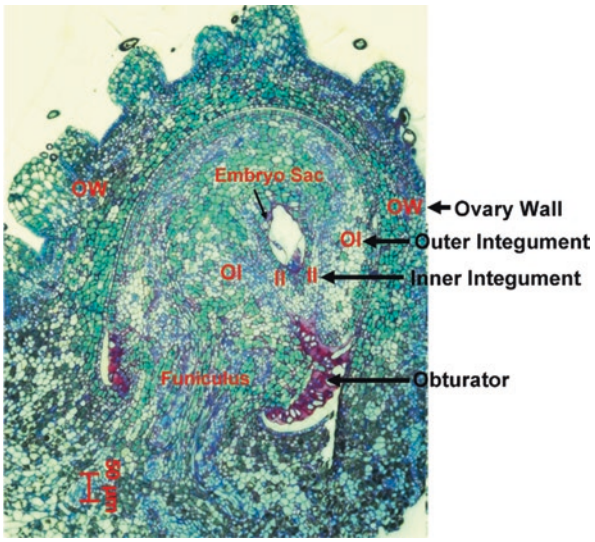

Fig. 1.1 Longitudinal section of a 3-day-old "Huaizhi" female flower. *OW* ovary wall, *OI* outer integument, *II* inner integument

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

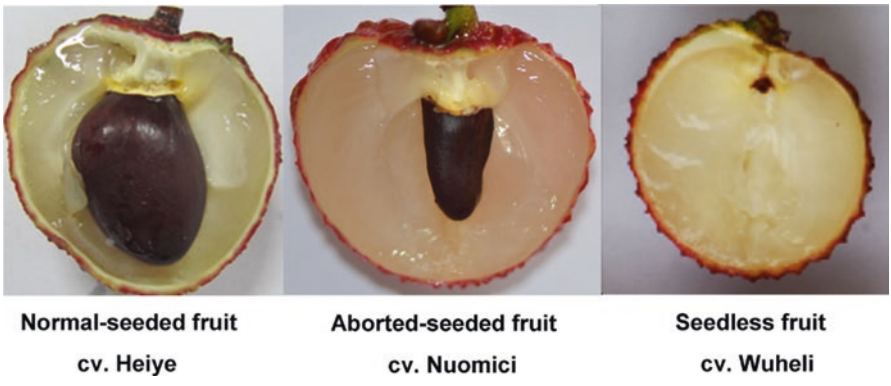

Fig. 1.2 Three different types of litchi fruit (The picture of cultivar Heiye cited from Wang et al. 2015)

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).

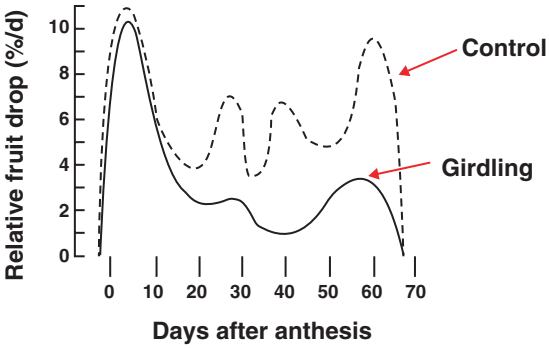

Fig. 1.4 The effects of girdling on the fruit drop of "Nuomici" (From Huang 2002)

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

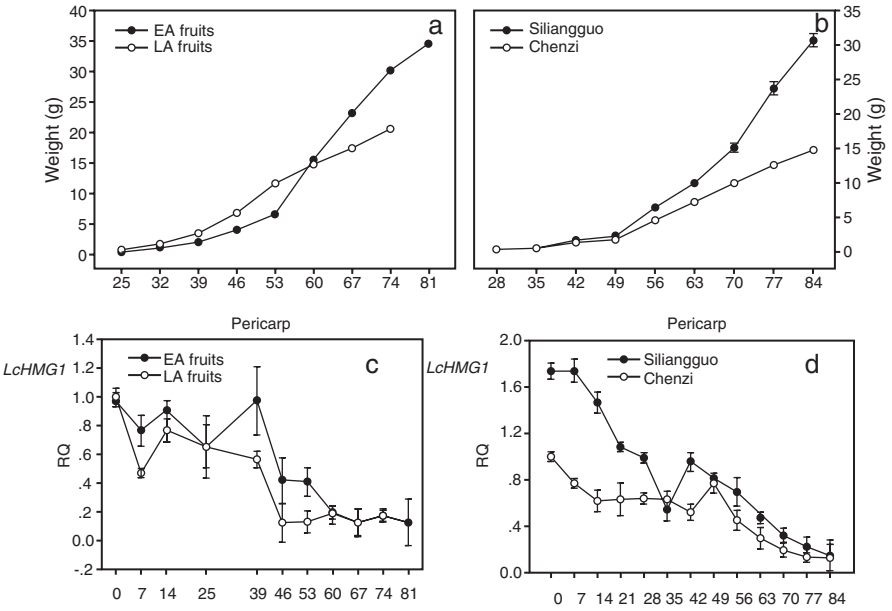

Fig. 1.5 Fruit development of early bloom and late bloom "Feizixiao" fruits (**a**) and large and small fruits of different genotypes (**b**) and expression analysis of *LcHMG1* (c and d) during fruit development in the pericarp of different types (From Xia et al. 2012)

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/

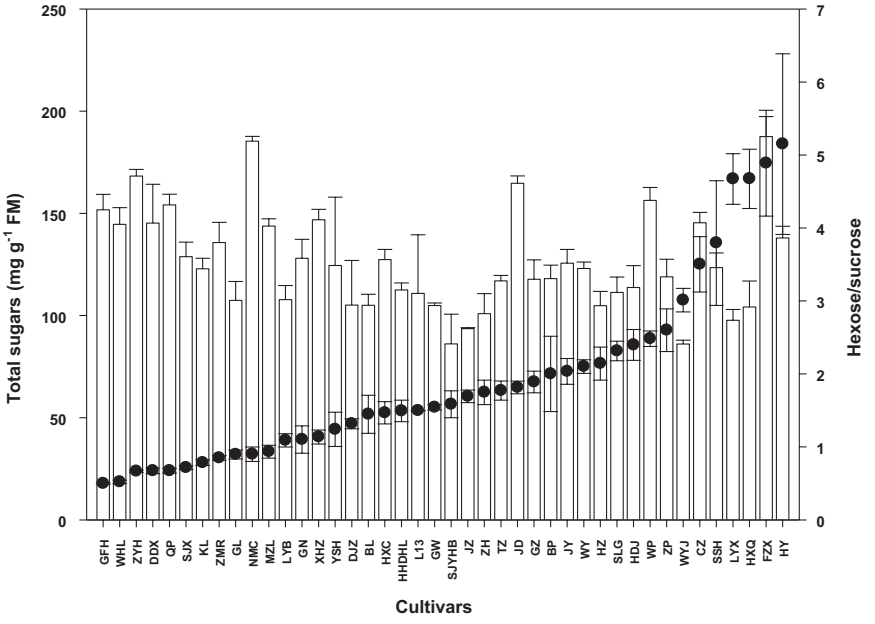

Fig. 1.6 Total soluble sugars and hexose/sucrose ratios in the arils of 42 cultivars at maturity. The *vertical bars* represent the standard error of three replicates (From Yang et al. 2013)

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

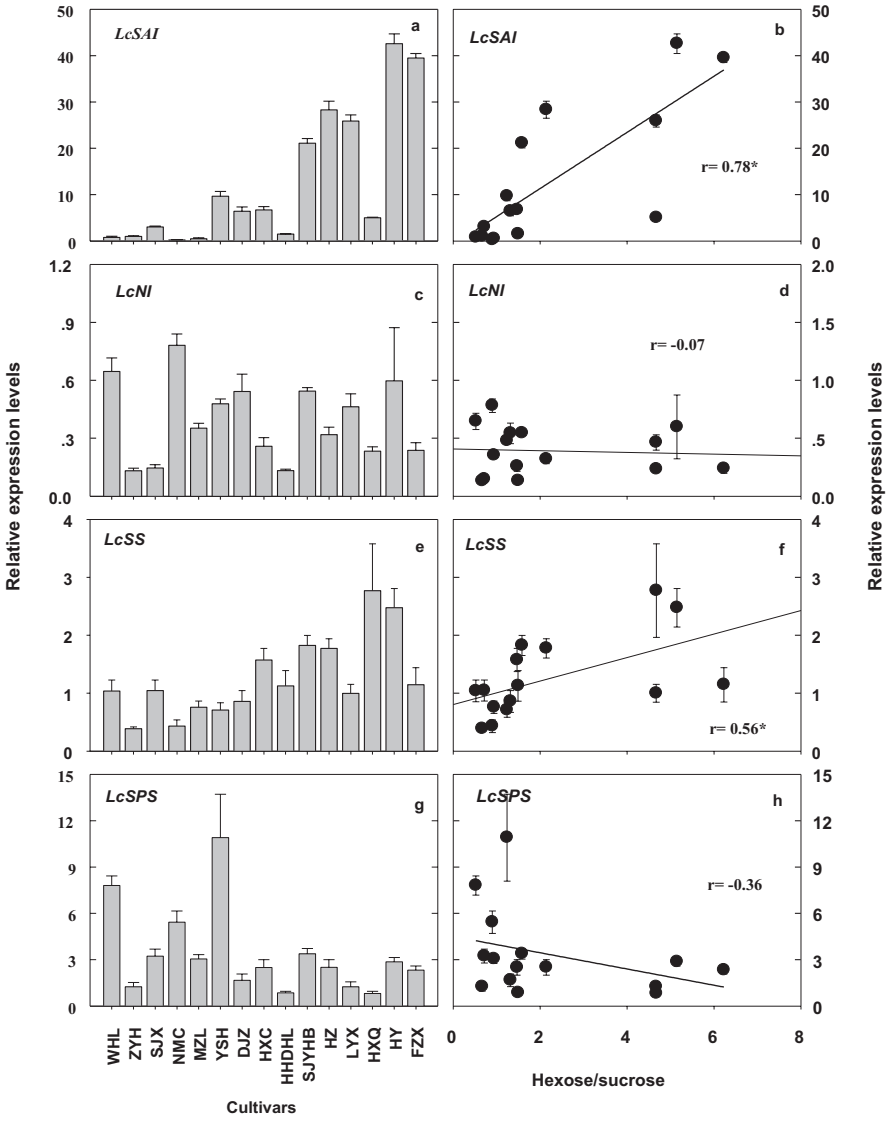

Fig. 1.7 Activities of CWAI, SAI, NI, SS, and SPS (**a**, **c**, **e**, **g**, and **i**) and their correlations with the hexose/sucrose ratio (**b**, **d**, **f**, **h**, and **j**) in the mature arils of 15 cultivars. The *vertical bars* represent the standard error of three replicates. Correlation 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.

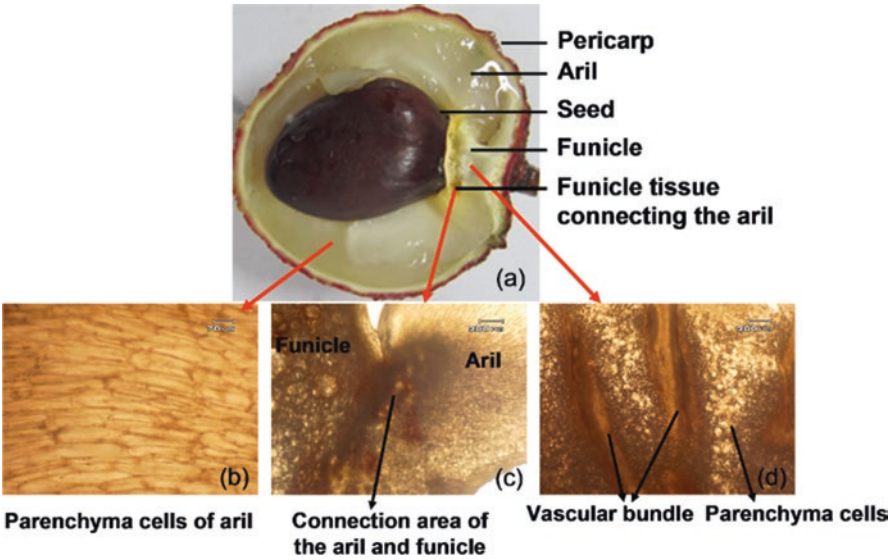

Fig. 1.8 Structure of litchi fruit. (**a**) Longitudinal section of a mature litchi fruit, showing various fruit structures. (**b**) A longitudinal section of the aril with no vascular bundle. (**c**) A longitudinal section of the funicle adjoining the aril with no vascular bundle. (**d**) A longitudinal 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

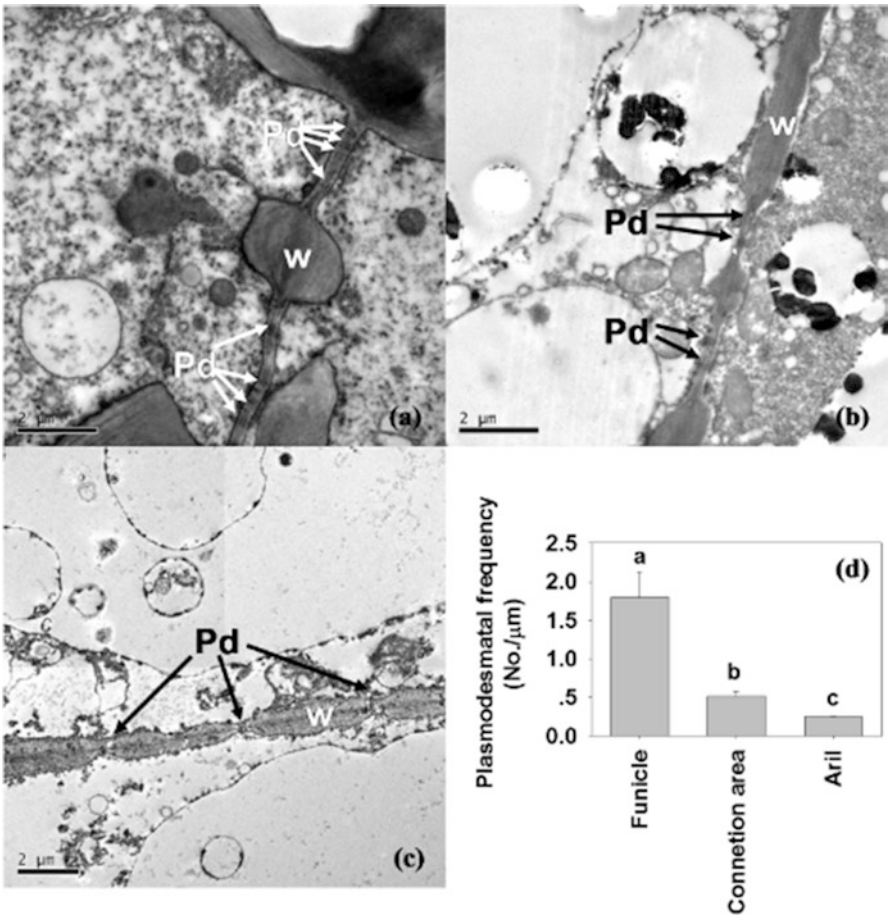

Fig. 1.9 The ultrastructure of the funicle (**a**), the funicle tissue adjoining the aril (**b**) and the aril (**c**), and the plasmodesmatal frequencies between parenchyma cells of these tissues in cultivar Feizixiao. The *vertical bars* represent the standard error of five replicates (From Wang et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

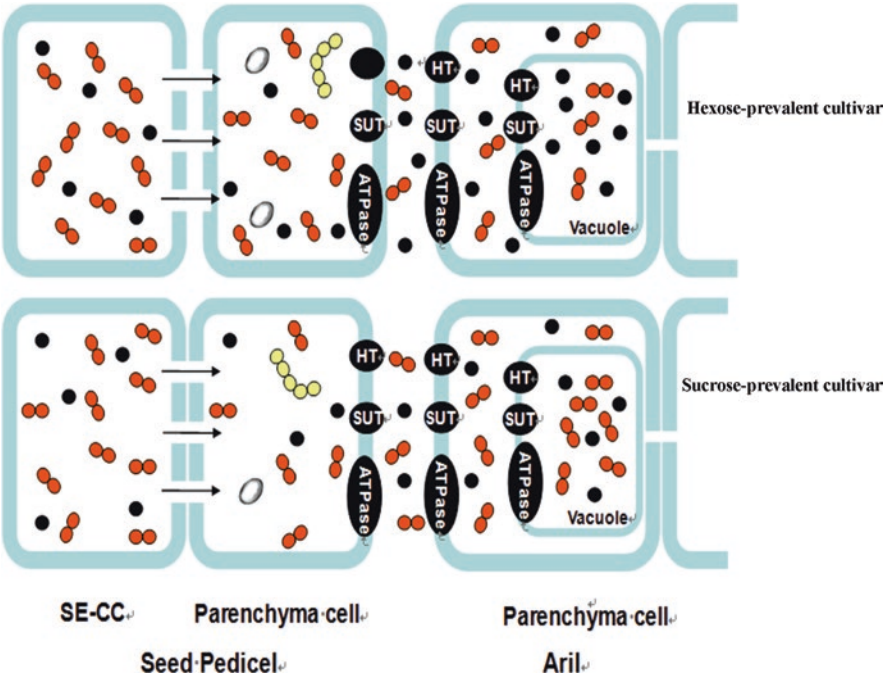

Fig. 1.10 Sugar accumulation strategy in the aril of hexose-prevalent and sucrose-prevalent cultivars. ● represents hexose, ●● represents sucrose, ●●●●● represents pentasaccharide, ⬭ represents starch, HT represents hexose transporter, and SUT represents sucrose transporter

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

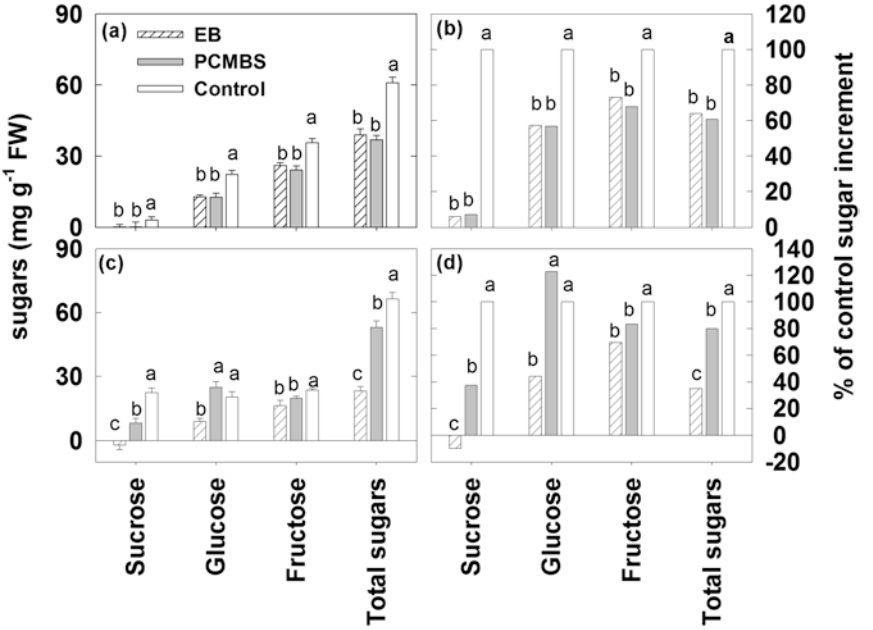

Fig. 1.11 Effects of EB and PCMBS on the sugar accumulation in the aril of cultivar Feizixiao and Nuomici. (**a**) Sugar increment in the aril of FZX. (**b**) Sugar increment in the aril of NMC. (**c**) % control sugar increments in the aril of FZX. (**d**) % control sugar increments in the aril of NMC.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

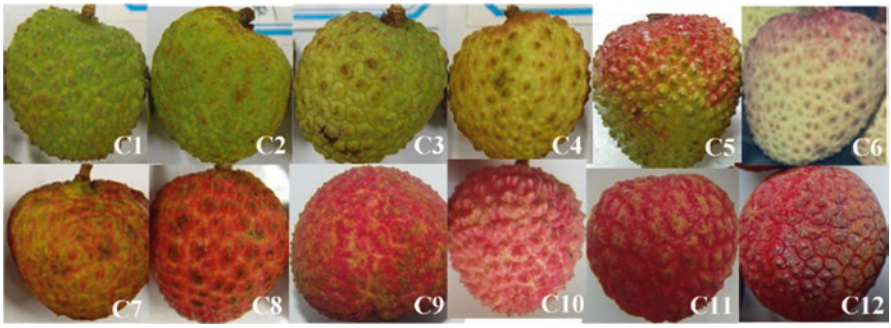

Fig. 1.12 Appearance of 12 litchi cultivars. C1, "Kuixingqingpitian"; C2, "Xinqiumili"; C3, "Yamulong"; C4, "Yongxing No. 2"; C5, "Feizixiao"; C6, "Sanyuehong"; C7, "Meiguili"; C8, "Baila"; C9, "Baitangying"; C10, "Guiwei"; C11, "Nuomici"; C12, "Guinuo" (From Wei et al. 2011)

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).

Anthocyanin biosynthesis is a well-studied plant secondary metabolism pathway. A series of structural genes coding for enzymes including chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase (F3′H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) involved in the anthocyanin biosynthetic pathway of litchi were isolated (Wei et al. 2011). Among of these structural genes, only the expression of *LcDFR* and *LcUFGT* were consistent with the degree of anthocyanin concentration in different color genotypes of the 12 litchi cultivars mentioned above (Wei et al. 2011). UFGT, an enzyme stabilizes anthocyanidin through attaching sugar moieties to the anthocyanin aglycone, is a rate-limiting gene involved in anthocyanin biosynthesis. In the pericarp of 15 cultivars with different color phenotypes, their UFGT activities were found strong positive correlate with anthocyanin accumulation capacity (Li et al. 2015b). The UFGT present in the pericarp of litchi is specific for UDP-glucose (Fig. 1.13). *LcUFGT1* involved in the formation of cyanidin glucoside, the major anthocyanin in the pericarp of litchi fruit (Zhao et al. 2012; Li et al. 2015b). In litchi, anthocyanins are synthesized in the cytosol and then sequestrated to vacuoles by LcGST4, an anthocyanin-related transporter (Hu et al. 2016). Recently, more structural gene's family members of anthocyanin biosynthesis pathway were identified by high-throughput sequencing and de novo assembling (Lai et al. 2015).

Many studies on various plant species demonstrate that anthocyanin biosynthesis is controlled at the transcriptional level. Three kinds of transcription factors including MYB, basic helix-loop-helix (bHLH), and WD40 form a regulatory complex to regulate the expression of anthocyanin biosynthesis pathway genes. One gene coding for R2R3-MYB transcription factor *LcMYB1* was isolated from litchi fruit pericarp (Lai et al. 2014). The phylogenetic analysis indicates that LcMYB1 is closely related to the subgroup 6 MYBs (anthocyanin regulator subgroup) in *Arabidopsis*. The expression of *LcMYB1* was highly correlated with anthocyanin concentration in different litchi tissues, developmental stages, and cultivars (Fig. 1.14). Overexpression of *LcMYB1* in tobacco resulted in pigmented leaf, pedicel, ovary, seed, filament, and highly pigmented petals. Late anthocyanin biosynthesis pathway genes (*NtDFR*, *NtANS*, and *NtUFGT*) and endogenous bHLH transcription factors (*NtAn1a* and *NtAn1b*) were activated by overexpressing of *LcMYB1* in tobacco (Lai et al. 2014). These results indicated that *LcMYB1* is the key transcription factor regulating anthocyanin biosynthesis in litchi fruit pericarp.

Three basic helix-loop-helix transcription factors (bHLHs) as candidate partners of LcMYB1 also isolated from litchi, LcbHLH1, LcbHLH2, and LcbHLH3 (Lai et al. 2016). LcbHLH1 and LcbHLH3 were hylogenetically clustered with bHLH proteins involved in anthocyanin biosynthesis in other plants and were found to localize in the nucleus and physically interact with LcMYB1. Unlike LcMYB1, the transcription levels of all these bHLHs were not coordinated with anthocyanin accumulation in different tissues and during development. However, when co-infiltrated with LcMYB1, both LcbHLH1 and LcbHLH3 enhanced anthocyanin accumulation in tobacco leaves.

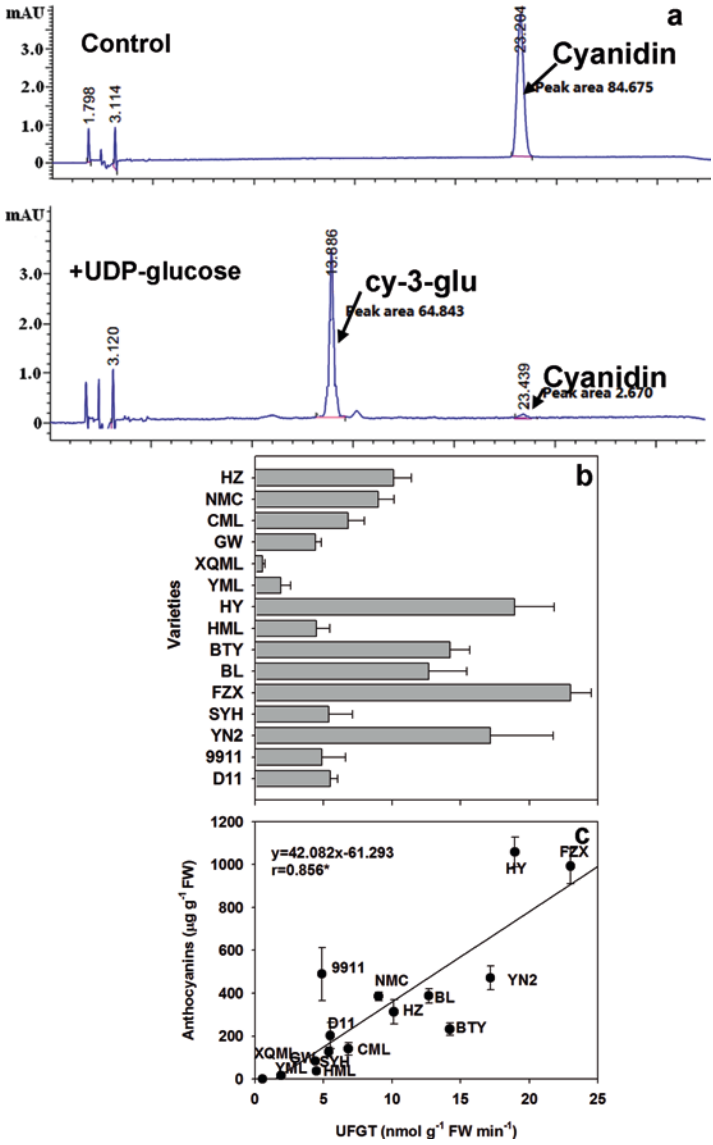

Fig. 1.13 Litchi UFGT activity analysis. (**a**) HPLC chromatogram of the following reactions at 520 nm: cyanidin + distilled water (control); cyanidin + UDP-glucose. (**b**) Activities of UFGT in the pericarp of 15 litchi cultivars and their correlation with pericarp anthocyanin contents. The *vertical bars* represent standard error of three biological replicates. Relative coefficient *r* with "*" indicates significant correlation at $p < 0.05$ (From Li et al. 2016b)

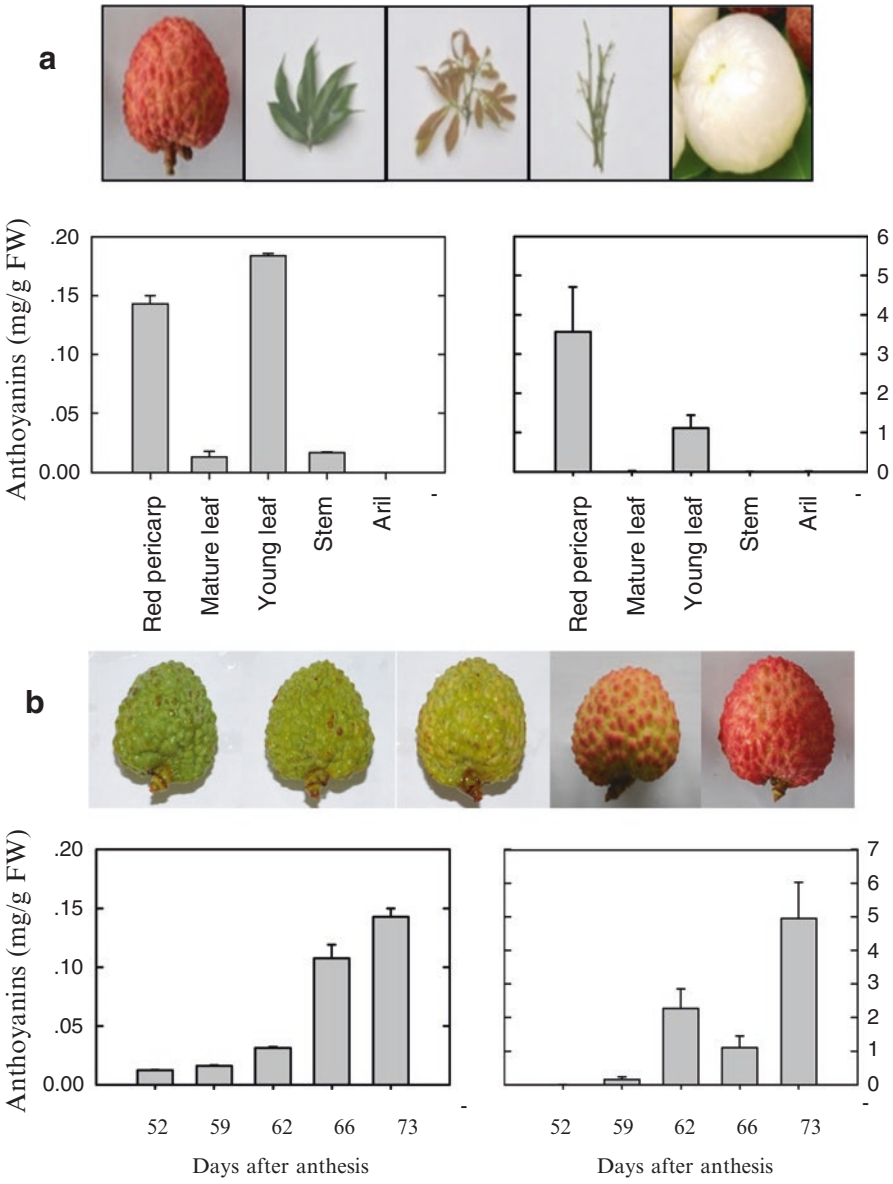

Fig. 1.14 Anthocyanin contents and *LcMYB1* expressions in different tissues (**a**) and developmental stages (**b**) (From Lai et al. 2014)

Light is one of the most important environmental factors regulating anthocyanin biosynthesis. Light exposure enhanced, while shading retarded the accumulation of anthocyanins in the pericarp of litchi (Wei et al. 2011; Zhang et al. 2016). A total of 16,622 light-responsive genes encode photoreceptors, light signal transduction elements, TFs, and structural genes involved in the anthocyanin biosynthetic pathway were detected by Zhang et al. (2016).

1.6.3 Chlorophyll Degradation

The degradation of chlorophylls is the prerequisite for the strong coloration of fruit crops. During litchi coloration, a visible degreening process associated with chlorophyll degradation was noticed (Wang et al. 2005; Lai et al. 2015). The degradation of chlorophylls was associated with the breakdown of chloroplast (Wang et al. 2002). Approximately 80% of the total chlorophyll content broke down during the pericarp degreening process of "Nuomici" (Lai et al. 2015). The concentrations of chlorophyll a and b in the pericarp of stay-green cultivar "Feizixiao" were significantly higher than those in degreening cultivar "Nuomici" during fruit maturation, while the chlorophyll derivatives in the former were obviously lower than in the latter. These results suggested that chlorophyllase was the key factor in the regulation of the chlorophyll loss in the pericarp (Wang et al. 2005). Recently, putative chlorophyll degradation relative genes, including chlorophyll catabolic enzyme genes and a chloroplast localized protein, stay green (SGR), were cloned from the pericarp of litchi. Their expression patterns and heterogenous expression assays suggested SGR is a crucial gene in the loss of chlorophyll in the litchi pericarp during maturation (Lai et al. 2015).

1.7 Mechanism of Fruit Maturation

Fruit maturation occurs during the later part of fruit development, along with the proliferation and enlargement of the aril cells. Akamine and Goo (1973) classified the fruit as non-climacteric, based on observations that ripening in "Tai So" was not accompanied by increased respiration and ethylene production. And this was confirmed by Jiang et al. (1986) by monitoring the release of CO_2 and ethylene and the absorption of O_2 by "Huaizhi" and "Nuomici." Wang et al. (2007) found that ethylene production declined from high to low values and recorded a small peak when the pericarp of "Nuomici" turned red at around 3 weeks before harvest, whereas ethylene was low and no peak was observed in "Feizixiao" (Fig.1.15).

Although litchi has been classified as a non-climacteric fruit and its ripening is thought to be ethylene independent, a transient increase of endogenous ethylene production was observed just around the degreening of "Nuomici" (Fig. 1.15). The activities of ACO and ethylene production in the pericarp were ten times higher than those in the aril (Jiang et al. 1986; Wang et al. 2007). Thus, the pericarp could undoubtedly be considered as the main tissue that produced ethylene. Ethrel

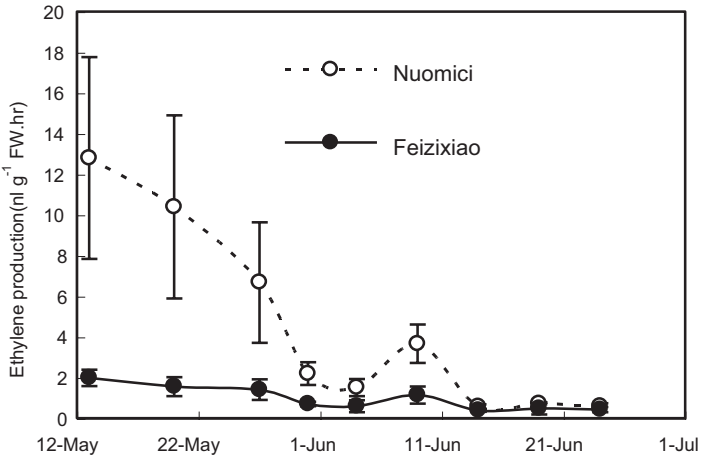

Fig. 1.15 Ethylene production of cvs. Nuomici and Feizixiao during fruit development and maturation. *Vertical bars* represent SE ($n = 3$) (From Wang et al. 2007)

treatment 3 weeks before harvest accelerated the fruit maturation in cv. Shashi (Sharma et al. 1986). Ethrel at 800 mg l^{-1} accelerated chlorophyll degradation and anthocyanin synthesis in the pericarp of "Feizixiao" (Wang et al. 2007). Ethylene action inhibitor, silver thiosulfate (STS), delayed the ripening of litchi (Yin et al. 2001). Although AOA infiltration significantly reduced the concentration of ACC in the aril, sugar accumulation in the aril was unaltered (Wang et al. 2007). Based on these data, we concluded that litchi fruit maturation may not be considered strictly independent of ethylene and that ethylene itself must have its subtle role in the litchi fruit maturation, especially in the chlorophyll degradation.

In non-climacteric fruits, solid evidences indicate that ABA is involved in the fruit maturation. In the arils and pericarp of both litchi cultivars "Nuomici" and "Feizixiao," endogenous ABA concentrations increased in parallel with aril sugar accumulation and pericarp pigmentation (Fig. 1.16). Exogenous application of ABA significantly enhanced sugar accumulation in the aril and anthocyanin synthesis in the pericarp and 6-benzyl aminopurine (6-BA) retarded sugar accumulation and pigmentation probably by decrease endogenous ABA (Table 1.3). These results suggest that ABA plays a more important role than ethylene in regulating the maturation of litchi fruit.

It is generally believed that low levels of endogenous cytokinins (CTKs) and gibberellins (GAs) plus a high level of ABA is the hormonal balance required for the onset of fruit maturation. Changes in endogenous auxin, CTKs, and GAs in litchi fruit during development thus far are inconsistent among researches (Qiu et al. 1998; Zhou et al. 1998; Li et al. 2005). Exogenous auxins (naphthalene acetic acid, NAA, and 3,5,6-trichloro-2-pysidyl-oxyacetic acid, 3,5,6-TPA) promote fruit coloration in addition to increase fruit set and size (Li et al. 2005; Stern et al. 2001). Tomer et al. (2001) report the GA_3 spray had no significant effect on ripening, but Dhua et al. (2005) reported GA_3 treatment increased fruit size and delayed color

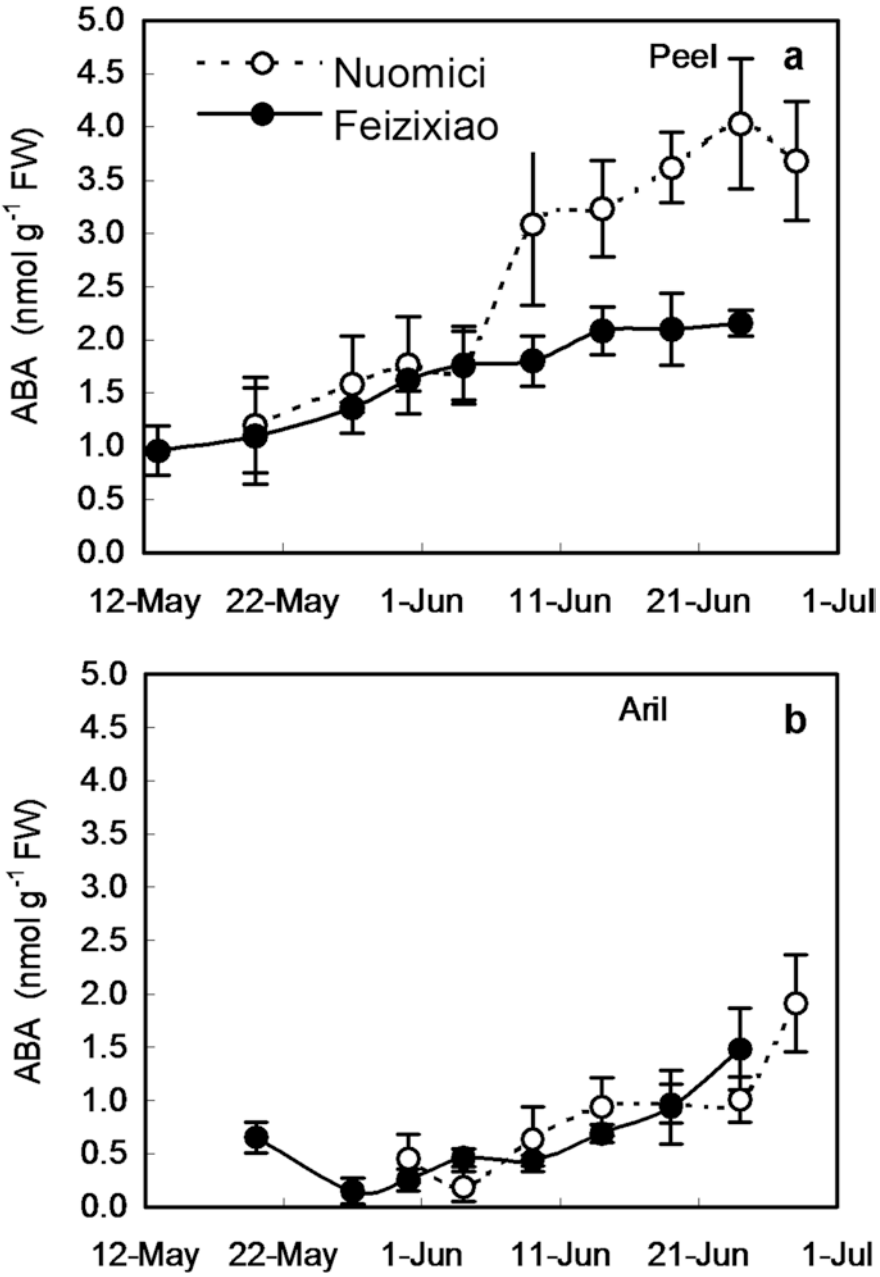

Fig. 1.16 Concentration of abscisic acid (ABA) in the peel (**a**) and aril (**b**) during fruit development in litchi cvs. Nuomici and Feizixiao. *Vertical bars* represent SE (n = 5) (From Wang et al. 2007)

Table 1.3 Effects of growth regulator transfusion treatments on pericarp chlorophyll and anthocyanin concentrations and aril sugar accumulation in "Feizixiao" litchi fruit (From Wang et al. 2007)

Treatments	Chlorophyll concentrations (mg g^{-1} FW)	Anthocyanin concentrations (U g^{-1} FW)	Total sugars (mg g^{-1} FW)
Control	0.080b	42.9a	115.3b
6-BA (400 mg l^{-1})	0.078b	29.7a	105.8a
ABA (800 mg l^{-1})	0.058a	76.9b	130.4c
AOA (300 mg l^{-1})	0.083b	34.3a	113.2b

Different letters behind the figures indicate significant difference at $P < 0.05$ among treatments according to Duncan's multiple new range test

changes by 4–5 days. GA_3 spray (50–100 mg L^{-1}) at early stage of inflorescence development (1 cm) delayed fruit harvest by 6–7 days (Mitra and Mandal 2014). These studies mainly investigate the effects of growth regulator on the pigmentation of litchi. The effects on the sugar accumulation and titratable acid decline might be more precise parameters reflecting their role in regulation maturation.

1.8 Fruit Compositions and Health Benefit Compounds

1.8.1 Compositions in the Aril

The drastic compositional changes in the aril give the delicious taste and aroma of litchi (Paull et al. 1984). Aril sugar accumulation is accompanied by acid loss. Total acids keep declining from the initiation of rapid aril growth to full maturity (Wang et al. 2006; Wu et al. 2016). Paull et al. (1984) detected abundant succinic acid, which was more than ten times higher than malic acid in the aril of litchi cv. Groff. However, the existence of abundant succinic acid in litchi was negated by Wang et al. (2006), who found malic acid (around 20.0 mg g^{-1} FW at the early aril development and 1.5 mg g^{-1} FW at maturity) was the major acid in the aril of litchi. Ascorbic acid is the other major acid in the aril of litchi, which ranges from 0.1 to 0.4 mg g^{-1} FW at maturity depended on cultivars (Wu et al. 2016).

At maturity, sucrose, fructose, and glucose (around 15.6–65.4 mg g^{-1} FW) are the predominant soluble sugars found in the aril of litchi, and their proportion differs among cultivars (Wang et al. 2006; Yang et al. 2013). Galactose is also present as a major constituent with contents ranging from 4.2 to 10.3 mg g^{-1} FW. L-Quebrachitol (1.6–6.4 mg g^{-1} FW), methyl-inositol (0.71–1.51 mg g^{-1} FW), and myoinositol (0.28–0.78) were found as minor constituents in the aril of litchi (Fig. 1.17, Wu et al. 2016).

Based on the study of Wu et al. (2016), γ-aminobutyric acid (GABA) was the most abundant amino acid with contents varying from 1.7 to 3.5 mg g^{-1} FW

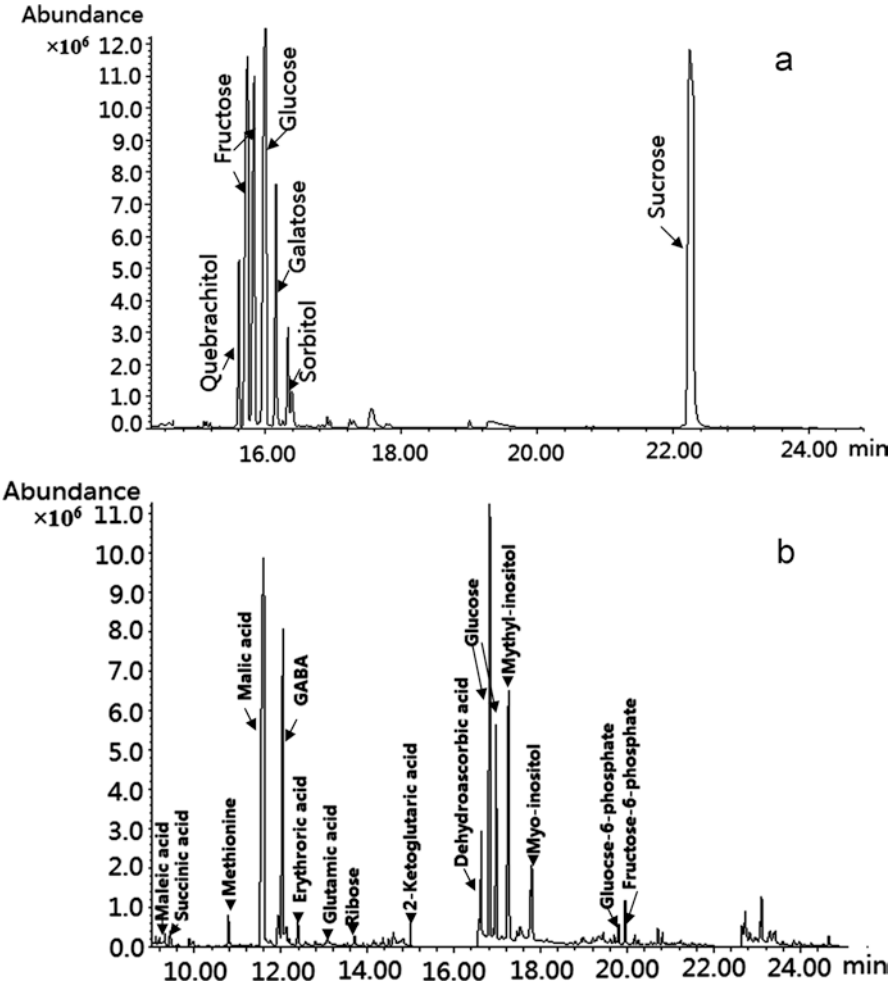

Fig. 1.17 GC-MS total ion chromatogram of the MSTFA derivatives of the combined sugars, organic acids, and amino acids of litchi flesh sample. Sorbitol was added as internal standard. (**a**) Abundant components in the flesh of litchi. (**b**) Less abundant components detected using higher concentration extract and avoiding the overload of major sugars (From Wu et al. 2016)

depended on cultivars. Inconsistent with the dramatic increased in total sugars but decreased in titratable acids, the contents of GABA remained relative constant throughout fruit development and maturation. The concentration of GABA in the aril was much higher than other amino acids reflecting by the overload of GABA but much small peaks of other amino acids including Asp, Glu, Asn, Ser, Gln, Arg, Ala, Tyr, Val, and Met (Fig. 1.18). The concentration of GABA in the flesh of litchi was about 25–55 times higher than Glu, the second most abundant amino acid.

The unique litchi aroma is related to the volatile substances produced in the aril. Chyau et al. (2003) identified a total of 25 volatile compounds including one ester,

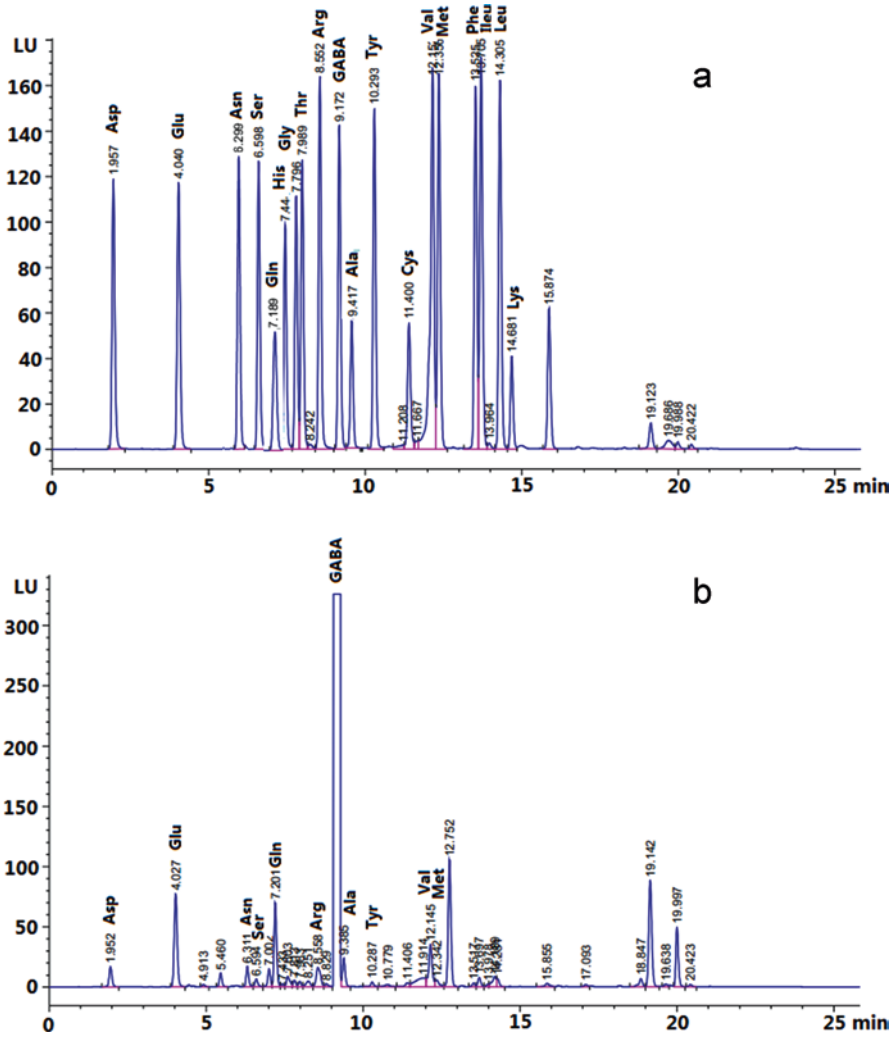

Fig. 1.18 HPLC chromatograms corresponding to (**a**) 19 amino acid standards, (**b**) litchi flesh sample showing the overload of GABA and the peaks of other amino acids (From Wu et al. 2016)

14 alcohols, two aldehydes, four acids, two ketones, and two terpenes. The volatile substances exist in free and glycosidically bound forms. In the free fraction, the major volatile compounds include acetoin, geraniol, 3-methyl-2-buten-1-ol, octanoic acid, 2-phenyethanol, cis-ocimene, and butyric acid, and their combination gave rise to the characteristic aroma of litchi flesh. The glycosidically bound volatile compounds are odorless but can be released upon hydrolysis by ß-glucosidase with production of the characteristic litchi aroma (Chyau et al. 2003).

The total phenolic compounds ranged from 0.47 to 1.6 mg g^{-1} FW in the aril of ten different litchi cultivars tested (Wu et al. 2016). Flavonoids were the major

phenolic compounds in the flesh of litchi with a relative content against total phenolic compounds ranged from 56 to 85%. Total phenolics and total flavonoids in the flesh of litchi increased as fruit developed toward maturity, which were inconsistent with Chyau et al. (2003), who proposed a loss of soluble tannins and total phenols during fruit develop toward maturity.

1.8.2 Health Benefit Compounds

The fruit of litchi has long been used as an alternative medicine for over 1000 years in China due to its loads of healthy nutrients. According to "Compendium of Materia Medica," an ancient monograph of herbal medicines written by Shizhen Li in Ming dynasty (1368–1644), litchi fruit displays a wide range of physiological effects. The pericarp of litchi can be used to cure dysentery, metrorrhagia, and eczema; the seed of litchi can be used for alleviating stomach pains and hernia; and the flesh of litchi is traditionally used as a tonic for the heart, brain, and liver. The medicinal uses, photochemistry, and pharmacology of litchi pericarp and seed were reviewed recently by Saudi Arabia authors Ibrahim and Mohamed (2015). The seed and pericarp of litchi possess a series of bioactivities including hypoglycemic, anti-cancer, antibacterial, antihyperlipidemic, antiplatelet, antitussive, analgesic, anti-pyretic, hemostatic, diuretic, and anti-viral properties and have been widely used in many cultures for the treatment of cough, flatulence, stomach ulcers, diabetes, obesity, testicular, swelling, hernia-like conditions, and epigastric and neuralgic pains (Ibrahim and Mohamed 2015).

For the pericarp of litchi, sound evidences of its health-promoting and medical functions have been obtained via modern phytochemistry and pharmacology studies, and functional compounds have been discovered. Abundant phenolic compounds (51–102 g kg^{-1} DW) were detected in the pericarp of litchi, and these phenolic compounds exhibited considerably bioactivities including high ferric reducing anti-oxidant power (FRAP) and strong activities of 1,1-diphenyl-2-picryhydrazyl (DPPH) scavenging, lipid peroxidation inhibiting, and oxidative DNA damage protection (Wang et al. 2011). The main phenolics in the pericarp of litchi are condensed tannins, epicatechin, anthocyanidin A2, anthocyanin, quercetin 3-rutinoside, and quercetin glucoside (Sarni-Manchado et al. 2000). In addition, Jiang et al. (2013) isolated a novel phenolic, 2-(2-hydroxyl-5-(methoxycarbonyl) phenoxy) benzoic acid, together with kaempferol, isolariciresinol, stigmasterol, butylated hydroxytoluene, 3,4-dihydroxyl benzoate, methyl shikimate, and ethyl shikimate from litchi pericarp methanol extracts. Anthocyanin and other polyphenolic compounds in the pericarp of litchi display significant activities of lipolysis, anti-oxidant, and anti-inflammatory (Ogasawara et al. 2009; Jiang et al. 2013; Yamanishi et al. 2014). Kong et al. (2010) isolated large numbers of polysaccharides with high anti-oxidant activities from the pericarp of litchi.

The aril of litchi is rich in nutritional and functional compounds according the data released by USDA (http://ndb.nal.usda.gov/ndb/foods/show/2311?manu=&fgcd=). One hundred grams of litchi flesh contains 16.5 g

sugars, 66 calories, 0.83 g protein, 0.44 g fat, 0.44 g ash, 1.3 g edible fiber, 5 mg Ca, 0.31 mg Fe, 171 mg K, 0.07 mg Zn, 71.5 mg vitamin C, 0.011 mg thiamin, 0.065 mg riboflavin, 0.603 mg niacin, 0.1 mg vitamin B6, 0.014 mg folate, 0.07 mg vitamin E, 0.007 mg tryptophan, 0.041 mg lysine, and 0.009 mg methionine. Zhang et al. (2013) identified six individual phenolics including gallic acid, chlorogenic acid, (+)-catechin, caffeic acid, (−)-epicatechin, and rutin in litchi pulp by HPLC. The flesh extract of litchi cultivars "Gimjeng" and "Chakapat" contained phenolics like trans-cinnamic acid (9.80 ± 0.21 mg GAE/g extract) and pelargonidin-3-*O*-glucoside (19.56 ± 0.4 mg GAE/g extract) (Bhoopat et al. 2011). These two compounds exhibited a trolox equivalent anti-oxidant capacity around 10 g/mg trolox. Lü et al. (2014) reported the effects of phenolic-rich litchi pulp extracts on glucose consumption in human HepG2 cells. The anti-lipid peroxidation and anti-apoptosis effects of litchi flesh as evidenced by the vitamin C and phenolic compounds might help to explain the hepatoprotective effects in CCl_4-induced hepatotoxicity in rats (Bhoopat et al. 2011). Polysaccharide-enriched fractions with anti-oxidant activity were also extracted from the flesh of litchi (Kong et al. 2010; Huang et al. 2015). However, these components seem insufficient to interpret the wide and significant physiological effects of litchi flesh. Recently, Wu et al. (2016) detected abundant quebrachitol and GABA in the aril of litchi. These two compounds are uncommon in the flesh of fruit species and display important health benefits and therefore might be the important contributors for the unique health benefits of litchi flesh.

References

Akamine EK, Goo T (1973) Respiration and ethylene production during ontogeny of fruit. J Am Soc Hort Sci 98:381–383

Batten DJ, McConchie CA, Lloyd J (1994) Effects of soil water deficit on gas exchange characteristics and water relations of orchard lychee (*Litchi chinensis* Sonn.) trees. Tree Physiol 14:1177–1189

Bhoopat L, Srichairatanatkool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T (2011) Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): a combination of anti-oxidant and anti-apoptotic activities. J Ethnopharmacol 136:55–66

Chang JC, Lin TS (2003) Effect of inflorescence thinning on flower sex ratio, fruit set and fruit quality in 'Yu Her Pau' litchi (*Litchi chinensis* Sonn.). J Agr Assoc China 4:418–428

Chen PA, Roan SF, Lee CL et al (2013) The effect of temperature during inflorescence development to flowering and inflorescence length on yield of 'Yu Her Pau' litchi. Sci Hortic 159:186–189

Chen PA, Lee CL, Roan SF, Chen LZ (2014) Effects of GA3 application on the inflorescence and yield of 'Yu Her Pau' litchi. Sci Hortic 171:45–50

Choudhury SR, Roy S, Sengupta DN (2009) A comparative study of cultivar differences in sucrose phosphate synthase gene expression and sucrose formation during banana fruit ripening. Postharvest Biol Technol 54:15–24

Chu YC, Lin TS, Chang JC (2015) Pollen effects on fruit set, seed weight, and shriveling of '73-S-20' litchi with special reference to artificial induction of parthenocarpy. HortSci 50:369–373

Chyau CC, Ko PT, Chang CH, Mau JL (2003) Free and glycosidically bound aroma compounds in lychee (*Litchi chinensis* Sonn.). Food Chem 80:387–392

Degani C, Stern RA, El-Batsri R et al (1995) Pollen parent effect on the selective abscission of Mauritius and Floridian lychee fruitlets. J Amer Soc Hort Sci 120:523–526

Dhua RS, Roychoudhury R, Kabir J, Ray SKD (2005) Stagger the lychee fruit harvest. Acta Hortic 665:347–354

Feng W, Zhang L, Li SG, Chen YY, Luo SR (2010) Comparison on embryonic development process of three cultivars of *Litchi chinensis*. Subtrop Plant Res Comm 31:736–739 (Chinese with English abstract)

Goncalves VD, Pires MC, Yamanishi OK (2014) Synthetic auxin 3-5-6 TPA increased fruit size and retention of 'Bengal' lychee in Brazil. Acta Hortic 1042:65–72

Hieke S, Menzel CM, Doogan VJ, Ludders P (2002) The relationship between yield and assimilate supply in lychee (*Litchi chinensis* Sonn.). J Hortic Sci Biotechnol 77:326–332

Hu B, Zhao JT, Lai B et al (2016) LcGST4 is an anthocyanin-related glutathione S-transferase gene in *Litchi chinensis* Sonn. Plant Cell Reports. doi:10.1007/s00299-015-1924-4

Huang HB (2002) Unfruitfulness of young litchi trees in relation to their peculiar root growth behavior: an overview. Acta Hortic 575:737–743

Huang HB (2005) Fruit set, development and maturation. In: Menzel CM, Waite GK (eds) Litchi and longan botany, production and uses. CABI, Wallingford

Huang HB, Qiu YX (1987) Growth correlations and assimilate partitioning in the arillate fruit of *Litchi chinensis* Sonn. Aust J Plant Physiol 14:181–188

Huang HB, Xu JK (1983) The developmental patterns of fruit tissues and their correlative relationships in *Litchi chinensis* Sonn. Sci Hortic 19:335–342

Huang HB, Jiang SY, Xie C (1983) The initiation of aril and ontogeny of fruit in *Litchi Chinese* Sonn. J South China Agric College 4:78–83 (Chinese with English abstract)

Huang F, Zhang RF, Dong LH, Guo JX, Deng YY, Yi Y, Zhang MW (2015) Anti-oxidant and anti-proliferative activities of polysaccharide fractions from litchi pulp. Food Funct 6:2598–2606

Ibrahim SRM, Mohamed GA (2015) *Litchi chinensis*: medicinal uses, phytochemistry, and pharmacology. J Ethnopharmacol. doi:10.1016/j.jep.2015.08.054

Jiang JP, Su MX, Lee PM (1986) The production and physiological effects of ethylene during ontogeny and after harvest of litchi fruits. Acta Phys Sin 12:95–103 (Chinese with English abstract)

Jiang SY, Xu HY, Wang HC, Hu GB, Li JG, Chen HB, Huang XM (2012) A comparison of the costs of flowering in 'Feizixiao' and 'Baitangying' litchi. Sci Hortic 148:118–125

Jiang G, Lin S, Wen L et al (2013) Identification of a novel phenolic compound in litchi (*Litchi chinensis* Sonn.) pericarp and bioactivity evaluation. Food Chem 136:563–568

Joubert AJ (1986) Litchi. In: Monselise SP (ed) Hand book of fruit set and development. CRC press, Boca Raton, pp 233–246

Komatsu A, Takanokura Y, Moriguchi T, Omura M, Akihama T (1999) Differential expression of three sucrose-phosphate synthase isoforms during sucrose accumulation in citrus fruits (*Citrus unshiu* Marc.). Plant Sci 140:169–178

Komatsu A, Moriguchi T, Koyama K, Omura M, Akihama T (2002) Analysis of sucrose synthase genes in citrus suggests different roles and phylogenetic relationships. J Exp Bot 53:61–71

Kong F, Zhang M, Liao S, Yu S, Chi J, Wei Z (2010) Anti-oxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi chinensis* Sonn. Molecules 15:2152–2165

Kuang JF, Wu JY, Zhong HY, Li CQ, Chen JY, Lu WJ et al (2012) Carbohydrate stress affecting fruitlet abscission and expression of genes related to auxin signal transduction pathway in litchi. Int J Mol Sci 13:16084–16103

Lai B, Li XJ, Hu B, et al. (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS One, 9(1): e86293.

Lai B, Hu B, Qin YH et al (2015) Transcriptomic analysis of *Litchi chinensis* pericarp during maturation with a focus on chlorophyll degradation and flavonoid biosynthesis. BMC Genomics 16:225. doi:10.1186/s12864-015-1433-4

Lai B, Du LN, Liu R et al (2016) Two LcbHLH transcription factors interacting with LcMYB1 in regulating late structural genes of anthocyanin biosynthesis in nicotiana and *Litchi chinensis* during anthocyanin accumulation. Front Plant Sci 7:166 Doi.org/10.3389/fpls.2016.00166

Lee HS, Wicker L (1991) Anthocyanin pigments in the skin of lychee fruit. J Food Sci 56:466–468

Li JG (2001) Ontogenetic studies of fruit development and minimizing cracking in *Litchi Chinese* Sonn. Ph.D. thesis, South China Agricultural University, Guangzhou, China. (Chinese with English abstract)

Li JG, Huang HB (1994) Analysing the cause of litchi fruit cracking increased by rain after a long period of drought. In: Zhang SL, Chen KS (eds) 481 Advances in Horticulture (I). China Agri. Press, Beijing, pp 361–364

Li JG, Zhou BY (2015) Comparison on fruit development and changes in endogenous hormone contents in pericarp between large- and aborted –seeded litchi (Litchi chinensis Sonn. c.v Guiwei). Comm Plant Physiol 41(5):587–590 (Chinese with English abstract)

Li JG, Huang XM, Huang HB (2010) An overview of factors relaled to fruit size in *Litchi chinensis* Sonn. Acta Hort 863:477-482

Li JG, Huang XM, Huang HB, Zhou BY (2002) A cytological and physiological study of large-fruited and small-fruited litchi cultivars. J Fruit Sci 19:158–162 (Chinese with English abstract)

Li JG, Huang HB, Huang XM (2003a) Re-evaluation of the division of developmental stages in litchi fruit. Acta Hort Sinica 30(3):307–310 (Chinese with English abstract)

Li JG, Huang HB, Huang XM (2003b) Relationship between nutrient competition and differential fruit sizing of 'Feizixiao' litchi fruit from early and late blooms. J Fruit Sci 20(3):195–198 (Chinese with English abstract)

Li JG, Huang HB, Huang XM (2004) Effects of bark ring incision on fruit sizing and fruit cracking in 'Nuomici' litchi (*Litchi chinensis* Sonn.). J Fruit Sci 21(u):379–381 (Chinese with English abstract)

Li JG, Zhou BY, Huang XM, Huang HB (2005) The roles of cytokinins and abscisic acid in the pericarp of litchi (*Litchi chinensis* Sonn.) in determining fruit size. J Hortic Sci Biotechnol 80:587–590

Li C, Wang Y, Ying P, Ma W, Li J (2015a) Genome-wide digital transcript analysis of putative fruitlet abscission related genes regulated by ethephon in litchi. Front Plant Sci 6:502

Li C, Wang Y, Huang X, Li J, Wang H, Li J (2015b) An improved fruit transcriptome and the identification of the candidate genes involved in fruit abscission induced by carbohydrate stress in litchi. Front Plant Sci 6:439. doi:10.3389/fpls.2015.00439

Li XJ, Lai B, Zhao JT, Qin YH, He JM, Huang XM, Wang HC, Hu GB (2016a) Sequence differences in LcFGRT4 alleles are responsible for the diverse anthocyanin composition in the pericarp of Litchi chinensis. Mol Breed 36:93. doi:10.1007/s11032-016-0518-3

Li XJ, Zhang JQ, Wu ZC, Lai B, Huang XM, Qin YH, Wang HC, Hu GB (2016b) Functional characterization of a glucosyltransferase gene, LcUFGT1, involved in the formation of cyanidin glucoside in the pericarp of Litchi chinensis. Physiol Plant 156:139–149

Liu SS, Ye YC, Zhao XD, Ye YX (1999) An embryological study of seed abortion in a seedless litchi strain. J South China Agric College 20(2):41–46 (Chinese with English abstract)

Lü LX, Chen JL, Chen XJ (1985) An observation on the process of embryo development in litchi. Subtrop Plant Res Comm 1:1–5 (Chinese with English abstract)

Lü Q, Si MM, Yan YY, Luo FL, Hu GB, Wu HS, Sun CD, Li X, Chen KS (2014) Effects of phenolic-rich litchi (*Litchi chinensis* Sonn.) pulp extracts on glucose consumption in human HepG2 cells. J Funct Foods 7:621–629

Menzel CM, Oosthuizen JH, Roe DJ, Doogan VJ (1995) Water deficits at anthesis reduce CO_2 assimilation and yield of lychee (*Litchi chinensis* Sonn.) trees. Tree Physiol 15:611–617

Mitra SK, Mandal D (2014) Delaying the harvesting of litchi by using gibberellic acid. Acta Hortic 1024:183–187

Ngugen-Quoc B, Foyer CH (2001) A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. J Exp Bot 52:881–889

Ogasawara J, Kitadate K, Nishioka H, Fujii H, Sakurai T, Kizaki T, Izawa T, Ishida H, Ohno H (2009) Oligonol, a new lychee fruit-derived lowmolecular form of polyphenol, enhances lipolysis in primary rat adipocytes through activation of the ERK1/2 pathway. Phytother Res 23:1626–1633

Patrick JW (1997) Phloem unloading: sieve element un-loading and post-sieve element transport. Annu Rev Plant Physiol 48:191–222

Paull RE, Chen NJ, Deputy J, Huang HB, Cheng GW, Gao FF (1984) Litchi growth and compositional changes during fruit development. J Amer Soc Hort Sci 109:817–821

Peng G, Wu JY, Lu WJ, Li JG (2013) A polygalacturonase gene clustered into clade E involved in lychee fruitlet abscission. Sci Hortic 150:244–250

Qiu YP, Zhang ZW, Qiu RX (1994) Study on embryo and endosperm development of *Litchi chinensis* Sonn. Chinese Bull Bot 11:45–47 (Chinese with English abstract)

Qiu YP, Xiang X, Wang BQ, Zhang ZW, Yuan PY (1998) Endogenous hormone balance in three types of litchi fruit and their fruit set mechanism. J Fruit Sci 15:39–43 (Chinese with English abstract)

Qiu YP, Ou LX, Li ZQ, Xiang X, Chen JZ, Wang BR (2006) Efects of pollinator on fruit quality of Guiwei litchi cultivar. J Fruit Sci 23:703–706 (Chinese with English abstract)

Ruan YL, Jin Y, Yang YJ, Li GJ, Boyer JS (2010) Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Mol Plant 3:942–955

Sarni-Manchado P, Le Roux E, Le Guerneve C, Lozano Y, Cheynier V (2000) Phenolic composition of litchi fruit pericarp. J Agric Food Chem 48:5995–6002

Sharma SB, Ray PK, Rai R (1986) The use of growth regulators for early ripening of litchi (*Litchi chinensis* Sonn.). J Hort Sci 61:533–534

Shi BM, Chen YR (2000) Observation on the embryo sac development of litchi. China Hort 46:359–368 (Chinese with English abstract)

Stern RA, Gazit S (1996) Lychee pollination by the honeybee. J Amer Soc Hort Sci 121:152–157

Stern RA, Gazit S (2003) The reproductive biology of the lychee. Hortic Rev 28:393–453

Stern RA, Gazit S, El-Batsri R, Degani C (1993) Pollen parent effect on outcrossing rate, yield, and fruit characteristics of 'Floridian' and 'Maritius' lychee. J Amer Soc Hort Sci 118:109–114

Stern RA, Stern D, Harpaz M, Gazit S (2000) Applications of 2,4,5-TP, 3,5,6-TPA and combinations thereof increase lychee fruit size and yield. HortSci 35:661–664

Stern RA, Stern D, Miller H, Xu H, Gazit S (2001) The effect of the synthetic auxins 2,4,5-TP and 3,5, 6-TPA on yield and fruit size of young 'Fei Zi Xiao' and 'Hei Ye' litchi trees in Guangxi Province, China. Acta Hortic 558:285–288

Subhadrabandhu S, Stern RA (2005) Taxonomy, botany and plant development. In: Menzel CM, Waite GK (eds) Litchi and longan botany, production and uses. CABI, Wallingford

Tomer E, Zipori I, Goren M, Shooker S, Ripa M, Foux Y (2001) Delaying the ripening of 'Mauritius' litchi fruit (preliminary results). Acta Hortic 558:315–317

Underhill S, Critchley C (1994) Anthocyanin decolorisation and its role in lychee pericap browning. Aust J Experi Agric 34:115–122

Vizzotto G, Pinton R, Varanini Z, Costa G (1996) Sucrose accumulation in developing peach fruit. Physiol Plant 96:225–2230

Wang HC, Huang XM, Huang HB (2002) A study on the causative factors retarding pigmentation in the fruit of 'Feizixiao' litchi. Acta Hortic Sinica 19:408–412 (Chinese with English abstract)

Wang HC, Huang XM, Hu GB, Yang Z, Huang HB (2005) A comparative study of chlorophyll loss and its related mechanism during fruit maturation in the pericarp of fast- and slow-degreening litchi pericarp. Sci Hortic 106:247–257

Wang HC, Huang HB, Huang XM, Hu ZQ (2006) Sugar and acid compositions in the arils of *Litchi chinensis* Sonn.: cultivar differences and evidence for the absence of succinic acid. J Hortic Sci Biotechnol 81:57–62

Wang H, Huang H, Huang X (2007) Differential effects of abscisic acid and ethylene on the fruit maturation of *Litchi chinensis* Sonn. Plant Growth Regul 52:189–198

Wang HC, Hu ZQ, Wang Y, Chen HB, Huang XM (2011) Phenolic compounds and the anti-oxidant activities in litchi pericarp: difference among cultivars. Sci Hortic 129:784–789
Wang TD, Zhang HF, Wu ZC, Li JG, Huang XM, Wang HC (2015) Sugar uptake in the aril of litchi fruit depends on the apoplasmic post-phloem transport and the activity of proton pumps and the putative transporter *LcSUT4*. Plant Cell Physiol 56:377–387
Wei YZ, Hu FC, Hu GB, Li XJ, Huang XM, Wang HC (2011) Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PLoS One 6:e19455
Wu SX (1998) Encyclopedia of China fruits: Litchi. China Forestry Press, Beijing
Wu DY, Lin XD, Ye QH, Wang WH (2000) Improvement of fruit-set in secondary panicles of Feizixiao litchi by removal of the primary panicles. J South China Agric Univ 21:19–21 (Chinese with English abstract)
Wu ZC, Yang ZY, Li JG, Chen HB, Huang XM, Wang HC (2016) Methyl-inositol, γ-aminobutyric acid and other health benefit compounds in the aril of litchi. Int J Food Sci Nutr 67:762–772
Xia R, Li CQ, Lu WJ, Du J, Wang ZH, Li JG (2012) 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*HMG1*) is highly associated with the cell division during the early stage of fruit development which determines the final fruit size in *Litchi chinensis*. Gene 498:28–35
Xiang X, Ou LX, Qiu YP, Yuan PY, Chen JZ (2001) Embryo abortion and pollen parent effects in 'Nuomici' and 'Guiwei'. Acta Hortic 558:257–260
Yamanishi E, Yoshigai T, Okuyama M, Mori H, Murase T, Machida T, Okumura M, Nishizawa M (2014) The anti-inflammatory effects of flavanol-rich lychee fruit extract in rat hepatocytes. PLoS One 9:e93818
Yang ZY, Wang TD, Wang HC, Huang XM, Qin YH, Hu GB (2013) Patterns of enzyme activities and gene expressions in sucrose metabolism in relation to sugar accumulation and composition in the aril of *Litchi chinensis* Sonn. J Plant Physiol 170:731–740
Yang ZY, Zhang JQ, Wang TD, Huang XM, Hu GB, Wang HC (2014) Does acid invertase regulate the seed development of Litchi chinensis? Acta Hortic 1029: 301–307
Ye XL, Wang DX, Qian NF (1992) Embryological studies of *Litchi Chinese*. Acta Bot Yunnanica 14:59–65 (Chinese with English abstract)
Yin JH, Gao FF, Hu GB, Zhu SH (2001) The regulation of litchi maturation and coloration by abscisic acid and ethylene. Acta Hortic 558:293–296
Yuan RC, Huang HB (1988) Litchi fruit abscission: its patterns, effect of shading and relation to endogenous abscisic acid. Sci Hortic 36:281–292
Yuan RC, Huang HB (1992) Improvement of fruit-set in *Litchi chinensis* Sonn. through regulation of source-sink relationship. J South China Agric Univ 13:136–142 (Chinese with English abstract)
Yuan WQ, Huang XM, Wang HC, Li JG, Chen HB, Yin JH (2009) The correlation of carbon nutrient reserves dynamics and fruit set of 'Nuomici' litchi. Acta Hortic Sin 36:1568–1574 (Chinese with English abstract)
Zhang Z, Xuequn P, Yang C, Ji Z, Jiang Y (2004) Purification and structural analysis of anthocyanins from litchi pericarp. Food Chem 84:601–604
Zhang R, Zeng Q, Deng Y et al (2013) Phenolic profiles and anti-oxidant activity of litchi pulp of different cultivars cultivated in Southern China. Food Chem 136:1169–1176
Zhang HN, Li WC, Wang HC, Shi SY, Shu B, Liu LQ, Wei YZ, Xie JH (2016) Transcriptome profiling of light-regulated anthocyanin biosynthesis in the pericarp of litchi. Front Plant Sci 7:963. doi:10.3389/fpls.2016.00963
Zhao ZC, Hu GB, Hu FC et al (2012) The UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT) gene regulates anthocyanin biosynthesis in litchi (*Litchi chinesis* Sonn.) during fruit coloration. Mol Biol Rep 39:6409–6415
Zhou BY, Ji ZL, Ye YC, Zhao XD (1998) Changes of endogenous hormones in litchi fruits during fruit development. Acta Hortic Sin 23:13–18 (Chinese with English abstract)

Litchi Breeding and Plant Management in Taiwan

2

Jer-Way Chang, Po-An Chen, and Iou-Zen Chen

Abstract

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

Keywords

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

J.-W. Chang
Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan

P.-A. Chen
Plant Technology Labourites, Agricultural Technology Research Institute, Hsinchu, Taiwan

I.-Z. Chen (✉)
Department of Horticulture and Landscape Architecture, National Taiwan University, Taipei, Taiwan
e-mail: chenyo@ntu.edu.tw

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2.1 Introduction

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

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

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

2.2 Industry Status in Taiwan

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

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

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

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

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

shriveled seed ratio, and it is prone to fruit cracking (Chang et al. 2009a, b). It has been assumed that "73-S-20" is unlikely to be genetically identical to the original China cv. "No Mai Tsz" (Yen 1995). But it most likely is "Guiwei" in China when you judge it from the fruit appearance and characteristics data.

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

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

2.3 Breeding Objective and Strategies

2.3.1 Breeding Objectives

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

2.3.2 Breeding Strategies

There are different breeding strategies applied for litchi to improved traits, such as conventional breeding, genetic engineering, in vitro mutagenesis, and molecular-assisted breeding (Sarin et al. 2009). However, conventional breeding is still the main approach in Taiwan (Chang et al. 2013). The molecular technologies are only

used to assist litchi breeder to identify the genetic relationship among varieties, currently (Lee et al. 2007; Wu 1998; Kumar et al. 2006).

2.4 Germplasm Resources

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

2.4.1 Parental Choice

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

2.4.1.1 The Utilization of Varieties with Special Characteristics

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

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

2.4.2 The Utilization of Varieties with Special Characteristics

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

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

2.4.3 Assess the Genetic Dynamics of Offspring

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

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

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

2.4.4 Seedlings Selection from Open Pollination

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

2.4.5 Controlled Hybridization

The hybridization technique used in Taiwan was as follows

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

2.5 Breeding Procedure

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

2.5.1 Seedlings Management

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

2.5.2 Primary Selection

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

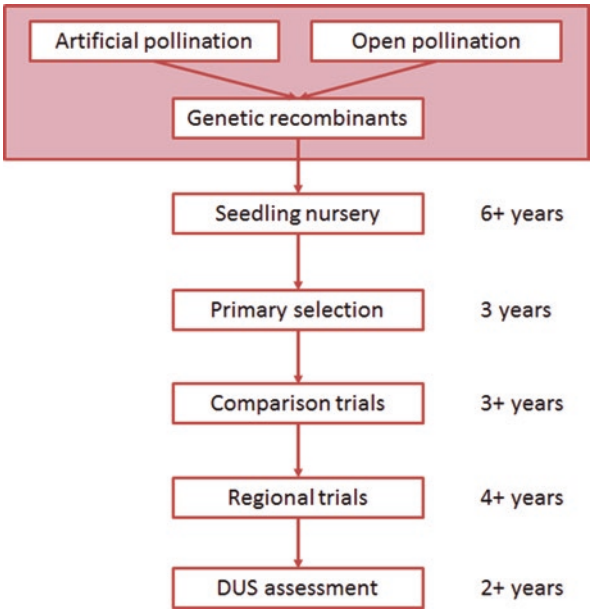

Fig. 2.1 The breeding procedure in Taiwan

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

2.5.3 Comparison Trials

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

2.5.4 Regional Trials

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

2.5.5 Other Related Trials

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

2.5.6 Apply and Get "the Plant Variety Right"

In Taiwan, the law of "the plant variety and plant seed act" was promulgated in 2004. The breeder would apply "the plant variety right" to acquire the protection

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

2.5.7 Achievements

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

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

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

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

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

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

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

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

2.6 Biotechnology

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

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

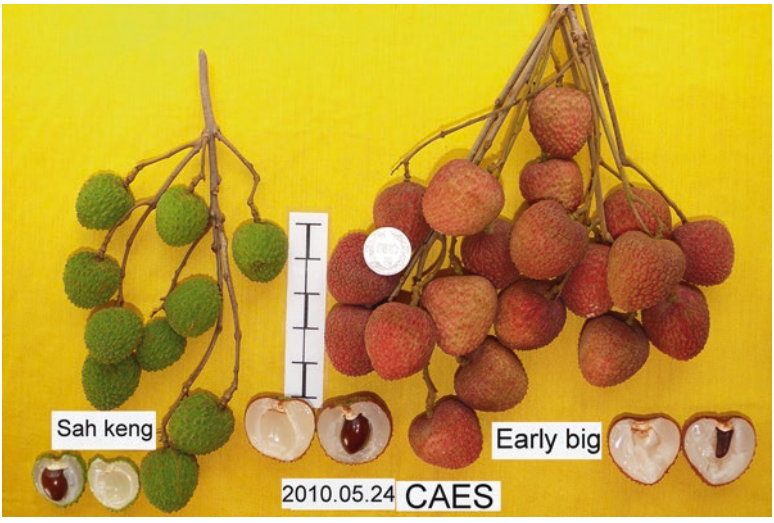

Fig. 2.6 Tainung No. 7 ("Early Big") is known for its early-maturing trait and large fruit size compared with other commercial varieties. The harvest of Tainung No. 7 is 17–21 days earlier than "Sah Keng" grown in the same region (Adapted from Chang, Jer-Way)

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

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

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

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

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

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

2.7 Plant Management

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

2.7.1 Growth Rhythm

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

2.7.2 Flower Bud Formation

Poor litchi flowering is a worldwide problem (Menzel 1983, 1984; Galan 1989), especially in regions where the weather during the induction period is too warm (Groff 1943; Young 1970; Menzel 1983). Cool and dry weather from late autumn to winter is the basely environmental condition for litchi flower bud formation, but Nakata and Suehisa (1969), Huang and Weng (1978), and Teng (1988) depicted that even under flower bud formation condition, immature flush, with the leaves still purple red to light green, could not change from vegetative to reproductive statute. Huang and Weng (1978) and Chang et al. (2014a, b) found that in Taiwan climate condition, late flushes should fully mature before November, or most of them would

form vegetative flush in next year spring. So, dry weather in autumn is a benefit for shoot to cease growth and to move to the vegetative dormancy stage and then increased the flowering intensity at next season. In Israel, got similar results from the study on "Mauritius" and "Floridian" litchi.

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

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

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

2.8 Cultural Practices Affected to Flower Bud Formation

2.8.1 Nitrogen Fertilizer Dosage and Application Season

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

2.8.2 Girdling, Cincturing, and Strangulation

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

2.8.3 Late Flush and Panicle Pruning and Panicle Length Control

2.8.3.1 Late Shoot Pruning

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

2.8.3.2 Panicle Thinning

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

2.8.4 Plant Growth Regulators

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

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

2.8.5 Irrigation

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

2.9 Floret Development

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

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

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

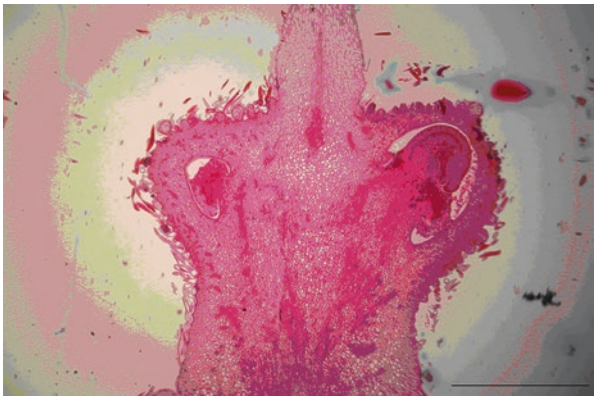

Fig. 2.7 The embryo sac of "Yu Her Pau" litchi pseudohermaphrodite flower. Normally, litchi pseudohermaphrodite flowers have two embryo sacs, but their developments are asynchronized. Bar =150 μm (Adapted from Chen, Iou-Zen)

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

2.10 Pollination and Pollen Storage

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

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

2.11 Fruit Set and Growth

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

In "73-S-20" litchi trees require a minimum number of three flushes for adequate fruit production (Chang and Lin 2008). Batten et al. (1994) found that fruit shedding

was significantly less in irrigated trees. The reasons may be water deficits at anthesis, reduced ratio of daytime stomatal conductance, and CO2 assimilation [Batten et al. 1994, Menzel and Simpson 1990).

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

2.12 Seed Storage

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

2.13 Vegetative Propagation

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

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

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

2.14 Conclusions

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

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

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

References

Abrol DP (2006) Diversity of pollinating insects visiting litchi flowers (*Litchi chinensis* Sonn.) and path analysis of environmental factors influencing foraging behavior of four honey-bee species. J Apic Res 45(4):180–187

Batten DJ, McConchie CA, Lloyd J (1994) Effects of soil water deficit on gas exchange characteristics and water relations of orchard lychee (*Litchi chinensis* Sonn.) trees. Tree Physiol 14(10):1177–1189

Bhat SK, Raina BL, Chogtu SK, Muthoo AK (1997) Effect of exogenous auxin application on fruit drop and cracking in Litchi (*Litchi chinensis* Sonn) cv. Deh-radun. Adv Plant Sci 10:83–86

Chaikiattiyos S, Menzel CM, Rasmussen TS (1994) Floral induction in tropical fruit trees: effects of temperature and water supply. J Hortic Sci 69(3):397–415

Chang CC (1961) The lychee growing in Taiwan. J Agric Assoc China 33:51–63

Chang JW (1999) Regulation of flowering in litchi (*Litchi chinensis* Sonn). Ph.D. dissertation, Department of Horticulture, National Taiwan University, Taipei, Taiwan, R.O.C. 136 pp

Chang JW, Chang JY (2015) Litchi world trade and general direction of table fruit export of Taiwan. Biotechnology of Plant Seed and Seedling 41:146–151

Chang JW, Cheng CY (2002) Effect of nitrogen concentrations of hydroponic media on flowering and fruiting of litchi (*Litchi chinensis* Sonn.). J Chin Soc Hortic Sci 48:1–8

Chang JC, Lin TS (2008) Fruit yield and quality as related to flushes of bearing shoots in litchi. J Am Soc Hortic Sci 133(2):284–289

Chang JW, Chao CN, Chen IZ, Cheng CY (1997) Effects of temperature and water stress on flowering of litchi (*Litchi chinensis* Sonn). J Chin Soc Hortic Sci 43:322–329

Chang JW, Chao CN, Yen CR (2001) Litchi (*Litchi chinensis* Sonn.) breeding - the influences of mother plant on juvenile period, fruit maturity season and fruit characters, and the selection of promising lines. J Agric Res China 50(4):59–65

Chang JW, Cheng YH, Yen CR, Hsu HT, Chao CN, Tien YJ, Ho CC, Lin CY (2005) A new variety of li-tchi (*Litchi chinensis* Sonn.) Tainung NO. 1. J Taiwan Agric Res 54:43–53

Chang JC, Lin TS, Yen CR, Chang JW, Lee WL (2009a) Litchi production and improvement in Taiwan. J Agric Assoc Taiwan 10:63–67

Chang JW, Yen CR, Hsu HT, Wang WL, Tsai WH, Cheng YH, Ho CC (2009b) A new variety of litchi (Li-tchi chinensis Sonn.)"Tainung NO. 3" Rose Red. J Taiwan Agric Res 58:208–218

Chang JW, Yen CR, Wang WL, Liu MN (2010) A new litchi cultivar: 'Tainung No.5 (Ruby)'. J Taiwan Agric Res 59:197–208

Chang JW, Teng YS, Yen CR (2012) Description and performance of new litchi varieties. In: Fang HH, Teng YS, Lee WL (eds) Proceedings of the symposium on litchi industry development in Taiwan, Taichung, pp 25–37

Chang JW, Teng YS, Yen CR (2013) Litchi breeding in Taiwan. In: Ke LS, Yen CR (eds) Proceedings of a symposium on breeding of fruit crops in Taiwan, Pingtiung, pp 115–129

Chang JY, Chang YA, Fang MY, Lin ML, Chang JW (2014a) Estimation of the putative marginal timing for subsequent in the last flush of litchi (*Litchi chinensis* Sonn). J Taiwan Soc Hort Sci 60:27–39

Chang JW, Yen CR, Wang WL, Liu MN, Chang JY (2014b) Breeding of novel litchi cultivar 'Tainung No. 7 (Eary Big). J Taiwan Agric Res 63:43–56

Chang JY, Chang YA, Tang L, Chang JW (2015) Characterization of generative development in early maturing litchi 'Early Big', a novel cultivar in Taiwan. Fruits 70(5):289–296

Chen MC, Hsiung TC, Chang TL, Lee CL, Chen IZ, Roan SF (2011) Effects of girdling and strangulation on root growth and nutrient concentrations of 'Haak Yip' litchi leaves and roots. J Taiwan Soc Hort Sci 57:231–242

Chen PA, Roan SF, Lee CL, Chen IZ (2013) The effect of temperature during inflorescence development to flowering and inflorescence length on yield of 'Yu Her Pau' litchi. Sci Hortic 159:186–189

Chen PA, Lee CL, Roan SF, Chen IZ (2014) Effects of GA3 application on the inflorescence and yield of 'Yu Her Pau' litchi. Sci Hortic 171:45–50

Chen PA, Roan SF, Lee CL, Chen IZ (2016a) Temperature model of litchi flowering—from induction to anthesis. Sci Hortic 205:106–111

Chen Z, Zhao J, Qin Y, Hu G (2016b) Study on the graft compatibility between 'Jingganghongnuo' and other litchi cultivars. Sci Hortic 199:56–62

Cheng YH, Chang TL, Chen IZ (2005) Effect of girdling on flower differentiation of litchi *(Litchi chinensis* Sonn.). J Chin Soc Hortic Sci 51:155–164

Chu YC, Lin TS, Chang JC (2015) Pollen effects on fruit set, seed weight, and shriveling of '73-S-20' litchi - with special reference to artificial Induction of parthenocarpy. HortSci 50(3):369–373

Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell, Tissue and Organ Cult (PCTOC) 109(2):315–325

Das DK, Rahman A, Kumari D, Kumari N (2016) Synthetic seed preparation, germination and plantlet regeneration of Litchi (*Litchi chinensis* Sonn.). Am J Plant Sci 7(10):1395

Degani C, Beiles A, El-Batsri R, Goren M, Gazit S (1995a) Identifying lychee cultivars by isozyme analysis. J Am Soc Hortic Sci 120(2):307–312

Degani C, Stern RA, El-Batsri R, Gazit S (1995b) Pollen parent effect on the selective abscission of 'Mauritius' and 'Floridian' lychee fruitlets. J Am Soc Hortic Sci 120(3):523–526

Degani C, Deng J, Beiles A, El-Batsri R, Goren M, Gazit S (2003) Identifying lychee (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using inter simple sequence repeat (ISSR) markers. J Am Soc Hortic Sci 128(6):838–845

Dwivedi AK, Mitra SK (1996) Divergence analysis of litchi (*Litchi chinensis* Sonn.) cultivars growth in west Bengal. The Indian J Genet Plant Breed 56(4):486–489

Fu L (1990) Litchi (*Litchi chinensis* Sonn.): In vitro production of Haploid plants. In: Haploids in crop improvement I. Springer Berlin/Heidelberg, pp 264–274

Fu L, Tang D (1983) Induction pollen plants of litchi tree (*Litchi chinensis* Sonn.). Acta Genet Sin 20(5):369–374

Fu JR, Zhang BZ, Wnag XP, Qiao YZ, Huang XL (1990) Physiological studies on desiccation wet storage and cryopreservation of recalcitrant seeds of three fruit species and their excised embryonic axes. Seed Sci Technol 18:743–754

Galan Sauco V, Menini UC (1989) Litchi cultivation. Food and Agriculture Organization of the United Nations, Rome, 136 pp

Groff GW (1943) Some ecological factors involved in successful lychee culture. Proc Florida State Hort Soc 56:134–155

Huang PC (1966) The lychee. National Chung-Hsing University, Taichung, 160 pp

Huang XM (2002) Lychee production in China. In: Minas KP, Frank JD (eds) Lychee production in the Asia-Pacific region. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, pp 41–54

Huang PC, Weng SW (1978) Effect of delayed shoot treatment of fruit bearing of litchi tree (I). J Chin Soc Hortic Sci 24:121–126
Jain MS (2000) A review of induction of mutations in fruits of tropical and subtropical regions. Int Symp Trop Subtrop Fruits 575:295–302
Jain SM (2001) Tissue culture-derived variation in crop improvement. Euphytica 118(2):153–166
Kumar M, Gupta M, Shrivastava D, Prsad M, Prsad US, Sarin NB (2006) Genetic relatedness among Indian li-tchi accessions (*Litchi chinensis* Sonn.) by RAPD markers. Int J Agric Res 1:390–400
Lee WL, Teng YS, Lin RQ (2007) Using RAPD markers to study genetic variation among lychee Cultivars. J Taiwan Agric Res:281–288
Liang GJ, Yu GX (1991) Effects of PP333 on mature, non-flowering and leaflet-on-panicle litchi trees. Sci Hortic 48(3–4):319–322
Liao YW, Ma SS (1998) Adventitious embryogenesis of *Litchi chinensis*. J Chin Soc Hortic Sci 44:29–40
Liu W, Xiao Z, Bao X, Yang X, Fang J, Xiang X (2015) Identifying litchi (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using single nucleotide polymorphism (SNP) markers. PLoS One 10(8):e0135390
Long Y, Cheng J, Mei Z, Zhao L, Wei C, Fu S, Khan MA, Fu J (2015) Genetic analysis of litchi (*Litchi chinensis* Sonn.) in southern China by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence re-peat (ISSR). Mol Biol Rep 42(1):159–166
Madhou M, Normand F, Bahorun T, Hormaza JI (2013) Fingerprinting and analysis of genetic diversity of litchi (*Litchi chinensis* Sonn.) accessions from different germplasm collections using microsatellite markers. Tree Genet Genomes 9(2):387–396
McConchie CA, Batten DJ (1989) Floral biology and fruit set in lychee. In: Proceedings of the 2nd national Lychee seminar, Cairns/Australia, pp 71–74
McConchie CA, Vithanage V, Batten DJ (1994) Intergeneric hybridization between litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour.). Ann Bot 74(2):111–118
Menzel C (1983) The control of floral initiation in lychee: a review. Sci Hortic 21(3):201–215
Menzel CM (1984) The pattern and control of reproductive development in lychee: a review. Sci Hortic 22(4):333–345
Menzel CM (2002a) Origin, distribution, production and trade. In: Overview of lychee production in the Asia-Pacific region. Lychee Production in the Asia-Pacific Region. Food and Agricultural Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, pp 1–6
Menzel CM (2002b) Lychee production in Austraia. In: Lychee production in the Asia-Pacific region. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, pp 14–27
Menzel CM, Paxton BF (1986) The effect of cincturing at different stages of vegetative flush maturity on the flowering of litchi (*Litchi chinensis* Sonn.). J Hortic Sci 61(1):135–139
Menzel CM, Simpson DR (1987a) Effect of cincturing on growth and flowering of lychee over several seasons in subtropical Queensland. Anim Prod Sci 27(5):733–738
Menzel CM, Simpson DR (1987b) Lychee nutrition: a review. Sci Hortic 31(3):195–224
Menzel CM, Simpson DR (1988) Effect of temperature on growth and flowering of litchi (*Litchi chinensis* Sonn.) cultivars. J Hortic Sci 63(2):349–360
Menzel CM, Simpson DR (1990) Performance and improvement of lychee cultivars:a review. Fruit Var J 44(4):197–215
Menzel CM, Simpson DR (1992) Effects of environment on floral sex ratios in lychee (*Litchi chinensis* Sonn.). Acta Hortic 321:616–620
Menzel CM, Simpson DR (1995) Temperatures above 20 °C reduce flowering in lychee (*Litchi chinensis* Sonn.). J Hortic Sci 70:981–987
Menzel CM, Rasmussen TS, Simpson DR (1989) Effects of temperature and leaf water stress on growth and flowering of litchi (*Litchi chinensis* Sonn.). J Hortic Sci 64(6):739–752
Menzel CM, Oosthuizen JH, Roe DJ, Doogan VJ (1995) Water deficits at anthesis reduce CO2 assimilation and yield of lychee (*Litchi chinensis* Sonn.) trees. Tree Physiol 15(9):611–617

Menzel CM, Huang X, Liu C (2005) Cultivars and plant improvement. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CABI Publishing, Queensland, pp 59–86
Mitra SK (2002) Overview of lychee production in the Asia-Pacific region. In: Lychee production in the Asia-Pacific region. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, pp 5–13
Mustard MJ (1960) Megametophytes of the lychee (*Litchi chinensis* Sonn.). J Am Soc Hortic Sci 75:292–304
Nakata S, Suehisa R (1969) Growth and development of *Litchi chinensis* as affected by soil-moisture stress. Am J Bot:1121–1126
Puchooa D (2004) In vitro regeneration of lychee (*Litchi chinensis Sonn*.). Afr J Biotechnol 3(11):576–584
Ray PK (2002) Litchi. Breeding tropical and subtropical fruit. Narosa Publishing House, Samastipur, pp 129–142
Ray PK, Sharma SB (1987) Growth, maturity, germination and storage of litchi seeds. Sci Hortic 33:213–221
Robbertse H, Fivaz J, Menzel C (1995) A reevaluation of tree model, inflorescence morphology, and sex ratio in lychee (*Litchi chinensis* Sonn.). J Am Soc Hortic Sci 120(6):914–920
Sanyal D, Mitra SK (2000) Application of the polyamine putrescine increased yield of 'Mauritius' litchi (*Litchi chinensis* Sonn.). J Hortic Sci Biotechnol 75(5):612–614
Sarin NB, Prasad US, Kumar M, Jain SM (2009) Litchi breeding for genetic improvement. In: Jain SM, Priyadar-shan PM (eds) *Breeding plantation tree crops: tropical species*. Springer, Heidelberg, pp 217–245
Shih PM (2000) Embryo sac development in litchi (*Litchi chinensis* Sonn.) and the effect of temperature on embryo sac development. Master thesis, National Taiwan University, Taipei, Taiwan. 130 pp
Shih PM, Chen IZ (2000) Observation of embryo sac development of 'Yuh Her Pau', 'Haak Yip', and 'No mi Tsz' litchi (*Litchi chinensis* Sonn.). J Taiwan Soc Hortic Sci 46:359–368
Stern RA, Gazit S (1996) Lychee pollination by the honeybee. J Am Soc Hortic Sci 121(1):152–157
Stern RA, Gazit S (1997) Effect of 3, 5, 6-trichloro-2-pyridyl-oxyacetic acid on fruitlet abscission and yield of 'Mauritius' litchi (*Litchi chinensis* Sonn.). J Hortic Sci 72(4):659–663
Stern RA, Gazit S (1998) Pollen viability in lychee. J Am Soc Hortic Sci 123(1):41–46
Stern RA, Gazit S (2003) The reproductive biology of the lychee. Hortic Rev 28:393–453
Stern RA, Adato I, Goren M, Eisenstein D, Gazit S (1993) Effects of autumnal water stress on litchi flowering and yield in Israel. Sci Hortic 54(4):295–302
Stern RA, Eisenstein D, Voet H, Gazit S (1996) Anatomical structure of two day old litchi ovules in relation to fruit set and yield. J Hortic Sci 71(4):661–671
Stern RA, Eisenstein D, Voet H, Gazit S (1997a) Female 'Mauritius' litchi flowers are not fully mature at anthesis. J Hortic Sci 72(1):19–25
Stern RA, Nadler M, Gazit S (1997b) 'Floridian' litchi yield is increased by 2, 4, 5-TP spray. J Hortic Sci 72(4):609–615
Stern RA, Stern D, Harpaz M, Gazit S (2000) Applications of 2, 4, 5-TP, 3, 5, 6-TPA, and combinations thereof increase lychee fruit size and yield. HortSci 35(4):661–664
Sun QM, Ou LX, Xu X, Chen JZ, Qiu YP, Li ZQ, Cai CH (2010) Progress in breeding for litchi (Lichi chinensis). J Fruit Sci 23(5):790–796
Taiwan Agricultural Statistic YearBook (2015) Council of agriculture, Taipei
Teng YS (1988) Development method for a stable production in lychee (*Litchi chinensis* Sonn) cv. Yu Ho Pau. Master thesis, National Taiwan University, Taipei
Teng YS (1996) Pruning and regulation of blooming time in high density planting of lychee (*Litchi chinensis* sonn) cv. Yu Her Pau. Ph.D. dissertation, National Taiwan University, Taipei
Teng YH, Chen KS (2011) Introduction to the new litchi cultivar Tainung No. 6 Colorful Lychee. Taiwan Agric Res Inst Tech Serv Q 87:5–8

Teng YH, Liu ZG (2007a) A new litchi cultivar Tainung No. 2 Wuang Lee. Taiwan Agric Res Inst Tech Serv Q 71:6–9
Teng YH, Liu ZG (2007b) A new litchi cultivar Tainung No. 4 Lucky. Taiwan Agric Res Inst Tech Serv Q 72:4–7
Teng YS, Chang JW, Wang YT (2004) Litchi. In: Taiwan agricultural encyclopedia, Crop edn, vol 2. Agricultural Publisher Councils, Taipei, pp 39–52
Vos JE, Du Preez RJ, Froneman I, Hannweg K, Hus-selman J, Rheeder S (2009) Mutation breeding in South Africa (2003–2004). Induced mutation in tropical fruit trees, 123
Wang WL, Chang JW (2010) Using RAPD and ISSR markers to identify the genetic variation among lychee Cultivars. In: Chen JJ (eds) Taiwan agricultural research institute year-book. Taichung, pp 16–18
Wang L, Wu J, Chen J, Fu D, Zhang C, Cai C, Ou L (2015) A simple pollen collection, dehydration, and long-term storage method for litchi (*Litchi chinensis* Sonn.). Sci Hortic 188:78–83
Wu SX (1998) Encyclopedia of china fruits: litchi. Chin For Press, Beijing, pp 20–43
Yen CR (1995) Litchi. In: Taiwan agricultural encyclopedia, Crop edn. vol 2. Agricultural Publisher Councils, Taipei. pp 35–42
Yen CR, Liao YW, Tien YJ (1984) The cultivars of litchi (*Litchi chinensis* Sonn.) and their improvement in Taiwan. J Chin Soc Hortic Sci 30:210–222
Young TH (1970) Some climatic effects on flowering and fruiting of ‘Brewster’ lychees in Florida. Proc Fla State Hortic Soc 83:362–367
Zhao YH, Hu YL, Guo YS, Zhou J, Fu JX, Liu CM, Zhu J, Zhang MJ (2008) Inter-generic hybrids obtained from cross between litchi and longan cultivars and their molecular identification. J Fruit Sci 25(6):950–952

Biotechnological Advances in Lychee (*Litchi chinensis*) and Their Future Implication in Improvement of Crop

3

Devendra Kumar Pandey, Abhijit Dey, and Joginder Singh

Abstract

Lychee (*Litchi chinensis* L.) belongs to family Sapindaceae and cultivated in tropical and subtropical countries. The fruit is an excellent source of nutrients, i.e., vitamin C, and polyphenols, and other parts of plants, i.e., leaves and stem, have huge medicinal value and are used to cure various ailments. *Litchi chinensis* is facing several agronomic and horticultural problems such as susceptibility to many pathogens damaging preharvest and postharvest fruits, uneven fruit growth, short shelf life of fruits, high seed content, and high yielding variety. There is limited scope of conventional breeding techniques in improvement of lychee due to self-incompatibility, to long juvenile period, and to heterozygous nature. Biotechnology can complement conventional breeding and enhance the lychee improvement programs. Studies involving in vitro culture, screening, micropropagation, embryo rescue, genetic transformation, marker-assisted characterization and DNA fingerprinting, and QTL are underway at different centers worldwide for the improvement of lychee. In vitro culture, callus induction, cell suspension culture, and somatic embryogenesis of several different genotypes have been achieved. Protocols for protoplast culture and somatic hybridization of protoplast of lychee and longan have also been achieved. Isozyme markers and DNA markers offer means for gaining more insights in the genetics of the crops and identifying genes that could lead to accelerate lychee improvements. Genetic transformation using *Agrobacterium tumefaciens* and physical and chemical methods has been reported. Genes that are involved with fruit ripening, disease

D.K. Pandey (✉) • J. Singh
Department of Biotechnology, Lovely Faculty of Technology and Sciences,
Lovely Professional University, Phagwara 144411, Punjab, India
e-mail: dkpandey1974@yahoo.com

A. Dey
Department of Life Sciences, Presidency University,
86/1 College Street, Kolkata 700073, West Bengal, India

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resistant, and enhancement of shelf life have been cloned, and there have been attempts to deliver these genes into plants. Transgenic lychee was developed by introducing the SAMDC genes from *Datura stramonium* responsible to increase the shelf life. Studies on genetic diversity of lychee cultivars by molecular markers are found to be effective in comparison to morphological markers. The purpose of this review is to focus upon contemporary information on biotechnological advances made in lychee by overcoming the problems encountered during in vitro propagation, generation of disease resistant, and enhanced shelf life cultivars.

Keywords
Litchi/lychee • Heterozygous • QTL: transgenic • Morphological markers • Disease resistant

3.1 Introduction

Lychee (*Litchi chinensis* Sonn.), known as "the queen of fruit" (Menzel and Waite, 2005), is an important fruit crop, originated in China and widely cultivated in the tropical and subtropical regions of the world (Menzel 1983). It belongs to family Sapindaceae of order Sapindales and class Magnoliopsida. The cultivable lychee is *Litchi chinensis* subsp. *chinensis*, while wild are the *Litchi chinensis* subsp. *philippinensis* and *Litchi chinensis* subsp. *javensis.* Lychee fruit has a succulent edible aril surrounded by a thin, leathery, and indehiscent pericarp and a large dark brown seed. China is the leading producer of lychee followed by India (Huang et al. 2005a, b). Lychee fruit has gained popularity as an exotic fruit in temperate regions and is prized on world markets with strong demand for its desirable flavor and semitranslucent to white aril (Jiang et al. 2002). The presence of vitamin C, anthocyanin, polyphenols, minerals, and amino acids in the fruits of lychee makes it beneficial to human health and in the treatment of various diseases. The fruit has been used to cure neural pain and swelling traditionally in Chinese medicinal formulation. In recent years, the extract from lychee fruit, including aril, pericarp, and seed, has exhibited excellent anti-oxidant ability and good anti-tyrosinase and anti-cancer activities. Thus, lychee fruit can be used as a potential source of natural anti-oxidants and can be exploited by food or pharmaceutical industries.

The efficiency of conventional breeding programs in lychee is generally limited due to high degree of heterozygosity, short life span of lychee seeds, long generation period, and little knowledge of the genetic background. Chapman (1984) used air-layering or marcottage method of vegetative propagation which are not effective and applicable. Thorpe (1990) reported that root cutting is often characterized by a rapid loss of rooting capacity of the cutting with increasing age of parent plant. The planting of extensive new orchards of vegetatively propagated clones of some tropical fruits has sometimes been limited by pathogens. This leads researchers to explore and develop a variety possessing desirable horticultural attributes such as

good fruit quality, increasing yields, disease resistance, longer shelf life of fruits, high vitamin C and pectin content, good aroma, attractive skin, flesh color, and small or no seeds. Such an ideal phenotype cannot be met by conventional breeding and hampers breeding programs due to floral structure (epigynous flower, with abundant incurred stamens of various sizes), long juvenile period, self-incompatibility, and heterozygous nature (Amin and Razzaque 1992). Lychee fruits that have aborted seeds are preferred by consumers since they have a high flesh to seed ratio. When cultivars, having a strong tendency to produce fruits with abortive seeds, are chosen as parents in hybridization breeding, few fertile seeds can be harvested, thus making crossbreeding very difficult. Despite these problems, a few successful reports on lychee breeding have also appeared (Ribeiro and Pommer 2004; Pommer and Murakami 2008). In addition, the world lychee trade is narrowed to a great extent owing to the rapid perishable nature of fruits (Lizada 1993). Lychee is a climacteric fruit and long-distance transport is sometimes a problem. The main postharvest problems of litchi fruit are loss of red color by high dehydration rates and browning of the rind. Worldwide, one of the most common postharvest diseases of lychee is anthracnose, caused by the fungal pathogen *Colletotrichum gloeosporioides* and occasionally *C. acutatum* (Coates et al. 2005). Browning of lychee fruit was caused by *Bacillus* and *Lactobacillus* and many other pathogens affecting the significant production and postharvest problem (Dodd et al. 1998).

Clonal propagation using cell, tissue, and organ culture techniques has considerable potential for the improvement of economically important trees within a limited time frame (Giri et al. 2004; Singh et al. 2004). In this context, protoplast-based technologies, such as somatic hybridization, cytoplasmic recombination, micronucleus transfer, direct DNA uptake, transformation, and mutation selection, may offer new possibilities for breeding. However, to apply these technologies, a regeneration system for protoplasts is often a prerequisite. In lychee, callus induction, somatic embryogenesis, and plant regeneration from immature zygotic embryos and anthers have been reported (Yu and Chen, 1997; Zhou et al. 1996; Kantharajah et al. 1992; Fu and Tang, 1983). Yu and Chen (1998) reported the development and maintenance of highly embryogenic suspensions and protoplast isolation for several lychee cultivars.

This chapter not only highlights the major biotechnological advances made in lychee during past years but also suggests how present technologies in tissue culture and genetic engineering might affect the direction of future research. Moreover, molecular methods are useful for taxonomical characterization to understand the regulation and expression of important traits/genes, phenotypic and genotypic traits. The attempted and potential biotechnological interventions are depicted in Fig. 3.1.

3.2 Taxonomy, Origin, and Distribution of Lychee

All the cultivars of the lychee are native to Asia. Many researchers found out that China is the place where lychee was originated. Xu and Peng (1964) and Fu and Yuan (1983) confirmed that wild litchi was found in the middle-south of Hainan.

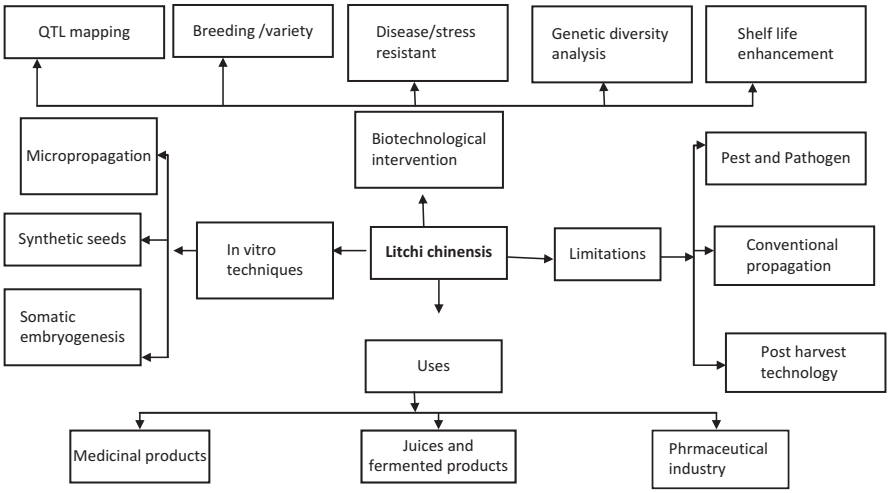

Fig. 3.1 Biotechnological interventions in different sectors

The extensive work on origin of lychee was further confirmed in the east-south of Guangxi (Zhong and Chen 1994), south Yunnan (Pei 1974), and the west-south of Guangdong. Some authors (Peng et al. 2005) suggested that wild litchi growing in tropical and subtropical rain forest not only contains primitive litchi gene but also should be treated as a separate ancient species due to long period of community succession and natural selection. In Table 3.1, the distribution of lychee cultivars was discussed.

3.3 Phytochemistry and Pharmacology

3.3.1 Phytochemicals

Phytochemical screening of numerous types of extracts (crude and/or fractioned) obtained from different plant parts of lychee revealed the presence of myriads of components like carbohydrates, phenolics, flavonoids, terpenoids, vitamins, and amino acids along with coumarins, lignans, sterol, proanthocyanidins, and anthocyanins (Irene et al. 2012). The most important flavonoids present in leaves and flowers are epicatechin and proanthocyanidin A2, two major compounds contributing toward anti-oxidant efficacies of lychee plant extracts (Yang et al. 2012; Hwang et al. 2013; Castellain et al. 2014). Other flavonoids detected via spectroscopic and NMR techniques are quercetin, phlorizin, onychin, nairutin, narcissi, kaempferol-7-*O*-*β*-D-glucopyranoside,2*S*, pinocembrin-7-*O*-(6″-*O*-*α*-L-arabinosyl-*β*-D-glucopyranoside), pinocembrin-7-*O*-glucoside,pinocembrin-7-*O*-[(6″-*O*-*β*-D-glucopyranoside)-*β*-D-glucopyranoside], and pinocembrin-7-*O*-[(2″,6″-di-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside] (Shen et al. 2013). D-mannitol, 2,5-dihydroxybenzoic acid, delphinidin 3-*O*-β-galactopyranoside-39,59-di-*O*-β-glucopyranoside,

Table 3.1 Production and distribution of lychee cultivars

Country	Production (tonnes)	Provinces	Cultivars	References
China	950,000	Guangdong	Baila, Baitangying, Heiye, Feizixiao, Gwiwei (M), Nuomici (M)	Wu (1998)
		Fujian	Lanzhu, Souey Tung, Haak Yip, Tai So, and Brewster	Chen and Huang (2001)
India	429,000	Bihar, West Bengal Uttar Tripura, Orissa, Punjab, Himachal Pradesh, Assam, and the Nilgiri Hills in the south	Shahi, Bombai, China, Deshi, Calcutta, Rose Scented, and Mazaffarpur	Ghosh et al. (2001)
Thailand	85,083	Lowland lychee or the tropical lychee	Haak Yip, Tai So, and Wai Chee (locally known as Baidum, Hong Huey, and Kim Cheng)	Phun and Dhu (2000)
		Subtropical group of lychee grows	Haak Yip, Tai So, and Wai Chee (locally known as Baidum, Hong Huey, and Kim Cheng)	
Vietnam (Hanoi)	27,000	Ha Tay Province and Ha Giang	The main cultivars are Thiew Thanh Ha (90%), hybrid lychee, and Phu Ho	Trung (2000)
Taiwan	108,668	Taiwan Province	Hap Ip, Yu Her Pau, No Mi Tsu	Yen (2001)
Bangladesh	12,755	Dinajpur, Rangpur, and Rajshahi districts		
Australia		Northern Queensland	Fay Zee Siu, Tai So, Bengal, Wai Chee, Kwai May Pink, and Salathiel	Menzel (2000)
Madagascar, South Africa, Mauritius		Transvaal-Lowveld Region (SA); east coastal belt (Madagascar)	Tai So	
USA		Hawaii, Florida	Tai So, Kaimana Brewster	

and delphinidin 3-*O*-β-galactopyranoside-39-*O*-β-glucopyranoside were isolated from alcoholic fractions and extracts of litchi fruit (Lee et al. 2009). Numerous volatile compounds have been isolated from *L. chinensis* among which geraniol, linalool, *β*-citronellol, *α*-terpineol, *p*-cymene, and 1-octanol are important (Mahattanatawee et al. 2007).

3.3.2 Pharmacology

3.3.2.1 Anti-oxidant Activity

Fractions of *lychee* fruit polysaccharides exhibited strong scavenging activity against DPPH, superoxide, and hydroxyl radicals (Kong et al. 2010). The acetone extract and ethyl acetate fraction of lychee flower showed DPPH radical scavenging activity and inhibited low-density lipoprotein (LDL) oxidation (Yang et al. 2012). Aqueous extract of lychee fruit has shown increased anti-oxidative properties against liver damage or altered level of serum lipids and MMP-9 activities in hamsters consuming high-fat diet (Chang et al. 2013).The aqueous and organic (methanol, 1-butanol, and ethyl acetate) extracts of leaves of *Litchi chinensis* have shown significant anti-oxidative efficacy using the ABTS (2,2′-azinobis (3-ethylbenzothia zoline-6-sulphonic acid)) decolorization assay, the ferric reducing anti-oxidant power (FRAP) assay, the DPPH (2.2′-diphenyl-1-picrylhydrazil) assay, and the total phenolic content (TPC) assay (Castellain et al. 2014). Comparative antiradical activity among three cultivars of lychee, viz., Hemaoli, Feizixiao, and Lanzhu, has showed strong DPPH and ABTS free radical scavenging except the Lanzhu cultivar with relatively low efficacy (Lv et al. 2014).

3.3.2.2 Hepatoprotective Activity

The chloroform and methanolic leaf extracts have shown hepatoprotective activity against paracetamol-induced liver damage by altering level of serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total protein, bilirubin, cholesterol, and triglycerides and liver biochemical parameters such as lipid peroxidation, reduced glutathione (GSH) content, and catalase (CAT) (Basu et al. 2012). Histopathological and immunohistochemical assays conducted on male Wistar albino rats have shown protective effect of *lychee* pulp extract against CCl_4-induced hepatotoxicity when compared to standard drug silymarin (Bhoopat et al. 2011), and aqueous extract of pulp (500 mg/kg) has shown protective activity comparable to the drug LIV-52 (Souza et al. 2006).

3.3.2.3 Anti-inflammatory and Analgesic Activity

The phytochemical analysis of hydroalcoholic extract of *Litchi chinensis* leaves (HLCL) has shown presence of terpenoids, flavonoids, phenols, tannins, and saponins, and anti-inflammatory activity was evaluated by carrageenan-induced paw edema model in rats observed after 4 h of carrageenan administration, and analgesic activity was evaluated by acetic acid-induced writhing test and hot plate method in mice (Chauhan et al. 2014). The effects of flavonoid-rich fruit extract decreased the

mRNA and protein expression of the iNOS gene, suppressed NO production, inhibited the phosphorylation of NF-kB inhibitor, and reduced the mRNA levels of NF-kB target gene, TNF-α (Yamanishi et al. 2014).

3.3.2.4 Cardiovascular Activity

Aqueous extract of flower exhibited cardioprotective efficacy based on lipid homeostasis in the high-fat/high-cholesterol dietary hamsters by enhancing the overall trolox equivalent anti-oxidant capacity (TEAC) of the serum, thus lowering serum lipid peroxidation [malondialdehyde (MDA) content]. The extract also normalized gene expression of LDL receptor gene, whereas it downregulated expression of FAS gene and upregulated peroxisome proliferator-activated receptor alpha (PPAR-α) gene expression (Yang et al. 2010).

3.3.2.5 Anti-lipase Activity

The anti-lipase activity of water extract of *lychee* flower has been evaluated in hypercaloric diet-induced rats, which has reduced the sizes of livers and perirenal and epididymal adipose tissues and normalized level of cholesterol and serum liver lipid. The significant alteration in liver tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) values was found to be ameliorated by aqueous extract of flower which has been identified to possess a wide range of botanicals (Wu et al. 2013).

3.3.2.6 Cytotoxicity

Aqueous extract of lychee flower was evaluated against heavy metal-induced hepatocytotoxicity. It has caused dose-dependent decrease of the Cd- and Pb-induced lipid peroxidation and DNA fragmentation, suppressed transforming growth factor b1 (TGF-b1)-induced activation of hepatic stellate cells (HSCs), downregulated expression of smooth muscle a-actin (aSMA), and increased cell viabilities (Hwang et al. 2013).

3.3.2.7 Aldose Reductase Activity

Alcoholic fruit pulp extract of lychee and delphinidin 3-*O*-β-galactopyranoside-39-*O*-β-glucopyranoside isolated from this extract showed remarkable in vitro inhibition of rat lens aldose reductase (RLAR) activity (Lee et al. 2009).

3.3.2.8 Anti-viral Activity

Oligonol, a purified phenolic compound isolated from *Litchi chinensis* fruit pulp, inhibited the replication of *Betanodavirus* of family Nodaviridae, which is the causal organism of viral nervous necrosis (VNN) disease of marine farmed fishes. Oligonol also inhibits attachment of the virion to the cell (Ichinose et al. 2013).

3.3.2.9 Nootropic Activity

Aqueous and alcoholic fruit extracts of *L. chinensis* were evaluated for nootropic activity in three models, namely, passive avoidance model (PAM), diazepam-induced amnesia model (DAM), and sodium nitrite-induced hypoxia model (SHM) in mice using piracetam as standard compound (Irene et al. 2012).

3.3.2.10 Antimicrobial Activity

The seed extract of *L. chinensis* exhibited significant inhibitory activity against gram-negative bacterial strain of *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* and gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (Singh et al. 2013). Aqueous pericarp extract of this plant also possesses antimicrobial efficacy which was examined by agar well diffusion method in vitro against *Salmonella typhi*, *Vibrio cholerae*, *Shigella dysenteriae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans* (Putta and Sastry 2014).

3.3.2.11 DNA Protective Activity

Lychee pericarp extract fermented with *Aspergillus awamori* showed enhanced DNA protection and anti-oxidant activity detected via DNA cleavage assay (Lin et al. 2012).

3.3.2.12 Anti-cancer Activity

Antiproliferative activity of pericarp extract of *L. chinensis* was observed against both positive and negative breast cancer cells in vitro by upregulation (CYP1A1 and ADPRTL1) and downregulation (BIRC3, ADAM9, and HMMR) of multiple genes and exhibited apoptosis induction and a strong inhibitory action in vivo in human estrogen receptor (ER) cell line (Wang et al. 2006a, b).

3.4 Problem to Be Addressed in Lychee Production

In spite of being an economically and ecologically important plant, lychee which is originated from China is now cultivated in tropical and subtropical part of the world. There are many problem associated with lychee production in spite of its huge demand in international market such as pre- and postharvest management, biotic and abiotic stresses, fruit quality, conventional breeding, and in vitro propagation. Some important aspects which need to be addressed immediately are summarized.

3.4.1 Quality of Lychee Fruit

3.4.1.1 Color of Fruit

Lychee is non-climacteric fruit as is ripened on trees only and physiological maturity before harvest for proper quality and shelf life is utmost important (Chen et al. 2001). Red cultivars, due to the presence of anthocyanin, are the most demanding among all the color varieties of lychee in international markets. Cyanidin-3-rutinoside and cyanidin-3-glucoside have been reported as the major pigments in the pericarp (Lee and Wicker, 1991; Rivera-López et al. 1999). Reichel et al. (2010) reported the impact of picking maturity, i.e., lychee maturity index (LMI) and postharvest litchi color index (PLCI) on pericarp color, eating quality, and shelf life of cold-stored lychee fruit which was evaluated for the Thai cultivars ‘Hong Huey’ and

'Chacapat.' The main postharvest problems of lychee fruit are loss of red color by high dehydration rates and browning of the rind (Scott et al. 1982; Underhill and Critchley 1994). The main reason behind browning of fruit was enzymatic degradation by anthocyanase of the anthocyanins to anthocyanidins (Holcroft and Mitcham, 1996; Zhang et al. 2000; Jiang et al. 2004) and further degradation of anthocyanidins and phenolic compounds to o-quinones by polyphenol oxidases (PPO) and/or peroxidases (POD).

The rapid postharvest browning of pericarp reduced the commodity value of the fruit and limited the expansion of the lychee trade and was considered as the most important limitation to the continued development of the lychee industry. Many conventional postharvest management has been implemented which control the activity of PPO expression such as ethylene treatment and fungicides. The browning of the lychee pericarp is slowed down by controlling the PPO activity, which is inhibited by fruit treatments such as cold storage, chemical preservative, and coating. In the era of genomics, biotechnological intervention can be the best method to improve the quality of fruits. Inhibition of PPO gene expression in plant tissues reduces the PPO activity and tissue browning. Already the lychee PPO gene, LcPPO, was cloned in order to investigate the relationships among LcPPO expression and PPO activity in many lychee cultivars and postharvest browning process (Wang et al. 2014).

3.4.1.2 Fruit Size and Fruit Number of Lychee

Li et al. (2010) documented various extrinsic, i.e., pruning and girdling, nutrient, temperatures, and water regime, and intrinsic factors, i.e., genetic characteristics, pericarp development, cytology, phytohormones, and plant regulators. Li et al. (2004) in his studies on 'Feizixiao' lychee determined that the unsynchronized flowering is responsible for uneven fruit sizes. Li et al. (2002) and Rue et al. (2012) finally concluded it is the cell number not cell volume which play crucial role in fruit size on his studies on activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR, EC: 1.1.1.34). Two HMGR genes (LcHMG1 and LcHMG2) were isolated from fruits of a subtropical fruit crop lychee, and their expression profiles were compared between fruits of different phenotypes or genotypes. It was found that LcHMG genes are the key factor affecting the final fruit size in early fruit growth, and LcHMG2 was associated with biosynthesis of isoprenoid compounds required for later cell enlargement.

3.4.2 Conventional Propagation Method

Lychee is openly pollinated and highly heterozygous and is not propagated from seed. Seedlings are genetically diverse and have a long juvenile period and poor fruit quality (Loebel, 1976; Pandey and Sharma, 1989). Some cultivars are highly sensitive to moisture content, and their life spans are very short which was presumably the key factor responsible for slow spread of lychee from China (Menzel 1985; Ray and Sharma 1985, 1987; Nijjar 1981). Furthermore lychee recalcitrant seeds

(Hanson 1984; Chin et al. 1984) rapidly lose their germination capacity in the open environment (Kumari-Singh and Prasad 1991; Xia et al. 1992a, b; Prasad et al. 1996). Seeds are planted for the selection and breeding purposes or for rootstocks. Development of new cultivars is a slow process because it may take up to 10 years or even more before the trees begin to bear fruit (Hamilton and Yee 1970; Menzel 1983).

The most common asexual mode of propagation of elite lychee cultivars are grafting, stem cutting, air layering or marcotting, and budding (Menzel 1985; Pandey and Sharma 1989; Ram and Majundar 1981), but the problem was the depletion of branches in great numbers from the mother trees (Ray and Sharma 1987) affecting the fruit production but not the quality. Also conventional vegetative propagation methods are slow and inefficient (Chapman 1984) but still a widespread practice in many countries. Pandey and Sharma (1989) have used auxins (IBA and NAA) to promote root initiation in stem cuttings. The alternative approach to vegetative propagation is in vitro culture and regeneration of litchi plants for mass-scale propagation of desirable genotypes.

3.4.3 Breeding

Lychee is an openly pollinated tree and has a long juvenile period (7–8 years). Litz and Raharjo (2005) proposed that fruit size, quality, maturation period, dwarfness, regularity of bearing, wider adaptability, tree characters and resistance to physiological disorders, shelf life, disease resistance, higher yield, annual bearing, and seedlessness are the primary breeding objectives of lychee. Clone can be produced by asexual means, while variations would arise only through sexual reproduction. Thus, there is an urgent need for breeding work and raising plants through seeds. But the main problem with the lychee is that the seeds are recalcitrant which make breeding programs difficult. Efforts are in progress to produce disease-resistant, fast-growing varieties having strong adaptability and higher content of nutritional substances, aroma (flavor), and biotic and abiotic resistance through conventional and molecular breeding and to harness maximum economic gains from lychee. The conventional breeding programs for improving quantitative traits are labor intensive and time-consuming. Marker-assisted selection (MAS), which uses DNA markers, is an excellent tool for selecting beneficial genetic traits for lychee. Thrust is given more on the development of QTLs for quality characters of lychee.

3.4.4 Flowering in Lychee

The inflorescence of lychee is panicle and has male, partial male, and partial female flowers. When the cultivars and environment are controlled, the partial female flowers could blossom (Stern and Gazit 2003). The pseudohermaphrodite flower number (Chang and Lin 2008; Hieke et al. 2002; McConchie and Batten 1991; Menzel 1984; Nakasone and Paull 1998; Roe et al. 1997), high embryo sac abortion rate

(Cheng 2003; Shih and Chen 2006), and nutrient set (Teng 1996) are responsible for fruit yield in lychee cultivar 'Yu Her Pau' from Taiwan. Lee (2006) and Lin (1994) proposed that pruning would increase yield. Although pruning reduced the size of inflorescences and nutritional competition and increased fruit set, the larger inflorescences would bear more "female flowers" that could set (Chadha and Rajpoot 1969; Menzel and Simpson 1992; Nakasone and Paull 1998; Stern et al. 1996). Also temperature plays a crucial role in the number of pseudohermaphrodite flower (functional female flower) which is higher in late inflorescence (smaller in size) than the early inflorescence (larger in size) (Chen and Cheng 1996, 1997; McConchie and Batten 1991; Menzel and Simpson 1992; Teng 1999 and Lee 2004; Cheng 2003; Shih and Chen 2006; Stern et al. 1996) and also influences pollen germination and fertilization (Chen and Weng 2000; Lee 1992; Lin 1994). High yield is achieved with a balance between fruit set and fruit retention.

3.4.5 Abiotic (Environmental Factors) and Biotic Stress (Insect Pest and Pathogen)

3.4.5.1 Abiotic Stress

Lychee is highly influenced by the growing environment and nutrition. Abiotic stresses lead to morphological, physiological, and biochemical changes that adversely affect plant growth and fruit production. The adverse climatic conditions, i.e., frost and salt stress, induce cellular damage and cause a reduction in the physiological growth processes in the plant body. Babita and Kumar (2008) reported the effect of overfertilization on the loss of the leaves. Heat stress, i.e., high temperature, can cause significant damage to shoot and root growth inhibition, sunburn on leaves and maturing fruits, drying of branches and stems, anticipated leaf senescence and abscission, reproductive development, extent of pollination, fruit discoloration, and damage including fruit cracking, fruit drop, and retarded/underdeveloped fruits (Menzel and Waite 2005; Menzel and Paxton 1986). The mechanisms of the physiological basis of abiotic stress tolerance/resistance or to avoid/escape the stress need to be understood using biotechnological tools.

3.4.5.2 Biotic Stress

3.4.5.2.1 Insect Pest

A considerable fruit loss is caused by insects and birds. Vevai (1971) reported 40 insect species on lychee, out of which deleterious insects were eriophyid mites (*Aceria litchii*, syn. *Eriophyes litchii*) and bark-eating caterpillars (Butani 1977), scale insects, leaf miners, bugs, weevils, fruit and seed borers, and eriophyid mite (Das and Chowdhary 1958; Chang 1970; Villiers and Mathee 1973; Rai and Bhandary 1973). Huang et al. (2005a, b) reported 193 species of lychee pests in China which include fruit and flower borers, stem borers, fruit-piercing moths, defoliators, sucking bugs, and erinose mites. The lychee fruit borers (or litchi stem-end borers) *Conopomorpha sinensis* Bradley and *Conopomorpha litchiella* Bradley

(Lepidoptera: Gracillariidae) (Yu et al. 1995; Chen and Yao 2001); lychee longhorn beetles (also known as lychee bark miners) *Aristobia testudo* (Voct), *Arbela dea* (Swinhoe), and *Arbela baibarana* (Mats) (Coleoptera: Longicornidae) (Tan et al. 1999; Huang et al. 2005a, b); leaf-eating chafer beetles (Coleoptera: Scarabaeidae) and chrysomelid beetles (Coleoptera: Chrysomelidae) (Pu et al. 1992; Huang et al. 2005a, b); geometrid moths *Thalassodes immissaria* (Walker) and *Buzura suppressaria* (Guenée) (Lepidoptera: Geometridae); etc., cause damage when the larvae eat fresh shoots, leaves, flowers, and young fruits (Zhou and Deng 2006; Chen et al. 2010). Lychee leaf gall midges, *Mayetiola* spp. (Diptera: Cecidomyiidae), and the eriophyid mite *Aceria litchii* (Kiefer) (Acari: Eriophyidae) cause leaf galls (known as "erinose" galls) and leaf loss (Yu et al. 1995; Shen 2006; Su et al. 2004). However, the most common and most significant pest in terms of pesticide usage is the lychee stink bug, *Tessaratoma papillosa* Drury (Hemiptera: Pentatomidae). It is highly mobile and occurs in all litchi orchards in southern China and must be closely monitored and controlled during almost the entire year (Poo et al. 1965; Poo 1992; Chiu and Chen 1987; Chen 2009). The total annual loss of lychee to *T. papillosa* is 30,000–60,000 tons, equivalent to 0.2–0.4 billion (He et al. 2001).

3.4.5.2.2 Pathogen

Fungal diseases, like dieback, leaf spots, and leaf blight caused by *Phomopsis* sp. and by *Gloeosporium* sp. and leaf diseases by *Colletotrichum* are responsible for huge loss of litchi (Johnson and Sangchote 1994). Gupta et al. (1997) reported red rust by *Cephaleuros virescens (algae)* on stem and leaf causing canker in fruit of lychee in the Kangra Valley of Himachal Pradesh (India). Postharvest diseases by fungal pathogens, i.e., *Aspergillus*, *Penicillium*, *Botryodiplodia*, *Pestalotiopsis*, *Fusarium*, *Trichoderma*, etc., after fruit harvest in several countries were reported (Pandey and Sharma (1989) and Underhill et al. (1997)). Roth (1963) reported yeasts and bacteria are also damaging lychee fruits. Physical damage of fruits, i.e., micro-cracks during fruit development and postharvest handling, can provide a port of entry for decay pathogens that colonize the fruit surface (Sivakumar et al. 2005). In Guangdong and Hainan (China), Liu et al. (2006) reported *C. gloeosporioides* as the main pathogen causing postharvest decay, arising predominantly from fruits with latent infection prior to harvest.

The predominant fungal genera associated with lychee in South Africa were *Phomopsis*, *Pestalotiopsis*, *Penicillium*, *Trichoderma*, *Alternaria*, *Botryosphaeria*, and *Fusarium* spp. (DeJager et al. 2003). *Penicillium* spp. and *Alternaria alternata* have been isolated before and after harvest from lychee (Jacobs and Korsten 2004).

The control of postharvest decay and browning of lychee fruits was done by application of sodium metabisulphite dip followed by hydrochloric acid dip or vitafilm (Duvenhage 1993), benomyl, prochloraz, imazalil (Pandey and Sharma 1989; Underhill et al. 1997), refrigeration, heat treatment, and orchard hygiene.

Biotechnological method complemented with integrated pest management appears a sound future prospect that emphasizes the need for development of transgenic lychee trees resistant to insect pests. Not only genes encoding proteins and

amylase inhibitor have been isolated and cloned, but also transgenic crops using these genes have been developed (Chrispeels et al. 1998; Ussuf et al. 2001). Though the diseases are controllable by fungicides, considering the cost and safety of the application, resistant varieties offer a viable alternative. Some attempts to produce transgenic lychee using transformation of zygotic embryos have been attempted. Das and Rahman (2010) reported on the use of *Agrobacterium* method to overexpress bacterial chitinase gene (ChiB) in *Litchi chinensis* cv. 'Bedana.' The transgenic plants that exhibited higher chitinase activity than non-transgenic plants showed increased resistance to dieback, leaf spot, and blight.

3.4.6 In Vitro Propagation

Lychee crop improvement by biotechnological methods requires the development of an efficient regeneration protocol which is jeopardized by phenol exudation, medium discoloration, explant browning, deep-seated systemic contamination, and in vitro recalcitrance of tissues. The problem associated with in vitro culture and solution to overcome was discussed below.

3.4.6.1 Phenol Exudation, Media Browning, and Explant Necrosis

Lychee has been a hard-to-deal crop compared to most other horticultural crops. Several attempts have been made to regenerate lychee using leaf and shoot explants other than zygotic embryo and anther due to the availability of these parts all around the year. But the major challenges are high phenolic exudation which leads to explant browning, deep-seated contamination, medium discoloration, and slow in vitro growth which can be overcome by pretreatment of leaf explants using liquid shaker culture (Kumar 2001). Sodium hypochlorite (1.5% and treatment time of 15 min) including Tween 20 proved to be the best all-round sterilant for the successful sterilization of young leaf explants of lychee. Browning can be controlled by the use of a combination of ascorbic acid (225 mgL^{-1}) and citric acid (225 mgL^{-1}) (Kantharajah et al. 1992; Das et al. 1999a, b; Amin and Razzaque 1995), PVP, or activated charcoal (Puchooa 2004).

3.4.6.2 Latent Systemic Contamination

Latent fungal infections reported in lychee are *Alternaria alternata* (Prusky et al. 1983), *Colletotrichum* spp. (Peterson 1986), *Dothiorella* spp. (Johnson et al. 1991), *Fusarium subglutinans* (Ploetz 1994), and *Phomopsis litchi* (Kumar 2006) which are present in the shoot tips and in different parts of the panicle (Kumar 1983) affecting the culture's rapid growth and development within host tissues. Thomas and Ravindra (1997) and Chandra et al. (2004) tried without success to remove deep-seated endogenous contamination by frequent sterilization. The application of antibiotics (Reuveni and Golubowicz 1997), imidazole sprays, Bavistin, and streptomycin (Hare Krishna 2006) reduces the infection.

3.4.6.3 In Vitro Recalcitrance

Recalcitrance is the inability of plant cells, tissues, and organs to respond to tissue culture. Tissue culture responses are greatly influenced by three main factors, viz., "whole plant" physiology of donor, in vitro manipulation, and in vitro stress physiology (Benson 2000). Maneuvering different components, such as inorganic, organics, amino acids, enzymes, phytohormones, carbon source, gelling agents, and other media additives, helps alleviating recalcitrance. One of the most important approaches for overcoming this problem is to optimize the plant growth regulator regime (Gaspar et al. 1996). Sowa and Roos (1991) stored lychee by application of nitrous oxide (N_2O) which act as an anesthetic agent.

3.4.7 Morphological Markers

There has been a great degree of confusion in the nomenclature of lychee varieties due to the use of synonyms for a single cultivar, which add difficulties in identifying varieties. Moreover, for efficient and effective utilization of plant genetic resources, the characterization of germplasm is inevitable. Though morphological markers have been in use to assess the genetic diversity, they had limited application in breeding as they are few in numbers as well as season and developmental stage specific/dependent. The tree phenotype is influenced by environmental factors (Anuntalabhochai et al. 2002) reflecting inefficiency of morphological traits for identification of desired cultivars. Moreover, there is widespread confusion over the identities of lychee cultivars that have been identified only by their morphological traits (Degani et al. 2003). Some researchers successfully identified the desired cultivars (Chen et al. 2004 and Wang et al. 2006a, b), but majority failed (Ding et al. 2000; Yi et al. 2003; Liu and Mei 2005; Menzel et al. 2005). The rich germplasm available in Southeast Asian countries have provided interesting plant material for lychee breeding programs, especially in China and also in different countries with subtropical climates mainly South Africa, Israel, the USA (Hawaii and Florida), and Australia (Menzel et al. 2005 and Sarin et al. 2009). However, the mislabeling of litchi cultivar names and/or homonymies (cultivars under the same name with different genetic profiles) and synonymies (cultivars under different names with the same genetic profile) in different places and even within a given country is an obstacle to design appropriate breeding programs, to optimize lychee germplasm management, and to compare the results obtained in different litchi-growing countries. To overcome the abovementioned problem, the molecular marker-based identification of cultivars will be the only solution for screening the elite cultivars for mass propagation and QTL.

3.5 Achievements Made in Lychee Through Modern Biotechnological Tools

The efficient regeneration of plants from cell, tissue, and organ culture is recognized as prerequisite for application of most modern genetic and biotechnological approaches to crop improvement (Litz and Gray 1992). Several workers have recognized that the two patterns of in vitro differentiation, i.e., organogenesis and somatic embryogenesis, are distinctly different process.

3.5.1 In Vitro Culture of Lychee

Lychee is difficult to propagate using in vitro techniques, but there would be many advantages in clonal propagation since traditional propagation methods are slow and inefficient (Chapman 1984). In recent years, several reports have been published on regeneration of lychee through organogenesis and somatic embryogenesis (Tables 3.1, 3.2).

3.5.1.1 Direct Organogenesis and Callus-Mediated Organogenesis

Anther Culture

Fu and Tang (1983) attempted anther cultures of two lychee varieties. In their work on lychee anther culture, Fu and Tang (1983) used 2,4-D and NAA for callus induction and a different cytokinin (kinetin) but the same auxin (IAA) for regeneration. The results of this study, therefore, show that, although auxin (2,4-D) on its own can be used for callus initiation in lychee, a low concentration of 2,4-D in combination with cytokinin (BAP) was more appropriate than subsequent proliferation and differentiation of shoots from such calli using again BAP but in combination with IAA. While haploid plants have little use, the potential from this technique does exist if diploid plants could be regenerated from haploid callus. There are many problem associated with raising plants by pollen culture such as heterogeneous nature of pollens in anther leads to chimeric plants. The regeneration frequency of the two cultivars used by Fu and Tang (1983) was very low.

3.5.1.2 Embryo Culture

Kantharajah et al. (1992) attempted in vitro methods on different lychee cultivars, i.e., Bengal, Kwai May Pink, and Wai Chee, by rescuing and culturing immature embryos and achieving multiplication through induction of adventitious buds from the embryonic shoots. One of the problems encountered by these workers was the browning of very young tissues. Although no plantlets were obtained, Kantharajah et al. (1992) believe that the induction of multiple shoots from lychee provides one method of clonal propagation and, if successfully applied, could produce up to 15 plants from a single embryo. Das et al. (1999a, b) managed to induce multiple shoot in five genotypes of *Litchi chinensis* by direct germination of lychee seeds in MS liquid medium supplemented with benzyl adenine (BA) (20 mgL^{-1}) and supported on filter-paper bridge. Contamination and browning were again the major problems

Table 3.2 Clonal propagation of lychee through in vitro shoot proliferation

Clone	Explant	Mature/juvenile	Results	Medium + PGR shoot multiplication	Rooting	References
Bengal, Kwai May Pink, and Wai Chee	Embryonic shoots	Juvenile	Adventitious shoots formation 15 plants from a single embryo	MS + 2% sucrose + 5 mg l^{-1} benzyl aminopurine (BAP) or MS+ 100 or 50 mg 1^{-1} kinetin or MS+ 50 mg 1^{-1} 2-isopentenyl adenine (2iP)	MS salts + 2% sucrose, 0–8% agar, and 0–5 mg 1^{-1} NAA either with or without 1% activated charcoal	Kantharanjah et al. (1992)
Litchi chinensis Sonn.	Seeds and axillary buds		Multiple shoot induction and plant	Seeds + 6-benzylaminopurine (20 mg l^{l}) + MS liquid medium +6-benzylaminopurine (100 µg on alternate days) of the axillary bud regions of plants	The shoots elongated and rooted directly in vermiculite after a pulse treatment with IBA (25 mg/ml) for 15 min	Das et al. (1999a, b)
Litchi chinensis Sonn.		Juvenile	Tissue culture of lychee (*Litchi chinensis* Sonn.)			Anon (1991)
Litchi chinensis Sonn.	Pollen	Mature	Introduction pollen plants of litchi tree (*Litchi chinensis*)			Fu and Tang (1983).
Xiafanzhi' and 'Chen Zi'	Zygotic embryo	Microcalli induction	Embryogenic suspension culture and protoplast isolation in lychee	MS basal and solid media containing silver thiosulfate	NA	Yu and Chen (1998).

'Huaizhi'	Leaf explants	Mature	Leaf callus induction and suspension culture establishment in lychee (*Litchi chinensis* Sonn.) cv. 'Huaizhi'	MS media + 2 mg/L 2,4-D, 0.5 mg/L NAA, 2 mg/L KT, and 200 mg/L AC	After the leaf calli were transferred and subcultured 2–3 times on the MS medium with IAA and BAP, friable calli were obtained	Ma et al. 2009
	Nodal explant	Mature	Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.)	(1) Direct germination of litchi seeds in 6-benzylaminopurine (20 mg L^{-1}) supplemented with MS liquid medium and supported on a filter-paper bridge and (2) in planta treatment with 6-benzylaminopurine (100 µg on alternate days) of the axillary bud regions	The shoots elongated and rooted directly in vermiculite after a pulse treatment with IBA (25 mg mL^{-1}) for 15 min	Khan and Ahmad 2005
'Xiafanzhi' and 'Chen Zi'('Brewster')	Zygotic embryo and anther	Juvenile	Induction of litchi embryogenic calli by immature embryos and anther culture in vitro			Yu and Chen 1998
'Brewster'	Embryo tissue	Juvenile	Embryogenic suspension culture and protoplast isolation in lychee	MS media CPW salts, 11% mannitol + 0.8% cellulose		Yu et al. 1996

(continued)

Table 3.2 (continued)

Clone	Explant	Mature/juvenile	Results	Medium + PGR shoot multiplication	Rooting	References
Litchi chinensis Sonn.	Pollen	Mature	Introduction pollen plants of litchi tree (*Litchi chinensis* Sonn.)	MS medium added with KT 2 mg l^{-1}, 2,4-D 2 mg l^{-1}, NAA 0.5 mg l^{-1}, 3% sucrose, and 0.7% agar		Fu and Tang (1983)
Litchi chinensis Sonn.	Protoplast	Juvenile	Fusion of protoplast of lychee and longan			Lai et al. (2000)
Litchi chinensis Sonn.	Shoot bud			MS media 0.2 mg L^{-1} BA, 0.1 mg L-^{1}IAA, 0.5 mgL^{-1} GA_3		Chandra and Padaria (1999)
Litchi chinensis Sonn.	Nodal part			MS media, BAP, 2ip, Kin, and other additives	IBA for rooting	Kumar et al. (2006)
'Feizixiao' litchi	Pollen in the anther of monocytes					Wang et al. (2006a, b)
Litchi (*Litchi chinensis* Sonn.) cv. 'Bedana'	Seeds and axillary bud		Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.)	MS+ BA (20 mg L^{-1}) and MS + 100 µg	MS + IBA (25 mg mL^{-1})	Khan and Ahmad 2005

encountered by these workers. Kantharajah et al. (1992) and Amin and Razzaque (1995) in their work on lychee also observed the beneficial effect of BAP.

Previously, embryogenic lychee cultures have been induced from immature zygotic embryos of 'Xiafanzhi' and 'Chen Zi' (also known as 'Brewster') (Yu and Chen 1997; Yu et al. 2000; Zhou et al. 1996); however, regeneration of plants from mature phase trees has not been reported.

3.5.1.3 Somatic Embryogenesis

The efficient regeneration of elite cultivars is an important prerequisite for applying biotechnology procedures to improve clonally propagated lychee. The establishment of a regeneration system in vitro for lychee bioengineering breeding is vital. Deng (2005), Xie et al. (2006), and Wang et al. (2013) reported on successful somatic embryogenesis and plantlet regeneration in lychee by anther culture. Somatic embryos derived from anthers gave 24 plantlets via organogenesis from pollen callus (Fu and Tang 1983). By "Heli" anther culture, Guo et al. (2007 and 2014) obtained somatic embryos with root and no sprout. The protocol for plant regeneration from litchi-protoplast-derived somatic embryos greatly improved the efficiency of plant recovery. One of the first works on somatic embryogenesis in lychee without plantlets was reported by Amin and Razzaque (1995) from zygotic embryos of lychee using BA (5 mgL^{-1}) and activated charcoal (1 gL^{-1}),while Zhou et al. (1996) had some difficulty in germinating the somatic embryos from zygotic embryos of lychee. On the other hand, Yu and Chen (1998) reported the development and maintenance of highly embryogenic suspensions and protoplast isolation for several lychee cultivars. Yu and Chen (1997), working with embryogenic cultures derived from zygotic embryos of lychee, used silver thiosulfate to inhibit somatic embryo maturation and obtain friable cultures. Yu et al. (2000) cultured lychee protoplasts of the cultivar 'Xiafanzhi' from protoplast suspensions by using Ca-alginate beads with very less plants. Raharjo and Litz (2007) successfully managed somatic embryogenesis from leaflets from the compound leaves of new vegetative flushes of mature (>100-year-old) trees of litchi cultivars 'Brewster' ('Chen Zi') and 'Mauritius' ('Da Zao'). Somatic embryogenesis of lychee from leaves of mature trees can play significant role in genetic transformation and in vitro mutagenesis to improve existing cultivars (Litz et al. 2005). Modern breeding techniques such as gene manipulation have the advantages of high efficiency and directional improvement of specific traits, providing a new way for the improvement of litchi cultivars (Das and Rahman 2012). Puchooa (2004) used young unvaccinated 'Tai So' leaves as explants to study various factors on the regeneration of the leaf blade and eventually obtained regenerated plants. However, Huang and You (1990), Yu (1991), and Guo et al. (2014) reported that the stem sprout buds failed to produce. All the results showed in Table 3.2 reflect that the regeneration of different lychee varieties is inconsistent with their medium. The same culture medium plays different roles on the regeneration of different litchi varieties. Therefore, different genetic backgrounds of lychee varieties have a remarkable effect on in vitro regeneration ability. To date, only a few cultivars, such as 'Nuomici' (Kuang et al. 1996), 'Xiafanzhi' (Lai and Sang 2003), and 'Hushanjiaohe' (Fu and Tang 1983), have

been successfully regenerated in vitro. 'Feizixiao' is an early maturity variety with a tender, juicy, sweet aril and high and stable yield. In vitro regeneration of 'Feizixiao' is potentially a valuable method for conservation, mass propagation, and genetic transformation (Deng 2005) (Table 3.3).

3.5.2 Synthetic Seed

Das et al. (2016) generated artificial seeds in seedless or functionless seeds of litchi varieties by encapsulating 4–9 mm long immature cotyledonary stage somatic embryos (originating from immature zygotic embryos of cv. 'Bedana') to mature somatic embryos (longer than 2.5 cm) and studied the effect of ABA on germination and plantlet regeneration from encapsulated somatic embryos. Yu et al. (2000) isolated protoplasts from embryogenic suspension to generate somatic embryos from Ca-alginate beads.

3.5.3 Protoplast Culture and Somatic Hybridization

Yu et al. (2000) described the isolation and culture of protoplasts from embryogenic litchi suspensions. Four-day-old suspensions were collected by low-speed centrifugation, plasmolyzed for 1 h in CPW (see Frearson et al. (1973), as modified by Grosser and Gmitter (1990)), and supplemented with 13% w/v mannitol. Preliminary studies on the fusion of protoplasts of lychee and longan have been reported (Lai and Chen 2001). Although limited division of somatic hybrid cells was described, the recovery of somatic embryos was not reported. The utility of lychee + longan somatic hybrids is uncertain, due to their polyploidy and the consequent problems of introgression of useful genes into either parent. McConchie et al. (1994) reported that litchi and longan can be sexually hybridized, with hybrids recovered if lychee is used as the female parent.

3.5.4 Achievements Made in Lychee Through Molecular Approach

Molecular approaches are useful for characterizing the genetic diversity among different cultivars or species, identifying genes of commercial interest, creation of variations in existing cultivars in vitro, overcoming reproductive isolation barrier via protoplast fusion, and improvement through genetic transformation technology. Some of the important achievements made in lychee breeding employing biotechnological tools are presented in Table 3.4.

3.5.4.1 Molecular Markers for Assessment of Lychee Diversity

The use of molecular markers, which comprise isozyme and DNA markers, can be used for cultivar identification. Another promising application could be marker-aided selection (MAS) to expedite the breeding program. Molecular genetic marker

Table 3.3 Somatic embryogenesis and synthetic seed formation

Species	Explant	Results/response	Medium + plant growth hormones				References
			Callus	Induction	Maturation	Germination	
'Feizixiao' litchi	Pollen	Higher number of SEs	MS + NAA+ 2,4-D+ KT + BA	MS + 4.52 µM 2,4-D	MS+ 0.54 µM NAA+ 23.23 µM KT+ 0.4 g/L LH + 0.56 µM inositol + 10% (w/v) CW (coconut water) + 6% sucrose	1/2 MS + 1.44 µM GA3	Wang et al. (2006a, b)
Xiafanzhi	Embryogenic suspension culture	100% SE from synthetic seeds and 33% plants	MS salts and B5 vitamins with 2 mg l^{-1} 2,4-D, 50 g l^{-1} sucrose, and 8 g l^{-1} agar (pH 5.8)	MS+ 8% sucrose +0.1 M citric acid +B3 MS salts + B5 vitamins with kinetin 1 mg l^{-1}, NAA 0.1 mg l^{-1},glutamine 500 mg l^{-1}, 8% (w/v) sucrose, and 15 g l^{-1} agar (pH 6.2)	B5 major + MS minor consisted of MS salts and B5 + vitamins with kinetin 1 mg l^{-1}, GA 5 mg l^{-1}, coconut water 50 mli^{-1}, 3% (w/v) sucrose, and 7 g l^{-1} agar (pH 5.8)	B5 + MS medium, 100% white somatic embryos produced roots and 33.1% formed shoots	Yu et al. (2000)
Brewster	Mature		B5 medium +400 mg l^{-1} glutamine + 200 mgl^{-1} casein hydrolysate + 30 g l^{-1} sucrose + 4.52 µM 2,4-D+ 9.30 µM kinetin + 3 g l^{-1} gellan gum	Semisolid MS medium +4.52 mM 2,4-D + 0.91 mM zeatin	Cell suspension culture of semisolid MS medium +4.52 mM 2,4-D + 0.91 mM zeatin semisolid MS medium +4.52 mM 2,4-D + 0.91 mM zeatin	Semisolid MS + 30 mg/L sucrose and 3 g/L gellan gum	Simon et al. (2007)

(continued)

Table 3.3 (continued)

Species	Explant	Results/response	Medium + plant growth hormones				References
			Callus	Induction	Maturation	Germination	
Lychee	Mature		MS+ 8.0 mg·L ~ (−1) 2,4-D + 0.2 mg·L ~ (−1) NAA and 5% sucrose	MS agar medium supplemented with 1.0 mg·L ~ (−1) 2,4-D	0.2 mg·L ~ (−1) NAA + 0.1 mg·L ~ (−1) IBA and 3% sucrose for liquid shake culture	1/2MS agar medium supplemented with 0.2 mg·L^{-1} NAA + 1.0 mg·L^{-1} IBA and 2% sucrose	Zhou et al. (1996)
Litchi chinensis Sonn.	Young embryo		8.0 mg·L^{-1} 2,4 D + 0.2 mg·L^{-1} NAA and 5% sucrose	MS agar medium supplemented with 1.0 mg·L^{-1} 2,4 D	0.2 mg·L^{-1} NAA +0.1 mg·L^{-1} IBA and 3% sucrose	1/2 MS + 0.2 mg·L^{-1} NAA + 1.0 mg^{-1} IBA and 2% sucrose	Linong et al. (1996)
Litchi chinensis Sonn.				MS medium with 5 mg L^{-1} ZT, 60 g L sucrose, and 10 g L agar	MS + 10% coconut water + 6% sucrose		Lai et al. (2002)
Litchi chinensis Sonn.	Zygotic embryos		Zygotic embryos	BA (5 mgL^{-1}) and activated charcoal (1 gL^{-1})		No plantlets	Amin and Razzaque (1995)
Yuherbau	Primary somatic embryo			0.05 mg L NAA, 0.05 mg L 2-ip, 0.2 mg L ABA			Liao and MA (1998)
Litchi chinensis Sonn.	Embryogenic callus			MS+ BA 0.5 mg·L^{-1} and KT 0.5 mg·L^{-1}	MS + BA 0.5 mg·L^{-1} + KT 0.5 mg·L^{-1} + LH 500 mg·L^{-1} + sucrose 60 g·L^{-1} + agar 10 g·L^{-1}		Deng et al. (2007)

Litchi chinensis Sonn.	Immature embryos		MS medium with 5 mg L^{-1} ZT, 60 g L sucrose, and 10 g L agar	MS medium with 10% coconut water and 6% sucrose		Medium free of phytohormones	Lai and Sang (2003)
Litchi chinensis Sonn.	Immature embryos, adult tree leaves		MS medium added 2,4-D	MS medium added 5% sucrose, 2,4-D 2 mg/L, and LH 500 mg/L	MT medium added 5% sucrose and 10% CW (coconut water)	MT medium added 5% sucrose and BA 1 mg/L	Su et al. (2004)
Litchi chinensis Sonn.	Pollen		MS medium added with KT 2 mg l^{-1}, 2,4-D 2 mg l^{-1}, NAA 0.5 mg l^{-1}, 3% sucrose, and 0.7% agar	MS of modified 85 medium containing KT 0.5 mg l^{-1}, NAA O.l mg/l, bee royal jelly 400 mg l^{-1}, LH 500 mg l^{-1}, 3% sucrose, and 0.7% agar	MS or modified 85 medium containing bee royal jelly 400 mg l^{-1}, L-glutamine 500 mg l^{-1}, and 3% sucrose	Modified 85 medium or white medium + KT 20.1 mg/l, IAA 0.01 mg/l, LH 500 mg l^{-1}, L-glutamine 1600 mg l^{-1}, 1% sucrose, and 0.7% agar	Fu and Tang (1983)

Table 3.4 Diversity assessment of lychee by molecular markers

Tools	Achievements	References
Isozyme	Identification of lychee cultivars	Degani et al. (1995)
Isozyme	Identification of a 28 kDa lychee allergen as a triose phosphate isomerase	Hoppe et al. (2006)
Isozyme	Isozyme variation in lychee (*Litchi chinensis* Sonn.)	Aradhya et al. (1995)
Microsatellite SSR	Development, characterization, and variability analysis of microsatellites in lychee (*Litchi chinensis* Sonn., Sapindaceae)	Viruel and Hormaza (2004)
SSR	Transferability of SSR markers from lychee (*Litchi chinensis* Sonn.) to pulasan (*Nephelium ramboutan-ake* L.)	Sim et al. (2005)
SSR	Development and characterization of SSR markers in lychee (*Litchi chinensis*)	Mingfang et al. (2006)
EST-SSR	Developing a core collection of litchi (*Litchi chinensis* Sonn.) based on EST-SSR genotype data and agronomic traits	Sun et al. (2012)
SSR	34 litchi accessions from three different agroclimatic regions. Phenotypic and morphological characters and microsatellite markers	Madhou et al. (2010)
SSR	Fingerprinting and analysis of genetic diversity of litchi (*Litchi chinensis* Sonn.) accessions from different germplasm collections using microsatellite markers	Madhouet al. (2013)
SSR	Genetic diversity of germplasm resources of litchi and longan using SSR analysis	Jia-xin et al. (2011)
SSR	Development, characterization, and variability analysis of microsatellites in lychee (*Litchi chinensis* Sonn., Sapindaceae)	Viruel and Hormaza (2004)
SSR	Development and characterization of SSR markers in lychee (*Litchi chinensis*)	Li et al. (2006)
SSR	Comparison of accessions conserved in different litchi germplasm collections using microsatellite markers	Madhou et al. (2012)
SSR	Identification of a new litchi variety Hongdenglong by EST-SSR markers	Xiang et al. (2010)
RAPD	Genetic diversity within lychee (*Litchi chinensis* Sonn.) based on RAPD analysis	Anuntalabhocha et al. (2002)
RAPD-HAD	Hybrid detection in lychee (*Litchi chinensis* Sonn.) cultivars using HAT-RAPD markers	Chundet et al. (2007)
RAPD-SCAR	Development and significance of RAPD-SCAR markers for the identification of *Litchi chinensis* Sonn. by improved RAPD amplification and molecular cloning	Cheng et al. (2015)

(continued)

Table 3.4 (continued)

Tools	Achievements	References
RAPD	RAPD analysis of genetic relationship among partial litchi germplasms in Hainan Island	Yeyuan et al. (2004)
AFLP	Construction of a high-density molecular linkage map for lychee based on AFLP and RAPD markers	Liu et al. (2008)
AFLP	Studies on the application of AFLP molecular markers on genetic diversity and classification of good and rare litchi resources in Guangxi	Xiang et al. (2006)
AFLP	Determination of genetic diversity and relationships among Thai litchi accessions by RAPD and AFLP markers	Tongpamnak et al. (2002)
AFLP	Amplified fragment length polymorphism fingerprinting to identify genetic relatedness among lychee cultivars and markers associated with small-seeded cultivars	Pathak et al. (2014)
ISSR	Genetic analysis of litchi (*Litchi chinensis* Sonn.) in southern China by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR)	Long et al. (2015)
RAPD	Morphological and molecular diversity of *Colletotrichum* spp. causing pepper spot and anthracnose of lychee (*Litchi chinensis*) in Australia	Anderson et al. (2012)
SNP	Identifying litchi (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using single nucleotide polymorphism (SNP) markers	Liu et al. (2015)
SRAP	Genetic diversity in litchi and longan germplasm as determined by SRAP markers	Jia et al. (2011)
RAPD	High-quality genomic DNA extraction protocol from litchi (*Litchi chinensis* Sonn.)	Kumar et al. (2012)
SSR	Construction of ore collection of lychee by SSR marker	Wang et al. (2012)

technology provides the most direct means for cultivar identification and genetic relationship analysis. A number of systems have been used including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR), and simple sequence repeat polymorphism (SSR). The possibility of studying the genetic diversity among cultivars and populations will benefit lychee breeding programs. The presence of a low level of polymorphism due to narrowness of genetic base prompted workers to rely more on DNA markers.

3.5.4.1.1 Isozyme Markers

Isozyme fingerprinting has revealed many instances of apparent synonymy or mislabeling of cultivar names (Aradhya et al. 1995). For example, 'Kwai Mi' in Hawaii was found to be identical to 'Mauritius' and 'Kim Jee' in Hawaii and to 'Tai So' in Australia but different from 'Kwai Mi' from China and Taiwan. Liu et al. (1989) and Zhou et al. (2001) analyzed the PER isozyme of 24 Guangxi lychee cultivars and 35 Guangdong lychee cultivars, respectively. Liu et al. (1989) also noted that litchi's PER isozyme pattern was not affected by the age of leaves. Peng et al. (1997) analyzed the PER isozyme of five late-bearing Guangxi lychee cultivars and proposed that 'Shatouchili' and 'Jiangkouli' were actually the sport of 'Heli (Huaizhi).' Stern et al. 1993 reported polymorphism of phosphoglucose isomerase (PGI) isozyme system and demonstrated its use for the unequivocal identification of selfed or outcrossed lychee fruits produced in adjacent blocks of 'Mauritius' and 'Floridian'. Degani et al. 2003 studied leaf isozyme banding patterns and polymorphism in aconitase, aspartate aminotransferase, isocitrate dehydrogenase, phosphoglucomutase, shikimate dehydrogenase, superoxide dismutase, and triose phosphate isomerase in 30 cultivars and selections of lychee *(Litchi Chinensis* Sonn.) by means of starch gel electrophoresis.

3.5.4.1.2 DNA-Based Molecular Markers

Several studies have used molecular tools to standardize litchi cultivar names (Aradhya et al. 1995; Anuntalabhochai et al. 2002; Degani et al. 2003; Viruel and Hormaza 2004; Liu and Mei 2005; Madhou et al. 2010). Such confusions were also revealed by the use of DNA molecular markers such as RAPDs, microsatellites, and ISSRs (Degani et al. 2003; Anuntalabhochai et al. 2002; Viruel and Hormaza 2004).

3.5.4.1.2.1 Single Sequence Repeats (SSRs)

Simple sequence repeats or SSRs, also known as microsatellites as DNA markers, are advantageous over many other markers as they are highly polymorphic, highly abundant, codominant inheritance, analytically simple, and readily transferable. Microsatellites are reported to be more variable and deliver more information per unit assay than other marker system like RFLPs or RAPDs due to their high levels of allelic variation and their codominant character and have been widely utilized and popular in plant genomic studies (Weber 1990; He et al. 2003). Moreover, as they are assayed using PCR, only small amounts of tissue are required (Rakoczy-Trojanowska and Bolibok 2004). Madhou et al. (2010) performed SSR in screening and characterization of elite lychee cultivars of Mauritius and Réunion Islands which are in huge demand in European countries. Virual and Hormanza (2003) developed a genomic library enriched for CT/AG repeats, and 12 polymorphic microsatellites have been developed and screened in a sample of 21 lychee cultivars. Li and Zheng (2004) performed SSR on lychee for screening the elite cultivars.

3.5.4.1.2.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP markers are quite suitable for cultivar identification, estimation of genetic relationship, and mapping of QTLs in lychee. AFLP (Yi et al. 2003; Peng et al. 2006) was performed on lychee for screening the elite cultivars. This PCR-based technique allows inspection of polymorphism at fairly a large number of loci within a very short span of time and at the same time requires a very small amount of DNA.

3.5.4.1.2.3 RAPD Markers

For the classification of litchi cultivars, the most commonly used DNA marker is RAPD (Ding et al. 2000; Chen et al. 2004, 2005; Liu and Mei 2005; Wang et al. 2006a, b). However, for most lychee cultivars tested, classification results obtained from different DNA markers are often in disagreement with each other, and even those obtained from the same DNA marker are not totally identical. But the results of RAPD and AFLP analysis both indicate that the genetic diversity within litchi collections at molecular level seems to be limited (Ding et al. 2000;Yi et al. 2003; Chen et al. 2004). As to identification of lychee cultivars, six pairs of accessions are reported to be synonyms: 'Nongmei No. 9' and 'Qiongshan No. 27'(Chen et al. 2004); 'Dazao' and 'Zaohong,' 'Baiye' and 'Guahong,' 'Feizixiao' and 'Zhimali,' and 'Ziniangxi' and 'Zengchengdaguoli' (Liu and Mei 2005); and 'Fengshuang' and 'Tunchangfengshuang' (Wang et al. 2006a, b). But some authors' data (Ding et al. 2000) showed that 'Dazao' and 'Zaohong' are two different cultivars. The research results of Peng et al. (2000) support that 'Qinzhouhongli' is a natural hybrid progeny of 'Heiye' and 'Xiangli.' Ding et al. (2001) reported that the segregation pattern of most RAPD markers in F1 population of 'Wuye' and 'Yu hebao' was consistent with Mendel's law of segregation. Liu and Mei (2003) developed an F1 population of 'Maguili' and 'Jiaohesanyuehong' and constructed a linkage map based on RAPD markers. Ouyang et al. (2005) have carried out selection and breeding based on the collection of early-bearing litchi germplasm since 1999. But now most new cultivars are selected from superior seedlings obtained after open pollination or sport, and crossbreeding is not commonly used since the genetic background of most litchi germplasm is not yet clear. Wang et al. (2003a, b) identified two lychee cultivars (Li No. 13 and A4) by using RAPD, which were selected from seedlings.

3.5.4.1.2.4 Inter-Simple Sequence Repeats (ISSR)

Amplification of inter-simple sequence repeats (ISSRs) is a relatively novel technique and has proven to be a powerful, rapid, simple, reproducible, and inexpensive way to assess genetic diversity or to identify closely related cultivars in many species, including fruit trees. Long et al. (2015) performed ISSR for the genetic improvement of lychee.

3.5.4.2 Identification of Cultivars with Disease Resistant Varieties

In addition to identification and characterization of diversity and elite cultivars screening in lychee, the modern biotechnological tools can be utilized for determining etiology of dreaded diseases, which may sabotage lychee cultivation if not controlled in time. One of such disease is lychee malformation, which appears both at vegetative and flowering stages. It is inevitable to confirm etiology of disease before working out for its control. Although confusion exists regarding the etiology of the disease, Anderson et al. (2012) provided unequivocal evidence that the *Colletotrichum gloeosporioides* is indeed a causal agent of pepper spot and anthracnose disease in lychee fruit.

3.6 Cloning of Useful Gene(s)

In nature, gene transfer is pretty ambiguous which makes the percentage recovery of desired gene combination subject to efficient screening and selection. Additionally, their range in terms of species involved is dependent on sexual compatibility. These delimit the movement of gene across different taxa. On the other hand, advances made in the field of biotechnology have made gene transfer a reality. This entails targeted manipulation of the genetic material toward a desired end through a predetermined way. In lychee, however, gene cloning technique is mostly confined to fruit ripening, floral induction, and shelf life. Wang et al. (2006a, b) cloned and analyze expression of the litchi polyphenol oxidase gene (PPO gene, LcPPO (2120 bps length, ORF of 1800 bps)) in order to investigate the relationships among LcPPO expression, the pericarp PPO activity, and the postharvest browning process. The LcPPO expression was tissue specific and was the highest in the flower and the leaf, followed by the seed and the root and the young stem, pericarp, and pulp. The LcPPO expression level was significantly different in the tissue parts, indicating that PPO may play different role. Yao et al. (2014) reported that miRNAs are actively involved in litchi fruit senescence during ambient storage and post-cold-storage shelf life by regulating the upstream transcription factor genes. Through sequencing and analysis, 296 miRNAs belonging to 49 conserved miRNA families and 11 novel miRNAs were first obtained in litchi. Among these, 170 miRNAs were identified to cleave 202 targets. After a series of analyses, 14 miRNA-target pairs were found to be involved in fruit senescence. The advantage of specific miRNAs as a marker for early senescence prediction and further as the candidate for miRNA-based posttranscriptional gene silencing to delay fruit senescence. Lai et al. (2014) performed transcriptomic analysis of *Litchi chinensis* pericarp during maturation with a focus on chlorophyll degradation and flavonoid by identifying approximately 70% of the unigenes (34,705) which could be annotated based on public protein databases. The total of 3649 genes were significantly differentially expressed between any two coloration stages, while 156 genes were differentially expressed among all three stages of coloration of fruit. Genes encoding enzymes involved in chlorophyll degradation

and flavonoid biosynthesis were identified in the transcriptome dataset. The transcript expression patterns of the Stay-Green (SGR) protein suggested a key role in chlorophyll degradation in the litchi pericarp, and this conclusion was supported by the result of an assay overexpressing LcSGR protein in tobacco leaves. The expression levels of most genes especially late anthocyanin biosynthesis genes were coordinated upregulated coincident with the accumulation of anthocyanins and that candidate MYB transcription factors that likely regulate flavonoid biosynthesis. Liu et al. (2013) cloned ROS-responsive genes responsible for floral induction of lychee to overcome defective flowering affecting quality fruit production.

Three XET genes from lychee fruit were identified and then examined by Lu et al. (2006) for their expression profiles in pericarp and aril tissues at different development stages using a cracking-resistant cultivar, 'Huaizhi', and a cracking-susceptible cultivar, 'Nuomici.' LcXET1 are more likely to play a role in reducing lychee fruit cracking than LcXET2 and LcXET3. Xyloglucan endotransglycosylase (XET) catalyzes the transglycosylation of xyloglucan, the major hemicellulose polymer, which has been thought to mediate the cross-linking of cellulose microfibrils in cellular walls and proposed to be involved in the control of cell wall relaxation. A full-length ABA senescence and ripening inducible gene named LcAsr from lychee genomic DNA based on a cDNA fragment originated from a single clone of a suppression subtractive hybridization (SSH) cDNA library of lychee fruit (Wang 2007). Liu et al. 2013 in his studies on lychee showed that LcAsr expression, which was upregulated in postharvest uncovered fruit under 25 °C, was inducible by dehydration. Zao et al. (2014) developed the molecular genetic map construction and QTL analysis for fruit maturation period in lychee. A hybrid F1 population of 'Maguili × Jiaohesanyuehong' was created by crossing between the very late ripening cultivar 'Maguili' as the maternal parent and the very early ripening cultivar 'Jiaohesanyuehong' as the paternal parent.

3.7 Genetic Transformation

Genetic transformation opens the opportunity for genetic manipulation of plants at cellular level and provides the means for modifying single horticultural traits. Genetic transformation opens the opportunity for genetic manipulation of plants at cellular level and provides the means for modifying single horticultural traits without significantly altering other aspects of the phenotype (Singh et al. 2004; Krishna and Singh 2007). The main target of gene transfer techniques is to produce improved varieties through the incorporation of horticulturally important genes into existing cultivars (Singh et al. 2004). Fruit trees are considered to be recalcitrant material for genetic transformation studies and the main impediment for genetic transformation is the regeneration of transformed plantlets. Choice of explants having competence for transformation and regeneration is a crucial factor. Hence, efficient tissue culture techniques become the base for genetic transformation studies (Giri et al. 2004).

The development of recombinant DNA technology has not only extremely impacted on our understanding of gene structures, functions, and regulations but also greatly facilitated gene cloning, characterization, and their expression into target species. *Agrobacterium*-mediated transformation is the key method for raising the transgenics in lychee. The majority of transgenic studies are based on the use of *Agrobacterium tumefaciens*. The gene transfer to litchi has been achieved till date by *Agrobacterium*-mediated transformation (Das et al. 1996; Puchooa 2004). *Agrobacterium*-mediated transformation has been most widely used and is compatible with both the productions of cell suspension cultures and the regeneration of transgenic plants from a variety of lychee cultivars. Previously bacterial chitinase (ChiB) gene were expressed into litchi cv. 'Bedana' which however showed low level of chitinase activity, and consequently transgenic plants showed partial resistance against *Phomopsis* sp. pathogen (Das and Rahman 2010). Sinha and Das (2013) transformed lychee by gly I and II genes together and manipulated the glyoxalase pathway for enhancing salinity tolerance in lychee. The transgenic that developed showed higher salt tolerance as compared to the wild type, observed by less reduction in chlorophyll content in the leaf disk. Also lychee has been transformed with two genes, i.e., nptII and uidA genes that are associated with resistance to pathogens (Witjaksono and Litz 1999). Embryogenic cultures derived from zygotic embryos of open-pollinated 'Brewster' were incubated for 3 days in liquid maintenance medium at 100 rpm, with acetosyringone-activated *Agrobacterium tumefaciens* strain LBA4404. *Agrobacterium tumefaciens* was electroporated with three different gene constructs: pBI121 containing the selectable marker, neophosphate transferase (nptII), and the scorable marker, b-glucuronidase (gus or uidA); pGPTV-BAR, in which chitinase and b-1,6-glucanase genes were inserted in tandem; and the antifungal protein gene. The nptII and uidA genes in pBI121 were driven by the 35S promoter. The chitinase, b-1,6-glucanase, and antifungal protein genes and uidA in pGPTV-BAR were driven by the double 35S promoter. *Agrobacterium tumefaciens* was then eliminated by incubating the cultures for 2 weeks in maintenance medium supplemented with 200 mg l^{-1} cefotaxime. Following the removal of *A. tumefaciens*, the cultures were transferred on to semisolid maintenance medium supplemented with 2 g l^{-1} glufosinate ammonium to select for pGPTV-BAR and 100 mg l^{-1} kanamycin to select for pBI121. Cefotaxime at 200 mg l^{-1} was incorporated into the medium. Expression of the uidA gene by the X-Gluc histochemical reaction (Jefferson 1987), 2 weeks after the end of selection, showed differential transformation efficiency among the constructs. Transformation with pBI121 was most efficient, whereas pGPTV-BAR with chitinase and b-1,6-glucanase were least efficient. *Agrobacterium tumefaciens*-mediated transformation of 'Brewster' ('Chen Zi') lychee (*Litchi chinensis* Sonn.) with the *PISTILLATA* cDNA in antisense. Posttranscriptional silencing of the lychee PI homologue induced by an antisense-oriented transgene could be a successful strategy; however, silencing in floral primordia to produce parthenocarpic fruits can only be confirmed in mature plants after several years.

3.8 In Vitro Germplasm Conservation and Cryopreservation

Lychee seeds are highly recalcitrant and cannot be stored. Hence, tissue culture methods can be an ideal approach. Cryopreservation, storage of living cells at 196 °C in liquid nitrogen, is the only reliable and low-cost, long-term conservation method available for recalcitrant seed and vegetatively propagated crops. Cryopreservation requires the use of either shoot tips or embryos (zygotic or somatic). Padilla et al. (2009, 2013) reported a reliable protocol for the cryoconservation by using vitrification solution (PVS2) of embryogenic lychee cultures obtained from mature trees and the possible effect of cryoprotectants and cryoconservation on the reversion of hyperhydricity. The ability to store embryogenic lychee cultures for extended periods is critical for many cell manipulation studies, because embryogenic cultures lose their morphogenic competence over time. Preliminary studies have demonstrated that embryogenic longan and lychee cultures can be cryopreserved by stepwise or slow cooling at −1 °C/min down to −196 °C (Matsumoto et al. 2004). The optimum cryoprotectant was 0.5% (v/v) glycerol with 5% (v/v) DMSO (dimethyl sulfoxide). Embryogenic cultures (approx. 100 mg) were suspended in 2 ml of sterile cryoprotectant in cryogenic vials and plunged into liquid nitrogen in a cryogenic storage tank. The cryogenic vials were removed from the liquid nitrogen after 48 h and rapidly thawed in a water bath at 40 °C. Embryogenic cultures were transferred on to maintenance medium, and growth resumed. The standardization of this protocol should have an important impact on the storage of experimental material and for use as a backup for germplasm collections.

3.9 Conclusion and Future Thrusts

Biotechnology holds several promises in lychee improvement. Tissue culture techniques like anther and zygotic embryo culture can be exploited for raising homozygous lines. Likewise, genetic transformation to raise stable transformants for different characters is gradually been explored. Genetic markers are of special significance as it can aid in conventional breeding approaches of lychee varieties. Considerable success has been achieved in the development of regeneration protocols in several lychee cultivars. Transformation of lychee through repetitive somatic embryogenesis has also successfully been accomplished. Despite the successful regeneration of different genotypes, the conversion rate of somatic embryos into normal plantlets remains low. Future research must be focused on enhancing conversion frequency of somatic embryos into normal plantlets and regeneration of plantlets from shoot/nodal segments. Most of the important lychee varieties which dominate the world lychee have higher shelf life, aroma, flavor, and seedless fruits which can be promoted. Introduction of beneficial gene(s) from elite cultivars could be a solution to induce better quality fruits. Likewise, the advances made in the field of biotechnology can also meet the challenges of abiotic and biotic stresses.

Biotechnology is an important and invaluable asset to the breeders, which hold the greater promise to revolutionize the lychee industry by development of altered variety(s) intended to serve the specific purpose through precise genetic manipulation, which was hitherto unachievable through conventional breeding.

References

Amin MN, Razzaque MA (1992) Induction of somatic embryogenesis in the cultures of zygotic embryos of lychee. Bangladesh J Bot 24:25–29

Amin MN, Razzaque MA (1995) Induction of somatic embryogenesis in the cultures of zygotic embryos of lychee. Bangladesh J Bot 24:25–29

Anderson JM, Aitken EAB, Dann EK, Coates LM (2012) Morphological and molecular diversity of Colletotrichum spp. causing pepper spot and anthracnose of lychee (Litchi chinensis) in Australia. Plant Pathol 62(2):279–288

Anon (1991) Tissue culture of lychee (*Litchi chinensis* Sonn.) CSFRI Inf Bull 229:1–2

Anuntalabhochai R, Chundet R, Chiangda J, Apavatjrut P (2002) Genetic diversity within lychee based on RAPD analysis. Acta Hortic 575:253–259

Aradhya MK, Zee FT, Manshardt RM (1995) Isozyme variation in lychee (*Litchi chinensis* Sonn). Sci Hortic 63:21–35

Babita S, Kumar R (2008) Feed your litchi orchard properly. Indian Hortic 53(3):18–20

Basu S, Haldar N, Bhattacharya S, Biswas S, Biswas M (2012) Hepatoprotective activity of *litchi chinensis* leaves against paracetamol-induced liver damage in rats. Am-Euras J Sci Res 7:77–81

Benson EE (2000) In vitro recalcitrance: an introduction. Special symposium: in vitro plant recalcitrance. In Vitro Cell Dev Biol Plant 36:141–148

Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T (2011) Hepatoprotective effects of Litchi (*Litchi chinensis* Sonn.): a combination of anti-oxidant and anti-apoptotic activities. J Ethnopharmacol 136:55–66

Butani DK (1977) Pests of litchi in India and their control. Fruits 32:269–273

Castellain RC, Gesser M, Tonini F, Schulte RV, Demessiano KZ, Wolff FR et al (2014) Chemical composition, anti-oxidant and antinociceptive properties of *Litchi chinensis* leaves. J Pharm Pharmacol 66:1796–1807

Chadha KL, Rajpoot MS (1969) Studies on floral biology, fruit set, and its retention and quality of some litchi varieties. Indian J Hortic 26:124–129

Chandra R, Padaria JC (1999) Litchi shoot bud culture for micropropagation. J Appl Hort Lucknow 1(1):38–40

Chandra B, Palni LMS, Nandi SK (2004) Micropropagation of *Picrorhiza kurrooa* Royle ex Benth., an endangered alpine herb, using cotyledonary node and shoot tip explants. Phytomorphology 54(3 & 4):303–316

Chang KP (1970) A Study of Anoplophora macularia, a serious insect pest of litchi. Taiwan Agric Q 6:133–143

Chang JC, Lin TS (2008) Fruit yield and quality as related to flushes of bearing shoots in litchi. J Am Soc Hortic Sci 133:284–289

Chang Y, Yang D, Chiu C, Lin Y, Chen J, Chen Y (2013) Antioxidative and anti-inflammatory effects of polyphenol-rich litchi (*Litchi chinensis* Sonn.)-flower-water extract on livers of high-fat-diet fed hamsters. J Funct Foods 5:44–52

Chapman KR (1984) Sapindaceae. In: Page PE (ed) Tropical tree fruits for Australia. Queensland Department of Primary Industry, Queensland, pp 179–191

Chauhan S, Kaur N, Kishore L, Singh R (2014) Pharmacological evaluation of anti-inflammatory and analgesic potential of *Litchi chinensis*. Int J Pharm Pharm Sci 6:116–119

Chen X (2009) Small RNAs and their roles in plant development. Annu Rev Cell Dev Biol 25(1):21–44
Chen IZ, Cheng CY (1996) Effect of root temperature on flowering, root growth and leaf mineral nutrients and carbohydrate content of mango (*Mangifera indica* L.) J Taiwan Soc Hortic Sci 42:131–141
Chen HB, Huang HB (2001) China litchi industry: development, achievement and problems. Acta Hortic 558:31–39
Chen MC, Weng SW (2000) Study on morphology and germination of pollen in litchi. Hortic NCHU 25:27–41
Chen SP, Yao CM (2001) Occurrence and control of main pests on litchi in Guangzhou. Guangdong Agric. Sci. 4:37–39 (in Chinese)
Chen W, Wu Z, Ji Z, Su M (2001) Postharvest research and handling of litchi in China – a review. Acta Hortic 558:321–329
Chen J, Lin H, Hu M (2005) Absorption and metabolism of genistein and its five isoflavone analogs in the human intestinal Caco-2 model. Cancer Chemother Pharmacol 55(2):159–169
Chen YY, Deng SS, Zhang X, Wei SX, Gao AP, Wang JB et al (2004) RAPD analysis of genetic relationship among partial litchi germplasms in Hainan Island. Acta Hortic Sin 31:224–226
Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT et al (2005) Real-time quantification of miRNA by stem-loop RT-PCR. Nucleic Acids Res 33:e179. doi:10.1093/nar/gni178
Chen JN, Zeng XN, Chen BX, Dong YZ, Lu H (2010) Occurrence and control of geometrid moth in litchi orchard. Chin J Trop Crops 31:1564–1570
Cheng YH (2003) Effect of girdling on flower differentiation of Litchi (Litchi chinensis Sonn.). II. Effect of root temperature on development of Embryo Sac of Litchi (Litchi chinensis Sonn.). M.S. thesis, Department of Horticulture, National Taiwan University, Taipei, Taiwan
Chin HF, Hor YL, Lassim MBM (1984) Identification of recalcitrant seeds. Seed Sci Technol 12:429–436
Chrispeels MJ, Grossi MF, Higgins TJV (1998) Genetic engineering with alpha amylase inhibitor seeds resistant to bruchids. Seed Sci Res 8:257–263
Chundet R, Cutler RW, Tasanon M, Anuntalabhochai S (2007) Hybrid detection in lychee (*Litchi chinensis* Sonn.) cultivars using HAT-RAPD markers. Sci Asia 33:307–311
Coates LM, Zhou E, Sittigul C (2005) Diseases. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CABI Publishing, Wallingford, pp 261–272
Das CS, Chowdhary KR (1958) Floral biology of litchi (*Litchi chinensis* Sonn.) South Indian Hortic 6:17–622
Das DK, Rahman A (2010) Expression of a bacterial chitinase (*ChiB*) gene enhances antifungal potential in transgenic *Litchi chinensis* Sonn. (cv. Bedana). Curr Trends Biotenol Pharm 41:820–833
Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell Tissue Organ Cult 109:315–325
Das DK, Shiva Prakash N, Sarin NB (1996) Regeneration and Transformation of Litchi (*Litchi chinensis* Sonn.). In Vitro Cell Dev Biol Plant 32:63A
Das DK, Shiva Prakash N, Sarin NB (1999a) Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.). Plant Cell Rep 18:691–695
Das DK, Prakash NS, Sarin NB, et al. (1999b) Multiple-shoot induction in *Litchi chinensis* – a tropical tree species. In: Proceedings of Plant tissue culture and biotechnology: emerging trends, Hyderabad, India, 29–31 January, 1997, pp 166–170
DeJager ES, Wehner FC, Korsten L (2003) Fungal post-harvest pathogens of litchi fruit in South Africa. SA Litchi Growers' Assoc Year B 15:24–23
Degani C, Beiles A, El-Bastri R, Goren M, Gazit S (1995) Identifying lychee cultivars by isozyme analysis. J Am Soc Hortic Sci 120:307–312
Degani C, Deng J, Beiles A, El-Batsri R, Goren M, Gazit S (2003) Identifying lychee cultivars and their genetic relationships using intersimple sequence repeat markers. J Am Soc Hortic Sci 128:838–845

Deng CJ (2005) Studies on high frequency somatic embryogenesis and regeneration culture in litchi. Master's thesis of Hunan Agricultural University, Changsha, Hunan, China.
Deng Chao-jun, YI Gan-jun, Zeng Ji-wu, Zhang Qiu-ming, Liu Wei-guo (2007) Application of orthogonal experiment on induction of somatic embryogenesis in Litchi. Fujian Fruits 2007-02
Ding XD, Lu LX, Chen XJ, Guan X (2000) Identifying litchi cultivars and evaluating their genetic relationships by RAPD markers. J Trop Subtrop Bot 8:49–54
Ding XD, Lu LX, Chen XJ (2001) Segregation patterns of RAPD markers in an F1 population of *Litchi chinensis* Sonn. Acta Hortic 558:167–172
Dodd JC, Prusky D, Jeffris P (1998) Fruits diseases. In: Litz RE (ed) The mango-botany, production and uses. CAB International, Wallingford/Oxon, pp 257–280
Duvenhage JA (1993) Control of post-harvest decay and browning of litchi fruit by sodium metabisulphite and low pH dips. SA Litchi Growers' Assoc Year B 5:31–32
Frearson EM, Power JB, Cocking EC (1973) The isolation, culture and regeneration of Petunia leaf protoplasts. Dev Biol 33:130–137
Fu L, Tang DY (1983) Induction of pollen plants of Litchi trees (*Litchi chinensis* Sonn.) Acta Genet Sin 10:369–374
Fu LF, Tang TY (1983) Plantlets formed from litchi pollen. Plant Physiol Commun Zhiwu Shenglixue Tongxun No.2, 28–29
Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA (1996) Plant growth regulators in plant tissue culture. In Vitro Cell Dev Biol Plant 32:272–289
Ghosh B, Mitra SK, Sanyal D (2001) Litchi cultivars of West Bengal, India. Acta Hortic 558:107–114
Giri CC, Shyankumar B, Anjaneyulu C (2004) Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an Overview. Trees 18:115–135
Grosser JW, Gmitter FG Jr (1990) Protoplast fusion and citrus improvement. Plant Breed Rev 8:339–374
Gupta D, Bhatia R, Sharma NK, Chandel JS, Sharma R (1997) Incidence and management of red rust of litchi in lower hills of Himachal Pradesh. Pest Manag Hortic Ecosyst 3:70–74
Guo Y-Q, Lai Z-X, Lü L-X (2007) Cryopreservation of embryogenic calli by vitrification and plant regeneration via somatic embryogenesis in litchi. J Fujian Agric Forestry Uni Nat Sci Edn 2007–01
Guo SY, Peng HX, He XH, Ding F, Li HL, Qin XQ, Xu N (2014) Callus induction from different explants of litchi. Southwest China J Agric Sci 27(2):748–753
Hanson J (1984) The storage of seeds of recalcitrant tree fruits. In: Allen G (ed) Crop genetic resources: conservation and evaluation. Unwin, London, pp 53–62
Hamilton RA, Yee W (1970) Lychee cultivars in Hawaii. Proc Trop Am Soc Hortic Sci 14:7–12
He DP (2001) An overview of integrated management of insect pests in litchi orchards of Guangdong. Acta Hortic 558:401
Hieke S, Menzel CM, Doogan VJ, Lüdders P (2002) The relationship between fruit and leaf growth in lychee (*Litchi chinensis* Sonn.) J Hortic Sci Biotechnol 77:320–325
Hieke S, Menzel CM, Doogan VJ, Lüdders P (2002) The relationship between yield and assimilate supply in lychee (*Litchi chinensis* Sonn.) J Hortic Sci Biotechnol 77:326–332
He G, Meng R, Newman M, Gao G, Pittman RN, Prakash CS (2003) Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biol 3:3
Holcroft DM, Mitcham EJ (1996) Postharvest physiology of litchi: a review. Postharvest Biol Technol 9:265–281
Hoppe S, Neidhart S, Zunker K, Hutasingh P, Carle R (2006) The influences of cultivar and thermal processing on the allergenic potency of lychees (*Litchi chinensis* SONN.). Food Chem 96(2):209–219
Huang ZZ, You KZ (1990) Primary study on the litchi shoots induction. Guangdong Agric Sci 3:27–29

Huang X, Zeng L, Huang HB (2005a) Lychee and longan production in China. Acta Hortic 665:27–36

Huang YZ, Yang CJ, Jiang SH (2005b) The preliminary analysis on the geographical division of litchi pest in China. J Cent S Forest Univ 25:78–80 (in Chinese with English abstract)

Hwang JY, Lin JT, Liu SC, Hu CC, Shyu YS, Yan DJ (2013) Protective role of litchi (*Litchi chinensis* Sonn.) flower extract against cadmium- and lead-induced cytotoxicity and transforming growth factor b1-stimulated expression of smooth muscle a-actin estimated with rat liver cell lines. J Funct Foods 5:698–705

Ichinose T, Musyoka TM, Watanabe K, Kobayashi N (2013) Evaluation of anti-viral activity of Oligonol, an extract of *Litchi chinensis*, against betanodavirus. Drug Discov Ther 7:254–260

Irene PR, Babu DJ, Rao NV, Sheikh RA (2012) Nootropic activity of fruit extracts of *Litchi chinensis* Sonn (Sapindaceae). Int J Phar Tech 4:4795–4804

Jefferson RA (1987) Assaying chimeric genes in plants. The GUS gene fusion system. Plant Mol Biol Report 5:387–405. http://dx.doi.org/10.1007/BF02667740

Jia HF, Chai YM, Li CL, Lu D, Luo JJ et al (2011) Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiol 157:188–199

Jiang YM, Yao LH, Lichter A, Li JR (2002) Postharvest biology and technology of litchi fruit. Food. Agric Environ 1:76–81

Jiang Y, Duan X, Joyce D, Zhang Z, Li J (2004) Advances in understanding of enzymatic browning in harvested litchi fruit. Food Chem 88:443–446

Johnson GI, Sangchote S (1994) Control of post-harvest diseases of tropical fruits: challenges for the 21st century. In: Champ BR, Highley E, Johnson GI (eds) Post-harvest handling of tropical fruit. Australian Centre for International Agricultural Research, Canberra, pp 140–161

Johnson GI, Cooke AW, Mead AJ, Wells LA (1991) Stem end rot of mango in Australia: causes and control. Acta Hortic 291:288–295

Kantharanjah AS, Mc Conchie CA, Dodd WA (1992) In vitro embryo culture and induction of multiple shoots in lychee. Ann Bot 70:153–156

Kantharajah AS, McConchie CA, Dodd WA (1992) *In vitro* embryo culture and induction of multiple shoots in lychee *(Litchi chinensis* Sonn.). Ann Bot 70(2):153–156

Khan MA, Ahmad I (2005) Multiple shoot induction and plant regeneration in Litchi (*Litchi chinensis* Sonn.). Int J Agric Biol

Kong F, Zhang M, Liao S, Yu S, Chi J, Wei Z (2010) Anti-oxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *litchi Chinensis* sonn. Molecules 15:2152–2165

Krishna H (2006) Studies on in vitro propagation of mango (*Mangifera indica* L.). Ph. D. thesis, I.A.R.I.

Krishna H, Singh S (2007) Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement - a review. Biotechnol Adv 25:223–243

Kuang ZS, Zhou LN, Ma XJ, Chen JQ, Cao J (1996) Study on the types of embryoid in tissue culture on Litchi chinensis Sonn. J Fruit Sci 13:25–28

Kumar M (2006) Mass scale propagation of (*Litchi chinensis* Sonn.) through in vitro Technoques. Ph.D. Thesis, Bhagalpur University, Bhagalpur

Kumari-Singh A, Prasad OS (1991) Dehydration pattern and viability loss in seeds of two cultivars of litchi *(Litchi chinensis* Sonn.). Seed Res 19:41–43

Kumar V, Kumar G, Sharma PD (2012) Osmotic dehydration of litchi pulp as a pretreatment for drying processes. Agric Eng Int CIGR J 14(3):146–151

Lai ZX, Chen ZG (2001) Electrofusion of protoplasts between longan and litchi. J Fujian Agric Uni 30(3):347–352

Lai ZX, Sang QL (2003) Optimization of somatic embryogenesis system from embryogenic callus and plant regeneration from transformed *hpt*-resistant calli of litchi (*Litchi chinensis* Sonn.). Chin J App Environ Biol 9(2):131–136

Lai Z, Chen C, Zheng L, Chen Z (2000) Somatic embryogenesis in longan (*Dimocarpus longan* Lour.). In: Jain SM, Gupta PK, Newton RJ (eds) Somatic embryogenesis in woody plants. Kluwer Academic Publishers, Dordrecht, pp 415–431

Lai ZX, Sang QL, Pan DM (2002) Establishment of transgenic system of embryogenic callus via particle bombardment in litchi, J Fujian Agric Uni Nat Sci, 2002–03

Lai B, Li XJ, Hu B, Qin YH, Huang XM, Wang HC, Hu GB (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS ONE 9:e86293

Lee KT (1992) The study of In vitro Litchi (*Litchi chinensis* Sonn.) Anther, pollen morphology and pollen germination characteristics. M.S. Thesis. Department of Horticulture, National Taiwan University, Taipei, Taiwan

Lee SR (2004) The enabling technology of the flowering and fruit seeting of 'YuHer-Pao' Litchi (*Litchi chinensis* Sonn.). Kaohsiung District Agricultural Research and Extension Station, Pingtung, Taiwan

Lee SR (2006) Flowering and Fruit Set in 'Yu-Her-Pao' Litchi (*Litchi chinensis* Sonn.) in Southern Taiwan. Res Bull KDARES 17:9–19

Lee HS, Wicker L (1991) Quantitative changes in anthocyanin pigments of lychee fruit during refrigerated storage. Food Chem 40:263–270

Lee SJ, Park WH, Park SD, Moon HI (2009) Aldose reductase inhibitors from *Litchi chinensis* Sonn. J Enzyme Inhib Med Chem 24:957–959

Li M, Hoch G, Körner C (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. Trees 16:331–337

Li M, Zheng X (2004) Research progress of regulatory mechanism of flowering and fruit setting in Litchi and its Germplasm analysis. Chin J Trop Agric:20–24

Li XY, Chen WX, Liu AY (2006) Effects of Colletotrichum gloeosporioides infection on physiological and biochemical changes of litchi fruit. Subtrop Plant Sci 1:1–4

Li X, Gao MJ, Pan HY, Cui DJ, Gruber MY (2010) Purple canola: Arabidopsis PAP1 increases anti-oxidants and phenolics in *Brassica napus* leaves. J Agric Food Chem 58:1639–1645. doi:10.1021/jf903527y

Liao YW, Ma SS (1998) Adventitious embryogenesis of *Litchi chinensis*. J Chin Soc Hortic Sci 44(1):29–40

Lin MW (1994) Studies on the preharvest fruit dropping of litchi cv. Yu-Her-Pau. In: Lin HS, Chang LR (eds) Proceeding of a symposium on The Pracical Aspects of Some Economically Fruits in Taiwan. Taichung District Agriculture Improvement Station, Taichung, pp 99–107

Lin S, Yang B, Chen F, Jiang G, Li Q, Duan X et al (2012) Enhanced DPPH radical scavenging activity and DNA protection effect of litchi pericarp extract by *Aspergillus awamori* bioconversion. Chem Cent J 6:108

Litz RE, Gray DJ (1995) Somatic embryogenesis for agricultural improvement. World J Microbiol Biotechnol 11:416–425

Litz RE, Raharjo SHT (2005) *Dimocarpus longan* longan and Litchi chinensis litchi. In: Litz RE (ed) Biotechnology of fruit and nut crops. CABI, Wallingford, pp 628–636

Litz RE, Raharjo S, Matsumoto KW (2005) Biotechnology. In: Menzel CM, Waite GK (eds) Longan and litchi botany production and uses. CABI, Wallingford, pp 49–48

Liu C, Mei M (2003) Construction of a lychee genetic linkage map based on rapd MARKERS. Acta Hortic (ISHS) 625:131–136

Liu C, Mei M (2005) Classification of lychee cultivars with RAPD analysis. Acta Hortic 665:149–160

Liu Q, Cai W, Shao X (2008) Determination of seven polyphenols in water by high performance liquid chromatography combined with preconcentration. Talanta 77:679–683

Liu WW, Kim HJ, Chen HB, Lu XY, Zhou BY (2013) Identification of MV-generated ROS responsive EST clones in floral buds of *Litchi chinensis* Sonn. Plant Cell Rep 32:1361–1372

Liu Y, Zhu XQ, Li WD, Wen H, Liu CS (2015) Enhancing ergosterol production in Pichia pastoris GS115 by overexpressing squalene synthase gene from Glycyrrhiza uralensis. Chin J Nat Med 13:338–345

Lizada C (1993) Mango. In: Seymour GB, Taylor JE, Tucker GA (eds) Biochemistry of fruit ripening. Chapman and Hill, London, pp 255–271
Loebel R (1976) The litchi. Trap Fruit Bull NSW Dept Agric 6:1–12
Long Y, Cheng J, Mei Z (2015) Genetic analysis of litchi (*Litchi chinensis* Sonn.) in southern China by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR). Mol Biol Rep 42:159
Lv Q, Si M, Yan Y, Luo F, Hu G, Wu H, Sun C, Li X, Chen K (2014) Effects of phenolic-rich litchi (*Litchi chinensis* Sonn.) pulp extracts on glucose consumption in human HepG2 cells. J Funct Foods 7:621–629
Ma XY, Yi GJ, Huang XL (2009) Leaf callus induction and suspension culture establishment in lychee (*Litchi chinensis* Sonn.) cv. Huaizhi. Acta Physiol Plant 31:401–405
Madhou M, Bahorun T, Hormaza JI (2010) Phenotypic and molecular diversity of litchi cultivars in Mauritius. Fruits 65:141–152
Madhou M, Normand F, Bahorun T, Hormaza JI (2013) Fingerprinting and analysis of genetic diversity of litchi (*Litchi chinensis* Sonn.) accessions from different germplasm collections using microsatellite markers. Tree Genet Genomes 9:387–396
Mahattanatawee K, Perez-Cacho PR, Davenport T, Rouseff R (2007) Comparison of three lychee cultivar odor profiles using gas chromatography-olfactometry and gas chromatography-sulfur detection. J Agric Food Chem 55:1939–1944
Matsumoto K, SHT R, Dhekney S, Moon PA, Litz RE (2004) Cryopreservation and somatic embryogenesis from *Dimocarpus longan* calli. Pesq Agropec Brasil Brası'lia 39:1261–1263
McConchie CA, Batten DJ (1991) Fruit set in lychee (*Litchi chinensis* Sonn.). Variation between flowers, panicles and trees. Aust J Agric Res 42:1163–1172
McConchie CA, Vithanage V, Batten DJ (1994) Intergeneric hybridization between litchi (*Litchi chinensis*, Sonn.) and longan (*Dimocarpus longan* Lour.). Ann Bot 74:111–118
Menzel CM (1983) The control of floral initiation in lychee: a review. Sci Hortic 21:201–215
Menzel CM (1984) The pattern and control of reproductive development in lychee—a review. Sci Hortic 22:333–345
Menzel CM (1985) Propagation of lychee: a review. Sci Hortic 25:31–48
Menzel C (2000) The physiology of growth and cropping in lychee. Abst. 1st Int. Symposium on litchi and longan. Guangzhou, China, p. 37
Menzel CM, Paxton BF (1986) The effect of cincturing at different stages of vegetative flush maturity on the flowering of litchi (*Litchi chinensis* Sonn.) J Hortic Sci 61:135–139
Menzel CM, Simpson DR (1992) Flowering and fruit-set in lychee (*Litchi chinensis* Sonn.) in subtropical Queensland. Aust J Exp Agric 32:105–111
Menzel CM, Waite GK (2005) Litchi and lonngan: botany, production and uses. CABI Publishing, Wellingford
Menzel CM, Huang X, Liu C (2005) Cultivars and plant improvement. In: Menzel CM, Waite GK (eds) Litchi and longan. Botany, production and uses. CAB International, Wallingford, pp 59–86
Mingfang L, Xueqing Z, Yongqiang Z, Xiangshe W, Suyu L, Lei L, Xingrong W (2006) Development and characterization of SSR markers in lychee (*Litchi chinensis*). Mol Ecol Resour 6:1205–1207
Nakasone HY, Paull RE (1998) Tropical fruits. CAB International, Wallingford, 445 pp
Nijjar GR (1981) Litchi cultivation. Punjab Agricultural University, Ludhiana, pp 1–3
Ouyang R, Hu GB, Wang ZH (2005) The exploration, research and exploit of early-bearing litchi germplasm in China. China Trop Agric 5:30–32
Padilla G, Moon P, Perea I, Litz RE (2009) Cryopreservation of embryogenic cultures of 'Brewester' Litchi (Litchi chinensis. Son) and its effect on hyperhydric embryogenic cultures. CryoLetters 30(1):55–63
Padilla G, Pérez JA, Perea-Arango I, Moon PA, Gómez-Lim MA, Borges AA, Expósito-Rodríguez M, Litz RE (2013) *Agrobacterium tumefaciens*-mediated transformation of 'Brewster' ('Chen Tze') litchi (*Litchi chinensis* Sonn.) with the *PISTILLATA* cDNA in antisense. In Vitro Cell Dev Biol Plant 49(5):510–519

Pandey RM, Sharma HC (1989) The litchi. ICAR, New Delhi, pp 1–79
Peng HX, Cao HQ, Zhu JH, Liao HH, Huang FZ, Li JZ et al (2006) Studies on the application of AFLP molecular markers on genetic diversity and classification of good and rare litchi resources in Guangxi. Southwest China J Agr Sci 19:108–111
Peterson RA (1986) Mango diseases. Proceedings of the CSIRO First Australian Mango Research Workshop. pp 233–247
Ploetz RC (1994) Distribution and prevalence of Fusarium subglutinans in mango tree affected by malformation. Can J Bot 72:7–9
Poo ZL, Mai SH, Huang MD (1965) The initially experimental report of utilizing *Anastatus* sp. to control *Tessaratoma papillosa*. Acta Phys Sin 1:301–306
Prasad LS, Kumar R, Mishra M, Singh AK, Prasad US (1996) Characteristics of litchi seed germination. Hortic Sci 31:1187–1189
Pu ZL (1992) Utilizing Eupelmid wasp Anastatus sp to control litchi stink bug Tessaratoma papillosa. In: Sun Yat-sen University, Guangdong Scientech Association (eds) Selected works of Pu Zhelong. Sun Yat-Sen University press, Guangzhou, pp 135–169 (in Chinese)
Puchooa D (2004) In vitro regeneration of lychee (Litchi chinensis Sonn.). Afr J Biotechnol 3:576–584
Putta EK, Sastry N (2014) Screening of methanolic fruit pericarp extract of *Litchi chinensis* against various disease conditions. AJPHR 2:300–306
Raharjo SHT, Litz RE (2007) Somatic embryogenesis and plant regeneration of litchi (*Litchi chinensis* Sonn.) from leaves of mature phase trees. Plant Cell Tissue Organ Cult 89:113–119
Rai PS, Bhandary KR (1973) Occurrence of Xyleborus fornicatus Eichhoff (Coleoptera: Scolytidae) on litchi (*Litchi chinensis* Sonn.) in India [Abstract]. Curr Sci 42(10):369
Ram M, Majundar PK (1981) A note on propagation of litchi(*Litchi chinensis* Sonn.) by stolling. Sci Cult 47:332–334
Rakoczy-Trojanowska M, Bolibok H (2004) Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. Cell Mol Biol Lett 9:221–228
Ray PK, Sharma SB (1985) Viability of *Litchi chinensis* seed when stored in air and water. J Agric Sci (Camb) 104:247–248
Ray PK, Sharma SB (1987) Growth, maturity, germination and storage of litchi seeds. Sci Hortic 33:213–221
Reichel M, Carle R, Sruamsiri P, Neidhart S (2010) Influence of harvest maturity on quality and shelf-life of litchi fruit (*Litchi chinensis* Sonn.) Postharvest Biol Technol 57:162–175
Reuveni O, Golubowicz S (1997) Trials of using in vitro techniques for vegetative propagation of mangoes. Acta Hortic 455:496–504
Ribeiro IJA, Pommer CV (2004) Breeding guava (*Psidium guajava*) for resistance to rustcaused by Puccinia psidii. Acta Hortic Leuven 632:75–78
Rivera-López J, Ordorica-Falomir C, Wesche-Ebeling P (1999) Changes in anthocyanin concentration in Lychee (Litchi chinensis Sonn.) pericarp during maturation. Food Chem 65:195–200
Roe DJ, Menzel CM, Oosthuizen JH, Doogan VJ (1997) Effects of current CO_2 assimilation and stored reserves on lychee fruit growth. J Hortic Sci 72:397–405
Roth G (1963) Post harvest decay of litchi fruit (Litchi chinesis Sonn.). Technical Commun., Dept. Agric. Tech. Serv., South Africa, 16 p
Sarin NB, Prasad US, Kumar M, Jain SM (2009) Litchi breeding for genetic improvement. In: Jain SM, Priyadarshan PM (eds) Breeding plantation tree crops: tropical species. Springer, New York, pp 217–245
Scott KJ, Brown BL, Chaplin GR, Wilcox ME, Bain JM (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic films. Sci Hortic Amsterdam 16:253–262
Shih PM, Chen IZ (2006) Observation of embryo sac development of litchi (*Litchi chinensis* Sonn.). J Taiwan Soc Hortic Sci 46:359–368
Sim CH, Mahani MC, Choong CY, Salma I (2005) Transferability of SSR markers from lychee (*Litchi chinensis* Sonn.) to pulasan (*Nephelium ramboutan-ake* L.). Fruits 60:379–385

Singh JP, Chandel R, Mishra B, Suneetha V (2013) Evaluation of antimicrobial and anti-oxidant property of lychee's seed for therapeutic purpose. Int J Pharm Sci Rev Res 19:72–76
Singh UP, Singh DP, Singh M, Maurya S, Srivastava JS, Singh RB, Singh SP (2004) Characterization of phenolic compounds in some Indian mango cultivars. Int J Food Sci Nutr 55(2):163–169
Sinha S, Das DK (2013) Transformation of Litchi (*Litchi chinensis* Sonn.) with Gly I + II gene leads to enhance salt tolerance. Int J Biotechnol 1(11):483–495
Sivakumar G, Kim SJ, Hahn EJ, Paek KY (2005) Optimizing environmental factors for large-scale multiplication of Chrysanthemum (Chrysanthemum grandiflorum) in balloon-type bioreactor culture. In Vitro Cell Dev Biol Plant 41:822–825
Souza M, Singh R, Reddy P, Hukkeri V, Byahatti V (2006) Hepatoprotective activity of fruit pulp extract of *Litchi chinensis* Sonner on carbon tetrachloride induced hepatotoxicity in albino rats. Int J Alt Med 4:1–5
Sowa S, Roos EE (1991) Anesthetic storage of recalcitrant seed: nitrous oxide prolongs longevity of lychee and longan. Hortic Sci 26(5):597–599
Stern RA, Gazit S (2003) The reproductive biology of lychee. Hortic Rev 28:393–453
Stern RA, Gazit S, El-Batsri R, Degani C (1993) Pollen parent effect on outcrossing rate, yield and fruit characteristics of 'Floridian' and 'Mauritius' lychee. J Amer Soc Hort Sci 118:109–114
Stern RA, Eisenstein D, Voet H, Gazit S (1996) Anatomical structure of two day old litchi ovules in relation to fruit set and yield. J Hortic Sci 71:661–671
Su MS, Lin SQ, Chen ZG, Yu CH, Lai ZX, Chen XJ (2004) Callus induction of litchi from flower. South China Fruit 33(6):62–63
Sun J, Li C, Prasad KN, You X, Li L, Liao F, Peng H, He X, Li Z, Zhang Y (2012) Membrane deterioration, enzymatic browning and oxidative stress in fresh fruits of three litchi cultivars during six day storage. Sci Hortic 148:97–103. doi:10.1016/j.scienta.2012.09.023
Tan SD, Wei JD, Lan RX, Wei JX (1999) Study on the structure and dynamics of pest community in lichee orchard. J Plant Prot 26:213–218
Teng YH (1996) Pruning and regulation of blooming time in high density planting of Lychee (*Litchi chinensis* Sonn.). Ph.D. Thesis. Department of Horticulture, National Taiwan University, Taipei, Taiwan
Thorpe TA (1990) The current status of plant tissue culture. In: Bhojwani SS (ed) Plant tissue culture: applications and limitations. Elsevier, Amsterdam, pp 1–33
Thomas P, Ravindra MB (1997) Shoot tip culture in mango; Influence of medium, genotype, explant factors, season and decontamination treatments on phenolic exudation, explant survival and axenic culture establishment. Hortic Sci 72:713–722
Tongpamnak P, Patanatara A, Srinivas P, Babprasert C (2002) Determination of genetic diversity and relationshiops among Thai litchi accessions by RAPD and AFLP markers. Kasetsart J (Nat Sci) 36:370–380
Underhill S, Critchley C (1994) Anthocyanin decolorisation and its role in lychee pericarp browning. Aust J Exp Agric 34:115–122
Underhill PA, Jin L, Lin AA, Mehdi SQ, Jenkins T, Vollrath D, Davis RW, Cavalli-Sforza LL, Oefner PJ (1997) Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography. Genome Res 7:996–1005
Ussuf KK, Laxmi NH, Mitra R (2001) Proteinase inhibition: plant derived genes of insecticide protein for developing in sect resistant transgenic plants. Curr Sci 80:847–853
Vevai EJ (1971) Know your crop, its pest problems and control of minor tropical fruits. Pesticides 5:31–54
Viruel MA, Hormaza JI (2004) Development, characterization and variability analysis of microsatellites in lychee. Theor Appl Genet 108:896–902
Villiers E, De A, Mathee WA (1973) The occurrence of bark borers on litchi and other fruit trees. Citrus Growers Subtrop Fruit J 477:17–19
Wang JB (2007) Analysis of physiological changes and differential gene expression during pericarp senescence of postharvest Litchi. Dissertation, South China University of Tropical Agriculture

Wang HC, Huang XM, Hu GB, Huang HB (2003a) Studies on the relationship between anthocyanin biosynthesis and related enzymes in litchi pericarp. Sci Agric Sin 37:2028–2032
Wang XH, Chen YG, Hua M, He F, Han J, Miao PS (2003b) Identification of some litchi germplasms by RAPD analysis. Chin J Trop Agric 23(2):1–4 (in Chinese)
Wang X, Yuan S, Wang J, Lin P, Liu G, Lu Y (2006a) Anti-cancer activity of litchi fruit pericarp extract against human breast cancer *in vitro* and *in vivo*. Toxicol Appl Pharmacol 215:168–178
Wang JB, Deng SS, Liu ZY, Liu LZ, Du ZJ, Xu BY, Chen YY (2006b) RAPD analysis on main cultivars of litchi (Litchi chinensis Sonn.) in Hainan. J Agric Biotechnol 14(3):391–396 (in Chinese)
Wang G, Li HL, Wang JB (2013) Primary study on the callus induction from anther of two litchi (*Litchi chinensis* Sonn.) cultivars. Chin J Trop Crops 34(4):669–674
Wang F, Wang JX, Li Q, He M (2014) Effects of GA3 and IAA on the germination of four species of Clematis seeds. Pratacultural Sci 31(4):672–676
Weber JL (1990) Human DNA polymorphisms based on length variations in simple-sequence tandem repeats. In: Davis KE, Tilghman SM (eds) Genome analysis, Genetic and physical mapping, vol 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 159–181
Witjaksono, Litz RE (1999) Induction and growth characteristics of embryogenic avocado cultures. Plant Cell Tissue Organ Cult 58:19–29
Wu SX (1998) Encyclopaedia of China fruits: Litchi. China Forestry Press, Beijing, 221 p
Wu YH, Chiu CH, Yang DJ, Lin YL, Tseng JK, Chen YC (2013) Inhibitory effects of litchi (*Litchi chinensis* Sonn.) flower-water extracts on lipase activity and diet-induced obesity. J Funct Foods 5:923–929
Xia Q, Chen HRZ, Fu JR (1992a) Effect of desiccation, temperature and other factors on the germination of lychee (*Litchi chinensis* Sonn.) and longan (*Euphoria longan steud*.) seeds. Seed Sci Technol 20:119–127
Xia Q, Chen HRZ, Fu ZJR (1992b) Moist storage of lychee (*Litchi chinensis* Sonn.) and longan (*Euphoria longan* steud.) seeds. Seed Sci Technol 20:269–279
Xiang X, Ou LX, Chen HB, Sun QM, Chen JZ, Cai CH et al (2010) EST-SSR analysis of genetic diversity in 96 litchi (*Litchi chinensis* Sonn.) germplasm resources in China. Genomics Appl Biol 29:1082–1092
Xie YM, Yi GJ, Zhang QM, Zeng JW (2006) Somatic embryogenesis and plantlet regeneration from anther of Feizixiao litchi. Chin J Trop Crops 27(1):68–72
Yamanishi R, Yoshigai E, Okuyama T, Mori M, Murase H, Machida T et al (2014) The anti-inflammatory effects of flavanol-rich lychee fruit extract in rat hepatocytes. PLoS One 9:e93818
Yang DJ, Chang YY, Hsu CL, Liu CW, Wang Y, Chen YC (2010) Protective effect of a litchi (*Litchi chinensis* Sonn.)-flower-water-extract on cardiovascular health in a high-fat/cholesterol-dietary hamsters. Food Chem 119:1457–1464
Yang DJ, Chang YZ, Chen YC, Liu SC, Hsu CH, Lin JT (2012) Anti-oxidant effect and active components of Litchi (*Litchi chinensis* Sonn.) flower. Food Chem Toxicol 50:3056–3061
Yi GJ, Huo HQ, Chen DC, Huang ZR, Cai CH, Qiu YP (2003) Studies on genetic relationship among litchi varieties by using AFLP. Acta Hortic Sin 30:399–403
Yu YB (1991) Study on some factors in tissue culture of lychee(*Litchi chinensis*). Fujian Agric Sci Tech 5:17–18
Yu CH, Chen ZG (1997) Induction of litchi embryogenic calli by immature embryos and anthers culture in vitro. J Fujian Agr Univ 26:168–172
Yu CH, Chen Z (1998) Embryogenic suspension culture and protoplast isolation in lychee. Chinese J Trop Crops 19:16–21 (in Chinese with English abstract)
Yu CH, Chen ZG, Lu LX (1996) Regeneration of plantlets from protoplasts culture of litchi. J Fujian Agric Univ 25(3):386
Yu SQ, Fang LX, Tang WQ, Xue Y, Liu JF, Wang CX, Li DS, Liu ZC (1995) Research on using green technologies in controlling major litchi insect pests and diseases. Guangdong Agric. Sci. 3:38–40 (in Chinese)

Yu C, Chen Z, Lu L, Lin J (2000) Somatic embryogenesis and plant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Organ Cult 61:51–58

Zhang D, Quantick PC, Grigor JM (2000) Changes in phenolic compounds in Litchi (Litchi chinensis Sonn.) fruit during postharvest storage. Postharvest Biol Technol 19:165–172

Zhou T, Chu C-L, Liu WT, Schaneider KE (2001) Postharvest control of blue mold and gray mold on apples using isolates of Pseudomonas syringae. Can J Plant Pathol 23:246–252

Zhou LN, Kuang ZS, Ma XJ, Chen JQ, Cao J (1996) The study of factors affecting somatic embryogenesis in young embryo culture of *Litchi chinensis*. J Agric Biotechnol 4:161–165

Biotechnological Interventions in Litchi (*Litchi chinensis* Sonn.) for the Improvement of Fruit Quality and Postharvest Storage

4

Annamalai Muthusamy, Puthanvila Surendrababu Swathy, and Kodsara Ramachandra Kiran

Abstract

Litchi (*Litchi chinensis* Sonn.) is an evergreen subtropical fruit, which is well acclaimed for its delicious, juicy aril and refreshing taste. Litchi fruit cultivation became an integral part of many Southeast Asian country's economy and has a tremendous demand in domestic and export market. Insufficient genetic data about the native cultivar, poor knowledge and availability of the superior cultivars, lack of pest management control, and technological deficiency for the production and postharvest storage are the major constraints in litchi production and development all over the world. Biotechnological interventions have been successfully introduced in the field of litchi production for the massive micro-propagation, *in vitro* generation, and improvement of the quality of the available cultivars to produce superior cultivars with high yield. Widening of the genetic base of native cultivars using different molecular markers, introduction of genetic engineering to produce promising hybrids with large fruit, resistance to pericarp browning, and long life-span are highly recommended in this field with the help of biotechnological tools. In the present review, we have attempted to overview the combining research and development for the improvement of fruit quality and postharvest storage using various conventional as well as biotechnological tools.

Keywords

Litchi • Subtropical fruit • Biotechnological intervention • Micro-propagation • Postharvest

A. Muthusamy (✉) • P.S. Swathy • K.R. Kiran
Department of Plant Sciences, School of Life Sciences, Manipal University, Manipal 576 104, Karnataka, India
e-mail: amsamy20@gmail.com

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4.1 Introduction (Distribution and Cultivation)

Litchi or lychee (*Litchi chinensis* Sonn.) belongs to the family Sapindaceae and subfamily Nephelaceae which has 125 genera and nearly 1000 species. The genus, *Litchi*, has two species, *Litchi philippinensis* and *Litchi chinensis*, usually known as Pearl of India and is an important fruit tree in tropical and subtropical region of the world. It ranks next to citrus and avocado in popularity for its better quality characteristic features; juicy refreshing summer fruits with significant taste; potential nutritional values; pleasant flavor and elegant color with its high sugar, protein, fat, mineral, and vitamin C, A, B1, and B2 contents; and value-added products such as squash, pickles, preserves, nuts, wine, canned fruit, and dried litchi making it prevalent in national as well as international markets. The fruit crop pays substantially to the employment and economy of millions of people in Southeast Asia (Li et al. 2013; Maity and Mitra 1996; Ochese et al. 1961; Singh and Singh 1964). Litchi belongs to non-climacteric fruit which contains a single large, brown, shiny inedible stone or seed with leathery scaly skin enclosing white, translucent, firm flesh which has sweetness and fragrance (Thompson 2003). Commercial industries have been established for litchi in India, China, Taiwan, Thailand, South Africa, Australia, among others. The anatomy of litchi tree and inflorescence was described by Hallè et al. (1978) and Weberling (1992). The lychee inflorescence is heterocladic pleiothyrsoid, with the development of an additional paracladia from the serial bud below the first-order paracladia. The flowers appear as three different form in an irregular sequence as male flowers, hermaphrodite that acts as male and female (Morton 1987). Litchi flowers are small and not suitable for hand pollination (Stern and Gazit 1998). Based on the cultivar and time, the position and gender of the flowers vary (Robbertse et al. 1995). The functionally active male and female flowers with a superior bicarpelar ovary with two ovules, rarely three, produce a single fruit at a time. The young litchi fruit is elongated and transformed to heart-shaped fleshy fruit when it attains maturity with one or two seeds attached to the internal angle of the marginal sutures. The fruit is covered with red-colored, thin, rough, and wrinkled pericarp because of the presence of brachisclereides immediately below the epidermis. The initial development of the fruit is with the formation of pericarp with three layers as epicarp, mesocarp, and endocarp (Nacif et al. 2001).

Litchi fruit crop is ethnic to China especially the southern part, Hainan Island, and northern Vietnam where litchi trees flourish along the rivers and near the seacoast. It is introduced to other parts of world since 400 years for tropical and subtropical climates (Huang et al. 2005a, b; Menzel 1995; Morton 1987). Currently China, India, Thailand, and Vietnam are the leading producers of litchi fruit (Menzel 2002), and more specifically, China holds the largest industry in the world, where litchi being cultivated for more than 2300 years (Huang et al. 2005a, b; Wu 1998). The major litchi producers in the world are China, Vietnam, Thailand, India, Bangladesh, and Nepal which constitutes 95% of the litchi production. Owing to long period of cultivation of litchi in China, the National Litchi Germplasm Gene Bank at Institute of Fruit Tree Research, Guangdong Academy of Agricultural Science, Guangzhou, China, has the largest litchi germplasm gene bank with more

Table 4.1 Commercial varieties of litchi from different states of India

State	Commercial varieties
Bihar	Shahi, Rose Scented, China, Kasba, Purbi, Early Bedana, Late Bedana
Utter Pradesh	Rose Scented, Dehradum, Calcutta
West Bengal	Bombay Green, Kalyani Selection
Punjab	Muzaffarpur, Dehradun, Seedless, Late Bedana

Source: Vision 2050, NRC on Litchi, ICAR

than 500 accessions (Li 2008). Among the accessions, the best cultivars are fruit which contains large edible portion with good sweet flavor and small seeds (Huang et al. 1990). In India, litchi is being cultivated in Bihar, Utter Pradesh, West Bengal, Uttarakhand, Assam, Punjab and Tripura with different morphological features due to genetic factors as well as climatic factors. The names of Indian commercial varieties from four states of India are depicted in Table 4.1, and area of litchi cultivation and production are shown in Fig. 4.1. The better scope for expansion of area under cultivation of lychee in Terai region of West Bengal is due to its prevalent appropriate agroclimatic condition (Khurshid et al. 2004).

Wild lychees account for 50% of the virgin forest composition and major population in several lowland rainforest areas on Hainan Island. In eastern and southern Asia, litchi has been widely cultivated. The history of litchi cultivation is more than 300 years in Taiwan. Due to the good fruit quality and better crop yield, "Hak Ip" is the most cultivated litchi cultivar in the region, which constitutes 70% of the total litchi production in Taiwan (Chang et al. 2012). The flower initiation and development of fruits are critically controlled by several environmental factors, genetic makeup and physiology, and various pests and pathogens. Therefore, the imbalance between the supply and demand of fresh fruit since the prevalence of dominant single cultivar which confines the litchi harvest by June in Taiwan (Batten and Lahav 1994; Groff 1943; McConchie and Batten 1991; Yuan and Huang 1998). Litchi flowering requires cool temperature and flush maturity. Proportionate amount of pesticides, pest management, and disease control is essential for the development and good quality fruit (Chang et al. 2014; Menzel and Simpson 1995). The shelf life of litchi fruit is estimated to be around 25–30 days fewer than in 2–5 °C storage temperatures (Chang et al. 2014; Chang and Lin 2008; Hieke et al. 2002; Holcroft and Mitcham 1996; Stern and Gazit 1996, 1998; Stern et al. 1996). Litchi grows in sandy loam to loam soils. In the Indian scenario, litchi cultivation is higher in the soil with calcium carbonate and in loamy soil with high moisture content. The pH of the soil is also to be considered for the proper development and growth.

The acidic soil is suitable for the tree growth in comparison with neutral or alkaline conditions in South Africa. In Indian condition also, the soil pH with 5.5–7.0 is optimum for the litchi plant development. Litchi has been widely established in Thailand, and the most cultivated cultivars are Tai So a lesser degree Wai Chee, Baidum, and Chacapat (Singh et al. 2012). The litchi cultivation was extended to

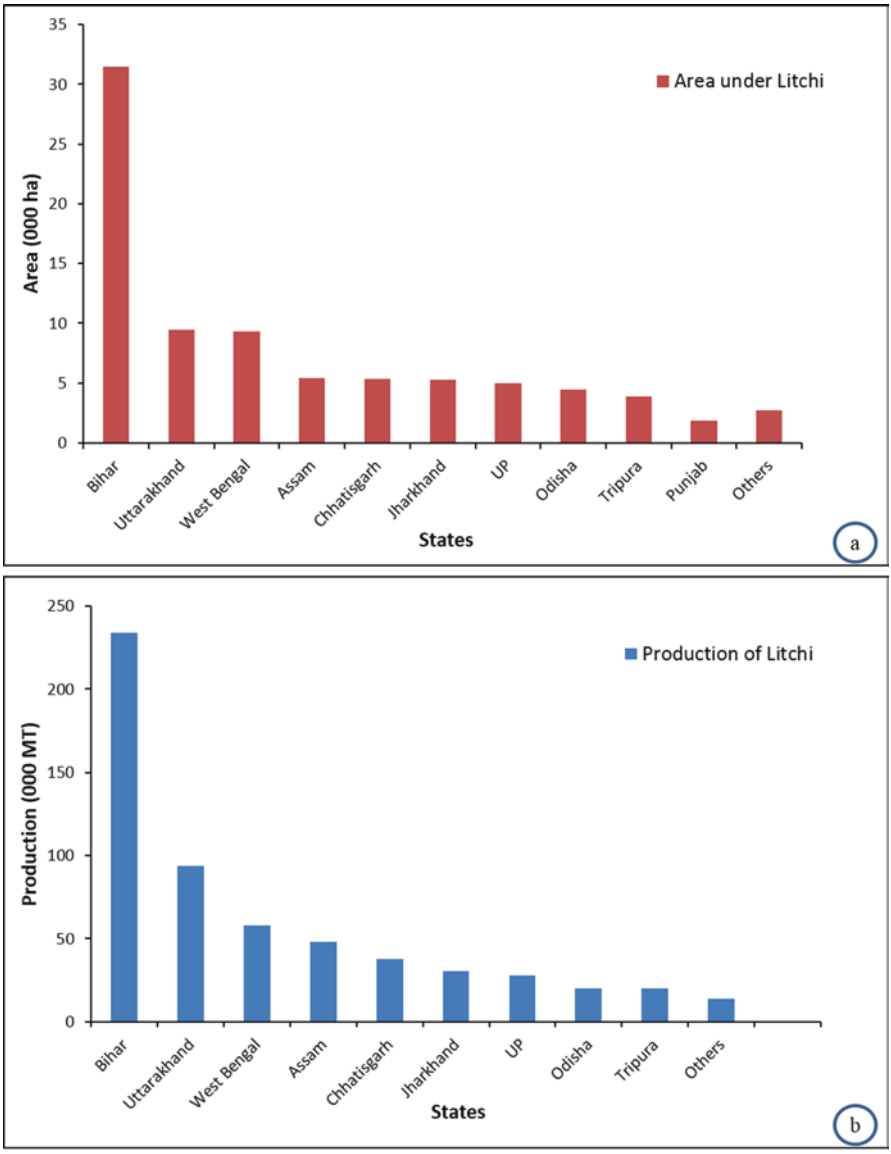

Fig. 4.1 Area (000 ha) and production (000 MT) in different states of India during 2013–2014 (Source: Vision 2050, NRC on Litchi, ICAR, Muzaffarpur, Bihar)

other countries such as South Africa in the early 1860s. The major cultivation in South Africa is depending on a single cultivar, named as H.L.H. Mauritius (Oosthuizen 1991). In the United States, Hawaii and Florida are the major centers for litchi production. In addition, to the Tai So cultivar, Brewster, Haak Yip, and Sweetcliff are well established in Hawaii, whereas Brewster is the main cultivar in

Florida. India and China is responsible for 91% of litchi production in the world and domestic markets. The annual production of litchi in India is about 585,300 metric tons encompassing an area of 84,170 ha. Due to the climatic needs, the production in India is restricted to few states with Bihar responsible for 40% of production. Litchi cultivation and production has become a major source of income for millions of people in Bihar. The annual productivity of litchi in Bihar is 6.95 tons/ha.

4.2 Nutrients of Litchi Fruits

Litchi is a delicious and important subtropical fruit with rich source of natural products and nutrients which is being used worldwide. Due to the high demand, the nutrient composition and compound profiling of the fruits has been studied in detail. The edible part of fruit is translucent juicy aril (Chadha and Rajpoot 1969), which is an organ without vascular tissue and stored sugars that accounts for 15–20% of the fresh mass. The quality and flavor are significant factors which is determined by sugar content and other compositions of the fresh fruits. Glucose, fructose, and sucrose are major sugar in aril of litchi, and the accumulation of sugar content varies among litchi cultivars (Yang et al. 2013). The aril initiates from seed stalk or funicle which acts as bridging tissue between the aril and the vascular pedicel. Several studies have proved that it acts as a tonic for the brain, heart, and liver (Syamal and Mishra 1984). The fresh lychee fruit is a good source of vitamin C, vitamin B complex, and other anti-oxidant molecules. About hundred grams of fruit contains 72 mg of vitamin C which is essential for 86% of daily value. Litchi is also rich of carbohydrates, proteins, and other dietary mineral contents. A 100 g litchi fruit consists of 4 mg calcium, 0.7 mg iron, 171 mg potassium, and 10 mg magnesium. The nutrient components of litchi fruit (100 g FW) are depicted in Table 4.2. A bioactive molecule named α-(methylenecyclopropyl)glycine, an analogue of hypoglycin A, was isolated from litchi seeds and showed hypoglycemic activity in animals (Gray and Fowden 1962). The other parts such as leaves, flowers, and bark also have exhibited various medicinal properties.

The bright color and delicious taste makes the litchi very popular in international market. Litchi fruit alone provides 2–4% of daily nutritional requirements. Apart from the fresh fruit market, litchi is processed into juices, purees (Neidhart et al. 2007), and canned (Hoppe et al. 2006), frozen, and dried fruit (Nagle et al. 2011). Using litchi vinegar, a fermentation product of fruit, is rich in vitamins, minerals, and amino and has many health benefits. Production of litchi vinegar has dual advantage since it helps to improve the litchi industry as well as vinegar types. Recently Qiu et al. (2015) reported the standardized protocol for alcohol fermentation of litchi with 16% original sugar content, 5% yeast inoculation, pH value of 3.5, and fermentation temperature of 30–32 °C. They are optimized for acetic acid fermentation with 10% inoculation, 7.0% of original alcohol content, and fermentation temperature of 30 °C to obtain a yield of 52.45 g/L acetic acid through response surface methodology.

Table 4.2 Nutritional composition of litchi fruits

Nutritional components	(Per 100 g FW)
Water	82%
Protein	0.7 g
Fat	0.1 g
Carbohydrate	15 g
Vitamin C	72 mg
Vitamin E	0.07 mg
Thiamine	0.02 mg
Niacin	1.1 mg
Riboflavin	0.07 mg
Phosphorus	32 mg
Iron	0.7 mg
Calcium	4 mg
Copper	0.15 mg
Potassium	171 mg
Magnesium	10 mg

Source: http://www.nutrition-and-you.com/Lychee.html

4.3 Bioactive Molecules and Medicinal Properties

Flavonoids, phenolic acids, anthocyanins, coumarin, lignans, chromanes, sesquiterpenes, fatty acids, sterols, and triterpenes are the major compounds found in the litchi fruit (Table 4.3).

Phenolics is a major class of plant secondary metabolites which exhibits several properties useful for human health. Litchi fruit and its secondary metabolites have been used in traditional medicine as anti-cancer, anti-inflammatory, antifungal, antiviral, antiplatelet, anticoagulant, anti-diabetic, and anti-oxidant. The biological activities of phenolics include high anti-oxidant, anti-inflammatory, and antimicrobial effects. In China and Southeast Asia, the litchi fruit has been consumed for its edible part which is rich in polyphenols, oligonol, and proanthocyanidin content since the time immemorial (Brat et al. 2006; Fujii et al. 2007; Miura et al. 2010; Sarni-Manchado et al. 2000). The aril part of litchi fruit contains 12.1–14.8% total sugars, 9–13.7% reducing sugar, 1.0–3.4% nonreducing sugar, 0.22–0.36% citric acid, and 34.5–45.4 mg/100 g of fruit. The plant also contains several organic acids such as levulinic, malic, lactic, malonic, fumaric, succinic, phosphoric, and glutaric acids. The bark of litchi tree consists of friedelin and stigmasterol, and 42.0% fatty acids such as cyclopropanoic, 27.0% oleic, 12.0% palmitic, and 11.0% linoleic have been reported (Khare 2007). Volatile categories are organic acids, fatty acids, amino acids, saponins, flavonoids and sugar. Besides the potential medicinal value, it is also used in cosmetic industry and as biopesticides, or fertilizers from the residue of

Table 4.3 Bioactive components of litchi fruits

Group	Compound	References
Anthocyanins	Cyanidin-3-glucoside	Li et al. (2012)
	Cyanidin-3-rutinoside	
	Malvidin-3-glucoside	
Proanthocyanidins	Procyanidin B2	Lv et al. (2015)
	Epicatechin	Zhou et al. (2011)
	A-type procyanidin trimer	Lv et al. (2015)
	Procyanidin A2	Sun et al. (2010)
	Epicatechin-(4β-8, 2β-O-7)-epicatechin-(4β-8)-epicatechin	Liu et al. (2007)
Flavonoids	Methylene-linked flavan-3-ol dimer, bis(8-epicatechinyl)methane	Qiu et al. (2015)
	Kaempferol	Jiang et al. (2013)
	Quercetin-3-O-rutinoside-7-O-α-L-rhamnoside	Su et al. (2014)
	Quercetin-3-O-rutinoside	Su et al. (2014)
Phenolics and phenolic acids	Catechin, caffeic acid, chlorogenic acid	Zhang et al. (2013)
	Epicatechin glucoside	Zhou et al. (2011)
	Epiafzelechin	
	Epicatechin glucoside	

peels, seeds, and wastes from extraction process (Deerasamee and Chaisawadi 2014). The parts of lychee fruit such as flesh, peel, and seeds have several health benefits with its anti-oxidants, dietary fiber, and vitamins and are used to treat various diseases such as oxidative stress, hyperuricemia, fatigue, analgesic, visceral fat, viral disease, cardiovascular and brain diseases, cancer, neurodegenerative disorders, cataracts, inflammatory markers, and immunomodulation (Aruoma 1999; Chang et al. 2013; Chauhan et al. 2014, Ghosh and Scheepens 2009; Govindarajan et al. 2005; Hwang et al. 2013; Kang et al. 2012; Lee et al. 2010; Li et al. 2012; Moriwaki et al. 2011; Nagasawa et al. 2010; Nishizawa et al. 2011; Ohno et al. 2008; Wen et al. 2012; Yang et al. 2006, 2011; Zhao et al. 2007). The progressive association was noted with consumption of food rich in phenolics and low prevalence of degenerative diseases such as cancer, heart disease, inflammation, arthritis, brain dysfunction, and cataracts (Kris-Etherton et al. 2002). Various studies have found out the anti-oxidant activity of litchi fruit pericarp and pulp using DPPH scavenging assays. The proanthocyanidins from the litchi fruit pericarp showed high anti-oxidant activities, and several studies exhibited that the presence of polyphenolic compounds and polysaccharides with high degree of free radical scavenging effects

(Liu et al. 2007; Yang et al. 2006; Zhao et al. 2006). Among the proanthocyanidins, the anti-oxidant capability was higher in proanthocyanidin B2 than epicatechin and proanthocyanidin B4 (Duan et al. 2007). Various extraction of litchi fruit pericarp also exhibits anti-cancer properties against breast cancer cell lines MCF7 and human embryonic lung fibroblast (HELF) by inhibiting the uncontrolled division of cancer cells and activating apoptosis signaling pathways (Wang et al. 2006a, b; Li and Jiang 2007). In addition to the complete extract of the fruit pericarp with hexane, water, and ethyl acetate fraction, other studies have been performed to evaluate the anti-cancer activity of combined or single bioactive molecules isolated from fruit pericarp. For example, epicatechin, procyanidin B2, and procyanidin B4 exhibited dose- and time-dependent inhibition of the proliferative cancer cells (Li and Jiang 2007). The aqueous extract of litchi fruit pericarp also exhibited hepatoprotective activities by gradual decrease in the apoptotic cells with morphological changes (Bhoopat et al. 2011). A study showed the anti-inflammatory activity of flavonoids present in the litchi fruit extract by suppressing the inflammation (Nishizawa et al. 2011). In addition, the litchi fruit extracts exhibited anti-diabetic activity (Lee et al. 2009), antiplatelet activity (Sung et al. 2012), and anti-viral activity (Xu et al. 2010a, b; Gangehei et al. 2010).

The litchi seeds have showed several pharmacological effects in regulation of qi, dissipating cold and emancipating pain. It has been used in the treatment of epigastric pain, qi and liver stagnation, hernia pain, qi stagnancy and blood stasis of women, testicular swelling (Castellain et al. 2014; Hwang et al. 2013; Jiang et al. 2013), modulation of blood glucose and lipid, and anti-oxidant, anti-virus, anti-tumor, liver injury (Lin et al. 2013; Wen et al. 2014; Zhao et al. 2007). The anti-tumor activity of litchi extracts has been studied in detail. The litchi extract suppresses the proliferation of MCF cells, S180 sarcoma, Ehrlich ascites carcinoma (EAC) (Lin et al. 2008). The study in HepG2 cell showed a dosedependant induction of apoptotic cell death (Hsu et al. 2012; Xiong et al. 2008). The ethanolic extract of litchi fruit pericarp exhibited inhibitory effect on MDA-MB-231 cells (Wang et al. 2006a, b). Litchi seeds also have shown anti-tumor potential on cell proliferation of CNE-2Z of nasopharynx (Wang et al. 2007; Zhang et al. 2012, cervical and lung cancer (Lv et al. 2014; Wen et al. 2014). Xu et al. (2010a, b) have reported that two new A-type trimericproanthocyanidins with two doubly bonded interflavanoid linkages, litchitannin A1 [epicatechin-(2β → O → 7,4β → 6)-epicatechin-(2β → O → 7,4β → 8)-catechin] and litchitannin A2 [epicatechin-(2β → O → 7,4β → 6)-epicatechin-(2β → O → 7,4β → 6)-epicatechin], were isolated from lychee (*Litchi chinensis* Sonn. cv. Heiye) seeds together with aesculitannin A, epicatechin-(2β → O → 7,4β → 8)-epiafzelechin-(4α → 8)-epicatechin, proanthocyanidin A1, proanthocyanidin A2, proanthocyanidin A6, epicatechin-(7,8-bc)-4β-(4-hydroxyphenyl)-dihydro-2(3H)-pyranone, and epicatechin from litchi seeds with potent anti-oxidant activity, in vitro anti-viral activity of litchitanin, and anti-herpes simplex virus activity of aesculitannin and proanthocyanidin. Lin et al. (2015) have observed seven new δ-tocotrienols, designated litchtocotrienols A–G

(1–7), together with one glorious macrocyclic analogue, macrolitchtocotrienol A, and one new meroditerpene chromane, cyclolitchtocotrienol A, with anti-cancer activity against gastric adenocarcinoma and hepatoma carcinoma cell line. Ma et al. (2014) have reported the anti-oxidant activity of bis(8-epicatechinyl)methane and epicatechin from litchi. Hsu et al. (2012) have reported the induced apoptosis, cell death, and cell cycle arrest of colorectal carcinoma cells through suppression of cyclins, elevation of Bax/Bcl-2 ratio, and caspase-3 activity in dose-dependent manner. Zhou et al. (2012) have reported the bioactive compounds, hydrobenzoin and 5-hydroxymethyl-2-furfurolaldehyde, for the first time from ethyl acetate extract of litchi fruits with stimulation of prostaglandin E2 and nitric oxide production, regulation of cyclooxygenase-2, nitric oxide synthase mRNA expression, and activation of NF-$_{\kappa}$B (p50). Lin et al. (2012) have showed enhanced anti-oxidant activity and DNA protection effect of litchi pericarp extract after bioconversion by *Aspergillus awamori*, and they suggested the potential way to utilize the fruit pericarp as easily available sources of natural anti-oxidants as effective application of fruit by-products for food industry. The phytochemical investigation of litchi pericarp reveals a novel compound, benzoic acid, with seven known compounds, and benzoic acid showed substantial anti-oxidant activity (Jiang et al. 2009, 2013; Yang et al. 2008; Zhao et al. 2006).

4.4 Improvement of Fruit Quality and Postharvest Storage

Maximum number of species of tropical fruits is found in Africa and tropical America (about 1200 and 1000 species, respectively). Asia harbors about 500 species and Indian subcontinent has about 300 species of the tropical fruits. Banana has the biggest annual production (107 metric tons in 2013) among freshly consumable fruits, whereas mango, pineapple, papaya, and avocado account for 75% in 2013 (FAOSTAT 2013). In spite of its commercial importance, sophisticated studies on breeding and genetics of tropical fruits are not developed. The breed improvement is mostly by the natural means like selection of chance seedlings, open-pollinated seedlings, or mutations. Crop improvement has been achieved in pineapple, papaya, and avocado by controlled pollination. Some tropical crops, like Cavendish bananas which has high commercial value in international market, are inherently difficult to breed due to its triploidy. Mango trees has few flowers, which in turn bear very few pollen grains that results in the production of less number of seeds by controlled pollination. Mangosteen (*Garcinia mangostana* L.) sets fruits parthenocarpically, and male flowers have not been reported, which made cross-pollination impractical. Some tropical fruits like durian (*Durio zibethinus* Murray) have very long juvenile period which extends up to 7–10 years (Paull and Duarte 2011). Even although India may be the world's second largest producer of litchi and growers have much experience with the crop, yields are low. Poor productivity is a direct result of issues associated with land tenure and the selection of inappropriate planting material.

Farmers are unwilling to sacrifice production in the short term in order to replace or to rework trees with superior planting material. Furthermore, the necessary postharvest infrastructure and transportation are not available to move the fruit from the main producing area to the international airport (Batt and Cadilhon 2007).

The genetic enhancement of temperate fruit tees was established and achieved than tropical trees which have limitation. Substantial resources are very imperative to develop the breeding programs of new cultivars of tropical fruit tree which are adapted to modern shipping and storage requirements. The limitations in the recombination mapping and recurrent selection have been solved by using molecular markers in tropical tree breeding (Arias et al. 2012).

4.5 Fruit Quality

The increased production of sufficient, nutritionally adequate, and culturally acceptable food in sustainable manner is today's most urgent effort for an active and healthy life of ever-growing population, while tremendous efforts are being taken to conserve the natural plant genetic resources for future. Among the food, the vegetables are dynamic sources of carbohydrates, minerals, dietary fibers, and vitamins and play indispensable role in providing nutrition to human health (Kirti Singh 2004). The fruits and vegetables are being cultivated worldwide to meet the demands in major part of human as well as animal diet. They provide wide range of minerals, dietary fiber, bioactive molecules/phytochemicals, and particularly potential sources of vitamins A, B1, B6, B9, C, and E and play imperative role as human nutrition (Dias and Ryder 2011). The daily diet from vegetables and fruits has highly significant impact on human health in general and improvement of gastrointestinal health, proper vision, reduction in cardiovascular diseases, stroke, diabetes, and different forms of cancer (Keatinge et al. 2010). The worldwide consumption of plant-based foods especially vegetable and fruit is increasing constantly with the reflection of enhanced income of consumer's, desire of food diversity, and awareness of nutritional benefits and its imperative role in preventing human diseases than animal-based foods (Kays and Dias 1995; Kays 2011).

Constant research efforts being taken to enhance the nutritional status and quality of plants have its own traditional limitation due to dearth of fundamental knowledge on interlinks of plant metabolic pathways and its products. However, with the availability of advanced molecular biological tools in the field of metabolomics, proteomics, genomics, as well as bioinformatics, we have great prospective in screening and identification of genes involved in various complex metabolic pathways. The tools of plant metabolic engineering will increase our understanding of the regulation and function of over- or under-expressed genes/enzymes and their products in major as well as underutilized crops for the enhancement of nutritional content which is very imperative for human and animal health and well-being (McGloughlin 2010).Currently several strategies and methods including new tools from conventional breeding and modern recombinant DNA technology have been implemented to improve the fruits and vegetables for its quality, bioactive

molecules, processing of fresh-cut fruits, shelf life, as well as important traits such as browning reactions, texture, tissue degradation, delayed or inhibited senescence, and inhibition of proliferation of the harmful microbes (Rodov 2007).

4.5.1 Conventional Breeding

The diminutive life-span and loss of seed viability, lower growth rate of seedlings, and long period of vegetative growth are severe factors which accompany with the development of seedlings. Diverse plant breeding strategies are applied for the progression of the litchi cultivars with enhanced traits. The improvement in the litchi cultivars majorly consists of the identification and selection of open-pollinated seedling from existing cultivars. The new cultivars should possess high crop yield capacity, better fruit quality, small and flat seeds, cracking resistant, more desirable flavor and taste, bigger size, early maturity cultivars, and high resistance to microbes and pests (Pivovaro 1974; Singh and Singh 1952). Conventional plant breeding is mainly through the hybridization or the cross breeding of the two superior varieties to obtain the highly qualified traits. It mainly involves the selection from mutations of existing cultivars and seedlings from open pollination. The pollination between two major litchi varieties to obtain superior hybrid is also in practice. For example, the two varieties Purbi and Bedana were pollinated for a variety named "SabourMadhu." The major cultivars produced in the past several years are "Salathiel" in Australia, "SahKeng" from "Haak Yip" in Taiwan Province of China, "Bengal" from "Purbi" in India, and several new types from Guangdong. The generation of plants through asexual produce is identical parental types without any variation in the traits. Various breeding programs have been carried out in Australia (Dixon et al. 2005), India (Singh and Singh 1952; Thapa et al. 2014), China (Huang et al. 2005a, b), Hawaii, and South Africa (Froneman and Oosthuizen 1995). Cross-breeding is also an approach to improve the litchi cultivars. In a recent study, 76 clones were selected from F1 generation of hybrids between Maguili and Jiaohesanyuehong to obtain an improved quality cultivar (Zhao et al. 2014). Seven novel litchi cultivars were developed in Taiwan popularly known as "early bird" which have the several advantageous traits like early maturation, large fruit size, improved flowering and fruit set ratio (Chang et al. 2015). Genetic diversity in morphological characteristics of four cultivars of litchi, i.e., Bedana, Bombay, Calcutti, and Gola, growing under the agroclimatic conditions of Multan was studied (Khurshid et al. 2004). Cryogene bank was established for litchi, and 19 cultivars have been stored to facilitate the long-term breeding program. Thapa et al. (2014) reported the morphological features of seven cultivars of litchi during 2013–2014 and selected the cultivar litchi cv. China shows substantial responses in terms of plant height, number of shoots/tree, and average of length of new shoots. The major aim of these entire breeding programs is to produce new cultivars with improved and promising traits with less harvesting time. The time-consuming process to produce new cultivar through convention breeding leads the breeders to combine

advanced techniques including transgenic crop technology and molecular-assisted breeding in the litchi breeding programs.

4.5.2 Role of Plant Cell, Tissue, and Organ Culture

The mature phase or elite cultivars lack the de novo in vitro regeneration which impede the improvement of existing litchi cultivars through several biotechnological approaches. Though the propagation of litchi cultivars using conventional methods such as air layering or marcottage, grafting, and budding (Menzel 1985) is incompetent and time-consuming (Chapman 1984; Sarin and Prasad 2003), it is unattractive *via* seed propagation since the existing cultivars are highly heterozygous (Sarin and Prasad 2003) which challenges the task in development of new litchi cultivars via conventional breeding (Litz 1988). Plant cell, tissue, and organ culture techniques offer numerous benefits over usual conventional breeding in field using biochemical and other selection procedure and recovery of unique metabolic mutants (Mann 1997). The protocol for plant cell, tissue, and organ culture, plantlet regeneration and development of transgenic litchi that was established by several researchers for direct organogenesis are depicted in Table 4.4 and somatic embryogenesis/suspension culture from somatic cells/tissues in Table 4.5 and embryogenesis/organogenesis from gametic cells in Table 4.6. The present well-established in vitro regeneration protocol can contribute to (1) micro-propagate the unique litchi varieties in large scale via organogenesis, (2) produce artificial seeds via somatic embryogenesis to help the farmers with fresh plantlets against sudden loss and damage of plants due to unpredictable environmental factors, (3) induce somaclonal variations by in vitro mutagenesis, and (4) develop transgenic plants for higher yield, fruit quality, biotic disease resistance, and abiotic stress tolerance.

4.5.3 Transgenic Approaches

Genetically modified crops or transgenic crops are vital in the field of plant breeding since it improves the quality of the cultivars considerably and assures high resistance to insects and plant pathogens. The attempt of the scientific community, to produce vegetables and crops with advanced characters like seedless fruit, high nutritional content, and resistant to disease, was highly fulfilled through genetic modification. A review carried out by Dias and Ortiz (2014) showed that for the past several years, the genetic modification was performed on various crops such as tomato, eggplant, potato, cucurbits, brassicas, lettuce, alliums, sweet corn, cowpea, cassava, sweet potato, and carrots. The main outcome of the transgenic studies was the enhanced resistance of these crops against various pathogens, insects, nematodes, fungi, bacteria, and viruses. In addition, the transgenic crops exhibits high nutritional status, extended shelf life, and better quality and flavor, thereby increasing the economic value of the crop. Genetically modified crops with resistant to abiotic stress are not only beneficial to the farmers but also to the environment. The

Table 4.4 Direct organogenesis and plantlet regeneration of litchi

Explant	Regeneration method	Observation/benefits	References
Direct organogenesis			
	In vitro clonal multiplication	Responses from seedlings and no response from mature tissues	Wolf (1987)
Shoot, node, and adventitious buds	Axillary shoot proliferation, shoot organogenesis	Enhanced shoot proliferation	Kane (1996)
Seeds	Direct germination	Multiple shoot induction	Das et al. (1999)
Cotyledonary node and axillary bud	Multiple shoot induction	Plantlet regeneration	Das et al. (1999)
Shoot bud	Bud proliferation	Shoot differentiation and growth	Chandra and Padaria (1999)
Leaf	Leaf culture	Callus, proliferation, and plant regeneration	Puchooa (2004a, b)
		Callus induction	Raharjo and Litz (2007)
		Well-established plantlets	Kumar (2006)
Nodal Stem sprout buds	Multiple shoot formation	Plantlets	Yu (1991), Kumar et al. (2004), and Guo et al. (2014))

amount of insecticide spraying on the vegetable crops during season could be gradually decreased by the use of transgenic crop with host plant resistance. The use of insecticide in India and the Philippines was reduced to 50–80 times due to the use of genetically modified eggplant with Bt cry gene which has resistant to shoot borer in the eggplant. The modification in the metabolic pathway that facilitates the production of crop carotenoid is also promising trait to increase the economical and medicinal significance of many vegetables. The transgenic carrots with high calcium content help to reduce the incidence of osteoporosis, genetically modified lettuce with zinc to overcome its deficiency that severely impairs organ function, and transgenic lettuce with high content of tocopherol and resveratrol to resist coronary diseases and arteriosclerosis are promising. The folate deficiency can overcome with genetically modified tomatoes, to cope up with the non-nutritional diet. The feasibility of transgenic crop is vast in the area of food safety and nutritional status. Conventional plant breeding is incapacitated to address some of the biotic and abiotic confronts in the vegetable and food production, which could be overcome through the advance techniques in genetic engineering.

Due to the high cost, and the minimal resources invested, the horticulture is still the primary choice over transgenic crop technology among the breeders. The major research in the transgenic crop technology has been carried out by the multinational seed corporations. Even though the cost of genetically modified crops with resistant to pest and insects and valuable marketable product is reduced, the regulatory

Table 4.5 Somatic embryogenesis/suspension culture and plantlet regeneration of litchi

Explant	Regeneration method	Observation/benefits	References
Somatic embryogenesis/suspension culture			
	Embryogenic callus	Somatic embryogenesis and plant regeneration	Merkle (1995) and Yu et al. (2000)
	Embryogenic calli	Adventitious embryogenesis	Liao and Ma (1998)
Leaf	Callus		Puchooa (2004a, b)
Leaf	Globular embryo production	Somatic embryogenesis	Ma et al. (2009)
Callus	Suspension culture	Fast growth, well disparity, fine texture, and more use in biotechnology	Raharjo and Litz (2007)
Protoplast	Semisolid/suspension	SE and plantlet regeneration	Yu et al. (2000)
Protoplasts		SE and plantlet regeneration	Yu and Chen (1998)
Leaflets		Maintenance of embryogenic suspensions	Raharjo and Litz (2007)
		Embryogenic suspension	
	Embryogenic calli	Genetic variations	Wang and Wang (2010a, b)
	Embryogenic calli	Induction, maturation, and regeneration of somatic embryos	Lin et al. (2010)
	Embryogenic callus	Enzymatic activity and isozymes bands with high temperature stress	Shao et al. (2010)

package system remains expensive. Due to this reason, the multinational seed corporation dropped the production of more transgenic crops. The other disadvantage of transgenic crop is that introducing a transgene can be complicated in crops with difficulty for using backcrossing (e.g., banana, cassava, potato, or sweet potato).

Among the genetic manipulation strategies, the antisense RNA technique has been used widely not only for the study on gene function via gene silencing but also for breeding of specific traits in fruit crops such as mango (Cruz-Hernández et al. 1997), citrus (Wong et al. 2001), strawberry (Jimenz-Bermudez et al. 2002), papaya (Magdalita et al. 2002), apple (Kotoda et al. 2006), pear (Gao et al. 2007), and plum (Callahan and Scorza 2007). The genetic manipulation shows several advantages of high efficiency, and enhancement of yield-specific traits through directional approach provides a platform for the development of elite litchi cultivars (Das and Rahman 2012). Ouyang and Zheng (1985) described induced tumor formation in *Litchi chinensis* using four strains (C58, B3.73, T37, and ACH5) of *Agrobacterium*

Table 4.6 Gametic embryogenesis, organogenesis, and plantlet regeneration of litchi

Explant	Regeneration method	Observation/benefits	References
Organogenesis from gametic cells			
Anther	Organogenesis	Plantlets	Fu and Tang (1983)
	Callus	SE with root and no sprout	Deng (2005), Xie et al. (2006), Wang et al. (2013), Guo et al. (2014), and Xu and Lai (2013)
	Callus	Embryogenic callus	
Immature embryos	Multiplication of embryonic shoots	Plant regeneration	Kantharajah et al. (1992), Zhou et al. (1996)
	Embryoids		Zhou et al. (1996), Yu and Chen (1997), Yu et al. (2000), and Chawla (2004)
	Embryogenic calli	Slow growth	Wang and Wang (2010a, b)
Zygotic embryos		Haploid plant production	Amin and Razzaque (1995)
Anther and embryos	Callus, embryoids	Developmental studies	Kuang et al. (1996)
Pollen	Somatic embryogenesis	Plant regeneration	Wang et al. (2016)
Leaf	*Suspension culture*	Cell mass in suspension culture	Ma et al. (2009)
	Friable calli, suspensions		

tumefaciens and confirmed the presence of nopaline gene. The established protocol and development of transgenic litchi are available for few cultivars using *Agrobacterium*-mediated transformation (Liu-hui and Liu-xin 2001; Puchooa 2004a, b) which is widely used in crop, medicinal and aromatic plants, as well as horticultural plants. The expression of GFO was observed in transgenic tissues of litchi, and no plantlets were regenerated (Puchooa 2004a, b) and suggested the use of GFP with combinations of suitable selection mark genes to detect the efficiency of transformation as well as establish widespread protocol for transformation of litchi which is confirmed to be challenging than any other crops. Das and Rahman (2010) reported the transgenic litchi with bacterial chitinase (ChiB) and observed the resistance partially against *Phomopsis* sp. Raharjo and Litz (2007) reported the establishment of embryogenic cultures from leaves of mature phase "Brewster" trees which facilitates the development of litchi cultivars through in vitro mutagenesis and genetic transformation. Lai et al. (2010) reported the establishment of transgenic resistant embryogenic callus on selection medium. Transgenic plant was developed using zygotic embryos of litchi with rice chitinase gene with maize-ubiquitin promoter and showed deferred onset of dieback, leaf spots, and blight diseases (Das and Rahman 2012). Padilla et al. (2013) reported the transgenic litchi with posttranscriptional silencing of litchi Pistillata (PI) homologue induced by antisense strategies for the development of parthenocarpic fruits of litchi.

4.5.4 Molecular Approaches

Molecular marker analysis is the most advanced technology to understand the phylogenetic relationships and cultivar identification. Due to the diversity of the litchi cultivars worldwide, the scientific community is forced to perform various genetic analysis in order to identify the cultivar variety. RAPD, SSR, AFLP, SRAP, RAPD-SCAR, and SNPs are the well-advanced and established molecular markers. Random amplified polymorphic DNA (RAPD was for the phylogenetic analysis in Southern China with six types of cultivars (Long et al. 2015)) combined with Inter-simple sequence repeat (ISSR) indicated 72.7% polymorphism in the samples with an index of similarity coefficient ranging from 0.59 to 0.87. In a recent study (Cheng et al. 2015), the improved RAPD markers of seven different cultivars were cloned, sequenced, and converted to sequence-characterized amplified region (SCAR) markers which are a new insight in the field of genetic analysis in the plant system. Kumar (2006) reported first time the genetic relatedness among Indian litchi (*Litchi chinensis* Sonn.) cultivars using random amplified polymorphic DNA (RAPD) markers. Fourteen RAPD primers which produced consistent profiles were chosen, resulting in amplification of 77 reproducible polymorphic bands. The RAPD analysis produced an average of 15.8% polymorphic and 0.10% monomorphic markers. Using the RAPD markers, all the accessions were classified into different groups despite of their same or different geographical origins and climatic adaptations. Hybrid plants via distant hybridization were established, and the intergeneric hybrids were confirmed by SRAP-PCR analysis (Zhao et al. 2010), and high-density molecular genetic map was constructed to study on the separation patterns and genetic variation for fruit quality traits which is very imperative for QTL location and breeding of new litchi cultivars (Fu et al. 2010a, b). Tongpamnak et al. (2002) studied the genetic diversity and relationships of Thai litchi cultivars using RAPD and AFLP markers. Similarly, SNP markers were developed for longan fruit tree, and 60 SNP was validated (Wang et al. 2015a, b), SSR loci were used to study molecular polymorphisms for 88 litchi accessions of Mauritius, Reunion, and Spain, and 67 amplification fragments were detected associated with 6.1 bands per SSR loci (Madhou et al. 2013). Several observations was reported to standardize the litchi nomenclature with Isoenzyme fingerprinting studies (Aradhya et al. 1995) and RAPDs, microsatellites and ISSRs (Anuntalabhochai et al. 2002; Degani et al. 2003; Liu and Mei 2005; Madhou et al. 2010; Viruel and Hormaza 2004) and genetic diversity in morphological features (Khurshid et al. 2004). The genetic relationships of 96 litchi accessions was studied and 90 SNPs with minor allele frequencies was noted among 155 SNPs and the study will be useful for litchi germplasm conservation and breeding program (Liu et al. 2015). Higher polymorphism level with low gene diversity from 20 litchi cultivars of Indian peninsula using ISSR (Bajpai et al. 2016) and phenotypic differences among litchi cultivars perhaps due to environmental factors rather than genetic factors (Madhou et al. 2010). These molecular markers used extensively for the comparative study to standardize the nomenclature of litchi cultivars in different countries.

4.5.5 Genomics and Proteomics

The diversity analysis of litchi germplasm and clonal fingerprinting is based on the development of isozyme and dominant PCR-based markers which has principally restricted the wide range of genomics and its application in the improvement of fruit crops. The dominant markers and their information are used limitedly in genomics application; however, it has assisted in our understanding of genetic diversity in germplasm and their collection as well as few genetic recombination maps. The codominant microsatellite markers (SSRs) have recently been established for their imperative role in parentage analysis, clonal finger printing, analysis of genetic diversity, and various tropical fruit crops. The advantages of improved capacity in sequencing of second- and third-generation pyrosequencing are not only useful in sequencing of several transcriptomes of tropical fruit crops in coming years, and also the developed new information will facilitate the interest which will create new prospects to intensify the rate of molecular breeding programs (Arias et al. 2012; Dutt et al. 2014). Several genes for pigment production were identified and characterized in fruit crops especially during ripening phase (Bogs et al. 2007; Palapol et al. 2009; Rahim et al. 2014; Takos et al. 2006). The ethephon- and ethylene-induced upregulation of *LcPG1*, a pectin-degrading enzyme, was identified with their role in litchi fruit abscission (Peng et al. 2013), whereas increased 1-aminocy clopropane-1-carboxylic acid oxidase (*ACO*) gene expression was observed with foliar spray of NAA (Wu et al. 2013). The light and ABA-mediated upregulation of transcription factor *LcMybA1* was characterized from litchi pericarp and identification of light, hormone, and abiotic stress-responsive cis-elements and their role in positive as well as negative regulation of gene expression through promoter analysis of *LcMybA1* gene (Lai et al. 2014). Similarly, 2730 significant litchi fruit abscission genes were identified through genome-wide digital transcript abundance analysis, and up- or downregulation of 1867 early-responsive genes was noted from 0 to 1 day after ethephon treatment (Li et al. 2015a, b). Recently Elitzur et al. (2016) reported the characterization of two banana E class MADS box genes (*MaMADS1* and *MaMADS2*) in transgenic banana with delay in ripening and increased shelf life phenotypes and also showed the evidence of ancient activity of MADS gene family. The litchi polyphenol oxidase gene (*LcPPO*, JF926153) was cloned and revealed higher expression patterns in flower and leaf and in pericarp of freshly harvested fruits than developing fruits (Wang et al. 2014). Furthermore, the expression of putative aril vacuolar membrane sucrose transporter gene (*LcSUT4*) and its critical role in apoplasmic transport and sugar accumulation in aril part of litchi fruit were identified. Currently, the researchers have made an attempt for novel screening and selection principles or genetic strategies using molecular techniques to enhance the soluble sugar content in litchi fruit with the revealed soluble sugar accumulation and their associated transport and metabolic pathways (Wang et al. 2015a, b).

The proteomics approach will facilitate the investigation of global changes in protein expression which leads to reveal the complex molecular mechanism in fruit developmental biology. Currently the identification and characterization of fruit ripening and senescence-related proteins using two-dimensional gel electrophoresis

(2-DE) coupled with liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) is in proceeding with both climacteric and non-climacteric fruits (Huerta-Ocampo et al. 2012). Recently Li et al. (2015a, b) observed 61 proteins through LC-ESI-MS/MS out of differentially expressed 64 spots by 2-DE and classified all identified proteins based on their cellular component, biological process, and molecular function especially in regulation litchi fruit senescence using Blast2Go.

Recently the combined techniques using flow cytometry, cellular imaging, and molecular analysis were established to study the developmental patterns, cell size and volume, nuclear endo-reduplication with varied gene expression, and mechanisms of growth control of fruits in general and tomato in particular as a model plant. The study has provided the new information on adjustment of cell cytoplasmic volume to their nuclear DNA content and further reveals karyoplasmic ratio theory (Pirrello et al. 2014).

4.6 Postharvest Storage

The production rate and access to food pose a task to food security against the rapid growing human populations with challengeable environment of declining land and water resources. The production of vegetables, fruits, flowers, spices, medicinal and aromatic plants, and plantation crops collectively known as horticulture has developed as vital economic activity in Asia and Pacific regions. More than 50% of the global production of tropical and subtropical fruits, such as passion fruit, litchi, guavas, pineapples, and papayas, and a wide diversity of fruits like mango, grapes, apples, bananas, and oranges have been produced in Asia-Pacific region. The environmental factors, soil properties, cultural practices, stage of maturity, harvesting methods, postharvest handling, and storage conditions decide the nutritional content and bioactive molecules of vegetables and fruits (Choudhury 2006). The current changes in dietary patterns and regional economic growth lead to increasingly important in both production and consumption of fruit and vegetables. The important horticultural sector, vegetables and fruits, has a prominent role in improved farm income, alleviation of poverty, food security, and overall sustainable agriculture in developing countries of Asia. The diverse climatic conditions in India support the production of wide variety of fruits and vegetables which accounts for 1% of global production and second major producer of vegetables. There are 86.602 million metric tons (MMT) of fruits from 6.110 million hectare and 169.478 MMT of vegetables 9.542 million hectare produced during 2014–2015 according to the National Horticulture Database of the National Horticulture Board (http://www.apeda.gov.in/apedawebsite/six_head_product/FFV.html).

In spite of its prominence, the horticultural sector suffers 30–40% of postharvest losses in fruit and vegetables which results in reduction of income for producers. The rejection rate of fruits and vegetables is increasing due to unapproved pesticides, pesticide residues beyond acceptable limits, and insufficient labeling and packaging. Furthermore, the food-borne diseases and poisoning have been growing

distress due to epidemics of *E. coli* and *Salmonella* species. Recently, the postharvest management of fruits and vegetables is a very important concern in most of the developing countries, and it decides the food quality and safety, attractiveness in the market, and income for producers and is major bottlenecks for the farmers and scientist. The main constrains of postharvest management are ineffective treatment and transport, deprived technologies for processing, storage and packaging, association of wide range of manpower, and improper infrastructure (Choudhury 2006). It is reported that 30–35% of total vegetable production in India is lost due to the lack of proper postharvest storage and among the vegetable production in India, only less than 2% is processed commercially than other countries like Brazil and the United States which have 70 and 65%, respectively (Ahsan 2006).

Currently the exposure of fruits and vegetables to heat as the most common method for postharvest processing primarily leads to inactivation of microbes and enzymes of spoilages. Several studies have observed the influence of both conventional thermal processing (blanching, pasteurization and sterilization and thermal drying) and nonthermal processing (dense phase CO_2, pulsed electric field, hydrostatic pressure, ozone ultrasound, and radiation) on stability of nutrients and micronutrients of foods. Apart from the conventional thermal processing techniques, wet or dry blanching is extensively used to inactivate the oxidative enzymes and release of gas as well as to prevent the microbial growth. The vegetables and fruits were processed with microwave, infrared, and ionizing radiation such as gamma rays, photochemical treatment, and fluidized bed drying and noted with the enhancement of carotene content, total phenolic scavenging activity and anti-oxidant activity, and decrease in anti-nutritional factors. The electromagnetic radiation (EMR)-based dry blanching such as microwave, infrared, and superheated steam inactivation of enzymes on product quality was evaluated and compared among different blanching methods for the postharvest storage of green bell pepper. The ascorbic acid (91.1%), soluble protein (91.6%), β-carotene (131.2%), and chlorophyll (136.4%) show higher retention and firmer vegetables followed by superheated steam-blanching slices compared to unbalanced control. Hence the blanching technique is very important of the preprocessing phases before drying, freezing, and canning for all perishable vegetables (Jeevitha et al. 2015). The dry-blanching method has several advantages than conventional water and steam blanching with no/minimal leaching of vitamins, flavors, pigments, carbohydrates, and water-soluble components with speedy inactivation of enzyme complexes and retention of nutrients with few observation in important vegetables using microwave, photochemical, fluidized bed, electron beam irradiation, and infrared (Bhat and Sridhar 2008; Jeevitha et al. 2013; Hebbar and Ramesh 2006; Murthy and Joshi 2007; Ramesh et al. 2002; Vishwanathan et al. 2013). The combination treatments of calcium ascorbate and gamma irradiation, anti-oxidant and anti-browning agents, irradiation, and high-pressure processing technology have also been extensively used to preserve the quality of marginally processed fruits and vegetables (Hussain et al. 2014). Ionizing radiation increased shelf life, and control of spoilage microbes was noted in carrot, sweet onions, lettuce and strawberries, minimally processed watercress, green onion, garlic, fresh celery and soybean sprouts, cucumber, bitter gourd, cabbage, and carrot (Baskaran

et al. 2007; Bhat et al. 2007; Chatterjee et al. 1999; Chaudry et al. 2004; Chikkasubbanna 2006; Sajilata and Singhal 2006; Variyar et al. 2004). The postharvest techniques like refrigeration, modified atmosphere storage, and freeze and drying methods can be used to increase the shelf life of fruits and vegetables (Arvanitoyannis et al. 2005; Doymaz and Göl 2011; Jha and Matsuoka 2002).

Furthermore, the multidirectional research on postharvest storage of vegetables and fruits is undertaken by several universities and research institutes, like IARI, New Delhi; CMRS, Lucknow; IIHR, Banglore, and YSPUHF, Solan; TNAU, Periyakulam; KKV, Dapoli; HAU, Hisar; and ICAR-RC, Shillong. During the seventh and eighth plans, four more centers were added, i.e., BCKVV, Kalyani; MPKVV, Rahuri; and RAU, Pusa, Bihar; CFTRI, Mysore; RRL, Jammu, and BARC, Mumbai; CSIR laboratory, Palampur; and the DFRL, Mysore through All India Coordinated Project. National Horticulture Board has initiated project to establish and improve the infrastructure which provide the assistance to create center for grading/packaging, retail outlets, cold storage, purchase of plastic crates and transport vehicles, access to market information, production of beverages from juice/fruits, practical training to farmers, marketing infrastructure via soft loans, and introduction of concepts, new techniques, and methods for postharvest storage of major fruits and vegetables (Choudhury 2006).

Among the several methods, irradiation of vegetables and fruits is established technology which has been validated by number of outstanding international health and food specialists. Being nonthermal treatment technology, the gamma irradiation inactivates spoilage and food-borne pathogens effectively in many fruits and vegetables including marginally treated foods (Niemira et al. 2003; Prakash et al. 2002). Gamma irradiation of marginally processed fresh fruits and vegetables is an effective technique to maintain their textural, sensory, nutritional, and hygienal qualities. Effective hygiene of marginally processed fresh fruits and vegetables and intact nutritional, textural, and sensory qualities was noted with gamma irradiation (Dhokane et al. 2006; Mishra et al., 2004; Song et al. 2007). The irradiation technique was used mainly to eradicate insect pests and microbial infection from fresh vegetables and fruits, and it delays ripening and senescence which leads to extension of shelf life without harmful effects on the quality. The irradiation substantially reduced the population of aerobic mesophiles, yeasts, molds, and microbial populations especially fungi and bacteria in three varieties of garden eggs, tomato, and carrot which leads to improved microbiological quality (Adu-Gyamfi 2009; Chervin and Boisseau 1994; Schmidt et al. 2006). The irradiation splits the water molecules in fruits and vegetables into hydrogen (H^+), hydroxyl (OH^-), and oxygen radicals (O^{2-}) which react with microbial DNA, proteins, and cell organelles of microbes (Niemira and Sommers 2006).

Though most of the vegetables and fruits are perishable, only a small proportion is being processed. Majority of vegetables and fruits are marketed and consumed fresh as shorter time lag between the harvest and consumption will assure the optimal quality of vegetables. Due to their cultivation intensity, fruits and vegetables face much biotic stress induced by pathogens, pests, and weeds. Stress loads also vary and are very complex because of the high diversity of crops. Application of

pesticides and weedicides is the commonly used measure to control the pests and weeds. The use of synthetic pesticides has been eminently successful since the 1950s, in controlling crop losses due to insect infestations and weed growth. Global pesticide market is predominantly occupied by the vegetable crops. Annually, about 20% of the pesticide expenditure (amounting to US $8.1 billion) is used for controlling pests on vegetable cultivation (Krattiger 1998). The insecticides are useful against insect pests which malign the crop either by directly feeding the plants or by transmitting the pathogens like viruses. Globally, preharvest loss by insect pests, pathogens, and weeds is estimated to be 15%, 13%, and 12%, respectively (Pimentel 1997). Indiscriminate use of insecticides and pesticides is the matter of concern since the residual chemicals can affect the health of farmers as well as consumers and can also contaminate the environment. The pesticide residue and biological contamination are a serious issue as the vegetables are being consumed fresh. Therefore, consumers are increasingly concerned about the quality and safety of their food, as well as the ecological condition in which it is produced. Vegetable cost will therefore increase by enhancing their quality and safety (Dias and Ryder 2011).

4.6.1 Postharvest Processing of Litchi Fruits

Litchi is a tropical and subtropical fruit with a high commercial value in the international market. Litchi fruit ripens without production of ethylene and respiration burst, typical of non-climacteric fruit. It is a highly perishable fruit which starts deteriorating immediately after harvest. Adopting postharvest techniques is essential to minimize the postharvest crop loss. The preharvest loss is controlled or managed by adopting integrated management techniques. Mulching the tree basins and proper irrigation during the growth and development of fruit are essential for minimizing the preharvest losses (Mitra et al. 2011). The use of biofertilizers having *Azotobacter* helps in better canopy spread, fruit weight, and bearing (fruits/panicle) of heavier fruits (Kumar et al. 2010). Postharvest techniques are the processes used to maintain the quality of the fruits (appearance, texture, color, flavor, and nutritive value), to protect the food safety issues, and to minimize the losses between harvest and consumption. Depending on the age of the tree, litchi has four growth phases, namely, young nonbearing stage (1–3 years), young bearing stage (6–10 years), junior adult bearing stage (11–20 years), and senior adult bearing stage (21 years and above). The plant starts bearing fruits after 5–6 years of age. Most of the Indian varieties take 70–100 days for fruit to ripe. The yield per tree depends on variety, season of harvesting, nutritional supplement, and age of the tree. Harvesting is done usually in May and June. Matured, red-colored fruits are harvested in bunches along with a portion of branch and a few leaves which serves dual purpose and helps in extending the shelf life of fruit, and at the same time trees gets a mild pruning. Quality of litchi fruit can be assessed by external criteria, internal criteria, and maturity indices. External quality criteria are skin color and fruit size. Internal criteria include seed size and sweetness of the pulp. Litchi is a non-climacteric fruit; hence

the fruit should be harvested at ripened stage. The ripened fruit is red in color and is marked by flattening of tubercles. Matured fruit which is red in color without brown coloration, with optimum sugar-to-acid ratio, free from micro-cracking, microbial decay, and physical damage, has the good commercial value.

The crop loss includes both qualitative loss and quantitative loss. Decreased crop yield results in quantitative loss which is mostly due to nonscientific agricultural practices and environmental factors. Qualitative loss refers to loss in nutritional quality, caloric value, edibility, and consumer acceptability. It is essential to understand the factors, both environmental and biological factors, involved in postharvest decay and to use suitable postharvest techniques which slow down the decay process and maintain quality and safety of the product. A considerable amount of crop loss occurs during harvesting, sorting, transportation, and storage. Disease, infestation with pests, high temperature during harvesting, and mishandling are major reasons for crop loss during harvesting. The shelf life of the fruit is very short due to microbial decay by fungal and bacterial pathogens and brown coloration of pericarp. Microbial pathogens entered through the micro-crack occurred during the preharvest or postharvest as a consequence of desiccation can cause the deterioration of the fruit. Postharvesting brown coloration of red pericarp is the main cause for sharp decrease in commercial value of the fruit. Brown coloration starts between day 3 and 8 followed by the loss of moisture and thickness. Due to the senescence and membrane breakdown, anthocyanin pigment, polyphenols, and polyphenol oxidase (PPO) come into contact which results in color change from red to brown due to discoloration of anthocyanin pigments and oxidation of phenols by PPO. Along with the enzymatic reaction, loss of water is the main element which leads to browning of the fruit. Since the commercial value declines rapidly due to loss of quality during storage, the adaptation of appropriate postharvest technique is essential to get a good market value. Inhibition of lipid peroxidation, which reduces membrane fluidity and increases membrane permeability, delays pericarp browning and extends the storage life of litchi fruit.

The two round flowering systems potentially reduce the fruit set and retention in the first round. These issues should be considered to develop field strategies to improve yield in early maturing cultivars of litchi (Chang et al. 2015). In order to improve the fruit quality and to reduce postharvest losses, different techniques have been employed (Table 4.7) by the following methods.

4.6.2 Gamma Radiation

Radiation treatment is compulsory quarantine treatment for exporting to many countries. Treatment with ionizing radiation results in reduced microbial load on the fruit. Low dose of gamma radiation combined with low temperature storage helps in delayed browning of pericarp and extends the shelf life of litchi fruit up to 28 days (Hajare et al. 2010). Radiation dose of 1 KGy combined with wax coating results in extended shelf life of the fruit without negatively affecting the quality of the fruit (Pandey et al. 2013).

Table 4.7 Major postharvest techniques employed to improve the shelf life of litchi

Sl. No.	Technique employed	Response	References
1	Gamma radiation	Improves shelf life, decreases fungal infection	Hajare et al. (2010) and Pandey et al. (2013)
2	Sulfur fumigation	Increases shelf life and decreases fungal infection	Jiang et al. (2003) and Lichter et al. (2000)
3	Heat treatment	Helps to remove dirt, dust, and fungal spores from the fruit skin	Lichter et al. (2000)
4	Acid dip	Technique is useful when supplemented with other postharvest techniques	Bhushan et al. (2015)
5	Oxalic acid	Reduces browning of pericarp by inhibiting peroxidases and anthocyanin degradation	Zheng and Tian (2006) and Marboh et al. (2012)
6	Salicylic acid	Reduces browning of pericarp and maintains quality in combination with iso-ascorbic acid	Kumar et al. (2013)
7	Modified atmosphere packaging	Increases shelf life by preventing pericarp browning by both enzymatic browning and desiccation browning	Sivakumar and Korsten (2006)
8	Nitric oxide	Preserves bioactive molecules	Barman et al. (2014)

4.6.3 Sulfur Fumigation

The fumigation with SO_2 is considered as one of the practical and most effective postharvest techniques to control the deterioration of litchi fruit. In this method, sulfur powder is burnt at ambient temperature or sulfur vapors are applied in pressurized cylinders or sulfite compounds are desiccated. The sulfur fumigation can be followed by acid dip, since SO_2 aids in ion leakage through cell membrane, which facilitates the accumulation of acid particles in the pericarp and thus by retaining the red pigment. In the recent years, the sulfur residues that remained on the pericarp are a concern (Jiang et al. 2003; Lichter et al. 2000).

4.6.4 Heat Treatment

The heat treatment technique was developed to prevent the usage of potentially hazardous chemicals like SO_2. The fruits are sprayed with hot water followed by dipping in the hydrochloric acid. Alternatively, litchi fruits are rinsed with hot water at 55 °C, then dipped in the acid solution, dried, and packed. This technique is very useful to preserve the quality of the fruits by maintaining the uniform red color for a period of 35 days (Lichter et al. 2000).

4.6.5 Acid Dip

This technique is applied in combination with the other postharvest techniques such as precooling, hot treatment, and fumigation. The precooled fruits are treated with disinfectants like sodium metabisulfite followed by dipping in 2% HCl solution for 10 min which is helpful in maintaining the fruit quality (Bhushan et al. 2015).

4.6.6 Oxalic Acid

It is the final metabolite of the plant having many biological role. It is thought to be the vital molecule in the early defense response in many organisms (Lehner et al. 2008). It is used as anti-browning agent in apples and banana (Yoruk et al. 2002). The oxalic acid helps in maintaining the membrane integrity. The application of oxalic acid to the litchi fruit results in increased shelf life by decreasing the anthocyanin degradation and oxidation and inhibiting the peroxidase activity (Zheng and Tian 2006). Oxalic acid application along with the hot water treatment is also useful in increasing the shelf life of the fruit up to 18 days (Marboh et al. 2012).

4.6.7 Salicylic Acid

Salicylic acid is a plant hormone known to involve in many physiological, growth, and developmental processes such as photosynthesis, transpiration, ion uptake, and transport. The application of salicylic acid helps in reducing the pericarp browning and ion leakage and inhibits PPO activity, thus increasing the shelf life and quality of the fruit (Kumar et al. 2013).

4.6.8 Modified Atmosphere Packaging (MAP)

Modified atmosphere can be created by regulating the respiration of packed fruits. The technique is employed to maintain optimal CO_2 and O_2 concentration and high relative humidity (RH) inside the package. The appropriate packing material is selected so that it withholds the internal environment in preferable condition. The browning of pericarp due to desiccation was prevented by maintaining high RH. Low humidity negatively affects the weight and firmness of the fruit. Browning due to enzymatic reaction and fermentation by anaerobic microbes can be negotiated by maintaining high O_2 concentration (5–17%) (Sivakumar and Korsten 2006). Combination of calcium chloride along with MAP is also helpful in reducing the loss of fruit firmness, rate of ethanol production, growth of bacteria and molds, and PME and PG activities and helps in maintaining the microstructure of the fruit by reducing the loss of cell turgor during storage (Punumong et al. 2016).

4.6.9 Nitric Oxide

Nitric oxide is a stable free radical and bioactive molecule initially assumed to be a pollutant. It plays a pivotal role in the plant system as a signaling molecule. NO interacts with cellular targets through either redox or additive chemistry, mediating physiological and developmental processes including triggering the expression of defense-related genes and programmed cell death. NO has shown to have inhibitory function on many enzymes like POD, PPO, and phenylalanine ammonia lyase (PAL). The short-term treatment with NO helps in water retention of in the litchi fruit. It delays the enzymatic browning of pericarp by inhibiting PPO and POD. Treatment with low concentration of sodium nitroprusside, donor of NO, increases the shelf life and quality of the fruit. The treatments results in retention the higher concentration of bioactive molecules like phenolic compounds and ascorbic acid (Barman et al. 2014).

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References

Adu-Gyamfi A (2009) Irradiation of fresh fruits and vegetables for improved quality. Ghana J Hortic 35:165–173

Ahsan H (2006) Status report of India (1). In: Rolle RS (ed) Reports of the APO seminar on reduction of postharvest losses of fruit and vegetables. Asian Productivity Organization Japan and Food and Agriculture Organization of the United Nations Italy, 131–142

Amin MN, Razzaque MA (1995) Induction of somatic embryogenesis in the cultures of zygotic embryos of lychee. Bangladesh J Bot 24:25–29

Anuntalabhochai R, Chundet R, Chiangda J, Apavatjrut P (2002) Genetic diversity within lychee based on RAPD analysis. Acta Hortic 575:253–259

Aradhya MK, Zee FT, Manshardt RM (1995) Isozyme variation in lychee (*Litchi chinensis* Sonn). Sci Hortic 63:21–35

Arias RS, Borrone JW, Tondo CL, Kuhn DN, Irish BM, Schnell RJ (2012) Genomics of tropical fruit tree crops. In: Schnell RJ, Priyadarshan PM (eds) Genomics of tree crops. Springer, New York/London, pp 209–239

Aruoma OI (1999) Free radicals oxidative stress and anti-oxidants in human health and disease. J Am Oil Chem Soc 75:199–212

Arvanitoyannis IS, Khah EM, Christakou EC, Bletsos FA (2005) Effect of grafting and modified atmosphere packaging on eggplant quality parameters during storage. Int J Food Sci Technol 40:311–322

Bajpai A, Muthukumar M, Singh A, Nath V, Ravishankar H (2016) Narrow genetic base of Indian litchi (*Litchi chinensis*) cultivars based on molecular markers. Indian J Agric Sci 86:448–455

Barman K, Siddiqui MW, Patel VB, Prasad M (2014) Nitric oxide reduces pericarp browning and preserves bioactive anti-oxidants in litchi. Sci Hortic 171:71–77

Baskaran R, Devi AU, Nayak CA, Kudachikar V, Prakash MK, Prakash M, Ramana K, Rastogi N (2007) Effect of low-dose γ-irradiation on the shelf life and quality characteristics of minimally processed potato cubes under modified atmosphere packaging. Radiat Phys Chem 76:1042–1049

Batt PJ, Cadilhon JJ (2007) Fresh produce supply chain management: overview of the proceedings and policy recommendations In: Batt PJ, Cadilhon J-J (eds) Proceedings of the international symposium on fresh produce supply chain management FAO AFMA Curtin University of Technology Department of Agriculture of the Thai Ministry of Agriculture and Cooperatives, p 10

Batten DJ, Lahav E (1994) Base temperature for growth processes of lychee a recurrently flushing tree are similar but optima differ. Aust J Plant Physiol 2:589–602

Bhat R, Sridhar KR (2008) Nutritional quality evaluation of electron beam irradiated (*Nelumbo nucifera*) seeds. Food Chem 107:174–184

Bhat R, Sridhar KR, Bhushan B (2007) Free radicals in velvet bean seeds (Mucunapruriens L DC) and their status after γ-irradiation and conventional processing LWT. Food Sci Technol 40:1570–1577

Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T (2011) Hepatoprotective effects of lychee (*Litchi chinensis* Sonn): a combination of anti-oxidant and antiapoptotic activities. J Ethnopharmacol 136:55–66

Bhushan B, Pal A, Narwal R, Meena VS, Sharma PC, Singh J (2015) Combinatorial approaches for controlling pericarp browning in Litchi (*Litchi chinensis*) fruit. J Food Sci Technol 52:5418–5426

Bogs J, Jaffe´ FW, Takos AM, Walker AR, Robinson SP (2007) The grapevine transcription factor VvMYBPA1 regulates proanthocyanidin synthesis during fruit development. Plant Physiol 143:1347–1361

Brat P, George S, Bellamy A, Du Chaffaut L, Scalbert A, Mennen L, Arnault N, Amiot MJ (2006) Daily polyphenol intake in France from fruit and vegetables. J Nutr 136: 2368–2373

Callahan A, Scorza R (2007) Effects of a peach antisense ACC oxidase gene on plum fruit quality. Acta Hortic 738:567–573

Castellain RC, Gesser M, Tonini F (2014) Chemical composition anti-oxidant and antinociceptive properties of *Litchi chinensis* leaves. J Pharm Pharmacol 6:1796–1807

Chadha KL, Rajpoot MS (1969) Studies on floral biology, fruit set, and its retention and quality of some litchi varieties. Indian J Hortic 26:124–129

Chandra R, Padaria JC (1999) Litchi shoot bud culture for micropropagation. J Appl Hortic 191:38–40

Chang JC, Lin TS (2008) Fruit yield and quality as related to flushes of bearing shoots in litchi. J Am Soc Hortic Sci 133:284–289

Chang JW, Teng YS, Yen CR (2012) Description and performance of new litchi varieties. In: Fang HH, Teng YS, Lee WL (Eds) Proceedings of the symposium on litchi industry development in Taiwan Taichung, Taiwan, pp 25–37

Chang YY, Yang DJ, Chiu CH, Lin YL, Chen JW, Chen YC (2013) Antioxidative and anti-inflammatory effects of polyphenol-rich litchi (*Litchi chinensis* Sonn)-flower-water extract on livers of high-fat-diet fed hamsters. J Funct Foods 5:44–52

Chang J, Chang YA, Fang MY, Lin ML, Chang JW (2014) Estimation of the putative marginal timing for subsequent flowering in the last flush of litchi. J Taiwan Soc Hortic Sci 60:27–39

Chang J, Chang YA, Tang L, Chang JW (2015) Characterization of generative development in early maturing litchi 'Early Big' a novel cultivar in Taiwan. Fruits 70:289–296

Chapman KR (1984) Tropical fruit cultivars collecting in SE Asia and China. Queensland Department of Primary Industries, p 123

Chatterjee S, Desai SRP, Thomas P (1999) Effect of g-irradiation on the anti-oxidant activity of turmeric (*Curcuma longa* L) extracts. Food Res Int 32:487–490

Chaudry MA, Bibi N, Khan M, Badshah A, Qureshi MJ (2004) Irradiation treatment of minimally processed carrots for ensuring microbiological safety. Radiat Phys Chem 71:171–175

Chauhan S, Kaur N, Kishore L, Singh R (2014) Pharmacological evaluation of anti-inflammatory and analgesic potential of *Litchi chinensis Gaertn* (Sonn). Int J Pharm Pharm Sci 6:116–119

Chawla HS (2004) Introduction to plant biotechnology, 2nd edn. Science Publishers, Beijing

Cheng J, Long Y, Khan A, Wei C, Fu S, Fu J (2015) Development and significance of RAPD-SCAR markers for the identification of *Litchi chinensis* Sonn. by improved RAPD amplification and molecular cloning. Electron J Biotechnol 18:35–39

Chervin C, Boisseau P (1994) Quality maintenance of "ready-to-eat" shredded carrots by gamma irradiation. J Food Sci 59:359–361

Chikkasubbanna V (2006) Status report of India (2). In: Rolle RS (ed) Reports of the APO seminar on reduction of postharvest losses of fruit and vegetables. Asian Productivity Organization Japan and Food and Agriculture Organization of the United Nations Italy, pp 143–151

Choudhury ML (2006) Recent developments in reducing post-harvest losses in the Asia-Pacific region. In: Rolle RS (ed) Reports of the APO seminar on reduction of postharvest losses of fruit and vegetables. Asian Productivity Organization Japan and Food and Agriculture Organization of the United Nations Italy, pp 15–22

Cruz-Hernández A, Gómez-Lim M, Litz RE (1997) Transformation of mango somatic embryos. Acta Hortic 455:292–298

Das DK, Rahman A (2010) Expression of a bacterial chitinase (ChiB) gene enhances antifungal potential in transgenic *Litchi chinensis* Sonn (cv. Bedana). Curr Trends Biotechnol Pharm 4:820–833

Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv Bedana). Plant Cell Tissue Organ Cult 109:315–325

Das DK, Shiva Prakash N, Sarin NB (1999) Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn). Plant Cell Rep 18:691–695

Deerasamee O, Chaisawadi S (2014) Clean production of freeze-dried lychee powder for medicinal herb and nutritional health benefits. Acta Hortic 1023:59–62

Degani C, Deng J, Beiles A, El-Batsri R, Goren M, Gazit S (2003) Identifying lychee cultivars and their genetic relationships using intersimple sequence repeat markers. J Am Soc Hortic Sci 128:838–845

Deng CJ (2005) Studies on high frequency somatic embryogenesis and regeneration culture in litchi. Master's thesis of Hunan Agricultural University Changsha Hunan, China

Dhokane V, Hajare S, Shashidhar R, Sharma A, Bandekar J (2006) Radiation processing to ensure safety of minimally processed carrot (*Daucus carota*) and cucumber (*Cucumis sativus*): optimization of dose for the elimination of *Salmonella Typhimurium* and *Listeria monocytogenes*. J Food Prot 69:444–448

Dias JS, Ortiz R (2014) Advances in transgenic vegetable and fruit breeding. Agric Sci 5:1448–1467

Dias JS, Ryder E (2011) World vegetable industry: production breeding trends. Hortic Rev 38:299–356

Dixon T, Batten D, Mcconchie C (2005) Genetic improvement of lychee in Australia ISHS. Acta Horticulture II nd international symposium on Lychee Longan Rambutan and other Sapindaceae plants held at Chiang Mai Thailand

Doymaz I, Göl E (2011) Convective drying characteristics of eggplant slices. J Food Process Eng 34:1234–1252

Duan XW, Jiang YM, Su XG, Zhang ZQ, Shi J (2007) Anti-oxidant properties of anthocyanins extracted from litchi (*Litchi chinenesis* Sonn) fruit pericarp tissues in relation to their role in the pericarp browning. Food Chem 101:1365–1371

Dutt M, Dhekney SA, Soriano L, Kandel R, Grosser JW (2014) Temporal and spatial control of gene expression in horticultural crops. Horm Res 1:14047

Elitzur T, Yakir E, Quansah L, Zhangjun F, Vrebalov JT, Khayat E, Giovannon JJ, Friedman H (2016) Banana MaMADS transcription factors are necessary for fruit ripening and molecular tools to promote shelf-life and food security. Plant physiol 171:380–391

Froneman JJ, Oosthuizen JH (1995) ETSG Litchi breeding programme. Yearbook-South African Litchi Growers Association 7:35–37

Fu LF, Tang DY (1983) Induction pollen plants of litchi tree (*Litchi chinensis* Sonn). Acta Genet Sin 10:369–374

Fu JX, Guo YS, Zhao HY, Xie JH, Liu CM, Hu YL, Ouyang R, Wang HC, Zhao YH, Zhou J, Huang SS (2010a) A study on the genetic effects on fruit quality traits and selection of promising individuals from a mapping population of litchi. Acta Hortic 863:175–182

Fu JX, Guo YS, Zhao HY, Xie JH, Liu CM, Hu YL, Ouyang R, Zhao YH, Zhou J, Huang SS (2010b) A study on the genetic effect and MAS exploration of juvenile phase and fruit maturation period in a mapping population of litchi. Acta Hortic 863:195–200

Fujii H, Nakagawa T, Nishioka H, Sato E, Ueno Y, Sun B, Nonaka GI (2007) Preparation characterization and anti-oxidative effects of oligomeric proanthocyanidin-L-cysteine complexes. J Agric Food Chem 55:1525–1531

Gangehei L, Ali M, Zhang W, Chen Z, Wakame K, Haidari M (2010) Oligonol a low molecular weight polyphenol of lychee fruit extract inhibits proliferation of influenza virus by blocking reactive oxygen species dependent ERK phosphorylation. Phytomedicine 17:1047–1056

Gao M, Matsuta N, Murayama H, Toyomasu T, Mitsuhashi W, Dandekar AM, Tao R, Nishimura K (2007) Gene expression and ethylene production in transgenic pear (*Pyrus communis* cv 'LaFrance') with sense or antisense cDNA encoding ACC oxidase. Plant Sci 173:32–42

Ghosh D, Scheepens A (2009) Vascular action of polyphenols. Mol Nutr Food Res 53:322–331

Govindarajan R, Vijayakumar M, Pushpangadan P (2005) Anti-oxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J Ethnopharmacol 99:165–178

Gray DO, Fowden L (1962) α-(Methylenecyclopropyl) glycine from Litchi seeds. Biochem J 82:385–389

Groff GW (1943) Some ecological factors involved in successful lychee culture. Proc Florida State Hortic Soc 56:134–155

Guo SY, Peng HX, He XH, Ding F, Li HL, Qin XQ, Xu N (2014) Callus induction from different explants of litchi. Southwest China J Agric Sci 27:748–753

Hajare SN, Saxena S, Kumar S, Wadhawan S, More V, Mishra BB, Parte MN, Gautam S, Sharma A (2010) Quality profile of litchi (*Litchi chinensis*) cultivars from India and effect of radiation processing. Radiat Phys Chem 79:994–1004

Hallè F, Oldeman RAA, Tomlinson PB (1978) Tropical trees and forests. An architectural analysis. Springer, New York

Hebbar UH, Ramesh MN (2006) An improved process for the retention of nutrients during dry blanching of vegetables using infrared radiation. In: Indian Patent Application #602/DEL/2006

Hieke S, Menzel CM, Doogan VJ, Lüdders P (2002) The relationship between fruit and leaf growth in lychee *(Litchi chinensis* Sonn). J Hortic Sci Biotechnol 77:320–325

Holcroft DM, Mitcham EJ (1996) Postharvest physiology and handling of litchi (*Litchi chinensis* Sonn). Postharvest Biol Technol 9:265–281

Hoppe S, Neidhart S, Zunker K, Hutasingh P, Carle R, Steinhart H, Paschke A (2006) The influences of cultivar and thermal processing on the allergenic potency of lychees (*Litchi chinensis* Sonn). Food Chem 96:209–219

Hsu CP, Lin CC, Huang CC (2012) Induction of apoptosis and cell cycle arrest in human colorectal carcinoma by litchi seed extract. J Biomed Biotechnol Article ID: 341479

Huang XY, Kang DM, Ji ZL (1990) The optimum storage temperature for litchi fruits and chilling injury of them. J South China Agric Uni 11:13–18

Huang XM, Subhadrabandhu S, Mitra SK, Ben-Arie R, Stern RA (2005a) Origin history production and processing. In: Menzel CM, Waite GK (eds) Litchi and longan. Comwell Press, Trowbridge, pp 1–23

Huang X, Zeng L, Huang HB (2005b) Lychee and Longan production in China ISHS Acta Horticulturae 665: II nd international symposium on Litchi Longan Rambutan and other Sapindaceae Plants held at Thailand

Huerta-Ocampo JÁ, Osuna-Castro JA, Lino-López GJ, Barrera-Pacheco A, Mendoza-Hernández G, De León-Rodríguez A, De la Rosa APB (2012) Proteomic analysis of differentially accumulated proteins during ripening and in response to 1-MCP in papaya fruit. J Proteomics 75:2160–2169

Hussain PR, Omeera A, Suradkar PP, Dar MA (2014) Effect of combination treatment of gamma irradiation and ascorbic acid on physicochemical and microbial quality of minimally processed eggplant (*Solanum melongena* L). Radiat Phys Chem 103:131–141

Hwang JY, Lin JT, Liu SC (2013) Protective role of Litchi (*Litchi chinensis* Sonn) flower extract against Cadmium- and Lead-induced cytotoxicity and transforming growth factor β1-stimulated expression of smooth muscle-actin estimated with rat liver cell lines. J Funct Foods 5:698–705

Jeevitha GC, Hebbar HU, Raghavarao KSMS (2013) Electromagnetic radiation-based dry blanching of red bell peppers: a comparative study. J Food Process Eng 36:663–674

Jeevitha G, Anto A, Chakkaravarthi A, Hebbar H (2015) Application of electromagnetic radiations and superheated steam for enzyme inactivation in green bell pepper. J Food Process Preserv 39:784–792

Jha S, Matsuoka T (2002) Surface stiffness and density of eggplant during storage. J Food Eng 54:23–26

Jiang Y, Yao L, Lichter A, Li J (2003) Post-harvest biology and technology of litchi fruit. Food Agric Environ 1:76–81

Jiang G, Jiang YM, Yang B, Yu CY, Tsao R, Zhang HY, Chen F (2009) Structural characteristics and anti-oxidant activities of oligosaccharides from longan fruit pericarp. J Agric Food Chem 57:9293–9298

Jiang G, Lin S, Wen L (2013) Identification of Anovel Phenolic Compound in Litchi (*Litchi chinensis* Sonn) Pericarp and Bioactivity Evaluation. Food Chem 136:563–568

Jiménez-Bermúdez S, Redondo-Nevado J, Munoz-Blanco J, Caballero JL, Lopez-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiol 128:751–759

Kane ME (1996) Propagation from preexisting meristems. In: Plant tissue culture concepts and laboratory exercises. CRC Press, Boca Raton, pp 61–71

Kang SW, Hahn S, Kim JK, Yang SM, Park BJ, Chul Lee S (2012) Oligomerized lychee fruit extract (OLFE) and a mixture of vitamin C and vitamin E for endurance capacity in a double blind randomized controlled trial. J Clin Biochem Nutr 50:106–113

Kantharajah AS, McConchie CA, Dodd WA (1992) *In vitro* embryo culture and induction of multiple shoots in lychee (*Litchi chinensis* Sonn). Ann Bot 70:153–156

Kays SJ (2011) Cultivated vegetables of the world: a multilingual onomasticon. Wageningen Academic Publishers Wageningen, pp. 828

Kays SJ, Dias JS (1995) Common names of commercially cultivated vegetables of the world in 15 languages. Econ Bot 49:115–152

Keatinge JDH, Waliyar F, Jammadass RH, Moustafa A, Andrade M, Drechsel P, Hughes JA, Kardivel P, Luther K (2010) Re-learning old lessons for the future of food: by bread alone no longer—diversifying diets with fruit and vegetables. Crop Sci 50:51–62

Khare CP (2007) Indian medicinal plants- an illustrated dictionary. Springer, New York, p 379

Khurshid S, Ahmad I, Anjum MA (2004) Genetic diversity in different morphological characteristics of litchi (*Litchi chinensis sonn*). Int J Agric Biol 6:1062–1065

Kirti S (2004) Vegetable research in India: some issues. In: Sant Kumar P, Joshi K, Pal S (eds) Impact of vegetable research in India. National Centre for Agricultural Economics and Policy Research, New Delhi, pp 3–8

Kotoda N, Iwanami H, Takahashi S, Abe K (2006) Antisense expression of MdTFL1 a TFL1-likegene reduces the juvenile phase in apple. J Am Soc Hortic Sci 131:74–81

Krattiger AF (1998) The importance of ag-biotech to global prosperity. International Service for the Acquisition of Agri-Biotech Applications

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88

Kuang ZS, Zhou LN, Ma XJ, Chen JQ, Cao J (1996) Study on the types of embryoid in tissue culture on *Litchi chinensis* Sonn. J Fruit Sci 13:25–28

Kumar M (2006) Mass scale propagation of Litchi (*Litchi chinensis* Sonn.) through in vitro techniques. Ph.D thesis, Bhagalpur University, Bhagalpur, India

Kumar M, Shiva Prakash N, Muthusamy A, Prasad US, Sarin NB (2004) Problems and prospective of mass scale production of litchi (*Litchi chinensis* Sonn) using *in vitro* cultures. In: Proceedings of national seminar on recent advances in production and post-harvest technology of Litchi for export. Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252, Nadia, West Bengal, pp 12–17

Kumar M, Gupta M, Shrivastava D, Prasad M, Prasad US, Sarin NB (2010) Genetic relatedness among Indian litchi accessions *(Litchi chinensis* Sonn) by RAPD markers. Int J Agric Res 5:805–815

Kumar D, Mishra DS, Chakraborty B, Kumar P (2013) Pericarp browning and quality management of litchi fruit by anti-oxidants and salicylic acid during ambient storage. J Food Sci Technol 50:797–802

Lai CC, Lai ZX, Sang QL, Huang ZY, Huang Q (2010) Long-term subculture and maintenance of transgenic resistant embryogenic callus in litchi (*Litchi chinensis* Sonn). Acta Hortic 863:135–140

Lai B, Li XJ, Hu B, Qin YH, Huang XM, Wang HC, Hu GB (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS One 9:e86293

Lee SJ, Park WH, Park SD, Moon HI (2009) Aldose reductase inhibitors from *Litchi chinensis* Sonn. J Enzyme Inhibit Med Chem 24:957–959

Lee JB, Shin YO, Min YK, Yang HM (2010) The effect of Oligonol intake on cortisol and related cytokines in healthy young men. Nutr Res Pract 4:203–207

Lehner A, Meimoun P, Errakhi R, Madiona K, Barakate M, Bouteau F (2008) Toxic and signalling effects of oxalic acid: oxalic acid—natural born killer or natural born protector? Plant Signal Behav 3:746–748

Li JG (2008) The litchi. China Agriculture Press, Beijing

Li J, Jiang Y (2007) Litchi flavonoids: isolation identification and biological activity. Molecules 12:745–758

Li W, Liang H, Zhang MW, Zhang RF, Deng YY, Wei ZC, Zhang Y, Tang XJ (2012) Phenolic profiles and anti-oxidant activity of litchi (*Litchi chinensis* Sonn) fruit pericarp from different commercially available cultivars. Molecules 17:14954–14967

Li CQ, Wang Y, Huang XM, Li J, Wang HC, Li JG (2013) De novo assembly and characterization of fruit transcriptome in *Litchi chinensis* Sonn and analysis of differentially regulated genes in fruit in response to shading. BMC Genomics 14:552

Li C, Wang Y, Ying P, Ma W, Li J (2015a) Genome-wide digital transcript analysis of putative fruitlet abscission related genes regulated by ethephon in litchi. Front Plant Sci 6:502

Li T, Zhu H, Wu Q, Yang C, Duan X, Qu H, Yun Z, Jiang Y (2015b) Comparative proteomic approaches to analysis of litchi pulp senescence after harvest. Food Res Int 78:274–285

Liao YW, Ma SS (1998) Adventitious embryogenesis of *Litchi chinensis*. J Chin Soc Hortic Sci 44:29–40

Lichter A, Dvir O, Rot I, Akerman M, Regev R, Wiesblum A, Fallik E, Zauberman G, Fuchs Y (2000) Hot water brushing: an alternative method to SO_2 fumigation for color retention of litchi fruits. Postharvest Biol Technol 18:235–244

Li-hui Z, Liu-xin LU (2001) Transformation and transgenic plantlets regeneration of litchi (*Litchi chinensis* Sonn) with LEAFY gene. J Fujian Agric Forestry Uni 30:563–564

Lin N, Xiao LY, Pan JQ (2008) Effects of semen Litchi on the expressions of S180 and EAC tumor cells and Bax and Bcl-2 proteins in rats. China Pharmacy 19:1138–1140

Lin XL, Lai ZX, Xu QF (2010) High frequency plant regeneration via *in vitro* somatic embryogenesis in fifteen cultivars of *Dimocarpus longan* lour. Acta Hortic 863:155–160

Lin S, Yang B, Chen F, Jiang G, Li Q, Duan X, Jiang Y (2012) Enhanced DPPH radical scavenging activity and DNA protection effect of litchi pericarp extract by *Aspergillus awamori* bioconversion. Chem Cent J 6:108

Lin CC, Chung YC, Hsu CP (2013) Anti-cancer potential of Litchi seed extract. World J Exp Med 3:56–61
Lin YC, Chang JC, Cheng SY, Wang CM, Jhan YL, Lo IW, Hsu YM, Liaw CC, Hwang CC, Chou CH (2015) New bioactive chromanes from *Litchi chinensis*. J Agric Food Chem 63:2472–2478
Litz RE (1988) Somatic embryogenesis from cultured leaf explants of the tropical tree *Euphoria longan* Stend. J Plant Physiol 132:190–193
Liu C, Mei M (2005) Classification of lychee cultivars with RAPD analysis. Acta Hortic 665:149–160
Liu L, Xie B, Cao S, Yang E, Xu X, Guo S (2007) A-type procyanidins from *Litchi chinensis* pericarp with anti-oxidant activity. Food Chem 105:1446–1451
Liu W, Xiao Z, Bao X, Yang X, Fang J, Xiang X (2015) Identifying litchi (*Litchi chinensis* Sonn) cultivars and their genetic relationships using single nucleotide polymorphism (SNP) markers. PLoS One 10:e0135
Long Y, Cheng J, Mei Z, Zhao L, Wei C, Fu S, Khan MA, Fu J (2015) Genetic analysis of litchi (*Litchi chinensis* Sonn) in southern China by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat. Mol Biol Rep 42:159–166
Lv Q, Si M, Yan Y (2014) Effects of phenolic-rich Litchi (*Litchi chinensis* Sonn) pulp extracts on glucose consumption in human HepG2 cells. J Funct Foods 7:621–629
Lv Q, Luo F, Zhao X, Liu Y, Hu G, Sun C, Li X, Chen K (2015) Identification of proanthocyanidins from Litchi (*Litchi chinensis* Sonn) pulp by LC-ESI-QTOF-MS and their anti-oxidant activity. PLoS One 10:e0120480
Ma XY, Yi GJ, Huang XL, Zeng JW (2009) Leaf callus induction and suspension culture establishment in lychee (*Litchi chinensis* Sonn) cv Huaizhi. Acta Physiol Plant 31:401–405
Ma Q, Xie H, Li S, Zhang R, Zhang M, Wei X (2014) Flavonoids from the pericarps of *Litchi chinensis*. J Agric Food Chem 62:1073–1078
Madhou M, Bahorun T, Hormaza JI (2010) Phenotypic and molecular diversity of litchi cultivars in Mauritius. Fruits 65:141–152
Madhou M, Normand F, Bahorun T, Hormaza JI (2013) Fingerprinting and analysis of genetic diversity of litchi (*Litchi chinensis* Sonn) accessions from different germplasm collections using microsatellite markers. Tree Genet Genomes 9:387–396
Magdalita PM, Laurena AC, Yabut-Perez BM, Mendoza EMT, Villegas VN, Botella JR (2002) Progress in the development of transgenic papaya: transformation of Solo papaya using ACC synthase antisense construct. Acta Hortic 575:171–176
Maity SC, Mitra SK (1996) Litchi. In: Fruits: tropical and subtropical. Naya Prakash, Calcutta, pp 420–448
Mann C (1997) Reseeding the green revolution. Science 277:1038–1043
Marboh ES, Lal RL, Mishra DS, Goswami AK (2012) Effect of hot water treatment and oxalic acid on colour retention and storage quality of litchi fruit cv rose scented. Indian J Hortic 69:84–488
McConchie CA, Batten DJ (1991) Fruit set in lychee (*Litchi chinensis* Sonn.) variation between flowers panicles and trees. Aust J Agric Res 42:1163–1172
McGloughlin MN (2010) Modifying agricultural crops for improved nutrition. New Biotechnol 27:494–504
Menzel CM (1985) Propagation of lychee: a review. Sci Hortic 25:31–48
Menzel CM (1995) Lychee: its origin distribution and production around the world. West Australian Nut and Tree Crop Association Year book 1941–48
Menzel C (2002) The lychee crop in Asia and the Pacific. FAO, Bangkok
Menzel CM, Simpson DR (1995) Temperature above 20 °C reduce flowering in lychee (*Litchi chinensis* Sonn). J Hortic Sci 70:981–987
Merkle SA (1995) Strategies for dealing with limitations of somatic embryogenesis in hardwood trees. Plant Tissue Cult Biotechnol 1:112–121

Mishra B, Gautam S, Sharma A (2004) Shelf-life extension of fresh ginger (*Zingiber officinale*) by gamma irradiation. J Food Sci 69:274–279

Mitra SK, Dutta Ray SK, Mandal D (2011) Control of fruit cracking and sun-burning in litchi by irrigation and moisture conservation. In International Symposium on Tropical and Subtropical Fruits, 1024, pp 177–181

Miura T, Kitadate K, Fujii H (2010) The function of the next generation polyphenol "Oligonol". In: Bagchi D, Lau FC, Ghosh DK (eds) Biotechnology in functional foods and nutraceuticals. CRC Press, Boca Raton, pp 91–101

Moriwaki Y, Okuda C, Yamamoto A, Ka T, Tsutsumi Z, Takahashi S, Yamamoto T, Kitadate K, Wakame K (2011) Effects of Oligonol an oligomerized polyphenol formulated from lychee fruit on serum concentration and urinary excretion of uric acid. J Funct Foods 3:13–16

Morton J (1987) Pomegranate. In: Fruits of warm climates. Julia F Morton, Miami, pp 532–534

Murthy ZVP, Joshi D (2007) Fluidized bed drying of aonla (*Emblica officinalis*). Dry Technol 25:883–889

Nacif SR, Sartori Paoli AA, Chamhum Salomãoa LC (2001) Morphological and anatomical development of the litchi fruit (*Litchi chinensis* Sonn cv Brewster). Fruits 56:225–233

Nagasawa J, Sugiyama K, Uchimaru J (2010) Oxidative stress in hypobaric and normobaric hypoxia and anti-oxidant effect of Oligonol. Japanese J Mountain Med 30:118–124

Nagle M, Habasimbi K, Mahayothee B, Haewsungcharern M, Janjai S, Müller J (2011) Fruit processing residues as an alternative fuel for drying in northern Thailand. Fuel 90:818–823

Neidhart S, Hutasingh P, Carle R (2007) Innovative strategies for sustainable lychee processing. In: Heidhues F, Herrmann L, Neef A, Neidhart S, Pape J, Sruamsiri P, Thu DC, Valle Zárate A (eds) Sustainable land use in mountainous regions of southeast Asia Meeting the challenges of ecological socioeconomic and cultural diversity. Springer, Berlin, pp 147–158, 163–171

Niemira BA, Sommers CH (2006) New applications in food irradiation. In: Heldman DR (ed) Encyclopedia of agricultural food and biological engineering Dekker Encyclopedias. Taylor & Francis publishers, New York

Niemira BA, Fan X, Sokorai KJ, Sommers CH (2003) Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and ATCC 51742 inoculated on endive (Cichorium endiva). J Food Prot 66:993–998

Nishizawa M, Hara T, Miura T, Fujita S, Yoshigai E, Ue H, Hayashi Y, Kwon AH, Okumura T, Isaka T (2011) Supplementation with a flavanol-rich lychee fruit extract influences the inflammatory status of young athletes. Phytother Res 25:1486–1493

Ochese JJ, Soule MJ, Dhaiman MJ, Wehilburg C (1961) Other fruit crops. In: Tropical and subtropical agriculture, vol 1. MacMillan Publ Co, New York

Ogata T, Yamanaka S, Shoda M, Urasaki N, Yamamoto T (2016) Current status of tropical fruit breeding and genetics for three tropical fruit species cultivated in Japan: pineapple mango and papaya. Breed Sci 66:69–81

Ohno H, Sakurai T, Hisajima T (2008) The supplementation of Oligonol the new lychee fruit-derived polyphenol converting into a low-molecular form has a positive effect on fatigue during regular track-and-field training in young athletes. Adv Exercise Sports Physiol 13:93–99

Oosthuizen JH (1991) Lychee cultivation in South Africa. Yearbook Australian Lychee Growers Association 1:51–55

Ouyang S, Zheng X (1985) T-DNA transfer and tumor formation induced by *Agrobacterium tumefaciens* on *Litchi chinensis*. Acta Genet Sin 12:42–45

Padilla G, Pérez JA, Perea-Arango I, Moon PA, Gómez-Lim MA, Borges AA, Expósito-Rodríguez M, Litz RE (2013) *Agrobacterium* tumefaciens-mediated transformation of 'Brewster' ('Chen Tze') litchi (*Litchi chinensis* Sonn) with the PISTILLATA cDNA in antisense *In vitro*. Cellular Dev Biol Plant 49:510–519

Palapol Y, Ketsa S, Lin-Wang K, Ferguson IB, Allan ACA (2009) MYB transcription factor regulates anthocyanin biosynthesis in mangosteen (*Garcinia mangostana* L) fruit during ripening. Planta 229:1323–1334

Pandey N, Joshi SK, Singh CP, Kumar S, Rajput S, Khandal RK (2013) Enhancing shelf life of litchi (*Litchi chinensis)* fruit through integrated approach of surface coating and gamma irradiation. Radiat Phys Chem 85:197–203

Paull RE, Duarte O (2011) Litchi and longan. In: Paull RE, Duarte O (eds) Tropical fruits 2nd ed. CABI, Oxfordshire, pp 221–251, 400 pp

Peng G, Wu J, Lu W, Li J (2013) A polygalacturonase gene clustered into clade E involved in lychee fruitlet abscission. Sci Hortic 150:244–250

Pimentel D (1997) Techniques for reducing pesticide use: economic and environmental benefits. Wiley, New York

Pirrello J, Bourdon M, Cheniclet C, Bourge M, Brown SC, Renaudin JP, Frangne N, Chevalier C (2014) How fruit developmental biology makes use of flow cytometry approaches. Cytometry A 85:115–125

Pivovaro SZ (1974) MSc thesis, Hebrew University of Rehovat, pp 139

Prakash A, Manley J, Decosta S, Caporaso F, Foley D (2002) The effects of gamma irradiation on the microbiological physical and sensory qualities of diced tomatoes. Radiat Phys Chem 63:387–390

Puchooa D (2004a) *In vitro* regeneration of lychee (*Litchi chinensis* Sonn). Afr J Biotechnol 3:576–584

Puchooa D (2004b) Expression of green fluorescent protein gene in litchi (*Litchi chinensis* Sonn) tissues. J Appl Hortic 6:11–15

Punumong P, Sangsuwan J, Kim SM, Rattanapanone N (2016) Combined effect of calcium chloride and modified atmosphere packaging on texture and quality of minimally-processed Litchi fruit. Chiang Mai J Sci 43:556–569

Qiu S, Wang Y, Zhou R, Yin A, Zhou T (2015) Optimization of cultural conditions for vinegar of Litchi (*Litchi chinensis* Sonn) in liquid state fermentation. J Food Nutr Res 3:641–647

Raharjo SHT, Litz RE (2007) Somatic embryogenesis and plant regeneration of litchi (*Litchi chinensis* Sonn) from leaves of mature phase trees. Plant Cell Tissue Org Cult 89:113–119

Rahim MA, Busatto N, Trainotti L (2014) Regulation of anthocyanin biosynthesis in peach fruits. Planta 240:913–929

Ramesh MN, Wolf W, Tevini D, Bognar A (2002) Microwave blanching of vegetables. J Food Sci 67:390–398

Robbertse H, Fivaz J, Menzel C (1995) A reevaluation of tree model inflorescence morphology and sex ratio in lychee (*Litchi chinensis Sonn*). J Am Soc Hortic Sci 120:914–920

Rodov V (2007) Biotechnological approaches to improving quality and safety of fresh-cut fruit and vegetable products. Acta Hortic 746:181–194

Sajilata MG, Singhal RS (2006) Effect of irradiation and storage on the antioxidative activity of cashew nuts. Radiat Phys Chem 75:297–300

Sarin NB, Prasad US (2003) *In vitro* regeneration and transformation of litchi (*Litchi chinensis* Sonn). In: PK Jaiwal, RP Singh (eds), Plant genetic engineering improvement of fruits, vol 6. Sci Tech Publishing LLC, 211–222

Sarni-Manchado P, Roux EL, Guerneve CL, Lozano Y, Cheynier V (2000) Phenolic composition of litchi fruit pericarp. J Agric Food Chem 48:5995–6002

Schmidt HM, Palekaer MP, Maxim JE, Castillo A (2006) Improving the microbiological quality and safety of fresh-cut tomatoes by low-dose electron beam irradiation. J Food Prot 69:575–581

Shao W, Lai ZX, Huang Q, Guo YQ (2010) Changes of apx isozymes under high-temperature stress and cloning of an APX functional fragment in embryogenic callus of litchi. Acta Hortic 863:183–188

Singh SN, Singh SP (1952) Studies on the storage and longevity of some fruits and vegetables. J Agric Animal Husbandry 2:3–11

Singh LB, Singh UP (1964) The Litchi Supdt Printing and Stationary, Uttar Pradesh, Allahabad

Singh A, Pandey SD, Vishal N (2012) The world Litchi cultivars technical bulletin 007, NRC for Litchi Mushahari, Muzaffarpur, pp 1–65

Sivakumar D, Korsten L (2006) Influence of modified atmosphere packaging and postharvest treatments on quality retention of litchi cv Mauritius. Postharvest Biol Technol 41:135–142
Song HP, Byun MW, Jo C, Lee CH, Kim KS, Kim DH (2007) Effects of gamma irradiation on the microbiological nutritional and sensory properties of fresh vegetable juice. Food Control 18:5–10
Stern RA, Gazit S (1996) Lychee pollination by the honeybee. J Am Soc Hortic Sci 120:152–157
Stern RA, Gazit S (1998) Pollen viability in lychee. J Am Soc Hortic Sci 123:41–46
Stern RA, Eisenstein D, Voet H, Gazit S (1996) Anatomical structure of two day old litchi ovules in relation to fruit set and yield. J Hortic Sci 71:661–671
Su D, Ti H, Zhang R, Zhang M, Wei Z, Deng Y, Guo J (2014) Structural elucidation and cellular anti-oxidant activity evaluation of major anti-oxidant phenolics in lychee pulp. Food Chem 158:385–391
Sun J, Jiang Y, Shi J, Wei X, Xue SJ, Shi J, Yi C (2010) Anti-oxidant activities and contents of polyphenol oxidase substrates from pericarp tissues of litchi fruit. Food Chem 119:753–757
Sung YY, Yang WK, Kim HK (2012) Antiplatelet anticoagulant and fibrinolytic effects of *Litchi chinensis* Sonn Extract. Mol Med Rep 5:721–724
Syamal MM, Mishra KA (1984) Litchi cultivation in India. Farmer Parliament 15–16:27–28
Takos AM, Robinson SP, Walker AR (2006) Transcriptional regulation of the flavonoid pathway in the skin of dark-grown 'Cripps' Red' apples in response to sunlight. J Hortic Sci Biotechnol 81:735–744
Thapa N, Bhowmick DK, Ghosh SK, Deb P (2014) Performance of Litchi (*Litchi chinensis Sonn*) 2014 Cultivars in Non-bearing Stage under Terai Region of West Bengal. Int J Agric Food Sci Technol 5:531–536
Thompson AK (2003) Postharvest technology of fruits and vegetables. In: Thompson (ed) Fruit and vegetables: harvesting handling and storage. Blackwell Publishing Ltd, Oxford, pp 253–255
Tongpamnak P, Patanatara A, Srinives P, Babprasert C (2002) Determination of genetic diversity and relationships among Thai litchi accessions by RAPD and AFLP markers *Kasetstart*. J Nat Sci 36:370–380
Variyar PS, Limaye A, Sharma A (2004) Radiation-induced enhancement of anti-oxidant contents of soybean (*Glycine max* Merrill). J Agric Food Chem 52:3385–3388
Viruel MA, Hormaza JI (2004) Development characterization and variability analysis of microsatellites in lychee. Theor Appl Genet 108:896–902
Vishwanathan KH, Girish KG, Umesh H (2013) Infrared assisted dry-blanching and hybrid drying of carrot. Food Bioprod Process 91:89–94
Wang JF, Wang ZQ (2010a) Studies on *in vitro* germplasm conservation of litchi. Acta Hortic 863:111–116
Wang ZQ, Wang JF (2010b) Genetic evaluations of *in vitro* conserved embryogenic calli of litchi. Acta Hortic 863:149–154
Wang X, Wei Y, Yuan S, Liu G, Zhang YL, Wang W (2006a) Potential anti-cancer activity of litchi fruit pericarp extract against hepatocellular carcinoma *in vitro* and *in vivo*. Cancer Lett 239:144–150
Wang X, Yuan S, Wang J (2006b) Anti-cancer activity of Litchi fruit pericarp extract against human breast cancer *in vitro* and *in vivo*. Toxicol Appl Pharmacol 215:168–178
Wang X, Xiao L, Pan J (2007) Experimental studies of effects of anti-tumor of Litchi seed Ke Li and the activity of in the tissue-end of EAC S180 and hepatic carcinoma of rats. China Healthcare Innovation 2:54–56
Wang G, Li HL, Wang JB (2013) Primary study on the callus induction from anther of two litchi (*Litchi chinensis* Sonn) cultivars. Chin J Tropical Crops 34:669–674
Wang J, Liu B, Xiao Q, Li H, Sun J (2014) Cloning and expression analysis of Litchi (*Litchi chinensis* Sonn) polyphenol oxidase gene and relationship with postharvest pericarp browning. PLoS One 9:e93982

Wang B, Tan HW, Fang W, Meinhardt LW, Mischke S, Matsumoto T, Zhang D (2015a) Developing single nucleotide polymorphism (SNP) markers from transcriptome sequences for identification of longan (*Dimocarpus longan*) germplasm. Horm Res 2:14065

Wang TD, Zhang HF, Wu ZC, Li JG, Huang XM, Wang HC (2015b) Sugar uptake in the Aril of litchi fruit depends on the apoplasmic post-phloem transport and the activity of proton pumps and the putative transporter LcSUT4. Plant Cell Physiol 56:377–387

Wang G, Li H, Wang S, Sun J, Zhang X, Wang J (2016) *In vitro* regeneration of Feizixiao litchi (*Litchi chinensis* Sonn). Afr J Biotechnol 15:1026–1034

Weberling F (1992) Morphology of flowering plants. Cambridge University Press, Cambridge

Wen LR, Yang B, Cui C, You LJ, Zhao MM (2012) Ultrasound-assisted extraction of phenolics from longan (*Dimocarpus longan* Lour) fruit seed with artificial neural network and their antioxidant activity. Food Anal Methods 5:1244–1251

Wen L, Wu D, Jiang Y (2014) Identification of flavonoids in Litchi (*Litchi chinensis* Sonn) leaf and evaluation of anti-cancer activities. J Funct Foods 6:555–563

Wolf LM (1987) *In Vitro* propagation of Lychee (*Litchi chinensis*). Honours Thesis, University of Queensland, Brisbane, Australia, 40 pp

Wong WS, Li GG, Ning W, Xu ZF, Hsaio WLW, Zhang LY, Li N (2001) Repression of chilling induced ACC accumulation in transgenic citrus by overproduction of antisense 1-aminocyclop ropane-1-carboxylate synthase RNA. Plant Sci 161:969–977

Wu SX (1998) Encyclopedia of China fruits: litchi. China Forestry Press, Beijing

Wu JY, Li CQ, Lu WJ, Li JG (2013) Cloning of Lc-ACO1 and its expression related to fruitlet abscission in litchi. J Fruit Sci 30:207–213

Xie YM, Yi GJ, Zhang QM, Zeng JW (2006) Somatic embryogenesis and plantlet regeneration from anther of Feizixiao litchi. Chin J Tropical Crops 27:68–72

Xiong AH, Shen WJ, Xiao LY (2008) Effect of semen Litchi containing serum on proliferation and apoptosis of HepG2 cells. J Chin Med Mater 31:1533–1536

Xu SS, Lai ZX (2013) Induction and maintenance of the embryogenic callus in anther of some ancient litchi tree in Fuzhou City. Subtropical Agric Res 9:123–126

Xu X, Xie H, Hao J (2010a) Eudesmane sesquiterpene glucosides from Lychee seed and their cytotoxic activity. Food Chem 123:1123–1126

Xu X, Xie H, Wang Y, Wei X (2010b) A-type proanthocyanidins from Lychee seeds and their antioxidant and anti-viral activities. J Agric Food Chem 58:1667–11672

Yang B, Wang J, Zhao M, Liu Y, Wang W, Jiang Y (2006) Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn) fruit in relation to their anti-oxidant activities. Carbohydr Res 341:634–638

Yang B, Zhao MM, Shi J, Jiang YM, Yang N (2008) Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. Food Chem 106:685–690

Yang B, Jiang YM, Shi J, Chen F, Ashraf M (2011) Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour) fruit – a review. Food Res Int 44:1837–1842

Yang Z, Wang T, Wang H, Huang X, Qin Y, Hu G (2013) Patterns of enzyme activities and gene expressions in sucrose metabolism in relation to sugar accumulation and composition in the aril of *Litchi chinensis* Sonn. J Plant physiol 170:731–740

Yoruk R, Balaban MO, Marshall MR, Yoruk S (2002) The inhibitory effect of oxalic acid on browning of banana slices (30G-18). In: Annual meeting and food expo Anaheim CA

Yu YB (1991) Study on some factors in tissue culture of lychee (*Litchi chinensis*). Fujian Agric Sci Technol 5:17–18

Yu CH, Chen ZG (1997) Induction of litchi embryogenic calli by immature embryos and anthers culture *in vitro*. J Fujian Agric Uni 26:168–172

Yu CH, Chen ZG (1998) Embryogenic suspension culture and protoplast isolation in litchee. Chin J Tropical Crops 19:16–20

Yu C, Chen Z, Lu L, Lin J (2000) Somatic embryogenesis and plant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Org Cult 61:51–58

Yuan R, Huang H (1998) Litchi fruit abscission: its patterns effect of shading and relation to endogenous abscisic acid. Sci Hortic 36:281–292

Zhang N, Zhou Z, Feng X (2012) Comparison and elevation on anti-tumor activity *in vitro* of the Litchi seeds and Longan seeds water extract hunan. J Tradit Chin Med 28:133–135

Zhang R, Zeng Q, Deng Y, Zhang M, Wei Z, Zhang Y, Tang X (2013) Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. Food Chem 136:1169–1176

Zhao MM, Yang B, Wang JS, Li BZ, Jiang YM (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their anti-oxidant activities. Food Chem 98:539–544

Zhao M, Yang B, Wang J, Liu Y, Yu L, Jiang Y (2007) Immunomodulatory and anti-cancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn) pericarp. Int Immunopharmacol 7:162–166

Zhao YH, Hu YL, Guo YS, Zhou J, Fu JX, Liu CM, Zhu J, Zhang MJ, Huang SS (2010) The creation and molecular identification of intergeneric hybrids between litchi and longan. Acta Hortic 863:129–134

Zheng X, Tian S (2006) Effect of oxalic acid on control of postharvest browning of litchi fruit. Food Chem 96:519–523

Zhou LN, Kuang ZS, Ma XJ, Chen JQ, Cao J (1996) The study of factors affecting somatic embryogenesis in young embryo culture of *Litchi chinensis*. J Agric Biotechnol 4:161–165

Zhou H, Lin Y, Li Y, Li M, Wei S, Chai W, Tam NF (2011) Anti-oxidant properties of polymeric proanthocyanidins from fruit stones and pericarps of *Litchi chinensis* Sonn. Food Res Int 44:613–620

Zhou Y, Wang H, Yang R, Huang H, Sun Y, Shen Y, Lei H, Gao H (2012) Effects of *Litchi chinensis* fruit isolates on prostaglandin E2 and nitric oxide production in J774 murine macrophage cells. BMC Complement Altern Med 12:1

Lychee Biology and Biotechnology

5

Bhupendra Koul and Joginder Singh

Abstract

Lychee (*Litchi chinensis* Sonn.) is one of the revered members of the soapberry family Sapindaceae which includes 150 genera and 2000 species. It is a tropical and subtropical fruit tree which is native to Fujian and Guangdong regions of China and is cultivated as an important commercial fruit crop in many parts of the world. It is famous for its fragrant and sugary flavour. After China, India is at the second position in the production of lychee in the world. The varieties with large pulp, small seeds and noteworthy flavour are of great interest among the consumers and farmers. Lychee fruit took tremendous attention of scientists as it contains ample amounts of anti-oxidants, vitamins and fibre. Moreover, the plant parts possess considerable anti-pyretic, anti-inflammatory, anti-cancer, anti-diabetic, anti-tumour and anti-oxidant properties. 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. Very limited efforts have been made in its varietal improvement through hybridization and modern breeding techniques. 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*.

Keywords

Lychee • Subtropical fruit • Anti-oxidant • Lychee propagation • Hybridization

B. Koul • J. Singh (✉)
School of Bioengineering and Biosciences, Lovely Professional University,
Phagwara 144411, Punjab, India
e-mail: joginder.15005@lpu.co.in

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5.1 Origin and History

Lychee (*Litchi chinensis* Sonn.) is one of the precious and economically important fruit crops of the world. The species belongs to the family Sapindaceae, which comprises about 2000 species of tropical and subtropical trees, shrubs and vines that have been classified into 140 genera (Chapman 1984). Lychee has been shown to possess variable diploid chromosome numbers where 2n = 28, 30, 32. The variation in chromosome number is thought to be because the modern species had more than one wild progenitor. Lychee cultivation was reported since 1500 BC by the people of Malayan descent and has been growing for thousands of years in southern Guangdong province of China. The first reference to this fruit is available in the literature of the Han dynasty (140 BC to 86 BC). It is sure that lychee is native of South China, but, according to Blume, Cochin-China and the Philippine islands are the lands of its origin (Popenoe 1920). It is also reported to have originated in China's Kwangtung and Fukien provinces and have been cultivated in China for about forty centuries (Ochse et al. 1961). A monograph written by Tsai Hsiang in 1059 AD is considered to be the first publication in the world devoted to this fruit. However, according to Walter T. Swingle, the first published work of fruit culture was written by a Chinese scholar in 1056 AD, on the varieties of lychee.

From China it reached Burma (Myanmar) by the end of the seventeenth century and was introduced in India about 100 years later. Lychee reached Madagascar and Mauritius around 1870 and was introduced in Hawaii in 1873 by a Chinese trader. It arrived in Florida, from India, between 1870 and 1880 and was introduced in California in 1897. Lychee was reported to be brought to Australia by Chinese migrants in 1954 and arrived in Israel sometime between 1930 and 1940. Presently, lychee is grown in Central and South America, parts of Africa and throughout Asia. China, India, South Africa, Australia, Mauritius, Madagascar and Thailand are now the major lychee-producing countries in the world.

5.2 Production

Lychee plantation requires a warm subtropical to tropical climate (Rivera-Lo'pez et al. 1999). Besides China and India, lychee fruit is also grown as a commercial crop in subtropical Asia, Hawaii, Israel, Mexico, Australia and South Africa (Jiang et al. 2001). India is the second largest producer of lychee after China with an annual production of 428,900 metric tons from 56,200 ha (Fig. 5.1). Lychee is mostly grown in Eastern India, and Bihar state alone contributes to 74% of Indian lychee production (Fig. 5.2). As lychee is an introduced fruit crop, it has great potential of yield in India. Figure 5.3 reveals a trend in the year-wise expansion in the area under lychee cultivation which has increased from 58,100 to 84,200 ha in 1991–1992 to 2013–2014 with a similar trend in the production from 355,900 MT to 585,300 MT in the last decades.

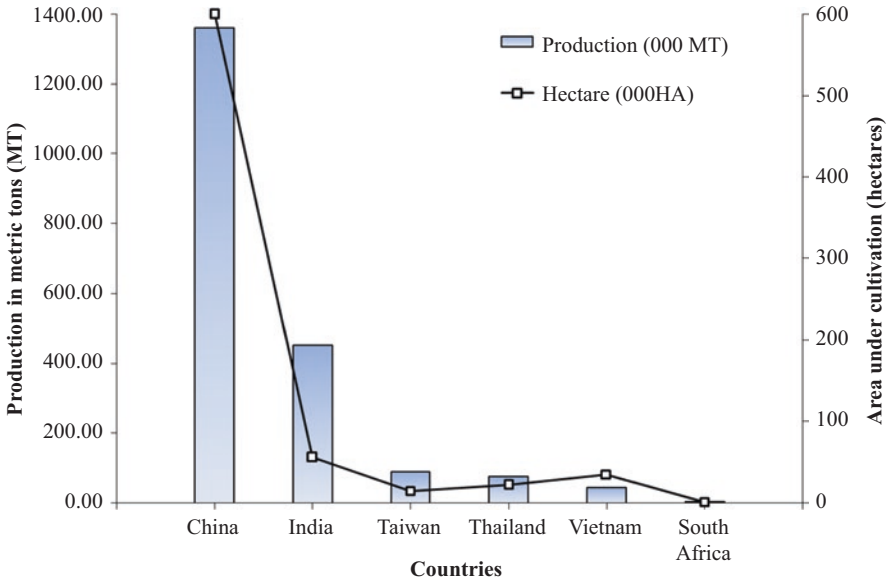


Fig. 5.1 World lychee production

5.3 Classification of Major Lychee Cultivars

The lychee (*Lychee chinensis* Sonn.) is a non-climacteric fruit of Southeast Asian origin (Nakasone and Paull 1998). It is covered by a pink or red leather-like pericarp due to the presence of anthocyanins (Lee and Wicker 1991; Rivera-Lo'pez et al. 1999) as shown in Fig. 5.4. When distinguishing the cultivars, the shape of fruit skin segments and protuberances are reliable and stable genetic characteristics (Fig. 5.5). Fruit size, shape and taste are also variables but are influenced by other than genetic factors. Chinese researchers report that the shape of the skin segments and protuberances are more reliable characteristics than fruit size, shape or taste, to identify cultivars. The lychee cultivars vary greatly in vegetative flushing patterns, flush colour and flowering ability. The leaf of the Rose Scented is boat shaped, while China has a distinctive twist along the length curved upwards from the midrib and down along its length. Small leaflets in Bedana are oval shaped. The fruit shape of lychee is very distinguishing. The round shape of Bedana is distinguished from oblong shape of China or Shahi. The fruit is smooth and pulp is even or uneven. The apex of the fruit can be round, obtuse, blunt as in Shahi or pointed as in China (Fig. 5.5).

The varieties can also be distinguished depending on the colour of new flush and season of flowering. Shahi produces very light-coloured flush, while China has pinkish flush. Bedana produces bright red or copper-coloured flush and short compact panicles. The fruit colour varies in different varieties and is also influenced by growing conditions. Skin thickness depends on cultivars. Bedana and China have

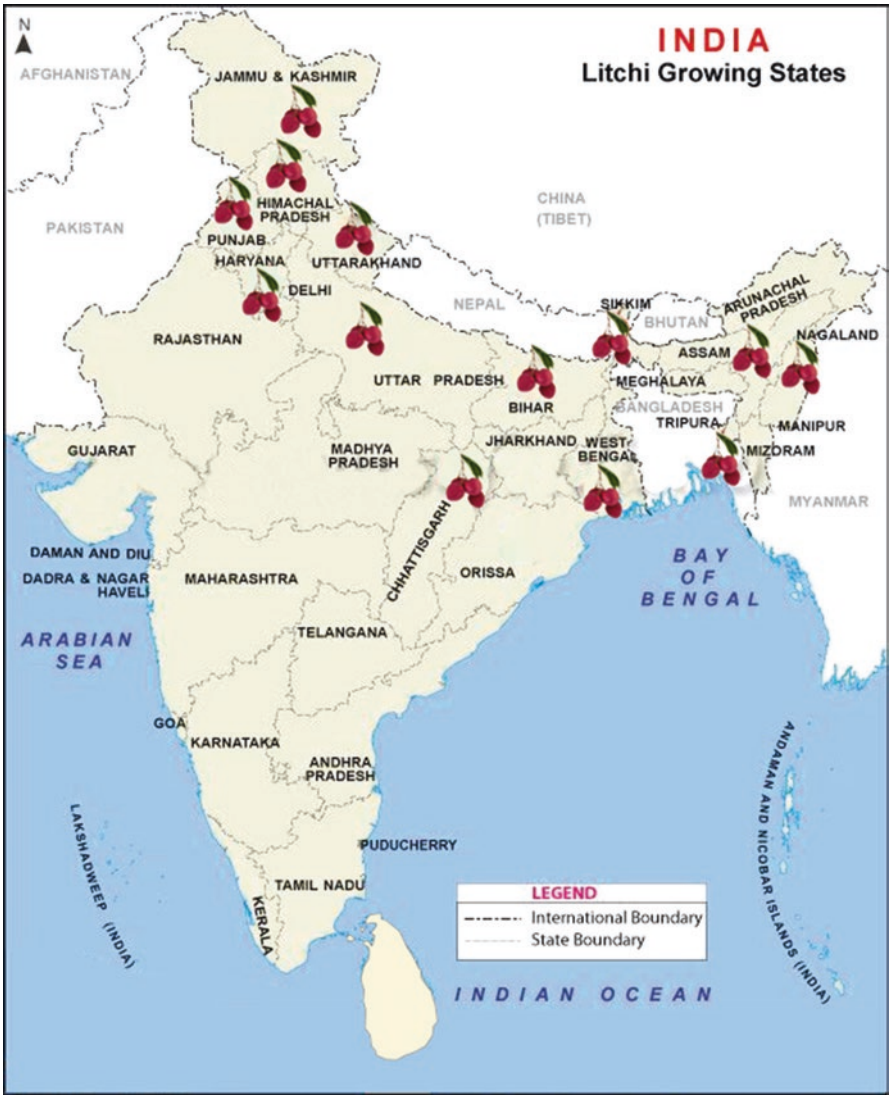


Fig. 5.2 Map showing lychee-producing states in India

very thick skin, whereas Rose Scented and Shahi have thin skin. Skin surface at maturity also varies being smooth, swelling and sharp pointed. Protuberances of pericarp (skin) can be smooth as in Bedana or sharply pointed as in China. The presence and absence of seed as well as structure and size of seeds also vary from cultivar to cultivar, but it is also influenced by the environmental conditions. In Rose Scented and Bedana, a high proportion of chicken-tongued seeds is observed, while China has bold seeds.

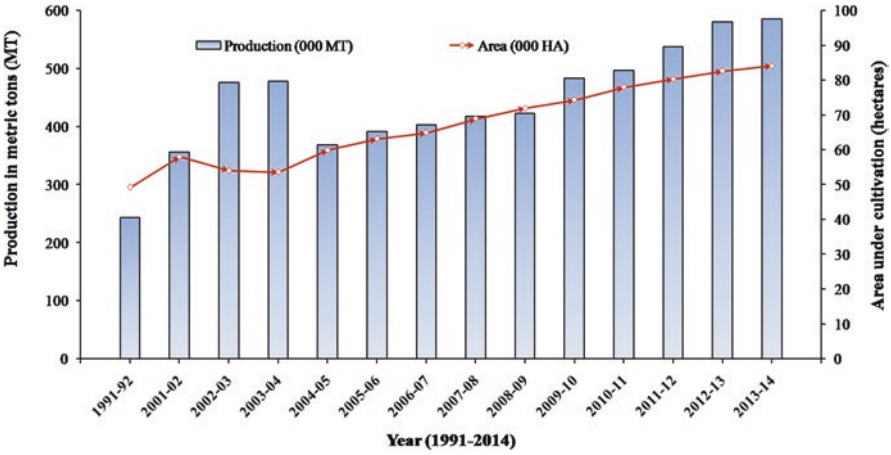


Fig. 5.3 Production of lychee in India (Indian horticulture database 2014)

Fig. 5.4 Lychee tree with ripened fruits

The harvest season lasts 5–10 weeks for a range of cultivars in any one location. Lychee cultivars can be broadly classified as early, mid or late maturing, although the order varies from year to year, depending on seasonal conditions. Table 5.1 enlists the lychee cultivars grown in different countries. According to the Indian Horticulture Database (2014), Indian lychee is exported to China (87 MT), Thailand (19 MT) and Mauritius (1MT).

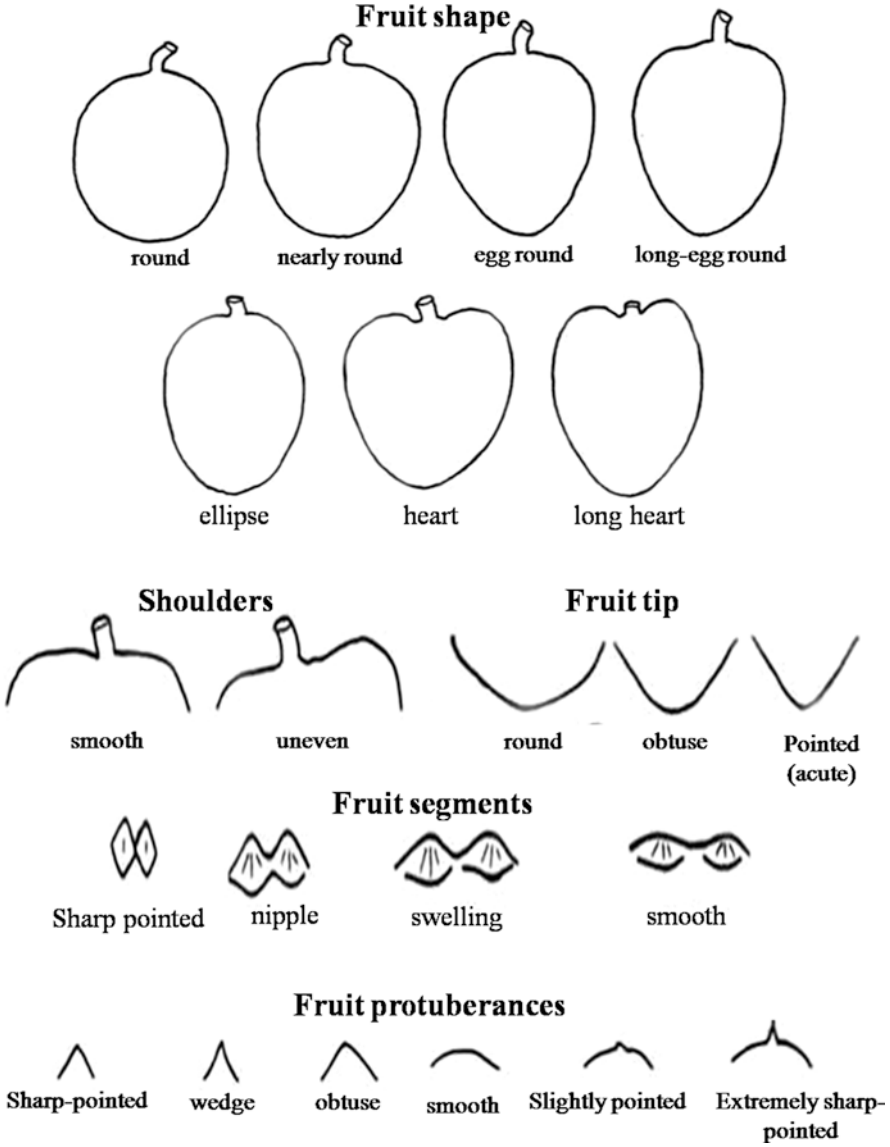


Fig. 5.5 Lychee fruit characteristics

Table 5.1 Major lychee cultivars grown in different countries

Country	Cultivars
Australia	Kwai May Pink, Tai So, Souey Tung, Fay Zee Siu, Salathiel, Wai Chee
Bangladesh	Bombai, Muzaffarpur, Bedana, China 3
Brazil	Bengal
China	Early: Sanyuehong, Baitangying
	Mid: Dazao, Heiye, Baila, Feizixiao and Shuidong, Tianyan, Chenzi
	Late: Xiangli, Guiwei, Noumici, Huaizhi, Xuehuaizi, Lanzhu, Bobaitangbo, Yuanhong, Xiafanzhi, Nanmuye
Florida, USA	Mauritius and Brewster
India	Bihar/Jharkhand: Deshi, Purbi, China, Kasba, Bedana, Early Bedana, Late Bedana, Dehra Rose, Shahi, Manragi, Maclean, Longia, Kaselia and Swarna Rupa, Ajhauli, Green, Mandraji, Rose Scented
	Uttar Pradesh/Uttrakhand/Himachal Pradesh: Early Large Red, Early Bedana, Late Large Red, Rose Scented, Late Bedana, Calcuttia, Extra Early, Gulabi, Pickling, Khatti, Dehra Dun, Piyazi
	West Bengal/Assam: Bombai, Ellaichi Early, China, Deshi, Purbi and Kasba, Kalyani Selection
	Haryana/Punjab: Early Seedless, Late Seedless, Seedless-1 and Seedless-2, Calcuttia, Muzaffarpur
	Chhattisgarh: Sarguja-1, Sarguja-2
Indonesia	Local Selections
Israel	Mauritius, Floridian
Madagascar	Mauritius
Nepal	Mujafpuri, Raja Saheb, Dehradun, Calcuttia, China
Philippines	Sinco, Tai So, Ulpb Red
South Africa	Mauritius, Mclean Red
Thailand	Tai So, Chacapat, Wai Chee, Haak yip, Khom
Vietnam	Thieuthauhha

5.4 Botanical Description

Tree Lychee is a long-lived, evergreen tree up to 30 m tall in old specimens, with a short stocky trunk. In some cultivars, the branches are crooked or twisting and spreading forming a crown broader than high, while in other cultivars, the branches are fairly straight and upright forming a compact, rounded crown. The varieties can be identified by using tree characteristics. However, they change with weather, soil and culture. Differences in the tree size and shape, length and spread of branches are commonly seen. For example, Brewster is vigorous and erect, with very wide strong crotch angles; Tai So is vigorous, with a spreading habit and sharp crotch angles, while Wai Chee is slow, compact and dome shaped.

Leaves The leaves are alternative and compound, with two to five leaflets. The leaflets are oblong and 5–15 cm long. The foliage comprises 2–4 pairs of leaflets, which are 3–6 in. long, coriaceous, elliptic-oblong to lanceolate, shortly acute, glabrous and shining above and glaucescent beneath. The new flushes are a distinctive red brown when immature and light to dark green as they mature.

The leaf characteristics include leaf size, shape and colour, e.g. Tai So has large, glossy, dark green leaflets that have an upward curl from the midrib to be almost canoe shaped. Bengal has large leaflets, mid-green in colour with a distinctive twist along their length. Haak Yip has dark, glossy green leaflets that are long, narrow pointed and slightly curled at the tip. Wai Chee leaflets are small, oval shaped and curve upwards from the midrib and down along their length. The new flush of growth is red in Wai Chee and Kwai May Pink and green bronze in Tai So.

Flower The inflorescences are many branched panicles, each with one or more leaves and up to 3000 flowers, and form 5–80 fruits at harvest. The flowers are small, yellowish white, functionally male or female and apetalous. Functionally, male flowers have 6–10 stamens. There are usually two stages of male flowering overlapping with the female cycle: a true male flower first and then a functionally male flower that opens towards the end of the flowering period. The second male flower has a rudimentary bicarpellate pistil. This is absent in the first stage. Functionally, female flowers have 6–10 staminodes and a functional, bicarpellate pistil (Fig. 5.2). The last stage of male flowering generally supplies most of the pollen used to fertilize the female flowers. The ovary is bilobed, compressed and silky; usually only one lobe develops into a fruit. The stigma is bilobed. According to their hermaphrodite nature, flowers are classified into three classes, viz. type I, type II and type III. Type I and type III flowers are male, while type II flowers function as a fruit-bearing female. Most of the flowers are of type III and only 20 % of the flowers are of fruit producing type II females.

Fruit Fruits are highly variable, depending on the cultivar (Fig. 5.3). They can be round, ovoid or heart shaped and from 2.0 to 3.5 cm in diameter. The skin can be smooth or rough with distinct protuberances, thick or thin and pink red, bright red or purple red. The flesh or aril is an outgrowth of the outer cells of the seed coat (outer integument), and in good cultivars may comprise 80% of fruit weight. The aril is generally translucent white, juicy or firm and sweet and aromatic in better cultivars. Many cultivars can be distinguished by their flavour and aroma. The fruit contains a single dark brown seed 6–12 mm wide and 10–23 mm long. Some cultivars have a high proportion of aborted seeds and thus a high flesh recovery. They are popular in the marketplace, especially in Asia. There are a few cultivars that produce nearly seedless fruit, although the fruit usually weighs less than 10 g. The fruit shape of some cultivars is very distinctive. The round fruit of Kwai May Pink distinguishes it from the egg shape of Tai So or the heart shape of Haak Yip. The shoulders of the fruit can be smooth or flat as in Wai Chee and Kwai May Pink or uneven as in Souey Tung and Bengal. The apex or tip of the fruit can be round as in Kwai May Pink and Wai Chee, obtuse or blunt as in Souey Tung and Brewster or

pointed as in Bengal. The fruit colours are bright red (Bengal), dull red (Wai Chee), purple red (Haak Yip) or pink red (Brewster). The skin can be thick as in Wai Chee, Bengal and Kwai May Pink or thin as in Haak Yip and Souey Tung. Skin segments at full maturity can be smooth (Haak Yip), swelling (Wai Chee) or sharp pointed (Kwai May Red). Similarly, the protuberances on each segment can be smooth as in Haak Yip, sharp pointed as in Kwai May Red and Bengal or hairlike and sharp as in Tai So. The presence or absence of an obvious suture line can distinguish some cultivars such as Haak Yip and Souey Tung.

The texture, juiciness, taste and aroma of the flesh can aid description, although experience is needed to make clear distinctions. For example, Wai Chee is watery, Kwai May Red is firm, Kwai May Pink is spicy, and Bengal is very sweet. The proportion of small or shrivelled seeds is important but varies with season and orchard. Cultivars with a high proportion of chicken-tongue seeds are favoured. Salathiel produces nearly always fruit with small seeds, while Bengal, Souey Tung, Haak Yip and Wai Chee produce hardly any. Other varieties such as Tai So and Kwai May Pink vary. Description to major lychee cultivars grown in different countries is shown in Table 5.2.

Table 5.2 Description of the lychee cultivars/breeding lines

Variety	Origin/cultivation	Characteristics	Reference(s)
Aili	Hainan Province, China	Selected from local *Litchi chinensis*; dwarf selection; average fruit weight 24.8 g; cultivated in Hainan Province (China)	Miao et al. (1998)
Ajhauli	Ajhauli village, Bihar, India	Early maturing; selected from Ajhauli village; a 16-year-old tree yields 80–100 kg fruit; average fruit weight 15–18 g; big seeds; fruits highly susceptible to cracking; proper irrigation can minimize cracking	Singh and Babita (2001)
Amboina	–	Bright red medium-sized fruits; borne in clusters of 6–20; slow-growing tree of warm climate	Singh et al. (2012)
Bah Lup	Dian Bai and Gao Zhan, Guangdong, China	Productive Chinese cultivar; important export variety; dome-shaped tree; narrow, long, glossy, dark green and pointed leaves; heart-shaped fruits; average weight 20–29 g; brilliant red to light purple soft skin; obtuse protuberances	
Bai-Teng-Ying	–	Early-maturing cultivar; dwarf tree; good-quality fruits; tolerant to various environmental stress; floral differentiation occurs between October and February	Ooyang et al. (1994)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Bengal	India	Seedling of an Indian cultivar 'Purbi'; selected in Florida; vigorous trees but with thin branches; resistant to wind damage; leaves are large, green, with prominent curl or twist along their length; attractive fruits with pleasant taste and large seed; fruits borne in cluster of 50 or more; outer skin is thick, rough and attractive red in colour; fruit shape is lopsided heart shaped to egg shaped; pulp is sweet, soft and juicy; usually aril is undeveloped and does not cover the seed at pointed end; flesh recovery is lower than 50%; not a good marketing type variety	Morton (1987)
Bombai	India	Important cultivar from West Bengal; vigorous trees yield 80–90 kg fruits; fruits are obliquely heart shaped with brilliant deep red in colour with greyish-white, juicy, soft and sweet pulp; average fruit weight is 15–20 g	Anonymous (2001) and Bose (2001)
Brewster	Florida	Brewster (Chen family purple) variety obtained from Fujian; propagated in Florida; small and upright trees with strong and wide crotch angles; dense foliage; distinct corky outgrowths and lenticels are present on branches; leaves are green, large and pointed; heart-shaped fruits with a pink-red, thick and rough skin; fruits borne in small loose clusters; full-seeded fruit has rounded tip; chicken-tongue fruit has pointed tip; mature fruit pulp is juicy and sweet; flesh recovery is 65–70%	Anonymous (2001), Morton (1987), and Chauhan (2001)
Calcutta	India	Successful variety for hot and dry areas; less vigorous trees with height of 4 m; yields 80–100 kg fruits/tree; fruits are deep carmine to red in colour with lopsided to oblong in shape; average fruit weight is 22 g; flesh is creamish white, juicy, soft and sweet; bold seed; fruit is less prone to cracking and sunburn	Bose (2001)
Chacapat/ Chakrapad	Thailand	Grown in Thailand; imported to Australia; trees are erect, vigorous, dense foliage with long branches; leaves are green, small, narrow and pointed; from the midrib leaves curl upwards and downwards towards tip; fruits are slightly heart shaped to rounded; skin is deep red and soft with juicy pulp; average fruit weight is 28–32 g; large seeds; flesh recovery is 60–70%	Anonymous (2001)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
CHES-2	Ranchi, India	Late-maturing variety developed from Bombaia cultivar; canopy bearing habit helps in decreasing sunburn and fruit cracking; fruits are deep red and borne in clusters of 15–20; average fruit weight is 21.3 g	Rai et al. (2001) and Singh and Babita (2001)
China	India	Late-maturing variety; tolerant to hot winds, soil moisture fluctuations and fruit cracking; trees are high yielding but short; oblong to conical-shaped fruit with dark-pink colour skin, fruit weighing about 20–25 g, aril sweet juicy aroma with excellent quality, heavy yielder, resistant to fruit cracking and sunburning; fruits are large sized of weight 22.0 g; pulp is soft, juicy and sweet; seeds are dark chocolate, glaucous and oblong to concave in shape	Singh and Babita (2001) and Rai et al. (2001)
China-3	India	Best variety in Bangladesh; late maturing; average height of tree is 5–6 m; leaves are small; globose fruits with a mixture of red-orange and green patches; average fruit weight is 25 g; flesh is soft, juicy and creamy white in colour; small seed; pulp/seed ratio is 15:1	Siddiqui (2002)
Chu Ma lsu or Chu Ma Isz (China grass fibre)	–	Trees with lush green foliage; leaves are overlapping, large with long petioles; fruits with rough skin; pulp is fragrant but of inferior flavour	Morton (1987)
Dahong Nuomizi	Guangdong, China	Matures in late June to early July; average fruit weight is 20–15 g; bright red fruit with small seed; sweet and juicy pulp; poor transportability and unstable yield	Li (1996)
Dahongpao	Eastern Sichuan province, China	Matures in mid-late July; large fruit clusters of weight 500–1000 g; good eating quality	Wong (1999)
Dazao (Tai So, Hong Huai, Mauritius) 'Tai So'	China, Thailand, South Africa, Florida, Israel, Australia	Trees have insufficient number of female flowers; vigorous trees with open crown; leaves are glossy, large and dark green; leaves have an upward curl from midrib; fruits are large and egg shaped with rounded tip; fruit skin is dull red at maturity; pulp is sweet when fully ripe but bland when overripe flesh recovery is 60–70%	Degani et al. (2003) and Singh et al. (2012)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Dehra Dun (Dehra Rose, Dehra Dhun)	India, Pakistan	Important cultivar in India and Pakistan; late-maturing cultivar; attractive bright rose-colour fruits are medium to large in size and small seeded; greyish-white, soft and juicy pulp; total sugar content is 10.4%; acidity is 0.72%; seeds are small (2.4 cm long, 1.4 cm diameter) shrunken, oblong, dark chocolate in colour and shrunken; fruits are most prone to sunburn and cracking	Anonymous (2001), Morton (1987), Rai et al. (2001), Chauhan (2001), Singh and Babita (2001), and Bose (2001)
Deshi	India	Early-maturing cultivar, medium-size trees of height 5.5 m and spread of 6.5 m; high fruit yield; oblong to conical in shape bright rose-pink-colour fruits; fruit pulp is greyish white, juicy and soft; oblong in shape, dark chocolate smooth seeds; fruits are less susceptible to sunburn and fruit cracking	Chauhan (2001)
Dong Si Ji Li	China	Rare lychee variety which is used in hybridization programmes; uneven, elongated and oval-shaped fruits with soft aril; flowering for the whole year; this cultivar is used as a parent in breeding programmes because of high TSS and vitamin C (53.7 mg/100 g)	Rai et al. (2001)
E Dan Li	China	This cultivar ripens during late June; sparkling and spotless aril makes it more suitable for canning; fruits are reddish yellow in colour, oval or cordate in shape; 15.3–18.00 brix; 22.1–27.6 mg/100 g of vitamin C	Rai et al. (2001)
Early Bedana	India (Uttar Pradesh, Uttrakhand, Punjab, Bangladesh)	Early-maturing cultivar with average tree height is 5.0 m and spread of 6.2 m; yields about 50–60 kg/tree; medium-sized heart-shaped or oval fruits with deep red and rough skin; good fruit quality; pulp is soft, juicy, creamy white and sweet with 19.50 brix TSS; sugar content is 13.91; seeds are small, shrunken, dirty chocolate and glabrous; average seed weight is 1.47 g	Singh and Babita (2001) and Rai et al. (2001)
Early Large Red	–	Early-maturing cultivar; obliquely heart-shaped, red-colour fruits with firm, rough and leathery skin; pulp is greyish white and sweet	Morton (1987)
Edanli	Hainan Province of China	Local cultivar of China; large-sized and good-quality fruits; average fruit weight is 52 g; colour of fruit is greenish red	Li et al. (2003)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Elachi (Elaichi, Ellaichi)	India (West Bengal)	Important cultivar for commercialization; vigorous tree with height of 5–6 m and spread 6–7 m; fruit yield is 50–60 kg/tree; cone-shaped fruits with average size 12–15 g; pulp is creamy white, soft and juicy; 18.00 brix TSS; sugar content is 1.5%; acidity is 0,45%; seeds are small and shining with average weight of 1.5–2.0 g; fruits are less prone to cracking and sunburn	Bose (2001), Rai et al. (2001), and Singh and Babita (2001)
Emperor	Florida and California	Tree is slow grower; not a commercial variety; variety of largest fruits of size about golf ball; fruit is hard with acid flavour; fruit skin with distinct bumps	Degani et al. (2003) and Singh et al. (2012)
Extra Early Green	–	Fruit is heart shaped, rarely rounded and 3.2 cm long; skin is rough, yellowish red and leathery; pulp is firm, creamy white, slightly acidic and of good quality; seeds are flat, oblong or cylindrical	Morton (1987)
Feizixiao (Fay Zee Siu) 'Fay Zee Siu'	South Africa, China	Vigorous tree; one of the best export cultivar of China; average fruit weight is 24–32 g; amber-colour fruit with size of about goose egg; pulp is fragrant, sweet, delicious and very fragrant; early-maturing variety with good storage quality	Froneman (1999) and Anonymous (2001)
Fei Tsu Hsiao or Fi Tsz Siu (imperial concubine's laugh or smile)	–	This cultivar has large, amber-coloured, thin-skinned fruits, with very sweet and fragrant flesh. The seeds vary from large to very small. It ripens early in the season	Morton (1987)
Feizixiao	–	Early-maturing cultivar; stable and high yield; vigorously grown trees; large fruits of average weight 60 g; pulp is juicy, sweet and of good quality; best grows at altitude between 600m and 1300 m	Wu and Zhang (1997) and Zhuang (1999)
Fengli	Hainan Province of China	Selected from local *Litchi chinensis* seedlings; average yield is 11.6 kg/tree	Miao et al. (1998)
Groff	–	Selected from seedling of Haak Yip cultivar; late-maturing cultivar; upright tree of medium vigour; medium-sized rose-red-colour fruit with yellowish-green tinges at the apex of tubercle; firm and white pulp with sweet and subacid flavour; seeds are abortive and chicken tongue	Morton (1987)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Guiwei	Eastern Sichuan province of China	Late-maturing cultivar; trees are productive and precocious; large fruits of average weight 24 g; fruit skin is dark red; pulp is juicy and pure white; TSS is 18.20 brix; vitamin C content is 58.96 mg/100 ml	Wong (1999), Zhu and Yuan (1999), and Yuan and Zhu (2001)
Gulabi	North India	Late-maturing cultivar; profusely branched and medium vigour (7 m spread and 6 m height) tree; yield about 90–100 kg/tree; medium-sized fruits of average weight 20.0 g; variable fruit shape; fruit colour is pinkish to red colour; pulp is greyish white, firm and sweet; TSS is 18.20 brix; seeds are heavy, big, chocolate-coloured and shiny	Rai et al. (2001), Morton (1987), Chauhan (2001), and Singh and Babita (2001)
Haak Yip (Haak Yip, Hei Yeh, Black Leaf)	China, Taiwan, Thailand	Medium vigour trees, dense foliage, long and thin branches; leaves are dark green, long, glossy and narrowly pointed; medium-sized fruits borne in compact clusters; skin of fruit is thin, soft and more prone to insect attack; pulp is sweet, crisp and of excellent quality; good marketing type cultivar	Anonymous (2001), Chauhan (2001), and Morton (1987)
Hongxin	China	Promising cultivar selected from Dahongpao cultivar; fruits are large sized of average weight 24.2 g; TSS is 17.4–18.10 brix	Li et al. (1999)
Hsi Chio Tsu or Sai Kok Tsz (rhinoceros horn)	–	Early-maturing cultivar; fruit is rough, large, narrow at apex and broad from base; fruit skin is tough and fibrous; pulp is fragrant and sweet	Morton (1987)
Hsiang Li or Heung Lai (fragrant lychee)	–	Erect trees, leaves pointing upwards; fruit is rough, small and prickly; seed are small; pulp is of superior taste and high-quality aroma	Morton (1987)
Huai Chih or Wai Chi (the Wai River lychee)	–	Late-maturing cultivar; blunt leaves of medium size; round fruits with smooth skin	Morton (1987)
Huazhi (Wai Chee)	China, Thailand, Australia	Dome-shaped trees with thick branches, compact foliage with many growing points; trees are more prone to wind damage; oval-shaped leaves curved upwards from the midrib; fruits borne in small clusters; average fruit size is 16–18 g; fruit skin is of medium texture; pulp is juicy, soft and sweet; fully developed seeds; large seeds decrease the eating quality	Degani et al. (2003) and Singh et al. (2012)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Jiangmiaolan	Eastern Sichuan province of China	Dark red-coloured fruits which mature in late July	Wong (1999)
Kaimana or Poamoto	Hawaii, Australia	Selected from Haak Ip cultivar; medium-sized trees with long and strong branches; leaves are long, elongated and mid-green in colour; fruits are heart shaped, large and purple red in colour; pulp is sweet, crispy and of excellent quality; medium-sized seeds	Anonymous (2001)
Kasba	India (Bihar)	Selected from Kasba village for its large-sized fruit; mid-late-maturing cultivar; large compact tree with long and broad leaves; yield is 85–100 kg/tree; average fruit weight is 23–27 g; pulp is juicy soft and greyish white in colour; TSS 16.80 brix; acidity is 1.14%; fruits are less prone to sunburn and cracking; seed is shiny, smooth and dark in colour	Singh and Babita (2001) and Chauhan (2001)
Kaselia (Khatti, Pickling)	–	Late-maturing cultivar; medium-sized tree; pink-coloured fruits; large seeds hence low pulp content; no commercial value	Singh and Babita (2001)
Khom	China, Thailand, Australia	Popular tropical cultivar; high yielding; not a good marketing type because of small size and poor flavour fruits; trees are erect, vigorous, compact foliage with long and strong branches; leaves are pointed, narrow, dark green in colour and of medium size; fruits borne in small loose clusters; fibrous pulp; small-sized fruits with chicken-tongue seeds; flesh recovery is 60–80%	Anonymous (2001)
Kwa Luk or Kua Lu (Hanging green)	–	Famous lychee cultivar; large red fruits with green tip having superior fragrance and flavour	Morton (1987)
Kwai May Pink	China, Australia	Large, erect trees having thin and long branches; leaves are long, narrow, shiny and oval shaped; round-shaped, rough skin medium-sized fruits; pulp is sweet, juicy, crispy and aromatic; seeds are of variable size; average fruit weight is 18–22 g	Anonymous (2001)
Kwai May Red	China	Trees have long and thin branches; leaves are oval shaped, small and shiny green in colour; good-quality fruits; pulp recovery is 70–80%; fruits have good aroma	Anonymous (2001)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Kwai Mi or Kue Wei (Cinnamon flavour)	Hawaii	Trees with upwardly curved branches; leaves curl inwards from midrib; heart-shaped small fruits with red skin; pulp is very fragrant and sweet; seeds are small in size; only 10% of the fruits have chicken-tongue seeds	Degani et al. (2003) and Singh et al. (2012)
Late Bedana (Late Seedless)	North India	Late-maturing cultivar; vigorously grown trees with an average height of 5.5 m and spread of 7.0 m; annual yield is 80–100 kg/tree; compact panicle; fruits are carmine in colour and conical in shape; fruit skin is rough and firm; flesh is creamy white; juicy and very soft; TSS is 19.50 brix; total sugar is 13.00%; seeds are glabrous, small, shrunken, chocolate colour and of fusiform shape; good-quality fruit	Rai et al. (2001), Morton (1987), Chauhan (2001) and Singh and Babita (2001)
Late Long Red or Muzaffarpur	India (Bihar, Punjab, Uttarakhand)	Heavy bearer and late-maturing cultivar; good-quality fruits; fruits are less than 4 cm in length; fruit shape is conical to oblong; fruit colour is dark red with greenish lines; fruit skin is firm, leathery and rough; pulp is greyish white, sweet and soft; fully developed cylindrical seeds	Morton (1987)
Liquili	Guangxi Province of China	Late-maturing cultivar; trees yield fruit after 3 years of planting; average fruit weight is 15.68–21.3 g; fruit contains 15.02–18.45% soluble solids, 37–38 mg ascorbic acid/100 g of fruit pulp and 13.5–14.9% sugar; yield is more and stable; resistant to various adverse environmental conditions	Xie (1995)
Longia	North Bihar (India)	Late-maturing cultivar; small-sized tree; small leaves of light colour; compact panicles; medium-sized fruit with excellent fragrance	Singh and Babita (2001)
Madras	South Africa (Nelspruit)	Heavy bearer cultivar; bright red-colour fruits with rough skin; pulp is sweet and juicy	Morton (1987)
Maguili	–	Late-maturing variety; trees are precocious; large-sized fruit with average weight of 39.6 gm; pulp is pure white in colour; TSS is 17–21 brix; ascorbic acid is 50.2 mg/l; good-quality fruit	Ooyang et al. (2002)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Mandraji	–	Vigorously grown trees with an average height of 6.0 m; fruits borne in clusters; average weight of fruit is 22–26 g; fruit skin is bright red in colour and rough; TSS is 19.50 brix; seeds are smooth, shiny and light chocolate in colour	Chauhan (2001)
Mianbaoli	Hainan Province of China	Selected from local lychee seedling; fruits having soluble solid content of 17.5%	Miao et al. (1998)
Mombaia (Mumbai)	India (West Bengal)	Early-maturing variety; vigorously grown trees with an average height of 6–7 m; large-sized heart-shaped fruits with an average weight of 15–20 g; fruit colour is attractive carmine; each fully developed fruit has a small underdeveloped fruit attached to stalk; pulp is greyish white, soft and juicy; TSS is 20.50 brix; total sugar is 11.68%; seeds are elongated, large, smooth, shiny and chocolate colour; seed having 2.3 cm length, 1.6 cm diameter and weight 3.83 g	Rai et al. (2001) and Chauhan (2001)
Muzaffarpur	India (Bihar)	One of the best litchi cultivar; trees are of medium vigour with an average height of 5.5 m and spread of 6.0 m; average yield is 80–100 kg/tree; fruits are less susceptible to cracking; large fruit with an average weight of 18.2 g; fruit shape is oblong conical to oval; pulp is juicy, white and soft; TSS of pulp is 17.70 brix; acidity is 0.48%; seeds are large with an average weight of 4.5 g and dark chocolate in colour	Chauhan (2001) and Bose (2001)
Muzaffarpuri	India, Bangladesh	Medium vigour tree with an average height of 5 m; pink-coloured oval-shaped fruits; average fruit weight is 20 g; pulp is soft and sweet; TSS of pulp is 17–18 brix; big seeds; seed/pulp ratio is 4.75:1	Degani et al. (2003) and Singh et al. (2012)
Nafarpal	India (West Bengal)	Important cultivar of West Bengal; not a commercial cultivar; fruits resemble with Chinese cultivar	Pereira et al. (2005)
Nanmuye	Sichuan province of China	Highly productive trees; yellowish-red fruits matures in mid-August; fruits borne in large clusters of an average weight 400–1 1100 g; soluble solid content is 15.4 brix	Wong (1999)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
No Mai Chee	China (Taiwan)	Highly prized and late cultivar; large trees with dense canopy; leaves are small soft with thin edges; large fruits with chicken tongues; pulp recovery is 75–85%; pulp is smooth, clean and firm with excellent aroma	Anonymous (2001)
No Mai Tsze or No Mic Tsz (glutinous rice)	China	Leading variety of China; late-maturing cultivar; fruits are red, dry, large and clean; best cultivar for drying; seeds are shrivelled and small	Morton (1987)
Nuomizi		Late-maturing cultivar; grows best at an altitude between 800–1400 m	Zhuang (1999)
O-Hia ('Baidum')	China	Third most cultivar after Tai So and Wai Chee; trees with dense foliage and long and thin branches; leaves are large, dark green and narrow; fruits mature in mid-season; medium-sized heart-shaped fruits; pulp is sweet and juicy; flesh recovery is 65–75%	Anonymous (2001)
Olan	Philippines	Selected from seeds brought from Thailand; fruit is oval shaped and of average weight 26 g; TSS is 17.5 brix	Sotto (2001)
Pai La Li Chih or Pak Lap Lai Chi (White wax lychee)	–	Late-maturing variety; large fruits with pink, rough outer skin; pulp is not sweet	Morton (1987)
Panjore Common	India (Punjab)	Trees bear heavily with longest fruit season; fruit is heart shaped, large and pink coloured; fruit skin is thin and rough	Morton (1987)
Pat Po Heung (eight precious fragrances)	Hawaii	Slow-growing tree of spreading habit; not a commonly planted cultivar; skin of fruit is thin and purple red in colour; pulp is soft, sweet and juicy; juice leaks from the broken fruit skin	Morton (1987)
Peerless	Florida	Selected from seedling of Brewster; good productivity of average productivity 174 kg/tree; abortive seed ranged from 62 to 80%	Morton (1987)
Purbi	Australia, India	Vigorously grown tree with an average height of 6.5 m and spread of 7.5 m; also called Bengal in West Bengal; large fruit of an average weight 23–27 g; fruit borne in clusters of 50 or more fruits; yield is 90–100 kg/tree; heart-shaped fruits with uneven shoulders; pulp is juicy, soft with 19.00 brix TSS; seeds are shiny, smooth and dark chocolate in colour; fruits are less prone to cracking	Chauhan (2001)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Pyazi	–	Early-maturing variety; fruits are 3.4 cm long, heart shaped to oblong conical; fruit skin is leathery and adhering; greyish-white pulp, sweet and with flavour of boiled onion; fully developed and cylindrical seeds; poor-quality fruits	Morton (1987)
Qinzhou red	–	Derived from spontaneous mutation of Black Leaf cultivar; early maturing and high yielding; big fruits of excellent quality	Peng et al. (2001)
Qinzhouhongli	–	Promising cultivar; large bright red fruits with an average fruit weight of 44.7 g; pulp is white, sweet and crispy; good eating quality	
Rose Scented	India (North Bihar, Jharkhand, Uttarakhand and Uttar Pradesh)	Fruits with distinct aroma hence called Rose Scented; vigorously grown trees with an average yield of 80–90 kg/tree; fruits are medium to large in size; mature fruits are susceptible to cracking; pulp is soft, juicy and sweet; seeds are small, shining, smooth, round and dark chocolate in colour; fruits are moderately susceptible to cracking	Rai et al. (2001) and Chauhan (2001)
Sah Keng	Taiwan, Australia	Medium-sized dome-shaped trees with fragile branches; heavy yielding cultivar; leaves are mid-green and 6–8 cm long; large, purple-red heart-shaped fruits; pulp is sweet and soft; seeds are small; flesh recovery is 75%	Degani et al. (2003) and Singh et al. (2012)
Saharanpur	India	Heavy bearing and early-maturing cultivar; matures in the first week of June; large, pink-coloured heart-shaped fruits; fruit and plant characters show similarity with Panjore and Large Red cultivar; TSS content is 19.80 brix; average fruit weight is 17.6 g	Bose (2001) and Lal and Nirwan (1980)
Salathiel	Australia	Small compact trees with undeveloped leaves and long branches; leaves are broad, small and curved inwards from the tip; tip of the leaf is round; egg-shaped small fruits which borne in clusters; tip of the fruit is obtuse; pulp is juicy, thick and sweet; sometimes, fruit is completely seedless; important variety in domestic markets; also exported to Asia	Degani et al. (2003) and Singh et al. (2012)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
San Yueh Hung or Sam Ut Hung (third month red)	–	Popular early-maturing cultivar; grown along Dykeys; brittle branches of trees; large-sized fruits with rough, thick and tough skin; pulp is sweet and juicy; seeds are long but aborted	Morton (1987)
Seedless Late	–	Vigorous trees with an average height of 7.5 m; average yield is 80–100 kg/tree; fruit attains maturity at the third week of June; cone- to oval-shaped fruits; average fruit weight is 29.0 g; pulp is creamy white, juicy and soft; TSS is 18.00 brix; sugar content is 13.8%; acidity is 0.44%; seeds are shrivelled, chocolate coloured, small and glabrous; average seed weight is 0.85 g	Bose (2001)
Shan Chi or Shan Chih (mountain lychee)	–	Grows wild in the hills; trees with pointed, short-petioled leaves and erect branches; fruits are bright red, rough skin, elongated in shape and acidic in flavour	Morton (1987)
Shatouli	–	Late-maturing cultivar; fruit attains maturity in early August; small fruits with an average weight of 21.6 g; fruit skin is crispy and red; pulp is sweet and white in colour; good eating quality; soluble solid content is 18.5%	
Sheung shu wai or Shang hou huai (President of a Board's embrace)	–	Late-maturing cultivar with small leaves tree; large-rounded fruit with many dark spots; flesh is scented and sweet; size of seed is variable	Morton (1987)
Shuidong	–	Early-maturing cultivar; grows best at an altitude of 1000 m	Zhuang (1999)
Shuyou	China	Promising cultivar selected from cultivar Dahongpao; productive and produce large fruits of average size 24.2 g; TSS is 17.4–18.10 brix	Li et al. (1999)
Sinco	Philippines	Important cultivar; selected from local seedling of China; fruits are dull red and ovate to round in shape	Sotto (2001)
Songmei 9	Hainan Province of China	High yielding and stable production cultivar	Miao et al. (1997)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Souey Tung	China, Australia, Fujian	Tree is short with long, thin, open and spreading branches; leaves are flat, large, glossy green and pointed; medium-sized heart-shaped fruits of an average weight 20–22 g; fruit skin is dull, thin dark red to purple; tip of the fruit is obtuse; pulp is juicy, soft and of high quality; seeds vary in size; flesh recovery is 65–75%; 5–10% seeds are abortive	Anonymous (2001)
Sum Yee Hong	Guangdong province of China	Early-maturing cultivar; medium-sized tree with spreading habit and long, fragile, thin branches; leaves are narrow, long, dark green and thick; large fruits with an average weight of 26–42 g; skin of the fruit peels off easily; pulp is juicy, soft and sweet acid; seeds are large	Anonymous (2001)
Swarna Roopa	India	Selected by clonal selection of seedless cultivar; medium-tall tree with dense foliage; panicle is compact; leaves are similar to Bedana cultivar; mid-season-maturing cultivar; medium-sized attractive red-coloured fruits with an average fruit weight is 8–20 g; seeds are small; TSS of pulp is 19.00 brix; total sugar is 12.5%; skin/pulp/seed ratio is 8.7:76.62:16.36; fruit is resistant to cracking; commercial cultivar	Rai et al. (2001) and Singh and Babita (2001)
Sweet Cliff	–	Small pink fruit; good eating quality; not planted anymore because of many other superior varieties	Degani et al. (2003) and Singh et al. (2012)
Sweetheart	–	Finest lychee; consistent bearer cultivar; large heart-shaped fruits with chicken-tongue seeds; variety of choice because of its superior quality	Singh et al. (2012)
T and Po or T Ong Pok (pond embankment)	–	Tree with small leaves; small fruit with red and rough skin; pulp is juicy and acidic; early-maturing cultivar	Morton (1987)
T Im Ngan or T Ien Yeh (Sweet cliff)	Kwangtung (China)	Common lychee variety; not a commercial cultivar	Morton (1987)
Ta Tsao or Tai Tso (large crop)	Canton	Widely grown cultivar; egg-shaped fruits; fruit skin is bright red, rough with dense dots; pulp is crispy, sweet, firm and yellow near the seed; juice leaked out from the broken skin	Morton (1987)

(continued)

Table 5.2 (continued)

Variety	Origin/cultivation	Characteristics	Reference(s)
Tatuo	–	Large fruits of average size 25.6 g with pink red and TSS is 19.30 brix	Yuan and Zhu (2001)
Trikolia	East Champaran	Similar to Shahi cultivar; good fruit retention capacity; average fruit weight is 18–20 g	
UPLB Red	Philippines, Thailand	Trees bear fruit after 3–4 years of planting; fruits harvested from April to May; red-coloured fruits which are dark red when fully matured; average fruit weight is 14 g	Sotto (2001)
Yuan Gyang Hong (Yuan Yang Mi)	Dahongpao (China)	Promising lychee cultivar with high soluble solid content (19.50 brix); small fruits of an average weight of 23.4 g	Li et al. (1999)
Zeng Cheng Gua Li	China	Excellent cultivar of China; fruits are oval to rounded with an average weight of 14.4–29.5 g; pulp is sweet, crispy and fragrant; TSS is 17–21.50 brix; vitamin C content is 13.4–31.2 mg/100 g; fruits attain maturity during the last week of June to the first week of July	Rai et al. (2001)
Ziniangxi	Hainan Province of China	Selected from local lychee seedlings; large fruits of high quality; average fruit weight is 52 g; fruit colour is purple red	Li et al. (2003) and Miao et al. (1998)

5.5 Nutritional Composition

Lychee fruit is classified as drupe and has a large seed, white translucent edible aril (flesh) and thin, tough, corky pericarp (skin). The pericarp of the mature fruit varies from pink red to plum, depending on the cultivar, while the aril is succulent, translucent cream or white and with sweet citrus flavour. Lychee is mainly consumed fresh, but different products like canned lychee, squash, cordial, syrup, jam, jelly and juice are also manufactured and marketed. It can be dried or dehydrated (lychee nuts) or used in ice creams and sorbets (Hui, 2008; Salunke and Desai 1984). Dried lychee is very popular among the Chinese. In China, various other products such as pickles and wine are also made from lychee. The food value of lychee mainly lies in its sugar content which varies from variety to variety. Depending upon the variety and climate, the fruit contains 60% juice, 8% rag, 19% seed and 13% skin. Apart from proteins, fats, carbohydrates, minerals, fibrous matter, calcium, phosphorus, iron and carotene, the fruit is also rich in vitamin B1, riboflavin and vitamin C (Table 5.3). Lychee is also an excellent source of anti-oxidants which protects the body from harmful free radicals. Lychee flesh is loaded with nutritional and functional compounds. According to the data released by USDA, 100 g of lychee flesh contains 16.5 g sugars, 276 kilojoule energy, 0.83 g protein, 0.44 g fat, 0.44 g ash, 1.3 g edible

Table 5.3 Nutritive value per 100 g of lychee fruit (*Litchi chinensis*)

Principle	Nutrient value	Percentage of RDA*
Energy	66 kcal	3.3%
Carbohydrates	16.53 g	12.7%
Protein	0.83 g	1.5%
Total fat	0.44 g	2%
Dietary fibre	1.3 g	3.5%
Vitamins		
Folates	14 µg	3.5%
Niacin	0.603 mg	3.5%
Choline	7.1 mg	1%
Pyridoxine	0.100 mg	9%
Riboflavin	0.065 mg	5%
Thiamin	0.011 mg	1%
Vitamin C	71.5 mg	119%
Vitamin E	0.07 mg	0.5%
Vitamin K	0.4 µg	0.3%
Electrolytes		
Sodium	1 mg	0%
Potassium	171 mg	3.5%
Minerals		
Calcium	5 mg	0.5%
Copper	0.148 mg	16%
Iron	0.31 mg	4%
Magnesium	10 mg	2.5%
Manganese	0.055 mg	2.5%
Phosphorus	31 mg	4.5%
Selenium	0.6 µg	1%
Zinc	0.07 mg	0.5%

Source: USDA National Nutrient Database
RDA * Recommended dietary allowance

fibre, 5 mg Ca, 0.31 mg Fe, 10 mg Mg, 31 mg P, 171 mg K, 1 mg Na, 0.07 mg Zn, 71.5 mg vitamin C, 0.011 mg thiamin, 0.065 mg riboflavin, 0.603 mg niacin, 0.1 mg vitamin B6, 14 mg folate, 0.07 mg vitamin E, 0.007 mg tryptophan, 0.041 mg lysine and 0.009 mg methionine. Taking 100 g lychee flesh satisfies 2–4% of the daily requirement for P, K, Mg, Fe, Zn and Mn and 22% for Cu (Wall 2006). Apart from nutritional value, lychee flesh improves digestion and blood circulation, moistens skin and alleviates symptom of anaemia (Chi et al. 2005). However, active compounds and mechanisms of these functions are unknown. Anti-oxidant activity of lychee flesh is well documented. In addition to vitamin C and E, lychee flesh contains anti-oxidant polysaccharides (Wu et al. 2004) and flavonoids including procyanidin A2 and leucocyanidin (Rooyen and Redelinghuys 1983). Polysaccharides in lychee flesh are effective to eradicate O_2 and reported to significantly suppress lipid peroxidation in rat liver (Wu et al. 2004). However, based on Chinese traditional

medicine, lychee flesh is a typical 'heating' food. Excessive taking causes 'heating symptoms' including sore and swell in the throat, boils in the mouth, tongue and face and tonsillitis. 'Heating effect' of lychee, which is only shown in some individuals, is not understood in terms of mechanism and effective compounds.

5.6 Phytochemistry and Functional Activities

Nowadays, HPLC (high-performance liquid chromatography) and HPTLC (high-performance thin-layer chromatography) have become regular analytical techniques due to their efficiency in quantitation of analytes at micro- or even nanogram levels and cost-effectiveness. Leaf, root, seed, fruit and pericarp extracts of various lychee varieties have been subjected to HPLC and HPTLC followed by pharmacological analyses. The recent reports reveal a total of 50 bioactive compounds from different parts of the lychee plants (Table 5.4). These compounds have been categorized under flavonoids, glycosides, phenolic aldehyde, monoterpenes, anthocyanins amino acid, phenolic compounds and fatty acids (Fig. 5.6).

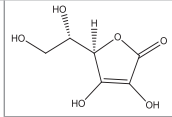
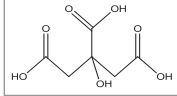
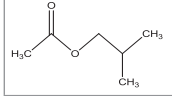
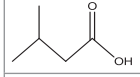
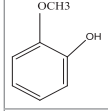
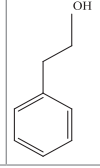
5.6.1 Functional Activities and Compounds in Lychee Skin

Anti-oxidant

Traditionally, lychee skin is useful to prevent the 'heating effect' from taking litchi flesh, but the mechanism is still unknown. However, the anti-oxidant activity of litchi skin is well defined (Guo et al. 2003a, b; Surinut et al. 2005). Lychee skin contains free-radical scavenging compounds like ascorbic acid, glutathione, carotenoids, polysaccharides (Huang and Wu 2006; Yang et al. 2006) as well as rich phenolic substances including flavonoids (flavonols and anthocyanins) and phenolic acids (Li and Jiang 2007). Zhang et al. (2000) found epicatechin, procyanidin B2, epigallocatechin and procyanidin B4 are among the major flavonoids in fruit skin of 'Huaizhi'. Analysis conducted by Sarini-Manchado et al. (2000) showed that polymerized tannins (procyanidins) were the most abundant (0.4% fresh weight) in 'Guiwei' skin, followed by epicatechin (0.17%), procyanidin A2 (0.07%), anthocyanins (0.04%) and flavanols (0.04%). Two flavonoids in lychee skin especially anthocyanins and procyanidins contribute to the major part of its anti-oxidant activity (Luximon-Ramma et al. 2003). Zhao et al. (2006) found procyanidin B2 was stronger in scavenging hydroxyl free radical and superoxgen anion than procyanidin B4 and epicatechin, while epicatechin is more active in eradicating DPPH than the other two flavonoids.

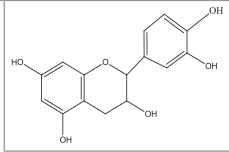
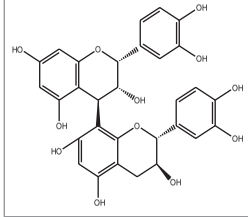
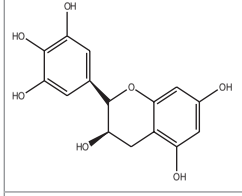
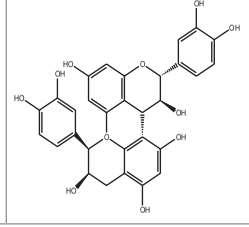
Lychee fruit development is accompanied by changes in chemical compositions including phenolic substances (Huang and Wu 2006). Hence, anti-oxidant activity in the skin at different maturity differs. Zheng et al. (2003) found skin of immature fruit had a much stronger anti-oxidant activity than that of mature fruit. Cultivars also differ in quantity and quality of phenolics including flavonoids, so do their anti-oxidant activities.

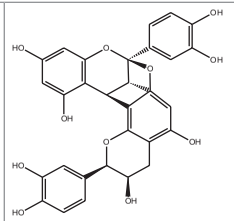
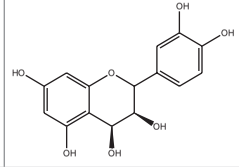
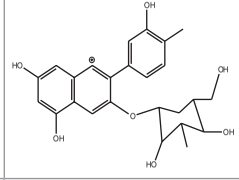
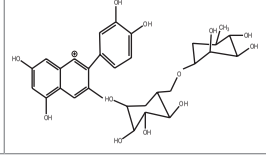
Table 5.4 List of bioactive compounds reported in *Litchi chinensis*

S.no.	Name of compound	Class	Structure	Property	Reference(s)
1	Ascorbic acid	Organic compound		Growth and repair of tissues in all parts of the body	Ong and Acree (1999), Huang and Wu (2006), Yang et al. (2006) and Wu et al. (2009)
2	Citric acid			Anti-bacterial; anti-fungal; anti-oxidant	
3	Isobutyl acetate			Antibacterial	
4	Isovaleric acid			Antibacterial	
5	Guaiacol			Antimicrobial activity	
6	2-phenyl ethanol			Anti-tyrosinase; antimicrobial	

(continued)

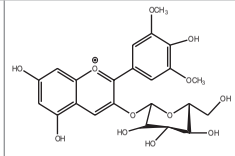
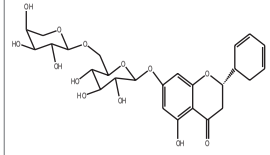
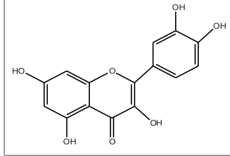
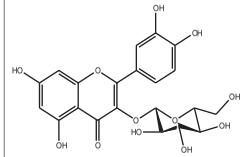
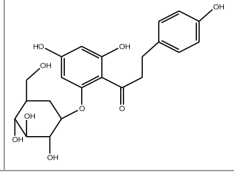
Table 5.4 (continued)

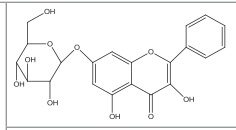
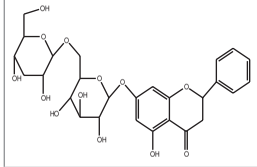
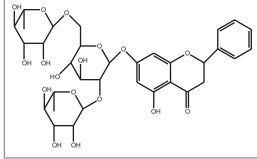
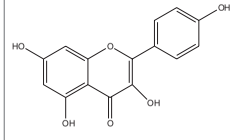
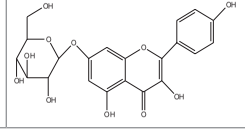
S.no.	Name of compound	Class	Structure	Property	Reference(s)
7	Epicatechin	Flavonoids		Anti-oxidant; free-radical scavenging activity; reduce blood sugar level; anti-diabetic; anti-cancer	Van Rooyen and Redelinghuys (1983), Ding (1999), Ong and Acree (1999), Sarini-Manchado et al. (2000), Luximon-Ramma et al. (2003), Luo et al. (2006), Liang et al. (2006), Gong et al. (2008), Shen et al. (2013), Wu et al. (2009), Reichel et al. (2014), and Su et al. (2016)
8	Procyanidin B2			Anti-oxidant activity; prevents malignancies	
9	Epigallocatechin			Chemoprevention and anti-cancer activities	
10	Procyanidin B4			Possess anti-oxidant activity; inhibition of proliferation and induction of apoptosis in cancer cells through up- and downregulation of multiple genes	

11	Procyanidin A2			Prevents hyperglycemia and type 2 diabetes	
12	Leucocyanidin			Protects the stomach lining	
13	Cyanidin-3-O-glu			Free-radical scavenging activity	
14	Cyanidin-3-O-rut			Free-radical scavenging and anti-platelet aggregating activity	

(continued)

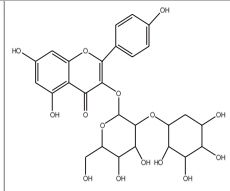
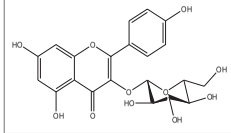
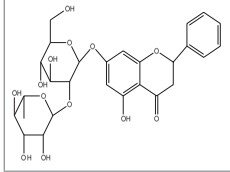
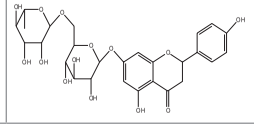
Table 5.4 (continued)

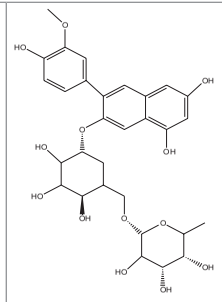
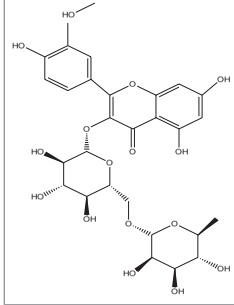
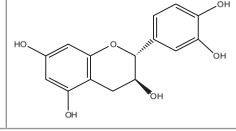
S.no.	Name of compound	Class	Structure	Property	Reference(s)
15	Malvidin-3-acetyl-O-gluoenin			Anti-oxidant properties	
16	(2S)-pinocembrin-7-O-(6′′-O-α-L-arabinosyl-β-D-glucopyranoside)			Anti-diabetic property	
17	Quercetin			Supports normal respiratory health; supports cardiovascular health; promotes balanced blood pressure; offers protection against stress; offers nutritional support for overall health	
18	Quercetin 3-O-glucoside				
19	Phlorizin			Anti-oxidant; anti-diabetic	

20	Pinocembrin-7-O-glucoside			Anti-oxidant activity; used to treat cerebral ischaemia, neurodegenerative diseases, cardiovascular diseases and atherosclerosis	
21	Pinocembrin-7-O-[(6′′-O-β-D-glucopyranoside)-β-D-glucopyranoside]				
22	Pinocembrin-7-O-[(2′′,6′′-di-O-α-L-rhamnopyranosyl)]-β-D-glucopyranoside]				
23	Kaempferol			Anti-oxidant; anti-cancer	
24	Kaempferol-7-O-β-D-glucopyranoside				

(continued)

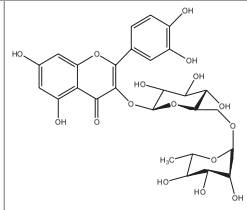
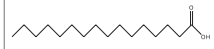
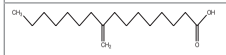
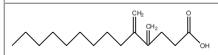
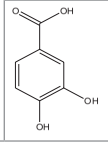
Table 5.4 (continued)

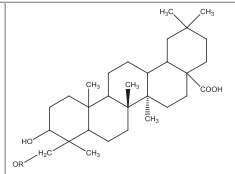
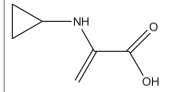
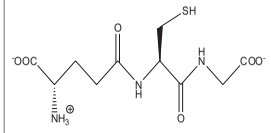
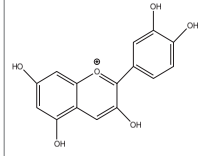
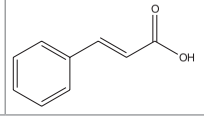
S.no.	Name of compound	Class	Structure	Property	Reference(s)
25	Kaempferol 3-O-rutinoside				
26	Kaempferol 3-O-glucoside				
27	Onychin			Anti-oxidant; anti-cancer	
28	Nairutin			Anti-oxidant	

29	Peonidin 3-O-rutinoside			Anti-oxidant	
30	Narcissin (Isorhamnetin-3-O-rutinoside)			Anti-oxidant	
31	Catechin			Anti-oxidant	

(continued)

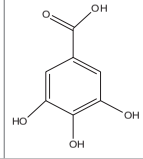
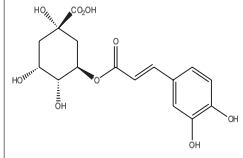
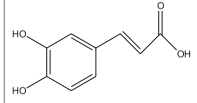
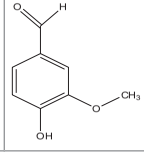
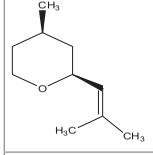
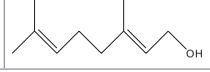
Table 5.4 (continued)

S.no.	Name of compound	Class	Structure	Property	Reference(s)
32	Rutin			Anti-oxidant; helps the body to utilize vitamin C and produce collagen; heals conditions such as haemorrhoids and high blood pressure and reduces cholesterol levels	
33	Palmitic acid	Fatty acids		Blood lipid-reducing activity	Ding (1999) and Ning et al. (1996)
34	Linoleic acid			Anti-oxidant; anticarcinogenic	
35	Dihydrosterculic acid			Anti-cancer; anti-tumour	
36	8-methylenehexadecanoic acid			Antibacterial	
37	Cis-5,6-methylenetetradecanoic acid			Antibacterial	
38	Cis-3,4-methylenedodecanoic acid			Antibacterial	
39	Protocatechuic acid			A major metabolite of anti-oxidant polyphenols; possess anti-cancer property	

40	Saponin	Glycoside		Cholesterol reduction; anti-oxidant; reduce cancer risk; immunity booster; reduce bone loss; anti-oxidant	Yang et al. (2004), Guo et al. (2003a, b), Yang and Liang (2004), and Jiang et al. (2008)
41	α-Methylenecyclopropylglycine	Amino acid		Possesses hypoglycaemic activity	Huang (1994)
42	Glutathione			It is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals	
43	Cyanidin glycoside	Anthocyanin		Anti-oxidant; anti-ageing	Sarni-Manchado et al. (2000)
44	Trans-cinnamic acid	Phenolic acid		Anti-oxidant; antimicrobial	

(continued)

Table 5.4 (continued)

S.no.	Name of compound	Class	Structure	Property	Reference(s)
45	Gallic acid	Phenolic acid		Anti-tumour; anti-oxidant; anti-inflammatory	
46	Chlorogenic acid	Phenolic compounds		Anti-oxidant; blood pressure-lowering effect; laxative effect	
47	Caffeic acid (3,4-dihydroxycinnamic acid)	Phenolic compounds		Anti-oxidant; antihypertension; antithrombosis; anti-fibrosis; anti-virus and anti-tumour	
48	Vanillin	Phenolic aldehyde		Anti-oxidant; antibacterial	
49	Cis-rose oxide	Monoterpene		Anti-inflammatory	
50	Geraniol			Anti-oxidant; anti-cancer properties	

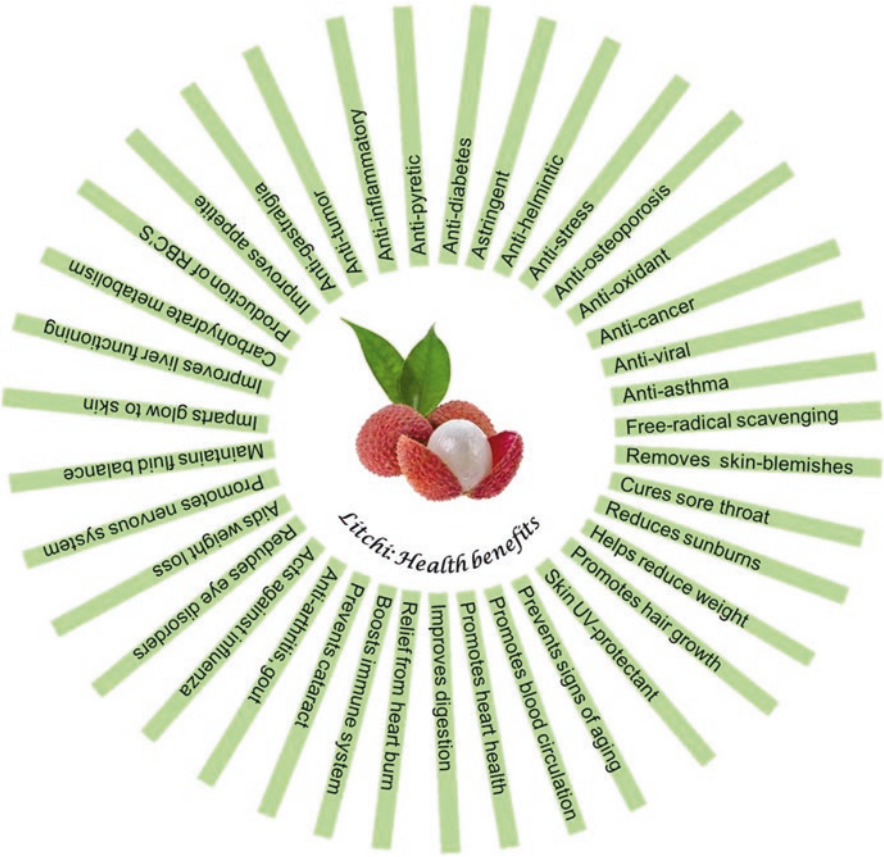


Fig. 5.6 Pharmacological activities of *lychee*

Anti-cancer Activity

Lychee skin is rich in insoluble fibre (40% dry weight), which prevents rectum cancer, diabetes and haemorrhoids (Li et al. 2006). Wang et al. (2006a) reported that water soluble alcohol extract from lychee skin significantly inhibited growth of human hepatoma cells in vitro and that feeding mice carrying liver cancer with lychee skin extract suppressed cancer development. In both cases, the anti-cancer effect was dosage and time dependent. Dosages at 0.14, 0.3 and 0.6 g/kg/day led to a cancer inhibiting rate of 17.3, 30.8 and 44.0%, respectively. Wang et al. (2006b) further found lychee skin extract also effective to suppress breast cancer. They found lychee skin extract caused changes in gene expression pattern, induced programmed cell death and suppressed multiplication in cancers cells (Wang et al. 2006a, b). However, active ingredients in lychee skin were not indicated in their studies. A study made by Zhao et al. (2007) indicated that flavonoids (epicatechin and procyanidin B2) were effective to suppress human breast cancer cells and human lung fibroblast (HLF), although their toxicity to cancer cells was lower than that of paclitaxel.

5.6.2 Functional Activities and Compounds in Lychee Seed

Chemical Composition of Lychee Seed

Lychee kernel contains starch (40.7%), crude fibre (24.5%), proteins (4.93%) and minerals including Mg (0.28%), Ca (0.21%) and P (0.11%). Fatty acids include 12% palmitic acid, 27% linoleic acid, 11% linoleic acid and 42% cyclopropanoic fatty acids (CPFA), among which dihydrosterculic acid accounts for 37%, cis-7,8-methylenehexadecanoic acid 4%, cis-5,6-methylenetetradecanoic acid 0.4% and cis-3,4-methylenedodecanoic acid 0.1% (Ding, 1999). Lychee seed contains also phenolic acids and flavonoids such as methyl 5-O-p-coumaroylquinate, protocatechuic acid, cyanidin-3-O-glu, cyanidin-3-O-rut and malvidin-3-acetyl-O-gluoenin (Ding 1999). Twenty-one amino acids were detected by Huang and Chen (2007), among which four were unknown. There were reports indicating lychee seed contains a special amino acid, α-methylenecyclopropylglycine (Ding 1999). Volatile compounds including ketones, aldehydes, esters, alcohols, enes and terpenoids with unknown functions were also detected in lychee seed (Ding 1999; Le and Fu 2001; Chen et al. 2005; Guo and Pan 2006). Tu et al. (2006) found sterol derivatives, while Yang et al. (2004) measured crude saponin in lychee seeds.

5.6.2.1 Functional Activities and Pharmacological Studies

Dried lychee seed is characterized by traditional Chinese medicine as slightly bitter, warming, qi flow promoting, cold driving, painkilling and liver and kidney tonifying (Tian 2005). There is abundant information on the health-promoting and medical functions of lychee seed, including anti-oxidant, anti-cancer, anti-virus, controlling diabetes and reducing blood lips.

Anti-oxidant Activity

As mentioned above, lychee seed contains flavonoids, which contribute to the anti-oxidant activity of the seed. Water and ethanol extracts from lychee seed were found to reduce the damage caused by free radicals and promoted SOD activity in alloxan monohydrate (ALX)-treated mice (Pan et al. 1999).

Anti-cancer Effect

Xiao et al. (2007) and Wang et al. (2007) reported in the same year that water extract of lychee seed or lychee seed pellets were effective to suppress tumour and hepatoma. Water extract of lychee seed at dosage of 62.5 kg/kg.d obtained 30% suppression on hepatoma tumour in mice (Wang et al. 2007). Xiao et al. (2007) found that extract from lychee seed inhibited the formation of telomere in hepatoma cells and thus their cell division.

Reducing Blood Sugars and Lipids

There have been reports about the effects of lychee seed in reducing blood sugars and lipids and in promoting the function of the liver (Wu et al. 1991; Zheng et al. 1998; Pan et al. 1999; Guo et al. 2003a, b). Results obtained by Wu et al. (1991), Zheng et al. (1998) and Pan et al. (1999) showed that the water extracts of lychee

seeds reduced blood sugar in rat suffering from diabetes induced by ALX and that the effect was similar to that of the anti-diabetic drug, biguanides. And it was found that lychee seed extract was safer and the effect lasted longer (over 1 week) than biguanides (Zheng et al. 1998). Pan et al. (1999) suggested that lychee seed extract reduced blood sugar because it inhibited glucose uptake by blood capillary but promoted glucose uptake in ambient tissues. Guo et al. (2003a, b) also found that lychee seed extract alleviated sugar metabolism disorder and improved sensitivity to insulin in rat suffering from insulin-resistant type 2 diabetes (T2DM) induced by streptomycin and therefore reduced blood sugar. There is little information about active antidiabetes and lipid-reducing substances in lychee seed. Ning et al. (1996) attributed the abundant unsaturated fatty acids to blood lipid-reducing effect of lychee seed. Some authors suggested that α-methylenecyclopropylglycine in lychee seed was effective to reduce the blood sugar and glycogen in liver in mice treated with ALX (Huang 1994), while others believed antidiabetes activity was related to saponins (Guo et al. 2003a, b; Yang and Liang 2004).

Anti-virus Effects

There have been not a few reports about the anti-virus effects of lychee seed extracts, which were effective to hepatitis B virus (Zheng and Zheng 1992; Li 1997; Pan et al. 2000; Xu et al. 2004; Xiao et al. 2005; Jiang et al. 2008), respiratory syncytial virus (RSV) (Liang et al. 2006), influenza virus (Luo et al. 2006) and SARS coronavirus (Gong et al. 2008). Zheng and Zheng (1992) found that lychee seed was the second most effective to control hepatitis B among 1000 tested herbal medicines. Pan et al. (2000) found direct inhibition on in vitro expression of HBsAg and HBV-DNA. Yang et al. (2001) showed that both water and alcohol (50%) extracts from lychee seeds were effective to inhibit HBsAg and HbeAg, but water extract was more effective on HbeAg, while alcohol extract was more effective on HBsAg. Xu et al. (2004) examined six extraction fractions of lychee seed, all showing strong effects in inhibiting the expression of HBsAg and HbeAg in Hep G 2.2.15 cell line, with an inhibiting rate reaching 90.9 and 84.3% on HBsAg and HbeAg, respectively. Most authors attribute anti-virus effect of lychee seed extracts to its flavonoids (Luo et al. 2006; Liang et al. 2006; Gong et al. 2008), while Jiang et al. (2008) suggested that saponins in lychee seed were the effective component.

Other Functions

Lychee seed is especially effective to cure haemorrhoids (Deng 2006).

5.7 Postharvest Strategies

The lychee fruit is highly prized, especially in Asia, and is a valuable international commodity. It is, however, also very perishable. This limits marketing in many countries without good storage facilities. The perishable nature of lychee (*Litchi chinensis* Sonn.) poses a serious problem in its transportation and marketing also. Lychee is delicate, so minimal handling is preferred. Ideally, fruit should be shipped

on the day of harvest. The fruit must also be marketed and consumed quickly. Research into the best handling practice for lychee is still in its infancy, and no accepted protocol exists. It is likely to begin with some form of antifungal treatment in the orchard prior to harvest. The harvested fruit would be initially placed in a coolroom to remove the field heat and then sorted on a roller conveyor in the packhouse. The optimum temperature for storage of lychee is approximately 5 °C (Huang and Wang 1990), although fruit stored at 10 °C can last almost as well (Olesen and Wiltshire 2000), with less risk of condensation in the pack. A modified atmosphere of 3–5% O_2 and 3–5% CO_2 was mentioned earlier, but other mixtures, and gases such as nitrous oxide (Qadir 2001), may also be used.

The shelf life of lychee at ambient temperature (26 ± 2 °C) is less than 72 h. Postharvest losses of lychee are estimated to be 20–30% of the harvested fruit and could reach as high as 50% (Jiang et al. 2001). As fruits start deteriorating quickly upon plucking, they are graded, packed in boxes with green leaves as cushioning materials and immediately routed to wholesale and retail markets (Shi et al. 2001). Besides postharvest decay, pericarp browning is another problem limiting market value of lychee. Much work has been done on the roles of pigments, plant growth regulators and other factors responsible for pericarp browning (Zhang and Quantick 1997). Optimizing suitable temperature and chemicals to inhibit or delay pericarp browning during postharvest is necessary (Paull and Chen 1987, Jiang and Fu 1999). It has been reported that pericarp browning of harvested lychee is due to a rapid degradation of anthocyanidin by polyphenol oxidase (PPO) and peroxidase (POD) (Akamine 1960; Chen and Wang 1989; Lee and Wicker 1991). Dehydration also contributes to pericarp browning (Scott et al. 1982; Underhill and Simons 1993) and leads to 40% decrease in water content after 48 h storage at 25 °C, 60% relative humidity (Underhill and Critchley 1994). Postharvest decay also occurs due to bacteria, yeast and fungi.

Various techniques to reduce browning, control postharvest decay and extend storage life of lychee fruit include sulphur fumigation, fungicide dips, application of plant growth substances, waxes and chitosan coating, use of microbial antagonists (e.g. *Bacillus subtilis*), irradiation and heat treatments (Table 5.5). Of these, only sulphur fumigation and fungicide dips have been used commercially (Jiang et al. 2003). Alternative procedures to sulphur dioxide (SO_2) fumigation of lychee fruits have been proposed. These include team treatment (Kaiser et al. 1995) or hot benomyl dipping (Scott et al. 1982), but so far, no method has been widely accepted or established commercially (Lichtera et al. 2000). SO_2-fumigated fruits absorb 30–65% of applied SO_2. In recent years, there has been an increasing concern about sulphur residues in fruit, particularly when some consumers are sensitive to sulphites. A maximum residue limit of 10 ppm sulphur is set in Europe, Australia and Japan, while in the USA, sulphur is only registered for postharvest use on grape (Paull et al. 1995). Similarly, a range of fungicides has been evaluated for disease control in lychee fruit, including benomyl, thiabendazole, iprodione and prochloraz (Huang and Scott 1985; Scott et al. 1982; Wong et al. 1991). Among these fungicides, benomyl is known to have a strong and broad spectrum of fungicidal activities and has been shown to be effective for control of lychee fruit decay, but it is no

Table 5.5 Overview of strategy used for postharvest management of lychee fruit

Treatments	Result	References
Sodium metabisulphite	Slowing browning and decay	Liang et al. (2012)
Hydro-cooling for 30 min	Prolonged storage life and suppressed fruit decay	Liang et al. (2013)
Nitric oxide (SNP 2 mM)	Extended shelf life up to 8 days	Barman et al. (2014)
Chitosan 1%	Shelf life extension up to 5 days	Lin et al. (2011)
Cold storage, anaerobic and pure oxygen environment	Short-term anaerobic treatment has been found effective than pure oxygen	Liu et al. (2011, 2014)
Gamma radiations (400Gy)	Maintaining quality attributes	(Gautam et al. 2013)
Inhibitory chemicals (butanol or hexanal) treatment	Oxidative stress management	Sun et al. (2011) and Sharma et al. (2010)
Ascorbic acid treatment and chitosan coating	5 mg/l ClO_2 solution significantly inhibited lychee anthracnose spore germination. In addition, treatments with 80 and 120 mg/l of ClO_2 significantly reduced postharvest decay and peel browning of the fruit	Sun et al. (2010)
Combinatorial treatment of sodium metabisulphite, acid dips and perforated LDPE storage	Enhanced shelf life of 9 days at ambient temperature	Neog and Saikia (2010)
Modified atmosphere at low temperature	Maximum retention of pericarp colour	Semeerbabu et al. (2007)
Apple polyphenols	Controlling enzymatic browning	Zhang et al. (2015)
Hot water brushing with micro-polyethylene film	Accumulated acetaldehyde and ethanol inhibited fungal growth	Pesis et al. (2002)
1-Methylcyclopropane	PPO, POD and browning decreased	Reuck et al. (2009)

longer registered as a postharvest chemical in many countries due to potential oncogenic risks (National Research Council 1987). Irradiation of fruit is considered to reduce browning and postharvest losses. Storage temperature of 2–5 °C is considered to extend the shelf life. Uses of perforated polythene bags (0.2% ventilation) and storage at 3 °C have also been reported to increase shelf life.

Ascorbic acid treatment of lychee fruit has been reported to increase the antioxidation capacity, and chitosan coating inhibits dehydration and microbial attack. Recently, a novel strategy of the combinatorial use of both the aforesaid treatments has been proposed. It includes treatment of the harvested fruits with 1.0% ascorbic acid (w/v) and 40 nmol/l chitosan solution (Sun et al. 2010).

5.8 Lychee Diseases

There are a few diseases affecting leaves, flowers and fruit and some others causing tree deaths or decline. However, no major disease currently limits production in the region. Brown blight (*Peronophythora Lycheei*) infects leaves, panicles and fruit in China and Thailand but can be controlled with metalaxyl. Anthracnose (*Colletotrichum gloeosporoides*) and similar diseases also attack fruit in China, India and Australia. Parasitic algae and nematodes affect some orchards but can be readily controlled with available chemicals. Various organisms have been associated with tree deaths or decline in Asia and Australia, although their pathogenicity is yet to be proven.

Regardless of where lychee is grown, several insect groups attack the flowers, fruit, leaves and branches. Lepidopterous fruit borers are generally the most important pests affecting production. Other important species include various leaf- and flower-eating caterpillars and beetles, bark borers, scales, leaf mites, fruit-sucking bugs, fruit-piercing moths and fruit flies (Table 5.6).

5.9 Lychee Biotechnology

Since lychee is a cross-pollinated plant, it is highly heterozygous, and the progeny is not true to the parental type. Conventional vegetative propagation methods currently being used, air layering or marcottage, are slow and inefficient (Chapman 1984). Hence, in vitro techniques have potential use in lychee propagation for the large-scale cloning of elite plants. However, lychee has so far proven to be a difficult material for propagation using in vitro culture. Attempts to regenerate plants from explants derived from mature trees have failed to give satisfactory results (Kantharajah et al. 1989). The biotechnological research on lychee, including tissue culture, anther culture, protoplast culture and lychee biopharming, are still in infancy but progressing (Table 5.7). Lychees are now widely grown in tropical and subtropical regions of the world. However, irregular and poor yields are commonly reported, and there is considerable scope for improving fruit quality and marketability through biotechnology (Menzel 1983; Galan Saúco and Menini 1989).

Table 5.6 Diseases of *Lychee chinensis* caused by various classes of organisms

Disease	Causative organism	Symptoms	Images	Control measures	Reference(s)
Algal	*Cephaleuros virescens*	Velvety, cushionary, reddish-brown or orange-coloured patches appear on leaves surface		–	Papademetriou and Dent (2002)
Fungal	*Botryosphaeria* spp.	Sunken, shrinking, irregular and dying tissues and expose the inner-side wood		Wound paint should be applied on the cut surface	
	Colletotrichum gloeosporoides	Anthracnose fruit rot: brownish spots appear on the fruit surface; mycelial mat of white colour also appears on mature fruit skin		5 mg/l ClO_2 solution could significantly inhibit lychee anthracnose spore germination. In addition, treatments with 80 and 120 mg/l of ClO_2 significantly reduce postharvest decay and peel browning of the fruit	
	Colletotrichum gloeosporoides (leaf necrosis)	Lesions appear on the leaf surface		–	
	Diplodia spp. (lychee dieback)	Wood becomes shrivelled and changes to black or brown in colour		Pruning of trees	
	Gloeosporium spp. (leaf blight)	Light brown-colour spots are visible on leaf surface		–	

(continued)

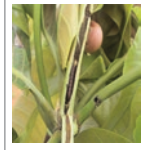
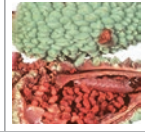
Table 5.6 (continued)

Disease	Causative organism	Symptoms	Images	Control measures	Reference(s)
	Phoma spp. (corky bark lesions)	Rough and brown-coloured lesions appear on the branches		–	
	Phomopsis spp. (dieback, canker and leaf spot)	Branch tip starts dying, red-colour spots appear on leaves		–	
	Phyllosticta spp. (Phyllosticta leaf rot)	Large, round, brownish-black-coloured spots appeared along the margins and tips of leaves which lead to wilting of leaves		–	
	Pythium spp. (root rot)	Root tips are rotten, the number of secondary roots is lesser, pale yellow leaves		Destroy the infected trees	
	Rhizoctonia solani (root rot)	Rotten roots turn dark brown in colour		Destroy the infected trees	
	Spheropsis spp. (Spheropsis dieback)	Cankers appeared on the branches and form witches broom		Remove the brooms from the tree	

	Peronophythora lychee	Fruit rotting		Crude extract of *Bacillus subtilis* used as antifungal against *Peronophythora lychee*	Jiang et al. (2001)
		Affect fruits, leaves and panicles, immature fruit turns brown		Spraying of copper oxychloride in winters and copper sulphate in spring	Menzel (2002)
	Colletotrichum gloeosporoides	Affect leaves, branches, flowers and fruits, small light grey lesions appear on leaf surface		Spray of copper oxychloride and copper hydroxide	
	Peronophythora lychee (downy blight)	Brown and withering spots appear on panicles, fruits and shoots		Application of fungicide mandipropamid protects the plant from downy blight	Tang et al. (2011)
	Colletotrichum spp.	Browning of pericarp and fruit decay		Application of chlorine dioxide (ClO_2) inhibits the spore germination	Wu et al. (2011)

(continued)

Table 5.6 (continued)

Disease	Causative organism	Symptoms	Images	Control measures	Reference(s)
Insect pests (fruit borers)	*Conopomorpha sinensis*	Lays yellow-coloured scale like eggs on fruits, leaves and shoots. Larva penetrates into fruit which leads to fruit fall		Insecticides (permethrin, cypermethrin, deltamethrin, carbofuran or fenthion)	
	Conopomorpha Lycheeella	Lays eggs on shoots, larva penetrates into leaf blades, midrib and veins and leads to wilting of shoots		Insecticidal spray	
	Argyroploce illepida	Lays eggs on fruit surface, larva penetrates into fruit which results in fruit fall		Spray of triflumuron (insecticide) 40 days before harvest	
Insect pests (Fruit-piercing moths)	*Eudocima fullonia*, *E. salaminia*, *E. jordani*	Sucks fruit juice through hole, contamination of fruit with yeast and bacteria damage the fruit		Trap system is used to capture the moths	
Insect pests (leaf feeding caterpillar)	*Oxyodes scrobiculata* F., *Oxyodes tricolor*	Severe defoliation occurs		Spray of carbaryl (insecticide) on young larvae	
Insect pests (leaf-rollers)	*Olethreutes perdulata* Meyr., *Platypeplus aprobola*, *Adoxophyes cyrtosema* Meyr., *Homona coffearia*, *Isotenes miserana*	Rolling of leaf and then leaf fall		Rolled leaves are removed manually or spraying of insecticides (phosphamidon, fenitrothion or endosulfan) for heavy infestation	

Insect pests (bark borers)	*Aristobia testudo*, *Anoplophora*, *Maculate*	Kill branches and shoot tips		Beetles can be picked manually, or dichlorvos (insecticide) is injected into the tunnels made by beetles	
Scarab beetles	*Xylotrupes gideon*	Damage the fruit		Can be picked manually, chemical control is not satisfactory	
Soft scales	*Pulvinaria psidii*, *Coccus hesperidum*, *Parasaissetia nigra*, *Saissetia coffeae*	Affects the leaves, flowers, twigs and young fruits, discolouring of fruits		Application of methidathion (insecticide)	
Bugs	*Tessaritoma papillosa*, *Tessaritoma javanica* Thunberg, *Tessaritoma quadrata* Distant	Causes severe fruit fall		Application of two sprays of endosulfan after every 2 weeks	
Gall flies	*Dasineura* spp.	Formation of galls on the leaf surface which later turn brown and fall off		Burn or remove the infected leaves, methyl parathion (2.5%) applied under trees or spraying of isofenphos (0.001%)	
Fruit flies	*Bactrocera tryoni* (Froggatt)	Damage the fruit pericarp by laying eggs		Spray of protein hydrolysate in combination with trichlorfon or mercaptothion is used	

(continued)

Table 5.6 (continued)

Disease	Causative organism	Symptoms	Images	Control measures	Reference(s)
Mites	*Aceria litchii* (Keifer)	Bristles on entire leaf which leads to curling of leaf, fruit deforming		Infected leaves should be removed and burnt, spraying of dimethoate, dicofol, chlorpyrifos, omethoate and isocarbophos	
		Leaf galls are formed, which later fall down from tree after drying up		Burn the infected leaves, spraying of Kelthane or Neoron or Torque or wettable sulphur (2.0 ml/l) of water in April or May	Papadimitriou and Dent (2002)
Weevil/fruit borer	*Apoderous sp.*, *Conopomorpha cramerella*	Tan spots appear on the leaves surface which causes severe harm to new leaves, young trees of age less than 5 years are totally damaged		Use of biocontrol agents such as *Trichogramma* spp. and neem (*Azadiracta indica*)-based insecticidal sprays	Kumar et al. (2006)

Table 5.7 Tissue culture reports on *Lychee chinensis*

Explant	Media composition (mg/l or µM or %)	Response	Reference(s)
Embryo	MS media + sucrose (0.2 g/l) + royal jelly (400 mg/l)	Callus formation	Kantharajah et al. (1992)
Embryonic shoots	MS media + BAP (100.0 mg/l)	Shoot formation	
Shoots	MS media + NAA (0.5 mg/l) + sucrose (0.2 g/l) + agar (0.8%)	Root formation	
Young embryo	MS media + 2,4-D (8.0 mg/l) + NAA (0.2 mg/l) + sucrose (0.5 g/l)	Embryogenic callus formation	Zhou et al. (1996)
Embryogenic callus	MS media +2,4-D (1.0 mg/l)	Embryogenic callus was maintained	
Embryogenic callus	½ MS media + NAA (0.2 mg/l) + IBA (1.0 mg/l) + sucrose (0.3 g/l)	Germination	
Shoot buds	MS media + BA (0.2 mg/l) + IAA (0.1 mg/l) + GA3 (0.5 mg/l)	Shoot differentiation	Chandra and Padaria (1999)
Cotyledonary node	MS media + BAP (20.0 mg/l)	Multiple shoot induction	Das et al. (1999)
Shoots	MS media + IBA (25.0 mg/l)	Root induction	
Zygotic embryos	MS media +1 2,4- D (2.0 mg/l) + sucrose (50.0 g/l) + agar (8.0 g/l)	Embryogenic callus formation	Yu et al. (2000)
Protoplast	MS media + KIN (1.0 mg/l) + NAA (0.1 mg/l) + glutamine (500.0 mg/l) + sucrose (0.8 g/l) + agar (15.0 g/l)	Somatic embryo formation	
Somatic embryo	MS media + glutamine (500 mg/l) + coconut water (50.0 ml) + sucrose (0.5 g/l) + agar (0.9 g/l)	Maturation of somatic embryo	
Somatic embryo	MS media + GA (5.0 mg/l) + coconut water (50.0 ml) + sucrose (0.3 g/l) + agar (0.7 g/l)	Germination of somatic embryo	
Leaf	MS media + 2, 4-D (2.0 mg/l) + NAA (2.0 mg/l) + IAA (2.0 mg/l) + BAP (1.0 mg/l) + KIN (1.0 mg/l) + sucrose (0.3 g/l) + phytagel (0.25%) + ascorbic acid (225.0 mg/l) + citric acid (225.0 mg/l)	Callus induction	Puchooa (2004a)
Callus	MS media + BAP (2.0 mg/l) + IAA (3.0 mg/l) + sucrose (0.3 g/l) + phytagel (0.25%) + ascorbic acid (225.0 mg/l) + citric acid (225.0 mg/l)	Shoot induction	
Shoots	MS media + IBA (2.0 mg/l) + NAA (3.0 mg/l)	Root formation	

(continued)

Table 5.7 (continued)

Explant	Media composition (mg/l or μM or %)	Response	Reference(s)
Cotyledonary nodes	MS media + BAP (20.0 mg/l)	Shoot induction	Khan and Ahmad (2005)
Shoots	MS media + IBA (25.0 ml/l)	Root formation	
Embryo	MS media + BAP (0.5 mg/l) + KIN (0.5 mg/l) + LH (500.0 mg/l) + sucrose (60.0 g/l) + agar (10.0 g/l)	Somatic embryogenesis	Chao-jun et al. (2007)
Leaflet	B5 media + glutamine (400.0 mg/l) + 2,4-D (4.52 μM) + KIN (9.30 μM) + casein hydrolysate (200.0 mg/l) + sucrose (30.0 g/l) + gellan gum (3.0 g/l)	Embryogenic callus formation	Raharjo and Litz (2007a)
Embryogenic callus	MS media + sucrose (45.0 mg/l) + coconut water (20%) + gellan gum (3.0 g/l)	Somatic embryo development	
Somatic embryo	MS media + sucrose (30.0 mg/l) + gellan gum (3.0 g/l)	Germination	
Leaflet	B5 media + glutamine (400.0 mg/l) + 2,4-D (4.52 μM) + KIN (9.30 μM) + casein hydrolysate (200.0 mg/l) + sucrose (30.0 g/l) + gellan gum (3.0 g/l)	Embryogenic callus formation	Raharjo and Litz (2007b)
Embryogenic callus	MS media + sucrose (45.0 mg/l) + coconut water (20%) + gellan gum (3.0 g/l)	Somatic embryo development	
Somatic embryo	½ MS media + GA3 (14.4 μM) + activated charcoal (0.2 g/l)	Germination	
Leaflet	MS media +2,4-D (2.0 mg/l) + NAA (0.5 mg/l) + KIN (2.0 mg/l) + activated charcoal (200.0 mg/l) + sucrose (3.0 g/l) + agar (7.0 g/l)	Callus	Ma et al. (2009)
Callus	MS media + IAA (3.0 mg/l) + BAP (2.0 mg/l)	Friable callus formation	
Friable callus	MS media +2,4-D (2.0 mg/l) + NAA (0.5 mg/l) + KIN (2.0 mg/l) + activated charcoal (200.0 mg/l)	Suspension culture	

(continued)

Table 5.7 (continued)

Explant	Media composition (mg/l or µM or %)	Response	Reference(s)
Nodal explant	Modified liquid woody plant medium + BAP (11.0 µM) + KIN (2.30 µM) + GA3 (0.60 µM) + bavistin (30 µg/l) + polyvinyl polypyrrolidone (0.2%)	Callus formation	Kumar et al. (2006)
Callus	Modified liquid woody plant medium + BAP (11.0 µM) + KIN (2.30 µM) + GA3 (0.60 µM) + coconut water (15%)	Shoot formation	
Shoots	MS media + BAP (6.6 µM) + GA3 (0.15 µM) + SN (30 µM) + CH (300.0 mg/l)	Shoot multiplication and elongation	
Elongated shoots	MS media + IBA (20.6 µM) + lychee seed powder (1.0 g/l)	Root formation	
Zygotic embryos	MS media (modified)	Transgenic plantlet	Das and Rahman (2012)
Shoot tip	MS media + ascorbic acid (250.0 mg/l) + citric acid (250.0 mg/l) + sucrose (0.3 g/l)	Callus formation	Pankaj et al. (2014)

5.10 Conclusions

Lychee is a wonder-fruit with a blend of taste and medicinal value. Lychee postharvest research has been ongoing since the 1940s, and there has been a significant growth in the past 10 years. Most of the scientific papers relating to lychee have been published since 1985. Due to its increasing demand, the area under cultivation has increased manifold. But, there are several factors which have cumulatively hampered its production. These include irregular flowering and fruit-set, frost and wind damage, narrow cultivar base, poor growing techniques, high incidence of insect pests, short production season, variable yields, poor-quality fruit, short harvest season, lack of planting material and growing technology, high cost of planting material, lack of irrigation, lack of technical information for new growers, susceptibility of fruits to browning and rotting, short shelf life of fruits, inappropriate pruning, harvesting and postharvest management, high cost of fertilizers, inefficient marketing system and inadequate industry research and extension. The following suggestions can ensure a marked improvement in lychee production, storage and supply strategies.

- Lychee has a very narrow genetic base, which needs to be widened through selection of genotypes from the existing population. Target-oriented programmes must be launched so that germplasm is conserved and used. In this context of a

network programme, 'operation lychee production' (OLP) should be initiated, and exchange of information and cultivars among countries should be encouraged. Starting of this network programme would boost lychee production and ensure livelihood security of the people.

- It should also be possible to apply in vitro mutation induction and selection procedures to address certain fungal diseases that affect specific lychee cultivars.
- Genetic transformation could possibly be utilized to develop the preferred 'chicken-tongue' seeds, using the pistillate gene from *Arabidopsis thaliana*, which directs seedlessness.
- Protoplast technology could be harnessed to produce somatic hybrids between haploids and diploids, so as to develop seedless triploids.
- Suitable cultivars are needed for various climatic conditions. It is also essential to develop promising lines/hybrids, which have larger fruit size, small/chicken-tongued seeds and tolerance to pericarp splitting.
- Suitable agro-techniques particularly for production and consumption management, postharvest technology and effective marketing need due attention.
- A systematic approach for the description of cultivars is needed. Thus, a lychee descriptor needs to be developed for herbaria and agriculture.
- There is need to develop propagation technology for faster multiplication of quality plants.
- The development of nutrition management to maintain tree health and encourage successful flowering and fruiting quality in sustainable manner requires attention.
- For efficient fertilizer use, monitoring through leaf analysis should be encouraged.
- Integrated management of nutrient and water with efficient monitoring mechanisms would improve both production quantity and quality.
- Through effective recycling of residues coupled with organic manure, it is possible to improve soil health. Thus, there is an immense potential for organic production of lychee through integrated pest management (IPM) to improve productivity and reduce the cost of production.
- The infrastructure for postharvest management requires input for timely marketing and to reduce the storage losses.

References

Akamine EK (1960) Preventing the darkening of fresh lychees prepared for transport. Technical Progress Report of Hawaii Agricultural Experimental Station, University of Hawaii 127, pp. 17

Anonymous (2001) FAO Document Repository-files\show_cdr_head.com

Barman K, Siddiqui MW, Patel VB, Prasad M (2014) Nitric oxide reduces pericarp browning and preserves bioactive anti-oxidants in Lychee. Sci Hortic 171:71–77

Bose, TK (2001) Fruit: tropical and subtropical, 1, Naya Yug, p 721

Chandra R, Padaria JC (1999) Lychee shoot bud culture for micropropagation. J Appl Hortic 1(1):38–40

Chao-jun D, Gan-jun Y, JI-wu Z, Qiu-ming Z, Wei-guo L (2007) Application of orthogonal experiment on induction of somatic embryogenesis in Litchi. Fujian Fruits 2:004

Chapman KR (1984) Lychee, *Litchi chinensis* Sonn. In: Page PE (ed) Tropical tree fruits for Australia. Queensland Department of Primary Industries, Brisbane, pp 179–191

Chauhan KS (2001) Lychee: botany, production and utilization. Kalyani publishers, Ludhiana, p 228

Chen YZ, Wang YR (1989) Study on peroxidases in Lychee pericarp. Acta Bot Austro Sin 5:47–52

Chen L, Liu ZP, Shi WB, Liu L, Deng QY (2005) GC/MS identification of essential oils from the seed and the membrane of *Litchi chinesis* Sonn. Acta Scientiarum Naturalium Universitatis Sunyatseni 44(2):53–56

Chi JW, Tang XJ, Zhang MW, Xu ZH, Zhang Y, Wei ZC, Li JX, Zhang RF (2005) Study on the processing technology of Lychee health oral solution. Food Sci Technol 26(7):63–65

Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic Lychee (cv. Bedana). Plant Cell Tissue Organ Cult 109(2):315–325

Das DK, Prakash NS, Bhalla-Sarin N (1999) Multiple shoot induction and plant regeneration in litchi (Litchi chinensis Sonn.). Plant Cell Rep 18(7–8):691–695

Degani C, Deng J, Beiles A, El-Batsri R, Goren M, Gazit S (2003) Identifying Lychee (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using inter simple sequence repeat (ISSR) markers. J Am Soc Hortic Sci 129(6):838–845

Deng Z (2006) Alcohol and vinegar extract of Lychee seed is especially effective for external haemorrhoids. Rur New Tech 6:46

Ding G (1999) The research progress of *Litchi chinensis*. Lishizhen Med Materia Medica Res 10(2):145–146

Froneman S (1999) Two new Lychee cultivars released: new Lychee cultivars will lengthen the South African production season. Neltropika Bulletin No 305:3–6

Galan-Sauco V, Menini UG (1989) Lychee cultivation. FAO, Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes plant production, and protection, paper 83

Gautam S, Mishra BB, Hajare SN, Kumar S, Saxena S, More VS, Wadhawan S, Bandyopadhyay N, Sharma A (2013) Process for retaining pericarp color and extending shelf life of Lychee. BARC Newsletter Founder Day Special Issue:245–250

Gong SJ, Su XJ, Wu HP, Li J, Qin YJ, Xu Q, Luo WS (2008) A study on anti-SARS-CoV 3CL protein of flavonoids from *Litchi chinensis* sonn core. Chin Pharma Bull 24(5):699–700

Guo JW, Pan JQ (2006) Chemical compositions, biological activities and pharmacological effects of Lychee or Lychee seeds. Chin J New Drugs 15(8):585–588

Guo JW, Pan JQ, Qiu GQ, Li AH, Xiao LY, Han C (2003a) Effects of Lychee seed on enhancing insulin sensitivity in type 2 diabetic-insulin resistant rats. Chin J New Drug 12(7):536–539

Guo CJ, Yang JJ, Wei JY, Li YF, Xu J, Jiang YG (2003b) Anti-oxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res 23(12):1719–1726

Huang TK (1994) Handbook for component analysis and pharmacology of common chinese traditional medicine. Chinese Medical Science and Technology Press, 1292

Huang PY, Scott KJ (1985) Control of rotting and browning of Lychee fruit after harvest at ambient temperatures in China. Trop Agric 62:2–4

Huang CC, Wang YT (1990) Effect of storage temperature on the color and quality of Lychee fruit. Acta Hortic:269–307

Huang XM, Wu ZX (2006) Maturation and senescence of Lychee fruit and their regulations. In: Nouredddine B, Norio S (eds) Advances in postharvest technologies for horticultural crops. Research Sunpost, Kerala, pp 315–340

Huang XS, Chen J (2007) Determination of free amino acids in Litchi Stone [J]. Amino Acid Biotic Resour 2:003

Hui YH (2008) Lychee: handbook of fruits and fruit processing. Wiley, New Delhi, pp 606–611

Jiang YM, Fu JR (1999) Post harvest browning of Lychee fruit by water loss and its prevention by controlled atmosphere storage and high relative humidity. Lebensmittel-Wissenchaft Technol 32:278–283

Jiang YM, Zhu XR, Li YB (2001) Post harvest control of Lychee fruit-rot by *Bacillus subtilis*. LWT Food Sci Technol 34:430–436

Jiang Y, Yao L, Lichter A, Li J (2003) Post harvest biology and technology of Lychee fruit. J Food Agric Enviorn 1(2):76–81

Jiang WF, Chen JZ, Zhang J, Peng J (2008) Inhibitory effects of total saponin extracted from the semen Lychee against hepatitis B virus in vitro. J Four Military Med Univ 29(2):100–103

Kaiser C, Levin J, Wolstenholme BN (1995) Vapour, heat and low pH dips improve litchi (Litchi chinensis Sonn.) pericarp colour retention. J South Afri Soci Horti Sci 4:6–12

Kantharajah AS, Dodd WA, Mcconchie CA (1989) The possible contribution of tissue culture to the lychee industry. In: Proceedings of the second national Lychee seminar, Cairns, Sep, 59–65

Kantharajah AS, Mcconchie CA, Dodd WA (1992) In vitro embryo culture and induction of multiple shoots in Lychee (*Litchi chinensis* Sonn.). Ann Bot 70:153–156

Khan MA, Ahmad I (2005) Multiple shoots induction and plant regeneration in Lychee (*Lychee chinensis* Sonn.). Int J Agric Bot 7(3):524–526

Kumar M, Prakash NS, Prasad US, Sarin NB (2006) A noval approach of regeneration from nodal explants of field grown Lychee (*Litchi chinensis* Sonn.) fruit trees. J Plant Sci 5(3):321–327

Lal H, Nirwan RPS (1980) Saharanpur selection- a new Lychee cultivar. Progress Hortic 12(3):75–77

Le CG, Fu HL (2001) Volatile compounds in Lychee peel and seed. Chin Tradit Herb Drugs 32(8):688–689

Lee HS, Wicker L (1991) Anthocyanin pigments in the skin of lychee fruit. J Food Sci 56(2):466–468

Li XZ (1996) Dahong Nuomizi, a high quality Lychee cultivar. S Chn Fruits 25(2):42

Li W (1997) Experimental study of the inhibitory effect of Chinese herbal medicine on HBV-DNA. J Tradit Chin Med 39(1):46

Li J, Jiang Y (2007) Lychee flavonoids: isolation, identification and biological activity. Molecules 12:745–758

Li ZX, Liang CW, Wang EP (1999) Study on the selections of Dahongpao Lychee variety. South China Fruits 28(5):26

Li JG, Gao AP, Wang ZH, Chen YY (2003) Comparison of the fruit characteristics between Edanli and Ziniangxi Lychee varieties. South China Fruits 32(1):29–30

Li YH, Wang XL, Qiu N (2006) Extracting technology of dietary fibre from lichee shell. J Gui Univ of Technol 26(3):385–387

Liang RG, Liu WB, Tang ZN, Xu Q (2006) Inhibition on respiratory syncytial virus *in vitro* by flavonoids extracted from the core of *Lychee chinensis*. J Four Military Med Univ 27(20):1881–1883

Liang YS, Chen NL, Ke LS (2012) Influence of dipping in sodium metabisulfite on pericarp browning of Lychee cv. Yu Her Pau (Feizixiao). Postharvest Biol Technol 68:72–77

Liang YS, Wongmetha O, Wu PS, Ke LS (2013) Influence of hydrocooling on browning and quality of Lychee cultivar Feizixiao during storage. Int J Refrig 36:1173–1179

Lichtera A, Dvira O, Rota I, Akermana M, Regevb R, Wiesblumb A, Fallika E, Zaubermana G, Fuchsa Y (2000) Hot water brushing: an alternative method to SO_2 fumigation for color retention of Lychee fruits. Postharvest Biol Technol 18(3):235–244

Lin B, Du Y, Liang X, Wang X, Yang J (2011) Effect of chitosan coating on respiratory behavior and quality of stored Lychee under ambient temperature. J Food Eng 102:94–99

Liu H, Song L, You Y, Li Y, Duan X, Jiang Y, Joyce DC, Ashraf M, Lu W (2011) Cold storage duration affects Lychee fruit quality, membrane permeability, enzyme activities and energy charge during shelf time at ambient temperature. Postharvest Biol Technol 60:24–30

Liu T, Wang H, Kuang J, Sun C, Shi J, Duan X, Qu H, Jiang Y (2014) Short term anerobic pure oxygen and refrigerated storage conditions affect the energy status and selective gene expression in Lychee fruit. LWT Food Sci Technol 60(2):1254–1261
Luo WS, Gong SJ, Liang RG, Xu Q (2006) A study of the action of flavonoids from Lychee seed against influenza virus. Chi J Chin Materia Medica 31(6):1379–1380
Luximon-Ramma A, Bahorun T, Crozier A (2003) Anti-oxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. J Sci Food Agric 83:496–502
Ma XY, Yi GJ, Huang XL, Zeng JW (2009) Leaf callus induction and suspension culture establishment in lychee (Lychee chinensis Sonn.) Huaizhi. Acta Physiol Plant 31:401–405
Menzel CM (1983) The control of floral initiation of lychee- a review. Sci Hortic 21:201–215
Menzel C (2002) The lychee crop in Asia and the Pacific. Food and agriculture organization of the United Nations, Bangkok, Thailand
Miao PS, Wang MN, Huang YQ, Zhang DT, Li XQ (1997) Brief introduction to several rare and promising Lychee varieties in Hainan province. South China Fruits 26(5):30–31
Miao PS, Han J, Huang YQ, Lin D, Wang FN (1998) Introduction of 4 new Lychee selections. China Fruit 2:53
Morton J (1987) Lychee. In: Fruits of Warm Climates. Julia F. Morton, Miami, pp 249–259
Nakasone HY, Paull RE (1998) Tropical fruits, Crop Production Science in Horticulture Series. CAB International, Wallingford, pp 173–207
Neog M, Saikia L (2010) Control of post-harvest pericarp browning of Lychee (*Litchi chinensis*. Sonn). J Food Sci Technol 47(1):100–104
Ning ZX, Peng KW, Qing Y, Wang JX, Tan XH (1996) Effects of the kernel oil from *Litchi chinensis* Sonn. seed on the level of serum lipids in rats. Acta Nutrimenta Sinica 18(2):159–162
Ochse JJ, Soule MJ, Dijkman MJ, Wehlburg C (1961) Other fruit crops. Tropical and sub-tropical agriculture, vol 1. The McMillan Company, New York
Olesen T, Wiltshire N (2000) Post harvest result from the 1999/2000 lychee season. Living Lychee 23:16–22
Ong PK, Acree TE (1999) Similarities in the aroma chemistry of Gewurztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. J Agric Food Chem 47:665–670
Ooyang R, Liang YG, Liu CM, Liu WW, Pan DF, Lu HH (1994) Study on the major economic characteristics of Bai-Tang-Tang lychee, a famous early-maturing cultivar with excellent eating quality. J Sout China Agric Univ 15(2):32–37
Ooyang R, Liang YG, Liu CM, Wang ZH, Hu GB, Lin SX, Chen H (2002) Maguili, an extra late new Lychee variety. South China Fruit 31(4):42–44
Pan JQ, Liu HC, Liu GN, Hu YL, Chen LX, Qiu ZQ (1999) A study in blood sugar reducing, blood lipid controlling and anti-oxidant activities in Lychee seed. Guangdong Pharm J 9(1):47–50
Pan JQ, Gui JW, Han C, Liu HC (2000) Survey of pharmacological experimental studies in Lychee seed. Chin New Drug J 9(1):14–16
Pankaj K, Pandey SK, Androy KA (2014) Ascorbic and citric acids in combination resolve the problems encountered in micro- propagation of Lychee from shoot tips. J Cell Tissue Res 14(1):4159–4164
Papademetriou MK, Dent MJ (2002) Lychee production in the Asia-pacific region. Food and agriculture organization of the United Nations, Bangkok, Thailand
Paull RE, Chen NJ (1987) Effect of storage temperature and wrapping on the quality characteristics of Lychee fruit. Sci Hortic 33:223–236
Paull RE, Reyes MEQ, Reyes MV (1995) Lychee and Rambutan insect disinfestations treatment to minimize induced pericarp browning. Postharvest Biol Technol 6:139–148
Peng HX, Li WQ, Huang DJ, Su WQ, Liu RG (2001) Qinzhou red Lychee – a new big fruit form variety of Lychee. Acta Hortic Sin 28(3):276
Pereira LS, Pathak P, Mitra SK (2005) Relation between flushing cycles and flowering in different Lychee cultivars. Indian J Hortic 62(2):141–144

Pesis E, Dvir O, Feygenberg O, Arie RB, Ackerman M, Lichter A (2002) Production of acetaldehyde and ethanol during maturation and modified atmosphere storage of Lychee fruit. Postharvest Biol Technol 26:157–165

Popenoe W (1920) The Lychee manual of tropical and subtropical fruits. McMillan Company, New York, pp 312–325

Puchooa D (2004a) In vitro regeneration of lychee (*Litchi chinensis* Sonn.). Afr J Biotechnol 3(11):576–584

Qadir H (2001) Inhibition of postharvest decay of fruits by nitrous oxide. Post Biol Technol 22(3):279–283

Raharjo SHT, Litz RE (2007a) Clonal regeneration of Lychee (*Litchi chinensis* Sonn.) via somatic embryogenesis. Acta Hortic:738–761

Raharjo SHT, Litz RE (2007b) Somatic embryogenesis and plant regeneration of Lychee (*Litchi chinensis* Sonn.) from leaves of mature phase trees. Plant Cell Tissue Organ Cult 89(2):113–119

Rai M, Nath V, Dey P (2001) Lychee. CHES, Plandu, Rajaulatu, Ranchi, Jharkhand, pp 1–100

Reichel M, Triani R, Wellhöfer J, Sruamsiri P, Carle R, Neidhart S (2014) Vital characteristics of Litchi (*Litchi chinensis* Sonn.) pericarp that define postharvest concepts for Thai cultivars. Food Bioprocess Technol 6:1191–1206

Reuck KD, Sivakumar D, Korsten L (2009) Integrated application of 1-methylcyclopropene and modified atmosphere packaging to improve quality retention of Lychee cultivars during storage. Postharvest Biol Technol 52:71–77

Rivera-Lopez J, Ordorica-Falomir C, Wesche-Ebeling P (1999) Changes in anthocyanin concentration in Lychee (*Litchi chinensis* Sonn.) pericarp during maturation. Food Chem 65:195–200

Rooyen PV, Redelinghuys HJP (1983) Crystal structure and molecular conformation of proanthocyanidin-A2, a bitter substance in Lychee. South Afr Chem 36(2):49

Salunke DK, Desai B (1984) Postharvest biotechnology of fruits. CRC Press, Florida, pp 77–80

Sarni-Manchado P, Le Roux E, Le Guerneve C, Lozano Y, Cheynier V (2000) Phenolic composition of Lychee fruit pericarp. J Agric Food Chem 48:5995–6002

Scott KJ, Brown BL, Chaplin GR, Wilcox ME, Bain JM (1982) The control of rotting and browning of Lychee fruit by hot benomyl and plastic films. Sci Hortic 16:253–262

Semeerbabu MT, Kudachikar VB, Baskaran R, Ushadevi A, Matche RS, Ramana KVR (2007) Effects of post-harvest treatments on shelf life and quality of Lychee fruit stored under modified atmosphere at low temperature. J Food Sci Technol 44(1):106–109

Sharma M, Jacob JK, Subramanian J, Paliyath G (2010) Hexanal and 1-MCP treatments for enhancing the shelf life and quality of sweet cherry (*Prunus avium* L.). Sci Hortic 125:239–247

Shen REN, Duo-duo XU, Yang GAO, Yu-ting MA, Qi-pin GAO (2013) Flavonoids from Litchi (*Litchi chinensis* Sonn.) seeds and their inhibitory activities on α-Glucosidase. Chem Res Chin Univ 29(4):682–685

Shi J, Wang C, An X, Li J, Zhao M (2001) Postharvest physiology, storage and transportation of Lychee fruits–a review. Acta Hortic 558:387–391

Siddiqui SBMAB (2002) Lychee Production in the Asia-Pacific Region. Food and agriculture organization of the United Nations, Bangkok, Thailand

Singh HP, Babita S (2001) Lychee Production in India. In: FAO Corporate Document Repository Sout Chi Fru 30(5):22

Singh A, Pandey SD, Nath V (2012) The world Litchi cultivars. National Research Centre for Litchi. Muzaffarpur, Bihar

Sotto RC (2001) Lychee production in the Philippines. In: FAO corporate Document Repository Sout Chi Fru 30(5):22

Su WQ, Peng HX, Liu RG (2001) Evaluation of the promising Lychee selections. South China Fruit 30(2):30–31

Sun D, Liang G, Xie J, Lei X, Mo Y (2010) Improved preservation effects of Lychee fruit by combining chitosan coating with ascorbic acid treatment during postharvest storage. Afr J Biotechnol 9(22):3272–3279

Sun J, You X, Li L, Peng H, SuW LC, He Q, Liao F (2011) Effects of a phospholipase D inhibitor on postharvest enzymatic browning and oxidative stress of Lychee fruit. Postharvest Biol Technol 62:288–294

Surinut P, Kaewsutthi S, Surakarnkul R (2005) Radical scavenging activity in fruit extracts. Acta Hortic 679:201–203

Tang ZH, Wang HC, Hou YP, Zhang SP, Wang JX, Zhou MG (2011) Baseline and differential sensitivity to mandipropamid among isolates of *Peronophythora Lycheei*, the causal agent of downy blight on Lychee. Crop Prot 30:354–359

Tian J (2005) Progress in research of anti-diabetes herbal medicines. J Hang Teach Coll 4(6):470–472

Tu PF, Luo Q, Zheng JH (2002) Studies on chemical constituents in seed of Litchi chinensis. Chin Tradit Herbal Drugs 33(4):300–306

Underhill SJR, Critchley C (1994) Anthocyanin decolorisation and its role in Lychee pericarp browning. Aus J Exp Agric 34:115–122

Underhill SJR, Simons DH (1993) Lychee (*Litchi chinensis* Sonn.) pericarp desiccation and the importance of postharvest micro-cracking. Sci Hortic 54(4):287–294

Wall MM (2006) Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. J Food Compos Anal 19:655–663

Wang HC, Huang HB, Huang XM, Hu ZQ (2006a) Sugar and acid compositions in the arils of *Litchi chinensis* Sonn.: cultivar differences and evidence for the absence of succinic acid. J Hortic Sci Biotechnol 81(1):57–62

Wang X, Wei Y, Yuan S, Liu G, Zhang YLJ, Wang W (2006b) Potential anti-cancer activity of Lychee fruit pericarp extract against hepatocellular carcinoma in vitro and in vivo. Cancer Lett 239:144–150

Wang XY, Xiao LY, Pan JQ, Lu JH, Zhang SP (2007) Experimental studies of effects of anti-tumor of Lychee seed Ke li and the activity of in the tissue-end of EAC, S180 and hepatic carcinoma of rats. Chin Health Innov 2(12):54–56

Wong DX (1999) Introduction of several promising Lychee varieties. Chin Fruit 4:55

Wong LS, Jacobi KK, Gales JE (1991) The influence of hot benomyl dips on the appearance of cool stored lychee (Litchi chinensis Sonn.). Sci Hortic 46:245–251

Wu DY, Zhang HL (1997) The characters of "Feizixiao" Lychee variety. South China Fruit 26(5):26–27

Wu QH, Liang SM, Li YJ, Zhao SH, Jian ZH, Chen SY (1991) Screening study of TCM simple and proved recipes on diabetes. J Guangzhou Univ Tradit Chin Med Z1:218–223

Wu HH, Li XH, Qiu L (2004) Studies on superoxide scavenging capability on Lychee and longan fruit pulp by its polysaccharides. Food Sci Technol 25(5):166–169

Wu Y, Pan Q, Qu W, Duan C (2009) Comparison of volatile profiles of nine Lychee (*Lychee chinensis* Sonn.) cultivars from Southern China. J Agric Food Chem 57:9676–9681

Wu B, Li X, Hu H, Liu A, Chen W (2011) Effect of chlorine dioxide on the control of postharvest diseases and quality of Lychee fruit. Afr J Biotechnol 10(32):6030–6039

Xiao LY, Zeng WT, Ma PQ, Zhu KL, Liang JX, Huang YH, Yang HH, Ou YY (2005) Clinical study on the effect of Lychee seed pellet on chronic hepatitis B. Chin J Tradit Chin Med Pharm 20(7):444–445

Xiao LY, Hong HJ, Pan JQ, Lu JH, Zhang SP (2007) Anti-tumor effect of semen Lychee and its effect on telomerase activation of hepatoma tissue. Chin Pharm 18(18):1366–1368

Xie CP (1995) Liquili, a late maturing Lychee cultivar. Crop Genet Res 3:55–56

Xu Q, Chen QB, Yi XH, Chen ZY (2004) Inhibitory effect of extracts of the core of Lychee on the expression of HBsAg and HBeAg in the HepG 2.2.15 cells. Chin J Hospital Pharm 24(7):393–395

Yang YJ, Liang BM (2004) Determination of anti-diabete saponins from *Litchi chinensis* Sonn. Guangdong Pharm J 14(6):12–15
Yang Y, Yi XH, Chen QB, Tan M (2001) Inhibition of semen Lychee on HbsAG and HbeAG. Chem Indu Times 7:24–26
Yang Y, Luo ZH, Yan Q (2004) Estimation of content on total saponins from semen Lychee. Chem Indu Times 18(1):45–46
Yang B, Wang J, Zhao M, Liu Y, Wang W, Jiang Y (2006) Identification of polysaccharides from pericarp tissues of Lychee (*Litchi chinensis* Sonn.) fruit in relation to their anti-oxidant activities. Carbohydr Res 341(5):634–638
Yu C, Chen Z, Lu L, Lin J (2000) Somatic embryogenesis and plant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Organ Cult 61(1):51–58
Yuan P, Zhu L (2001) Preliminary report on trials of 4 high quality late Lychee varieties. South China Fruit 30(5):22
Zhang D, Quantick PC (1997) Effects of chitosan coating on enzymatic browning and decay during postharvest storage of Lychee (*Litchi chinensis* Sonn) fruit. Postharvest Biol Technol 12:195–202
Zhang DL, Quantick PC, Grigor JM (2000) Changes in phenolic compounds in Lychee (*Litchi chinensis* Sonn.) fruit during postharvest storage. Postharvest Biol Technol 19(2):165–172
Zhang Z, Huber DJ, Qu H, Yun Z, Wang H, Huang Z, Huang H, Jiang Y (2015) Enzymatic browning and anti-oxidant activities in harvested Lychee fruit as influenced by apple polyphenols. Food Chem 15(9):171–191
Zhao M, Yang B, Wang JS, Li BZ, Jiang YM (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their anti-oxidant activities. Food Chem 98:737–742
Zhao MM, Yang B, Wang JS, Liu Y, Yu LM, Jiang YM (2007) Immunomodulatory and anti-cancer activities of flavonoids extracted from Lychee (*Litchi chinensis* Sonn.) pericarp. Int Immunol pharmacol 7:162–166
Zheng MS, Zheng YF (1992) Experimental studies on the inhibition effects of 1000 Chinese medicinal herbs on the surface antigen of hepatitis B virus. J Tradit Chin Med 12(3):193
Zheng LY, Han C, Pan JQ (1998) Chemical, pharmacological and clinical studies of Lychee seed. Acta Chin Med Pharmacol 5:51–53
Zheng GM, Yi ZZ, Zhang JB, Zhong DH (2003) Studies on the antioxidative effect of extract from mature and premature Lychee pericarp. Nat Prod Res Dev 15(4):341–344
Zhou L, Zheshi K, Xuejun M, Junqiu C, Jing C (1996) The study of factor affecting somatic embryogenesis in young embryo culture of *Litchi chinensis*. J Agric Biotechnol 4(2):161–165
Zhu L, Yuan P (1999) Guiwei, a high quality late Lychee variety in Sichuan. South China Fruit 28(5):27
Zhuang XN (1999) Lychee cultivars suitable for growing in Yunnan province. South China Fruit 28(3):28

The Lychee Fruit: Post Harvest Handling Techniques

6

Manoj Kumar, Priyanku Teotia, Ram Prasad, Ajit Varma, and Vivek Kumar

Abstract

Lychee fruit (*Lychee chinensis* Sonn.) is harvested under vivid humid and subtropical conditions in India and China, economically a significant fruit crop at health scale due to its beautiful skin color and bizarre and outlandish flavor. The fruit has a coarse pericarp surrounding the juicy succulent, edible aril with a seed in center. Cultivators are still in fancy for its commercialization; the reasons are pericarp browning, postharvest decay, and minor racking among the numerous key constraints influencing the quality of lychee during storage and transportation. Aridness of pericarp invites inflammatory damage owing to untimely postharvest handling practices. Cracking is expected at preharvest and fruit developmental stages. Pre- and postharvest stages are owing to the modest handling practices and sorting line operations. We address cracking problems of lychee pericarp which offers points of entry to the invasion of postharvest microbial pathogens during cold storage and transport. It is advisable that pericarp skin browning which is triggered by withering is not only limited to the corporeal attributes of lychees, involuntary injury, and postharvest deterioration; rather it leads to deadly effects on sensory attributes of lychee aril. We tried to develop an explicit understanding on pericarp skin browning during postharvest and transportation and further suggest adoption mechanism of SO_2 fumigation in elite lychee cultivars. Moreover, we count the health issues on post fumigation which leaves the residual effects, changes fruit taste, and must be measured at ground level.

M. Kumar • R. Prasad • A. Varma • V. Kumar (✉)
Amity Institute of Minorbial Technology, Amity University, Noida, Uttar Pradesh, India
e-mail: vivekbps@gmail.com

P. Teotia
Laboratory of Microbiology, Department of Botany, CCS University, Meerut, India

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Lychee • Pericarp • Postharvest • Browning • Pathogen

6.1 Introduction

Lychee (*Lychee chinensis* Sonn.) belongs to the family Sapindaceae (soapberries), a tropical to subtropical fruit that is originated from northern Vietnam (then southern China, as quoted by Menzel 2001). This lychee fruit is originated through panicle and takes the various shapes from tapering to sphere shaped. Fruits have uneven and coarse indehiscent red-colored pericarp texture due to the presence of pigment succulent anthocyanins; palatable aril bears seed in the center with various sizes which signifies the varieties.

Due to a narrow gene pool, a least number of lychee cultivars are peculiar across the globe. The paucity of lychee quality is caused by natural defunct such as swelling and bulge type. Horticultural arrangement restricts to its least diversification and classification approach which further recognizes least at cultivar level. The old fashioned classification is still reliable on fruit outline, dimension, flavor palate, etc. The arrangements and characteristics of lychee cultivars are still based on obsolete parameters of taxonomy, e.g., round fruit of "Kwai May Pink" is distinguished from the egg-shaped "Tai So" variety or the heart-shaped cultivar "Haak Yip." Fruit carry approach is an even or smooth ("'Wai Chee" and "Kwai May Pink" varieties) or rough and rough ("Souey Tung" and "Bengal" cultivars) (Chapman 1985). The top or tip of lychee fruit is being round and curved ("Kwai May Pink" and "Wai Chee" cultivar), rounded ("Souey Tung" and "Brewster" cultivar), or sharp ("Bengal" cultivar). Characteristic insignia include bright red ("Bengal" cultivar), dull red ("Wai Chee" cultivar), violet red or plum red ("Haak Yip"), or pink red ("Brewster"). The covering layer is always thick ("Wai Chee," "Bengal," and "Kwai May Pink" cultivars) or skinny ("Haak Yip" and "Souey Tung" cultivar) (Gaur and Bajpai 1978).

The crust segments at full maturity even ("Haak Yip" cultivar) puffiness ("Wai Chee" cultivar) or sharp pointy ("Kwai May Red" cultivar) are found to be comparable to protuberances on each segment. There are very few cultivars ("Haak Yip" and "Souey Tung") which are differentiated by the incidence or absence of an evident junction line. The trace, surface, juiciness, palate, and fragrance of flesh have been reported quite helpful, e.g., "Wai Chee" is watery, "Kwai May Red" is stable and definite, "Kwai May Pink" is slight hot or spicy, whereas "Bengal" is loaded with sugar. From customer's point of view, a lychee peel is characteristically rose pink to bright red, depends on type of the cultivar, a sweet and tart merger of taste, savor and juicy, soft, lenient, and crunchy aril (Steyn and Robbertse 1992; Huang et al. 2005). The daily requirement of vitamin C for an average adult is reported as estimated source of 15–16 lychee fruits (Wall 2006). Though, an average amount of ascorbic acid from lychee is ca. 27.8 mg/100 g aril. The recommended consumption

of lychee fruit which meets 2–4% of dietary reference intakes (DRI) for P, K, Mg, Fe, Zn, and Mn and provides 22% of the DRI for Cu (Menzel and Simpson 1994). Matured lychee fruit pericarp changes its color from green to reddish pink due to decrease in chlorophyll content and increase in anthocyanin synthesis (Holcroft et al. 2005). The fruit is climacterically independent with relatively reduce level of production of ethylene after harvest. The fruit has least tendency of ripening during postharvest process where ethylene gas is produced in a steady manner at 1–3 °C storage temperature for 30 days (Schoeman et al. 2006). A radical decline in rate of respiration was reported during developmental stages of the fruit, especially in cv. "Huaizi"; rather it was reported that respiration rate is resumed with an exponential increase (Li et al. 2005). Though, significant data on respiratory act is not available with reference of other lychee cultivars.

Several researchers have highlighted the inherent problems in lychee such as pericarp browning (Lee and Wicker 1991; Kumari 1990), dryness-withering (Kumari 1993), postharvest deterioration (Underhill and Simons 1993), and minor cracking (Li et al. 2001; Huang et al. 2004a, b); these constrains are recognized as main limiting factors at industry level and further restricts the lychee exportation. Lychee industry mainly uses gas SO_2 to overcome these problems (Lemmer and Kruger 2000; Schoeman et al. 2005). In least lychee-growing countries like Israel and South Africa, SO_2-fumigated fruit is subjected to diluted HCl in order to restore the red color; following such practice is widely accepted (Lemmer and Kruger 2000; Kremer-Köhne 1993).

The main concern of SO_2 fumigation is some health issues due to the marginal carcinogenic residues (Lemmer and Kruger 2000; Kremer-Köhne 1993). Recent report says that rising concerns in SO_2 residue reaches the tolerance levels in the fruit; particularly it is more prone in importing countries (Europe, the USA, South America, and Japan). Updated international guideline in food standards has enforced sulfur concentration level max at 10 μg/g in eatable portion of the fruit (Lemmer and Kruger 2000). The FAO (UNO) has developed CODEX quality standards for such imports and exports of fresh delicate fruits.

Commercial aspect of lychee is still required at grower level; moreover China, Taiwan, South Africa, Israel, Bangladesh, Madagascar, Mauritius, Réunion, and the USA are putting steady efforts on lychee as a profitable and marketable crop. Though Australia, subtropical parts of India, Pakistan, Philippines, Thailand, Indonesia, and Brazil have ethnic approach on lychee (Menzel et al. 2005). China is the key player in lychee production worldwide with 950,000 tons of production with potential export cultivars in 2002 (Huang 2005). China and Taiwan export roughly 12,000–15,000 tons of lychees to international markets in Hong Kong and Singapore (Mitra 2006). On the other hand, European markets are having remarkable import capacity of lychee ca. 20,000 tons, whereas France alone imports 50%, and the rest is mainly imported by Germany and the UK. Highest lychee venders for Europe are established during Christmas and New Year season in countries like Madagascar and South Africa (Mitra 2006).

6.2 Fumigation of Lychee with SO_2

Fumigation of lychee with sulfur dioxide causes unsolicited and unappealing effects on the fruit value. The fruit taste is changed owing to the increased acidity and the lesser pH resulting from the straight penetration of sulfur dioxide from the outer or pericarp skin into the aril flesh, which ultimately decreased the pH (Huang and Qiu 1987; Kaiser 1995). The evaluation of sulfur dioxide fumigated lychee fruit of dissimilar cultivars showed a 12–14% mass damage at low-temperature storage (Kaiser 1998; Sivakumar et al. 2005). On the other hand, Sivakumar and Korsten (2004) have also reported that commercial sulfur dioxide fumigation enhanced minor cracking of the skin pericarp, comparable to observations on the grape fruits by Lin and Chiang (1998). The sulfur dioxide fumigation also results in health threats and risks for packhouse labors, consumers, and clients, which may lead to various allergic reactions and lung-related problems (Joas et al. 2005).

During fumigation, buildup of sulfur dioxide residues in the skin pericarp and aril depends on diverse aspects, such as damage to the pericarp RH and temperature of fruit storage. Huang et al. (Huang et al. 2004a, b) reported that the sulfur dioxide residue levels in the pericarp skin and aril (edible portion) of six cultivars ("Wai Chee," "Fay Zee Siu," "Kwai May Pink," "Haak Yip," "HLH Mauritius," and "McLean's Red") were ranging from 1000 to 1400 ppm in the skin pericarp and 10–14 ppm in the aril flesh just after the sulfur dioxide fumigation dropped to 200–250 ppm and 8–12 ppm, correspondingly during 1 °C of low-temperature storage (Underhill and Critchley 1992). The detected sulfur dioxide leftovers varied between the cultivars: the upper values were recorded in cv. "McLean's Red" than cv. "Mauritius." Additionally, greater sulfur dioxide remains were reported in the flesh of fruit of cv. "McLean's Red" and "Mauritius" exposed to an acid dip usage following sulfur dioxide smoking. In other studies, Lemmer et al. (2000) and Lemmer and Kruger (2000) also debated that peel injury caused by a lower pH usage can enable an augmented diffusion rate into the aril flesh, leaving less remains in the peel and subsequently greater remains in the aril flesh. Lemmer et al. (2000) and Lemmer and Kruger (2000) extra examined and investigated the effect of chemical Vapogard® preceding to the acid dip treatment to lessen sulfur dioxide remains in the pericarp skin and the aril flesh of "McLean's Red" and "Mauritius" varieties. Though, no buffering effect due to the covering was noticed in the remainder levels in the pericarp skin or aril flesh of both the varieties.

Likewise, more developed fruit (higher SSC/TA ratio) was likely to have greater sulfur dioxide remainder ratios between the aril flesh and pericarp skin owing to initiation of pericarp deprivation as a result of the senescence procedure. These factors then also lead to a greater sulfur dioxide diffusion rate through the pericarp skin, resulting in developed deposits (Lichter et al. 2000) in the aril. The relative humidity and storage temperature also influence the movement and absorption of sulfur dioxide in the lychee fruit; on the other hand, more storage temperatures with low relative humidity favored the accumulation of sulfur dioxide remains in the aril flesh (Lemmer et al. 2000; Lemmer and Kruger 2000). Time interval between picking and disinfection also influenced the sulfur dioxide deposit buildup in the aril

flesh (Lemmer et al. 2000; Lemmer and Kruger 2000). Interestingly, all these unwelcome effects of sulfur dioxide disinfection have demanded the development of other postharvest handlings to maintain overall excellence, superiority of fruit during storage, and conveyance (Lin et al. 1998).

6.3 Limitations in Lychee Exports

6.3.1 Browning of Pericarp

Skin pericarp browning is connected to the water loss or dryness and dehydration from the skin pericarp (Kaiser 1995; Joas et al. 2005). Cutting or automated injury, storing of lychee fruit at unwanted and unwelcomed low temperatures (chilling injury), pathogen or insect attack (Prusky and Keen 1995), and senescence can result in browning of the skin pericarp. Browning caused by temperature trauma, deterioration, and senescence (Sivakumar et al. 2005) is obvious as characteristic dark and water-soaked areas on skin pericarp, while browning owing to the desiccation is distinguished by a pale or yellow dry appearance of the skin pericarp. The findings of Huang et al. (2005) showed that browning is started after fruit harvest, and the skin pericarp becomes wholly brown within 3 days at room temperature and at 65–70% of relative humidity. The browning of fruit starts from the swellings of the skin pericarp and subsequently extends over whole skin pericarp surface, till the skin pericarp ultimately becomes dehydrated, hard, and fragile (Wang et al. 2001). Though the skin pericarp browning does not disturb the aroma and taste of the aril flesh, but it concerns the ornamental appearance of lychee fruit at the time of consumer choice (Fig. 6.1).

Rigorous research work has been conducted to find out the biochemical process underlying the lychee browning. Yin et al. (2001) suggested that pH of the skin pericarp tissue plays a chief role in the browning process. The dryness, dehydration, or moisture loss from the skin pericarp tends to upsurge the pH of pericarp (4.15–4.52 over 48 h at 25 °C and 60% relative humidity) (Hu et al. 2001). The anthocyanins pigments in the vacuoles of the skin pericarp cells are responsible for the red color of lychee fruit and are influenced by the pH change. At a higher pH (more than four), the anthocyanin pigment is converted to a colorless form, known as carbinol (Sharma et al. 1986). Consequently, the lychee aril pH is the chief aspect that governs the ratio between the flavylium cation and the colorless carbinol form of anthocyanin pigments. Other means and methods of lychee fruit pericarp browning are mainly accredited to the oxidation procedure of phenolics and the degradation of the anthocyanin pigment by enzymes known as polyphenol oxidase (PPO) or peroxidase (POD) (Paull et al. 1984; Wang et al. 2007) and along with formation of the brown polymeric pigments (o-quinones). Polyphenol oxidase activity was observed to be less at maturity, whereas an upsurge in activity happened during the first 2 days of stowage, but no noteworthy changes in the anthocyanin pigment was noticed during additional storage (Wang et al. 2005). Though the anthocyanin pigment content did not show substantial changes with respect to the enhanced browning (Cronje

Fig. 6.1 Pericarp browning of lychee

2008), the skin pericarp browning index augmented, while anthocyanin pigment degenerated during storing. This opinion was further supported by Li (2003) who also observed a deterioration in cyanidin-3-glucoside (major anthocyanin, representing 91.9% of the total anthocyanin pigment) with rising severity of fruit browning during storage. Moisture loss and enhanced pH in skin pericarp tissue are directly related to the polyphenol oxidase activity, which was observed to increase at the higher pH (7–7.4) and decrease at the lower pH, while no activity was observed below 4.2 pH (Zhang et al. 1997). On the other hand, Stern et al. (2000) established that the rise in pH from 4.15 to 4.52 during the desiccation process can arouse polyphenol oxidase activity. The anthocyanin pigments located chiefly in fruit epicarp and mesocarp and high polyphenol oxidase activity are observed in these two layers which led to the assumption that contribution of polyphenol oxidase activity in dryness facilitated the fruit browning (Kumari 1989). In whole tissues, the polyphenol oxidase is separated from pigment anthocyanin in the vacuole owing to compartmentation. Dehydration, dryness, or water loss causes quick injury to membrane integrity, bringing the polyphenol oxidase in close contact with substrate epicatechin to originate the browning process (Scheer 1994). The loss of membrane integrity was detected to be associated with enhanced electrolyte seepage after fruit harvest and during stowage (Shi et al. 2001).

6.3.2 Cracking of Fruit and Minute Cracking

In lychee fruit, the small cracking was reported by Underhill and Simons (1993) and Huang et al. (2004a, b) who suggested that it is caused by dryness and water loss (Fig. 6.2).

The splitting- or fracturing-resistant Chinese cv. “Huaizi” exhibited a lower rate of dehydration than the cracking vulnerable cv. “Nuomici.” Slight cracking is also one of the causes of skin pericarp browning (Li et al. 2001). Conferring to Underhill and Simons (1993) and Li et al. (2001), the minor cracks have been noted preceding to the harvest and were observed to intensify during the stowage. Slight cracking of the skin pericarp takes place at the early stages of the fruit development owing to the speedy expansion of the aril flesh (Huang et al. 2004a, b). As per the findings of

Fig. 6.2 Cracking of lychee fruit on tree

Maguire et al. (1999), the expanding aril flesh exerts an increased tension or turgor pressure against the already grown skin pericarp, which is comprised of three layers: exocarp, mesocarp, and the endocarp. Water scarcity is another main reason of skin pericarp cracking during the fruit development process, which leads to a loss of skin pericarp extensibility (Li et al. 2001). Skin pericarp structure development has been reported for Chinese (cv. "Huaizi" and "Nuomici"; Li et al. 2001; Huang et al. 2004a, b) and South African varieties (cv. "Mauritius" and "McLean's Red"; Sivakumar et al. 2005). Marginal cracking can also be present owing to mishandling or during packing line operations (Macfie 1955). The lychee varieties exhibited similar skin pericarp development, though differences in thickness of the cuticle and spongy layers were observed between dissimilar varieties (Underhill and Simons 1993; Li et al. 2001; Huang et al. 2004a, b). The soft tissue responsible for gas exchange in skin pericarp was thought to be accountable for water loss or dryness (Underhill and Simons 1993). However, as per the conclusions of Huang et al. (2004a, b) and Huang (2005) exhibited that cv. "Huaizi," which had a denser, soggy layer, exhibited less dryness. Huang (2005) further demonstrated that cuticle buildup pattern might help in explaining the vulnerability or opposition to the slight cracking in dissimilar cultivars. Moreover, differences in wax deposition distribution pattern were also observed on the skin pericarp between the development stages of South African lychee cv. "Mauritius" and "McLean's Red." Slight cracking has also been observed as a result of poor handling processes, and disturbances of surface were observed in freshly harvested lychee fruit (Azizah et al. 2009). Fruit falling during the separation process has also been observed to cause "splitting" damage in skin pericarp. Commercial sulfur dioxide disinfection was observed to exaggerate slight cracking in skin pericarp (Li et al. 2001). The lychee fruit cracking also influences the decorative and aesthetic appearance of the fruit in local and international market. It occurs during the fruit development process as a result of speedy growth of aril flesh, applying pressure on the skin pericarp, which is not growing anymore. The degree of sternness or injury, depends on the lychee cultivar, is known to intensify with dehydration and water loss. Changes in wet and dry periods or monsoon at late fruit development stages can also worsen fruit cracking. Connection between

fruit cracking and internal hormones concentration or mineral nutrition (such as Ca, Mg, and B) has been reported by Qui et al. (1999) in variety "Nuomici." Contribution to cracking opposition by mineral Ca is related to its structural role in the pericarp cell walls, and the obtainability of Ca during initial lychee fruit progress is significant for cracking defiance (Li et al. 2005; Huang et al. 2005). Variety "Huaizi" gathered more Ca than its physiological requirements, and the additional was stored as calcium oxalates, mostly in the epidermis, which provide resistance to cracking. In contrast, cv. "Nuomici" stored petite calcium oxalate. Higher levels of structural Ca in "Huaizi" were due to greater Ca availability and more Ca-binding sites. Owing to the greater amount of structural Ca and galacturonans, "Huaizi" variety exhibited a stouter pectic network, which improved cracking opposition. Structural Ca levels were reduced in fruit skin pericarp from 22 to 52 days after the anthesis process. Cell expansion in skin pericarp during this period resulted in dilution of structural Ca (Underhill and Simons 1993; Huang et al. 2004a, b), which ultimately amplified during aril flesh expansion. This upsurge in structural Ca could be owing to an augmented obtainability of Ca or owing to augmented Ca-binding sites in the pectin (Huang et al. 2005; Wang et al. 2005). Foliar brassinolide spray meaningfully affected the enzyme's activities and the Ca matter of the lychee fruit skin pericarp and lessened the fruit cracking. Application of brassinolide was observed to upsurge the activity of enzyme pectin methylesterase and polygalacturonase resulting in the rise of pectin metabolism, which is related to the cell division, elongation, and rapid fruit growth (Kaiser 1995; Peng et al. 2004). Enzyme polygalacturonase resulted in monogalacturonic acid capable of binding calcium and enabling the formation of junction zones. The increase in Ca during initial stages of fruit progress provides a good basis of fruit pericarp expansion and the final increase in protopectin content in skin pericarp which guarantees the good fruit pericarp excellence (Underhill and Simons 1993; Peng et al. 2004; Wang et al. 2005).

6.3.3 Postharvest Worsening

The postharvest deterioration and decline are the chief problems in postharvest fruit chain management, resulting in dropping the business value of the lychee fruit. Various types of fungal infections can cause deterioration of the lychee fruit (Revathy and Narasimham 1997; Underhill et al. 1997). The significant pathogens isolated from lychee fruit were identified as Peronophythora lychee (Prusky and Keen 1995). The major fungal genera accompanying with lychee in South Africa are *Phomopsis*, *Pestalotiopsis*, *Penicillium*, *Trichoderma*, *Alternaria*, *Botryosphaeria*, and *Fusarium* spp. (Qu et al. 2001) (Fig. 6.3).

Numerous *Penicillium* spp. have been isolated during pre- and postharvest from lychee fruit, of which *P. expansum* has been reported as chief pathogenic species (Jacobs and Korsten 2004). After sulfur dioxide fumigation, *Penicillium* spp. can become a chief problem in lychee fruit export industry (Qu et al. 2001). The sulfur dioxide fumigation influences the natural ecological balance and augments deterioration owing to saprophytic postharvest colonization of *Penicillium* spp. (Qu et al.

Fig. 6.3 Postharvest fungal infection to lychee fruit

2001). Slight cracks detected during the fruit development process and caused during postharvest handling can provide a point of entry for deteriorating pathogens that may colonize the fruit surface (Scott et al. 1982; Coates et al. 1994).

6.4 Substitution of SO_2 Fumigation to Improve Postharvest

Presently, there is a continuing search for substitute sulfur-free postharvest know-hows to retain the complete or total quality of the lychee fruit during transportation and cold storage to please the increasing consumer demand for high-quality fruit, which is free of possibly harmful chemicals and microorganisms. Search for appropriate replacements for sulfur dioxide fumigation was started in lychee fruit-growing countries in 1990s. Every part of the technologies developed are focused on avoiding or diminishing postharvest browning, dehydration, and deterioration, thereby extending the shelf life and storage life and retaining overall fruit quality (Underhill et al. 1997).

6.4.1 Heat Therapy

In this technique, the lychee fruit exposed to steam treatment at 98 °C for 30 s followed by water cooling in distilled aqua at 0 pH for 5 min preserved the red color of the skin pericarp during stowage. However, this technique has failed to reach commercial acceptance due to the steaming process which affected the edible portion (aril flesh) of the lychee fruit (Zauberman et al. 1990). This treatment was further improved by dropping the steam treatment to 2 s, cooling in water at 0 pH and coating the fruit with Vapogard® chemical (1%), an antitranspirant, which retained the red color of the skin pericarp without discoloration of the aril and also prevents water loss. It has been suggested that copigmentation or complexing of anthocyanin might increase the stability of the pigment (Kaiser 1995).However, Kaiser's cure failed to demonstrate direct indication of copigmentation, and the observation on red color retention could be owing to the straight effect of the pH (Joas et al. 2005).

Another heat treatment, known as vapor heat treatment at 45 °C temperature for approx. 42 min, has been reported to maintain the quality of "Tai So" and "Wai Chee" lychee varieties at 5 °C for 4 weeks, which retains the appearance and prevents disease control (Jiang et al. 1996). Though, the accomplishment of the vapor heat treatment is cultivar dependent, which relies on anatomical features of the skin pericarp such as fatness (Jacobi et al. 1993; Jiang et al. 1996). In vulnerable cultivars, such as "Kwai May Pink," the vapor heat treatment can result in loss of membrane integrity, electrolyte escape, polyphenol oxidase activation, fluctuation in pH, and skin pericarp browning (Wong et al. 1991; Jacobi et al. 1993). The Taiwanese lychee varieties have been reported to respond well to the vapor heat treatment which suggests that cultivars were more heat tolerant (Jiang et al. 2003). The lychee fruit sprayed with hot water by brushing, followed by treatment of HCl and prochloraz® (N-propyl-N-[2-(2,4,6-trichlorophenoxy) ethyl] imidazole-1-carboxamide) dips, upheld uniform red color and admirable eating quality in terms of palate and savor during storage for minimum of 35 days (Ketsa et al. 1992). The hot water brushing at 25 °C for 20 s has been observed to lessen or prevent polyphenol oxidase activity in skin pericarp by homogeneously exposing the fruit to the acid by a brushing action. Though, the accomplishment of the hot water brushing relies on fungicidal treatment also. However, the fungal growth is controlled by providing low temperatures; transfer of lychee fruit to market shelf temperature increases colonization by fungal pathogens. As per the opinion of Schlack (1991) and Lichter et al. (2000), the hot water brushing technique does not provide any antifungal protection to lychee fruit. Commercial application of the hot water brushing remains a question until a suitable alternative method is found to replace the prochloraz dip to control the deterioration at consumer level or market shelf. The hot water spray is preferred over the hot water dip; though both the methods are equally effective and good in retaining the red color of the skin pericarp, but fungal rot is a main problem since hot water does not provide extra protection, but it may deteriorate the flesh quality (Lichter et al. 2000; Olesen et al. 2004). Though, a contrary observation has been reported by Lee and Wicker (1991) on heat treatment methods of lychee cv. "Guiwei." The heat treatments amplified anthocyanase enzyme activity in the skin pericarp with a rapid discoloration and a successive decay in anthocyanin pigment. Additionally, the application of hot water dip treatments at 50 °C for 2 min, or at 55 °C for 1 min, caused harmful effects on the skin pericarp color, the quality parameters, and also the surface structure, e.g., flattening of highly ornamented pericarp surface, homogeneous with occasionally lifted wax plates owing to the melting of wax layer (Sivakumar and Korsten 2004).

6.4.2 Gamma Rays Treatment

Irradiation along with low-temperature storage may be suggested as an alternative to chemical fumigation during short-term storage process, which is around less than 10 days, Ilangantileke et al. (1993). The irradiation cure exhibited the differential responses with respect to lychee variety and dosage. Akamine and Goo (1977) and

Ilangantileke et al. (1993) reported irradiation up to 1 kGy dose, followed by treatment with low-temperature storage, which preserved the consumable quality of Thai lychee by lessening the loss of red pericarp color and decay. Though, it was unsuccessful to retain the complete fruit quality during prolonged cold storage process (Akamine and Goo 1977). Additionally, the irradiation method is not commercially practiced in many countries for fresh commodities owing to the psychological opinion of consumers and users, considering the safety of irradiated food for the human use (Holcroft et al. 2005). Since it is also an ineffective process in retaining the value attributes of lychee fruit during long-term stowage exceeding 16 days, therefore, the irradiation process would not be practical to employ (Ilangantileke et al. 1993).

6.5 Postharvest Dip Management

Application of dissimilar postharvest treatments has been investigated to upsurge the stowage life of lychee fruit at low temperatures (2–5 °C). Ethephon has been recommended for the retention of red color at low-temperature storage for 30 days (Sadhu and Chattopadhyay 1989). Chemicals such as polyamines such as putrescine, spermine, or spermidine (1 mmol/l) in combination with potential fungicides have been reported to postpone or lessen ethylene production and enzyme POD activity and also retain membrane integrity, which guaranteed the separation of the enzyme and the substrates. Kaiser et al. (1995) have recommended the pooled application of glutathione and citric acid to lessen the browning of the lychee fruit. Though use of glutathione is safe for human health, the blend treatment with citric acid caused inhibition of polyphenol oxidase and greater deposit levels of glutathione, which has been detected specially in the inedible portion of the fruit. This application was merely operative in controlling browning in storage up to 4 days. The lychee fruit cv. "Huaizi" dipped in 1% HCl for 6 min and stored at temperature of 25 °C and 80–90% relative humidity exhibited the best fruit color with minimal pericarp damage even after 1 day of storage (Jiang et al. 2004) (Fig. 6.4).

Fig. 6.4 Postharvest dip treatment of lychee fruit

As per the report of Duvenhage (1994), the treatment of HCl constrained polyphenol oxidase activity in the skin pericarp and maintained high anthocyanin pigment content, which retained the red color. The treatment of HCl stabilized the pH change which maintained the anthocyanin pigment content in the skin pericarp tissues (Zauberman et al. 1990). The HCl dip cure inhibited the enzyme anthocyanase activity in lychee cv. "Guiwei" (Lee and Wicker 1991; Hu et al. 2005).

In variety of "Mauritius," chemical chitosan at 0.1% concentration reduced microbial decay and showed the antimicrobial properties of chemical chitosan (Sivakumar et al. 2005). Bhushan et al. (2015) hypothesized that the filmogenic chitosan coating around the lychee fruit could modify its endogenous carbon dioxide and oxygen levels, which could result in a reduced supply of O_2 for the enzymatic oxidation reaction of the anthocyanin pigment. Chitosan coating, combined with acidification, formed an acid coat, which further stabilized the acidification of epicarp during the dipping treatment (Joas et al. 2005; Kumar et al. 2013). When the fruit was transported from cold storage to market shelf or consumer conditions at ambient temperature of (25 °C), the lychee skin pericarp turned brown, losing its visual quality, and therefore lost marketable appeal. On the other hand, Jiang et al. (2005) reported that the use of 2% chitosan coating soon after cold storage which extended the shelf life of fruit for 12 h at 25 °C. The chitosan dipping after cold storage protected the fruit skin pericarp from browning and deterioration and retained the physicochemical properties of the edible portion of the fruit (Jiang et al. 2003, 2005).

6.5.1 Chemical Management

Plentiful fungicides such as iprodione, benomyl, prochloraz, and thiabendazole are tested with variable levels of effectiveness, for control of postharvest diseases in lychee (Lichter et al. 2000). Though, escalating international unease over the random utilization of fungicides on the consumables and its ensuing damaging upshot on environment made the chemical control less popular (Jiang and Fu 1998; Kumar et al. 2013). Due to stringent policies, particularly in the European Union, the exercise of numerous chemicals is presently prohibited, and many have been withdrawn from the market. Eventually, their incessant application can result in building up of the pathogen resistance. The use of chemicals like SO_2 fumigation is habitually liable for elimination of the majority microbial growth on the surface of the fruit, leaving a space for the decay-causing organisms (Jiang et al. 1997; Kaiser 1998; Kumar et al. 2013).

6.5.2 Storage at Controlled Environment

The lychee fruit cv. "Huaizhi", stored under controlled environmental condition of 3–5% of carbon dioxide and oxygen level at 1 °C and 90% Relative humidity showed excellent browning control, even holding on to the fruit quality for about 30

days (Jiang and Fu 1998). Duan et al. (2004) suggested that Lychee cv. "Huaizhi" stored in 100% oxygen (pure oxygen) and 0% CO_2 for 6 days at a temperature of 28 °C demonstrated considerably diminished pericarp browning. It is apparent from their study that the pure oxygen subdued the actions of PPO and the anthocyanase concerned in enzymatic browning mechanism. Consequently, the atmosphere with 100% oxygen facilitates the prevention of the degradation of anthocyanin by averting the hydrolysis of the sugar moieties, i.e., anthocyanin to the anthocyanidin, and further the breakdown of anthocyanidin by the PPO to brown polymers. Pang et al. (2001) suggested that elevated levels of the anthocyanins in lychee pericarp at the end of 6 days of storage are present. Duan et al. (2004) showed that the appliance of the pure oxygen sustained raised levels of total soluble solids and the titratable acidity in aril of lychee. The application of elevated O_2 storage (70% oxygen + 0% CO_2) for 7 days followed with 5% oxygen and 5% CO_2 storage on 3 °C with 95% RH for 14, 24, and 48 days illustrated a noteworthy drop of the decay, whereas browning is amplified following 14 days in cv. "Heiye." Techavuthiporn et al. (2006) showed that the anthocyanidin content in pericarp declined slowly as compared with control and ethanol content accountable for rotten flavor was decreased when the fruit was exposed to the 70% oxygen for 7 days, followed by 5% oxygen and 5% CO_2 on 5 °C. According to Lonsdale and Kremer-Köhne (1991), the advantageous effects of elevated oxygen in the controlled atmosphere storage to limit PPO and POD activities maintained high anthocyanin levels, avoids decay, and retained superior fruit quality. The super atmosphere oxygen at 50% demonstrated a vital effect on the inhibition of browning in lychee cv. "Hong Huay" for 8 days more than the ambient temperature; however, amplified concentrations up to 70% do not illustrate extra control of browning (Lonsdale and Kremer-Köhne 1991; Techavuthiporn et al. 2006; Sangeeta and Chopra 2015). Nevertheless, the effect of the pure or greater oxygen concentrations for long-term storage on maintenance of the whole quality related to storage life and disease advancement requires additional inquiry.

6.6 Biocontrol Agents

The postharvest decompose control in lychee has lately become the focal point for the utilization of naturally occurring antagonists like nonpathogenic bacteria or yeasts, owing to the challenges allied with the chemical disease control. Utilization of antagonists to manage postharvest diseases is probably more effectual than in the field, since the storage atmosphere around the fruit could be administered without difficulty to favor the growth of antagonist. The biocontrol agent *Bacillus subtilis* was established to be effectual in the controlling of postharvest fester in lychee cv. "Madras" (Korsten et al. 1993) as well as "Huaizi" (Lemmer 2002; Sivakumar et al. 2007) when reserved in cold storage at 5 °C. The mechanism of antagonism of this microbe was accounted as the antibiotic action of iturin, a cyclic polypeptide (Gueldner et al. 1988; Sangeeta and Chopra 2015). Treating with the cell-free suspension (extract) of an antagonist was successful in controlling decay of fruit during storage period of 30 days at 5 °C. Even if appliance of antagonist does not alter

consumption quality considerably, it causes modest browning on pericarp. The surroundings for this reason need to be more favorable for both the antagonist survival and withholding fruit excellence. Additional research is needed on novel biocontrol mediators and their utilization as substitute to the chemical treatments in lychee industry, with the purpose of maintaining a defensive barrier, which does not permit the fungal infection and devoids negotiation with the fruit integrity (Sivakumar et al. 2007; Sangeeta and Chopra 2015).

6.7 Possible Future Postharvest Handling Practices

The postharvest handling approaches have to be improved throughout the system of fruit treatment, i.e., starting from harvesting practices, harvest time, pre-cooling, packing line operations, and storing at low temperature to the market and eventually to the consumer. Most significantly, the cold chain management has to be maintained nicely in order to guarantee the excellent fruit quality. The lychee fruit has to be harvested prior to high day temperatures that occur from late morning to early afternoon, which results in water loss. It has been shown that from the fruit water potential that a rapid loss of turgor occurs early in the morning (~8 am), with a recovery in the afternoon (~4 pm) (Wall et al. 2006). The loss of turgor can influence the fruit weight and appearance (Wall et al. 2006), with negative financial consequences. Research has also exhibited the potential to rehydrate the lychee fruit after harvest to avert or lessen the skin browning process. The potential to rehydrate the fruit has been observed to lessen during the first hour following the harvest (Olesen et al. 2003; Li et al. 2014). Still, it remains a query whether rehydration process can be used commercially, as the period from harvest to delivery to the packhouse may take longer than 1 h. The lychee fruit has to be pre-cooled instantaneously after collection to eradicate the field heat and deliver an effective cold chain management system during stowage and transport (Li et al. 2014). Application of water cooling treatment at 0–2 °C is also recommended to enhance shelf life of lychee. However, the fruit must be dried, or water droplets should be removed prior to packing after water cooling, since the presence of water droplets on the fruit surface can invite microbes and enhance decay development during stowage and transport. The use of forced air cooling has also been recommended, which will become more effective when the cold room has humidifiers to maintain around ~90% humidity to prevent dehydration during the required air cooling process. The maturity standards for harvesting of each variety must be adopted according to maturity standards developed, which relies on the growth conditions and the climatic factors. The ripeness standards in terms of SSC: TA has also affected the postharvest functioning with respect to dissimilar know-how. Selecting lychee varieties with good postharvest characters such as rigid pericarp and less browning or susceptibility for browning will be more useful and practical and finally beneficial for the future and for the end users (Li et al. 2014).

References

Akamine EK, Goo T (1977) Effects of gamma irradiation on shelf life of fresh lychees (*Litchi chinensis* Sonn.). Hawaii Agric Exp Sta Bull 169:20–24

Azizah O, Saari N, Saleh R, Bakar J, Zainal ND, Yacob M (2009) Postharvest handling practices on selected local fruits and vegetables at different levels of the distribution chain. J Agribus Mark 2:39–53

Bhat RS, Al-daihan S (2014) Antimicrobial activity of *Litchi chinensis* and *Nephelium lappaceum* aqueous seed extracts against some pathogenic bacterial strains. King Saud Univ J King Saud Univ–Sci 26:79–82

Bhushan B, Pal A, Narwal R, Meena VS, Sharma PC, Singh J (2015) Combinatorial approaches for controlling pericarp browning in Litchi (*Litchi chinensis*) fruit. J Food Sci Technol 52(9):5418–5426

Chapman KR (1985) Sapindaceae. In: Tropical tree fruits for Australia. Queensland Department of Primary Industries, Brisbane, pp 179–191

Coates L, Johnson GI, Sardsud U, Cooke AW (1994) Postharvest diseases of lychee in Australia, and their control. ACIAR Proc 58:68–69

Cronje RB (2008) Effect of fruit development, maturity and harvesting of litchi (*Litchi chinensis* Sonn.) on postharvest fruit quality. Stewart Postharvest Rev 3:3–10

Duan XW, Jiang YM, Su XG, Zhang ZQ (2004) Effects of pure oxygen atmosphere on enzyme browning of harvested litchi fruit. J Hortic Sci Biotechnol 79:859–862

Duvenhage A (1994) Control of postharvest decay and browning of litchi fruit by sodium metabisulphite and low pH dips-an update. Yearbook S Afr Litchi Growers' Assoc 6:36–38

Gaur GS, Bajpai PN (1978) Some aspects of developmental physiology of the litchi fruit. Indian J Hortic 35:173–177

Gueldner RC, Reilly CC, Pusey PL, Costello CE, Arrendale RF, Cox RH, Himmelsbach DS, Crumley FG, Cutler HG (1988) Isolation and identification of iturines as antifungal peptides in biological control of peach-brown rot with Bacillus subtilis. J Agric Food Chem 36:366–370

Holcroft DM, Lin H, Ketsa S (2005) Harvesting and storage. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CAB International, Wellingford, pp 273–295

Hu HC, Chen DC, Li P, Ouyang R, Gao FF, Wang HC, Dong J (2001) Effects of bagging on fruit coloration and phenylalanine ammonia lyase and polyphenol oxidase in 'Feizixiao' litchi. Acta Hortic 558:273–278

Hu WR, Pang XQ, Liu SZ, Zhang ZQ, Ji ZL (2005) Effect of postharvest handling on the anthocyanin content and anthocyanase activity in litchi pericarp (English Abstract). J Fruit Sci 22:224–228

Huang H (2005) Fruit set, development and maturation. A. Litchi. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CAB International, Wellingford, pp 115–140

Huang H, Qiu Y (1987) Growth correlations and assimilate partitioning in the arillate fruit of *Litchi chinensis* Sonn. Aust J Plant Physiol 14:181–188

Huang X, Yuan W, Wang H, Li J, Huang H (2004a) Early calcium accumulation may play a role in spongy tissue formation in litchi pericarp. J Hortic Sci Biotechnol 79(6):947–952

Huang X, Yuan W, Wang H, Li J, Huang H, Shi L, Jinhua Y (2004b) Linking cracking resistance and fruit desiccation rate to pericarp structure in litchi (*Litchi chinensis* Sonn.). J Hortic Sci Biotechnol 79(6):897–905

Huang XM, Subhadrabandhu S, Mitra SK, Ben-Arie R, Stern RA (2005) Origin, history, production and processing. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CAB International, Wellingford, pp 1–23

Ilangantileke SG, Noomhorn A, Upadhyay IP, Srinivas Rao M (1993) Effect of irradiation and storage temperature on the shelf life and quality of Thai lychee. In: Champ BR, Highley E, Johnson GI (eds) Postharvest handling of tropical fruits, ACIAR Conference Proceedings, Chang Mai, Thailand, 50:352–354

Jacobi KK, Wong LS, Giles JE (1993) Lychee (*Litchi chinensis* Sonn.) fruit quality following vapour heat\ treatment and cool storage. Postharvest Biol Technol 3:119.

Jacobs R, Korsten L (2004) Preliminary identification of Penicillium species isolated through the litchi export chain from South Africa to distribution centres in the Netherlands and United Kingdom. In: South African litchi growers' association yearbook, vol 16. pp 34–39

Jiang YM, Fu JR (1998) Inhibition of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. Food Chem 62:49–52

Jiang YM, Liu SX, Li YB, Chen F (1996) A reassessment of heat treatment as a means of reducing the occurrence of browning of litchi (*Litchi chinensis* Sonn.) fruit. Inter. J Trop Agric 14:163–167

Jiang YM, Liu SX, Chen F, Li YB, Zhang DL (1997) The control of postharvest browning of litchi fruit by sodium bisulphite and hydrochloric acid. Trop Sci 37(3):189–192

Jiang Y, Yao L, Lichter A, Li J (2003) Postharvest biology and technology of litchi fruit. Food Agric Environ 1(2):76–81

Jiang YM, Li Y, Li J (2004) Browning control, shelf life extension and quality maintenance of frozen litchi fruit by hydrochloric acid. J Food Eng 63:147–151

Jiang YM, Li J, Jiang W (2005) Effects of chitosan coating on shelf life of cold-stored litchi fruit at ambient temperature. Lebensm Wiss Technol 38:757–761

Joas J, Caro Y, Ducamp MN, Reynes M (2005) Postharvest control of pericarp browning of litchi fruit (*Litchi chinensis* Sonn cv Kwai Mi) by treatment with chitosan and organic acids I. Effect of pH and pericarp dehydration. Postharvest Biol Technol 38:128–136

Kaiser C (1995) Litchi (*Litchi chinensis* Sonn.) pericarp colour retention – alternatives to sulphur. S Afr Lychee Growers' Assoc Yearbook 7:47–52

Kaiser C (1998) An overview of postharvest storage of mauritius litchi fruit. J South Afr Soc Hortic Sci 9:57–60

Kaiser C, Levin J, Wolstenholme BN (1995) Vapour, heat and low pH dips improve litchi (*Litchi chinensis* Sonri) pericarp colour retention. J S Afr Soc Hortic Sci 5:7–10

Ketsa S, Leelawatana K, Subhadrabandhu S (1992) Effect of pre and post storage acid dipping on browning of lychee fruits. Acta Hortic 321:726–731

Korsten L, De Villiers EE, De Jager ES, Van Harmelen MWS, Heitmann A (1993) Biological control of litchi fruit diseases. S Afr Litchi Growers' Assoc Yearbook 5:36–40

Kremer-Köhne S (1993) SO_2 residues in litchi fruit after post-harvest SO_2 treatments. S Afr Litchi Growers' Assoc Yearbook 5:15–16

Kumar D, Mishra DS, Chakraborty B, Kumar P (2013) Pericarp browning and quality management of litchi fruit by anti-oxidants and salicylic acid during ambient storage. J Food Sci Technol 50(4):797–802

Kumari A (1989) Nucleic acid, protein and amino acid metabolism in litchi seeds during maturation. Plant Physiol Biochem (New Delhi) 16(1):27–33

Kumari A (1990) Changes in pigmentation of seed coat of *Litchi chinensis* Sonn. during different stages of development. Soc Plant Physiol Biochem 2:1356–1360

Kumari A (1993) Changes in carbohydrate composition of seeds of *Litchi chinensis* Sonn. during maturation. J Sci Food Agric 61(2):205–210

Lee HS, Wicker L (1991) Anthocyanin pigments in the skin of lychee fruit. J Food Sci 56:466–468

Lemmer D (2002) Report on the Chinese litchi industry. S Afr Litchi Growers' Assoc Yearbook 13:1–4

Lemmer D, Kruger FJ (2000) Factors influencing SO_2 residues on commercially fumigated HLH Mauritius and McLean's Red litchi fruit. S Afr Litchi Growers' Assoc Yearbook 11:42–46

Lemmer D, Kruger FJ, Froneman IJ (2000) Evaluation of the postharvest characteristics of litchi fruit from the cultivar development programme. S Afr Litchi Growers' Assoc Yearbook 11:33–36

Li JG (2003) A revised division of the developmental stages in litchi fruit. Acta Hortic Sin 30(3):307–310

Li JG, Huang HB, Gao FF, Huang XM, Wang HC (2001) An overview of litchi fruit cracking. Acta Hortic 558:205–208

Li J, Zhou B, Huang X, Huang H (2005) The roles of cytokinins and abscisic acid in the pericarp of litchi (*Litchi chinensis* Sonn.) in determining fruit size. J Hortic Sci Biotechnol 80(5):571–574

Li WC, Wu JY, Zhang HN, Shi SY, Liu LQ, Shu B, Liang QZ, Xie ZH, Wei YZ (2014) De novo assembly and characterization of pericarp transcriptome and identification of candidate genes mediating fruit cracking in *Litchi chinensis* Sonn. Int J Mol Sci 15(10):17667–17685

Lichter A, Dvir O, Rot I, Akerman M, Regev R, Wiesblum A, Fallik E, Zauberman G, Fuchs Y (2000) Hot water brushing: an alternative method to SO_2 fumigation for color retention of litchi fruits. Postharvest Biol Technol 18:235–244

Lin SC, Chiang HL (1998) Postharvest handling system for subtropical fruits in Taiwan. Ext Bull Food Fertil Technol Center Taiwan 268:10–17

Lin ZF, Li SS, Zhang DL, Liu SX, Li YB, Lin GZ, Chen MD (1998) The changes of oxidation and peroxidation in postharvest litchi fruit. Acta Bot Sin 30:383–387

Lonsdale JH, Kremer-Köhne S (1991) Maintaining market quality of fresh litchis during storage. Part 2: control of postharvest decay. S Afr Litchi Growers' Assoc Yearbook 10:18–20

Macfie GB Jr (1955) Wrapping and packaging of fresh lychee. Florida Lychee Growers Assoc Yearbook 1:25–28

Maguire KM, Lang A, Banks NH, Hall A, Hopcroft D, Bennett R (1999) Relationship between water vapour permeance of apples and micro-cracking of the cuticle. Postharvest Biol Technol 17:89–96

Menzel C (2001) The physiology of growth and cropping in lychee. In: The SA lychee growers assoc yearbook, vol 12. pp 9–14

Menzel CM, Simpson DR (1994) Lychee. In: Schaffer B, Andersen PC (eds) The handbook of environmental physiology of fruit crops. CRC Press, Boca Raton, pp 123–141

Menzel CM, Xuming H, Chengming L (2005) Cultivars and plant improvement. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CAB International, Wellingford, pp 59–86

Mitra SK (2006) Overview of lychee production in the Asia-Pacific region. Food Agric Org Publ 22(04):5–13

Olesen T, Wiltshire N, McConchie C (2003) Improved postharvest handling of lychee. Rural Industries Research and Development Corporation Publication 03/111

Olesen T, Nacey L, Wiltshire N, O'Brien S (2004) Hot water treatments for the control of rots on harvested litchi (*Litchi chinensis* Sonn.) fruit. Postharvest Biol Technol 32:135–146

Pang XQ, Zhang ZQ, Duan XW, Ji ZL (2001) Influence of pH and active oxygen on the stability of anthocyanins from litchi pericarp. Proceedings of the First International Symposium on Litchi and Longan, Guangzhou, China, 16–19 June 2000. Acta Hortic 558:339–342

Paull RE, Chen NJ, Deputy J, Huang H, Cheng G, Gao F (1984) Litchi growth and compositional changes during fruit development. J Am Soc Hortic Sci 109(60):817–821

Peng J, Tang X, Feng H (2004) Effects of brassinolide on the physiological properties of litchi pericarp (*Litchi chinensis* cv. Nuomoci). Sci Hortic 101:407–416

Prusky D, Keen NT (1995) Inducible preformed compounds and their involvement in the resistance of plant pathogens. In: Reuveni R (ed) Novel approaches to integrated pest management. Lewis Publishers, Boca Raton, pp 139–152

Qu HX, Sun GZ, Jiang YM, Zhu XR (2001) Induction of several pathogenesis related proteins in litchi after inoculation with *Peronophythora litchi*. Acta Hortic 558:439–442

Qui Y, Cheng J, Qu L, Wang B, Yuam P (1999) Relationship between fruit cracking and endogenous hormones in 'nuomoci' litchi variety. J Fruit Sci 16:276–279

Revathy J, Narasimham P (1997) Litchi (*Litchi chinensis* Sonn.) fruit: influence of pre and postharvest factors on storage life and quality for export trade – a critical appraisal. J Food Sci Technol 34:1–19

Sadhu MK, Chattopadhyay G (1989) Effect of a postharvest fruit dip in ethephon on the ripening of litchi fruits. J Hortic Sci 64:239–242

Sangeeta S, Chopra CS (2015) Reducing post-harvest losses of litchi by processing. Int J Processing Post Harvest Technol 6(2):184–189
Scheer A (1994) Reducing water loss of horticultural and arable products during long term storage. Acta Hortic 368:511–522
Schlack SHC (1991) Hot-water immersion disinfestation treatment for lychee infested with Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). University of Queensland, Gatton College, Gatton
Schoeman MH, Botha A, Kruger FJ (2005) Refinement of the maturity cut off points for sulphur fumigated HLH Mauritius litchi fruit. S Afr Litchi Growers' Association Yearbook 17:46–49
Schoeman MH, Botha A, Kruger FJ (2006) Prediction and improvement of storage potential of South African litchi fruit in the country of import. S Afr Litchi Growers' Assoc Yearbook 18:71–75
Scott KJ, Brown BI, Chaplin GR, Wilcox ME, Bain JM (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic film. Sci Hortic 16:253–262
Sharma SB, Ray PK, Rai R (1986) The use of plant growth regulators for early ripening of litchi (*Litchi chinensis* Sonn.). J Hortic Sci 61(4):533–534
Shi J, Wang C, An X, Li J, Zhao M (2001) Postharvest physiology, storage and transportation of litchi fruits – a review. Proceedings of the First International Symposium on Litchi and Longan, Guangzhou, China, 16-19 June 2000. Acta Hortic 558:387–391
Sivakumar D, Korsten L (2004) A preliminary study on the changes of epicuticular wax layer of litchi (cv. Mauritius) with respect to commercial packing line operations. S Afr Litchi Growers' Assoc Yearbook 16:31–33
Sivakumar D, Regnier T, Demoz B, Korsten L (2005) Effect of post-harvest treatments on overall quality retention in litchi fruit during low temperature storage. J Hortic Sci Biotechnol 80:32–38
Sivakumar D, Zeeman K, Korsten L (2007) Effect of a biocontrol agent (Bacillus subtilis) and modified atmosphere packaging on postharvest decay control and quality retention of litchi during storage. Phytoparasitica 35(5):507–518
Stern RA, Stern D, Harpaz M, Gazit S (2000) Application of 2,4,5-TP, 3,5,6-TPA, and combinations thereof increase lychee fruit size and yield. Hortic Sci 35:661–664
Steyn EMA, Robbertse PJ (1992) Is aril development in the litchi triggered by pollen tube growth? S Afr J Bot 58(4):258–262
Techavuthiporn C, Nivomlao W, Kanlavanarat S (2006) Superatmospheric oxygen retards pericarp browning of litchi cv. 'Hong Huay'. Acta Hortic 712:629–634
Underhill SJR, Critchley C (1992) The physiology and anatomy of lychee (*Litchi chinensis* Sonn.) pericarp during fruit development. J Hort Sci 67(4):437–444
Underhill SJR, Simons DH (1993) Lychee (*Litchi chinensis* Sonn.) pericarp desiccation and the importance of postharvest micro-cracking. Sci Hortic 54:287–294
Underhill SJR, Coates LM, Saks Y (1997) Litchi. In: Mitra SK (ed) Postharvest physiology and storage of tropical and subtropical fruits. CAB International, Wallingford, pp 191–208
Wall MM (2006) Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. J Food Compos Anal 19:655–663
Wang H, Huang X, Huang H (2001) Litchi fruit maturation studies: changes in abscisic acid and 1-aminocyclopropane-1-carboxylic acid and the effects of cytokinin and ethylene on coloration in cv. Feizixiao Acta Hortic 558:267–269
Wang H, Huang X, Hu G, Yang Z, Huang H (2005) A comparative study of chlorophyll loss and its related mechanism during fruit maturation in the pericarp of fast- and slow degreening litchi pericarp. Scientia Hortic 106:247–257
Wang H, Huang H, Huang X (2007) Differential effects of abscisic acid and ethylene on the fruit maturation of *Litchi chinensis* Sonn. Plant Growth Regul 52:189–198
Yin J, Gao F, Hu G, Zhu S (2001) The regulation of litchi maturation and coloration by abscisic acid and ethylene. Acta Hortic 558:293–296

Zauberman G, Ronen R, Akerman M, Fuchs Y (1990) Low pH treatment protects litchi fruit colour. Acta Hortic 269:309–314

Zhang Z, Yuan P, Wang B, Qiu Y, Li J (1997) Litchi pictorial narration of cultivation. Pomology Research Institute, Guangdong Academy of Agricultural Science, Guangdong, pp 123–129

Secondary Metabolite Credentials and Biological Properties of *Litchi chinensis*

7

Deepak Ganjewala

Abstract

Litchi (*Litchi chinensis* Sonn.) is a medium sized evergreen tree indigenous to China, Vietnam, Indonesia and Philippines. Litchi and its different parts viz., leaves, flowers, fruits, seeds, and pericarp contain significantly high amounts of phenolics and flavonoids compounds which are potential sources of natural anti-oxidants. Many previously published reports have demonstrated plenty of bioactivities, such as anti-oxidant, antimicrobial, hepato-protective, anti-cancer, cytotoxic, anti-inflammatory, analgesic, and immunomodulatory of litchi and its different parts. Litchi fruit is a good source of food nutrition and highly rich in natural anti-oxidants. It very much liked by consumers because of its delicious taste and beneficial health effects. Consumption of litchi and its products owing to their beneficial health promoting effects is rapidly increasing in humans. Litchi as an important plant of traditional medicine and its promising bioactivities has drawn much attention from researchers all over the world. In this chapter phytochemical composition and important bioactive properties of the litchi and different parts have been discussed with given emphasis to the mechanism of action of bioactive principle.

Keywords

Litchi chinensis • Phenolics • Flavonoids • Proanthocyanidins • Bioactivity • Anti-oxidant • Sapindaceae

D. Ganjewala (✉)
Amity Institute of Biotechnology, Amity University Uttar Pradesh,
Sector-125, Noida 201 303, UP, India
e-mail: dganjewala@amity.edu; deepakganjawala73@yahoo.com

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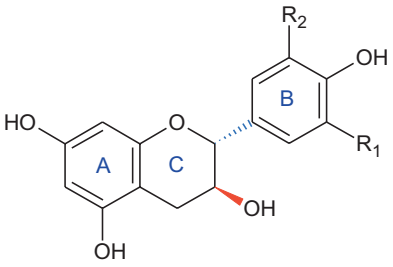
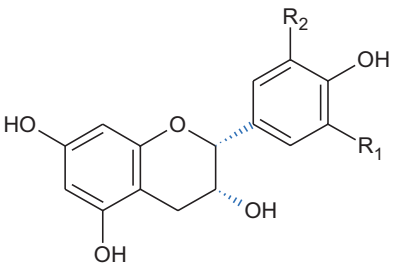
7.1 Introduction

Litchi (*Litchi chinensis* Sonn.) belongs to family Sapindaceae and is a medium-sized evergreen tree indigenous to China, Vietnam, Indonesia, and the Philippines. It has been grown for as long as 1766 B.C. (Menzel 2001). Litchi is cultivated commercially for its palatable sweet fruits all over the tropical and subtropical world (Gontier et al. 2000). China is the leading producer of litchi in the world with an annual production of 950,000 tons. Apart from China, South Africa, Israel, Madagascar, Mauritius, USA, Australia, India, Pakistan, the Philippines, Thailand, Taiwan, Indonesia, Vietnam, and Brazil also have considerable production of litchi (Menzel 2001; Lemmer 2002). In India, Dehradun in the state of Uttarakhand and Muzaffarpur in Bihar are popular for litchi production. There are many cultivars of litchi available which possess considerable genetic variations (Huang et al. 2004; Khurshid et al. 2004), resulting in marked differences in flushing pattern, flush color, flowering ability, fruit color, shape, and size (Waseem et al. 2002; Sivakumar et al. 2010) as well as fruit cracking susceptibility (Huang et al. 2004). Some commercially important varieties of litchi are Mauritius, McLean's Red, Muzaffarpur, Wai Chee, Kwai May Pink, Kwai May Red, Souey Tung, Bengal, and Haak Yip. They exhibit significant differences in nutritional, biochemical, and phytochemical compositions. Litchi fruit is a good source of food nutrition; it is very much liked by consumers because of its delicious taste and possible health benefits. Litchi fruit has medicinal values as it is tonic to the heart, brain, and liver (Painuly et al. 2012). The health effects of litchi fruits are associated with phenolics and flavonoids present in the fruit which are well-known for beneficial anti-oxidant activities (Morton et al. 2000). A number of studies carried out in litchi have revealed the presence of large quantities of phenolics and proanthocyanidins in pericarp, seeds, and pulp of litchi which are potential sources of natural anti-oxidants (Khan et al. 2009). Proanthocyanidins are oligomers and polymers consisted of basic flavon-3-ol units (Fig. 7.1). The major phenolic constituents of litchi leaf, pericarp, seeds, and fruit identified were proanthocyanidins with A- and B-type linkage; proanthocyanidins A1 and A2; procyanidins A2, B2, and D; luteolin; kaempferol; kaempferol 3-O-β-glucoside; kaempferol 3-O-α-rhamnoside; rutin; cinnamtannin B1; gallic acid; chlorogenic acid; caffeic acid; stigmasterol; pinocembrin-7-O-6-O-alpha-L-rhamnopyranosyl-beta-D-glucoside; phlorizin; 3,4-dihydroxyl benzoate; butylated hydroxytoluene; isolariciresinol; methyl shikimate; litchioside D; (−)-pinocembrin 7-O-neohesperidoside; (−)-pinocembrin 7-O-rutinoside; taxifolin 4′-O-β-d-glucopyranoside; kaempferol-7-O-neohesperidoside; and tamarixetin3-O-rutinoside (Castellain et al. 2014; Wen et al. 2015; Li et al. 2012; Jiang et al. 2013; Li and Jiang 2007; Xu et al. 2010, 2011). Chemical structures of major phenolics and other constituents of litchi are presented in Fig. 7.2. Litchi and its different parts due to the presence of the large quantities of phenolics demonstrated many useful bioactivities such as anti-oxidant, antimicrobial, hepatoprotective, anti-cancer/cytotoxic (Wen et al. 2014), anti-inflammatory, analgesic, anti-pyretic (Wang et al. 2006), and immunomodulatory (Zhao et al. 2007) (Table 7.1). Pareek (2015) has deliberately discussed nutritional and biochemical compositions of litchi cultivars, whereas

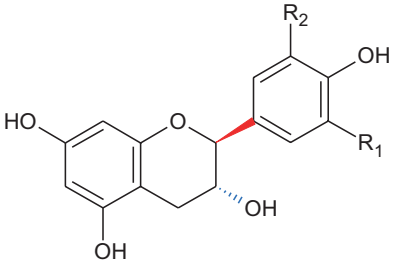


(+)-Afzelechin $R_1 = R_2 = H$
(+)-Catechin $R_1 = OH$, $R_2 = H$
(+)-Gallocatechin $R_1 = R_2 = OH$



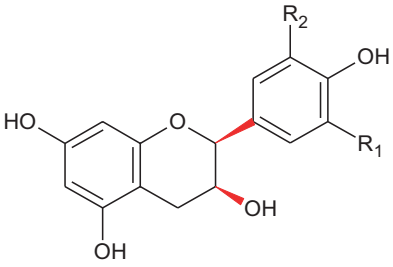
2R, 3R-Flavan-3-ol

(−)-Epiafzelechin $R_1 = R_2 = H$
(−)-Epicatechin $R_1 = OH$, $R_2 = H$
(−)-Epigallocatechin $R_1 = R_2 = OH$



2S, 3R-Flavan-3-ol

(−)-Afzelechin $R_1 = R_2 = H$
(−)-Catechin $R_1 = OH$, $R_2 = H$
(−)-Gallocatechin $R_1 = R_2 = OH$



2S, 3S-Flavan-3-ol

(+)-Epiafzelechin $R_1 = R_2 = H$
(+)-Epicatechin $R_1 = OH$, $R_2 = H$
(+)-Epigallocatechin $R_1 = R_2 = OH$

Fig. 7.1 Structures of the fundamental flavan-3-ol units of phenolic compounds

Kilari and Putta (2016) have covered various aspects like traditional medicinal uses, phytoconstituents, and pharmacological activities of various parts of litchi. Ibrahim and Mohamed (2015) have reviewed botany, distribution, traditional uses, chemical compositions, pharmacological and toxicity studies of litchi. The number of reports on phytochemical composition and bioactivities of litchi has been rapidly increasing as a result of more studies revealing new phytoconstituents with newer bioactivity from litchi and its different parts. The growing number of reports certainly reflected the increasing medicinal and pharmacological significance of the litchi. In the present chapter phytochemical compositions and major bioactive properties of

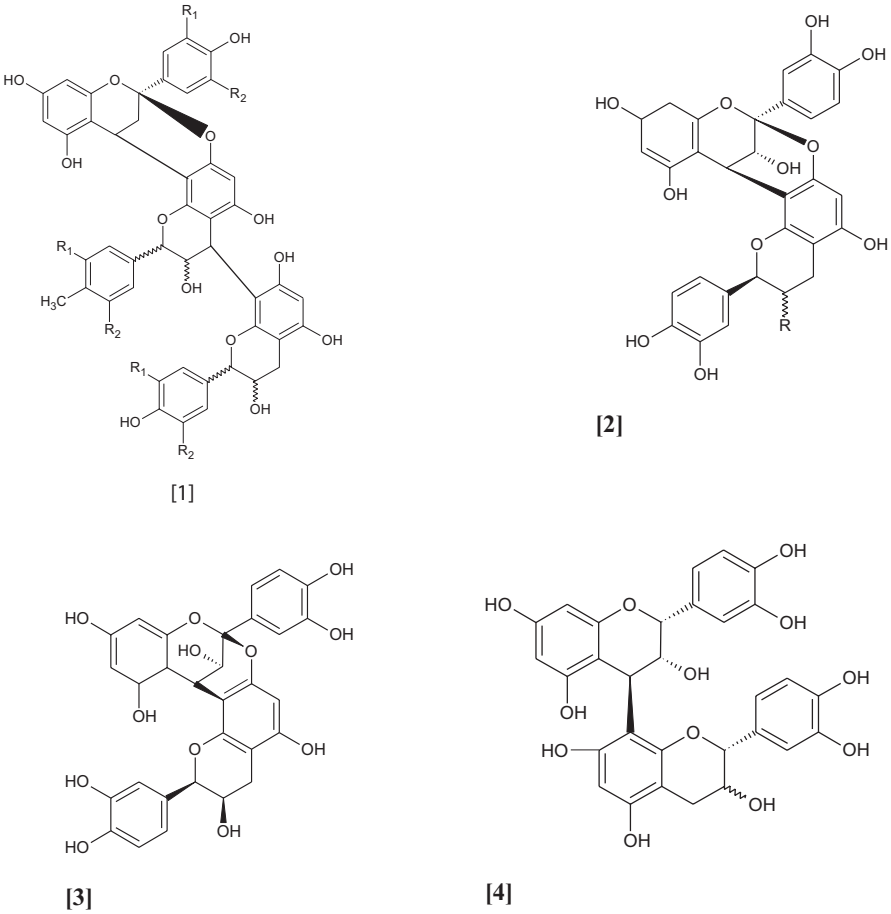
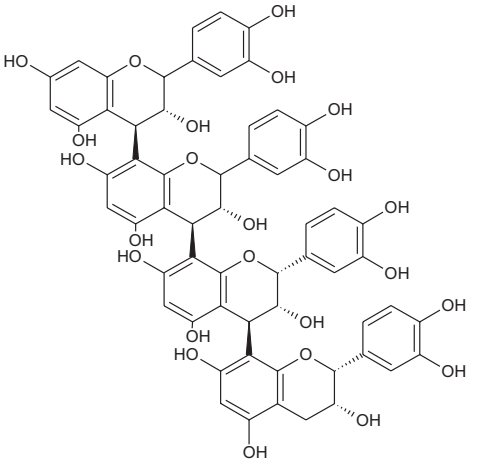


Fig. 7.2 Chemical structures of major constituents of *Litchi chinensis*, proanthocyanidins [1], proanthocyanidins A1 and A2 [2], procyanidin A2 [3], procyanidin B2 [4], procyanidin D [5], luteolin [6], kaempferol [7], kaempferol 3-O-*β*-glucoside [8], kaempferol 3-O-*α*-rhamnoside [9], rutin [10], (−)-secoisolariciresinol 9-*O*-α-L-arabinopyranoside [11], 4,7,7′,8′,9,9′-hexahydroxy-3,3′-dimethoxy-8,4′-oxyneolignan [12], cinnamtannin B1 [13], gentisic acid [14], gallic acid [15], chlorogenic acid [16], caffeic acid [17], 2-(2-hydroxyl-5-(methoxycarbonyl) phenoxy) benzoic acid [18], epicatechin–epicatechin–epicatechin [19], stigmasterol [20], pinocembrin-7-O-6-O--alpha-L-rhamnopyranosyl-beta-D-glucoside [21], 3,5-dihydroxybenzoic acid [22], 3,4-dihydroxybenzaldehyde [23], phlorizin [24], scopoletin [25], litchitannin A1[26], litchitannin A2 [27], aesculitannin A [28], epicatechin–epiafzelechin–epicatechin[29], proanthocyanidin A6 [30], epicatechin-(7,8-bc)-4β-(4-hydroxyphenyl)-dihydro-2(3H)-pyranone[31], 3,4-dihydroxyl benzoate [32], butylated hydroxytoluene [33], isolariciresinol [34], methyl shikimate [35], ethyl shikimate [36], (+)-isolariciresinol 9-*O*-α-L-arabinopyranoside [37], garcimangosone D [38], β-D-glucopyranosyldihydrophaseate [39], citroside [40], malvidin-3-glucoside [41], cyanidin-3-glucoside [42], cyanidin-3-rutinoside [43], quercetin 3-O-rutinoside-7-O-a-L-rhamnosidase [44], quercetin 3-O-glucoside [45], quercetin 3-O-rutinoside [46], litchioside D [47], (−)-pinocembrin 7-O-neohesperidoside [48], (−)-pinocembrin 7-O-rutinoside [49], taxifolin 4′-O-β-d-glucopyranoside [50], kaempferol-7-O-neohesperidoside [51], tamarixetin 3-O-rutinoside [52], dehydrodiepicatechin A [53], (−)-epicatechin, 8-(2-pyrrolidinone-5-yl)-(−)-epicatechin [54],



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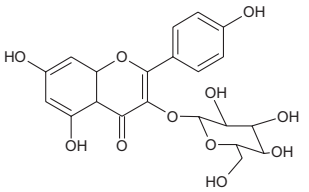

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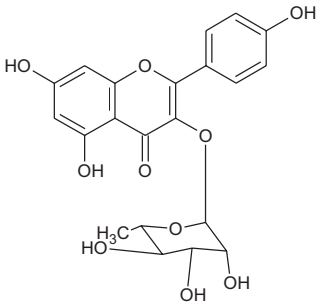

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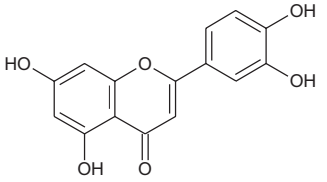

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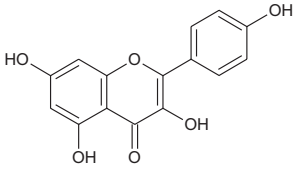

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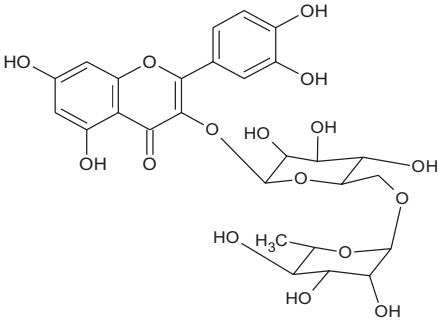
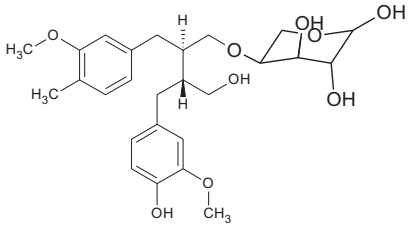


[10]

Fig. 7.2 (continued) (−)-epicatechin 8-C-β-D-glucopyranoside [55], naringenin 7-O-(2,6-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside [56], (2R)-naringenin-7-*O*-(3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) [57], genistein [58]



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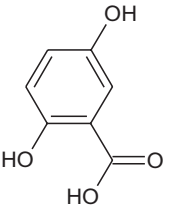

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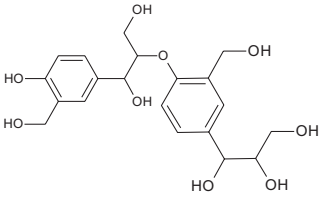

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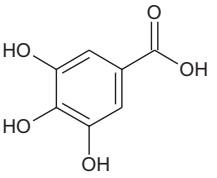

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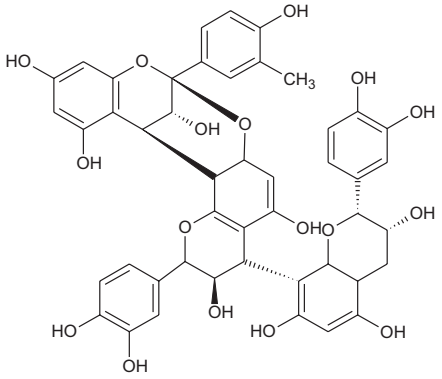

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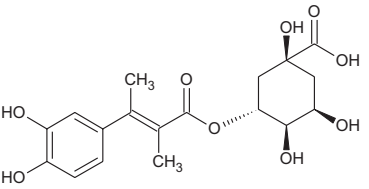

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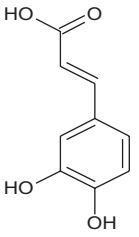

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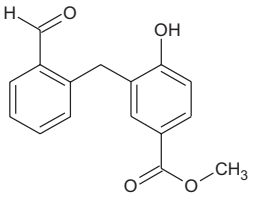

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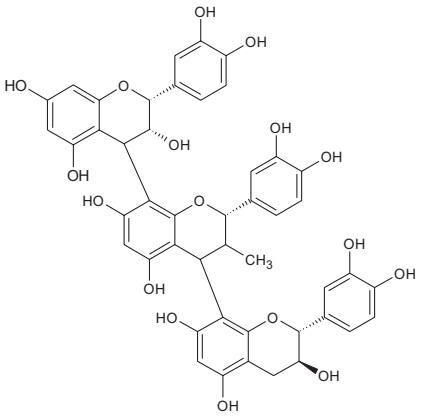

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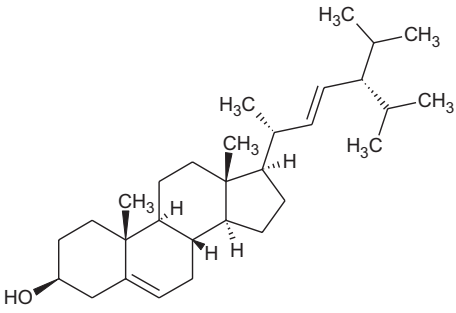

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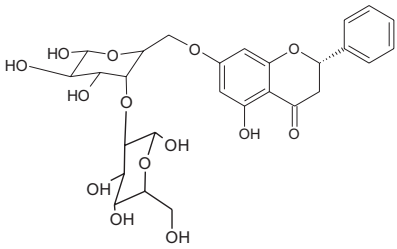

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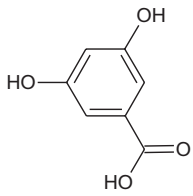

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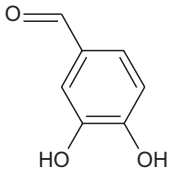

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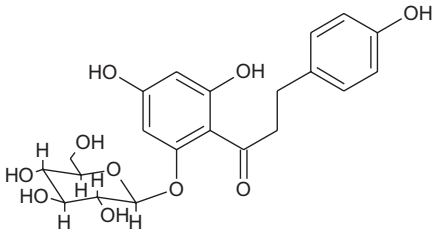

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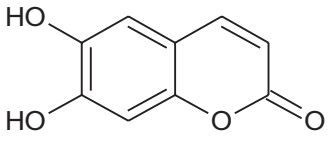

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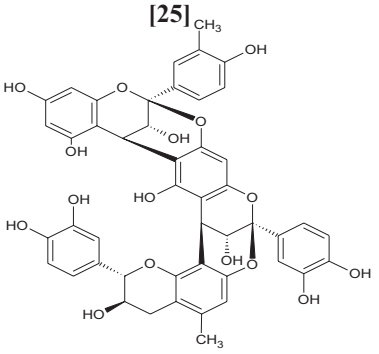

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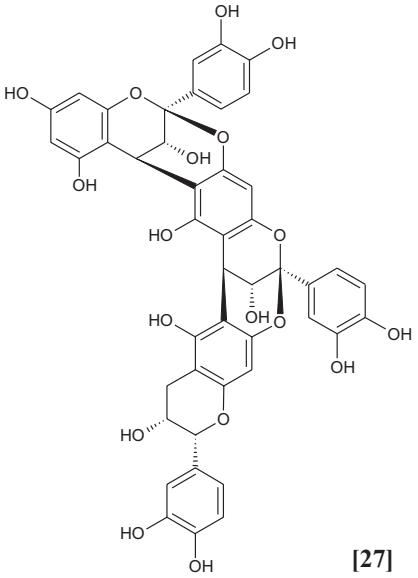

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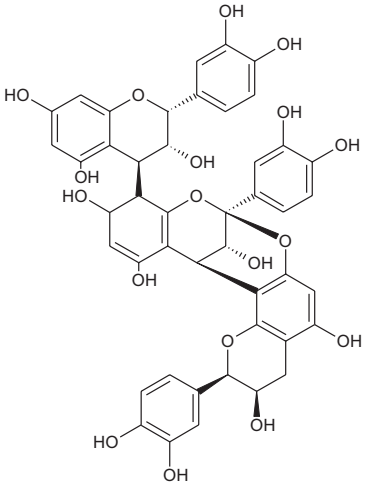

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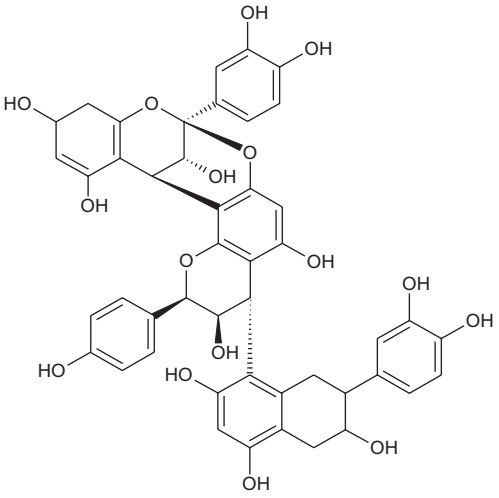

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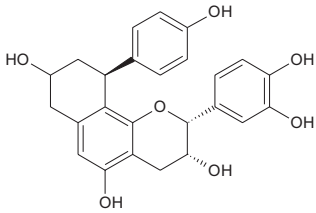

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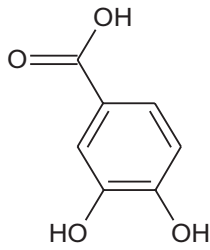

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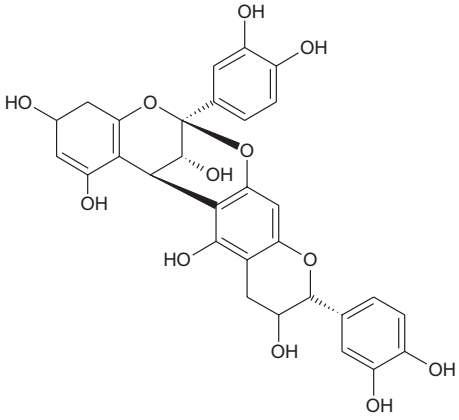

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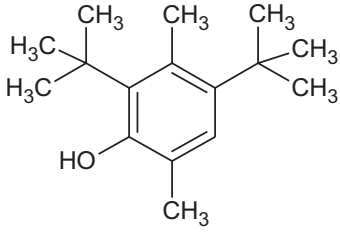

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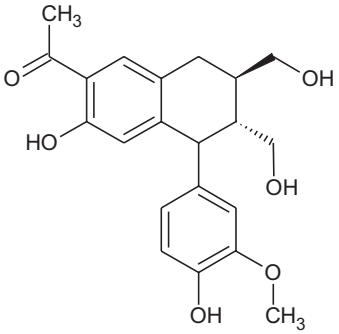

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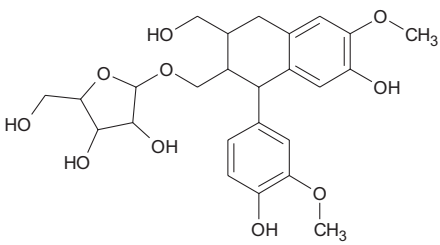

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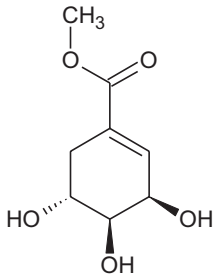

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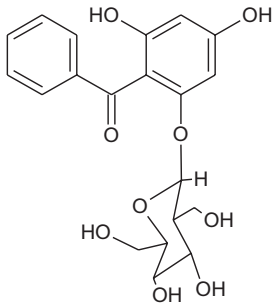

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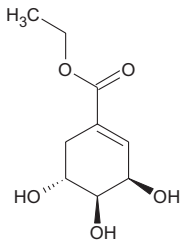

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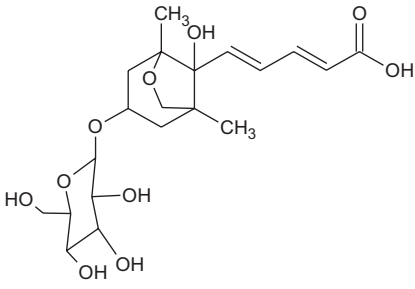

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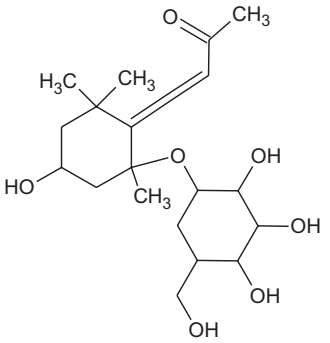

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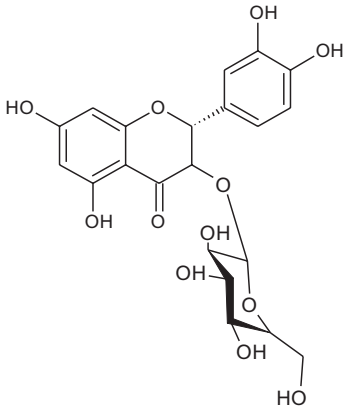

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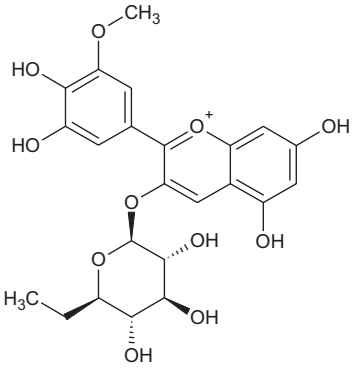

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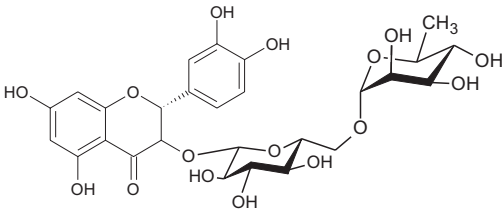

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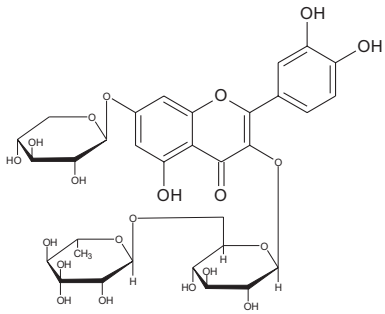

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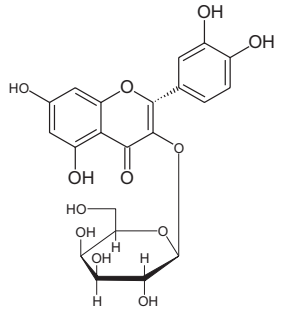

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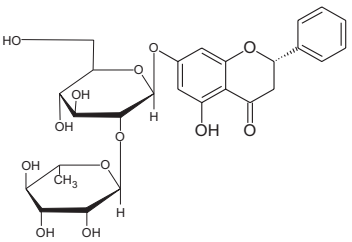

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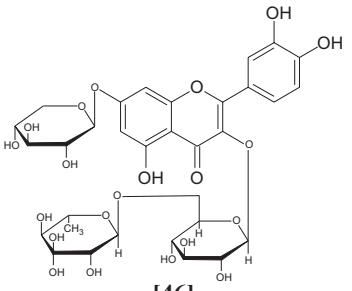

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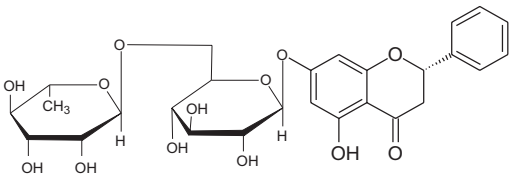

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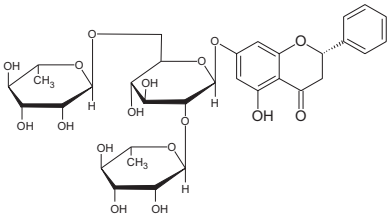

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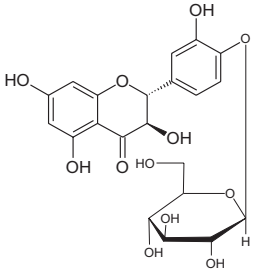

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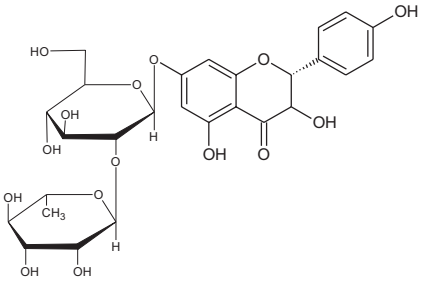

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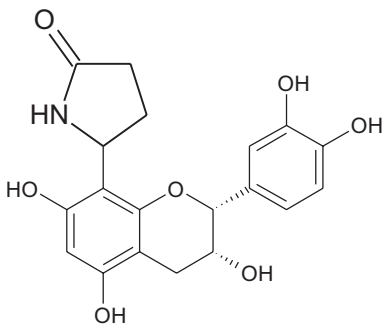

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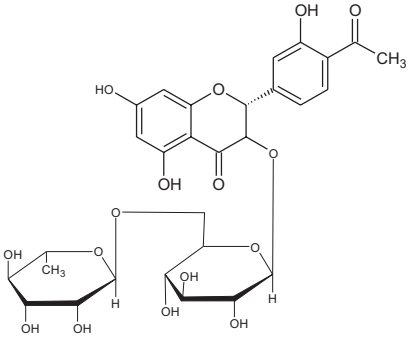

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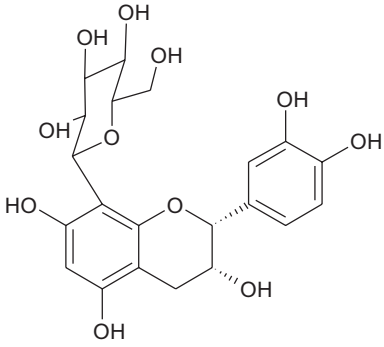

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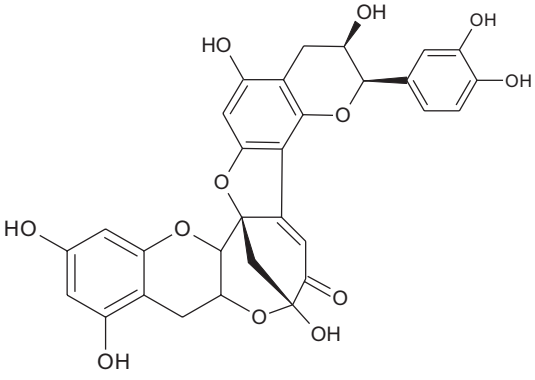

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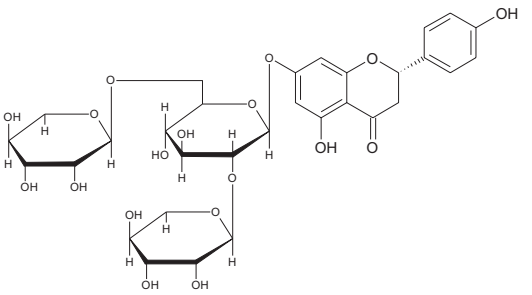

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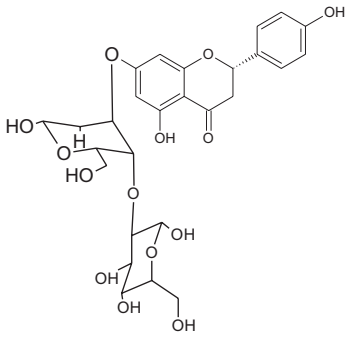

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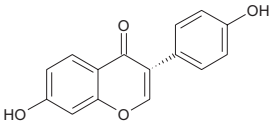

[58]

Fig. 7.2 (continued)

the litchi elucidated in the past 5 years have been discussed elaborately. Information provided in this chapter has been squeezed from rigorous analyses of reports published in reputed scientific journals, books, and worldwide scientific databases via a library and electronic search (PubMed, Elsevier, Google Scholar, Springer, Scopus, Web of Science, and ScienceDirect). The most relevant articles published during 2010–2016 were selected for this review.

Table 7.1 Phytochemical composition of different parts of *Litchi chinensis*

	Phytochemical compositions				
Parts	Phenolics	Phenolic glycosides	Proanthocyanidins	Lignans	Terpenes
Leaf	Epicatechin	Kaempferol 3-O-*β*-glucoside	Procyanidin A2 Procyanidin B2	N.R.	N.R.
	Luteolin				
	Rutin	Kaempferol 3-O-*α*-rhamnoside			
	4,7,7′,8′,9,9′-Hexahydroxy-3,3′-dimethoxy-8,4′-oxyneolignan	Ecoisolariciresinol 9′-*O*-*β*-D-xyloside			
	Cinnamtannin B1				
Flowers	Epicatechin	N.R.	Proanthocyanidin A2	N.R.	N.R.
Fruit	Catechin	N.R.	N.R.	N.R.	N.R.
	Epicatechin				
	Rutin				
	Gallic acid				
	Chlorogenic acid				
	Caffeic acid				
	Gentisic acid				
Seeds	3,5-Dihydroxybenzoic acid	(2R)-Naringenin-7-*O*-(3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside)	N.R.	N.R.	
	3,4-Dihydroxybenzaldehyde	(2S)-Pinocembrin-7-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside)			
	Procyanidin D				
	Cianidanol				
	Cinnamtannin B1				
	Procyanidin A1				
	Scopoletin				
	Rutin				
	Phlorizin				
	Epicatechin–epicatechin–catechin				

(continued)

Table 7.1 (continued)

Parts	Phytochemical compositions				
	Phenolics	Flavonoids	Proanthocyanidins	Lignans	Terpenes
Pericarp	(−)-Epicatechin,	8-(2-Pyrrolidinone-5-yl)-(−)-epicatechin, (−)-epicatechin 8-C-β-D-glucopyranoside	Proanthocyanidin A1, A2	(+)-Isolariciresinol 9-*O*-α-L-arabinopyranoside	β-D-Glucopyranosyldihydrophaseate citroside
	2-(2-hydroxyl-5-(methoxycarbonyl) phenoxy) benzoic acid		Procyanidin A2		
			Cyanidin-3-glucoside	Burselignan 9-*O*-α-L-arabinopyranoside	
	Kaempferol	Naringenin 7-O-(2,6-di-O-α-L--rhamnopyranosyl)-β-D-glucopyranoside	Cyanidin-3-rutinoside		
	Isolariciresinol		Malvidin-3-glucoside		
	Stigmasterol				
	Rutin			Secoisolariciresinol 9-*O*-α-L-arabinopyranoside	
	Butylated hydroxytoluene				
	3,4-Dihydroxyl benzoate				
	Methyl shikimate			Sisymbrifolin	
	Ethyl shikimate				
	Bis(8-epicatechinyl) methane dehydrodiepicatechin A				
	Epicatechin–epicatechin–epicatechin				

N.R. Not reported

7.2 Phytochemical Compositions

Litchi and its various parts have been extensively investigated for their phytochemical composition (Roux et al. 1998; Zhao et al. 2006; Li et al. 2012; Prasad et al. 2009; Xu et al. 2010; Yang et al. 2012). The major chemical constituents of litchi are the groups of phenolics and flavonoids (Table 7.1). In addition, lignans, sterols, and triterpenens have been identified from litchi (Table 7.1). Flavonoids are defined as a group of polyphenolic compounds containing a 15-carbon skeleton (C6-C3-C6), which consists of a heterocyclic ring (C) and two phenyl rings (A and B) (Fig. 7.1). The heterocyclic benzopyran ring is referred to as the C ring, the fused aromatic ring as the A ring, and the phenyl constituent as the B ring. They differ structurally according to the nature of the stereochemistry of the asymmetric carbons on the C rings and the number of hydroxyl groups on the B rings (Fig. 7.1). The major flavonoids identified in litchi were anthocyanins, flavones, flavonols, flavanols, chalcones, dihydrochalcones, dihydroflavonols, and isoflavonoids, all of which are beneficial for the plant as physiologically active compounds or stress-resistant agents (Treutter 2006). Flavonoids have shown a wide range of pharmacological and biological effects such as anti-oxidant, anti-inflammatory, immunomodulatory, anti-cancer, and antimicrobial effects hence, they attracted much attention in recent years (Costa et al. 2013; Yang et al. 2012). Several novel phenolic compounds have been discovered from different parts of litchi. Litchi also contains lignans which are widely distributed phenylpropanoid derivatives in plants. They are classified into five main structure types including lignans, oligomeric lignans, hybrid lignans, norlignans, and neolignans. The chemical composition of litchi reveals that it has 74.5% edible portion, 78.5% moisture, 1.2% citric acid, 0.69% ash, and 13.57% sugar (Cabin 1954). The litchi fruit is a rich source of vitamin C (Wall 2006) and phenolic compounds which are known natural anti-oxidants (Hu et al. 2010). Phytochemical analyses of flours from the skin and seeds of Bengal litchi revealed that the flour made from the skin has highest levels of flavonoids, vitamin C, phenolic compounds, anthocyanins, lipids, proteins, ash, and fiber, while the flour of seed stood out for the contents of potassium, sulfur, copper, and zinc (Queiroz et al. 2015). The flour of the skin also contains alkaloids. The fruit pulp of the litchi cultivar Tailandes grown in Brazil is a good source of dietary fiber, vitamin C, iron, magnesium, copper and potassium (Cabral et al. 2014).

As reported previously, litchi contains large quantities of phenolics called proanthocyanidins which are considered as the second most abundant groups of natural phenolics after lignins. Proanthocyanidins are oligomers and polymers of elementary flavan-3-ol units and highly distributed in plants, viz., apple, blueberry, chocolate, grape, and bark of pine (De Freitas et al. 1998; Prior et al. 2001; Lin et al. 2014; Jerez et al. 2009). They have been classified into several classes according to the hydroxylation patterns of their constitutive units: (1) procyanidins, proanthocyanidins exclusively constituted of (epi)catechin units and they are major constituents of plants; (2) propelargonidins, proanthocyanidins consisted of (epi)afzelechin units; and (3) prodelphinidins, proanthocyanidins consisted of (epi)gallocatechin units. Both propelargonidins and prodelphinidins are less common in plants (De Freitas et al. 1998;

Lin et al. 2014; Jerez et al. 2009). Flavan-3-ol units are usually linked via B-type bonds, i.e., C4 → C8 or C4 → C6 linkages. However, additional C2 → O7 or C2 → O5 linkage leading to doubly bonded A-type proanthocyanidins may occur (Prior and Gu 2005; Rasmussen et al. 2005). Proanthocyanidins owing to their beneficial health effects are occupying ever-increasing significance in human health (He et al. 2008). Like flavonoids they are also bestowed with many excellent bioactive properties such as antioxidant (González-Centeno et al. 2012), anti-diabetic (Jiao et al. 2013), anti-angiogenic (Pesca et al. 2013), anticarcinogenic (Actis-Goretta et al. 2008), anti-inflammatory (Bak et al. 2013), and cardioprotective activities (Bagchi et al. 2003). Phytochemical studies revealed the presence of large amounts of procyanidins in litchi pericarp, seeds and flowers (Roux et al. 1998; Zhao et al. 2006; Li et al. 2012; Prasad et al. 2009; Xu et al. 2010; Yang et al. 2012). Here, phytochemical compositions of the different parts of litchi have been discussed in detail.

7.2.1 Leaf and Flower

Leaves are much easier to collect than any other parts of the litchi plant still information on its phytochemical composition are limited as compared to the other parts. Two recently published reports have revealed the presence of phenolics and flavonoids in litchi leaf extracts prepared with methanol and ethyl acetate. The major phenolics and flavonoids identified were (−)-epicatechin (Fig.7.1), procyanidin A2, procyanidin B2, luteolin, kaempferol 3-O-β-glucoside, kaempferol 3-O-α-rhamnoside, and rutin (Castellain et al. 2014; Wen et al. 2014). Luteolin and kaempferol 3-O-α-rhamnoside were reported for the first time. In addition, three other phenolics reported from the litchi leaf were secoisolariciresinol 9′-O-β-D-xyloside, 4,7,7′,8′,9,9′-hexahydroxy-3,3′-dimethoxy-8,4′-oxyneolignan, and cinnamtannin B1 (Wen et al. 2015).

Litchi flower extract prepared with water contains significant quantities of phenolics, flavonoids, and tannins (Prakash et al. 2011). Major phenolics of litchi flower extract were (−)-epicatechin and proanthocyanidin A2 with observed concentration of 5.52 and 11.12 mg/g of dry weight, respectively (Yang et al. 2012), whereas flower acetone extract contains gentisic acid apart from the epicatechin and proanthocyanidin A2 (Hwang et al. 2013).

7.2.2 Fruits and Fruit Pulp

Recent works on litchi suggested that fruit pulp the most commonly consumed part of the litchi contains a large number of phenolics. However, structures of only few of phenolics have been identified so far and rest remains unidentified. Six phenolics isolated and identified from litchi pulp extract were gallic acid, chlorogenic acid, (+)-catechin, (-)-epicatechin (Fig. 7.1), caffeic acid, and rutin (Li et al. 2012). A recently published article reported the presence of three phenolics namely, quercetin 3-O-rutinoside-7-O-a-L-rhamnosidase, quercetin 3-O-rutinoside and

(-)-epicatechin (Fig. 7.1) in litchi pulp extract which possessed strong anti-oxidant activity (Su et al. 2014). The concentration of quercetin 3-O-rutinoside-7-O-a-L-rhamnosidase was 17.25 mg per 100 g of lychee pulp fresh weight.

7.2.3 Pericarp

The pericarp has been most vigorously investigated part of litchi plant for phytochemical compositions. It accounts approximately 15% of total weight of the whole fresh fruit and contains substantial amounts of phenolics. The pericarp is usually discarded as waste is however now being considered as new source of bioactive phenolics and flavonoids (Zhao et al. 2006). The total phenolic in dried litchi pericarp ranged from 51 to 102 g k g^{-1}. A number of previously published reports have documented the presence of epicatechin, epicatechin gallate (Fig. 7.1), procyanidin A2, procyanidin B2, rutin, epicatechin–epicatechin–epicatechin, malvidin-3-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, and quercetin 3-O-glucoside in the pericarp (Sun et al. 2010; Li et al. 2012). The A-type of procyanidin is accounted for 42% and B-type procyanidins 24.1% of the total phenolics of litchi pericarp. Jiang et al. (2013) have reported a novel bioactive phenolics called 2-(2-hydroxyl-5-(methoxycarbonyl) phenoxy) benzoic acid from litchi pericarp methanol extracts. Other constituents of the litchi pericarp were, i.e., kaempferol, stigmasterol, 3,4-dihydroxyl benzoate, butylated hydroxytoluene, isolariciresinol, methyl shikimate, and ethyl shikimate. A phytochemical study carried out by a group of Ma (2014a) have demonstrated the presence of four lignans, (−)-secoisolariciresinol 9-*O*-α-L-arabinopyranoside, (+)-isolariciresinol 9-*O*-α-L-arabinopyranoside, burselignan 9-*O*-α-L-arabinopyranoside, and sisymbrifolin; a chromane, 2α-methoxychroman-3α,5,7-triol; a benzophenone, garcimangosone D; and two sesquiterpenes, β-D-glucopyranosyldihydrophaseate and citroside, in the litchi pericarp. This group has further reported a new methylene-linked flavan-3-ol dimer, bis(8-epicatechinyl)methane from litchi pericarp along with rutin, dehydrodiepicatechin A, proanthocyanidin A1 and A2, (−)-epicatechin, 8-(2-pyrrolidinone-5-yl)-(−)-epicatechin, (−)-epicatechin 8-C-β-D-glucopyranoside, and naringenin 7-O-(2,6-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (Ma et al. 2014b). The amount of phenolics and flavonoids accumulated depends mainly on the developmental stages and type of the tissue. Besides, phenolic content is influenced by various factors such as the type of cultivars, geographical variation, and climatic and storage conditions. Zhang et al. (2013) have revealed significant variation in phenolics and flavonoids in pulp extract of prepared from 13 different cultivars of litchi grown in China. Sun et al. (2010) have studied that concentrations of the (−)-epicatechin and procyanidin- A2 in pericarp of postharvest litchi fruit decreased with increasing skin browning index during storage at 25 °C. Seasonal variations in phenolic contents of pericarp have also been reported from ten cultivars of litchi collected from two production seasons (Wang et al. 2011).

7.2.4 Seeds

The major flavonoids identified in litchi seeds were epicatechin (Fig. 7.1), procyanidin A1 and A2, rutin, phlorizin, litchioside D, and tamarixetin 3-O-rutinoside (Li and Jiang 2007). Xu et al. (2010) have reported two new A-type trimeric proanthocyanidins with two doubly bonded inter-flavonoid linkages, litchitannin A1 and litchitannin A2, from litchi seeds together with proanthocyanidin A1 and A2, aesculitannin A, epicatechin–epiafzelechin–epicatechin, proanthocyanidin A6, and epicatechin-(7,8-bc)-4β-(4-hydroxyphenyl)-dihydro-2(3H)-pyranone. The group of Xu et al. (2011) have reported several flavonoid glycosides, namely, phlorizin, litchioside D [47], (−)-pinocembrin 7-O-neohesperidoside [48], (−)-pinocembrin 7-O-rutinoside, taxifolin 4′-O-β-d-glucopyranoside, kaempferol 7-O-neohesperidoside, and tamarixetin 3-O-rutinoside, from litchi seeds. Two flavanones, (2R)-naringenin-7-O-(3-O-α-L-rhamnopyranosyl-β-D-glucopyranoside), a new natural product, and (2S)-pinocembrin-7-O-(6-O-α-L-rhamnopyranosyl-β-D-glucopyranoside), have been reported from the ethanol extract of litchi seeds (Ren et al. 2011). Some other constituents reported from litchi seeds were cianidanol or (+)-D-catechin (Fig. 7.1), procyanidin A [3], procyanidin D [5], rutin [10], cinnamtannin B1, epicatechin–epicatechin–catechin [19], 3,5-dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, phlorizin, and scopoletin (Man et al. 2016).

7.3 Bioactivities of Litchi

Litchi and its different parts, viz., leaf, flower, fruits, seeds, and pericarp, displayed many useful bioactivities such as anti-oxidant, anti-cancer, anti-inflammatory, antimicrobial, anti-viral, anti-diabetic, antiobesity, hepatoprotective, and immunomodulatory activities (Table 7.2). Major bioactivities of litchi have been discussed here.

7.3.1 Anti-oxidant Activities

Litchi is a very good source of phenolics and flavonoids compounds known for their excellent anti-oxidant properties. The anti-oxidant activities of litchi are often correlated with phenolics and flavonoids content (Shukla et al. 2014). The anti-oxidant potential of the plant extracts or the pure compound are evaluated using 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical, ferric reducing anti-oxidant power (FRAP), hydroxyl radical and superoxide radical scavenging assays. The (−)-epicatechin and procyanidin A2 [3] present in the litchi pericarp own anti-oxidant properties, however, the (−)-epicatechin has strong anti-oxidant potential than procyanidin A2 [3] (Sun et al. 2010). Methanol extracts of pericarp from ten different cultivars of litchi displayed anti-oxidant potential stronger even than the standard anti-oxidant butylated hydroxylytoluene [33] and catechin. Generally, the anti-oxidant potential of the pericarp extract was corresponding to

Table 7.2 Bioactivities and phytochemical composition of different parts of *Litchi chinensis*

Plant parts	Bioactivities	Composition	References
Leaves	Antimicrobial	Phenolics and flavonoids	Shukla et al. (2012)
	Antimicrobial	Luteolin	Wen et al. (2014)
	Anti-cancer	Procyanidins	
	Anti-oxidant and antinociceptive	Procyanidin A2, procyanidin B2, (−)-epicatechin	Castellain et al. (2014)
	Anti-oxidant	Phenolics	Shukla et al. (2014)
	Hepatoprotective	Triterpenoids, steroids, alkaloids, tannins	Basu et al. (2012)
	Anti-cancer and anti-oxidant	Luteolin, epicatechin, kaempferol 3-O-*β*-glucoside, kaempferol 3-O-*α*-rhamnoside, procyanidin A2, and rutin	Wen et al. (2015)
Flower	Cardioprotection effects	Phenols, flavonoids, tannins	Yang et al. (2010)
	Anti-oxidant	Gentistic acid and epicatechin	Chen et al. (2011)
	Anti-oxidant	(−)-Epicatechin and proanthocyanidin A2	Yang et al. (2012)
	Protective role against cadmium- and lead-induced cytotoxicity	Epicatechin, gentisic acid, and proanthocyanidin A2	Hwang et al. (2013)
	Lipase inhibitory	Crude extract	Wu et al. (2013)
	Antioxidative and anti-inflammatory	Gentisic acid and epicatechin	Chang et al. (2013)
	Cytotoxic	Proanthocyanidins	Lin et al. (2015)
Fruit	Anti-oxidant	Ascorbic acid, phenolics, and flavonoids	Saikia et al. (2016)
Peel and seeds	Anti-oxidant	Ascorbic acid and beta-carotene	Queiroz et al. (2015)
Pulp, peel, and seeds	Anti-oxidant and free radical scavenging	Phenolics	Prakash et al. (2012)
Seeds	α-Glucosidase inhibitory	(2R)-Naringenin-7-*O*-(3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) and (2S)-pinocembrin-7-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside)	Ren et al. (2011)

(continued)

Table 7.2 (continued)

Plant parts	Bioactivities	Composition	References
Pericarp	Anti-oxidant	2-(2-Hydroxyl-5-(methoxycarbonyl) phenoxy) benzoic acid, kaempferol, isolariciresinol, stigmasterol, butylated hydroxytoluene, 3,4-dihydroxyl benzoate, methyl shikimate, and ethyl shikimate	Jiang et al. (2013)
	Anti-oxidant	(+)-Isolariciresinol 9-*O*-α-L-arabinopyranoside, (−)-secoisolariciresinol 9-*O*-α-L-arabinopyranoside, garcimangosone D, β-D-glucopyranosyldihydrophaseate, and citroside A	Ma et al. (2014a)
	Anti-oxidant	Dehydrodiepicatechin A [53], proanthocyanidin A1, A2 [2], (−)-epicatechin, 8-(2-pyrrolidinone-5-yl)-(−)-epicatechin, (−)-epicatechin 8-C-β-D-glucopyranoside, naringenin 7-O-(2,6-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside, and rutin	Ma et al. (2014b)
	Anti-cancer and antibacterial	Cyanidin-3-rutinoside	Ni et al. (2016)
Pulp	Anti-oxidant	Polysaccharide-enriched fractions	Komg et al. (2010)
	Hepatoprotective	Vitamin C and phenolic compounds	Bhoopat et al. (2011)
	Anti-oxidant	Phenolics	Zhang et al. (2012)
	Anti-oxidant	(+)-Catechin, (−)-epicatechin, gallic acid, chlorogenic acid, caffeic acid, and rutin	Zhang et al. (2013)
	Anti-oxidant	Epicatechin, procyanidin B2, procyanidin C1	Lv et al. (2015)
Seeds	Anti-oxidant and anti-viral	Proanthocyanidins A	Xu et al. (2010)
	Antimicrobial and anti-oxidant	Phenolics	Singh et al. (2013)
	Antimicrobial	Phenolics	Bhat and Al-daihan (2014)
	Hypoglycemic	3,5-Dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, procyanidin D, cinnamtannin B1, procyanidin A1, scopoletin, rutin, phlorizin, and epicatechin–epicatechin–catechin	Man et al. (2016)

the concentrations of phenolics present in the extract. Zhang et al. (2013) have revealed the anti-oxidant activities of pulp extracts of 13 different cultivars of litchi from China. The study revealed marked variation in phenolics and flavonoids contents in all the 13 cultivars which were held responsible for variation in the anti-oxidant potential. Thus the anti-oxidant potentials were closely associated with phenolics and flavonoids contents. Two oligomeric phenolics, namely, (−)-epicatechin and procyanidin A2, and a trimer, epicatechin-(4β → 8, 2β → O → 7)-epicatechin-(4β → 8)-epicatechin, of litchi pericarp exhibited strong anti-oxidant properties (Li et al. 2012). A novel phenolic named as 2-(2-hydroxyl-5-(methoxycarbonyl) phenoxy) benzoic acid of pericarp showed an impressive anti-oxidant activity without inhibiting the tyrosinase and α-glucosidase enzyme activities (Jiang et al. 2013). Other active constituents of litchi pericarp like kaempferol, stigmasterol, 3,4-dihydroxyl benzoate, butylated hydroxytoluene, isolariciresinol, methyl shikimate, and ethyl shikimate demonstrated good anti-oxidant activities (Jiang et al. 2013). Butylated hydroxytoluene has been recognized as a natural anti-oxidant which is otherwise usually taken as a synthesized anti-oxidant. Some more phenolics from litchi pericarp such as proanthocyanidin A1, rutin, dehydrodiepicatechin A, (−)-epicatechin 8-C-β-D-glucopyranoside, and naringenin 7-O-(2,6-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside possessed anti-oxidant properties (Ma et al. 2014a). In addition to phenolics, the lignans, chromane, and sesquiterpenes present in pericarp can be attributed for the anti-oxidant effects of litchi (Ma et al. 2014a, b). The litchi flower extract showed the anti-oxidant properties which is attributed to the (-)-epicatechin and proanthocyanidin A2 [2] (Yang et al. 2012). Gentisic acid and epicatechin present in litchi flower water extract have also exhibited anti-oxidant and anti-inflammatory effects on the livers of high-fat-diet-fed hamsters (Chang et al. 2013). Besides pericarp, litchi leaves contain several flavonoids such as epicatechin, procyanidin A_2, luteolin, and rutin which displayed anti-oxidant properties stronger than the butylated hydroxytoluene used as authentic anti-oxidant (Wu et al. 2013; Castellain et al. 2014). Litchi fruit pulp is also capable of displaying anti-oxidant effects due to the presence of sufficient amounts of phenolics and proanthocyanidins. Litchi cultivars with high levels of proanthocyanidins are considered good resources of dietary anti-oxidants and offer important roles to play in human health. There are 32 cultivars of litchi reported to have high proanthocyanidins in the fruit pulp (Lv et al. 2015). Recently, Saikia et al. (2016) have reported the phytochemical composition and anti-oxidant activities of the litchi grown in various parts of India.

7.3.2 Hepatoprotective and Cardiovascular Properties

Hepatoprotective effects are combination of anti-oxidant and antiapoptotic activities. Litchi flower-water extract have protective effects on cardiovascular health in high-fat cholesterol-dietary hamsters which can be attributed to phenolics, flavonoids, and tannins (Prakash et al. 2012). Litchi fruit pulp extracts exhibited hepatoprotective effects on CCl_4-induced hepatotoxicity in male Wistar albino rats (Bhoopat et al. 2011). Litchi pulp extract tends to prevent increase in the serum GPT, GOT, and ALP level in CCl_4-induced hepatotoxicity in male Wistar albino rats. Pretreatment of CCl_4-induced rats with litchi pulp extract significantly decreased apoptotic cells and restored morphological changes favoring hepatoprotective effects of litchi pulp. Litchi contains adequate amounts of vitamin C and phenolic compounds which may stop lipid peroxidation and apoptosis in CCl_4-induced hepatotoxicity in rats. Litchi flower acetone extract has shown protective effects against Cd (cadmium)- and Pb (lead)-induced cytotoxicity and transforming growth factor β1-stimulated expression of smooth muscle α-actin in rat liver cell lines (Hwang et al. 2013). This protective effect has been correlated with the phenolics such as epicatechin, proanthocyanidin A2, and gentisic acid present in litchi flower. They decreased lipid peroxidation and DNA fragmentation which resulted in increase of the cell viabilities in Cd- and Pb-treated rat liver cell lines. In addition, phenolics present in flower acetone extract may suppress TGF-β1-induced activation of HSCs which has been evidenced from the downregulated expression of smooth muscle α-actin (αSMA). Hence, the protective roles of litchi flower extracts can be attributed to the anti-oxidant action of phenolics and flavonoids.

7.3.3 Anti-cancer or Cytotoxic Activities

Some recently conducted studies have unraveled anti-cancer potential of the litchi. A study by Wu et al. (2013) has revealed strong anti-cancer potential of procyanidin A2 found in litchi leaf extract against human hepatoma HepG2 and human cervical carcinoma HeLa cells. Another phenolic compound cinnamtannin B1 from litchi leaf has been reported as a potent inhibitor of cancer cell proliferation and a good intracellular anti-oxidant (Wen et al. 2015). Cinnamtannin B1 can be accounted for the upregulation of endogenous anti-oxidant enzyme activities such as superoxide dismutase, catalase, and glutathione peroxidase which resulted in inhibition of ROS generation. It also exhibits strong antiproliferative effects against $HepG_2$ and SiHa cell lines with no significant cytotoxicities (Wen et al. 2015). Three compounds, litchoside D, taxifolin 4′-O-β-d-glucopyranoside, and kaempferol 7-O-neohesperidoside, present in litchi seeds demonstrated in vitro anti-tumor activity against A549 (human lung adenocarcinoma, LAC), Hep-G2 (human hepatocellular carcinoma), and HeLa cell lines (Xu et al. 2011). Litchi flower extracts in ethanol also exhibited cytotoxic activity against A549, KB (human nasopharyngeal carcinoma), MCF-7 (human breast adenocarcinoma), and HepG2 cells (Lin et al. 2015). The anti-cancer activity of litchi flower was attributed to five flavonoids, nine

phenolic acids, and proanthocyanidin A2 [2] present in the flower ethanol extract. These phytochemicals may have effects on apoptosis; expressions of p53, tBid, Bad and Bax, and Bcl-2 and Bcl-xL; and the release of cytochrome c from mitochondria to cytosol. Additionally, it may be accompanied by activation of caspase-3, caspase-8, and caspase-9 and cleavage of poly (adenosine diphosphate (ADP)-ribose) polymerase (PARP) and downregulations of phosphoinositide 3-kinase (PI3K), Akt, Akt phosphorylation, and Bad phosphorylation (Lin et al. 2015).

7.3.4 Antimicrobial and Other Activities

Reports on antimicrobial activities of litchi are limited. Litchi leaf acetone extract and seed aqueous extract demonstrated antimicrobial activity against some pathogenic gram positive *Staphylococcus aureus*, Streptococcus pyogenes, Bacilllus subtillis and gram negative *Escherichia coli*, Pseudomonas aeruginosa bacteria (Shukla et al. 2012; Bhat and Al-Daihan 2014). Luteolin [6] present in the litchi leaf has been identified as a strong antimicrobial agent while others epicatechin, procyanidin A2 and rutin as weak antimicrobial agents (Wen et al. 2014). Ramis et al. (2015) have reported antibacterial and anti-oxidant activity of ethanolic and acetonic extracts of litchi pulp and waste including seeds and pericarp of fruit. Litchi waste and pulp extracts due to their strong antimicrobial potentials can be proposed as new potential sources of natural additives for food quality and preservation and/or pharmaceutical applications. Two flavanones namely, (2S)-pinocembrin-7-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) and (2R)-naringenin-7-*O*-(3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside), from litchi have α-glucosidase inhibitory activity (Ren et al. 2011). Litchi flower water extracts are also reported to be capable of inhibiting lipase activity in diet-induced obesity in male rats (Wu et al. 2013). Flower water extract mainly suppresses in vitro lipase activities, size of the liver, perirenal and epididymal adipose tissues, and cell sizes of epididymal adipose tissues in hypercaloric-diet-fed group of rats. Hence, litchi flower water extract has nutraceuticals potential for antiobesity effects. Litchi seeds also have anti-diabetic prospects as revealed from the study of Man et al. (2016) conducted on type 2 diabetic rats. Litchi seed extract showed hypoglycemic effect which has been attributed to phenolics, procyanidin A1, procyanidin D, rutin, cinnamtannin B1, epicatechin–epicatechin–catechin, 3,5-dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, phlorizin, and scopoletin. Litchi seed extract showed protective effects on pancreas, liver, and kidney tissue damage via improvement of glucose tolerance and insulin resistance. Besides, the seed extract also influenced lipid metabolism and increased mRNA levels of Bax and NF-κB, thereby inhibiting apoptosis-induced hepatic damage and inflammation to protect the body against diabetic exacerbation. Litchi peel extract when combined with the Tofu wastewater demonstrated synergistic anti-cancer and antibacterial activities with cordycepin (Ni et al. 2016). A phenolic, namely, cyanidin-3-rutinoside, present in litchi peel and genistein in Tofu wastewater is reported to inhibit activity of adenosine deaminase enzyme which regulates cellular levels of adenosine and deoxyadenosine and thus mediated

synergistic anti-cancer and antibacterial activities with cordycepin. Docking simulation suggested that cyanidin-3-rutinoside and genistein can enter into ADA active site, bind with its functional amino acids through H-bonds, and competitively inhibit the adenosine deaminase (Ni et al. 2016). Hence, cyanidin-3-rutinoside and genistein can be used as anti-inflammatory and anti-cancer drugs or degradation inhibitors of adenosine drugs.

7.4 Conclusion

The chapter provides detail insight into phytochemical compositions and various bioactive properties of litchi which has been reported in the past 5 years. It reflects ever increasing and vastly varied significance of litchi and its products in human health, food supplements, nutraceuticals and pharmaceuticals. A number of reports published showed that litchi and its various parts contain a large number of phenolics and flavonoid compounds which has been accountable for the tremendous anti-oxidant and various other bioactivities of litchi. Owing to the beneficial anti-oxidant potential of flavonoids and phenolics they offer promises for the development of functional foods, food supplements and nutraceuticals for humans for positive health effects. Litchi plant extract being a natural anti-oxidant can be used effectively to improve the quality, stability and safety of different meat and meat products. In view of the tremendous anti-oxidant potential of proanthocyanidins, development of new varieties and cultivars of litchi having high proanthocyanidin content is highly desirable which may enhance medicinal and a nutraceuticals value of litchi fruits and help advancement of litchi fruit industry. Litchi seeds have a positive anti-cancer effect through a variety of molecular and immune regulation mechanisms with no serious side effects. Therefore, combining litchi seeds medicine with radiotherapy and chemotherapy could be a novel application enhancing tumor treatment as well as improving the quality of life of cancer patients. Prospects of litchi flavonoids as the constituents of anti-breast cancer drugs may also be explored. For this more in vitro and pharmacokinetics studies would be required prior to development of novel anti-tumor drug. Litchi has promising anti-inflammatory and analgesic potential but the fundamental mechanism of these effects is needed to be elucidated. Luteolin a phenolic has strong antimicrobial activity against human pathogenic drug resistant bacteria may be used as a lead molecule for the synthesis of new effective antimicrobial drugs. Nevertheless, litchi as an important ingredient of traditional medicinal systems has drawn much attention due to its anti-oxidant and other useful bioactivities. Further studies may underpin discoveries of more phytoconstituents with new bioactive properties of litchi. After understanding the mechanism of action of the active constituents their therapeutic potentials can be utilized for the drug discovery program. Meanwhile, researchers may continue to develop new approach for a better utilization of litchi and its products for better human health and development of litchi fruit industry.

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References

Actis-Goretta L, Romanczyk LJ, Rodriguez CA, Kwik-Uribe C, Keen CL (2008) Cytotoxic effects of digalloyl dimer procyanidins in human cancer cell lines. J. Nutr Biochem 19:797–808

Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG et al (2003) Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. Mutat Res 523:87–97

Bak MJ, Truong VL, Kang HS, Jun M, Jeong WS (2013) Anti-inflammatory effect of procyanidins from wild grape (*Vitis amurensis*) seeds in LPS-induced RAW 264.7 Cells. Oxidative Med Cell Longev 2013:1–11

Basu S, Haldar N, Bhattacharya S, Biswas S, Biswas M (2012) Hepatoprotective activity of *Litchi chinensis* leaves against paracetamol-induced liver damage in rats. Am-Eur J Sci Res 7:77–81

Bhat RS, Al-daihan S (2014) Antimicrobial activity of *Litchi chinensis* and *Nephelium lappaceum* aqueous seed extracts against some pathogenic bacterial strains. J King Saud Univ Sci 26(1):79–82

Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T (2011) Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): a combination of anti-oxidant and anti-apoptotic activities. J Ethnopharmacol 136(1):55–66

Cabin M (1954) The lychee in Florida. Bulliten No. 546. Floride Fruit Research Institute, USA

Cabral TA, Cardoso L d M, Pinheiro-Sant'Ana HM (2014) Chemical composition, vitamins and minerals of a new cultivar of lychee (*Litchi chinensis* cv. Tailandes) grown in Brazil. Fruits 69(6):425–434

Castellain RC, Gesser M, Tonini F, Schulte RV, Demessiano KZ, Wolff FR et al (2014) Chemical composition, anti-oxidant and antinociceptive properties of *Litchi chinensis* leaves. J Pharm Pharmacol 66(12):17961–17967

Chang YY, Yang DJ, Chiu CH, Lin YL, Chen JW, Chen YC (2013) Antioxidative and anti-inflammatory effects of polyphenol-rich litchi (*Litchi chinensis* Sonn.)-flower-water-extract on livers of high-fat-diet fed hamsters. J Funct Foods 5(1):44–52

Chen YC, Lin JT, Liu SC, Lu PS, Yang DJ (2011) Composition of flavonoids and phenolic acids in Lychee (*Litchi Chinensis* Sonn.) flower extracts and their anti-oxidant capacities estimated with human LDL, erythrocyte, and blood models. J Food Sci 76(5):C724–C728

Costa AGV, Garcia-Diaz DF, Jimenez P, Silva PI (2013) Bioactive compounds and health benefits of exotic tropical red-black berries. J Funct Foods 5:539–549

De Freitas VAP, Glories Y, Bourgeois G, Vitry C (1998) Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. Phytochemistry 49:1435–1441

Gontier E, Boussouel N, Terrasse C, Jannoyer M, Menard M, Thomasset B, Bourgaud F (2000) *Litchi chinensis* fatty acid diversity: occurrence of unusual cyclopropanoic fatty acids. Biochem Soc Trans 28:578–580

González-Centeno MR, Jourdes M, Femenia A, Simal S, RosselloC TPL (2012) Proanthocyanidin composition and anti-oxidant potential of the stem winemaking byproducts from 10 different grape varieties (*Vitis vinifera* L.). J Agric Food Chem 60:11850–11858

He F, Pan Q-H, Shi Y, Duan C-Q (2008) Biosynthesis and genetic regulation of proanthocyanidins in plants. Molecules 13:2674–2703

Hu ZQ, Huang XM, Chen HB, Wang HC (2010) Anti-oxidant capacity and phenolic compounds in litchi (*Litchi chinensis* sonn.) pericarp. Acta Hortic 863:567–574

Huang XM, Yuan WQ, Wang C, Li JG, Huang HB, Shi L, Jinhua Y (2004) Linking cracking resistance and fruit desiccation rate to pericarp structure in litchi (*Litchi chinensis* Sonn.). J Hortic Sci Biotechnol 79:897–905

Hwang JY, Lin JT, Liu SC, Hu CC, Shyu YS, Yang DJ (2013) Protective role of litchi (*Litchi chinensis* Sonn.) flower extract against cadmium- and lead-induced cytotoxicity and transforming growth factor β1-stimulated expression of smooth muscle α-actin estimated with rat liver cell lines. J Funct Foods 5(2):698–705

Ibrahim SR, Mohamed GA (2015) *Litchi chinensis*: medicinal uses, phytochemistry, and pharmacology. J Ethnopharmacol 174:492–513

Jerez M, Sineiro J, Guitián E, Núñez MJ (2009) Identification of polymeric procyanidins from pine bark by mass spectrometry. Rapid Commun Mass Spectrom 23:4013–4018

Jiang G, Lin S, Wen L, Jiang Y, Zhao M, Chen F, Prasad KN, Duan X, Yang B (2013) Identification of a novel phenolic compound in litchi (*Litchi chinensis* Sonn.) pericarp and bioactivity evaluation. Food Chem 136(2):563–568

Jiao L, Zhang X, Huang L, Gong H, Cheng B, Sun Y et al (2013) Proanthocyanidins are the major anti-diabetic components of cinnamon water extract. Food Chem Toxicol 56:398–405

Khan IU, Asghar MN, Iqbal S, Bokhari TH, Khan ZUD (2009) Radical scavenging and anti-oxidant potential of aqueous and organic extracts of aerial parts of *Litchi chinensis* Sonn. Asian J Chem 21(7):5073–5084

Khurshid S, Ahmad I, Anjum MA (2004) Genetic diversity in different morphological characteristics of litchi (*Litchi chinensis* Sonn.). Int J Agric Biol 6:1062–1065

Kilari EK, Putta S (2016) Biological and phytopharmacological descriptions of *Litchi chinensis*. Pharmacogn Rev 10(19):60–70

Lemmer D (2002) Report on the Chinese litchi industry. S.A. Litchi Growers' Association Yearbook 13:1–4.

Li JR, Jiang YM (2007) Litchi flavonoids: isolation, identification and biological activity. Molecules 12:745–758

Li S, Xiao J, Chen L, Hu C, Chen P, Xie B, Sun Z (2012) Identification of A-series oligomeric procyanidins from pericarp of *Litchi chinensis* by FT-ICR-MS and LC-MS. Food Chem 135(1):31–38

Lin LZ, Sun JB, Chen P, Monagas MJ, Harnly JM (2014) UHPLC-PDA-ESI/HRMSn profiling method to identify and quantify oligomeric proanthocyanidins in plant products. J Agric Food Chem 62:9387–9400

Lin JT, Chang YY, Chen YC, Hu CC, Chang YP, Hsu SH, Yang DJ (2015) Induction of apoptotic death of human hepatocellular carcinoma (HepG2) cells by ethanolic extract from litchi (*Litchi chinensis* Sonn.) flower. J Funct Foods 19:100–109

Lv Q, Luo F, Zhao X, Liu Y, Hu G, Sun C, Li X, Chen K (2015) Identification of proanthocyanidins from Litchi (*Litchi chinensis* Sonn.) pulp by LC-ESI-Q-TOF-MS and their anti-oxidant activity. PLoS ONE 10(3):1–17

Ma Q, Xie H, Jiang Y, Wei X (2014a) Phenolics and sesquiterpenes from litchi pericarp. J Funct Foods 9:156–161

Ma Q, Xie H, Li S, Zhang R, Zhang M, Wei X (2014b) Flavonoids from the pericarps of *Litchi chinensis*. J Agric Food Chem 62(5):1073–1078

Man S, Ma J, Wang C, Li Y, Gao W, Lu F (2016) Chemical composition and hypoglycaemic effect of polyphenol extracts from *Litchi chinensis* seeds. J Funct Foods 22:313–324

Menzel CM (2001) The physiology of growth and cropping in lychee. South African Litchi Growers' Association Yearbook. 12:9–14

Morton LW, Caccetta RAA, Puddey IB, Croft KD (2000) Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin Exp Pharmacol Physiol 27(3):152–159

Ni H, Li YH, Hao RL, Hu SQ, Li HH (2016) Identification of adenosine deaminase inhibitors from Tofu wastewater and litchi peel and their synergistic anti-cancer and antibacterial activities with cordycepin. Int J Food Sci Technol 51(5):1168–1176

Painuly D, Shukla RK, Porval A, Shukla A (2012) Proximate analysis, nutritive value, total phenolic content and anti-oxidant activity of *Litchi chinensis* sonn. Nat Prod An Ind J 8(9):361–369

Pareek S (2015). Nutritional and biochemical composition of lychee (*Litchi chinensis* Sonn.) cultivars. Nutritional composition of fruit cultivars, Elsevier, Preedy V, Simmonds M (eds.), 24 pp

Pesca MS, Dal Piaz F, Sanogo R, Vassallo A, Bruzual de Abreu M, Rapisarda A et al (2013) Bioassay-guided isolation of proanthocyanidins with antiangiogenic activities. J Nat Prod 76:29–35

Prakash D, Upadhyay G, Pushpangadan P, Gupta C (2011) Anti-oxidant and free radical scavenging activities of some fruits. J Complement Integr Med 8(1):196–209

Prakash D, Upadhyay G, Gupta C, Pushpangadan P, Singh KK (2012) Anti-oxidant and free radical scavenging activities of some promising wild edible fruits. Int Food Res J 19:1109–1116

Prasad KN, Yang B, Yang S, Chen Y, Zhao M, Ashraf M et al (2009) Identification of phenolic compounds and appraisal of anti-oxidant and antityrosinase activities from litchi (*Litchi chinensis* Sonn.) seeds. Food Chem 116:1–7

Prior RL, Gu L (2005) Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry 66:2264–2280

Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF (2001) Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* Spp.) using high performance liquid chromatography/mass spectrometry. J Agric Food Chem 49:1270–1276

Queiroz EDR, Abreu CMPD, Oliveira KDS, Ramos VDO, Fráguas RM (2015) Bioactive phytochemicals and anti-oxidant activity in fresh and dried lychee fractions. Rev Ciênc Agron 46(1):163–169

Ramis ES, Ahmad AF, El-Chaghaby GA (2015) Screening for total anti-oxidant, antibacterial activities, total phenolic and total flavonoid contents of Litchi fruit parts extracted using two different solvents. Afr J Agric Sci Tech 3(12):520–526

Rasmussen SE, Frederiksen H, Struntze Krogholm K, Poulsen L (2005) Dietary proanthocyanidins: occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. Mol Nutr Food Res 49:159–174

Ren S, Xu D, Pan Z, Gao Y, Jiang Z, Gao Q (2011) Two flavanone compounds from litchi (*Litchi chinensis* Sonn.) seeds, one previously unreported, and appraisal of their α-glucosidase inhibitory activities. Food Chem 127(4):1760–1763

Roux EL, Doco T, Sarni-Manchado P, Lozano Y, Cheynier V (1998) A-type proanthocyanidins from pericarp of *Litchi chinensis*. Phytochemistry 48:1251–1258

Saikia S, Mahnot NK, Mahanta CL (2016) Phytochemical content and anti-oxidant activities of thirteen fruits of Assam, India. Food Biotechnol 13(1):15–20

Shukla RK, Painuly D, Shukla A, Porval A (2012) Phytochemical investigation and antimicrobial screening of extracts of *Litchi chinensis* leaves from Dehradun region, India. Environ Conserv J 13(1, 2):7–10

Shukla RK, Painuly D, Porval A, Shukla A (2014) Proximate analysis, nutritional value, phytochemical evaluation, and biological activity of *Litchi chinensis* Sonn. leaves. J Herbs Spices Med Plants 20(2):196–208

Singh JP, Chandel R, Mishra B, Suneetha V (2013) Evaluation of antimicrobial and anti-oxidant property of lychee's seed for therapeutic purpose. Int J Pharm Sci Rev Res 19:72–66

Sivakumar D, Terryb L, Korsten L (2010) An overview on litchi fruit quality and alternative postharvest treatments to replace sulfur dioxide fumigation. Food Rev Intl 26:162–188

Su D, Ti H, Zhang R, Zhang M, Wei Z, Deng Y, Guo J (2014) Structural elucidation and cellular anti-oxidant activity evaluation of major anti-oxidant phenolics in lychee pulp. Food Chem 158:385–391

Sun J, Jiang Y, Shi J, Wei X, Xue SJ, Shi J, Yi C (2010) Anti-oxidant activities and contents of polyphenol oxidase substrates from pericarp tissues of litchi fruit. Food Chem 119:753–757

Treutter D (2006) Significance of flavonoids in plant resistance: a review. Environ Chem Lett 4(3):147–157

Wall MM (2006) Ascorbic acid and mineral composition of longan (*Dimocarpus longan*). Lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. J Food Compos Anal 19:655–663

Wang XJ, Yuan SL, Wang J, Lin P, Liu GJ, Lu YR et al (2006) Anti-cancer activity of litchi fruit pericarp extract against human breast cancer *in vitro* and *in vivo*. Toxicol Appl Pharmacol 215:168–178

Wang HC, Hu ZQ, Wang Y, Chen HB, Huang XM (2011) Phenolic compounds and the anti-oxidant activities in litchi pericarp: difference among cultivars. Sci Hortic 129:784–789

Waseem K, Ghafoor A, Rahman SU (2002) Effect of fruit orientation on the quality of Litchi (*Litchi chinensis* Sonn) under the agro-climatic conditions of Dera Ismail Khan-Pakistan. Int J Agric Biol 4:503–505

Wen L, Wu D, Jiang Y, Prasad KN, Lin S, Jiang G, He J, Zhao M, Luo W, Yang B (2014) Identification of flavonoids in litchi (*Litchi chinensis* Sonn.) leaf and evaluation of anti-cancer activities. J Funct Foods 6:555–563

Wen L, You L, Yang X, Yang J, Chen F, Jiang Y, Yang B (2015) Identification of phenolics in litchi and evaluation of anti-cancer cell proliferation activity and intracellular anti-oxidant activity. Free Radic Biol Med 84:171–184

Wu YHS, Chiu CH, Yang DJ, Lin YL, Tseng JK, Chen YC (2013) Inhibitory effects of litchi (*Litchi chinensis* Sonn.) flower-water extracts on lipase activity and diet-induced obesity. J Funct Foods 5(2):923–929

Xu X, Xie H, Wang Y, Wei X (2010) A-type proanthocyanidins from lychee seeds and their anti-oxidant and anti-viral activities. J Agric Food Chem 58:11667–11672

Xu X, Xie H, Hao J, Jiang Y, Wei X (2011) Flavonoid glycosides from the seeds of *Litchi chinensis*. J Agric Food Chem 59(4):1205–1209

Yang D-J, Chang Y-Y, Hsu C-L, Liu C-W, Wang Y, Chen Y-Y (2010) Protective effect of a litchi (*Litchi chinensis* Sonn.)-flower-water-extract on cardiovascular health in a high-fat/cholesterol-dietary hamsters. Food Chem 119:1457–1464

Yang DJ, Chang YZ, Chen YC, Liu SC, Hsu CH, Lin JT (2012) Anti-oxidant effect and active components of litchi (*Litchi chinensis* Sonn.) flower. Food Chem Toxicol 50(9):3056–3061

Zhang R, Zeng Q, Deng Y, Zhang M, Wei Z, Zhang Y, Tang X (2013) Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. Food Chem 136(3–4):1169–1176

Zhao MM, Yang B, Wang JS, Li BZ, Jiang YM (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their anti-oxidant activities. Food Chem 98:539–544

Zhao MM, Yang B, Wang JS, Liu Y, Yu LM, Jiang YM (2007) Immunomodulatory and anti-cancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. Int Immunopharmacol 7:162–166

Abiotic Stress Management in Fruit Crop *Litchi chinensis*

8

Garima Malik and Priyanka Deveshwar

Abstract

Frequent variations in global climate patterns direct changes in soil-plant-atmosphere continuum. Abiotic stresses caused due to these changes are responsible for inducing various modifications at molecular and cellular level in plants that in turn cause irreversible damage to agricultural yields of several major fruit crops. Lychee is a delicious, juicy fruit packed with numerous health benefits. The lychee production on a commercial level in Southeast Asia is a source of livelihood for thousands of people. Being highly specific in its climatic prerequisite, lychee crop quality and yield is adversely affected by any alteration in the environmental factors causing serious economic loss for the growers. In the near future, this situation might become more critical due to scarcity of land and water resources and deterioration of growing conditions in many parts of the world. Thus, it is necessary 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, 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. In this chapter, we will focus on various abiotic stresses that interferes with lychee growth and development and affects its productivity as well as provide a detailed update on recent research which contributes to a better understanding of stress regulatory mechanism to combat abiotic stresses in lychee.

G. Malik
Gargi College, University of Delhi, Delhi, India

P. Deveshwar (✉)
Sri Aurobindo College, University of Delhi, Delhi, India
e-mail: deveshwar.priyanka@gmail.com

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Lychee/*Litchi* • Abiotic stress • Planta • Fruit

8.1 Introduction

Litchi chinensis, commonly known as lychee, is a subtropical evergreen fruit tree that belongs to family Sapindaceae and subfamily Napheleae. Lychee is native to southern China and is commercially cultivated in some subtropical countries of the world (Menzel 1985). Lychee tree is a slow-growing tree of medium height (less than 15 m) with short, stout trunk, spreading branches that are mostly twisted and dense round canopy (Zhang 1997; Subhadrabandhu and Stern 2005). Lychee fruits are produced in loose, pendent clusters of 2–30 and can be round, ovoid, heart, or kidney shaped. Ripe fruits are usually strawberry red or pinkish in color, measuring about 3–3.5 cm in diameter and covered with thin leathery skin. Lychee fruit is considered as "queen of fruits" and is famous for its juicy, scented pulp and its characteristic flavor. Lychee is a highly valued fruit in international market for its high nutrition and medicinal values. Edible portion of lychee is a thick juicy aril that possesses high moisture (81%), carbohydrate (18 g/100gm), vitamin C (49 mg/100gm), and heart-healthy polyphenol content. However, it contains insignificant amount of fat (0.30–0.50%), protein (0.8–0.9%), and minerals such as thiamine, riboflavin, calcium, phosphorus, and iron (Bose and Mitra 1990). Lychee fruit is a brilliant thirst quencher in scorching summer heat and known to have a beneficial effect on the brain, heart, and liver.

China is the largest producer of lychee followed by India, Taiwan, Thailand, and Vietnam (Pareek 2016). India and China together account for 91% of the total world lychee production. India enjoys an important place in the world's lychee map in terms of quality, production, and productivity. India accounts for about one-fifth of the lychee global production. In India, lychee fruit is very popular and in demand during the short period of harvesting season, ranging from the first week of May to end week of June. It is grown in the states of Bihar, West Bengal, Jharkhand, Assam, Uttar Pradesh, Punjab, Chattisgarh, Tripura, and Orissa [NHB 2015]. Bihar is the leading producer and contributes 74% of total lychee production in India, followed by West Bengal, Jharkhand, and Assam. Over the past decades, India has recorded a significant increase in area and production of lychee; however, it is mainly marketed locally due to expanded domestic market (Table 8.1). A large number of lychee cultivars are under cultivation in different regions of the country; however, only few of them are popular among farmers and are planted on a commercial level. The major cultivars of lychee in India are Kasba, Longia, Shahi, China, Bedana, Late Bedana (Late Seedless), Elaichi, Calcutta, and Bombai.

Lychee fruit crop has very specific climatic requirements for optimum vegetative growth and successful fruiting; that is why its cultivation is limited to a narrow range of climates in comparison to other subtropical fruit crops. The major commercial plantings of lychee are found at low altitude in the subtropical areas and at

Table 8.1 All India area, production, and productivity of lychee

Year	Area (in '000 ha)	% of total fruit area	Production (in '000 mt)	% of total fruit production	Productivity (in mt/ha)
1991–92	49.3	1.7	243.8	0.9	4.9
2001–02	58.1	1.4	355.9	0.8	6.1
2002–03	54.1	1.4	476.4	1.1	8.8
2003–04	53.7	1.1	478.5	1.0	8.9
2004–05	60.0	1.2	368.6	0.7	6.1
2005–06	63.2	1.2	392.1	0.7	6.2
2006–07	65.0	1.2	403.0	0.7	6.2
2007–08	69.0	1.2	418.0	0.6	6.1
2008–09	72.0	1.2	423.0	0.6	5.9
2009–10	74.4	1.2	483.3	0.7	6.5
2010–11	78.0	1.2	497.0	0.7	6.4
2011–12	80.4	1.2	538.1	0.7	6.7
2012–13	82.7	1.2	580.1	0.7	7.0
2013–14	84.2	1.2	585.3	0.7	7.0

Source: NHB_Database_2015

higher altitude in the tropics and hilly regions. Various climatic factors such as temperature, rainfall, light, wind velocity, and atmospheric humidity play crucial role in successful production of lychee in an area. Generally, lychee flourishes best in regions that have an absence of strong winds; short, dry, and frost-free winters (minimum temperatures between 8 °C and 14 °C); long and hot summers with copious rainfall (1250–1500 mm); and moist atmosphere (Menzel 1991). Lychee farming is very successful in regions having a minimum temperature of ~10 °C from December to February and 32–38 °C from April to June. In lychee-growing areas in India, the temperature varies from 25 °C to 38 °C during flowering and fruiting season. Lychee can be grown on a variety of soil types, which include sandy loams, laterite, alluvial sand, and calcareous soil; however, fairly deep (1–2 m), well-drained sandy loam or clayey loam (with a pH ranging between 5.5 and 7) rich in organic matter is an ideal soil for its cultivation (Sarin et al. 2009).

8.2 Abiotic Stress

Stress is defined as any ecological variable, which can stimulate a potentially damaging strain that diminish development and yield beneath ideal levels in plants (Tuteja and Gill 2016). Both abiotic (physical/chemical) and biotic (biological) stresses have significant impact on agricultural systems and may limit crop production by up to 70% (Boyer 1982). The various abiotic stresses such as drought, salinity, heat and cold stress, osmotic stress, etc. have an adverse influence on growth, development, and productivity of fruit crops. They are accountable for devastating monetary losses to growers and increased prices for customers. Plant reactions to

stress are dynamic and intricate and can be both reversible and irreversible (Cramer et al. 2011). In order to survive under stress conditions, plant responds by activating their inherent defense machinery and counteract through inducing various molecular and cellular responses. Whenever plants perceives any stress signals, its defense machinery activates appropriate stress-inducible genes. The products of these genes are either directly involved in protection against environmental stresses (e.g., osmotic regulatory protein; enzymes for synthesizing betaine, proline, and other osmoregulators; etc.), or they modulate the expression of genes and/or downstream signal transduction pathways involved in stress tolerance (e.g., various transcriptional elements) (Tuteja and Gill 2016). Certain stress hormones such as abscisic acid (ABA) and ethylene also play an important role in regulating abiotic stress responses in plants.

The changing environment variables pose grave and imminent dangers to worldwide agribusiness and place unparalleled pressures on the sustainability of crop industry. Plant diversity is the answer for the future agriculture scientists to safeguard fruit crops from various natural afflictions and to generate crops with enhanced qualities. Germplasm may have genes or traits that can be recombined to produce novel or improved traits. The genetic engineering technology and other biotechnological tools can be utilized to enhance the stress tolerance capacity of plants to combat the negative effect of abiotic stress variables. Therefore, it is crucial to comprehend the physiological and molecular aspects of plant function under stress conditions in order to raise genetically modified plants.

Globally lychee is considered as one of the most environmentally susceptible fruit crop adapted to tropics having hot wet and humid summers and cool dry winters and warm subtropic. Environmental conditions play a key role in determining the economic viability of lychee crop production and postharvest quality of fruit. Abiotic stresses can result in significant reductions in tree longevity along with yield and quality of fruit. We will further discuss the effect of various abiotic stresses on lychee crop and focus on recent scientific studies conducted to unravel the complex mechanisms or set of genes involved in combating these effects.

8.2.1 Effect of Drought on Vegetative Growth and Reproductive Development of *Litchi*

Water accessibility is an important variable for plant survival and almost all basic developmental processes can be constrained by water deficit. Drought is one of the most predominant factors that causes enormous loss of productivity of fruit crops. Water stress has an inhibitory effect on the plant vigor, stem extension, leaf expansion, functioning of the stomata, CO_2 assimilation, flowering, and fruit growth and yield (Menzel 2005). Lychee being an evergreen plant requires adequate amount of rainfall and soil moisture for optimum growth, development, and fruit production. Lychee varieties are found in hot and dry geographical regions and are considered as a hardy plant species, but they are highly affected by seasonal droughts. Since, water paucity is a genuine threat to lychee production, adequate supply of irrigation

water plays a vital role in maintaining the productivity and quality of lychee grown in tropical or subtropical areas (Mali et al. 2015).

Litchi varieties showed a range of complex responses at various levels of plant organization to drought stress. Lychee plants are highly susceptible to drought stress during their initial years of establishment (1–3 years old); however, adult plants are comparatively drought tolerant. It has been observed that shoot growth is very sensitive to drought stress as its growth decreases as the level and period of drought increases. Severe water stress leads to decrease in leaf water potential and in turn inhibits leaf expansion and photosynthesis in lychee. One of the recent study revealed that when lychee trees cv. Kwai¨ Mi were subjected to long term drought, the leaf mass-to-area ratio (LMA) increased as an adaptive mechanism to limit evaporation. The amount of starch per unit leaf area was found to be decreased sharply whereas the concentration of total soluble sugar increased with water stress. The decrease in photosynthetic capacity was also observed with increasing water stress, which was attributed to downregulation of photosynthetic electron transport and decreased activity of chief regulatory enzymes of the Calvin cycle (Damour et al. 2008).

The lychee tree starts flowering from the third week of February, and its fruit ripens in the month of May. High moisture preceding floral induction in lychee promotes vegetative growth and suppresses flowering. Several studies have indicated that flower initiation or reproductive phase is promoted in lychee by mild water stress during the preceding autumn and winter (Nakata and Suehisa 1969; Stern et al. 1998). However, the period from flowering to early fruit development is chiefly susceptible to water deficit. Lack of moisture for an extended time during this period may lead to poor fruit setting, reduced fruit size, and abnormally high fruit dropping (Mitra and Pathak 2010; Joshi et al. 2011). The availability of calcium, a structural component of the cell walls, during early fruit development is important for resistance to fruit cracking in the lychee fruit. The high level of water stress diminished calcium content of the lychee fruit which in turn reduces the strength of fruit skin and thus aggravate the fruit cracking and curtail the postharvest shelf life of fruits (Rab and Haq 2012). Once harvested, if lychee fruit is exposed to heat or dry air, it loses water rapidly, which cause pericarp dehydration and browning (Liu et al. 2013a).

Endogenous plant growth regulators play crucial role in managing plant responses to stress. Studies have reported many-fold increase in level of abscisic acid (ABA) and decrease in auxin and gibberellic acid content in lychee in order to reduce the damaging effect of drought and other abiotic stresses. Several stress-inducible genes in plants, including RAB18, RD29A, RD29B, are known to be expressed differentially when they are subjected to drought stress. One such plant-specific stress and developmentally regulated gene family, abscisic acid stress ripening (*ASR*), expression has been reported to be induced by several abiotic and biotic factors in tomato, maize, rice, lily, banana, and grapes (Liu et al. 2013a; Tiwari et al. 2015). The expression levels of ASR genes may vary according to plant tissue and growth conditions. Overexpression of the lily ASR in *Arabidopsis thaliana* and tomato ASR protein in *Nicotiana tabacum* resulted in high tolerance to drought and salt tolerance, respectively (Yang et al. 2005; Kalifa et al. 2004). The function of the ASR-1 protein in the regulation of expression of sugar transporters

and reallocation of sugar from the leaf to other organs has been reported in tobacco plants (Dominguez et al. 2013). Recently, an ABA senescence and ripening inducible gene, Lc*ASR*, was identified in lychee showing differential expression in roots, stems, leaves, flowers, pericarps, pulps, and seeds. Overexpression of Lc*ASR* (*Litchi* ASR) gene in *Arabidopsis* showed enhanced tolerance to dehydration (Liu et al. 2013a). Identification and characterization of more such drought stress-inducible gene in fruit crops in the near future will help scientists in raising transgenic lychee plants that are more resistant to water deficit.

8.2.2 Effect of High Temperature on Flowering

Fruits are the commercially important product of the lychee tree. Number of flowers formed is directly proportional to the yield of the fruit. Defective flowering is a cause of huge losses in lychee cultivation. Thus the transition from vegetative to reproductive phase during floral differentiation governs the lychee crop production. Flowering in lychee is affected by many factors, and temperature is one of the most crucial factors. Floral initiation in lychee is triggered by an inductive low temperature and enhanced by dry period during autumn and winter (Chen and Huang 2005; Menzel and Simpson 1988). Subsequently the apical bud of a shoot may break and elongate as air temperature and soil moisture increase. Thereafter the axillary or apical panicle primordia emerge and become visible as "whitish millets" and is called as "millet stage" (Huang and Chen 2005). At this stage, the floral head is a mixture of panicle primordia (axillary as well as apical), leaf primordia, and rudimentary leaves. Whether the development of mixed buds proceeds toward the development of flowers or toward the development of leaves is entirely dependent on prevailing environmental conditions. If the temperature is not too high, then under normal conditions, panicle primordia grow and develop functional flowers. Simultaneously the rudimentary leaves will abscise. On the contrary, if the temperature is high, the rudimentary leaves may develop into fully expanded leaves with no or little development of panicle primordia and hence no flowers and subsequently no fruits (Zhou et al. 2008). Thus, high temperature is detrimental to lychee crop production. Global warming has resulted in overall rise of temperature across the globe, resulting in warm winters and hot springs that presents very unfavorable conditions for lychee cultivation. Hence, it is essential to develop an understanding about the molecular mechanisms governing lychee panicle development and abscission of rudimentary leaves to deal with the defective flowering problem.

Studies have suggested that suppression of rudimentary leaf growth enhances panicle development. Two stress signaling molecules, viz., nitric oxide (NO) and reactive oxygen species (ROS), have been implicated in governing the abortion of rudimentary leaves and formation of functional panicle (Zhou et al. 2012). ROS are well-established stress signals that are recognized by an array of signal transduction processes. In the presence of light, ROS is produced in chloroplast and peroxisomes, whereas at night or in non-photosynthesizing tissues, they are produced in mitochondria (Rhoads et al. 2006; Sierla et al. 2013). Investigations have shown

that flowering inductive low temperature causes accumulation of H_2O_2 (a type of ROS) and NO in mixed buds. Similar results are observed in *Arabidopsis* wherein transition to flowering stage is associated with H_2O_2 accumulation in leaves, which is implicated as a senescence signal for leaves and initiation signal for reproductive structures (Banuelos et al. 2008; Zimmermann et al. 2006). Additionally, artificial production of H_2O_2 and NO, by treatment of lychee leafy panicle with methyl viologen dichloride hydrate (MV) which is a superoxide generator and with sodium nitroprusside (SNP) which is a NO donor, respectively, stimulated the abscission of rudimentary leaves and development of panicle in lychee. *APETALA* (*AP1*) and *LEAFY* (*LFY*) are very well-documented genes required during floral transition (Weigel and Meyerowitz 1993). Accumulation of ROS and NO showed increased expression of lychee homologues of LFY (*LcLFY*) and AP1 (*LcAP1*) (Zhou et al. 2012). The essential role of ROS and NO in flowering is further reinforced by experiments using inhibitors of H_2O_2 and NO production. Dimethylthiourea (DMTU) is a scavenger of hydroxyl radical and traps H_2O_2, whereas 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl3-oxide (PTIO) is a NO scavenger. Moreover N ω -nitro-L-arginine methyl ester (L-NAME) is an inhibitor of nitric oxide synthase. Treatment of lychee mixed buds with DMTU, PTIO, and L-NAME inhibited flowering even during chilling conditions. Further, these chemical also suppressed the expression of *LcLFY* and *LcAP1* (Zhou et al. 2012).

ROS affect the development of both the rudimentary leaves and the panicle primordia; thus, it is important to identify ROS-responsive genes in both these organs and understand the mechanism of their functioning. Two studies are available wherein whole-genome transcriptome profiling is done for ROS-treated rudimentary leaves and the panicle primordia. In the first study, a suppression subtractive hybridization (SSH) library was made using both MV treated and untreated panicle primordia to identify ROS-responsive genes (Liu et al. 2013b). SSH is an effective technique to isolate differentially expressed genes (Diatchenko et al. 1996). A total of 783 ESTs were found to be differentially regulated on treatment with MV for production of ROS. These ESTs were sequenced and accounted for 93 unique gene sequences. A large number of ROS-responsive genes at millet stage were found to be involved in transcription regulation (11%), transport facilitation (16%), and intracellular signaling (14%) (Liu et al. 2013b). The second study involved de novo transcriptome sequencing of ROS-treated and untreated rudimentary leaves (Lu et al. 2014). A sum of 5865 genes was found to be differentially regulated by ROS production in rudimentary leaves. Of these, 2052 genes were upregulated, i.e., either induced by or have an increase in mRNA expression by ROS treatment. 3035 genes showed downregulation. Among those induced by ROS included component genes of abscisic acid (ABA) and ethylene signal transduction pathways. Ethylene is a gaseous plant hormone and has proven roles in stress signaling (Wang et al. 2013). Earlier studies have determined that abortion of rudimentary leaves is associated with ethylene production. Furthermore, ethylene production is associated with increase in H_2O_2 levels (Biyan et al. 2013). ABA is also shown to promote flowering and diminish the growth of rudimentary leaves in lychee (Cui et al. 2013). Application of exogenous ABA to mixed buds of lychee tree reduced the number of

leaves per panicle and increased the number of axillary panicles per panicle, and therefore an increase in percentage of axillary panicles in relation to total nodes per panicle was seen. Early application of exogenous ABA to trees prior to floral primordium initiation also resulted in better floral output (Cui et al. 2013). Application of naproxen, an inhibitor of ABA biosynthesis, inhibited flower formations and also resulted in suppression of LcAP1. LcAP1 mRNA accumulated on ABA treatment, but the inductive effect of ABA was reversed in presence of DTMU hence showed that ABA directed LcAP1 transcript enhancement is via H2O2. On the other hand, ABA directed induction of LcAP1 was independent of NO as application of PTIO does not have any inhibitory effect. Interestingly, calcium also plays important role in ABA based enhancement of LcAP1. Application of a calcium chelator glycol-bis (β-amino ethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and calcium channel blocker LaCl3 to the panicle primordia in the presence of ABA suppressed the LcAP1 expression and thereby flower formation (Cui et al. 2013).

Here we summarize the complex signaling pathways governing the crucial transition of vegetative and reproductive stages in lychee. Fluctuations in temperature have serious effects on the productivity of lychee fruit crop (Fig. 8.1).

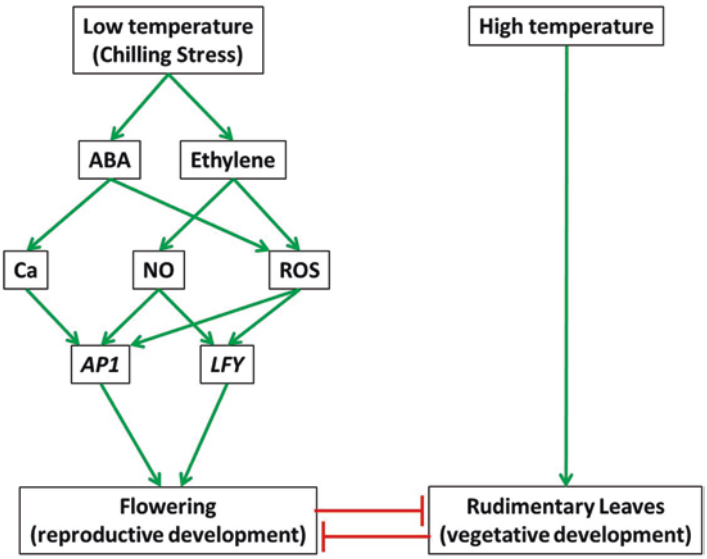

Fig. 8.1 Model depicts the signal transduction pathway for controlling flowering in *Litchi chinensis*. *Green arrows* show positive regulation*; Red lines with bars* show negative regulation. *ABA* Abscisic acid, *AP1* Apetalla1, *Ca* Calcium, *LFY* Leafy, *NO* Nitric Oxide, *ROS* Reactive oxygen species

8.2.3 Carbohydrate Stress Accelerates Fruitlets Abscission in Lychee

Fruit set is an important process for reproductive success and relies on the successful fulfillment of pollination and fertilization in flowering plants. After fruit sets, its growth increases until reaping; however, preharvest fruit shedding can happen amid fruit growth and development (Nartvaranant 2015). Abscission is a natural process developed by plants to shed unwanted, old, infected, ripened, or senescent vegetative or reproductive organs. Abscission takes place through dissolution and disintegration of the cell wall of a group of specialized cells, called as abscission zones (AZ), present between pedicel and fruitlets (Bonghi et al. 2000; Addicott 1982). The mechanisms that cause physiological shedding of young and juvenile fruit are highly intricate and variable among plant species.

The preharvest fruit drop or fruit abscission, occurring during fruit growth and development, is a major issue among lychee cultivators around the world. Fruit abscission is a well-synchronized developmental process that is both influenced and initiated in response to various kinds of exogenous (adverse environmental conditions) and endogenous (hormonal regulation, nutrient competition, etc.) cues in plants. There are three to four waves of abscission in lychee throughout fruit development depending on cultivars (Yuan and Huang 1988). The first wave of abscission occurs around 1 week after full bloom and mainly consists of abnormal and unpollinated flowers. The second wave appears after third week of full bloom, whereas third wave, also known as "June drop," occurs after sixth or seventh week of full bloom and is predominantly dependent on metabolic factors (Goldschmidt and Koch 1996). Fourth abscission wave that is specific to cultivars with aborted seeds takes place 2–3 weeks before fruit maturation. Overall, just 2–5% of the initial female flowers develop into mature fruit successfully (Stern et al. 1995; Mitra et al. 2005).

The crucial role of carbohydrate and various phytohormones in the regulation of fruit abscission is well documented in lychee (Li et al. 2004; Yuan et al. 2009; Zhou et al. 1999; Yuan and Huang 1992, 1993; Xiang et al. 1995). Competition between different vegetative and reproductive organs (e.g., fruit shoot) as well as among individual unit of same organ (e.g., fruit-fruit) of plants for photo-assimilates is well known (Goldschmidt and Koch 1996). During initial fruit development, active sinks, for instance, the growing shoots and fruit struggle for restricted supply of carbohydrate and nutrients. In citrus and other tree species, various studies on source-sink imbalances provided evidence that the carbohydrate content of fruitlets is a major factor determining abscission (Powell and Krezdorn 1977; Nzima et al. 1999; Mehouachi et al. 1995; Berüter and Droz 1991). After hormonal stimulation of initial fruit growth, successive fruit development is generally supported by nutrient supply (Talon et al. 1998). Thus, once mineral and water necessities are fulfilled, struggle for photo-assimilates is thought to be responsible for fruit abscission (Gómez-Cadenas et al. 2000; Moss et al. 1972; Powell and Krezdorn 1977). Depending on their concentration and their interactions with each other, hormones can either accelerate or delay/inhibit the abscission process. Phytohormones such as

ABA, cytokinin, and ethylene are known to accelerate plant organ abscission, whereas auxin, gibberellins, and polyamines act as inhibitors of abscission process (Taylor and Whitelaw 2001; Dal Cin et al. 2007; Ben-Cheikh et al. 1997; Aziz et al. 2001; Sipes and Einset 1983). It is generally assumed that the endogenous balance of phytohormone ethylene and auxin in the AZ influence organ abscission. Ethylene operates as the final effector in the organ abscission process and activates the transcription of genes that encodes synthesis and secretion of hydrolytic enzymes associated with cell wall dissolution in the AZ. Unlike ethylene, auxin retards abscission of reproductive structures in plants. Application of exogenous ethylene accelerates abscission, whereas auxin treatment decreases cell sensitivity to ethylene and delays or inhibits the process. It has been demonstrated in citrus that fruit abscission induced by carbon shortage is regulated by ABA, which acts as a sensor of the nutrient shortage intensity and modulates the level of ethylene (Gómez-Cadenas et al. 2000). A study in lychee demonstrated that the fruit set was greatly dependent on current photosynthates and the fruit abscission waves were in concomitant with ABA upsurge in the seed while auxin appeared to be antagonistic (Yuan and Huang 1988). Auxin can affect fruit abscission by influencing carbohydrate availability. Application of synthetic auxin 3,5,6-TPA (3,5,6-trichloro-2-pyridil-oxyacetic acid) on developing citrus fruitlet stimulated carbohydrate accumulation and hence reduce fruit abscission (Agustí et al. 2001). During lychee management practices, use of synthetic auxin like 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 2,4,5-TP (2-(2,4,5-trichlorophenoxy) propionic acid), 2,4-D and NAA to prevent fruit abscission is well known (Yuan and Huang 1991). Recently, it has been reported that girdling plus defoliation (GPD) treatment in lychee aggravated fruit abscission due to carbohydrate shortage and reduction in endogenous IAA content and thereby highlighting the importance of auxin in fruit retention in lychee crop. The quantitative real-time PCR studies to investigate the role of genes involved in auxin signal transduction pathway under the GPD treatment revealed that the transcript levels of three IAA-responsive genes (*LcAUX/IAA1*, *LcGH3.1*, and *LcSAUR1*) were increased as compared to the decreasing accumulation of auxin response factor (*LcARF1*) mRNA in AZ and other tissues (Kuang et al. 2012). One more study in lychee demonstrated that carbohydrate stress induced by GPD stimulated fruitlet abscission rate along with an increase in expression level of pectin-degrading enzyme gene (*LcPG1*) in AZ (Peng et al. 2013). The digital transcript abundance profile analysis under GPD-induced carbohydrate stress in lychee identified a total of 2771 differentially expressed genes as GPD-responsive genes. Out of 2771 genes, 857 genes were found to be involved in fruit abscission process through diverse metabolic processes and pathways, including carbohydrate metabolism, plant hormone synthesis, and signaling, transcription factor activity, and cell wall modification (Li et al. 2015a). A similar study conducted to study gene expression profile occurring in the fruit AZ-enriched pedicel during lychee fruit abscission induced by ethephon (an ethylene-releasing compound) on the genome-wide level revealed most affected genes were related to ethylene biosynthesis and signaling, auxin transport and signaling, transcription factors (TFs), protein ubiquitination, ROS response, calcium signal transduction, and cell wall modification (Li et al. 2015b).

The above studies indicate how fruit setting and development are greatly reliant on the carbohydrate supply. Similarly, if plants are subjected to shading during early fruit development phase, photosynthesis rate will be significantly altered along with availability of photo-assimilates. This will aggravate the competition for carbohydrates between active sinks. It was observed in apple trees that within 2 days of shading treatment, there was decline in the relative growth rate of fruits (Morandi et al. 2011). Fruit abscission begins within 1 week of shading and peaks after 2 weeks (Zhu et al. 2011; McArtney et al. 2004). The DTA profiling after shading treatment in lychee revealed that the expression of most of the 36 differentially expressed unigenes encoding enzymes involved in carbohydrate metabolisms was upregulated. The upregulated expression of these genes seems to be a direct or indirect early response of the lychee fruitlet to the carbohydrate shortage (Li et al. 2013).

Carbohydrate stress leads to the competition for phyto-assimilates among different organs of plants and thereby modify the nutritional equilibrium at the whole-plant level. A holistic approach to gain insight how different reproductive organs deal with carbohydrate stress might suggest the potential to identify new traits or genes that could be manipulated to improve their stress tolerance. Further studies should focus on development of new strategies to improve carbohydrate stress tolerance, notably with the use of genes involved in carbohydrate metabolism and hormone signaling pathways, implied to be involved in the carbohydrate stress response by stimulation of the carbon status. Global transcriptomic, metabolic, and hormonometer analysis should be developed in economically important fruit trees during flower and fruit development in order to provide more clues for a better understanding of the mechanisms of fruit abscission induced by carbohydrate stress.

8.3 Fruit Cracking in *Litchi*

Lychee is a highly prized and popular fruit in international market, owning to its characteristic features like bright color, exotic aroma, anti-oxidant properties, medicinal value, and good amount of vitamins and minerals. Brightly colored and intact pericarp is one the most desirable commercial features; hence, fruit cracking as a result of cracked pericarp causes huge losses to the farmers. Pericarp is the outer layer that covers the fleshy aril in lychee. Fruit cracking occurs when there are cracks and fractures in the outer layer (pericarp) that do not pierce through the fleshy part (aril). Fruit cracking is a common problem in other fruits also like pomegranate, wax apple, fresh fig, cherry tomato, nectarine, and sweet cherry. In lychee, some of the highly prized varieties like "nuomici" lychee (*L. chinensis* cv. nuomici) is seriously susceptible to fruit cracking (Peng et al. 2004). Therefore, researchers have contributed their efforts to understand the physiological, biochemical, and molecular mechanisms to understand the phenomenon of fruit cracking wherein the pericarp growth fails to keep pace with the aril growth (Peng et al. 2004; Lu et al. 2006; Yong et al. 2006; Li et al. 2014).

Plant cell wall is made up of cellulose, hemicellulose, pectin, and various structural proteins that are arranged as a complex network (Carpita and Gibeaut 1993).

During fruit development, the components of the cell wall loosen to allow growth. Primary cell wall is made up of cellulose and hemicellulose. Xyloglucan is a primary component of hemicellulose that is cross-linked with cellulose via hydrogen bonds in the cell wall. Alterations in these cross-link bonds control the cell extensibility. An enzyme called xyloglucan endotransglycosylase (XET) is a cell wall loosening enzyme that catalyzes the reversible formation of xyloglucan cross-links by breaking the xyloglucan polymer and facilitates insertion of new chains of xyloglucan and thereby loosens the cell wall (Campbell and Braam 1999; Kaku et al. 2004). Transcript expression of XET genes was checked in cracking susceptible ("Nuomici") and resistant ("Huaizhi") varieties of lychee in both aril and pericarp tissues at different developmental stages of fruit (Lu et al. 2006). Of the three XET transcripts (LcXET1, LcXET2, and LcXET3) isolated and identified from developing lychee fruit, one of them, LcXET1, showed fruit-specific expression. LcXET1 was differentially expressed in "Nuomici" and "Huaizhi" varieties. In susceptible "Nuomici," LcXET1 transcript expression was activated at 59 days after anthesis (DAA) in aril tissue in comparison to 73 DAA in pericarp tissue. On the other hand, in the resistant "Huaizhi" variety, the expression of LcXET1 transcript appeared simultaneously in both aril and pericarp at 66 DAA. Probably the late activity of XET in the pericarp in relation to aril in Nuomici results in lagging growth of pericarp which eventually results in pericarp cracking. Alpha-naphthalene acetic acid (NAA) is a synthetic plant hormone for auxin. In parts of India and China, application of NAA is done on the lychee trees to reduce fruit cracking especially in "Nuomici" variety (Li et al. 2001). NAA reduce fruit cracking by facilitating the growth of pericarp as auxins are involved in cell division and cell expansion. LcXET1 mRNA accumulated in the pericarp tissue after NAA treatment, whereas other XET genes (LcXET2, LcXET3) showed enhanced expression in aril tissue. Thus, it appears that LcXET1 play acritical role in pericarp extension that is regulated via auxin mediated signaling (Lu et al. 2006).

Pectin and related pectin substances are an important component of primary cell wall in plants. They make up almost one third of cell wall in dicot plants and are major component of the middle lamella. The most important role of pectin is to bind the adjacent cells. Calcium ions are known to be an important signaling component for cell elongation and stabilization of cell membrane during cell wall formation. Calcium also plays important structural roles in the cell wall of pericarp. Pectin requires calcium ions for cross-linking that is required for the adhesive properties of pectin that binds the cells together (Daher and Braybrook 2015). Deficiency of calcium is shown to cause severe pericarp cracking in lychee (Huang et al. 2005). Brassinosteroids are a class of hormones that are involved in cell division and cell growth. They are known to work in coordination with auxins. Foliar spray of brassinolide (a type of brassinosteroid) to lychee trees before anthesis is shown to significantly increase the concentration of calcium in fruit pericarp (Peng et al. 2004). Additionally, the content of water-insoluble pectin (protopectin) and water-soluble pectin also increased in response to the brassinolide treatment (Peng et al. 2004). Two enzymes, viz., pectin methylesterase (PME) and polygalacturonase (PG), are involved in pectin metabolism. A comparison of activities of PME and PG in the

pericarp of Nuomici and Huaizhi varieties showed that theses enzymes have higher catalytic activity in the cracking susceptible Nuomici variety (Li et al. 2003). Application of brassinolide to lychee increased the activity of PME and PG in the pericarp. Thus, considering together, the increased calcium content, increased PME and PG activity, and increased protopectin content in pericarp, brassinolide appears to function toward cell division and elongation in pericarp, hence assuring the good development and quality of pericarp in lychee and reduced cracking (Peng et al. 2004). Another cell wall metabolizing enzyme, cellulase, that degrades the cellulose also shows higher enzymatic activity in Nuomici compared to Huaizhi (Li et al. 2003). Brassinolide application considerably reduces the activity of cellulase in pericarp and further associate brassinolide treatment with reduced fruit cracking (Peng et al. 2004).

Expansins are a class of wall proteins that are involved in cell enlargement. They govern the extensibility of the cell wall in a pH-dependent manner (Cosgrove 2000). Two cDNAs encoding for expansin genes (LcExp1 and LcExp2) were identified from the developing lychee fruit (Yong et al. 2006). LcExp1 and LcExp2 had preferential expression in fruit and negligible expression in other tissues of the plant. LcExp1 showed pericarp specific expression with no transcript accumulation in aril tissue, both in Nuomici and Huaizhi varieties. LcExp1 showed strong expression in Huaizhi during the rapid growth phase of fruit development (59–80 DAA) with a steep upregulation during the last stages of fruit expansion. On the other hand, in Nuomici, LcExp2 expression did not shoot up instead showed a constant low mRNA accumulation in the pericarp. These results directly correlated with the inability of Nuomici to expand its pericarp during late stages of fruit development. LcExp2 on the other hand was predominantly expressed in aril of both Nuomici and Huaizhi at almost similar levels. However, LcExp2 mRNA showed no expression in Nuomici pericarp but low level expression was found in Huaizhi pericarp throughout the fruit development (Yong et al. 2006). Thus, clearly, both LcExp1 and LcExp2 are important factors governing the pericarp cracking.

A recent study involving whole-genome expression analysis of pericarp of cracking and non-cracking fruits using high-throughput RNA sequencing has allowed identification of an array of candidate genes that may govern mechanism of pericarp cracking in lychee (Li et al. 2014). The study involved de novo assembly of pericarp transcriptome. The comparative analysis of cracking and non-cracking fruits identified 1998 differentially regulated genes. Of these, 632 were upregulated and 1366 were downregulated in cracked fruits in comparison to non-cracked fruits (Li et al. 2014). Interestingly four genes with roles in water transport, viz., LcAQP1, LcPIP1, LcNIP1, and LcSIP1, were upregulated in cracked fruits. It has been reported previously that excess water absorbed by roots or by fruit surface increases the fruit cracking percentage (Measham et al. 2010). In some fruits, rainfall before harvest causes fruit cracking (Simmon 2006). Upregulation of water transport-related genes in cracked lychee fruit is postulated to be associated with increased water entry into the aril tissue, resulting in greater turgor and thereby rupturing of the pericarp of lychee. Earlier studies have also shown that active uptake of water led fruit osmotic potential is positively related to pericarp cracking in lychee (Li et al. 1996). Further

analysis of cracked and non-cracked pericarp transcriptome showed that genes driving the hormonal signaling were prominently represented in the set of differentially expressed genes. Important genes of ABA-mediated signal transduction (21 genes, viz., two LcCYP707A, nine LcGT, six Lcβ-Glu, two LcPP2C, one LcABI1, and one LcABI5) and gibberellin-mediated signaling (five genes, viz., two LcKS, two LcGA2ox, and one LcGID1) were differentially expressed in cracked fruits in comparison to non-cracked fruits. In agreement with the previous reports, pericarp transcriptome highlighted the role of calcium and showed differential expression of 13 genes involved in Ca transport during cracking (one LcTPC, three Ca2+/H+ exchanger, four Ca2 + −ATPase, two LcCDPK, and three LcCBL) (Li et al. 2014). Furthermore, as many as 24 genes encoding for enzymes involved in cell wall metabolism (five LcPG, one LcEG, three LcPE, five LcEXP, nine Lcβ-Galand, one LcXET) showed considerable change of expression when comparing pericarp of cracked and non-cracked fruits.

8.3.1 Pericarp Browning and Senescence in Lychee

Lychee, being a non-climacteric fruit, has a short shelf life under normal ambient condition and senesces quickly after harvest. Skin color loss or pericarp browning is one of the first visual signs of senescence of fruit in lychee. Once harvested, the bright red color of lychee gets lost within 2 days and pericarp turns brown. This browning is induced by degradation of anthocyanins, a group of secondary metabolites synthesized via the flavonoid pathway, by enzymes polyphenol oxidase (PPO) and peroxidase (POD) and subsequent production of brown colored by-products (Akamine 1960; Huang et al. 1990; Chen and Wang 1989; Nip 1988). Different methods such as wax coating, acid treatment, etc. have been tried by various researchers to solve the problem of postharvest pericarp browning. One of the methods where precooled lychee fruits were treated with 0.6% sodium metabisulfite solution for 10 min followed by dipping in 2% HCl solution for 5 min seems to be the best approach for delaying pericarp browning and extended their shelf life up to 9 days at ambient conditions (Neog and Saikia 2010).

Fruit senescence is controlled by various intrinsic (phytohormones, TFs, etc.) and extrinsic (sunlight, temperature, etc.) factors which upregulates a subset of senescence-associated genes (SAGs) involved in perception, signal transduction pathways, and downstream responses (Guo 2013). ABA is a signal molecule that accelerates fruit ripening as well as the accumulation of anthocyanin. ABA significantly enhances anthocyanin accumulation in lychee pericarp as evident by a study where the treatment of lychee with ABA 30 days before commercial harvest enhanced anthocyanin synthesis in the pericarp of lychee (Wei et al. 2011). MYBs, one of the major TFs, have been reported to play crucial role in regulating senescence. Along with senescence, MYB control anthocyanin accumulation by modulating the expression of structural genes in the anthocyanin biosynthetic pathway in various plants. In lychee, an R2R3-MYB gene, *LcMYB1*, is accountable for red pigmentation in the pericarp of lychee fruit. ABA and sunlight exposure promotes anthocyanin

accumulation by upregulating expression level of *LcMYB1* (Lai et al. 2014). The promoter region of *LcMYB1* revealed presence of cis-elements associated with light responsiveness and abscisic acid responsiveness. The overexpression of *LcMYB1*in tobacco plants induced anthocyanin accumulation in all tissues of transformed lines, validating the role of *LcMYB1* in the regulation of anthocyanin biosynthesis.

Several studies revealed that polyamines (PAs) inhibit senescence of leaves and fruit ripening by blocking ethylene biosynthesis (Kaur-Sawhney et al. 1982; Kakkar and Rai 1993). PAs inhibit ethylene biosynthesis by blocking the conversion of S-adenosylmethionine (SAM), a common precursor for biosynthesis of both polyamines and ethylene, to 1-aminocyclopropane-1-carboxylic acid (ACC) and of ACC to ethylene (Even-Chen et al. 1982; Apelbaum et al. 1981). Similarly, ethylene negatively regulates PAs synthesis as it is an effective inhibitor of two key enzymes in PA biosynthetic pathway, viz., arginine decarboxylase (ADC) and S-adenosylmethionine decarboxylase (SAMDC) (Icekson et al. 1985; Apelbaum et al. 1985). Recently, one study reported that overexpression of *Datura SAMDC* gene (dSAMDC) in lychee plants led to increase in PAs content, especially spermidine and spermine, and several anti-oxidant enzyme activities under high temperature condition in transgenic lines as compared to wild types. The transformed lychee plants also exhibited a significant increase in fruit flavor, color quality, including fruit ripening time (Das et al. 2016). Increased spermidine and spermine levels are usually associated with upgraded plant resistance to multiple abiotic stresses. Therefore, manipulation of genes involved in PAs biosynthesis pathways using genetic engineering technology can be used to increase shelf life of lychee by delaying the process of fruit ripening.

MicroRNAs (miRNAs) regulate various biological processes in plants by modulating gene expression either by mRNA degradation or by translational inhibition. Various studies have indicated the role of miRNA in mediating plant senescence. miR390 targets auxin response factors ARF2/3/4 by degrading its mRNA and thus in turn modulates the timing of senescence (Ellis et al. 2005). Another miRNA, miR164, has been found to downregulate the expression of ORE1, a NAC transcription factor, which positively regulates aging-induced cell death in *Arabidopsis*. miR164 expression gradually decreases with aging which leads to the upregulation of ORE1. *Arabidopsis* miR164 mutants exhibit early senescence as compared to wild-type plants (Kim et al. 2009). Recently, a study was conducted in lychee to understand the role of miRNAs in regulating the senescence of harvested lychee fruit (Yao et al. 2015). In this study, 49 known miRNA families and 11 lychee-specific miRNAs were identified in lychee. Data analysis revealed that lychee_43435 gene annotated as an MYB transcription factor was targeted by 31 miRNAs from the miR159 and miR319 families, suggesting a crucial role of miRNAs in regulating MYB TFs which, on the other hand, are known to regulate anthocyanin accumulation and senescence in lychee fruits (e.g., *LcMYB1*). Another miRNA, lch-miR393b, was found to target lychee_55456 gene annotated as auxin signaling F-box 2 (AFB2), known to act as auxin receptor (Parry et al. 2009). The expression level of miR393b was found to be downregulated fourfold after post-cold storage at 24 h, while the expression of its target gene AFB2 increased almost fourfold during the

same period, thereby suggesting a key role of miR393 in the postharvest senescence of lychee fruit by mediating auxin signaling (Yao et al. 2015). More such findings of miRNAs and their target genes in horticulture crops will help researchers to understand miRNA role in regulating fruit senescence and thereby its use for miRNA-based posttranscriptional gene silencing to delay fruit senescence.

8.4 Conclusions

In this chapter, we have tried to compile the studies on various factors that affect the commercially desirable traits of lychee. For any fruit crop, high fruit production; maintenance of color, flavor, and odor; and long shelf life are the most important features. But these traits are drastically affected by abiotic stresses like temperature fluctuations, scarcity as well as excess of water, and inadequate photosynthesis. Most of these stress signals are perceived by hormones. The studies till date have identified candidate genes that orchestrate the stress signaling pathways. The identification and understanding the functioning of these genes is an important step toward engineering plants with better resistance to the environmental stresses, so as to ensure consistent and good fruit yield.

References

Addicott FT (1982) Abscission. University of California Press, Berkeley

Agustí M, Almela V, Juan M, Alferez F, Tadeo FR, Zacarías L (2001) Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann Bot 88(3):415–422. doi:10.1006/anbo.2001.1482

Akamine EK (1960) Preventing the darkening of fresh lychees prepared for transport. Technical Progress Report of Hawaii Agricultural Experimental Station, vol 127. University of Hawaii

Apelbaum A, Burgoon AC, Anderson JD, Lieberman M, Ben-Arie R, Mattoo AK (1981) Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts. Plant Physiol 68(2):453–456

Apelbaum A, Goldlust A, Icekson I (1985) Control by ethylene of arginine decarboxylase activity in pea seedlings and its implication for hormonal regulation of plant growth. Plant Physiol 79(3):635–640

Aziz A, Brun O, Audran J-C (2001) Involvement of polyamines in the control of fruitlet physiological abscission in grapevine (*Vitis vinifera*). Physiol Plant 113(1):50–58. doi:10.1034/j.1399-3054.2001.1130107.x

Banuelos GR, Argumedo R, Patel K, Ng V, Zhou F, Vellanoweth RL (2008) The developmental transition to flowering in Arabidopsis is associated with an increase in leaf chloroplastic lipoxygenase activity. Plant Sci 174(3):366–373. doi:10.1016/j.plantsci.2007.12.009

Ben-Cheikh W, Perez-Botella J, Tadeo FR, Talon M, Primo-Millo E (1997) Pollination increases gibberellin levels in developing ovaries of seeded varieties of citrus. Plant Physiol 114(2):557–564

Berüter J, Droz P (1991) Studies on locating the signal for fruit abscission in the apple tree. Sci Hortic 46(3):201–214. doi:10.1016/0304-4238(91)90043-X

Biyan Z, Huang X, Chen H, Shu W, Hu Z, Liu W, Xiang C, Zhang S (2013) Effects of ethylene on rudimentary leaf and panicle primordium in Litchi: anti-oxidants, hydrogen peroxide and nitric oxide. In: Acta Horticulturae, 2013. International Society for Horticultural Science (ISHS). Leuven, p 247–254. doi:10.17660/ActaHortic.2013.975.27

Bonghi C, Tonutti P, Ramina A (2000) Biochemical and molecular aspects of fruitlet abscission. Plant Growth Regul 31(1):35–42. doi:10.1023/a:1006338210977
Bose TK, Mitra SK (1990) Fruits: tropical and subtropical. Naya Prokash, Calcutta
Boyer JS (1982) Plant productivity and environment. Science 218(4571):443–448. doi:10.1126/science.218.4571.443
Campbell P, Braam J (1999) Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall-modifying functions. Trends Plant Sci 4(9):361–366
Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3(1):1–30
Chen HB, Huang HB (2005) Low temperature requirements for floral induction in lychee. Acta Hortic 665:195–202
Chen YZ, Wang YR (1989) Study on peroxidases in litchi pericarp. Acta Batanica Austro Sinica 5:47–52
Cosgrove DJ (2000) Loosening of plant cell walls by expansins. Nature 407(6802):321–326. doi:10.1038/35030000
Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163. doi:10.1186/1471-2229-11-163
Cui Z, Zhou B, Zhang Z, Hu Z (2013) Abscisic acid promotes flowering and enhances LcAP1 expression in *Litchi chinensis* Sonn. S Afr J Bot 88:76–79. doi:10.1016/j.sajb.2013.05.008
Daher FB, Braybrook SA (2015) How to let go: pectin and plant cell adhesion. Front Plant Sci 6:523. doi:10.3389/fpls.2015.00523
Dal Cin V, Boschetti A, Dorigoni A, Ramina A (2007) Benzylaminopurine application on two different apple cultivars (*Malus domestica*) displays new and unexpected fruitlet abscission features. Ann Bot 99(6):1195–1202. doi:10.1093/aob/mcm062
Damour G, Vandame M, Urban L (2008) Long-term drought modifies the fundamental relationships between light exposure, leaf nitrogen content and photosynthetic capacity in leaves of the lychee tree (*Litchi chinensis*). J Plant Physiol 165(13):1370–1378. S0176–1617(07)00316–1 [pii], doi:10.1016/j.jplph.2007.10.014
Das DK, Prabhakar M, Kumari D, Kumari N (2016) Expression of SAMDC gene for enhancing the shelf life for improvement of fruit quality using biotechnological approaches into litchi (*Litchi chinensis* Sonn.) cultivars. Adv Biosci Biotechnol 7:300–310. doi:10.4236/abb.2016.76028
Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci U S A 93(12):6025–6030
Dominguez PG, Frankel N, Mazuch J, Balbo I, Iusem N, Fernie AR, Carrari F (2013) ASR1 mediates glucose-hormone cross talk by affecting sugar trafficking in tobacco plants. Plant Physiol 161(3):1486–1500. doi:10.1104/pp.112.208199
Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005) AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 132(20):4563–4574. doi:10.1242/dev.02012
Even-Chen Z, Mattoo AK, Goren R (1982) Inhibition of ethylene biosynthesis by aminoethoxyvinylglycine and by polyamines shunts label from 3,4-[(14)C]Methionine into spermidine in aged orange peel discs. Plant Physiol 69(2):385–388
Goldschmidt EE, Koch KE (1996) Citrus. In: Zaminski E, Schaffer AA (eds) Photoassimilate distribution plants and crops source-sink relationships. Marcel Dekker, New York, pp 797–823
Gómez-Cadenas A, Mehouachi J, Tadeo RF, Primo-Millo E, Talon M (2000) Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta 210(4):636–643. doi:10.1007/s004250050054

Guo Y (2013) Towards systems biological understanding of leaf senescence. Plant Mol Biol 82(6):519–528. doi:10.1007/s11103-012-9974-2

Huang HB, Chen HB (2005) A phase approach towards floral formation in lychee. Acta Hortic 665:185–194

Huang S, Hart H, Lee H, Wicker L (1990) Enzymatic and color changes during post-harvest storage of lychee fruit. J Food Sci 55(6):1762–1763. doi:10.1111/j.1365-2621.1990.tb03623.x

Huang X, Wang HC, Li J, Yin J, Yuan W, Lu J, Huang HB (2005) An overview of calcium's role in lychee fruit cracking. In: International Society for Horticultural Science (ISHS). Leuven, pp 231–240. doi:10.17660/ActaHortic.2005.665.26

Icekson I, Goldlust A, Apelbaum A (1985) Influence of Ethylene on S-adenosylmethionine Decarboxylase Activity in Etiolated Pea Seedlings. J Plant Physiol 119(4):335–345. doi:10.1016/S0176-1617(85)80101-2

Joshi G, Singh PK, Singh SK, Srivastava PC (2011) Effect of drip fertigation and mulching on water requirement, yield and economics of high density litchi. Progress Hortic 43(22):237–242

Kakkar RK, Rai VK (1993) The international journal of plant biochemistry plant polyamines in flowering and fruit ripening. Phytochemistry 33(6):1281–1288. doi:10.1016/0031-9422(93)85076-4

Kaku T, Tabuchi A, Wakabayashi K, Hoson T (2004) Xyloglucan oligosaccharides cause cell wall loosening by enhancing xyloglucan endotransglucosylase/hydrolase activity in azuki bean epicotyls. Plant Cell Physiol 45(1):77–82

Kalifa Y, Perlson E, Gilad A, Konrad Z, Scolnik PA, Bar-Zvi D (2004) Over-expression of the water and salt stress-regulated Asr1 gene confers an increased salt tolerance. Plant Cell Environ 27(12):1459–1468. doi:10.1111/j.1365-3040.2004.01251.x

Kaur-Sawhney R, Shih L-M, Flores HE, Galston AW (1982) Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol 69(2):405–410

Kim JH, Woo HR, Kim J, Lim PO, Lee IC, Choi SH, Hwang D, Nam HG (2009) Trifurcate feed-forward regulation of age-dependent cell death involving miR164 in Arabidopsis. Science 323(5917):1053–1057. doi:10.1126/science.1166386

Kuang JF, Wu JY, Zhong HY, Li CQ, Chen JY, Lu WJ, Li JG (2012) Carbohydrate stress affecting fruitlet abscission and expression of genes related to auxin signal transduction pathway in litchi. Int J Mol Sci 13(12):16084–16103. ijms131216084 [pii], doi:10.3390/ijms131216084

Lai B, Li X-J, Hu B, Qin Y-H, Huang X-M, Wang H-C, Hu G-B (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS One 9(1):e86293. doi:10.1371/journal.pone.0086293

Li C, Wang Y, Huang X, Li J, Wang H (2015a) An improved fruit transcriptome and the identification of the candidate genes involved in fruit abscission induced by carbohydrate stress in litchi. Front Plant Sci 6:439. doi:10.3389/fpls.2015.00439

Li C, Wang Y, Ying P, Ma W, Li J (2015b) Genome-wide digital transcript analysis of putative fruitlet abscission related genes regulated by ethephon in litchi. Front Plant Sci 6. doi:10.3389/fpls.2015.00502

Li JG, Huang HB, Gao FF, Huang XM, Wang HC (2001) An overview of litchi fruit cracking. In: International Society for Horticultural Science (ISHS). Leuven pp 205–208. doi:10.17660/ActaHortic.2001.558.28

Li J, Huang X, Huang H (2003) Comparison of the activities of enzymes related to cell-Wa metabolism in pericarp between litchi cultivars susceptible and resistant to fruit cracking. Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao 29(2):141–146

Li JG, Huang HB, Yuan RC, Gao FF (1996) Litchi fruit cracking in relation to fruit growth and water-uptake kinetics. J South China Agric Univ 13:129–135

Li J, Liu S, Wang Z (2004) Changes in endogenous polyamine contents during fruit development of litchi ({\sl *Litchi chinensis*}). Plant Physiol Commun 40(2):153–156

Li C, Wang Y, Huang X, Li J, Wang H (2013) De novo assembly and characterization of fruit transcriptome in *Litchi chinensis* Sonn and analysis of differentially regulated genes in fruit in response to shading. BMC Genomics 14:552. 1471–2164–14-552 [pii], doi:10.1186/1471-2164-14-552

Li WC, Wu JY, Zhang HN, Shi SY, Liu LQ, Shu B, Liang QZ, Xie JH, Wei YZ (2014) De novo assembly and characterization of pericarp transcriptome and identification of candidate genes mediating fruit cracking in *Litchi chinensis* Sonn. Int J Mol Sci 15(10):17667–17685. doi:10.3390/ijms151017667

Liu J, Jia C, Dong F, Wang J, Zhang J, Xu Y, Xu B, Jin Z (2013a) Isolation of an abscisic acid senescence and ripening inducible gene from litchi and functional characterization under water stress. Planta 237(4):1025–1036. doi:10.1007/s00425-012-1820-x

Liu WW, Kim HJ, Chen HB, Lu XY, Zhou BY (2013b) Identification of MV-generated ROS responsive EST clones in floral buds of *Litchi chinensis* Sonn. Plant Cell Rep 32(9):1361–1372. doi:10.1007/s00299-013-1448-8

Lu X, Kim H, Zhong S, Chen H, Hu Z, Zhou B (2014) De novo transcriptome assembly for rudimentary leaves in Litchi chinensis Sonn. and identification of differentially expressed genes in response to reactive oxygen species. BMC Genomics 15:805. doi:1471–2164–15-805 [pii],10.1186/1471–2164–15-805

Lu W, Wang Y, Jiang Y, Li J, Liu H, Duan X, Song L (2006) Differential expression of litchi XET genes in relation to fruit growth. Plant Physiol Biochem 44(11–12):707–713. doi:10.1016/j.plaphy.2006.09.020

Mali SS, Das B, Bhatnagar PR (2015) Effect of water application method and deficit irrigation on yield, quality and irrigation water use efficiency of litchi (*Litchi chinensis* Sonn.) cv Shahi. International Journal of Irrigation and Water Management

McArtney S, White M, Latter I, Campbell J (2004) Individual and combined effects of shading and thinning chemicals on abscission and dry-matter accumulation of 'Royal Gala' apple fruit. J Hortic Sci Biotechnol 79(3):441–448. doi:10.1080/14620316.2004.11511787

Measham PF, Gracie AJ, Wilson SJ, Bound SA (2010) Vascular flow of water induces side cracking in sweet cherry (*Prunus avium* L.). Adv Hortic Sci 24(4):243–248

Mehouachi J, Serna D, Zaragoza S, Agusti M, Talon M, Primo-Millo E (1995) Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of Citrus unshiu. Plant Sci 107(2):189–197. doi:10.1016/0168-9452(95)04111-7

Menzel CM (1985) Propagation of lychee: A review. Sci Hortic 25(1):31–48. doi:10.1016/0304-4238(85)90074-3

Menzel CM (1991) *Litchi chinensis* Sonn. In: Varheij EWM, Coronel RE (eds) Plant resources of South-East Asia, vol 2. Wageningen, Edible fruits and nuts. Pudoc, pp 191–195

Menzel C (2005) Photosynthesis and Productivity. In: Menzel C, Waite G (eds) Litchi and Longan: botany. CABI, Production and Uses, pp 153–182

Menzel CM, Simpson DX (1988) Effect of temperature on growth and flowering of litchi (*Litchi chinensis* Sonn.) cultivars. J Hortic Sci 63:349–360

Mitra SK, Pathak PK (2010) Litchi production in the Asia-Pacific Region. In: International Society for Horticultural Science (ISHS). Leuven, pp 29–36. doi:10.17660/ActaHortic.2010.863.1

Mitra SK, Pereira LS, Pathak PK, Majumdar D (2005) Fruit abscission pattern of lychee cultivars. In: International Society for Horticultural Science (ISHS). Leuven, pp 215–218. doi:10.17660/ActaHortic.2005.665.24

Morandi B, Zibordi M, Losciale P, Manfrini L, Pierpaoli E, Grappadelli LC (2011) Shading decreases the growth rate of young apple fruit by reducing their phloem import. Sci Hortic 127(3):347–352. doi:10.1016/j.scienta.2010.11.002

Moss GI, Steer BT, Kriedemann PE (1972) The regulatory role of inflorescence leaves in fruit-setting by sweet orange (*Citrus sinensis*). Physiol Plant 27(3):432–438. doi:10.1111/j.1399-3054.1972.tb03639.x

Nakata S, Suehisa R (1969) Growth and development of *Litchi chinensis* as affected by soil-moisture stress. Am J Bot 56(10):1121–1126. doi:10.2307/2440772

Nartvaranant P (2015) Endogenous hormonal status in Pummelo fruitlets cultivar Thong Dee: relationship with pre-harvest fruit drop. Songklanakarin J Sci Technol 37(5):539–544

Neog M, Saikia L (2010) Control of post-harvest pericarp browning of litchi (*Litchi chinensis* Sonn). J Food Sci Technol 47(1):100–104. doi:10.1007/s13197-010-0001-9

NHB (2015) Govt of India. http://nhb.gov.in/area-pro/NHB_Database_2015.
Nip WK (1988) Handling and preservation of lychee (*Litchi chinensis* Sonn.) with emphasis on color relation. Trop Sci 28:5–11
Nzima MDS, Martin GC, Nishijima C (1999) Effect of fall defoliation and spring shading on shoot carbohydrate and growth parameters among individual branches of alternate bearing `Kerman' Pistachio trees. J Am Soc Hortic Sci 124(1):52–60
Pareek S (2016) Nutritional and biochemical composition of lychee (*Litchi chinensis* Sonn.) cultivars. In: Simmonds M, Preedy V (eds) Nutritional composition of fruit cultivars. Academic Press, San Diego, pp 395–418. doi:10.1016/B978-0-12-408117-8.00017-9
Parry G, Calderon-Villalobos LI, Prigge M, Peret B, Dharmasiri S, Itoh H, Lechner E, Gray WM, Bennett M, Estelle M (2009) Complex regulation of the TIR1/AFB family of auxin receptors. Proc Natl Acad Sci U S A 106(52):22540–22545. doi:10.1073/pnas.0911967106
Peng J, Tang X, Feng H (2004) Effects of brassinolide on the physiological properties of litchi pericarp (*Litchi chinensis* cv. nuomici). Sci Hortic 101(4):407–416. doi:10.1016/j.scienta.2003.11.012
Peng G, Wu J, Lu W, Li J (2013) A polygalacturonase gene clustered into clade E involved in lychee fruitlet abscission. Sci Hortic 150:244–250. doi:10.1016/j.scienta.2012.10.029
Powell AA, Krezdorn AH (1977) Influence of fruit setting treatment on translocation of 14C-metabolites in citrus during flowering and fruiting. J Am Soc Hortic Sci 102:709–714
Rab A, Haq IU (2012) Irrigation intervals affect physicochemical quality attributes and skin cracking in litchi fruit. Turk J Agric For 36(5):553–564
Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol 141(2):357–366. doi:10.1104/pp.106.079129
Sarin NB, Prasad US, Kumar M, Jain SM (2009) Litchi breeding for genetic improvement. In: Jain SM, Priyadarshan PM (eds) Breeding plantation tree crops: Tropical species. Springer New York, New York, pp 217–245. doi:10.1007/978-0-387-71201-7_7
Sierla M, Rahikainen M, Salojarvi J, Kangasjarvi J, Kangasjarvi S (2013) Apoplastic and chloroplastic redox signaling networks in plant stress responses. Antioxid Redox Signal 18(16):2220–2239. doi:10.1089/ars.2012.5016
Simmon G (2006) Review on rain induced fruit cracking of sweet cherries (*Prunus aviuml*.), its causes and the possibilities of prevention. Int J Hortic Sci 12(3):27–35
Sipes DL, Einset JW (1983) Cytokinin stimulation of abscission in lemon pistil explants. J Plant Growth Regul 2(1):73–80. doi:10.1007/bf02042235
Stern RA, Kigel J, Tomer E, Gazit S (1995) `Mauritius' lychee fruit development and reduced abscission after treatment with the auxin 2,4,5-TP. J Am Soc Hortic Sci 120(1):65–70
Stern RA, Meron M, Naor A, Wallach R, Bravdo B, Gazit S (1998) Effect of fall irrigation level in `Mauritius' and `Floridian' lychee on soil and plant water status, flowering intensity, and yield. J Am Soc Hortic Sci 123(1):150–155
Subhadrabandhu S, Stern AS (2005) Taxonomy, botany and plant development. In: Menzel C, Waite G (eds) Litchi and Longan: botany, production and uses. CABI, Cambridge, MA, pp 25–34
Talon M, Tadeo FR, Ben-Cheikh W, Gomez-Cadenas A, Mehouachi J, Pérez-Botella J, Primo-Millo E (1998) Hormonal regulation of fruit set and abscission in Citrus: classical concepts and new evidence. In: International Society for Horticultural Science (ISHS), Leuven, pp 209–218. doi:10.17660/ActaHortic.1998.463.24
Taylor JE, Whitelaw CA (2001) Signals in abscission. New Phytol 151(2):323–340. doi:10.1046/j.0028-646x.2001.00194.x
Tiwari V, Chaturvedi AK, Mishra A, Jha B (2015) Introgression of the SbASR-1 Gene Cloned from a Halophyte *Salicornia brachiata* Enhances Salinity and Drought Endurance in Transgenic Groundnut (*Arachis hypogaea*) and Acts as a Transcription Factor. PLoS One 10(7):e0131567. doi:10.1371/journal.pone.0131567
Tuteja N, Gill S (2016) Abiotic stress response in plants. Wiley

Wang F, Cui X, Sun Y, Dong CH (2013) Ethylene signaling and regulation in plant growth and stress responses. Plant Cell Rep 32(7):1099–1109. doi:10.1007/s00299-013-1421-6

Wei Y-Z, Hu F-C, Hu G-B, Li X-J, Huang X-M, Wang H-C (2011) Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PLoS One 6(4):e19455. doi:10.1371/journal.pone.0019455

Weigel D, Meyerowitz EM (1993) Activation of floral homeotic genes in Arabidopsis. Science 261(5129):1723–1726. doi:10.1126/science.261.5129.1723

Xiang X, Qiu YP, Zhang ZW (1995) Endogenous hormones in the fruit of *Litchi chinensis* cv. Nuomici relating to fruit abscission. J Fruit Sci 12:88–92

Yang CY, Chen YC, Jauh GY, Wang CS (2005) A Lily ASR protein involves abscisic acid signaling and confers drought and salt resistance in Arabidopsis. Plant Physiol 139(2):836–846. doi:10.1104/pp.105.065458

Yao F, Zhu H, Yi C, Qu H, Jiang Y (2015) MicroRNAs and targets in senescent litchi fruit during ambient storage and post-cold storage shelf life. BMC Plant Biol 15:181. doi:10.1186/s12870-015-0509-2

Yong W, Wangjin L, Jianguo L, Yueming J (2006) Differential expression of two expansin genes in developing fruit of cracking-susceptible and-resistant litchi cultivars. J Am Soc Hortic Sci 131(1):118–121

Yuan R, Huang H (1988) Litchi fruit abscission: its patterns, effect of shading and relation to endogenous abscisic acid. Sci Hortic 36(3):281–292. doi:10.1016/0304-4238(88)90063-5

Yuan R, Huang H (1991) Effect of NAA, NAA plus nucleotides on fruit set of lychee. Austral Lychee Yearbook 1:46–50

Yuan RC, Huang HB (1992) Improvement of fruit-set in *Litchi chinensis* Sonn. Through regulation of source-sink relationships. J S China Agric Univ 13:136–141

Yuan RC, Huang HB (1993) Regulation of root and shoot growth and fruit drop of young litchi trees by trunk girdling in view of source-sink relationship. J Fruit Sci 10:195–198

Yuan W, Huang X, Wang H, Li J, Chen H, Yin J (2009) Seasonal changes in carbon nutrition reserve in Nuomici litchi trees and its relation to fruit load. Acta Horticulturae Sinica 36(11):1568–1574

Zhang ZW (1997) China, the native home of litchi. In: Zhang ZW, Yuan PY, Wang BQ, Qiu YP, Li JS (eds) Litchi pictorial narration of cultivation. China Books & Periodicals, Incorporated, Guangdong Sheng nong ye ke xue yuan. Pomology Research Institute, Guangdong, pp 12–17

Zhou XJ, Yan HD, Bai HH, Yao WD (1999) Carbohydrate and endohormone status in relation to fruit set as influenced by trunk spiral girdling of young litchi trees. Acta Hortic Sinica 26:77–80. doi:10.3321/j.issn:0513- 353X.1999.02.002

Zhou B, Chen H, Huang X, Li N, Hu Z, Gao Z, Lu Y (2008) Rudimentary leaf abortion with the development of panicle in litchi: changes in ultrastructure, anti-oxidant enzymes and phytohormones. Sci Hortic 117(3):288–296. doi:http://dx.doi.org/10.1016/j.scienta.2008.04.004

Zhou B, Li N, Zhang Z, Huang X, Chen H, Hu Z, Pang X, Liu W, Lu Y (2012) Hydrogen peroxide and nitric oxide promote reproductive growth in *Litchi chinensis*. Biol Plant 56(2):321–329. doi:10.1007/s10535-012-0093-3

Zhu H, Dardick CD, Beers EP, Callanhan AM, Xia R, Yuan R (2011) Transcriptomics of shading-induced and NAA-induced abscission in apple (*Malus domestica*) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. BMC Plant Biol 11:138. doi:10.1186/1471-2229-11-138

Zimmermann P, Heinlein C, Orendi G, Zentgraf U (2006) Senescence-specific regulation of catalases in *Arabidopsis thaliana* (L.) Heynh. Plant Cell Environ 29(6):1049–1060

Strategies to Retard Postharvest Pericarp Browning in Lychee Fruit

9

Sahana Basu and Gautam Kumar

Abstract

Lychee (*Litchi chinensis* Sonn.) is one of the major commercially valuable fruits with an attractive red colour, sweet taste and aroma. Postharvest pericarp browning is one of the main constraints reducing its market value. Desiccation of pericarp tissues is one of the leading factors for this problem. Desiccation ultimately results in direct interaction and oxidation of pericarp phenolics with polyphenol oxidase (PPO) and peroxidase (POD) enzymes due to loss of cell compartment. Lychee pericarp browning is an ABA-mediated oxidative process, including oxidation of lipids, polyphenols and anthocyanins. 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 proven considerably effective. A comprehensive genomic, transcriptomic and metabolomic analyses may help in revealing the molecular background of postharvest senescence of lychee.

Keywords

Lychee • Pericarp browning • Polyphenol oxidase (PPO) • Peroxidase (POD) • Postharvest cold-storage

S. Basu
Department of Biotechnology, Assam University, Silchar 788011, Assam, India

G. Kumar (✉)
Department of Life Science, Central University of South Bihar, Patna 800014, Bihar, India
e-mail: gautam@cub.ac.in

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9.1 Introduction

Lychee (*Litchi chinensis* Sonn.) is a non-climacteric tropical and subtropical fruit with bright red colour and exotic flavour. It has a rough indehiscent pericarp surrounding the translucent white, edible aril and a seed in the centre. It possesses a high commercial value in the international market due to its delicious sweet taste and high nutritional quality. Lychee fruits are highly senescent and have a very short shelf life both under ambient conditions and ambient conditions followed by pre-cold storage (approximately 4–6 days and 1–2 days, respectively). After harvest, lychee quickly deteriorates due to browning of pericarp and ultimately exhibits fruit senescence. Browning is initiated on the pericarp under ambient environments and within 3–4 days, it completely becomes brown, desiccated and brittle (Huang et al. 1990). Prolonged storage under low temperature slows down the deterioration process. But fruit exposed to pre-cold storage shows accelerated browning than that of the ambient conditions. Pericarp browning is mainly attributed to loss of moisture from the pericarp (Scott et al. 1982). It may also result from mechanical or chilling injury, pathogen attack and senescence. Postharvest pericarp browning and decay are the major limitations widely affecting lychee fruit quality and market value (Sivakumar et al. 2007). Here we cited country-wise cultivar distribution of lychee which has significant nomenclatures and commercial identification (Table 9.1).

Table 9.1 Country-wise commercially grown lychee cultivars

Country	Major lychee cultivars
Australia	Fay Zee Siu, Kwai May Pink, Salathiel, Souey Tung, Tai So and Wai Chee
China	Bah Lup, Baitang-ying, Fay Zee Siu, Haak Yip, Kwai May, (Red), Lanzhu, No Mai Chee and Wai Chee
India	Bedana, China, Culcuttia, Late Bedana, Longia, Shahi, Deshi, Purbi, Kasba, Dehra Rose, Manragi, Maclean, Kaselia, Swarna Rupa, Bombai, Ellaichi, Dehra Dun, Gulabi, Rose Scented, Khatti, Piyazi, Late Large Red
Indonesia	Local selection
Israel	Mauritius
Madagascar	Madras and Mauritius
Philippines	Sinco, Tai So and ULPB Red
South Africa	Mauritius, McLean's Red
Thailand	Chacapat, Haak Yip, Kom, Tai So, Wai Chee
USA	Brewster, Haak Yip, Kwai Wai, No Mai Chee, Shan Chi
Vietnam	Vaithieu

9.2 Biochemical Basis of Pericarp Browning in Lychee

Lychee fruit pericarp accounts for approximately 15% by weight of the whole fresh fruit and is considered as an important source of dietary flavonoids (Li and Jiang 2007). The red pericarp contains an abundance of phenolic compounds (51–102 g kg^{-1} dry weight), which inhibit fatty acid oxidation and act as free radical scavengers. Postharvest pericarp browning in lychee affects the appearance of the fruit at the trade market. Usually fruit having >25% brown pericarp is not accepted by the consumers. Pericarp browning is mainly caused by the degradation of anthocyanin. The pH of pericarp tissue has a major contribution in the browning process. Desiccation of the pericarp increases its pH (>4.0), at which anthocyanin is converted to colourless carbinol (Underhill and Critchley 1994).

Oxidation of polyphenols and the degradation of anthocyanins by polyphenol oxidase (PPO) and peroxidase (POD) result in the formation of polymeric brown substances (Jing et al. 2013). PPOs are a group of copper-binding metalloproteinases encoded by nuclear genes. The PPO family comprises monophenol oxidase (tyrosinase, EC 1.14.18.1), diphenol oxidase (catechol oxidase, EC 1.10.3.1) and laccase (EC 1.10.3.2) (Wang et al. 2014). PPOs mainly consist of catechol oxidase and laccase. They catalyse the oxidation of monophenolic hydroxyl group and dihydric phenol and produce odiphenol and o-quinone, respectively. Quinones on reaction with cellular amino acids and proteins of plant generate a brown substance, which leads to postharvest browning of pericarp (Liu et al. 2007). PPO can oxidise only the polyphenols having o-hydroxyl group, which recommends the enzyme to be a diphenol oxidase lacking cresolase and laccase activities (Jiang et al. 2004a, b; Fang et al. 2015). Further studies confirmed that PPO can oxidise most of the phenols including epicatechin and procyanidin A2 present in lychee pericarp (Zhang et al. 2000). PPO and its substrate phenols both are present in the epidermis and sclerenchyma cells of exocarp and some of the cells in the mesocarp and endocarp. PPO activity in the exocarp is higher than the meso- and endocarp. In the course of the postharvest ageing process, the pericarp PPO activity diffuses with the browning site, consistent with the browning process. Postharvest inhibition of the lychee PPO activity through cold storage and application of preservatives retard the pericarp browning (Wang et al. 2010).

Reactive oxygen species (ROSs) play a vital role in regulating plant senescence. Oxidative stress generated by senescence leads to excessive production of ROSs like superoxide radical, singlet oxygen, hydrogen peroxide, nitric oxide, peroxynitrite and hydroxyl ions. ROSs cause membrane lipid peroxidation, nucleic acid damage, protein oxidation, enzyme inhibition and ultimately result in cell death. Cold storage of lychee can increase ROS production and induce the expression of respiratory burst oxidases. These respiratory oxidases catalyse the reduction of molecular oxygen to hydrogen peroxide. Pre-cold storage increases ROS production via a small GTPase signalling pathway in lychee. Lipid peroxidation destabilises membrane compartmentalisation by decreasing membrane fluidity and enhances pericarp browning process. It can be elicited by ROS or lipoxygenase during senescence of plant cell. Loss of membrane integrity also plays a major role

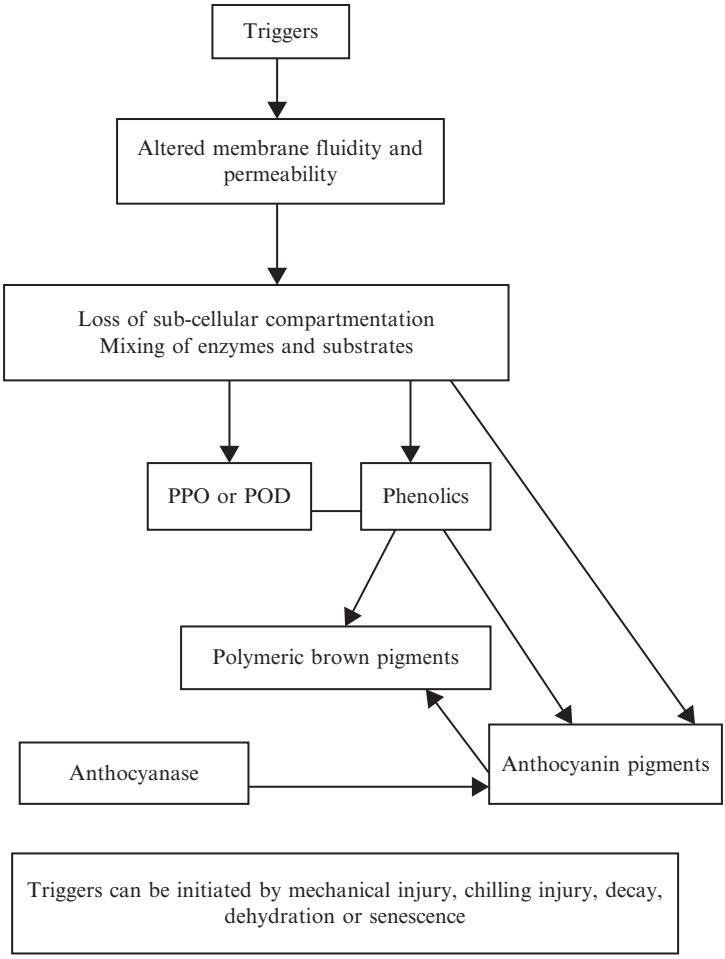

Fig. 9.1 Schematic diagram of lychee pericarp browning (Jiang et al. 2004a, b). Increase in pH of pericarp cell stimulates the PPO and POD activity. Water loss and senescence lead to loss of cell compartment. Interaction of the enzyme and phenolic substrate results in pericarp browning. Anthocyanase hydrolyses anthocyanins to anthocyanidins, which is oxidised to o-quinones by PPO/POD

in pericarp browning (Liu et al. 2011). Reactive oxygen species levels are generally governed by ROS-scavenging anti-oxidant enzymes including superoxide dismutase, catalase and peroxidase, which balance between the ROS production and scavenging in the fruit (Fig. 9.1).

High concentrations of chlorophyll in the pericarp mask the red colour by absorbing sufficient amount of incident red light and reducing the phytochrome-regulated anthocyanin biosynthesis. Thus breakdown of photosynthetic pigments contributes to a bright red coloration during fruit ripening. Sometimes lychee pericarp lacks its stronger coloration due to slow chlorophyll degradation. Chlorophyll degradation,

anthocyanin accumulation, increase in membrane permeability and cell wall disassembly coincide with the onset of lychee maturation.

Fruit ripening and senescence are unique biological processes observed in plants. Non-climacteric fruits are characterised by a non-significant increase in phytohormones. ABA and ethylene have differential effects on lychee pericarp coloration. ABA is more important in anthocyanin synthesis, while ethylene has a more significant role in chlorophyll degradation. ABA plays a key role in promoting senescence of lychee at ambient temperature. ABA-mediated fruit senescence includes oxidation of lipids, polyphenols and anthocyanins stimulated by peroxidase activity and increased ROS production. Phenolic compounds are oxidised to corresponding semiquinones and quinones by peroxidases. The main anthocyanin of lychee – cyanidin 3-rutinoside is also oxidised by peroxidases. However, the molecular mechanism behind the maturation of these non-climacteric fruits remains to be elucidated. Other signalling molecules including auxin, jasmonic acid and salicylic acid are also involved in lychee senescence.

Furthermore, several pathogens including *Alternaria* sp., *Aspergillus* sp., *Botryodiplodia* sp., *Colletotrichum* sp. and yeasts play a major role in the browning and senescence of lychee fruit. Plants develop a defence mechanism through the accumulation of callose (Benhamou 1996).

9.3 Molecular Background of Postharvest Pericarp Browning in Lychee

9.3.1 Transcriptomic Analysis

The lychee pericarp transcriptome was first assembled by Li et al. (2015). Anthocyanin present in the lychee pericarp vacuoles contributes to its attractive red colour. Flavonoid biosynthesis in lychee is influenced by two groups of genes – structural and regulatory genes. Structural genes are common in several species, whereas, regulatory genes control the temporal and spatial accumulation of pigments. Postharvest pericarp browning in lychee is associated with fruit senescence, which is governed by a group of senescence-associated genes (SAGs), which are up-regulated during the signalling cascade. Anthocyanin biosynthesis in lychee varies with cultivars, developmental stages and environmental factors. The variation is strongly determined by the structural gene (LcUFGT) and transcription factor (LcMYB1) (Lai et al. 2016). However, postharvest pericarp browning is caused by anthocyanin pigment degradation. A 50% decrease in anthocyanin concentration is responsible for pericarp coloration change from red to brown. Transcription factors (TFs) myeloblastosis (MYBs) have an essential role in the regulation – of anthocyanin metabolism and thereby affect fruit senescence and pericarp browning (Jiang 2000; Zhang et al. 2001). Cellular energy level also has a significant role in lychee senescence, and snf1-related kinase1 (SnRK1) acts as global energy regulator. Pathogen-induced infections are also responsible for browning and senescence of lychee, which have been regulated by various TFs.

Molecular mechanisms underlying the physiological changes in lychee pericarp have been revealed through transcriptomic analysis (Lai et al. 2015). Chlorophyll degradation, anthocyanin accumulation, increase in membrane permeability and cell wall disassembly coincide with the onset of lychee maturation. De-greening and pigmentation two obvious physiological alterations, take place parallel with chlorophyll degradation and anthocyanin accumulation. Genes encoding chlorophyll degrading and flavonoid synthesising proteins have been identified from the analysis. Chlorophyll catabolism enzymes (CCEs) and stay green (SGR) proteins play a prominent role in the chlorophyll degradation pathway, which has been supported by overexpressing LcSGR protein in tobacco leaves. The expression levels of different genes have also been studied. The late anthocyanin biosynthesis genes have been found to be up-regulated with the anthocyanin accumulation. The candidate MYB transcription factors have also been identified as regulator of flavonoid biosynthesis.

PPO plays a crucial role in the postharvest pericarp browning of lychee during early storage. Specific inhibition of PPO gene expression in lychee pericarp suppresses PPO activity and postharvest tissue browning. PPO activity and thereby the browning intensity are associated with PPO antisense RNA expression (Murata et al. 2001). The lychee PPO gene (LcPPO) is present in a single copy within the lychee genome. Cloning of the LcPPO gene infers that PPO activity is boosted, and thus LcPPO expression is up-regulated during early postharvest storage accelerating the pericarp browning (Wang et al. 2014). The LcPPO expression level in lychee pericarp shows similar variations to the PPO activity. LcPPO expression is observed in a number of tissues, but the level of expression varies, specifying different roles of PPO. LcPPO exhibits the maximum expression in flower and leaf, followed by seed, root, stem, pericarp and pulp. The LcPPO expression level also varies with developmental stages. During fruit development, the LcPPO shows lowest expression that increases fast after harvest. LcPPO expression level determines differences in the PPO activity. The level of expression differs with cultivars resulting in diverse pericarp browning index. At early postharvest storage, up-regulated LcPPO expression accelerates the synthesis of PPO protein, which enhances the PPO activity and pericarp browning.

9.3.2 Signal Transduction-Related Genes in Senescent Lychee Fruit

Genes involved in the metabolic pathways in lychee pericarp have been identified through transcriptome analysis. Structural and regulatory genes associated with pericarp colouring have also been identified based on a cDNA library of lychee pericarp. Analysis on genes involved in lychee senescence-related signal transduction suggests the pathway to be regulated by various factors including ABA, G protein-coupled receptor proteins, small GTPases and calcium ions. Most of the

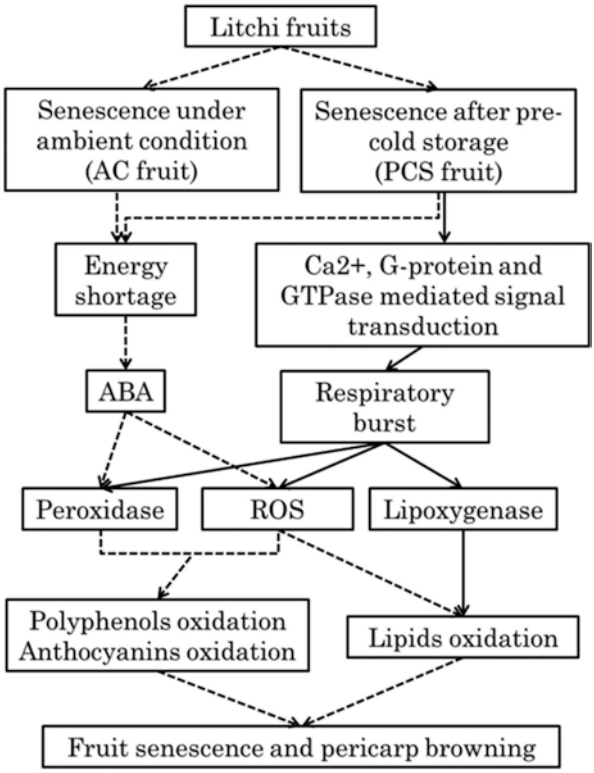

Fig. 9.2 Hypothetical model on senescence of lychee based on transcriptomic and metabolomic studies (Yun et al. 2016). Senescence of lychee under ambient condition (AC) is an ABA-regulated process. Pre-cold storage accelerates the senescence process. Solid arrows indicate activation specific to pre-cold stored (PCS) fruits. Arrows with broken shafts indicate events that occur in both AC and PCS fruits

genes related to the metabolic pathways in lychee pericarp show up-regulation during the pre-cold storage lychee senescence. The relative transcript levels of the targeted genes have been verified with real-time qRT-PCR. The genes are involved in signal transduction, transport, cell wall function, respiration, fatty acid and other types of metabolism (Fig. 9.2).

9.3.2.1 Genes Involved in Pericarp Maturation of Lychee

Ethylene response factors (ERFs) show differential expression during lychee pericarp maturation. Increased ethylene production enhances the degradation of chlorophyll in the pericarp (Wang et al. 2007). ABA plays a crucial role in lychee fruit pigmentation (Lai et al. 2014). Mitogen-activated protein kinases (MAPKs) also play important roles in the maturation of lychee fruit. Ubiquitin-protein ligase genes are also expressed during lychee pericarp maturation. Chlorophyll degradation promoted by SGR is essential for the strong coloration of fruit, and a de-greening process associated with chlorophyll degradation has been observed during lychee coloration.

9.3.2.2 Genes Involved in Flavonoid Biosynthesis

Anthocyanins, proanthocyanidins and flavonols are the predominant flavonoids in the lychee pericarp (Wang et al. 2011). Phenylalanine ammonia lyase (PAL) catalyses the initial step of the phenylpropanoid pathway, which leads to the synthesis of both lignin and flavonoids. UDP-flavonoid glucosyltransferase (UFGT) is considered to be a key enzyme in lychee anthocyanin biosynthesis (Wei et al. 2011). Glutathione S-transferase (GST) is necessary for the transport of anthocyanins from cytosol to the vacuole. Anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR) play important roles in the biosynthesis of proanthocyanidins, while flavonol synthase (FLS) is specifically involved in the biosynthesis of the flavonols catechin and epicatechin (Bogs et al. 2005). *Litchi chinesis* MYB1 (LcMYB1) has been reported to control anthocyanin biosynthesis in lychee (Lai et al. 2014). Lychee MYB TFs are involved in the regulation of flavonoid biosynthesis in the lychee pericarp.

9.3.2.3 ABA Signalling Pathway-Related Genes

The senescence of lychee is regulated by an ABA-mediated signalling pathway. The genes involved in the ABA signalling pathway are up-regulated in the senescent lychee.

9.3.2.4 Lipid Transport and Metabolism-Related Genes

Lipid transport and metabolism-related genes are found to be up-regulated during fruit senescence. Lipoxygenase, a key enzyme in lipid peroxidation, is also up-regulated in senescent lychee. Most of these genes involved in fatty acid oxidation or glycerophospholipid, sterol and isopentenyl metabolism are up-regulated in the pre-cold storage fruit senescence. Fatty acid oxidation includes α-oxidation, β-oxidation, unsaturated fatty acid β-oxidation and other oxidations.

9.3.2.5 Phenylalanine Metabolism-Related Genes

Phenylpropanoid metabolic process is the major pathway of anthocyanin and flavonoid synthesis. A large number of genes involved in the phenylpropanoid metabolic pathway are linked with cytochrome P450, which are up-regulated during fruit senescence and lead to enhanced polyphenol synthesis. The increased polyphenol is oxidised by ROS and peroxidases and causes pericarp browning.

9.3.2.6 Oxidation-Reduction-Related Genes

Genes involved in oxidation-reduction processes are differentially expressed in senescent lychee. Most of the up-regulated genes are expected to be peroxidases. Genes encoding a main subunit of nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase are anticipated to be respiratory burst oxidases.

9.3.2.7 Genes Related to Protein Phosphorylation

Protein phosphorylation genes are down-regulated in senescent fruit under ambient condition contrasting with pre-cold storage condition. In the latter condition, calcium signal-related genes, serine/threonine protein kinase-related genes, other protein kinase-related genes and ATP-binding proteins are up-regulated.

9.3.2.8 Genes Related to Energy Production and Conversion Processes

Energy production and conversion genes related to NADPH synthesis, ATP transport and adenosine 5′-triphosphatase (ATPase) activity are up-regulated during senescence of lychee. Genes involved in ATP synthesis and nicotinamide-adenine dinucleotide (NADH) synthesis and reduction are up-regulated in pre-cold stored fruits, and their corresponding proteins are anticipated to participate in energy production and conversion. The ATP transport-related genes that are members of the ABC transporter family are up-regulated in the pre-cold stored fruit. Genes involved in the tricarboxylic acid (TCA) cycle including malate dehydrogenases, isocitrate dehydrogenase, aldehyde dehydrogenase and 2-oxoglutarate dehydrogenase have been predicted to be up-regulated in senescent lychee fruits. Both aerobic and anaerobic respiration processes are established to be activated in lychee during senescence. Levels of ATP and ADP molecules involved in intracellular energy metabolism as well as the ATP/ADP ratio are decreased during fruit senescence under ambient temperature. Calcium ions (Ca^{2+}) also play important roles in the induction of cell death. It acts as secondary messengers during cold-induced signal transduction via a stimulus-specific increase in cytoplasmic Ca^{2+}. The calcium-mediated signal is accepted by the G protein-coupled receptor, whereas, the small GTPase positively regulates calcium ion signalling. Thus, Ca^{2+}, G protein- and GTPase-mediated signal transduction might be involved in the acceleration of PCS fruit senescence.

9.4 Metabolomic Analysis

Metabolomic studies of lychee pericarp have also been performed by gas chromatography (GC-MS) for different storage environments. Differential accumulation of metabolites have been observed during fruit senescence. The accumulated metabolites are classified into the following seven clusters: sugars, organic acids, fatty acids, alcohols, alkaloids, amino acids and others. Primary metabolites of lychee decrease during cold storage but increase after transfer to ambient temperature. Several metabolites, including D-(+)-turanose, threonic acid, butanedioic acid, 5α-androstan-17-one, trisiloxane, myo-inositol and butane increase in senescent lychee fruits both under ambient and pre-cold storage conditions. Secondary metabolism-related genes are differentially expressed during senescence of lychee fruit. The genes up-regulated during ambient and pre-cold storage fruit senescence are involved in fatty acid oxidation, gibberellin metabolism, ABA metabolism, redox reaction and ROS metabolism. The genes up-regulated during pre-cold storage fruit senescence and down-regulated during ambient fruit senescence are involved in phenylpropanoid, coumarin, lignin and flavonoid metabolism. Up-regulation of some cytochrome P450-related genes during both ambient and pre-cold storage fruit senescence suggests the key role of cytochrome P450 during lychee fruit senescence.

9.5 The miRNA and Pericarp Browning in Lychee

MicroRNAs (miRNAs) are endogenous non-coding small RNAs that play significant roles in plant development through the regulation of gene expression mediated by mRNA degradation or translational inhibition (Bartel 2004; Bushati and Cohen 2007). In plants, cleavage occurs in between the 10th and 11th nucleotides from 5′ end of the miRNA in the mRNA-miRNA duplex leaving a 5′ monophosphate at the 3′ fragment of the target mRNA that validates miRNA target (Rhoades et al. 2002; Llave et al. 2002). The rapid discovery of miRNAs and their targets with high throughput sequencing helps in understanding the miRNA-mediated gene regulation network (Chen 2012). Plant senescence is facilitated by miRNAs (Ellis et al. 2005). The number of miRNAs identified in lychee is limited as compared to the other plants (Chen 2012). Most of the lychee miRNAs exhibit stage-specific expression and the majority of these targets are conserved in plant species. Lychee miRNAs have been found to control transcription and stress responses during fruit senescence.

9.5.1 MicroRNAs Contribute in Proteolysis

Senescence in plants is closely related to proteolysis, which triggers a substantial degradation network of proteins and ultimately leads to cell death (Diaz-Mendoza et al. 2014). Cysteine protease1 (CP1) gene shows increased expression during shelf life after cold storage, which suggests that low temperature inhibits fruit senescence (Liu et al. 2011). One of the lychee miRNA, lch-miR396a has been recognised to target CP1 showing a conflicting expression (Yao et al. 2015).

9.5.1.1 MiRNAs Contribute in Hormonal Regulation

microRNAs have strong relation with plant hormone auxin that inhibits fruit ripening (Windels et al. 2014). Lychee miRNA393 plays a significant role in the postharvest lychee fruit senescence through auxin signalling.

9.5.1.2 MiRNA-TF Network Contributes in Lychee Fruit Senescence Regulation

Transcription factors are the main target genes for most of the miRNAs. A number of miRNAs regulating downstream TFs are associated with lychee senescence. Ethylene-responsive factors (ERF) are key elements in integrating ethylene and jasmonic acid pathways for fruit ripening and senescence (Zarei et al. 2015). Lychee miRNA396 is a transcription factor containing an AP2/ERF domain. Ethylene-responsive factors are regulated by lch-miR396 and participates in senescence of lychee.

9.6 Postharvest Handling and Sampling

9.6.1 The Effect of Pericarp Water Loss on the PPO Activity

Packaging treatment and pericarp coating can prevent postharvest pericarp water loss and delay the pericarp browning of fruits by suppressing the enzymatic reaction catalysed by pericarp PPO activity at the early stage of postharvest storage. It also reduces the pericarp LcPPO expression.

9.6.1.1 The Effect of Temperature on the PPO Activity

The low temperature storage reduces the pericarp PPO activity than at room temperature. LcPPO expression is also inhibited by low temperature. Postharvest cold storage prolongs the life of lychee fruit for up to 30 days.

9.7 Commercial Postharvest Management of Lychee

Desiccation of the lychee pericarp due to poor postharvest handling practices during the fruit export chain eventually results in pericarp browning. Postharvest losses during harvesting and transportation have been reported to be approximately 8.0% and 7.5%, respectively (Molla et al. 2010). Precise postharvest management strategies have the potential to extend the fruit shelf life without failing to maintain their quality during storage and transportation.

9.7.1 Sulphur-Dioxide Fumigation

In many lychee exporting countries, pericarp browning and postharvest decay are commonly controlled with sulphur dioxide (SO_2) fumigation. Sometimes this procedure is followed by the application of dilute hydrochloric acid. But this practice alters the fruit taste and results in health risks. Therefore, alternative treatments have been acquired to retain the fruit quality.

9.7.2 Gamma Irradiation

Gamma irradiation in combination with low temperature storage may be considered as an exceptional substitute to SO_2 fumigation. Pathogen-induced decay can be controlled by irradiation up to 1 kGy with no adverse effects on quality (Ilangantileke et al. 1993). But it cannot retain the fruit quality during prolonged cold storage. It is not commercially well practised regarding the safety issues with irradiated food for human consumption (Jiang et al. 2003).

9.7.3 Temperature Treatments

Exposure to higher temperature (45°C) for a short period (30 min) can also be used for disinfecting lychee fruits. Steam treatment (98°C) for 30 s followed by hydrocooling preserves the red colour of the pericarp during storage. The steaming process usually affects the edible aril of the fruit and has not been accepted (Kaiser et al. 1995).

9.7.4 Chemical Control

Various fungicides like benomyl, thiabendazole, prochloraz and iprodione prevent postharvest diseases of lychee (Korsten et al. 1993). But continuous use of fungicides can build resistant pathogen. Harmful effect of fungicide on food and environment has made chemical control less practicable.

9.7.5 Biochemical Treatments

Application of tea polyphenols markedly delays pericarp browning of lychee fruit even after 30 days of cold storage (4°C). Dipping of the fruits into 1% solution of tea polyphenol for 5 min before storage changes their browning index (Chen et al. 2014).

Anti-oxidant compound like pyrogallol also offers protection against microbial attack and delays pericarp browning during their storage at ambient (25°C) or low temperature (4°C) (Kumar et al. 2006a; b). Pyrogallol treatment reduces the incidence of disease and browning in lychee during short storage (2–4 days), transport or distribution under ambient temperature (Jing et al. 2013).

Polyamines like spermidine and putrescine also retain the red colour of lychee pericarp under low temperature storage for 30 days. They delay ethylene production and POD activity responsible for the browning.

1-Methylcyclopropane disturbs ethylene production and PPO and POD activities and delays the activity of anthocyanase in lychee (Hu et al. 2005).

9.7.6 Bio-control Agents

Bio-control agent like *Bacillus subtilis* under cold storage is effective in regulating postharvest decay of lychee (Jiang et al. 2001). The antagonist treatment is effective up to 30 days of storage at 5°C. Application of antagonist does not modify the food quality, but research is necessary for the optimisation of the environment.

9.7.7 Modified Atmosphere Storage

Modified atmosphere storage is a low cost and easy employable technique (Flores et al. 2004). It maintains a higher relative humidity inside the plastic sealed fruit and prevents browning induced by water loss (Persis et al. 2000). It also controls decay of fruit caused by higher O_2 or CO_2 composition.

9.7.8 Controlled Atmosphere Storage

Controlled atmosphere containing 3–5% oxygen (O_2) and 3–5% carbon dioxide (CO_2) at 90% relative humidity reduces pericarp browning caused by PPO activity. Pure O_2 inhibits anthocyanin degradation by preventing the PPO activity (Duan et al. 2004). However, the strategy has not been studied for long-term storage and disease development. Therefore, commercial use of modified atmosphere packaging is very limited. Different packaging types during long-term storage affect the overall fruit quality (Sivakumar and Korsten 2006).

References

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297

Benhamou N (1996) Elicitor-induced plant defence pathways. Trends Plant Sci 1:233–240

Bogs J, Downey MO, Harvey JS, Ashton AR, Tanner GJ, Robinson SP (2005) Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. Plant Physiol 139(2):652–663

Bushati N, Cohen SM (2007) microRNA functions. Annu Rev Cell Dev Biol 23:175–205

Chen X (2012) Small RNAs in development – insights from plants. Curr Opin Genet Dev 22(4):361–367

Chen W, Zhang Z, Shen Y, Duan X, Jiang Y (2014) Effect of tea polyphenols on lipid peroxidation and anti-oxidant activity of lychee (*Litchi chinensis* Sonn.) fruit during cold storage. Molecules 19:16837–16850. doi:10.3390/molecules191016837

Diaz-Mendoza M, Velasco-Arroyo B, Gonzalez-Melendi P, Martinez M, Diaz I (2014) C1A cysteine protease-cystatin interactions in leaf senescence. J Exp Bot 65(14):3825–3833

Duan XW, Jiang YM, Su XG, Zhang ZQ (2004) Effects of pure oxygen atmosphere on enzyme browning of harvested litchi fruit. J Hortic Sci Biotechnol 79:859–862

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005) AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 132(20):4563–4574

Fang F, Zhang X, Luo H, Zhou J, Gong Y, Li W, Shi Z, He Q, Wu Q, Li L, Jiang L, Cai Z, Oren-Shamir M, Zhang Z, Pang X (2015) An intracellular laccase is responsible for epicatechin-mediated anthocyanin degradation in lychee fruit pericarp. Plant Physiol 169:2391–2408. doi:10.1104/pp.15.00359

Flores FB, Martínez-Madrid MC, Amor MB, Pech JC, Latche A, Romojaro F (2004) Modified atmosphere packaging confers additional chilling tolerance on ethylene inhibited cantaloupe Charentais melon fruit. Eur Food Res Technol 219:431–435

Hu WR, Pang XQ, Liu SZ, Zhang ZQ, Ji ZL (2005) Effect of postharvest handling on the anthocyanin content and anthocyanase activity in litchi pericarp. J Fruit Sci 22:224–228

Huang S, Hart H, Lee H, Wicker L (1990) Enzymatic and colour changes during postharvest storage of lychee fruit. J Food Sci 55:1762–1763

Ilangantileke SG, Noomhorn A, Upadhyay IP, Srinivas Rao M (1993) Effect of irradiation and storage temperature on the shelf life and quality of Thai lychee. In: Champ BR, Highley E, Johnson GI (eds) Postharvest handling of tropical fruits, ACIAR conference proceedings, Chang Mai, Thailand, 50, pp 352–354

Jiang YM (2000) Role of anthocyanins, polyphenol oxidase and phenols in pericarp browning. J Sci Food Agric 80(3):305–310

Jiang YM, Zhu XR, Li YB (2001) Postharvest control of litchi fruit rot by *Bacillus subtilis*. Lebensm Wiss Technol. Food Sci Technol 34:430–436

Jiang YM, Yao L, Lichter A, Li J (2003) Postharvest biology and technology of litchi fruit. Food Agric Env 2:76–81

Jiang YM, Duan XW, Joyce D, Zhang ZQ, Li JR (2004a) Advances in understanding of enzymatic browning in harvested litchi fruit. Food Chem 88:443–446

Jiang Y, Li Y, Jianrong L (2004b) Browning control, shelf life extension and quality maintenance of frozen litchi fruit by hydrochloric acid. J Food Eng 63:147–151

Jing G, Huang H, Yang B, Li J, Zheng X, Jiang Y (2013) Effect of pyrogallol on the physiology and biochemistry of lychee fruit during storage. Chem Cent J 7:19–29. doi:10.1186/1752-153X-7-19

Kaiser C, Levin J, Wolstenholme BN (1995) Vapour, heat and low pH dips improve litchi (*Litchi chinensis* Sonn.) pericarp colour retention. J SA Soc Hort Sci 4:6–12

Korsten L, De Villiers EE, De Jager ES, Van Harmelen MWS, Heitmann A (1993) Biological control of litchi fruit diseases. S Afr Litchi Grower Assoc Yearbook 1993 5:36–40

Kumar M, Prakash NS, Prasad US, Sarin NB (2006a) A novel approach of regeneration from nodal explants of field grown litchi (*Litchi chinensis* Sonn.) fruit trees. J Plant Sci 5(3):321–327

Kumar M, Gupta M, Shrivastava D, Prasad M, Prasad US, Sarin NB (2006b) Genetic relatedness among Indian litchi accessions (*Litchi chinensis* Sonn.) by RAPD markers. Int J Agric Res 1:390–400

Lai B, Li XJ, Hu B, Qin YH, Huang XM, Wang HC et al (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS One 9(1):e86293

Lai B, Hu B, Qin YH, Zhao JT, Wang HC, Hu GB (2015) Transcriptomic analysis of *Litchi chinensis* pericarp during maturation with a focus on chlorophyll degradation and flavonoid biosynthesis. BMC Genomics 16:225–242. doi:10.1186/s12864-015-1433-4

Lai B, Du LN, Liu R, Hu B, Su WB, Qin YH, Zhao JT, Wang HC, Hu GB (2016) Two LcbHLH transcription factors interacting with LcMYB1 in regulating late structural genes of anthocyanin biosynthesis in *Nicotiana* and *Litchi chinensis* during anthocyanin accumulation. Front Plant Sci 7:166–180. doi:10.3389/fpls.2016.00166

Li J, Jiang Y (2007) Lychee flavonoids: isolation, identification and biological activity. Molecules 12:745–758

Li C, Wang Y, Ying P, Ma W, Li J (2015) Genome-wide digital transcript analysis of putative fruitlet abscission related genes regulated by ethephon in litchi. Front Plant Sci 6:502. doi:10.3389/fpls.2015.00502

Liu L, Cao S, Xie B, Sun Z, Li X et al (2007) Characterization of polyphenol oxidase from lychee pericarp using (-)-epicatechin as substrate. J Agric Food Chem 55:7140–7143

Liu H, Song LL, You YL, Li YB, Duan XW, Jiang YM, Joyce DC, Muhammad A, Lu WJ (2011) Cold storage duration affects lychee fruit quality, membrane permeability, enzyme activities and energy charge during shelf time at ambient temperature. Postharvest Biol Technol 60:24–30

Llave C, Xie ZX, Kasschau KD, Carrington JC (2002) Cleavage of scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. Science 297(5589):2053–2056

Molla MM, Islam MN, Nasrin TAA, Bhuyan MAJ (2010) Survey on postharvest practices and losses of litchi in selected areas of Bangladesh. Bangladesh J Agric Res 35(3):439–451

Murata M, Nishimura M, Murai N, Haruta M, Homma S et al (2001) A transgenic apple callus showing reduced polyphenol oxidase activity and lower browning potential. Biosci Biotechnol Biochem 65:383–388

Persis E, Dvir O, Feygenberg O, Ben Aire R, Ackerman M, Lichter A (2000) Production of acetaldehyde and ethanol during maturation and modified atmosphere storage of litchi fruit. Postharvest Biol Technol 26:157–165

Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP (2002) Prediction of plant microRNA targets. Cell 110(4):513–520

Scott KJ, Brown BI, Chaplin GR, Wilcox ME, Bain JM (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic film. Sci Hortic 16:253–262

Sivakumar D, Korsten L (2006) Influence of modified atmosphere packaging and postharvest treatments on quality retention of litchi cv Mauritius. Postharvest Biol Technol 41:135–142

Sivakumar D, Korsten L, Zeeman K (2007) Postharvest management on quality retention of litchi during storage. Fresh Prod 1(1):66–75

Underhill SJR, Critchley C (1994) Anthocyanin decolourisation and its role in lychee pericarp browning. Aus J Exp Agric 34:115–122

Wang H, Huang H, Huang X (2007) Differential effects of abscisic acid and ethylene on the fruit maturation of *Litchi chinensis* Sonn. Plant Growth Regul 52(3):189–198

Wang JB, Wang XS, Jin ZQ (2010) Enzymatic browning of postharvest lychee: a review. Acta Hortic 863:613–618

Wang H, Hu Z, Wang Y, Chen H, Huang X (2011) Phenolic compounds and the anti-oxidant activities in lychee pericarp: difference among cultivars. Sci Hortic 129(4):784–789

Wang J, Liu B, Xiao Q, Li H, Sun J (2014) Cloning and expression analysis of lychee (*Litchi Chinensis* Sonn.) polyphenol oxidase gene and relationship with postharvest pericarp browning. PLoS One 9(4):e93982. doi:10.1371/journal.pone.0093982

Wei YZ, Hu FC, Hu GB, Li XJ, Huang XM, Wang HC (2011) Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PLoS One 6(4):e19455

Windels D, Bielewicz D, Ebneter M, Jarmolowski A, Szweykowska-Kulinska Z, Vazquez F (2014) miR393 is required for production of proper auxin signalling outputs. PLoS One 9(4):e95972

Yao F, Zhu H, Yi C, Qu H, Jiang Y (2015) MicroRNAs and targets in senescent lychee fruit during ambient storage and post-cold storage shelf life. BMC Plant Biol 15:181–192. doi:10.1186/s12870-015-0509-2

Yun Z, Qu H, Wang H, Zhu F, Zhang Z, Duan X, Yang B, Cheng Y, Jiang Y (2016) Comparative transcriptome and metabolome provides new insights into the regulatory mechanisms of accelerated senescence in lychee fruit after cold storage. Sci Report 6:19356–19371. doi:10.1038/srep19356

Zarei A, Korbes AP, Younessi P, Montiel G, Champion A, Memelink J (2015) Two GCC boxes and AP2/ERF-domain transcription factor ORA59 in jasmonate/Yao et al. BMC Plant Biol. 15:181 Page 11 of 12 ethylene-mediated activation of the PDF1.2 promoter in *Arabidopsis*. Plant Mol Biol 2011 75(4–5):321–331

Zhang DL, Quantick PC, Grigor JM (2000) Changes in phenolic compounds in lychee (*Litchi chinensis* Sonn.) fruit during postharvest storage. Postharvest Biol Technol 19:165–172

Zhang ZQ, Pang XQ, Ji ZL, Jiang YM (2001) Role of anthocyanin degradation in lychee pericarp browning. Food Chem 75(2):217–221

Intellectual Property Rights Protection in Plants: Scopes in Lychee Commercialization

10

Niharika Sahoo and Sukanta S. Bhattacharya

Abstract

Global Patent Acts are the most popular and effective way to protect intellectual properties in academics, industry, corporate, etc. Patents are peculiarity abbreviated as IP law and implications of its civil rights are given to the inventor/assignee for a stipulated period of time with respect to inventions which are novel, are non-obvious, and have industrial utility. 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. Here we discuss the country's patent law, the scopes of patentable claims for lychee plants/products, and those that can popularize the lychee in international market.

Keywords

Global patent • Intellectual properties (IP) • Lychee biotechnology

10.1 Introduction

The rapidly growing sector of biotechnology has opened a plethora of options for farmers/breeders to develop new varieties of *L. chinensis* (Menzel 1985; Chen et al. 2016), catering to the increasing demand for lychee and its products and

N. Sahoo
IIT, Kharagpur, West Bengal 721302, India

S.S. Bhattacharya (✉)
University of Cincinnati, Cincinnati, OH 45267, USA
e-mail: bhattacharya.s.s@gmail.com

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circumventing the environmental challenges. Plant biotechnology, both traditional breeding techniques and newer strategies like recombinant DNA technology, has enabled development of new resistant varieties; biological pest control (Li et al. 2014); high-yielding cultivars, along with advances in the agriculture such as development of biofertilizers (Vassilev et al. 2015) such as vermicomposts; and postharvest technology (Martinez-Castellanos et al. 2011; Pandey et al. 2013). This has been critical in mitigating the production losses due to pests and diseases, abiotic stress such as droughts, enhancing productivity and quality in lychee. After the successful introduction of insect-resistant and herbicide-tolerant crops in 1996, many new crop varieties have been developed and made available to the farmers worldwide (Gottschalk et al. 2008). In 2012, the transgenic crops segment was identified as the biggest market segment of the global agricultural biotechnology industry which had reached a value of US$15,300 million in 2012 (Transperency Market Research 2015).

The global agricultural biotechnology industry was led by North America owing to the high cultivation of genetically modified crops. In 2012, the region held 32.5% share of the global market. Till 2012, 88% of the corn, 94% of the cotton, and 93% of the soybeans planted in the USA were varieties produced through genetic engineering. A large proportion of the production of other crops, such as alfalfa, papaya, and sugar beet, is also biotech derived (Jorge Fernandez-Cornejo et al. 2014).

In Europe, genetically modified crops are majorly used for animal feed, which are imported from the USA and Brazil. There is minimal production of these crops in Europe owing to strict regulatory policies and stern opposition from consumers. The agricultural biotechnology market in Asia Pacific is anticipated to reach US$7810.5 million by 2020. The rising demand for quality food in Asia added with fast growth in industrialization is likely to propel the agricultural biotechnology market in this region (Transperency Market Research 2015).

Companies such as Monsanto, DuPont Pioneer Hi-Bred, Certis USA, ADAMA Agricultural Solutions Ltd., KWS SAAT AG, Dow AgroSciences LLC, Evogene Ltd., Global Bio-chem Technology, Syngenta, Vilmorin, Rubicon, Bayer CropScience AG, Performance Plants Inc., and Mycogen Seeds are the key players of the global agricultural biotechnology market, and together they control major portion of the IPRs related to plant germplasms and related technologies for different varieties of plants. However, the seed market is very much consolidated in nature with top three market players (Monsanto, DuPont, and Syngenta) occupying around 42% of the overall agricultural biotechnology market in 2012 (Bonny 2014). Together they control 85% of the maize related patents and 69.6% of patents of other crops (Howard 2015).

In general development of a transgenic or genetically modified variety using biotechnology requires a great deal of time and money. Intellectual property rights (IPRs) basically act as a tool to gain returns on such invested efforts. These rights allow the inventor to restrict the use of the intellectual property. Once it is protected, no one can use, manufacture, grow, sell, or offer to sell the invention without the permission of the right holder. So basically to prevent the theft of long-term investment, special IPRs have been developed. There are different forms of IPRs

depending on types of innovations/inventions. Also various means and mechanisms are available for the effective use and management of IPRs at national and international level.

While developed nations like the USA and Europe are rapidly patenting technologies used in agri-biotech sector, developed countries are lagging far behind. For instance, the patents on CaMV 35S promoter are granted only in the USA and Europe (Binenbaum et al. 2003). However due to jurisdictional nature of IPRs, there is no restriction in use of such patented technologies in developing nations where the protection has not been sought. IP issues may arise when those crops are exported to the countries where the technologies are originally protected. So it is essential for both scientists and farmers to be aware of IPRs and its implications. In this context, this chapter provides a summary of various means of protection of IPRs related to plants available internationally and domestically.

10.2 Methods for Protection of IPR Related to Plants and Case Studies in Lychee

10.2.1 Patents: Utility Patents and Plant Patents

Worldwide patents are the most popular and effective method of intellectual property protection. Patents are the IPRs assigned to the inventor/assignee for a limited period of time with respect to inventions which are novel, are non-obvious, and have industrial utility. Patents can be given to a new product or process or both. However, depending on the country's patent law, the scopes of patentable claims for plants/plant products are different. In most countries, plants per se and inventions toward plants or plant products such as seed are not eligible for a patent. However, countries like the USA and Australia allow such patent claims provided the legal criteria for patentability are fulfilled. The USA is the first country to allow patents on plants per se since 1930 under the Plant Patent Act of 1930 (35 USC 131) (Yeo et al. 2008). It acted as an encouragement for the horticulture industry to engage in plant breeding research to increasing plant genetic diversity. The US Patent and Trademark Office (USPTO) did not grant patents for living matters prior to the 1980s. After the landmark decision of Diamond v. Chakrabarty in US Supreme Court which held that a genetically modified microorganism is a subject matter of patents, the USPTO allowed genetically modified plants and other living organisms as patentable claims (Diamond v. Chakrabarty 1980). These patents, also popularly called as the utility patents, are issued under the US patent law by the USPTO and can be granted for plant inventions (35 U.S.C. 101). In the plant inventions, patents can claim whole plants, seeds, plant varieties, plant parts (e.g., fruit and flowers), processes of producing plants, plant genes, and hybrids. The utility patents related to plants and varieties grants 20 years of protection to the inventor or owner. The utility patent can be classified as process patent or product patent or both depending on the nature of claims.

In India, patents are regulated by the Patent Act of 1972. The Section 3 of the Act specifies the inventions which are not patentable in India. Among other things

obvious to natural laws, mere discovery of new use of known substance or per se method of agriculture or horticulture is not patentable in India. Even plants, animals, and biological processes as such are not patentable in India. An overview of patent applications related to lychee in India shows that most of the patents applied are basically process patents covering different aspects of litchi beverage preparation or medicinal uses of litchi or plant parts thereof. Some of the examples of patents on litchi from India are as follows:

Patent No. 232574: An improved process for the preparation of lychee (*Litchi chinensis*) beverage (process patent)
Patent No. 240608: A process for preparation of shelf stable lychee juice and beverage (process patent)
Publication No. WO2005070234 A1: Process for the production of sediment free clarified fruit juices (process patent)
Publication No. WO 2013140382 A2: A fruit juice powder mix and process for making the same (product and process patent)

Most of the common claims in lychee patents are:

- Methods of anti-browning and color protection in lychee
- Methods of brewing lychee
- Methods of preparation of alcoholic beverages and fruit jam from lychee
- Method of lychee seed saponin extraction
- Lychee extract composition for treatment of diabetes
- Lychee extract composition for treatment of hyperlipidemia
- Technology for fresh keeping lychee

Examples of some US utility patents on lychee:

1. US Patent No. 7,651,692: Use of extracts of the plant *Litchi chinensis* Sonn.
   This patent claims both product and process. The main claims read as follows:

Claim 1. A skin and/or hair-treating composition comprising (b) a neutral extract from a pericarp of a *Litchi chinensis* Sonn. plant, said extracted being present in an amount effective to protect human skin and/or hair from environmental influences and aging
Claim 8. A process for protecting human skin and/or hair comprising contacting the skin and/or hair with a composition containing a neutral extract from a pericarp of a *Litchi chinensis* Sonn. plant

2. US Patent No. 5,086,043: Production of saponins of lychee
   This patent can be classified as process patent. The main claim reads as follows:

Claim 1. A process for producing saponins of lychee comprising (a) extracting dried and powdered seeds or fruits of *Litchi chinensis* Sonn. with 95% ethanol

China has the maximum patents for lychee which may be related to the fact that the plant has origin in China itself and its widespread cultivation in the country. While methods of horticulture are non-patentable in India, in China we may find many patents where methods of grafting are patented. Some of the examples of such patents are as follows:

- Patent CN104322290A – Litchi tree grafting method
- Patent CN103621321A – Litchi grafting method
- Patent CN104335823A – Litchi grafting method
- Patent CN104221730A – Method for overcoming graft incompatibility of lychee
- Patent CN 104322289A – Inferior litchi tree top grafting method
- Patent CN 104126426A – Beak litchi multi-stock grafting method
- Patent CN 1887048 B – Seedless litchi graft stock selecting technology

In plant-based agriculture, the revenue margins for most commodity crops are usually low. To gain more financial benefit in the current commercial world, the scope of patent needs to be broadened. The inventions may need to cover an entire variety, species, new crop uses, or novel crop management tools including herbicides or insecticides (Jefferson et al. 2015). For each of these purposes, patenting of both nucleotide and amino acid sequences is critical. As per patent laws, a gene per se is never patentable. However, method of isolation of gene or gene sequence or nucleotide sequence is patentable.

Biotechnological tools and materials such as genetically modified cells, plants, and animals are patented since the last few decades. However, the US Supreme Court recently narrowed the scope of patent protection by holding that naturally occurring DNA sequences as unpatentable as the laws of nature and abstract mental processes (Jefferson et al. 2015). On June 13, 2013, in the case of the Association for Molecular Pathology v. Myriad Genetics, Inc., the US Supreme Court held that human genes cannot be patented in the USA. The DNA is a product of nature which existed in nature and there is no novelty in gene discovery as nothing new is created. So there is no intellectual property to protect and patents cannot be granted. Because of this landmark ruling, all the earlier gene patents (nearly 4300 human genes were patented) were invalidated. This made the genes easily accessible for the research, diagnostic testing, and for other commercial genetic testing. However, the Court did not hold all the genes to be non-patentable. The ruling allowed the companies to patent the modified forms of the genes which are not originally found in nature such as the complementary DNA or cDNA, while genes with the same sequences found in cells were considered non-patentable. In the commercial cultivar of lychee, many genes are already patented; some of the examples are given in Table.10.1.

Table 10.1 Some gene patents in litchi

Genes	Patent publication number	Description/function/use of claimed gene	Filing year
Litchi R2R3-MYB gene LcMYB1 (Google Patents 2014a)	CN103725693 A	The LcMYB1 is verified to have the function of regulating and controlling the biosynthesis of the anthocyanins. Thus, the foundation for the application of the LcMYB1 for improving the accumulation of plant pigments is provided	2014
Litchi fruit senescence starting gene LcAtpB (Google Patents 2014b)	CN102943082 B	Use of cAtpB gene in the regulation of aging and ripe fresh litchi fruit postharvest application	2012
Litchi flower formation regulator gene LcFLC (Google Patents 2015)	CN105112427 A	The gene LcFLC has the function of retarding the flowering time of plants	2015
Litchi cell wall acid invertase gene ZcU/7, ZcU/it ZcU/J, ZcU/it ZcU/ (Google Patents 2016)	CN105420250 A	Use in prevention of embryo abortion	2015
Promoter from *Litchi chinensis* (Google Patents 2013)	CN102994504 B	The promoter provided by the invention can specifically express in pericarp and leaf of *Litchi chinensis* and can be used for cultivation of transgenic plants and control of tissue-specific expression of exogenous gene expression and especially tissue-specific expression in pericarp of *Litchi chinensis*	2012
Method for constructing litchi core collection by using EST-SSR (expressed sequence tag-simple sequence repeat) molecular marker (Google Patents 2011)	CN102174510 A	Provides a molecular fingerprint litchi core collection and construction method	2011
Plant cyclopropane fatty acid synthase genes (Gontier et al. 2008)	US20080155714 A1	Claims an isolated nucleic acid encoding a cyclopropane fatty acid synthase isolated from *Litchi sinensis*	2005
Lysophosphatidic acid acyltransferase genes (Thomasset et al. 2007)	WO2007141257 A1	An isolated nucleic acid from *Litchi sinensis* encoding a protein having LPA acyltransferase activity	2007

10.2.1.1 Plant Patents

In the USA, a plant patent can be granted to an inventor or the assignees upon inventing or discovering or asexually reproducing a distinct and new variety of plant, other than a tuber propagated plant or a plant found in an uncultivated state. The duration of the plant patent is 20 years from the date of filing the application. The patent provides the sole right to the inventor to exclude others from asexually reproducing, selling, or using the plant. Title 35 US Code, Section 161, lays down the various laws related to plant patents which states:

> *Whoever invents or discovers and asexually reproduces any distinct and new variety of plant, including cultivated sports, mutants, hybrids, and newly found seedlings, other than a tuber propagated plant or a plant found in an uncultivated state, may obtain a patent therefor, subject to the conditions and requirements of title.*

The plant patent must also satisfy the general requirements of patentability. The subject matter of the application can be a plant which is developed or discovered by applicant and which has been found stable by asexual reproduction. Acceptable modes of asexual reproduction method includes rooting, cuttings, apomictic seeds, division, layering, runners, tissue culture, grafting and budding, bulbs, slips, rhizomes, corms, and nucellar embryos. The asexual reproduction method helps in establishing the stability of the plant. This second step of the invention, i.e., the stability testing, must be performed with sufficient time prior to filing of application to allow the thorough evaluation of propagules or clones of the claimed plant for stability. It helps assuring that such varieties preserve the identical distinguishing characteristics of the original plant.

As per 35 USC 161, the patentability criteria also include:

- *That the plant was invented or discovered and, if discovered, that the discovery was made in a cultivated area.*
- *That the plant is not a plant which is excluded by statute, where the part of the plant used for asexual reproduction is not a tuber food part, as with potato or Jerusalem artichoke.*
- *That the person or persons filing the application are those who actually invented the claimed plant;* i.e.*, discovered or developed and identified or isolated the plant, and asexually reproduced the plant.*
- *That the plant has not been sold or released in the United States of America more than one year prior to the date of the application.*
- *That the plant has not been enabled to the public,* i.e.*, by description in a printed publication in this country more than one year before the application for patent with an offer to sale; or by release or sale of the plant more than one year prior to application for patent.*
- *That the plant be shown to differ from known, related plants by at least one distinguishing characteristic, which is more than a difference caused by growing conditions or fertility levels,* etc.
- *The invention would not have been obvious to one skilled in the art at the time of invention by applicant.*

Plant patents are quite limited in nature as it protects only a single plant or genome. The plant characteristics, mutants of the patented plant, and technologies associated with its cultivation do not get any protection when a variety is protected through plant patents. Only one claim per plant patent is permitted since plant patents are granted on the entire plant. The Plant Patent Act was amended on October 27, 1998, to extend the exclusive right to plant parts obtained from protected varieties, but it is not applied retroactively.

The disclosure of a new plant variety or a granted patent of the new variety in a foreign jurisdiction does not destroy the patentability of the invention if the disclosure occurred less than 12 months prior to the plant patent application being filed in the USA. As per Rule 35 U.S.C 102(b), a 12-month grace period applies to plant patents. However, if publication has occurred in any other country prior to the 12-month grace period, the plant patent application is refused.

The same plant can be protected under both utility patent and plant patent at the same time, provided all the requirements for patentability for both types of patents are fulfilled. It is interesting to note that most of the agro-biotechnology companies prefer utility patents than plant patents for their plants or seeds. The utility patents provide more extensive protection to the protected plants. Utility patents have the added advantage of covering inventions beyond plants. Utility patents may include modified genes, process of inserting a gene to plant genome, or process of modifying a gene in case of a genetically modified variety. Secondly, utility patents are considered to provide better defense in infringement issues. While plant patents allow licensee to sexually reproduce the plant for indefinite period (only for their personal use), utility patent prohibits the replantation of the seeds harvested from the licensed plant. So far no plant patent has been granted for litchi in the USA (Box 10.1).

Box 10.1 Summary of Utility Patent and Plant Patent

Utility Patent

Issued for the invention of a new and useful process, machine, manufacture, or composition of matter or a new and useful improvement thereof, it generally permits its owner to exclude others from making, using, or selling the invention for a period of up to 20 years from the date of patent application filing, subject to the payment of maintenance fees.

Plant Patent

Issued for a new and distinct, invented or discovered asexually reproduced plant including cultivated sports, mutants, hybrids, and newly found seedlings, other than a tuber propagated plant or plant found in an uncultivated state, it permits its owner to exclude others from making, using, or selling the plant for a period of up to 20 years from the date of patent application filing. Plant patents are not subject to the payment of maintenance fees.

10.2.2 Agreement on Trade-Related Aspects of Intellectual Property Rights: The TRIPS Agreement

The year 1995 marked a new chapter for protection of IP rights for new plant varieties with the coming of TRIPS Agreements which is the most effective multilateral agreement on intellectual property. As a part of the World Trade Organization (WTO), the TRIPS Agreement sets minimum national standards for levels of protection to the inventors of intellectual property. The TRIPS Agreement extended the requirement to protect plant varieties to all WTO members which was earlier limited to OECD (Organization for Economic Co-operation and Development) countries. Under TRIPS different areas which are relevant for agriculture can be protected under patents, plant variety protection, commercial marks such as trademarks and geographical indications, and trade secrets. The particular provision in Article 27.3(b) of the TRIPS Agreement, which was concluded on April 15, 1994, as part of the Marrakesh Agreement establishing the WTO, includes the plant varieties as a subject matter for IP protection (Takamiya et al. 2008).

As per the TRIPS Article 27:

1. *..., patents shall be available for any invention, ..., provided that they are new, involve an inventive step and are capable of industrial application ...*
2. *Members may exclude from patentability. [ordre public, morality, life or health, environment].*
3. *Members may also exclude from patentability (a) ... (b) plants and animals other than micro-organisms, ... However, Members shall provide for the protection of plant varieties either by patents or by an effective sui generis system or by any combination thereof ..."*

It is believed that the scope to develop a unique system to protect the plant varieties under article 27.3 (b) was very important for developing nations particularly for India. While it is essential to protect the interest of private entities to foster innovation catering the need of food and medicine at the same time, the fact cannot be ignored that more than 70% of the Indian populations are farming communities. Patenting of particular varieties may exclude farmers from their livelihood. Further the foreign patents on some of the Indian medicinal plant like neem, turmeric, and basil are continuously in public debate. So it was essential for India to develop a sui generis system to protect the rights of inventors of new distinct plant varieties.

10.2.3 Plant Breeder's Right (PBR) and UPOV (International Convention for the Protection of New Varieties of Plants)

Plant variety protection, also called a plant breeder's right (PBR), is a form of IPR, a patent-like system granted to the owners of a new plant variety. According to this right, certain uses concerning the exploitation of the protected variety require the prior consent of the breeder or owner.

Plant variety protection construed on the principles of International Convention for the Protection of New Varieties of Plants (UPOV Convention) is a sui generis form of intellectual property protection. It is specifically intended to reflect the particularities of breeding, cultivation, and uses of new varieties of plants which have been technologically upgraded in recent years. It has many features in common with other forms of intellectual property rights (Tang et al. 2006). Though the UPOV Convention is not overtly declared as a sui generis system, the majority of states which have ratified Article 27.3(b) have also adopted the UPOV system. The UPOV is the only internationally harmonized and effective sui generis system of plant variety protection. The UPOV aims to deliver and promote an effective system of plant variety protection to encourage the development of new varieties of plants for the overall benefit of the community.

10.2.3.1 Basic Features of the UPOV Convention

The UPOV system of plant variety protection was established with the adoption of the International Convention for the Protection of New Varieties of Plants at a Diplomatic Conference in Paris in 1961. The Convention sets the basic legal framework which countries may incorporate to their national laws to grant plant breeders IP rights for their invention. It particularly caters the requirements of the plant breeders and beneficiaries of new plant varieties, including farmers, growers, and producers. So far 74 countries have ratified UPOV convention. Fifteen states and one intergovernmental organization have initiated the procedure for agreeing to the UPOV Convention, and 23 states and one intergovernmental organization are in the process of development of laws based on the UPOV Convention (Tang et al. 2006). To date, only China, a member of UPOV, has litchi (*Litchi chinensis* Sonn.) as a protected plant (Caramelo et al. 1999) (Table 10.2).

10.2.3.2 Plant Breeder's Right (PBR): Protection Requirements

Under UPOV for a protected variety must meet five important requirements, namely, novelty, distinctness, uniformity, stability, and a suitable denomination (IUftPoNVoPU. Getting the Most out of your New Plant Variety 2016). The distinctness, uniformity, and stability are the main technical criteria and they are collectively known as DUS standard. As per UPOV, the detailed criteria are as follows:

Novelty As per UPOV convention, Act of 1991, Article 6, to be eligible for protection, "a variety must not have been sold, or otherwise disposed of, in the territory of the member of the Union concerned for more than one year prior to the application for a breeder's right, or more than four years (six years for trees or vines) in a territory other than that of this member of the Union" (As per UPOV convention, Act of 1991, Article 6).

Table 10.2 List of UPOV Members

African Intellectual	Germany	Romania
Property Organization	Hungary	Russian Federation
Albania	Iceland	Serbia
Argentina	Ireland	Singapore
Australia	Israel	Slovakia
Austria	Italy	Slovenia
Azerbaijan	Japan	South Africa
Belarus	Jordan	Spain
Belgium	Kenya	Sweden
Bolivia (Plurinational State of)	Kyrgyzstan	Switzerland
Brazil	Latvia	The former Yugoslav
Bulgaria	Lithuania	Republic of Macedonia
Canada	Mexico	Trinidad and Tobago
Chile	Montenegro	Tunisia
China	Morocco	Turkey
Colombia	Netherlands	Ukraine
Costa Rica	New Zealand	United Kingdom
Croatia	Nicaragua	United Republic of Tanzania
Czech Republic	Norway	United States of America
Denmark	Oman	Uruguay
Dominican Republic	Panama	Uzbekistan
Ecuador	Paraguay	Viet Nam
Estonia	Peru	
European Union	Poland	(Total 74)
Finland	Portugal	
France	Republic of Korea	
Georgia	Republic of Moldova	
States and intergovernmental organizations which have initiated the procedure for acceding to the UPOV Convention		
Armenia, Bosnia and Herzegovina, Egypt, Ghana, Guatemala, Honduras, India, Iran (Islamic Republic of), Kazakhstan, Malaysia, Mauritius, Philippines, Tajikistan, Venezuela (Bolivarian Republic of), Zimbabwe, as well as the African Regional Intellectual Property Organization (ARIPO)		

Source: UPOV 2016

Distinctness As per UPOV convention, Act of 1991, Article 7, "A variety is deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of filing of the application" (As per UPOV convention, Act of 1991, Article 7).

A variety common knowledge must fall within the definition of a variety set out in Article 1(vi) of the 1991 Act of the UPOV Convention and does not have to be a protected variety and includes ecotypes or landraces which fall within the definition of variety.

Uniformity "A variety is deemed to be uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics" (As per UPOV convention, Act of 1991, Article 8).

Stability "A variety is deemed to be stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle" (As per UPOV convention, Act of 1991, Article 9).

Denomination Each member of the Union must register the denomination of a new plant variety at the same time as it issues the title of protection for the new variety. Anyone who, within the territory of one of the members of the Union, offers material of the protected variety for sale or markets propagating material of the variety is obliged to use the denomination, even after the expiration of the breeder's right of that variety. The denomination is chosen by the breeder of the new variety but it must conform with all the criteria set out in Article 20 of the 1991 Act.

As per Act of 1991 of UPOV, the PBR protection period is 25 years in the case of trees and vines and for 20 years in the case of other crops from the date of grant of the PBR. The right is valid in the territory where it was granted and in the case of intergovernmental organizations which grant PBRs, validity applies in all the member states of that organization. For instance, the Community Plant Variety Office (CPVO) grants PBR, which are valid in all member states of the European Union.

10.2.4 Plant-Related Patents in EU

In EU, like any other technological field, biotechnological inventions have to meet the same criteria to qualify for patent protection, i.e., novelty, inventive step, and industrial application. However, certain specific rules are considered while deciding the patentability of an invention in this field due to the nature of biotechnology and its ethical implications. Articles 52 and 53 of the European Patent Convention (EPC) lay down what can and cannot be patented. It says no European patent can be granted for any of the following:

- *Any invention whose commercial exploitation would be contrary to order public or morality (Art. 53(a) EPC).*
- *Plant and animal varieties (Art. 53(b) EPC).*
- *Essentially biological processes for the production of plants and animals (Art. 53(b) EPC),* i.e. *classical breeding comprising crossing and selection. Discoveries (*e.g. *the mere discovery of natural substances, such as the sequence or partial sequence of a gene) are not patentable.*

In EU the debate on biotechnological patents started since the 1980s, and the member states adopted the Directive 98/44/EC on the legal protection of biotechnological inventions ("biopatent directive") in 1998 to harmonize the law related to

biotechnological inventions. As per the Directive, plants or animals are patentable if the technical feasibility of the invention (e.g., a genetic modification) is not confined to a particular plant or animal variety (Rule 27(b) EPC). Furthermore, an invention relating to gene sequences can be patented as long as the industrial application of the sequence is disclosed in the application and all other patentability criteria are fulfilled (Rule 29(3) EPC).

With regard to plant-related inventions, the Board of Appeal (EBoA) of the European Patent Office (EPO) decided in the case G 1/98 (Transgenic plant/Novartis II) that plants are in principle patentable if the technical teaching of the invention is not limited to a specific plant variety or varieties. This was also clearly codified in Rule 27(b) EPC in 1999. Similarly in 2010, in the "broccoli and tomato I case" (G 2/07 and G 1/08), the EBoA decided that a process for the production of plants comprising the steps of crossing and selection is excluded from patentability even if it contains an additional step of a technical nature, such as the use of molecular genetic markers. However, the products of such processes (i.e., plants or fruits) could still be patented if the conditions of patentability are fulfilled even if they are obtained from such a non-patentable method (decision of G2/12 and G2/13, known as "broccoli and tomato II" case, March 2015) (European Patent Office 2016).

10.2.4.1 Protection of New Plant Varieties in EU

The Community Plant Variety Office (CPVO) is the European Union agency responsible for implementing a system for the protection of plant varieties in European member states. Based on UPOV principles, the CPOV came to being in 1995 and it aimed at fostering innovation in plant varieties by high-quality processing of applications at affordable costs, while providing policy guidance and assistance in the exercise of these rights for the benefit of stakeholders (Bhatti et al. 2008). The CPOV follows the UPOV guidelines for application filing and grant of title. A plant variety right can be granted by the CPOV for a single plant variety generated by traditional breeding or through genetic engineering, provided that the variety fulfills the criteria of distinctness, uniformity, stability, and novelty (Fleck and Baldock 2003) (Box 10.2).

10.2.5 Plant Variety Protection in India: The *Sui Generis* Approach

As a step toward ratification of TRIPS Agreement, the Indian Parliament enacted the Protection of Plant Varieties and Farmers' Rights Act, in 2001(PPV&FR). This Act aimed for the establishment of an effective system for protection of new plant varieties and the rights of farmers and plant breeders and to encourage the development of new varieties of plants. The PPV&FR recognizes and protects the rights of the farmers in respect to their contribution made at any time in conserving, improving, and making available plant genetic resources for the development of the new plant varieties.

As per the Act, any person (farmer/breeder/successor thereof/public or private institution) may make an application for registration of any variety. The Act allows

Box 10.2 Intergovernmental Organizations Dealing with Plant Intellectual Property Rights

- The Convention on Biological Diversity (CBD)
- The Nagoya Protocol on Access and Benefit-sharing
- European Commission
- Directive 98/44/EC of the European Parliament and of the Council of July 6, 1998 on the legal protection of biotechnological inventions
- European Group on Life Sciences (EGLS)
- Food and Agriculture Organization of the United Nations (FAO)
- Organization for Economic Co-operation and Development (OECD)
- International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)
- International Union for the Protection of New Varieties of Plants (UPOV)
- United Nations Educational, Scientific and Cultural Organization (UNESCO)
- World Trade Organization (WTO)
- Article 27.3(b), protection for plants and animals, traditional knowledge and biodiversity

broadly four types of varieties: new variety, extant variety, essentially derived variety, and farmer's variety.

Under the Act, a plant variety is considered new if it conforms to the criteria of novelty, distinctiveness, uniformity, and stability.

As per PPV&FR ACT, Section 15(3), a new variety is considered to be novel if:

(a) *If, at the date of filing of the application, the propagating or harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purposes of exploitation of such variety.*
   (i). *In India, earlier than 1 year*
   (ii). *Outside India, in the case of trees or vines earlier than 6 years, or in any other case, earlier than 4 years, before the date of filing such application*

(b) *Distinct, if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application.*

(c) *Uniform, if subject to the variation that may be expected from the particular features of its propagation it is sufficiently uniform in its essential characteristics.*

(d) *Stable, if its essential characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle.*

(a) *Of such genera and species as specified section 29(2)*
(b) *Which is an extant variety: "extant variety" means a variety available in India which is*:
  (i) *Notified under section 5 of the Seeds Act, 1966*
  (ii) *Farmers' variety*
  (iii) *A variety about which there is common knowledge*
  (iv) *Any other variety which is in public domain*
(c) *Which is a farmers' variety: "farmers' variety" means a variety which*:
  (i) *Has been traditionally cultivated and evolved by the farmers in their fields*
  (ii) *Is a wild relative or land race of a variety about which the farmers possess the common knowledge*

Duration of Registration As per the Act, the certificate of registration for trees and vines is valid for 9 years, and for other crops, it is 6 years. The duration may be reviewed and renewed for the remaining period on payment of such fees as may be fixed by the rules made in this behalf. However, the total period of validity shall not exceed 18 years from the date of registration of the variety in the case of trees and vines and 15 years in the case of extant variety from the date of the notification of that variety by the Central Government under section 5 of the Seeds Act, 1966, and in other cases, 15 years from the date of registration of the variety.

One of the important features of the PPV&FR Act is the Farmers' Rights, i.e., the right to sell seed including the protected seed. As per the Act, the farmer can save, use, sow, resow, exchange, share, or sell his farm produce including seed of a protected variety in the same manner as he was entitled before the coming into force of this Act. The Act also recognizes the role of rural communities as contributors of landraces and farmer varieties in the breeding of new plant varieties. A breeder must take permission before using farmers' varieties for developing Essentially Derived Varieties (EDVs). Anyone can register a community's claim and have it duly recorded at a notified center. If the claim is found to be genuine, a share of profits made from the new variety has to go into a National Gene Fund. The Act seeks to protect farmers from exaggerated claims by seed companies regarding the performance of their registered varieties. The breeder is obliged to disclose the full agronomic performance under recommended condition to the farmers. Upon failing such disclosures, farmers may claim compensation from the breeding company through the Authority (Sahai 2001; Hammel and Cullen 2008). The PPV&FR Act is the first Act that integrated access benefit sharing along with breeder's right.

From the year 2007, around 611,663 applications have been received by the Indian plant authority of which 2193 certificates have been issued. It is interesting to note that maximum applications are filed by farmers (6828 applications) (Darah and Ibrahim 1998). So far no new variety of Litchi has been protected in India. Recently the PPV&FR Authority has issued draft guidelines for DUS testing in litchi (Protection of Plant Varieties and Farmers' Rights Authority (PPV, and FRA) Government of India ND 2016) (Table 10.3).

Table 10.3 A simple overview of alternative intellectual property protection systems in plant varieties[a]

Type of protection	Treaty/system	Germplasm (parents)	Novel (GM) rait[b]	Native Trait[c]	Breeding Method (Conventional, Biotech)	Variety Protection (Genotype)	Own Or License Seed? ('Bag Tag')	Farmers' Privilege
PBRS	UPOV (1961/1978), PVPA 1970	+	−	+	+	−	Own	+
	UPOV 1991, PVPA 1994	+ (not EDV)	+(if licensed)	+	+ (not EDV)	−	Own	Varies (for 'small farmers' in EU,general in USA)
Patents	US patent	−	−	−	−	−	License	−
	EPC patent	− (unclear)	−	−	− (not essentially biological process)	Patents usually extend to varieties with PBR	Own (unclear)	+ (like PBRs) + (in EU like PBRs in UPOV 1991)
	Patent with breeding exception (Europe)	− (unclear)	(+ for R&D, then if licensed)	(+ for R&D, then if licensed)	− (not essentially biological process)	Patents usually extend to varieties with PBR	Own (unclear)	(+,ike PBRs) + (in EU like PBRs in UPOV 1991)
Facilitated licensing	ILP-V, e-licensing	+	−	(+ for R&D < comma > then if licensed)	− (not essentially biological process)	Patents usually extend to varieties with PBR	(Unclear)	(+, like PBRs) + (in EU like PBRs in UPOV 1991)

Compulsory licensing	This proposal	+	+ (with license)	+	+ (with license, not essentially biological process)	Only PBRs,but patent fees added	Own	(+,with fee)
Open source	OSSI	+	+	+	+	+	Own	+
Biodiversity	CBD (Nagoya protocol)	− (license < comma >country of origin)	−	−	−	−	−	−
ITPGRFA	+ (facilitated access, SMTA)	+	+	+	+ (fee if patent, voluntary if PBR)	Own	Farmers' rights	

Source: Bjornstad (2016)

[a]+, freedom to operate; −, exclusive right requiring a license is needed from owner of intellectual property type

[b]Novel trait: not previously available in the gene pool, transferred by GM or NBTs

[c]Native trait: available in the gene pool but exceptional quantitative expression level satisfying "non-obviousness" in certain laws

[d]Essentially derived variety (EDV): obtained by repeated backcrossing or transformation. Defined by 'too close' genetic distance with no precise boundary

10.3 Concluding Remarks

Lychee being exacting in climatic requirement is confined to a few states in India with major production recorded in Bihar. In India lychee is the livelihood for millions of people as it provides both on-farm and off-farm employment. Small and marginal farmers get additional income from lychee plants in their farms. Considering the importance of this fruit crop, efforts are made to provide technological support through research and promoting production, postharvest management, and marketing, including export, through development programs. Further, educational programs on IPR can help in boosting interest of farmers/cultivators and related parties in lychee supply chain to adopt and generate new technologies.

Enhanced IPR protection mechanisms have no doubt provided an encouragement to the private sector to invest more in crop breeding and genetic engineering contributing higher-yielding new improved varieties and more agricultural productivity. It is also promoting industry competitiveness allowing improved access to foreign plant varieties. While it is important to incentivize the innovator to promote more research and development, care should be taken to avoid any monopolistic situation. Economics of innovation and societal harmony should be maintained simultaneously. Many critics argue that IPR does not recognize or reward the contribution of communities of farmers who have developed the landraces through natural process of conservation, selection, and breeding over long period of time. But the PPV&FR Act of India with extensive Farmers' Right is an example where a harmony is attempted between the rights of private breeder and that of a farmer. While the private players are flourishing, the farmers are being able to protect their rights to conserve, use, and develop their plant genome resource.

References

Bhatti HN, Akram N, Asgher M (2008) Optimization of culture conditions for enhanced decolorization of cibacron red FN-2BL by Schizophyllum commune IBL-6. Appl Biochem Biotechnol 149(3):255–264

Binenbaum E, Nottenburg C, Pardey PG, Wright BD, Zambrano P (2003) South-north trade, intellectual property jurisdictions, and freedom to operate in agricultural research on staple crops. Econ Dev Cult Change 51(2):309–335

Bjornstad A (2016) Do not privatize the Giant's shoulders': rethinking patents in plant breeding. Trends Biotechnol 34(8):609–617

Bonny S (2014) Taking stock of the genetically modified seed sector worldwide: market, stakeholders, and prices. Food Secur 6(4):525–540

Caramelo L, Martinez MJ, Martinez AT (1999) A search for ligninolytic peroxidases in the fungus pleurotus eryngii involving alpha-keto-gamma-thiomethylbutyric acid and lignin model dimers. Appl Environ Microbiol. 65(3):916–22. PMCID: 91123

Chen Z, Zhao JT, Qin YH, Hu GB (2016) Study on the graft compatibility between 'Jingganghongnuo' and other litchi cultivars. Sci Hortic-Amsterdam 199:56–62

Darah I, Ibrahim CO (1998) Laboratory-scale production of lignin-degrading enzymes by free and entrapped cells of Phanerochaete chrysosporium in a tubular air-lift bioreactor. Folia Microbiol (Praha) 43(2):161–168

Diamond v. Chakrabarty (1980) 16 Jun 1980. U S Rep U S Supreme Court. 1980;447:303–22

Fleck B, Baldock C (2003) Intellectual property protection for plant-related inventions in Europe. Nat Rev Genet. 4(10):834–838
Gontier E, Thomasset B, Wallington E, Wilmer J (2008) Plant Cyclopropane Fatty Acid Synthase Genes and Uses Thereof. Google Patents; 2008.
Google Patents (2011) Method for constructing lichi core collection by using EST-SSR (Expresses Sequence Tag-Simple Sequence Repeat) molecular marker
Google Patents (2013) Promoter from Litchi chinensis and application thereof
Google Patents (2014a) Litchi R2R3-MYB gene LcMYB1 and application thereof
Google Patents (2014b) Litchi chinensis fruit senescence start mark gene and application thereof
Google Patents (2015) Gene LcFLC for retarding flowering time of plants and application thereof
Google Patents (2016) 荔枝细胞壁酸性转化酶基因及其应用 Litchi cell wall acid invertase gene and its application
Gottschalk LM, Bon EP, Nobrega R (2008) Lignin peroxidase from Streptomyces viridosporus T7A: enzyme concentration using ultrafiltration. Appl Biochem Biotechnol. 147(1–3):23–32
Hammel KE, Cullen D (2008) Role of fungal peroxidases in biological ligninolysis. Curr Opin Plant Biol 11(3):349–355
Howard PH (2015) Intellectual Property and Consolidation in the Seed Industry. Crop Sci 55(6):2489–2495
IUftPoNVoPU. Getting the Most out of your New Plant Variety. [cited 2016 26/7]; Available from: http://www.wipo.int/sme/en/documents/upov_plant_variety_fulltext.html
Jefferson OA, Kollhofer D, Ehrich TH, Jefferson RA (2015) Gene patent practice across plant and human genomes. Nat Biotechnol 33(10):1033–1038
Jorge Fernandez-Cornejo SW, Mike Livingston, and Lorraine Mitchell (2014) Genetically Engineered Crops in the United States. Economic Research Report: United States Department of Agriculture, Service ER; 2014. Report No.: 162 Contract No.: Document Number|
Li DS, Liao CY, Zhang BX, Song ZW (2014) Biological control of insect pests in litchi orchards in China. Biol Control 68:23–36
Martinez-Castellanos G, Pelayo-Zaldivar C, Perez-Flores LJ, Lopez-Luna A, Gimeno M, Barzana E et al (2011) Postharvest litchi (Litchi chinensis Sonn.) quality preservation by Lactobacillus plantarum. Postharvest Biol Tec 59(2):172–178
Menzel CM (1985) Propagation of Lychee – a Review. Sci Hortic-Amsterdam 25(1):31–48
Pandey N, Joshi SK, Singh CP, Kumar S, Rajput S, Khandal RK (2013) Enhancing shelf life of litchi (Litchi chinensis) fruit through integrated approach of surface coating and gamma irradiation. Radiat Phys Chem. 85:197–203
Patents on Biotechnology. European Patent Office (2016) [updated 2016 6.3.2016; cited 2016 7/26]; Available from: https://www.epo.org/news-issues/issues/biotechnology.html
Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) Government of India ND. Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on Litchi (Litchi chinensis Sonn.) [cited 2016 27/7]; Available from: http://plantauthority.gov.in/pdf/Litchiguideline.pdf
Sahai S (2001) India's plant variety protection and farmers' rights act.
Takamiya M, Magan N, Warner PJ (2008) Impact assessment of bisphenol A on lignin-modifying enzymes by basidiomycete Trametes versicolor. J Hazard Mater 154(1–3):33–37
Tang L, Zeng GM, Shen GL, Zhang Y, Huang GH, Li JB (2006) Simultaneous amperometric determination of lignin peroxidase and manganese peroxidase activities in compost bioremediation using artificial neural networks. Anal Chim Acta 579(1):109–116
Thomasset B, Wallington E, Wilmer J, Gougeon S, Gontier E (2007) Lysophosphatidic acid acyltransferase genes and uses thereof. Google Patents; 2007
Transperency Market Research (2015) Global agricultural biotechnology market to be propelled by demand for quality food. [updated 2015; cited 2016 26/7]; Available from: http://www.transparencymarketresearch.com/article/agricultural-biotechnology-market.htm
Vassilev N, Vassileva M, Lopez A, Martos V, Reyes A, Maksimovic I et al (2015) Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Appl Microbiol Biotechnol 99(12):4983–4996
Yeo S, Kim MK, Choi HT (2008) Increased expression of laccase by the addition of phthalates in Phlebia tremellosa. FEMS Microbiol Lett 278(1):72–77

Genetic Manipulation of Litchi for Crop Improvement: Challenges and Possibilities

11

Anjana Rustagi, Garima Malik, Samira Chugh, Shachi Agrawal, Shashi Shekhar, and Nishakant Pandey

Abstract

Litchi fruit crop comprises a lucrative commodity, which significantly contributes to the economic sustainability and livelihood for millions of people in Southeast Asia. Litchi fruit is accepted globally due to its unique taste, rich nutritional value, and exotic aroma and flavor. However, its commercial production and geographical expansion remain constrained due to several reasons that include limited availability of suitable cultivars, irregular flowering, poor production, and unpredictable weather which limits pollination during blooming. At the present juncture, there is a lack of optimized breeding system for crop improvement. In vitro plant regeneration has been harnessed to give an impetus to production of litchi, but litchi being a recalcitrant plant and restrictions in explant collection slows the progress in this regard. Genetic transformation along with omics approach and biotechnology tools may immensely help in development of desired cultivars of litchi. In the present chapter, we discuss the challenges and possibilities of genetic manipulation of litchi.

Keywords

Litchi • *In vitro* plant regeneration • Genetic transformation

A. Rustagi (✉) • G. Malik • S. Chugh • S. Agrawal
Department of Botany, Gargi College, Sirifort Road, New Delhi, India
e-mail: anjana.chaurasia@gmail.com

S. Shekhar • N. Pandey
School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

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11.1 Introduction

Litchi, the queen of fruits, is a native of Southeast Asia and is an important economic fruit crop grown in tropical and subtropical regions of the world. Currently China, India, Thailand, and Vietnam are the leading litchi-producing countries in the world. Apart from being an edible fruit, it is also used in traditional medicine. The fruit and its secondary metabolic products are reported to possess anti-cancer, anti-inflammatory, antifungal, anti-viral, anti-oxidant, antiplatelet and anticoagulant, and anti-diabetic properties. Its bright color, exotic aroma, excellent taste, and rich nutritional value make this fruit very attractive and popular in international markets. Litchi fruit is a drupe, in which the edible part is the delicious and translucent aril. In its normal fruits, the presence of single large seed along with the pericarp reduces the edible flesh content of the fruit by 50–70% (Menzel et al. 2005). To overcome this problem, two types of seedless fruits (aborted seeded and parthenocarpic) are very much sought after. Litchi is also susceptible to an array of diseases especially fungal diseases (dieback, leaf spots, leaf blight). Due to its long reproductive cycle (juvenile period 7–8 years), low fertility, and heterozygous genetic background, new litchi cultivars are difficult to create via conventional breeding methods (Litz 1988; Gomez-Lim and Litz 2004; Raharjo and Litz 2007). Cross breeding is also not commonly used because the genetic background of most litchi germplasm is unclear (Wu et al. 2007). These limitations of conventional methods can be overcome by molecular breeding techniques such as genetic transformation (Kumar et al. 2006a, b). Thus, there is an urgent need for the biotechnology-assisted crop improvement, which aims to obtain new improved cultivars with novel traits (Petri and Burgos 2005).

Genetic transformation technology integrates gene with desired characteristics into different plant cells/tissues to produce transgenic plants with new improved traits. These biotechnological approaches have been recently applied to improve fruit genotypes with significant commercial properties such as increased stress tolerance (biotic or abiotic), nutrition, yield, and quality (delayed fruit ripening and longer shelf life). Genetic manipulation of host defense mechanism also offers a new perspective for introducing traits like disease resistance into the cultivars of litchi. The gene transfer to litchi has been achieved almost always by *Agrobacterium*-mediated transformation (Das et al. 1999; Puchooa 2004). In litchi somatic embryogenesis and plantlet regeneration can be done by culture of anthers and immature embryos. Protoplasts from embryogenic suspensions obtained from immature zygotic embryo (Yu et al. 2000) have also been reported to be an important transformation and regeneration tool for gene transfer in litchi. During the last two decades, the rapid advances in direct genetic manipulation techniques have provided new opportunities for improvement of many important tropical fruit species. The plant transformation technique has made it possible to generate transgenic plants for a number of fruit species such as citrus, strawberry, grape, cranberry, peach, and plum. Genetic transformation along with tissue culture technology is being used for alteration of plant genomes and has led to the development of new and improved

genotypes by the inclusion of different transgenes that could code for various desirable traits such as abiotic/biotic stress tolerance.

This chapter discusses different parameters that affect the genetic transformation of litchi, including the mode of transgene delivery, various explants used for transformation experiments, different media (composition and physical state), and different constructs and vectors used in litchi for crop improvement.

11.2 Different Types of Explants for Genetic Transformation of Litchi

Any successful transformation protocol requires an efficient regeneration system, which could be the regeneration of shoot buds or somatic embryos in case of litchi. Different explants show different potentials for regeneration and genetic transformation. Explant selection is thus a crucial step in production of transgenic plants. The type of explant (leaves, shoot tip, zygotic embryo, etc.) and also the age and physiological status of the donor plant are critical parameters to be considered before choosing an explant for transformation procedures (Piqueras et al. 2010). The first transformation experiment in litchi was reported by Puchooa (2004), wherein leaf tissues were used as explants. A survey of literature reveals that leaf explant has been most commonly used in transformation experiments in litchi as leaf explants could be readily obtained from in vitro grown plantlets. However, according to Yu et al. (2000), protoplasts from immature zygotic embryo are the most efficient and favored tool for gene transfer into litchi. Protoplasts offer single-cell system for gene transformation procedures, thereby reducing the chances of chimera formation.

Padilla et al. (2013) reported the *Agrobacterium tumefaciens*-mediated transformation of "Brewster" ("Chen Tze") litchi embryogenic cultures induced from leaf explants. Das and Rahman (2010, 2012) demonstrated the successful transformation of somatic embryos of *L. chinensis* cv. Bedana derived from zygotic embryos. They were successful in conferring enhanced resistance to *Phomopsis* sp. *spectrum* which causes dieback, leaf spots, and leaf blight. Their results demonstrated that zygotic embryos are well suited as explant material for *Agrobacterium tumefaciens*-mediated transformation of litchi using MS medium supplemented with BA, 2, 4-D and PVPP (as anti-oxidant) for cocultivation. Embryogenic calli formation has the limitation that it needs to be optimized for each cultivar and genotype. Also preparation of suitable explant for induction of embryogenic calli is a lengthy process, often requiring longer in vitro culture phase with high cytokinin and/or auxin supplementation, which may result in somaclonal variations. The embryogenic calli could be agitated in liquid medium to induce homogeneous embryogenic cell suspensions (ECS). From these ECS, plantlet could be regenerated from each potential embryogenic cell. Thus, single-cell origins of plantlet from ECS make them the most desired explant for genetic transformation experiments.

11.3 Effect of Physical State of Media on Genetic Transformation of Litchi

The physical state of media (gelled or liquid medium) can drastically change the in vitro performance of explants, even when using the same medium formulation, because of differences in microculture humidity and nutrient availability. Even though liquid culture systems can generate some disorders, such as hyperhydricity, they generally encourage faster growth and propagation thus scaling up the production. Additionally, liquid media are more amenable to automation (Munoz et al. 2009). It also removes the chances of impurities from the medium due to agar or any other solidifying medium and lessens the cost of the medium due to the removal of the solidifying agent (Ziv and Halvey 1983). Jackson et al. (1991) reported that agitation of liquid medium increases the availability of the nutrients to all parts of the explants. It also reduces the depletion zones around the explants, which are usually formed in solid media due to utilization by actively growing explant tissues.

One of the frequently encountered problems in the micropropagation of woody species including litchi is the secretion of polyphenols into the medium which become toxic to the tissues when oxidized and turns the medium brown (Kantharanjah et al. 1992; Das et al. 1999; Amin and Razzaque 1995; Puchooa 2004). It has been suggested to culture-excised explants in liquid medium supplemented with the two anti-oxidants for a limited period of time (1–2 h) to reduce browning of the medium. This allows most of the phenolics to be released into the medium and at the same time also facilitates a better contact between the explants and the anti-oxidants so that when they were subsequently cultured onto solidified medium, the inhibitory effects of the phenolics are minimized. It was concluded during the survey of available literature that almost all works on litchi micropropagation and genetic transformation report the use of liquid and semisolid state media in different steps of the protocol. Raharjo and Litz (2007) reported the induction of proembryonal masses on semisolid medium and then their transfer to liquid medium to initiate embryogenic suspension cultures. Das and Rahman (2012) advocate the use of the very efficient regeneration procedure (zygotic embryo-calli-protoplast-plant) given by Yu et al. (2000), which includes modified MS medium in liquid and semisolid states and also Ca alginate beads in different steps of the protocol.

Sinha and Das (2013) used the "zygotic embryo-calli-protoplast-plant" protocol with different media for each step to generate genetically engineered salt-tolerant litchi. Embryogenic callus was induced on modified MS medium, and 1.5 g of the embryogenic callus was transferred to liquid medium, in a rotary shaker maintained at 110 rpm in darkness at 26 ± 1 °C to obtain cell suspension culture. This cell suspension culture was later used for protoplast isolation. The protoplasts were suspended in Ca alginate beads in order to obtain protoplast-derived colonies to initiate somatic embryo development.

11.4 Different Constructs and Vectors Used for Genetic Transformation of Litchi

Enhancement of fruit crops by conventional breeding is hampered by various factors such as long adolescent periods, loss of desired genetic combination, self-incompatibility, and genetic restrictions (e.g., high heterozygosity, polyploidy, etc.) (Malony et al. 2010). Therefore, to obtain novel plant traits in economically important fruit crops as litchi, biotechnology-assisted crop improvement, i.e., genetic transformation, is very much required (Petri and Burgos 2005). Also genetic transformation is a better option in comparison to conventional breeding because it gives the possibility to transfer particular attributes into preferred genotypes without influencing their desirable genetic background (Pena and Seguin 2001). Currently, genetic transformation of *litchi* as a tool for lychee improvement is gaining popularity and has been reported by various researchers using several methods.

The selection of vector for genetic manipulation is one of the crucial factors for the successful transformation of candidate gene and its stable gene integration in the host genome. Vectors used for genetic transformation of plants are specifically designed plasmids that assist the creation of transgenic plants. Binary vectors are the most commonly used vectors for plant transformation. These vectors can replicate in both *E. coli* and *Agrobacterium tumefaciens* and contain three main elements: plasmid selection, plasmid replication, and transfer DNA (T-DNA) region. In *litchi*, no standard protocols have been formulated for the creation of transgenic plants till recently. However, introduction of various constructs/vectors via *Agrobacterium*-mediated transformation has been most widely used by researchers and is reported to be compatible with the regeneration of transgenic plants from a variety of litchi cultivars. T-DNA transfer and tumor formation induced by using four strains of *Agrobacterium tumefaciens* (C58, B3/73, T37, and ACH5) on *Litchi chinensis* were first reported in 1985 (Ouyang et al. 1985). Since then various transformation studies have been conducted in lychee (Table 11.1).

In 2001, a team from China reported the development of protocol of *Agrobacterium tumefaciens*-mediated transformation of litchi by optimizing the factors affecting gene transfer efficiency and the selection and regeneration of transformed cells. They used embryogenic calluses of litchi cv. Yuanhong as explant for transformation with a plant expression vector, pBILFY, constructed by inserting *A. thaliana* LFY gene into pBI121 driven by a CaMV35S (cauliflower mosaic virus) promoter. The results showed that among three strains of *Agrobacterium* used (viz., LBA4404, AGL-1, and EHA105), strain EHA105 had the strongest virulence to litchi (Li-hui and Liu-xin 2001). The introduction of pBin 35S mGFP 4 vector, containing the green fluorescent protein (GFP) transcriptionally controlled by the CaMV35S promoter, was attempted in lychee leaf explant of in vitro cultures of *Litchi chinensis* Sonn. variety "Tai So" (Haseloff et al. 1996; Puchooa 2004). The expression of GFP was reported in regenerated leaves and callus of lychee after 4 weeks of infection under a fluorescence microscope. Although no transgenic plantlets were obtained,

Table 11.1 Genetic transformation studies on *Litchi chinensis*

Litchi cultivar/ variety	Explant type	Gene/construct	Mode of transformation	References
Brewster ("Chen Tze") litchi	Embryogenic cultures	*PISTILLATA* (PI) cDNA in antisense orientation	*A. tumefaciens* mediated	Padilla et al. (2013)
Litchi chinensis Sonn.	Embryogenic calli	Gly I + II Gene	*A. tumefaciens* mediated	Sinha and Das (2013)
Litchi chinensis Sonn., variety "Tai So"	In vitro grown leaf tissues	Green fluorescent protein (GFP)	*A. tumefaciens* mediated	Puchooa (2004)
		T-DNA transfer and tumor formation	*A. tumefaciens* mediated	Ouyang et al. (1985)
Litchi (cv. Bedana)	Zygotic embryos	Rice chitinase gene driven by a maize ubiquitin promoter along with its first intron	*A. tumefaciens* mediated	Das and Rahman (2012)
Litchi (cv. Bedana)	Zygotic embryos	Bacterial chitinase gene and *GUS* gene	*A. tumefaciens* mediated	Das and Rahman (2010)
Litchi chinensis Sonn.	Embryogenic calli	*LEAFY* gene	*A. tumefaciens* mediated	Ceng (2003)
Litchi chinensis cv. Yuanhong	Embryogenic callus	*A. thaliana* LFY	*A. tumefaciens mediated*	Li-hui and Liu-xin (2001)
		Plasmid pBI121		
Litchi chinensis cv. "Bedana"	Zygotic embryos	*D. stramonium SAMDC*	*A. tumefaciens* mediated	Das et al. (2016)
		Plasmid pBI121		

the results demonstrate that the GFP gene can be expressed transiently and stably in lychee tissues using *Agrobacterium*, and it can be used as a suitable indicator of transformation in lychee (Puchooa 2004).

11.5 Genetic Transformation and Crop Improvement in Litchi

In the recent years, lychee has been reported to be genetically transformed with desirable candidate genes for imparting biotic and abiotic stress tolerance, for increasing the shelf life of the fruit, and for obtaining seedless fruits by inducing parthenocarpy.

Fungal diseases are one of the most important factor contributing to yield losses in major horticultural crops. To improve the antifungal response of *Litchi chinensis* Sonn., cv. "Bedana," zygotic embryos of lychee were transformed with the binary vector pGL2 having the rice chitinase gene driven by a maize ubiquitin promoter along with its first intron (CaMV-Ubi-RCC11) via *Agrobacterium tumefaciens*-mediated transformation. The transgenic lychee plants thus obtained displayed higher chitinase activity than the non-transgenic control plants (Das and Rahman 2012). The transgenic plants did not exhibit any phenotypic alterations and showed a delayed onset of the disease and increased resistance to blight pathogen

(*Phomopsis spectrum*). In an earlier experiment of transformation studies with rice chitinase, bacterial chitinase (ChiB) gene was also expressed in *Litchi* cv. Bedana. However, the bacterial chitinase-transgenic plants thus obtained exhibited limited resistance to *Phomopsis* due to low level of bacterial chitinase activity (Das and Rahman 2010).

In another study, Padilla et al. (2013) obtained transgenic plants of elite "Brewster" ("Chen Tze") litchi through *Agrobacterium*-mediated transformation to study the possibility of production of parthenocarpic fruits in lychee. Embryogenic cultures of "Brewster" ("Chen Tze") litchi were induced from the leaves of a mature tree and transformed with the binary vector pCambia3301 containing *A. thaliana* PISTILLATA (PI) cDNA in antisense orientation driven by CaMV 35S promoter (Padilla et al. 2013). PISTILLATA is a floral homeotic gene, first identified in *A. thaliana*, and is known to play important regulatory function during embryo development and in floral identity (Bowman et al. 1989; Meyerowitz et al. 1989; Lehti-Shiu et al. 2005). The expression of the litchi PI homolog was significantly lower in transgenic lines as compared to wild-type plants. The antisense approach for gene silencing is important not only for the study of gene function but also for specific breeding. This strategy has already been applied to a variety of fruit crops such as apple (Kotoda et al. 2006; Yao et al. 2001), pear (Gao et al. 2007), plum (Callahan and Scorza 2007), strawberry (Jimenez-Bermudez et al. 2002), citrus (Wong et al. 2001), mango (Cruz-Hernández et al. 1997), and papaya (Magdalita et al. 2002). By transforming litchi with the *A. thaliana* PI cDNA in antisense orientation, the homologous gene in litchi (PI-Lch) was suppressed. The candidate gene *PISTILLATA* was originally identified as a floral homeotic gene in *A. thaliana* (Bowman et al. 1989; Meyerowitz et al. 1989). Its expression is maximum during early floral development (Goto and Meyerowitz 1994), but *PI* gene also shows expression at low levels in young seedlings, embryos, and embryogenic cultures (Kinoshita et al. 2001; Lehti-Shiu et al. 2005). These findings suggest that the *PI* gene may have a regulatory function in embryo development, apart from its established role in floral identity (Lehti-Shiu et al. 2005). In the study of Padilla et al. (2013), it was demonstrated that PI antisense decreased PI-Lch mRNA levels, but whether silencing will be effective during flower development, when the expression level of endogenous genes is notably upregulated in comparison to embryonic stages, could not be concluded. Mature plants derived from transgenic somatic embryos will be available in another 3–4 years (typical juvenile period for litchi is 7–8 years) which will throw light if PI-Lch mRNA levels will be sufficiently decreased in floral primordial leading to development of parthenocarpic fruits.

The litchi crop is susceptible to salinity stress, and its productivity is reduced in high salt concentration. Sinha and Das (2013) manipulated the glyoxalase pathway for enhancing salinity tolerance in litchi. The embryogenic calli explants were infected with *Agrobacterium tumefaciens* containing *glyI-glyII* gene in plasmid Pbi121. The presence of transgene in the putative transgenic plantlets was confirmed by PCR analysis using glyI + glyII gene-specific primer. Glyoxalase activity of glyoxalase enzyme was measured in the transgenic lines. Enhanced glyoxalase activity was observed in the transgenic lines in comparison to untransformed control plants.

Thus expression of *glyI-glyII* gene reduced the toxicity caused by methyl glyoxal by glutathione-based detoxification, thereby enhancing the tolerance of transgenic litchi plants to salt stress. Litchi is a non-climacteric fruit and has a short shelf life under normal ambient conditions (Yun et al. 2016). High concentrations of polyamines are known to be associated with increased shelf life of harvested fruits. SAMDC (S-adenosyl-1-methionine decarboxylase) is one of the key regulatory enzymes in the biosynthesis of polyamines (Torrigiani et al. 2005). In one of the recent study, transgenic litchi plants were obtained by transforming zygotic embryos with *Agrobacterium tumefaciens* strain LBA4404 containing binary plasmid pBI121 with SAMDC gene from *Datura stramonium* under the control of CaMV35S promoter (Das et al. 2016). The results revealed a significant increase in polyamines accumulation, especially spermidine (spd) and spermine (spm), in the litchi transgenic plants as compared to wild-type plants under normal environmental. Increased Spd and Spm levels are generally linked with enhanced plant tolerance to abiotic stresses (Jimenez-Bremont et al. 2007). These results suggest that genetic engineering of the synthesis of polyamines in litchi plants is apparently a promising tool for improvement of abiotic tolerance and shelf life in litchi fruits.

11.6 Conclusion and Future Prospects

Genetic manipulation techniques have made it feasible to alter one or more horticulturally important traits, while retaining the distinctive individuality of the original cultivar. The above studies indicate how the advancement of genetic transformation techniques has opened new avenues for improvement in litchi crop.

Since Litchi species has not benefited much from conventional breeding, genetic transformation of lychee as a tool for lychee enhancement is picking up prominence. One of the major obstacles that have hindered genetic transformation studies in lychee is that de novo regeneration protocols for elite (mature phase) selections are not well defined. Generally, the leaf explants and embryogenic cultures derived from zygotic embryos have been used as explants for *Agrobacterium*-mediated transformation method. The prime objectives that are being addressed using genetic transformation of litchi include disease resistance, recovery of parthenocarpic fruits, and the control of fruit ripening to increase its shelf life. The recent studies unlock new frontiers to comprehend the role of genes regulating key biological processes and their potential use for lychee enhancement through genetic engineering. As a result, emerging biotechnological tools can now be employed to generate lychee varieties having improved fruit quality such as large fruit size, seedless fruits, bright red pericarp color, delayed ripening, and enhanced resistance to numerous biotic or abiotic stresses, which in turn will contribute in expanding marketing potential of lychee fruit in near future.

References

Amin MN, Razzaque MA (1995) Induction of somatic embryogenesis in the cultures of zygotic embryos of lychee. Bangladesh J Bot 24:25–29

Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in Arabidopsis. Plant Cell 1:37–52

Callahan A, Scorza R (2007) Effects of a peach antisense ACC oxidase gene on plum fruit quality. In: Litz RE, Scorza R (eds) Proceedings of the international symposium on biotechnology of temperate fruit crops and tropical species. Acta Horticulturae, vol 738. International Society for Horticultural Science, Leuven, pp 567–573

Ceng LH (2003) Agrobacterium tumefaciens-mediated genetic transformation of litchi (*Litchi chinensis* Sonn.) With LEAFY Gene. Global Thesis.com ID: 1103360065956300

Cruz-Hernández A, Litz RE (1997) Transformation of mango somatic embryos. Acta Hort 455: 292–298

Das DK, Rahman A (2010) Expression of a bacterial chitinase (ChiB) gene enhances antifungal potential in transgenic Litchi chinensis Sonn. (cv Bedana). Curr Trends Biotechnol Pharm 4(3):820–833

Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell Tissue Organ Cult 109(2). doi:10.1007/s11240-011-0097-2

Das DK, Shiva Prakash N, Bhalla-Sarin N (1999) Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.). Plant Cell Rep 18:691–695

Das DK, Prabhakar M, Kumari D, Kumari N (2016) Expression of SAMDC gene for enhancing the shelf life for improvement of fruit quality using biotechnological approaches into litchi (*Litchi chinensis* Sonn.) cultivars. Adv Biosci Biotechnol 07:300–310. doi:10.4236/abb.2016.76028

Gao M, Matsuta N, Murayama H, Toyomasu T, Mitsuhashi W, Dandekar AM, Tao R, Nishimura K (2007) Gene expression and ethylene production in transgenic pear (Pyrus communis cv. 'LaFrance') with sense or antisense cDNA encoding ACC oxidase. Plant Sci 173:32–42

Gomez-Lim MA, Litz RE (2004) Genetic transformation of perennial tropical fruit crops. In Vitro Cell Dev Biol Plant 40:442–449

Goto K, Meyerowitz EM (1994) Function and regulation of the *Arabidopsis* floral homeotic gene PISTILLATA. Genes Dev 8:1548–1560

Haseloff J, Siemering K, Hodge S, Golbik R, Prasher D (1996) The green fluorescent protein gene must be modified for use as a vital marker in *Arabidopsis thaliana*. Plant Physiol 111:17

Jackson MB, Abbott AJ, Belcher AJ, Hall KC (1991) Ventilation in plant tissue cultures effects of poor aeration on ethylene and carbon dioxide accumulation, oxygen depletion and explants development. Ann Bot 67:229–237

Jimenez-Bermudez S, Redondo-Nevado J, Munoz-Blanco J, Caballero JL, Lopez-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiol 128:751–759

Jimenez-Bremont JF, Oscar A, Ruiz OA, Rodriguez-Kessler M (2007) Modulation of spermidine and spermine levels in maize seedlings subjected to long-term salt stress. Plant Physiol Biochem 45:812–821. http://dx.doi.org/10.1016/j.plaphy.2007.08.001

Kantharanjah AS, Mc Conchie CA, Dodd WA (1992) *In vitro* embryo culture and induction of multiple shoots in lychee. Ann Bot 70:153–156

Kinoshita T, Harada JJ, Goldberg RB, Fischer RL (2001) Polycomb repression of flowering during early plant development. Proc Natl Acad Sci U S A 98:14156–14161

Kotoda N, Iwanami H, Takahashi S, Abe K (2006) Antisense expression of MdTFL1: a TFL1-like gene, reduces the juvenile phase in apple. J Am Soc Hortic Sci 131:74–81

Kumar M, Prakash NS, Prasad US, Sarin NB (2006a) A novel approach of regeneration from nodal explants of field grown litchi (*Litchi chinensis* Sonn.) fruit trees. J Plant Sci 5(3):321–327

Kumar M, Gupta M, Shrivastava D, Prasad M, Prasad US, Sarin NB (2006b) Genetic relatedness among Indian litchi accessions (*Litchi chinensis* Sonn.) by RAPD markers. Int J Agric Res 1:390–400

Lehti-Shiu MD, Adamczyk BJ, Fernandez DE (2005) Expression of MADS-box genes during the embryonic phase in Arabidopsis. Plant Mol Biol 58:89–107

Li-hui Z, Liu-xin L (2001) Transformation and transgenic plantlets regeneration of litchi (*Litchi chinensis* Sonn.) with LEAFY gene. J Fujian Agric Univ 30(4):563–564. (in Chinese)

Litz RE (1988) Somatic embryogenesis from cultured leaf explants of the tropical tree *Euphoria longan* Stend. J Plant Physiol 132:190–193

Magdalita PM, Laurena AC, Yabut-Perez BM, Mendoza EMT, Villegas VN, Botella JR (2002) Progress in the development of transgenic papaya: transformation of Solo papaya using ACC synthase anti- sense construct. Acta Hortic 575:171–176

Malony M, Boresjza-WysockaJohn EE, Norelli JL, Flaishman MA, Gidoni D, Aldwinckle HS (2010) Genetic transformation of apple (*Malus x Domestica*) without use of a selectable marker gene. Tree Genetics & Genomes 6(3):423–433

Menzel CM, Huang X, Liu C (2005) Cultivars and plant improvement. In: Menzel CM, Waite GK (eds) Litchi and longan: botany, production and uses. CABI, Wallingford, pp 59–86

Meyerowitz EM, Smyth DR, Bowman JL (1989) Abnormal flowers and pattern formation in floral development. Development 106:209–217

Munoz M, Seemann P, Jara G, Riegel R (2009) Influence of vessel type, physical state of medium and temporary immersion on the micropropagation of three *Rhodophiala* species. Chil J Agric Res 69:581–587

Ouyang S, Zheng X et al (1985) T-DNA transfer and tumor formation induced by *Agrobacterium tumefaciens* on *Litchi chinensis*. Acta Genet Sin 12:42–45

Padilla G, Jose AP, Perea-Arango I, Moon PA, Miguel AG-L, Borges AA, Exposito-Rodríguez M, Litz RE (2013) *Agrobacterium tumefaciens*-mediated transformation of 'Brewster' ('Chen Tze') litchi (*Litchi chinensis* Sonn.) with the PISTILLATA cDNA in antisense. In Vitro Cell De Biol—Plant 49:510–519. doi:10.1007/s11627-013-9533-9

Pena L, Seguin A (2001) Recent advances in the transgenic transformation of trees. Trends Biotechnol 19:500–506

Petri C, Burgos L (2005) Transformation of fruit trees useful breeding tool or continued future prospect? Transgenic Res 14:1526

Piqueras A, Alburquerque N, Folta KM (2010) Explants used for the generation of transgenic plants. In: Kole C et al (eds) Transgenic Crop Plants. Springer-Verlag, Berlin/Heidelberg

Puchooa D (2004) Expression of green fluorescent protein gene in litchi (*Litchi chinensis* Sonn.) tissues. J Appl Hortic 6(1):11–15

Raharjo SHT, Litz RE (2007) Somatic embryogenesis and plant regeneration of litchi (*Litchi chinensis* Sonn.) from leaves of mature phase trees. Plant Cell Tissue Organ Cult 89:113–119

Sinha S, Das DK (2013) Transformation of litchi (*Litchi chinensis* Sonn.) With gly I + II gene leads to enhanced salt tolerance. IJBAF 1(11): 483–495

Torrigiani P, Scaramagli S, Ziosi V, Mayer M, Biondi S (2005) Expression of an antisense *Datura stramonium* S-adenosylmethionine decarboxylase cDNA in tobacco: changes in enzyme activity, putrescine-spermidine ratio, rhizogenic potential, and response to methyl jasmonate. J Plant Physiol 162:559–571. http://dx.doi.org/10.1016/j.jplph.2004.10.008

Wong WS, Li GG, Ning W, Xu ZF, Hsaio WLW, Zhang LY, Li N (2001) Repression of chilling induced ACC accumulation in transgenic citrus by overproduction of antisense 1-aminocyclopropane-1-car- boxylate synthase RNA. Plant Sci 161:969–977

Wu Y, Yi G, Zhou B, Zeng J, Huang Y (2007) The advancement of research on litchi and longan germplasm resources in China. Sci Hortic 114:143–150

Yao J-L, Dong YH, Morris B (2001) Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. Proc Natl Acad Sci U S A 98:1306–1311

Yu CH, Chen ZG, Lu LX, Lin JW (2000) Somatic embryogenesis and plant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Organ Cult 61:51–55

Yun Z, Qu H, Wang H, Zhu F, Zhang Z, Duan X, Jiang Y (2016) Comparative transcriptome and metabolome provides new insights into the regulatory mechanisms of accelerated senescence in litchi fruit after cold storage. Sci Report 6:19356. http://doi.org/10.1038/srep19356

Ziv A, Halvey AH (1983) Control of oxidative burrowing and *in vitro* propagation of *Strelitzia reginae*. Hortic Sci 119:315–319

Regeneration from Nodal Explants of Field-Grown Litchi (*Litchi chinensis* Sonn.) Fruit Trees

12

Vivek Kumar, Priyanku Teotia, Ram Prasad, Ajit Varma, Neera Sarin, and Manoj Kumar

Abstract

Mass propagation of *Litchi chinensis* (Sonn.) via seeds is understood as detrimental because of the highly heterozygous nature of the plant due to cross-pollination. The conservative methods of vegetative propagation utilized for litchi are air layering or marcottage, grafting, and budding which are slow and incompetent as evinced by several reports. In the recent past numerous efforts for clonal propagation of litchi were made with marginal success. Earlier our group have reported multiple shoot induction and plant regeneration in litchi from the nodal cuttings and cotyledonary nodes and by *in planta* treatment of the axillary bud regions. In this, we highlight a research protocol which has a comprehensive discussion. It addresses the technical inputs for reproducible and efficient method of in vitro regeneration of elite litchi trees appropriate for clonal propagation. The protocol that has been referred has been proven advantageous to the horticulturists and the industry for recalcitrant trees, those that can be developed as true to the parental type.

Keywords

Litchi • Heterozygous • Grafting • Clonal propagation • True to the parental type

V. Kumar • R. Prasad • A. Varma • M. Kumar (✉)
Amity Institute of Minorbial Technology, Amity University, Noida, Uttar Pradesh, India
e-mail: mkumar9@amity.edu

P. Teotia
Laboratory of Microbiology, Department of Botany, CCS University, Meerut, India

N. Sarin
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

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12.1 Introduction

Litchi chinensis Sonn. (litchi) of the family Sapindaceae is an important horticultural crop which bears fruits that rank among the most delicious fruits possessing high nourishment and medicinal value. The aril of the fruit is glutinous and aromatic and contains high amount of vitamin C and phosphorus. The fruit is an excellent thirst quencher reportedly serving as a tonic for the brain, heart, and liver (Mishra and Syamal 1984). Litchi has various names such as litchi, lychee, lici, licy, le-ci, lichee, lichies, and leechee (Hayes 1957). It originated in Southern China and reached India first via Myanmar by the end of the seventeenth century (Goto 1960). Natural propagation of litchi occurs by seeds, and the reproductive phase normally begins after 8–10 years (Hamilton and Yee 1970). The seeds lose their viability within 5–6 days (Ray and Sharma 1985). Propagation via seeds is also undesirable because of the highly heterozygous nature of the plant due to cross-pollination (Kumar et al. 2006; Sarin and Prasad 2003). The conventional methods of vegetative propagation utilized for litchi are air layering or marcottage, grafting, and budding (Menzel 1985; Pandey and Sharma 1989) which are slow and inefficient (Chapman 1984; Sarin and Prasad 2003). In the recent past several attempts for clonal propagation of litchi were made with marginal success (Kantharajah et al. 1989; Chandra and Padaria 1999; Kumar et al. 2004; Yu 1991; Jiang and Fu 1999). Earlier we reported multiple shoot induction and plant regeneration in litchi from the nodal cuttings and cotyledonary nodes and by *in planta* treatment of the axillary bud regions (Kumar et al. 2006; Das et al. 1999). In this communication, it has been reported as a highly reproducible and efficient method of in vitro regeneration of elite litchi trees suitable for clonal propagation. The protocol that has been established could prove advantageous to the horticulturists and the industry for developing trees true to the parental type.

12.2 Plant Material

12.2.1 Sterilization and Preparation of Explants (Preparatory Phage)

This was the first crucial step toward clonal propagation. After removing the leaves, the cuttings were thoroughly washed first in running tap water, then a 5% solution of either extran or savlon (Johnson and Johnson), and finally in bavistin (1%, w/v), a fungicide, for 15–20 min. All subsequent operations were carried out inside a laminar airflow cabinet. The cuttings were given a quick (30 seconds) rinse in 70% ethanol, followed by washing in sterile distilled water. They were then surface-sterilized in 0.5% mercuric chloride solution for 5 min and rinsed thrice with sterile distilled water.

The cuttings were trimmed at both ends to expose fresh tissue before planting them on the medium. Filter paper bridge technique was followed to provide the support to the explants.

Table 12.2 Composition of media

Compound	Murashige and Skoog	Gamborg B5	Woody Plant Medium
Concentration (mg/l)			
NH_4NO_3	1650	–	400
KNO_3	1900	2500	–
$Ca(NO_3)2.4H_2O$	–	–	556
$(NH_4)2SO_4$	–	134	–
$MgSO_4$ $7H_2O$	370	250	370
$CaCl_2$ $2H_2O$	440	150	96
KH_2PO_4	170	–	170
NaH_2PO_4 H_2O	–	150	–
K_2SO_4	–	–	990
$FeSO_4$ $7H_2O$	27.8	27.8	27.8
Na_2EDTA	37.3	37.3	37.3
$MnSO_4$ H_2O	22.3	–	–
$ZnSO_4$ $7H_2O$	8.6	2.0	8.6
H_3BBO_3	6.2	3.0	6.2
KI	0.83	0.75	–
Na_2MoO_4 $2H_2O$	0.25	0.25	0.25
$CuSO_4$ $5H_2O$	0.025	0.025	0.25
$CoCl_2$ $6H_2O$	0.025	0.025	–
Myo-inositol	100	100	100
Nicotinic acid	0.5	1.0	0.5
Pyridoxine HCl	0.5	1.0	–
Thiamine HCl	0.1	10.0	1.6
Glycine	2.0	–	–
L-Glutamine	–	–	50
Casein hydrolysate	–	–	300–400
[a]Coconut water	–	–	15% (V/V)
[a]Sucrose	30,000	20,000	30,000

Kumar et al. 2006

[a]Added after two subcultures; pH ranging from 5.6 to 5.8; agar needed varies depending on the brand used; varying the hormones allows the above media to be used for micropropagation of litchi

12.2.2 Medium Used

For initiation and proliferation of the axillary buds from the nodal cuttings of litchi, the medium used is by Lloyd and McCown (1980), which has been referred to as Woody Plant Medium (WPM). Tested plant culture media for micropropagation of litchi (*Litchi chinensis* Sonn.) contain the following ingredients (Tables 12.2 and 12.3).

Table 12.3 Composition of media

(A) Major salts (x)		
NH_4NO_3	8.000 g	400 mg/l
K_2SO_4	19.800 g	990 mg/l
$CaCL_2$	1.920 g	96 mg/l
$MgSO_4$	7.400 g	370 mg/l
KH_2PO_4	3.400 g	170 mg/l
Dissolved in 500 ml of distilled water (DW). Take 25 ml/l		
Ca $(NO_3)_2$	11.120 g	556 mg/l
Dissolved in 250 ml of DW. Take 12.5 ml/l		
NaEDTA	37.3 g	746 mg/l
$FeSO_4$	27.8 g	556 mg/l
Dissolved in 250 ml of distilled water; take 12.5 ml/l		
(B) Minor (1x50X)		
H_3BO_3	6.2 g	310 mg/l
$MnSO_4$	22.3 g	1.15 mg/l
$ZnSO_4$	8.6 mg	4.30 mg/l
Na_2MoO_4	0.25 g	125 mg/l
$CuSo_4$	0.25 g	125 mg/l
Dissolved in 500 ml of DW. Take 10 ml/l		
(C) Vitamins		
Meso-inositol	100 mg/l	
Pyridoxine	50 mg/l	
Nicot. acid	50 mg/l	
Thiamine HCL	10 mg/l	
Glycine	200 mg/l	
Glutamine	20 mg/l	
Dissolved in 100 ml of DW. Take 10 ml/l		

Kumar et al. 2006

12.2.3 Preculture in Liquid Medium

Preculture Woody Plant Medium (WPM) was supplemented with BAP (11 µM), Kn (2.30 µM), GA_3 (0.60 µM), bavistin (30 µg/l), and PVP (0.2%). Sucrose was not added to the preculture medium. Different strengths, viz., MS full, $MS_{½}$, $MS_{1/3}$, and $MS_{1/4}$ of Murashige and Skoog (MS) medium (Murashige and Skoog 1962) and MS (full) supplemented with B5 vitamins and B5 medium (Gamborg medium) (Gamborg and Shyluk 1981), were also tested with different combinations of phytohormones for multiple shoot formation.

After adjusting the pH to 5.6, an aliquot of 20 ml liquid medium was dispensed into each Borosil rimless glass tube (150 × 25 mm). The culture tubes were plugged with non-adsorbent cotton wrapped in cheesecloth and autoclaved at 1.06 kg cm^{-2} at 121 °C for 18 min. All the cultures were incubated at 25 ± C under light provided

by Phillips cool white fluorescent lamps (40 W) at a photon fluence density of 50 μ mol m^{-2} s^{-1} for 16 h per day.

In experiments which involved frequent changes of the medium, it was difficult to transfer nodal explants from one solid medium to another without damage. Therefore, the following method of culturing the nodal cuttings in liquid medium was standardized.

Filter paper bridge technique was followed to provide support to nodal explant in liquid culture. Nodal cuttings were inserted into filter paper which was folded and immersed in the desired liquid medium in the culture tubes. The level of the medium was maintained by regular supplementation so that the nodal surface/cut ends were in constant contact with the medium. The liquid medium was decanted off from the tubes held in a slant and replaced with another. The excess liquid was allowed to drain off from the filter paper bridge before pouring the fresh medium. Following this method enabled frequent media changes possible for the bud induction from the nodal cuttings without causing any damage to them.

12.2.4 Induction

After a 2-week preculture on WPM liquid medium, the cultures were transferred to WPM agar medium supplemented with 3% sucrose, 15% deproteinized coconut water, casein hydrolysate (400 mg/l), and the same hormone combinations and concentrations as used in preculture. The concentration of PVP and bavistin was reduced to 0.02% and 10 μg/l, respectively. At the initial stage, rapid subculturing (at every 10-day interval) was essential to avoid the local deposition of phenolics. After two subcultures, bavistin and PVP were also excluded from the medium.

12.2.5 Multiplication and Elongation of Microshoots

Small (0.5 cm) shoots from 4–5-week-old cultures were excised carefully and transferred to full-strength liquid as well as semisolid MS medium supplemented with BAP (6.0 μM), GA_3 (0.15 μM), SN (30 μM), and CH (300 mg/l) for multiplication and elongation of shootlets. After 15–20 days of subculture, each axillary shoot which had elongated to ca. 7 cm was cut into small segments and transferred to fresh semisolid MS medium of the same composition for further multiplication.

12.2.6 Rooting and Transplantation

The elongated shoots (5–8 cm) were separated from the clump of multiple shoots by giving a gentle cut at the basal region. After giving a pulse treatment with IBA (100 mM) in liquid MS medium for 15 min. The shootlets were transferred to different strengths of MS major salts (full, three quarter, half, and one quarter) supplemented with 20 μM IBA or NAA along with 1 g/l of sterilized litchi seed powder

which was prepared from green seeds with soft seed coat. The seeds were collected from immature fruits before ripening, frozen at minus 80 °C, pulverized in liquid nitrogen, and ground to make a fine powder which was transferred to a glass bottle and autoclaved. Stock of sterilized seed powder was stored at room temperature and used in the medium when required. The rooted plantlets (1–2 inches long) were gently removed from the culture medium, and the roots were washed carefully in running tap water to remove agar. The plantlets were transferred to plastic pots (5 cm diameter) containing a mixture of vermiculite and litchi seed powder (3:1) and maintained at 25 ± 2 °C, 16 h photoperiod 200 μmol.m^{-2} s^{-1} light intensity, and 80% RH. These potted plants were covered with polythene bags to retain high humidity conditions and provided with either half-strength MS salt solution devoid of sucrose and myo-inositol or Hoagland's medium (Hewitt 1966) twice a week. After 25 days, polythene bags were removed from the plastic pots, and the plants were hardened for a week, before transfer to earthen pots (10 cm diameter) containing garden soil mixed with sand and vermiculite (1:1:2) and watered at regular intervals. The plants were transferred to the glass house before transplanting them in the nursery. At least five replicates were maintained for each treatment, and the experiments were repeated thrice. Observations on the number of cultures showing budbreak, shoot elongation, and rooting were made at regular intervals, and contaminated cultures were removed from time to time.

12.2.7 Establishment of Aseptic Nodal Segment Cultures

The nodal segments were cultured in various media (as described in material and methods). However, contamination was a major problem during initiation of cultures in vitro. The explants were mainly contaminated by endogenous pathogen which had to be isolated and identified for controlling it (Kumar et al. 2004). The extent of contamination as well as budbreak was highly dependent on the season in which the material was collected. Utilizing the elaborate sterilization procedure described in Sect. 12.2.2, the cultures initiated in the months February–May showed higher budbreak (45%) and less contamination (20%) than those raised later. Since June–August is the period that coincides with rainy season in the North, 90% explants collected in these three months were prone to infection. Therefore, the cultures were routinely raised from February to May. Sterilization steps for the explants which are extremely important for growing disease-free material were well standardized in the present investigation.

Seasonal effects on establishment of cultures have earlier been reported for other tree species, viz., neem (Chaturvedi et al. 2004), apple (Hutchinson 1984), papaya (Litz and Conover 1981), sweet gum (Sutter and Barker 1985), and guava (Amin and Jaiswal 1988). Yu and Chen (1998) reported that exudation of phenolics and contamination depended on the age of the mother plant from which the explants are taken as well as the growing conditions of the donor plants. They reported a contamination rate of 100% in test material taken after 10 continuous rainy days and 20% after 15 continuous sunny days and found the highest regeneration rate on

Table 12.1 A list of different cultivars of litchi plants used in the experiments and the sources from where they were obtained has been given below (Kumar et al. 2006)

Cultivars	Sources
Purbi, Deshi, Kasba, China, Ajholi, Late Large Red, Late Bedana, Bedana, and Dehra Rose	Bihar Agricultural College (Central-East India)
Seedless, Kalkattia, and Dehradun	Punjab Agricultural University, Punjab (Northern India)
Rose scented, Early Bedana, Late Bedana, Dehradun, and Kalkattia	Bihar Agricultural College (Central-East India)
Dehradun and Dumdum	Forest Research Institute, Uttarakhand (Mid-Northern India)
Seedless and Kalkattia	Regional Horticultural Division, Uttar Pradesh (Central India)
Seedless and Bedana	Regional Horticultural Division, Jharkhand (Central-East India)

half-strength MS medium fortified with 4.44 μM of BA, 1.142 μM of IAA, and 1.734 μM of GA3. In this investigation, 100% contamination was obtained during the rainy season (June–August). Therefore, to solve this problem of contamination, none of the explants were taken for in vitro culture during these months.

12.2.8 Axillary Shoot Proliferation

Axillary shoot proliferation from the nodal explants varied considerably on different strengths of inorganic salts and phytohormone concentration in WPM semisolid medium. Full-strength WPM supplemented with various growth regulators (L1 medium, Table 12.1) resulted in the best response of shoot bud formation in 53.3% of the cultures (Fig. 12.1a, b, adapted from Kumar et al. 2006) (Table 12.2). These explants were transferred to semisolid L2 medium (Table 12.1) after 3 weeks and to liquid L2 medium (on filter paper bridges) after 5 weeks. Though the shootlets elongated to ca. 2–3 cm, they appeared yellow and abscission of leaves was observed. Incorporation of filter-sterilized silver nitrate (30 μM) in the medium and reducing the concentration of CH to 300 mg/l helped in controlling abscission, improved the incidence of budbreak, and promoted shoot proliferation (Fig. 12.2a, b adapted from Kumar et al. 2006).

BAP was found to be optimum at 11.00 μM in inducing frequent shootlet formation from the nodal cuttings, but increasing concentration of cytokinins often led to lower regeneration rate and stunted growth (Fig. 12.3a, b adapted from Kumar et al. 2006).

On the different cytokinins tested, 2-iP at 6.0 μM with same concentrations of Kn and GA_3 as employed in case of L1 semisolid medium, it was found to trigger the apical shoot proliferations with an average of three shoots/explant (Fig. 12.4a, b). Elongation and multiplication frequency and efficiency of shootlets obtained from apical shoot meristems on L3 semisolid medium (Table 12.1) were quite slow

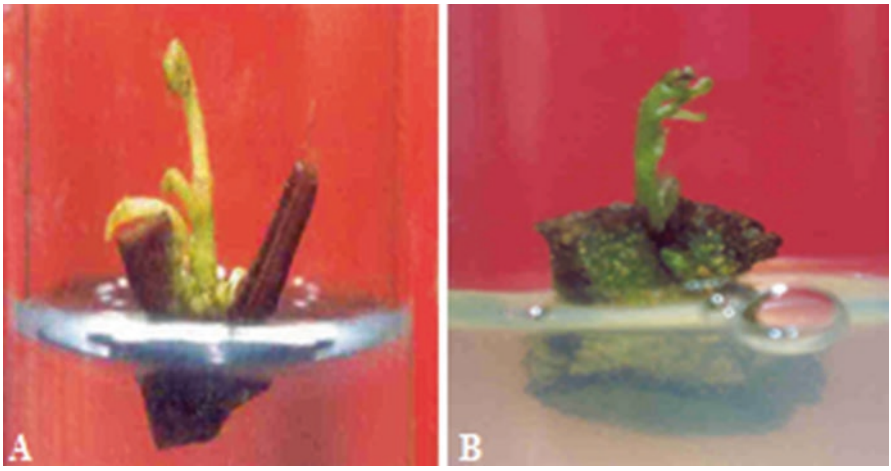


Fig. 12.1 (**a**, **b**) Induction of single shootlet formation from the nodal segments of litchi on L1 medium {WPM + BAP (11.00 µM) + Kn (2.3 µM) + GA3 (0.6 µM) + CH (400 mg/l) + CW(15%)}

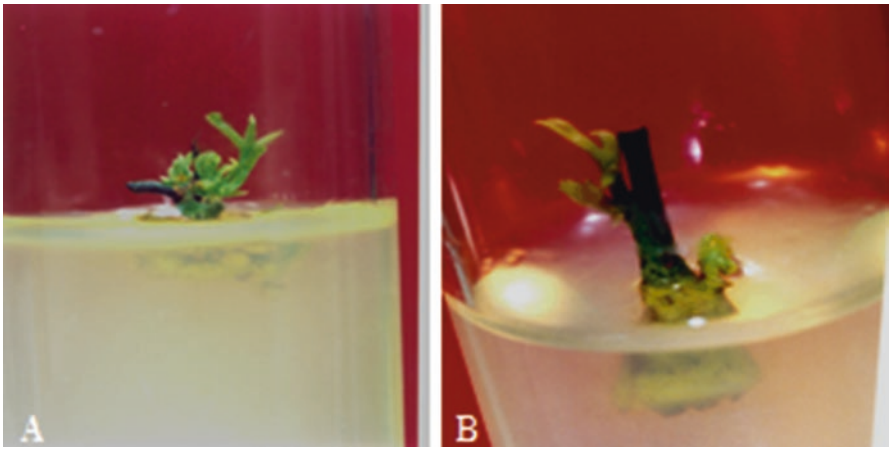


Fig. 12.2 (**a**, **b**) Multiple shoot formation and multiplication on MS1 semisolid medium {MS + BAP (6.6 µl) + GA3 (0.15 µM) + SN (30 µM) + CH (300 mg/l)}

and inefficient as compared to the shoots obtained from nodal segments on L1 semisolid medium (Table 12.3).

12.2.9 Elongation and Multiplication of Shootlets

Shootlets (0.5 cm long) were excised carefully from the explants and transferred to MS1 medium (Table 12.1). Each shootlet (Fig. 12.4a) produced a clump of 3–4 shootlets from the base after 6 weeks (Fig. 12.4b–d). Thus, three- to fourfold shoot

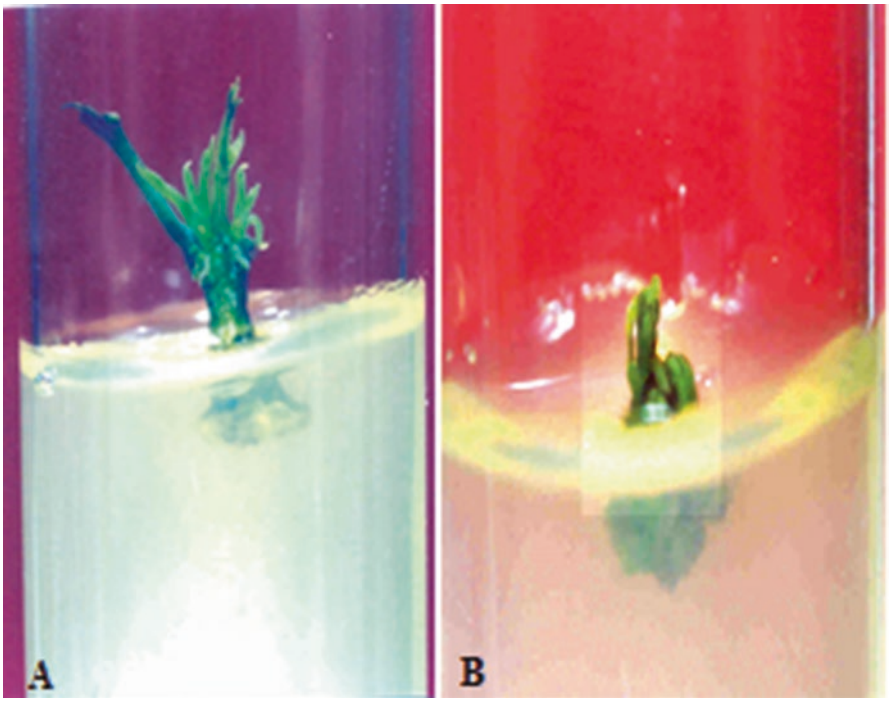


Fig. 12.3 (**a**, **b**) Apical shoot proliferation on L3 semisolid medium {WPM + 2-iP (6.0 μM) + Kn (2.3 μM) + GA3 (0.6 μM) + CH (400 mg/l) + CW (15%)}

multiplication could be achieved on MS1 medium. Yu (1991) also obtained the highest regeneration as well as multiplication rate only when the shootlets were excised from the mother explants. The medium composition used was, however, different [half-strength MS supplemented with BA (4.4 μM) + IAA (1.14 μM) + GA_3 (0.2 μM)]. Initiation and in vitro propagation of mature trees in general are difficult due to various problems which include mainly recalcitrance of the tissue, contamination, and field established.

The method of multiple shoot induction reported by Kantharajah et al. (1989) involved culturing immature embryos, which are difficult to obtain and tedious to culture because of the labor and time involved in isolating the immature embryos. Das et al. (1999) reported multiple shoot formation by direct germination of litchi seeds in MS liquid medium supplemented with 6-benzylaminopurine (20 mg l^{-1}) and supported on a filter paper bridge. In the same investigation they also reported *in planta* treatment of the axillary bud regions of plants germinated and maintained under sterile conditions with 6-benzylaminopurine (100 mg l^{-1}) on alternate days for multiple shoot induction. Both methods of multiple shoot induction were effective for the five genotypes of litchi tested (cv. Deshi, Kasba, Bedana, Purbi, and Shahi). Chandra and Padaria (1999) cultured shoot buds of litchi seedlings cv Seedless on MS medium supplemented with 0.2 mg/l BA +0.1 mg/l IAA + 0.5 mg/l

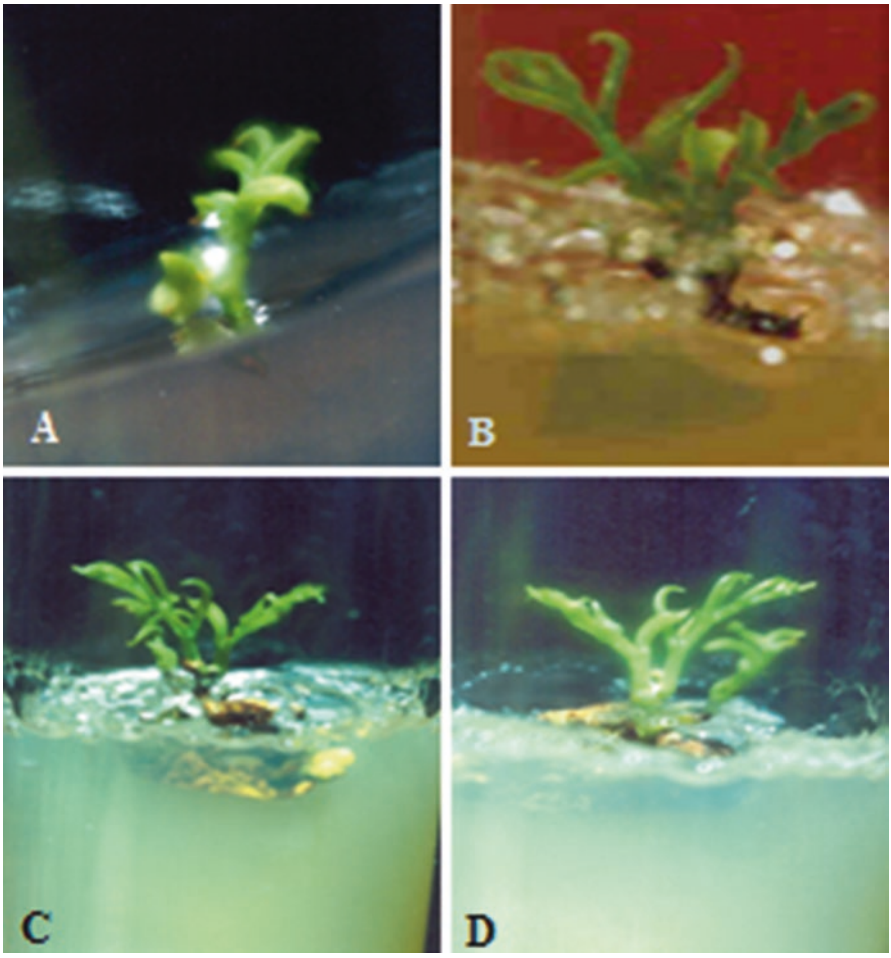


Fig. 12.4 (**a**–**d**) Multiplication and elongation of in vitro shoots on MSI semisolid medium {MS + BAP (6.6 µl) + GA3 (0.15 µM) + SN (30 µM) + CH (300 mg/l)}

GA3 and obtained shoot differentiation and growth. In the present investigation, an alternative method of direct regeneration of litchi plantlets from nodal explants taken from 3–4-year-old plants in the field has been demonstrated for the first time.

The discontinuity of shoot elongation and periodic growth are common problems in newly established woody plant cultures. It usually persists until explants are fully adjusted to the in vitro environment, which may take several subcultures to several years depending on the species, the age, and the source of explants as well as the culture medium (McCown and Sellmer 1987).

12.2.10 Root Initiation and Acclimatization

For rooting, MS and WPM were tested at full, half, one third, and quarter strength, respectively, of the major inorganic salts. All the media, liquid as well as semisolid, were supplemented with IBA at 20 μM and 1 g/l litchi seed powder. Before transfer of the shoots into MS2 semisolid medium, a short-pulse treatment with IBA (100 mM) for 15 min was essential. After the pulse treatment of basal part, the shoots were transferred to MS2 semisolid and liquid medium, respectively (Table 12.1). Rooting was induced directly at the base of the shoots on MS2 semisolid medium which proved to be the best rooting medium, 70% shoots formed root.

On this medium roots appeared after 4 weeks and maximum response was observed after 5–6 weeks. Liquid MS2 medium was equally good as the semisolid MS2 medium in terms of response percentage.

Generally, an auxin is essential for rooting either at a high concentration for a short duration pulse or in optimum concentration in the medium. For shoots obtained from cotyledonary nodes, a pulse treatment with IBA (25 mg/l) for 15 min, followed by implantation on vermiculite, resulted in the establishment of regenerated plantlets (Das et al. 1999). Kantharajah et al. (1989) observed adventitious rooting in 65% of shoot cultures on MS medium supplemented with NAA (0.5 mg/l) and activated charcoal (ca. 1%). Yu (1991) obtained plants with roots, stem, and leaves when the regenerated shoots were grafted on stocks cultured in tubes with a seedling age of 15–20 days. However, in the present study, rooting of the shoots proved particularly difficult as various media and grafting strategies were experimented. Finally 70% of the shoots could be rooted on MS medium supplemented with IBA (20 μM) + litchi seed powder (1 g/l) after a pulse treatment of IBA (100 μM) for 15 min. The alternate approach used in this investigation for root regeneration involved the use of litchi seed powder, which may serve as the natural source of rooting hormones. The use of litchi seed powder to promote establishment of roots is novel and could be used in a commercial venture for clonal propagation from adult trees.

12.2.11 Hardening and Transplantation

Following the protocol described above under the section of culture maintenance, the plants were hardened and successfully transferred to the nursery.

12.3 Factors Affecting Regeneration of Litchi from Nodal Cuttings

There are many potential variables which affect the regeneration from the explants in vitro. These include the physical factors (light, temperature, orientation, and positioning of the explants), media components (hormones, pH of the medium), physiological factors (the age of the donor plant, developmental stage of the

explant), and genotype factors. This information helps in the reproduction of the results independently in other laboratories. In addition, these studies may help in understanding the basic phenomenon of regeneration. The current method of litchi micropropagation described is explant specific or cultivar specific, unlike other explants (seeds, immature embryos, and juvenile leaves) belonging to the same genotypes which were not ever reported to show this kind of behavior and sharing almost common features in their developmental program. Knowledge of the critical factors affecting regeneration in litchi may also help in identification of the factors inherently limiting in other fruit crops and hence may lead to modified protocols suitable for the regeneration of those crop plants as well.

12.3.1 Effect of Media Type on Explant Establishment

In order to standardize the most suitable medium for explant establishment and multiple shoot induction, sterilized explants were inoculated into different media: WPM, MS (Murashige and Skoog 1962), B5 (Gamborg and Shyluk 1981), and 1/2 MS, containing BAP (11.00 μM) + Kn (2.3 μM) + GA3 (0.6 μM) + CH (400 mg/l) + CW (15%). The sucrose concentration was 3% in all the media tested. Percent survival, shoot number, and shoot length per explant were recorded after 2 weeks of incubation.

Nodal segments showed highest response in WPM as compared to MS medium**,** B5 medium, and 1/2 MS media. Cultures grown in WPM gave three shoots/explant, which were significantly higher than other tested media (Table 12.2).

Reducing the salt concentration in MS resulted in poor performance with regard to percent establishment and induction of shoot number as compared to WPM. Some tree species give similar response in all media, while others show preference for a specific medium for explant establishment and growth (McCown and Sellmer 1987). This is especially true for WPM that possesses lower nutrient content compared to other media. Conversely, high medium concentrations have been known to cause anatomical, morphological, and physiological disorders, commonly described as hyperhydration or vitrification (Gaspar et al. 1994). In the present study, WPM gave better results than B5, MS, and 1/2 MS medium. This is contrary to the earlier report by Nandwani (1994) who found best results in MS medium. Reports suggested that in B5, explants do not respond well due to the presence of high ammonium and nitrate content (Constabel 1984), which inhibit percent establishment and shoot multiplication. In the present study, the best results in WPM were probably due to lower concentration of salts and sucrose as compared to B5 and MS. Low concentration of sucrose is reported to reduce browning in *Pseudotsuga menziesii* cultures (Evers 1984), and weaker salt formulations promote axillary bud development in forest trees (McCown and Sellmer 1987).

12.3.2 Effect of Different Cytokinins

To determine the best cytokinin for the production of multiple shoots, preliminary experiments were conducted with different cytokinins (BAP, 2-iP, TDZ, zeatin, and kinetin) at various concentrations, viz., 11 μM, 6 μM, 0.5 μM, 1.0 μM, and 5 μM, respectively. The maximal response was obtained on BAP (53.5%) and 2-iP (50%), while few shoots were induced on kinetin, zeatin, and TDZ (10–15%). Though more shoots were produced on 2-iP or KIN and GA_3 (KIN:GA_3 in combination)-supplemented media than those on BAP, the shoots were thin, slender, and weak on 2-iP or KIN, while those on BAP were stout and robust. Therefore, further studies were performed with BAP as the cytokinin of choice.

Among the different cytokinins tested, BAP was found to be the most effective for regeneration (Table 12.3). The highest number of shoots per nodal segment was obtained on WPM supplemented with 10–11 μM BAP out of several concentrations like 0.6, 8, 11, 15, 25 μM BAP. Use of a lower and higher concentration of BAP resulted in the drastic decline in the number of shoots per explant.

BAP has been commonly used for the induction of micropropagation in tree species. However, comprehensive studies comparing BAP with other cytokinins for multiple shoot production in tree species are well studied. Effectiveness of BAP over other cytokinins for regeneration has been reported in several other systems. The stimulatory effect of BAP on budbreak and multiple shoot formation was reported on various tree spp. like *Anogeissus pendula*, (Joshi et al. 1991), *Withania somnifera* (Sen and Sharma 1991), *Ocimum* spp. (Pattnaik and Chand 1996), *Piper* species (Bhat et al. 1995), and *Vitex negundo*. The synergestic effect of BAP and Kn on shoot budbreak and multiple shoot formation is also well documented in many plant species such as *Dalbergia latifolia* (Raghava Swamy et al. 1992).

12.3.2.1 Effect of Higher Concentration of KIN and GA3

Increasing the level of kinetin in the medium decreased shoot length in all medium concentrations tested for shoot elongation, and a greater shoot length was obtained on kinetin-free MS medium. On the other hand, a cluster of shoots was obtained on WPM + KIN (11.5 μM) + GA_3 (6 μM), but shootlets were hurriedly elongated, chlorotic, and tip burned at a later stage**.** In general, results in this study show that regular strength of WPM in combination with 0.5 mg/l kinetin was the best for shoot induction, while GA_3 at lower concentration (0.2 mg/l) induced elongation which was found very effective with BAP.

According to Lesham et al. (1984), kinetin is needed to induce shoot proliferation, but the supraoptimal concentrations can be toxic and shoots are small with no expanded leaves. These morphological alterations have been reported to be detrimental to plantlet survival. Although the proliferation rate was lower at 0.5 mg/l kinetin in the presence of GA_3 (0.2 mg/l) and BAP (2.5 mg/l) as compared to 5.0 mg/l, it has resulted in better shoot growth, with no vitrification or other morphological alterations. A similar proliferation ratio was achieved with 1 mg/l kinetin and 0.5 mg/l GA3, but root formation was not observed. Kinetin at 0.5 mg/l has also

been recommended for shoot proliferation of other plant species like *Emblica officinalis*.

12.3.2.2 Effect of BAP and 2-iP on Shoot Proliferation and Elongation

Litchi explants receiving BAP and 2-iP showed different developments of new shoots. By day 45 explants propagated in the medium with BAP yielded shoot tips with multiple microshoots ranging from 1 to 4 cm.

Various concentrations of 2-iP (3–10 μM) were tried alone and in combination of BAP for multiple shoot production of litchi from nodal cuttings and apical shoot meristems. Single shoot with swellings at the base of apical shoot meristems was found on optimum concentration of 2-iP (6 μM). Similarly, WPM was found as effective as in the case BAP. 2-iP gave the least number of shoots (only one shoot per explant) with an average length of 1.6 cm. Explants in combination of 2-iP and BAP (1:1) gave no induction of multiple shoots. For nodal segments, the highest frequency (45%) of differentiation was obtained with BAP, while frequency was only 20% with 2-iP for apical shoot meristems. The maximum number of shoot buds (4 per explant) was produced by BAP alone, but shootlet obtained on 2-iP containing medium failed to multiply and elongate further.

In this investigation, BAP was found superior to 2-iP in giving more shoots per explant when same concentrations of the two plant growth regulators were compared. This holds true for all concentrations of plant growth regulators used throughout the investigation and showed the explants specificity also. BAP and 2-iP separately were found to be effective in nodal segments and apical shoot meristems, respectively. The result obtained here is similar to the results from Economou and Spanoudaki (1986).

12.3.3 Effect of Coconut Water and Casein Hydrolysate

Coconut water (15%) and casein hydrolysate (400 mg/l) used singly or in combination were tested for their effect on the multiple shoot induction from the nodal explants. The experimental results are summarized as follows: (1) Buds that exist in the dormant condition into the nodal meristems failed to give the multiple shoots on WPM without coconut milk. (2) When cultured on the medium supplemented with 15% coconut milk (autoclaved) alone, “nodal explants” were found responsive and gave 25% multiple shoot induction frequency. If casein hydrolysate (400 mg/l) was added with coconut milk, the response of the nodal explants and apical shoot meristems increased to 35%. In another series of experiments, the response of the young proliferated multiple shoots obtained from nodal explants amounted to 27.2% when the coconut water alone was supplemented, while it increased up to 53% when the casein hydrolysate was added at the same time.

Coconut water, as in earlier studies (Guha and Maheshware 1964), was found to be the most potent substance for inducing embryoids. To determine the optimal

concentration, it was tested at 5, 10, 15, and 20% levels. The best response was noted at 15% level.

12.3.4 Effect of Silver Nitrate ($AgNO_3$) as an Ethylene Inhibitor

Supplementing the regeneration medium with $AgNO_3$ led to an augment in the multiplication of microshoots. At 3o μg/l, 48% explants responded at an efficiency of 5.84 multiple shoots per explant. Further increase in $AgNO_3$ concentration was accompanied by a reduction in the regeneration response**.**

After multiple shoot formation from the nodal explants of litchi, it was observed that after a certain stage shootlets failed to elongate further due to abscission layer formation. Of the inhibitors of ethylene action used in the regeneration (elongation and multiplication) of litchi, the explants were highly sensitive to silver thiosulfate, while a significant improvement was recorded at low concentration of $AgNO_3$. This differential susceptibility of explants reflects that the $AgNO_3$ may be more beneficial as compared with STS owing to its affectivity and higher mobility in the medium. Nevertheless, the extreme sensitivity of explants proliferating in semisolid and liquid media to silver ion may limit the use of ethylene action inhibitors in litchi tissue culture. Therefore, its temporal use only at the initial stage of litchi tissue culture is recommended only.

Silver ion, potent inhibitor of the physiological action of ethylene, is known to influence the regeneration potential in a variety of plants (Songstad et al. 1988), whereas silver thiosulfate has also been used to enhance regeneration in *Solanum tuberosum* (Hulme et al. 1992).

12.3.5 Source Material and Choice of Explants

In field-grown litchi plants (3–4 years old) established in the nursery, young juvenile branches were the most preferable source of explants. The proper handling of source material is of utmost importance before in vitro culture initiation in the successful establishment and reduction of further contamination, especially in hardwood species. Among different explants (apical shoot meristem, internodal segments, and nodal segments), nodal segment bearing axillary bud was found to be the most responsive for multiple shoot production on optimized culture conditions. In nodal explants and apical shoot meristems, the maximum number of shoots was obtained on BAP, Kn, and GA_3 in combination (3.3 or cluster of shoots/explant), while kinetin and GA_3 separately induced the minimum number of multiple shoots (single shoot per explant). When clonal propagation is a part of the objective, apical shoot meristems and nodal segments consisting buds are generally preferred to initiate in vitro culture (*Vitis* spp.). Plant multiplication through nodal segment/axillary branching is the most reliable micropropagation method in terms of genetic stability of elite selections (George et al. 1993). With woody plants such as avocado, the morphogenetic capacity is generally lower in adult than in juvenile material

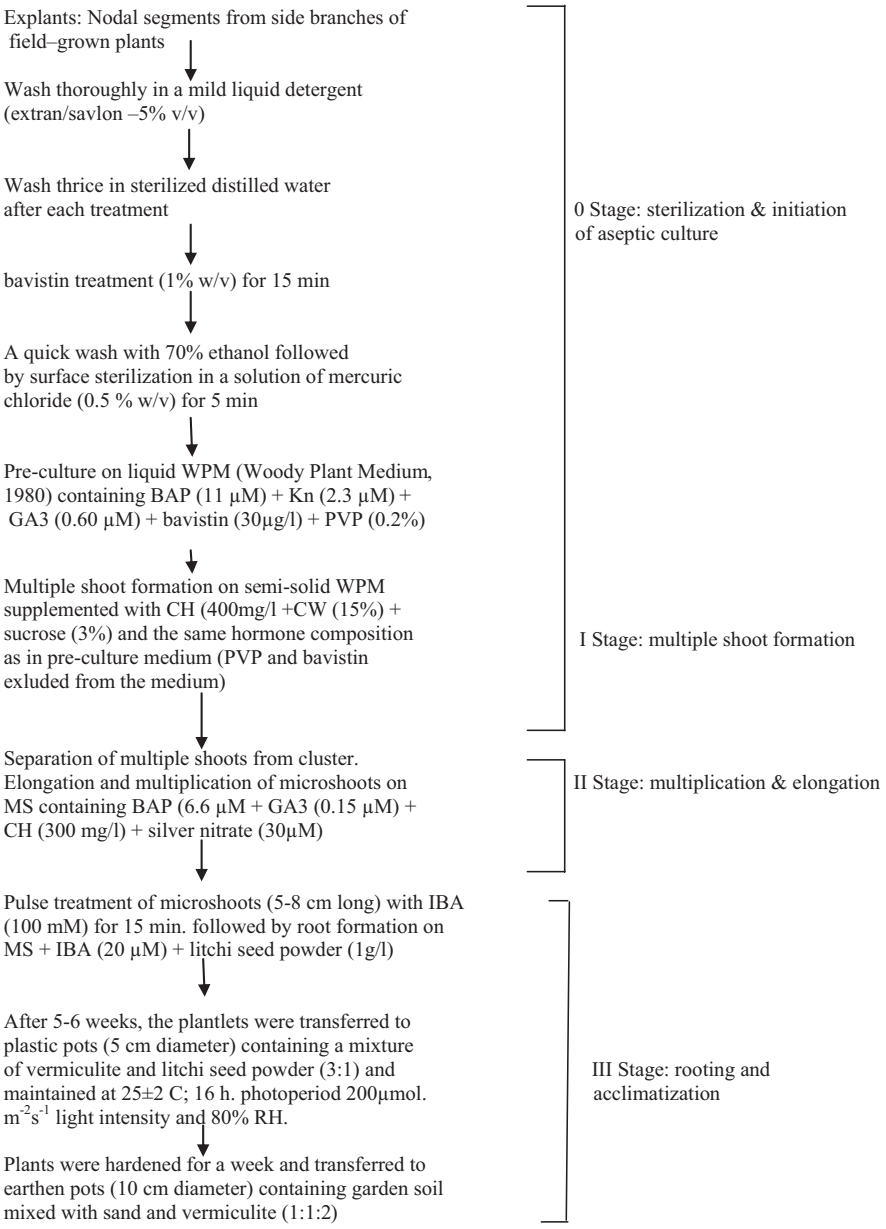
(Pliego-Alfaro and Murashige 1987). Hence, micropropagation is usually developed using juvenile explants, and the results can be used as a guide to propagating material of adult origin.

12.3.6 Genotype Specificity

Of the 18 different genotypes tested for regeneration, only three were capable of producing the frequent and healthy multiple shoots from nodal cuttings. Frequency and efficiency of regeneration varied with different cultivars. Significant differences were observed in the regeneration potential among the various cultivars of litchi which were tested (data not shown). The best response in terms of multiple shoot formation and the number of shoots/nodal explant (4 ± 1 or cluster) was obtained from litchi cultivars Deshi, Purbi, and Kasba. The rest tested cultivars (Table 12.1) produced only one unhealthy shoot/explant which was statistically significantly different from Deshi, Purbi, and Kasba.

It has been shown that regeneration potential is heritable and hence a genetically controlled trait. Therefore, most protocols are found to be very specific.

12.4 Conclusion: Schematic Representation of the Protocol Established for Regeneration from Nodal Explants of Field-Grown Trees of Litchi (*Litchi chinensis* Sonn.)



References

Amin MN, Jaiswal VS (1988) Micropropagation as an aid to rapid cloning of a guava cultivar. Sci Hortic 36:89–95

Bhat SR, Chandel KPS, Malik SR (1995) Plant regeneration from various explants of cultivated Piper species. Plant Cell Rep 14:395–402

Butcher FG (1957) Pollinating insects of lychee blossoms. Proc Fla State Hortic Soc 70:326–28

Chandra R, Padaria JC (1999) Litchi shoot bud culture for micropropagation. J Appl Hort 191:38–40

Chapman KR (1984) Sapindaceae. In: Page PE (ed) Tropical tree fruits for Australia. Queensland Dept. of Primary Industries, Queensland, pp 179–191

Chaturvedi R, Razdan MK, Bhojwani SS (2004) *In vitro* clonal propagation of an adult tree of neem (*Azadirachta indica*) by forced axillary branching. Plant Sci 166:501–506

Constabel F (1984) Callus culture: induction and maintenance. In: Vasil IK (ed) Cell culture and somatic cell genetics of plants, vol 1. Academic, New York, 79: 217–222

Das DK, Prakash NS, Sarin NB (1999) Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.). Plant Cell Rep 18:691–695

Economou AS, Spanoudaki MJ (1986) The influence of cytokinins and Gibberellic acid on gardenia tissue cultures. Sci Hortic 29:155–161

Evers P (1984) Growth and morphogenesis of shoot initials of Douglas fir, Pseudotsuga menzisii (Mirb.) Franco, in vitro 1. Plant nutrition and physical factors. Uitvoerig Verslag 16(1):1–47

Gamborg OL, Shyluk JP (1981) Methodology, nutrition, media, and characteristics of plant cell and tissue culture. In: Thorpe TA (ed) Plant tissue culture methods and application in agriculture. Academic, New York, pp 21–43

Gaspar PE, Reeves DL, Schumacher TE, Fixen PE (1994) Oat cultivar response to potassium chloride on soils testing high in potassium. Agron J 86:255–258.

George PS, Ravishankar GA, Venkataraman LV (1993) Clonal multiplication of *Gardenia jasminoides* Ellis through axillary bud culture. Plant Cell Rep 13:59–62

Goto YB (1960) Lychee and its processing. Pacific Region Food Conf.: 15-23.graftage onto juvenile rootstocks in vitro. Hortscience 22:1321–1324

Guha S, Maheshware SC (1964) *In vitro* production of embryos from anthers of Datura. Nature 204:497

Hamilton RA, Yee W (1970) Lychee cultivars in Hawaii. Proc Trop Am Soc Horticult Sci 14:7–12

Hayes WB (1957) Fruit growing in India (3rd rev ed). Kitabistan, Allahabad, pp 323–332

Hewitt EJ (1996) Sand and water culture methods used in the study of plant nutrition. Technical Communication No. 22. Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Maidstone, Kent, England

Hulme JS, Higgins ES, Shields R (1992) An efficient genotype independent method for regeneration of potato plants from leaf tissue. Plant Cell Tissue Organ Cult 31:161–167

Hutchinson JE (1984) Factors affecting shoot proliferation and root initiation in organ culture of apple 'Northern Spy'. Sci Hortic 22:347–358

Joshi R, Shekhawat NS, Rathore TS (1991) Micropropagation of *Anogeissus pendula* Edgen. – An arid Forest tree. Indian J Exp Biol 29:615–618

Kantharajah AS, McConchie CA, Dodd WA (1989) The possible contribution of tissue culture to the lychee industry. In: Proceedings of the second national lychee seminar, Cairns, pp 59–65

Kumar M, Shiva PN, Muthusamy A, Prasad US, Sarin NB (2004) Problems and perspectives of mass scale production of litchi using in vitro cultures. In: Proceedings of national seminar on recent advances in production and post harvest technology of litchi for Export. BCKV, West Bengal, June 24–26, pp 12–17

Kumar M, Prakash NS, Prasad US, Sarin NB (2006) A novel approach of regeneration from nodal explants of field grown litchi (*Litchi chinensis* Sonn.) fruit trees. J Plant Sci 5(3):321–327

Lesham YY, S Sridara, JE Thompson (1984) Involvement of calcium and calmodulin in membrane deterioration during senescence of pea foliage. Plant Physiol 75:329–335.
Litz RE, Conover RA (1981) Effect of sex type, season and other factors on in vitro establishment and culture of Carica papaya L. explants. J Am Soc Hortic Sci 106:792–794.
Lloyd G, McCown B (1980) Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Proc Int Plant Propagators 30:421–427
McCown BH, Sellmer JC (1987) General media and vessels suitable for woody plant cultures. In: Bonga JM, Durzan DJ (eds) Tissue culture in forestry – general principles and biotechnology, vol 2. Martinus Nijhoff Publ., Dordrecht/Boston, pp 4–6
Menzel CM (1985) Propagation of lychee: a review. Sci Hortic 21:201–215
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco cultures. Physiol Plant 15:473–497
Nandwani D (1994) Clonal propagation of *M. esculenta* (Box- berry) A fruit bearing tree of north-east India. Gartenbauwissenchaft (Horticultural Science) 59(6):264–267
Pandey RM, Sharma HC (1989) The litchi. ICAR, New Delhi, pp 1–79
Pattnaik SK, Chand PK (1996) *In vitro* propagation of the medicinal herbs *Ocimum americanum* L. Syn, *O. canum* Sims (hoary basil) and *Ocimum sanctum* L. (holy basil). Plant Cell Rep 15:846–850
Pliego-Alfaro F, Murashige T (1987) Possible rejuvenation of adult avocado by graftage onto juvenile rootstocks in vitro. Hortscience 22:1321–1324
Raghava Swamy BV, Himabindu K, Lakshmi Sita G (1992) *In vitro* micropropagation of elite rosewood (*Dalbergia latifolia* Roxb.). Plant Cell Rep 11:126–131
Ray PK, Sharma SB (1985) Viability of *Litchi chinensis* seed when stored in air and water. J Agric Sci (Camb), 104(1985): 247–248.
Sarin NB, Prasad US (2003) In vitro regeneration and transformation of litchi (*Litchi chinensis* Sonn.) In: Jaiwal PK, Singh RP (eds) Plant genetic engineering, improvement of fruits, vol 6. Sci Tech Publishing LLC, pp 211–222
Sen J, Sharma AK (1991) Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tissue Organ Cult 26:71–73
Songstad DD, Duncan DR, Widholm JM (1988) Effect of 1-aminocyclopropane-1-carboxylic acid, silver nitrate and norbornadiene on plant regeneration from maize callus cultures. Plant Cell Rep 7:262–265
Sutter EG, Barker PB (1985) In vitro propagation of mature Liquidambar styraciflua. Plant Cell Tissue Organ Cult 5:13–21
Mishra KA, Syamal MM (1984) Litchi cultivation in India. Farmer & Parliament 1915–16 and 27–28
Yu CH, Chen AG (1998) Embryogenic suspension culture and protoplast isolation in Litchee. Chinese J Trop Crops 19:16–20
Yu YB (1991) Study on some factors in tissue culture of lychee (Litchi chinensis). Fujian Agric Sci Technol 5:17–18

Bioactive Compounds and Medicinal Importance of *Litchi chinensis*

13

Devanshi Chandel Upadhyaya
and Chandrama Prakash Upadhyaya

Abstract

Litchi chinensis Sonn. has been widely used as anti-cancer, antiseptic, hypoglycemic, antihyperlipidemic, antiplatelet, antitussive, analgesic, anti-pyretic, diuretic, anti-viral, and hemostatic activities. Several bioactive compounds have been isolated and reported by the scientist using the modern techniques. Several new compounds are being reported. The litchi has now gained a status like other important medicinal plants because of its compound constituent as well as the activities like therapeutic drugs. The ethnomedical use of *L. chinensis* has been recorded in China, India, Vietnam, Indonesia, and Philippines. Phytochemical exploration has established that the key chemical constituents of litchi are flavonoids, sterols, triterpenes, phenolics, and other bioactive compounds. Crude extracts and pure compounds isolated from *L. chinensis* exhibited noteworthy anti-oxidant, anti-cancer, anti-inflammatory, antimicrobial, anti-viral, anti-diabetic, anti-obesity, hepato-protective, and immunomodulatory activities. The civilized cultures are using this since ancient time for the treatment of several diseases such as diabetes, stomach ulcers, cough, flatulence, obesity, testicular swelling, epigastric, and neuralgic pains. However, litchi extracts and fruit juices have been established to be secure at a dosage from the toxicological point of view.

Keywords

Litchi • Hypoglycemic • Anti-cancer • Pharmacological activities

D.C. Upadhyaya • C.P. Upadhyaya (✉)
Department of Biotechnology, Dr Harisingh Gour Central University,
Sagar 470003, Madhya Pradesh, India
e-mail: cpupadhyay@gmail.com

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13.1 Introduction

Litchi (*Litchi chinensis* Sonn.) is a member of family Sapindaceae also known as Chinese cherry, litchi, lychee, water lychee, and mountain lychee and is a non-climacteric subtropical fruit with high commercial value. It has been widely planted in Asia and Africa, especially in China, for many years. Due to the delicious taste and abundant nutrition, litchi fruit is accepted by consumers all over the world (Jiang et al. 2004). Interestingly Menzel and Waite (2005) have reported several synonyms of litchi, viz., *Dimocarpus lichi* Loureiro, *Euphoria didyma* Blanco, *Euphoria litchi* Desf., *Euphoria sinensis* Gmel., *Litchi chinensis* var. *euspontanea* H. H. Hsue, *Litchi litchi* Britt., *Litchi philippinensis* Radlk., *Nephelium chinense*, and *Scytalia litchi* Roxb. Litchi tree is medium-sized, evergreen, round top with grayish smooth stem. Plant height is usually smaller, but it may reach up to 10–15 m high. Leaves are 5–7 cm long, reddish when young, flashy at maturity, pinnate, divided into 4–8 pairs of elliptic or lanceolate, and glabrous leaflets, with bright and shiny green. Inflorescence is 5–30 cm long and has several branched panicles. Flowers are yellowish white in color, with tetramerous calyx, small in size, and functionally male or female without corolla. The fruits are oval and heart shaped covered by a rough leathery ring or pericarp which appears strawberry to red in color with approx. 2.5–3.0 cm in diameter. The edible portion (aril) is white, juicy, and translucent with delicious and sweet flavor. Seed is present inside the aril which varies considerably in size, 1–2 cm in length. The seeds appear brown or reddish brown in color and oblong egg in shape with smooth and glossy surface (Nacif et al. 2001; Menzel 2002). The litchi originated from southern China, particularly the provinces of Kwangtung and Fukien. However, spread of litchi to other parts of the world was relatively slow perhaps due to its climatic and soil requirements and short life span of the seeds. The Myanmar and northeast region of India are supposed to be the gateway for the introduction of litchi in India probably in the eighteenth century (Maity and Mitra 1990). Litchi is typically spread in Southeast Asia especially in Indonesia, Thailand, China, Vietnam, and Philippines. It is now grown as commercial crop in several countries throughout the world especially for its ripened sweet fruits (Gontier et al. 2000; Jiang et al. 2013; Ibrahim and Mohamed 2015).

Geographically the litchi is grown in low elevation and can be grown up to an altitude of 800 m. The plants are deeply rooted in loamy soil rich in organic matter and having pH in the range of 5.2–6.8. In India the litchi production is dominant in northern and northwestern part. It occupies an area of approx. 75,000 hectare in India where a number of varieties ranging from 20 to 35 varieties of litchi have been reported with annual production of 450,000 Mt. Among the northern states, Bihar is the largest producer of litchi in India followed by West Bengal, Western UP, and by Punjab (Saxena et al. 2011) (Table 13.1).

The translucent fleshy aril of the fruit is the edible part having a sweet odor of rose and delicious in taste with enough nutritional value (Bhoopat et al. 2011). The fruits are eaten as such or also used for making juice, vinegar, jelly, and wine (Alves et al. 2011). Litchi fruit and their processed products have been extensively accepted by consumers with traditional great recognition in the global market. The litchi fruit

Table 13.1 Compounds isolated from different parts of the litchi with references

S N	Compound	Plant parts	Molecular formula	Extract/fraction	Reference(s)
Group I: Coumarins					
1	Scopoletin	Seeds	$C_{10}H_8O_4$	n-BuOH	Wang et al. (2011)
Group II: Chromanes					
2	Litchtocotrienol A – G	Leaves	$C_{27}H_{42}O_4$	EtOAc	Lin et al. (2015)
3	Macrolitchtocotrienol A	Leaves	$C_{27}H_{40}O_4$	EtOAc	Lin et al. (2015)
4	Cyclolitchtocotrienol A	Leaves	$C_{27}H_{39}O_3$	EtOAc	Lin et al. (2015)
Group III: Fatty acids					
5	Methyl dihydrosterculate	Seeds	$C_{20}H_{38}O_2$	CHCl3:MeOH (2:1)	Stuart and Buist (2004)
6	2,5-Dihydroxy-hexanoic acid	Seeds	$C_6H_{12}O_4$	n-BuOH	Wang et al. (2011)
7	Litchioside C (3,12-dihydroxy-cis-3,4methylenedodecanoic acid 3O-β-D-glucopyranoside)	Seeds	$C_{19}H_{34}O_9$	EtOAc	Xu et al. (2011a)
Group IV: Lignans					
8	Schizandriside	Leaves	$C_{25}H_{32}O_{10}$	EtOAc	Wen et al. (2014b)
9	Isolariciresinol	Pericarps	$C_{20}H_{24}O_6$	EtOAc	Jiang et al. (2013)
Group V: Phenolics					
	1. Flavon-3-ols:				
10	Epicatechin	Leaves	$C_{15}H_{14}O_6$	EtOAc	Wen et al. (2014a)
	Epicatechin	Pericarps	$C_{15}H_{14}O_6$	Aqueous	Zhou et al. (2011a)
	Epicatechin	Pericarps	$C_{15}H_{14}O_6$	MeOH	Sun et al. (2010)
	Epicatechin	Pericarps	$C_{15}H_{14}O_6$	EtOAc	Zhao et al. (2007)
	Epicatechin	Pericarps	$C_{15}H_{14}O_6$	Aqueous	Sun et al. (2006)
	Epicatechin	Pulp	$C_{15}H_{14}O_6$	Acetone/water (80:20, v/v)	Su et al. (2014)
	Epicatechin	Pulp	$C_{15}H_{14}O_6$	80% Aqueous acetone	Zhang et al. (2013)
	Epicatechin	Seeds	$C_{15}H_{14}O_6$	Pet. ether	Xu et al. (2010a)

(continued)

Table 13.1 (continued)

S N	Compound	Plant parts	Molecular formula	Extract/fraction	Reference(s)
11	Catechin	Pulp	$C_{15}H_{14}O_6$	80% Aqueous acetone	Zhang et al. (2013)
12	Gallocatechin	Pericarps	$C_{15}H_{14}O_7$	Aqueous	Zhou et al. (2011a)
	Gallocatechin	Seeds	$C_{15}H_{14}O_7$	50% EtOH	Prasad et al. (2009)
13	Epiafzelechin	Pericarps	$C_{15}H_{14}O_5$	Aqueous	Zhou et al. (2011a)
14	Epicatechin glucoside	Pericarps	$C_{21}H_{24}O_{11}$	Aqueous	Zhou et al. (2011a)
15	Epicatechin-3-gallate	Seeds	$C_{21}H_{18}O_9$	50% EtOH	Prasad et al. (2009)
16	Epicatechin-(7,8-bc)-4β-(4hydroxyphenyl)-dihydro2(3H)-pyranone	Seeds	$C_{24}H_{20}O_8$	EtOAc	Xu et al. (2010a)
	2. Proanthocyanidins				
17	Proanthocyanidin A1	Seeds	$C_{31}H_{28}O_{12}$	Pet. ether	Xu et al. (2010a)
18	Procyanidin A2	Leaves	$C_{30}H_{24}O_{12}$	- EtOAc	Wen et al. (2014a)
	Procyanidin A2	Pericarps	$C_{30}H_{24}O_{12}$	MeOH	Sun et al. (2010)
	Procyanidin A2	Pericarps	$C_{30}H_{24}O_{12}$	EtOH/water (40:60 v/v)	Liu et al. (2007)
	Procyanidin A2	Pericarps	$C_{30}H_{24}O_{12}$	MeOH:1.5 N HCl (17:3)	Roux et al. (1998)
	Procyanidin A2	Seeds	$C_{30}H_{24}O_{12}$	Pet. ether	Xu et al. (2010a)
19	Proanthocyanidin A6	Seeds	$C_{31}H_{28}O_{12}$	EtOAc	Xu et al. (2010a)
20	Litchitannin A1	Seeds	$C_{45}H_{34}O_{18}$	EtOAc	Xu et al. (2010a)
21	Litchitannin A2	Seeds	$C_{45}H_{34}O_{18}$	EtOAc	Xu et al. (2010a)
22	Aesculitannin A	Seeds	$C_{45}H_{36}O_{18}$	- EtOAc	Xu et al. (2010a)
23	Epicatechin (2β→O→7,4β→8) epiafzelechin-(4α→8) epicatechin	Seeds	$C_{45}H_{36}O_{17}$	EtOAc	Xu et al. (2010a)
24	Epicatechin-(4β→8, 2β → O → 7)-epicatechin(4β → 8)-epicatechin	Pericarps	$C_{45}H_{36}O_{18}$	EtOH/water (40:60 v/v)	Liu et al. (2007)

(continued)

Table 13.1 (continued)

S N	Compound	Plant parts	Molecular formula	Extract/fraction	Reference(s)
25	Propelargonidin	Pulp	$C_{45}H_{36}O_{15}$	50% Aq. MeOH	Lv et al. (2015)
25	Procyanidin	Pulp	$C_{45}H_{36}O_{18}$	50% Aq. MeOH	Lv et al. (2015)
27	Prodelphinidin	Pulp	$C_{45}H_{36}O_{21}$	50% Aq. MeOH	Lv et al. (2015)
28	Proanthocyanidin B2	Leaves	$C_{30}H_{26}O_{12}$	EtOAc	Castellain et al. (2014)
	Proanthocyanidin B2	Pericarps	$C_{30}H_{26}O_{12}$	EtOAc	Zhao et al. (2007)
29	Proanthocyanidin B4	Pericarps	$C_{30}H_{26}O_{12}$	EtOAc	Zhao et al. (2007)
30	Cinnamtannin B1	Leaves	$C_{45}H_{36}O_{18}$	EtOAc	Wen et al. (2015)
	3. Anthocyanins				
31	Cyanidin-3-glucoside	Pericarps	$C_{21}H_{21}O_{11}$	80% Acetone	Li et al. (2012)
32	Cyanidin-3-rutinoside	Pericarps	$C_{27}H_{31}O_{15}$	80% Acetone	Li et al. (2012)
33	Malvidin-3-glucoside	Pericarps	$C_{23}H_{25}O_{12}$	80% Acetone	Li et al. (2012)
	4. Flavones				
34	Luteolin	Leaves	$C_{15}H_{10}O_6$	EtOAc	Wen et al. (2014a)
	5. Flavonols				
35	Kaempferol	Pericarps	$C_{15}H_{10}O_6$	EtOAc	Jiang et al. (2013)
36	Quercetin	Seeds	$C_{15}H_{10}O_7$	50% Aq. EtOH	Jiang et al. (2013)
37	Kaempferol-3-O-β-D--glucoside	Leaves	$C_{21}H_{20}O_{11}$	EtOAc	Wen et al. (2014a)
38	Kaempferol-7-O-β-D--glucopyranoside	Seeds	$C_{21}H_{20}O_{11}$	50% Aq. EtOH	Jiang et al. (2013)
39	Kaempferol-3-O-α-rhamnoside	Leaves	$C_{21}H_{20}O_{10}$	EtOAc	Jiang et al. (2013)
40	Kaempferol-7-Oneohesperidoside (34)	Seeds	$C_{27}H_{30}O_{15}$	n-BuOH	Xu et al. (2011b)
41	Quercetin-3-O-rutinoside	Pulp	$C_{27}H_{30}O_{16}$	Acetone/water (80:20, v/v)	Su et al. (2014)
	Quercetin-3-O-rutinoside	Pulp	$C_{27}H_{30}O_{16}$	80% Aq. acetone	Zhang et al. (2013)
	Quercetin-3-O-rutinoside	Leaves	$C_{27}H_{30}O_{16}$	EtOAc	Wen et al. (2014a)
42	Quercetin-3-O-rutinoside-7-O- α-L-rhamnoside	Pulp	$C_{33}H_{40}O_{20}$	Acetone/water (80:20, v/v)	Su et al. (2014)
43	Tamarixetin 3-O-rutinoside	Seeds	$C_{28}H_{32}O_{16}$	n-BuOH	Xu et al. (2011b)

(continued)

Table 13.1 (continued)

S N	Compound	Plant parts	Molecular formula	Extract/fraction	Reference(s)
	6. Flavanones				
44	Pinocembrin-7-O-(6-Oα-L-rhamnopyranosyl-β-D-glucopyranoside)	Seeds	$C_{27}H_{32}O_{13}$	n-BuOH	Ren et al. (2011)
45	(2R)-Naringenin-7-O-(3-O-αL-rhamnopyranosyl-β-D-glucopyranoside)	Seeds	$C_{27}H_{32}O_{14}$	n-BuOH	Ren et al. (2011)
46	Narirutin	Seeds	$C_{27}H_{32}O_{14}$	50% Aq. EtOH	Wang et al. (2011)
47	Narirutin	Seeds	$C_{27}H_{32}O_{14}$	n-BuOH	Wang et al. (2011)
48	Naringin	Seeds	$C_{27}H_{32}O_{14}$	n-BuOH	Wang et al. (2011)
49	(2R)-Pinocembrin-7neohesperidoside (43)	Seeds	$C_{27}H_{32}O_{13}$	n-BuOH	Wang et al. (2011)
50	Litchioside D	Seeds	$C_{33}H_{42}O_{17}$	n-BuOH	Xu et al. (2011b)
51	Pinocembrin-7-Oneohesperidoside (Onychin)	Seeds	$C_{27}H_{32}O_{13}$	n-BuOH	Xu et al. (2011b)
	7. Flavanonols				
52	Taxifolin-4`-O-β-D-glucopyranoside	Seeds	$C_{21}H_{22}O_{13}$	n-BuOH	Xu et al. (2011b)
	8. Dihydrochalcones				
53	Dihydrocharcone-4`-O-β-D-glucopyranoside	Seeds	$C_{21}H_{24}O_{10}$	n-BuOH	Wang et al. (2011)
54	Phlorizin	Seeds	$C_{21}H_{24}O_{10}$	Pet. ether	Xu et al. (2011b)
	9. Phenolic acids				
55	Gentisic acid	Flowers	C7H6O4	50% Aq. EtOH	Chang et al. (2013)
56	Protocatechuic acid	Seeds	C7H6O4	n-BuOH	Wang et al. (2011)
57	Coumaric acid	Seeds	C9H8O2	n-BuOH	Wang et al. (2011)
58	Caffeic acid	Pulp	C9H8O4	80% Aq. acetone	Zhang et al. (2013)
59	Methyl-3,4-dihydroxybenzoate	Pericarps	$C_8H_8O_4$	80% Aq. acetone	Jiang et al. (2013)
60	Chlorogenic acid	Pulp	$C_{16}H_{18}O_9$	80% Aq. acetone	Zhang et al. (2013)
61	Gallic acid	Seeds	$C_7H_6O_5$	50% EtOH extract	Prasad et al. (2009)

(continued)

Table 13.1 (continued)

S N	Compound	Plant parts	Molecular formula	Extract/fraction	Reference(s)
62	2-(2-Hydroxy-5(methoxycarbonyl) phenoxy)benzoic acid	Pericarps	$C_{15}H_{12}O_6$	EtOAc	Jiang et al. (2013)
63	Butylated hydroxytoluene	Seeds	$C_{14}H_{22}O$	EtOAc	Jiang et al. (2013)
Group VI: Sesquiterpenes					
64	Litchioside A	Seeds	$C_{31}H_{52}O_{10}$	EtOAc	Xu et al. (2010b)
65	Litchioside B	Seeds	$C_{30}H_{44}O_{10}$	EtOAc	Xu et al. (2010b)
66	Pumilaside A	Seeds	$C_{21}H_{38}O_8$	EtOAc	Xu et al. (2010b)
67	Funingensin A	Seeds	$C_{30}H_{44}O_{10}$	EtOAc	Xu et al. (2010b)
68	Pterodontriol-D-6-O-β-D-glucopyranoside	Seeds	$C_{21}H_{38}O_{18}$	n-BuOH	Wang et al. (2011)
Group VII: Sterols and triterpenes					
69	β-Sitosterol	Aerial parts	$C_{29}H_{50}O$	n-BuOH	Malik et al. (2010)
70	Stigmasterol	Pericarps	$C_{29}H_{48}O$	EtOAc	Jiang et al. (2013)
71	Lupeol	Aerial parts	$C_{30}H_{50}O$	EtOAc	Malik et al. (2010)
72	Betulin	Aerial parts	$C_{30}H_{50}O_2$	EtOAc	Malik et al. (2010)
73	Betulinic acid	Aerial parts	$C_{29}H_{48}O_3$	EtOAc	Malik et al. (2010)
74	3-Oxotrirucalla7,24 dien-21-oic acid	Seeds	$C_{30}H_{46}O_3$	EtOAc	Nimmanpipug et al. (2009)
75	Lup-12,20-diene-3-diols	Aerial parts	$C_{30}H_{48}O_2$	EtOAc	Malik et al. (2010)

and its processed seeds have shown to deter the growth of cancerous cells in laboratory conditions (Bhat and Al-daihan 2014). The presence of flavonoids in fruits and seeds is also reported to act as efficient in treatment of breast cancer (Xu et al. 2011b). The fruits and seeds also enclose abundant bioactivities such as anti-cancer, antiplatelet, antibacterial, hypoglycemic, antihyperlipidemic, and anti-viral (Chen et al. 2007; Xu et al. 2011b). The oligonol from litchi also converts the high-molecular-weight proanthocyanidins into low-molecular-weight proanthocyanidins to improve bioavailability (Ogasawara et al. 2009) which is supposed to be an important anti-cancer compound. The litchi fruits are also rich in polyphenol monomer (catechin, epicatechin etc.) and polyphenol dimer (procyanidin B2) As previous quantification of the identical procyanidins were estimated as procyanidin B2 termed as PB2E, the selective ion monitoring (SIM) mode was coined to select the

molecular properties / ions of the isomers from the procyanidins groups in lychee pulp. The United States Food and Drugs Administration Agency (US-FDA) has recognized the importance of litchi and certified as a new safe dietary ingredient. Litchi fruits and its processed products have also been shown to exhibit several other secondary health benefits such as defense against oxidative stress, prevention and management of hyperuricemia, and lessening of fatigue and visceral fat (Sakurai et al. 2008; Kang et al. 2012; Yamanishi et al. 2014). Litchi foodstuffs have also been revealed to restrain inflammatory markers following exercise with athletics (Nishizawa et al. 2011; Lee et al. 2010). There are ample reports where several researchers have reported the medicinal importance of litchi bioactive compounds such as anti-cancer, hepato-protective, anti-oxidant, antiplatelet, anti-viral, antimicrobial, antihyperlipidemic, antimutagenic, anti-pyretic, and anti-inflammatory. These studies have resulted in the isolation of several compounds, viz., anthocyanins, phenolic acids, flavonoids, tannins, sterols, and triterpenes which are listed in Table 13.2.

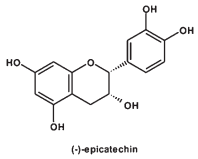
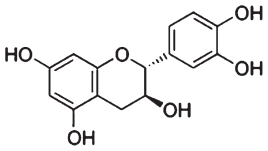
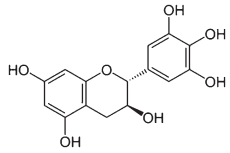
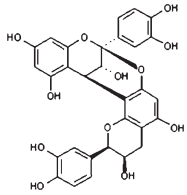
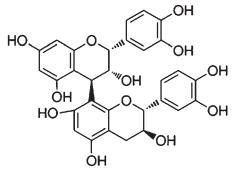
13.2 Bioactive Compounds of Litchi

Anthocyanins, alcohols, alkenes, aldehydes, coumarins, chromanes, esters, fatty acids, flavonoids, lignans, monoterpenes, phenolic acids, proanthocyanidins, sesquiterpenes, sterols, and triterpenes are the major groups of compound identified from *L. chinensis* (Ong and Acree 1998; Lee et al. 2008; Sivakumar et al. 2008; Li 2009; Wu et al. 2009; Wang et al. 2013). Additionally, over 96 other volatile components are reported to be present in almost all cultivars of litchi *which include* geraniol, *cis*-rose oxide, linalool, *β*-citronellol, *α*-terpineol, *p*-cymene, ethanol, 3-methyl-3-buten-1-ol, 1-hexanol, 3-methyl-2-buten-1-ol, (*E*)-2-hexen-1-ol, 1-octen-3-ol, 2-ethyl-1-hexanol, 1-octanol, *p*,*α*-dimethylstyrene, ethyl acetate, and 3-tert-butyl-4-hydroxyanisole. Chyau et al. (2003) have analyzed fresh clear litchi juice using an amberlite XAD-2 column and reported free and glycosidically bound volatile fractions and ester, 14 alcohols, four acids, two each of aldehydes, ketones, and terpenes. In addition, 51 odor-active compounds, eight volatile sulfur components, hydrogen sulfide, diethyl disulfide, dimethyl sulfide, 2-acetyl-2-thiazoline, 2,4-dithiopentane, 2-methyl thiazole, methional, and dimethyl trisulfide were also identified in all samples of litchi fruit (Mahattanatawee et al., 2007). These compounds are divided in seven diverse groups in accordance to their chemicals structures, which includes group I, coumarins; group II, chromanes; group III, fatty acids; group IV, lignans; group V, phenolics; group VI, sesquiterpenes; and group VII, sterols and triterpenes.

13.2.1 Group I: Coumarins

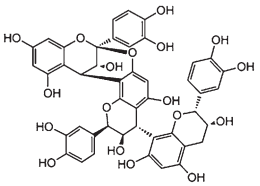
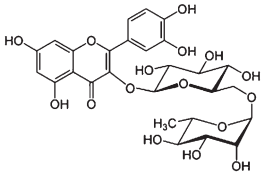
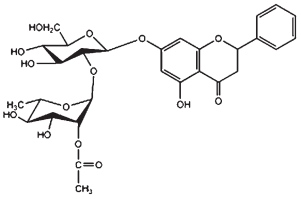
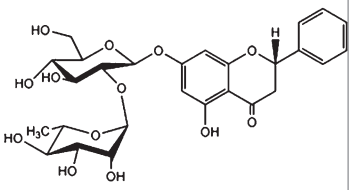
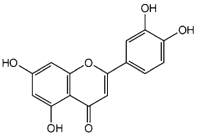
Coumarins or 1,2-benzopyrones are universally present in almost all higher plants originated from the phenylpropanoid pathway. Coumarins are reported to be involved possibly in hormonal regulation, protection against phytopathogens,

Table 13.2 Major bioactive compounds identified from litchi and their pharmacological activities

Compounds	Pharmacological activities	References
Epicatechin  (-)-epicatechin	Anti-oxidant	Wen et al. (2014a)
	Cytotoxicity	Zhou et al. (2011a)
	Anti-viral	Sun et al. (2010)
	Antimicrobial	Zhao et al. (2007), Sun et al. (2006), Su et al. (2014), Zhang et al. (2013) and Xu et al. (2010a)
Catechin 	Anti-oxidant	Zhang et al. (2013)
Gallocatechin 	Anti-oxidant	Zhou et al. (2011a) and Prasad et al. (2009)
Procyanidin A2 	Anti-oxidant	Wen et al. (2014a)
	Inhibition of LP	Sun et al. (2010)
	Cytotoxicity	Liu et al. (2007)
	Anti-viral	Roux et al. (1998)
	Antimicrobial	Xu et al. (2010a)
Proanthocyanidin B2 	Anti-oxidant	Castellain et al. (2014)
	Inhibition of LP	Zhao et al. (2007)

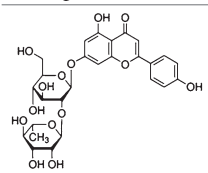
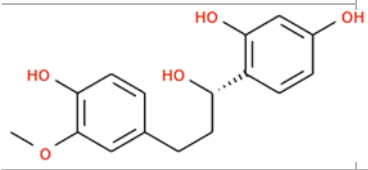
(continued)

Table 13.2 (continued)

Compounds	Pharmacological activities	References
Cinnamtannin B1	Anti-oxidant	Wen et al. (2015)
	Cytotoxicity	
Quercetin-3-*O*-rutinoside	Anti-oxidant	Su et al. (2014)
	Antimicrobial	Zhang et al. (2013)
	α-Glucosidase inhibitory effect	Wen et al. (2014a)
(2*S*)-Pinocembrin-7-*O*-(6-*O*-*α*-L-rhamnopyranosyl-*β*-D-glucopyranoside)	Anti-oxidant	Ren et al. (2011)
	Cytotoxicity	Wang et al. (2011)
	α-Glucosidase inhibitory effect	Xu et al. (2011b)
Pinocembrin-7-*O*-neohesperidoside (Onychin)	Cytotoxicity	Xu et al. (2011b)
	*α*-Glucosidase inhibitory effect	
Luteolin	Anti-oxidant	Wen et al. (2014a)
	Antimicrobial	Wen et al. (2014a)

(continued)

Table 13.2 (continued)

Compounds	Pharmacological activities	References
Kaempferol-7-*O*-neohesperidoside 	Cytotoxicity	Xu et al. (2011b)
Litchioside A and B 	Cytotoxicity	Xu et al. (2010b)
Litchiol A and B	Anti-oxidant	Wang et al. (2011)
Aesculitannin A	Anti-oxidant	Xu et al. (2010a)
	Anti-viral	Xu et al. (2010a)

regulation of oxidative stress, and responses to abiotic stresses. There is only one derivative of coumarins found in the *L. chinensis*. Wang et al. (2011) reported the presence of coumarin derivative called scopoletin in the seed of the litchi. Chemical analysis of the 95% ethanol extract of *Litchi chinensis* seeds led to the separation of scopoletin ($C_{10}H_8O_4$) (Table 13.1). The scopoletin is reported to control the blood pressure. When the blood pressure is high, scopoletin helps to lower it and when it is too low it can help to raise it. Scopoletin also regulates the hormone serotonin, which helps to diminish nervousness and despair and also play as an anti-inflammatory action used to treat bronchial illnesses and asthma. Scopoletin has also been reported to play as bacteriostatic action against various species of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

13.2.2 Group II: Chromanes

Chromane also called benzodihydropyran ($C_9H_{10}O$) is a heterocyclic compound exhibiting structural feature of complex compounds such as of vitamin E (tocotrienols) which is a wonderful anti-oxidant found in vegetable oils and fruits. The hydroxyl chromane ring and a hydrophobic farnesyl side chain are common features of tocotrienols. They can be classified into four different isomers according to number and position of methyl groups in the chromanol ring system (α-, β-, γ-, and δ-tocotrienol). Lin et al. (2015) reported the separation and detection of chromane derivatives from the leaves of *L. chinensis* and its evaluation of anti-cancer activities. The chromane derivatives isolated from the leaves of the litchi by Lin et al. (2015)

are listed in (Table 13.1). Major chromatin derivative isolated from leaves of *L. chinensis* using the combined methanol and ethyl acetate (EtOAc) extracts is litchtocotrienol and nine different chromane derivatives. Based on the position of the chromanol ring, five compounds are named as litchtocotrienols A to G. Another compound reported was macrolitchtocotrienol A and cyclolitchtocotrienol A.

13.2.3 Group III: Fatty Acids

Fatty acids have been reported only from the seed fraction of the litchi using different solvents. Fatty acid composition of litchi seed consisted of palmitic acid (12%), oleic acid (27%), linoleic acid (11%), and CPFAs (42%) as reported by Gaydou et al. (1993). The CPFA portion consisted of dihydrosterculic acid (37%), *cis*-7-8-methylenehexadecanoic acid (4%), *cis*-5,6-methylenetetradecanoic acid (0.4%), and *cis*-3-4-methylenedodecanoic acid (0.1%). Stuart and Buist (2004) reported the presence of fatty acid compound, methyl dihydrosterculate (chemical formula $C_{20}H_{38}O_2$), in the seed extract (using Chl3:MeOh (2:1) fraction. Wang et al. (2011) also reported the presence of the fatty acid derivative 2,5-dihydroxy-hexanoic acid in the seed extract using the extract n-BuOH. Xu et al. (2011a) also reported the presence of another fatty acid derivative litchioside C (3,12-dihydroxy-cis-3,4-methylenedodecanoic acid 3-D-glucopyranoid) in the seed extract using the EtOAc solvent. Details of chemical formula and information related to extraction are mentioned in Table 13.1.

13.2.4 Group IV: Lignans

The lignans are a group of chemical compounds found in plants. Plant lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols (see cinnamic acid), known as monolignols, to a dibenzylbutane skeleton. Schizandriside ($C_{25}H_{32}O_{10}$) is a lignin isolated and identified from leaves of litchi using EtOAc extract as reported by Wen et al. (2014a). Another lignin, isolariciresinol ($C_{20}H_{24}O_6$), was reported by Jiang et al. (2013) using the fruit pericarp extract with EtOAc solvent (Table 13.1).

13.2.5 Group V: Phenolics

Litchi is considered to be one of the most important sources of the phenolics compounds. Almost all the parts of the litchi including the leaves, pericarp, pulp, and seeds are rich source of the different phenolics. Nine major groups of the phenolics have been reported from the litchi which include flavon-3-ols, proanthocynadins, anthocyanins, flavones, flavonols, flavanones, flavanonols, dihydrochalcones, and phenolic acids (Table 13.1).

13.2.5.1 Flavon-3-ols

Epicatechin (C15H14O6) is the major phenolics present in almost all the parts of the litchi. The epicatechin in leaves is reported by Wen et al. (2014b) using the EtOAc extract. Epicatechin is reported from the pericarp of the fruit by several laboratories, viz., Zhou et al. 2011a; Sun et al. 2006, 2010; Zhao et al. 2007, using aqueous MeOH and EtOAc extracts. Epicatechin is also reported in the pulp of the fruit by Su et al. (2014). Zhang et al. (2013) use the water/acetone (20:80 v/v) and/or aqueous extracts. Xu et al. (2010a) also reported the presence of the epicatechin in the seeds of the litchi using the petroleum ether extracts. Other major flavon-3-ol present in the litchi is the catechin ($C_{15}H_{14}O_6$) which was reported by Zhang et al. (2013) in the pulp of the fruit using the 80% aqueous acetone. Gallocatechin ($C_{15}H_{14}O_7$) is another flavon-3-ol reported in the pericarps by Zhou et al. (2011a) using the aqueous extract. Prasad et al. 2009 also reported the gallocatechin and epicatechin-3-gallate ($C_{21}H_{18}O_9$) in the seed of the litchi using 50% EtOH extract. Zhou et al. (2011b) also reported other two flavon-3-ol called epiafzelechin ($C_{15}H_{14}O_5$) and epicatechin glucoside ($C_{21}H2_4O_{11}$) using the aqueous extract. Xu et al. (2010a, b) reported another compound epicatechin-(7,8-*bc*)-4*β*-(4-hydroxyphenyl)-dihydro-2(3H)-pyranone ($C_{24}H_{20}O_8$) in the seeds of litchi using the EtOAc extract.

13.2.5.2 Proanthocynadins

The litchi seeds were used to isolate different groups of proanthocynadins as reported by Xu et al. (2010b). They used different kinds of the extract ranging from petroleum ether to EtOAc. Major compounds present in the seeds of the litchi are reported to be proanthocyanidin ($C_{31}H_{28}O_{12}$), procyanidin A2 ($C_{30}H_{24}O_{12}$), proanthocyanidin A6 ($C_{31}H_{28}O_{12}$), litchitannin A1 ($C_{45}H_{34}O_{18}$), litchitannin A2 ($C_{45}H_{34}O_{18}$), aesculitannin A ($C_{45}H_{36}O_{18}$), and epicatechin-(2*β*→O→7,4*β*→8)-epiafzelechin-(4α→8)-epicatechin ($C_{45}H_{36}O_{17}$).

The pericarp of litchi fruit is also an important source of proanthocyanidins. Zhao et al. (2007) reported two major proanthocyanidin compounds from pericarp and identified as proanthocyanidin B2 ($C_{30}H_{26}O_{12}$) and proanthocyanidin B4 ($C_{30}H_{26}O_{12}$) using the EtOAc extract. Liu et al. (2007) reported another compound from the pericarp known as epicatechin-(4*β*→8,2*β*→*O*→7)-epicatechin-(4*β*→8)-epicatechin with molecular formula $C_{45}H_{36}O_{18}$ using the EtOH/water (40:60 v/v) extract. Sun et al. (2010) also reported another compound from the pericarp, identified as procyanidin A2 ($C_{30}H_{24}O_{12}$), using the MeOH extract.

The pulp of the litchi fruit is also a rich source of proanthocynadins. Lv et al. (2015) reported three major compounds from the pulp using the 50% Aq. MeOH extract and identified the compounds as propelargonidin ($C_{45}H_{36}O_{15}$), procyanidin ($C_{45}H_{36}O_{18}$), and prodelphinidin ($C_{45}H_{36}O_{21}$). Leaves were also reported to contain proanthocynadins. Recently Wen et al. (2014a) identified the compound as procyanidin A2 ($C_{30}H_{24}O_{12}$) using the EtOAc extract. Similarly, Castellain et al. (2014) also reported proanthocyanidin B2 ($C_{30}H_{26}O_{12}$) from leaves using the EtOAc extract.

13.2.5.3 Anthocyanins

Three major anthocyanins are reported from litchi pericarp and identified as cyanidin-3-glucoside ($C_{21}H_{21}O_{11}$), cyanidin-3-rutinoside ($C_{27}H_{31}O_{15}$), and malvidin-3-glucoside ($C_{23}H_{25}O_{12}$) using 80% acetone extract as reported by Li et al. 2012.

13.2.5.4 Flavones

Wen et al. (2014a) reported the isolation of one flavone from the leaves of the litchi and identified as luteolin ($C_{15}H_{10}O_6$) using the EtOAc extract.

13.2.5.5 Flavonols

The flavonols are reported in almost all parts of the litchi including seeds, leaves, pulp, and the pericarp of the litchi. The seeds are rich source of flavonols. Xu et al. (2011b) reported two major compounds from seeds using *n*-BuOH extract and identified as kaempferol-7-*O*-neohesperidoside ($C_{27}H_{30}O_{15}$) and tamarixetin 3-*O*-rutinoside ($C_{28}H_{32}O_{16}$). The presence of flavonols from seed was also reported using 50% Aq. EtOH extract and identified the compound as narcissin ($C_{28}H_{32}O_{16}$) and quercetin ($C_{15}H_{10}O_7$). Kaempferol ($C_{15}H_{10}O_6$) is the major flavonol reported from the pericarp of the litchi fruit by Jiang et al. (2013) using the EtOAc. Kaempferol-3-*O*-*β*-D-glucoside ($C_{21}H_{20}O_{11}$) and kaempferol-3-*O*-*α*-rhamnoside ($C_{21}H_{20}O_{10}$) were isolated from the leaf using the EtOAc extract as reported by Wen et al. (2014a). Recently Su et al. (2014) reported the isolation and identification of two major flavonols from the pulp of the fruit reported as quercetin-3-*O*-rutinoside ($C_{27}H_{30}O_{16}$) and quercetin-3-*O*-rutinoside-7-*O*- *α*-L-rhamnoside using the acetone/water (80:20, v/v) extract.

13.2.5.6 Flavanones

Litchi seeds are the major source of flavanones. Several research groups have identified different flavanones from the seeds. Ren et al. (2011) identified two flavanones from the seeds identified as (2*S*)-pinocembrin-7-*O*-(6-*O*-*α*-L-rhamnopyranosyl-*β*-D-glucopyranoside) ($C_{27}H_{32}O_{13}$) using the *n*-BuOH. Later several complex flavanones were also identified from the litchi seeds and identified as pinocembrin-7-*O*-[(2″,6″-di-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside] ($C_{33}H_{42}O_{17}$), pinocembrin-7-*O*-[(6″-*O*-*β*- *D*-glucopyranoside)-*β*-D-glucopyranoside ($C_{27}H_{32}O_{14}$), (2*S*)-pinocembrin-7-*O*-(6″-*O*- *α*-L-arabinosyl-*β*-*D*-glucopyranoside) ($C_{26}H_{30}O_{13}$), pinocembrin-7-*O*-glucoside ($C_{21}H_{22}O_8$), onychin ($C_{27}H_{32}O_{13}$), and narirutin ($C_{27}H_{32}O_{14}$) using 50% Aq. EtOH extract. Wang et al. (2011) also reported the extraction and identification of narirutin ($C_{27}H_{32}O_{14}$) and naringin ($C_{27}H_{32}O_{14}$), from the seeds of litchi using the *n*-BuOH as extract.

13.2.5.7 Flavanonols

Xu et al. (2011b) reported the extraction and identification of one flavanonol as taxifolin-4`-*O*-*β*-D-glucopyranoside ($C_{21}H_{22}O_{13}$) from the seed of litchi using the *n*-BuOH extract.

13.2.5.8 Dihydrochalcones

Wang et al. (2011) used the seeds using the *n*-BuOH extract and identified the dihydrocharcone-4′-*O*-*β*-D-glucopyranoside ($C_{21}H_{24}O_{10}$). Xu et al. (2011b) also identified one chalcone hydrate from the seed of litchi fruit known as phlorizin ($C_{21}H_{24}O_{10}$) using the petroleum ether as the solvent for extract.

13.2.5.9 Phenolic Acids

The phenolic acids have been identified from the seeds, pulp, pericarp, and also from the flower part of the litchi. Wang et al. (2011) identified two phenolic acids known as protocatechuic acid ($C_7H_6O_4$) and coumaric acid ($C_9H_8O_2$) from the seed portion of litchi using the *n*-BuOH as extract solvent. Gallic acid ($C_7H_6O_5$) (with 50% EtOH extract, Prasad et al., 2009) and butylated hydroxytoluene ($C_{14}H_{22}O$) (EtOAc extract, Jiang et al., 2013) were also extracted and identified from the seed of the litchi fruit.

13.2.6 Group VI: Sesquiterpenes

Almost all the sesquiterpenes have been extracted from the seeds of the litchi fruit. Xu et al. (2010b) have identified several terpenes from the seeds using the EtOAc extract. These include litchioside A ($C_{31}H_{52}O_{10}$), litchioside B ($C_{30}H_{44}O_{10}$), pumilaside A ($C_{21}H_{38}O_8$), and funingensin A ($C_{30}H_{44}O_{10}$). Wang et al. (2011) have also reported the extraction and identification of terpenes known as pterodontriol-D-6-*O*-*β*-D-glucopyranoside ($C_{21}H_{38}O_{18}$) from the seeds of litchi using the *n*-BuOH extract (Table 13.1).

13.2.7 Group VII: Sterols and Triterpenes

Litchiol A ($C_{21}H_{32}O_{10}$) and litchiol B ($C_9H_{12}O_6$) were reported by Wang et al. (2011) as the sterols from the seeds of the litchi fruit using the *n*-BuOH extract. Stigmasterol ($C_{29}H_{48}O$) was reported by Jiang et al. (2013) from the pericarp of the fruit with the EtOAc extract. Nimmanpipug et al. (2009) from a laboratory in Thailand reported the extraction and identification of the triterpenes known as 3-oxotrirucalla-7,24-dien-21-oic acid ($C_{30}H_{46}O_3$) from the seeds using the EtOAc extract. Additional details are provided in Table 13.1.

13.3 Litchi as a Traditional Medicine

Litchi fruits are balanced in the nutrition composed of good amounts of dietary fiber, vitamins, anti-oxidants, and about 60–80 calories per 100 g fruit which is better in comparison to that in the table grapes. The litchi fruit does not contain saturated fats or cholesterol and is also being used as traditional medicine since long time where people used the tree bark, flowers, and root decoction to treat the ailments of

the throat, coughing, tumors, gland enlargements, and several other diseases (Perry and Metzger 1980; Pandey and Sharma 1989; Cohen and Dubois 2010). Different parts of litchi have been found to be valuable for improvement of health due to its various nutritious compounds present in it such as amino acids, dietary fibers, linoleic acid, trace elements, vitamins, and added unsaturated fatty acids; hence it is also considered as a functional food by several scientists and agencies of the world (Wills et al. 1986; Wall 2006; USDA 2012). Ahmad and Sharma (2001) have reported the use of litchi seeds to be useful in treatments of intestinal troubles, ulcers, hernia, lumbago, neuronal disorders, and arthritis. The fruit peel and bark are boiled and taken as tea which is reported to overcome diarrhea and pox eruptions (Li 2009; Lim 2013). Moreover, litchi leaves are used for making moisturizing gel for treatment of skin ailments (Pandey and Sharma 1989). Leaf paste and extract are used for the management of ulcer, stomachache, flatulence, heat stroke, and detoxification (Wen et al. 2014b). The external coat of litchi fruit and leaves has been used to treat poisonous insect bites (Vardhana 2008). Boiled extracts of litchi fruits are used as a medicine for diarrhea, stomach ulcers, cough, diabetes, swelling and obesity, dyspepsia, diuretic, digestive, carminative, anti-febrile, and dysentery and also to kill intestinal worms (Morton 1987; Sayre 2001; Liu et al. 2007; Obrosova et al. 2010; Ahmad et al. 2012; Castellain et al. 2014). Powder form of litchi seeds is used as pain reliever and congealing cold stagnation. Yan et al. (1999) have reported the use of litchi seeds in management of premenstrual and postpartum abdominal pains. Lin et al. (2013) reported the use of mixture of litchi seeds, cumin, and peel of litchi leaf in relieving the pain of hernia or testicular swelling of Chinese tribal populations.

Parts of litchi fruit and seed have been used as therapeutic tablet to treat different kinds of diabetes, the pregnancy diabetes in particular. The blend of flower is used as a drink for joy or refreshment in Taiwan. The large populations in Vietnam have been using litchi to treat stomachache and the pain in small intestine (Yang et al. 2014). The litchi flesh is also being used since ancient time by Vietnamese to avert tiredness and to treat neck pain. In Taiwan and Vietnam, the fruit pulp is very popular among the Vietnamese and Taiwan population where they use it as an excellent thirst quencher and tonic for healthy liver and heart (Bhoopat et al. 2011; Bhalla-Sarin et al. 2003). Litchi parts have been used to heal the wounds in traditional medicine of Asia and Pacific region (Wiart 2006). The litchi seed powder is being used in Indian population as tea which is good to alleviate intestinal troubles and to reduce neuralgic pain and nerve irritation (Perry and Metzger 1980; Li and Jiang 2007; Miller 2011; Lim 2013). In Ayurveda, which is the ancient method of natural medicine, different parts of litchi fruit, leaf, and inflorescence have been used for treatment of several diseases, particularly in curing of digestive, excretory, and reproductive system disorders (Wang et al. 2011; Xu et al. 2011b; Lin et al. 2013). The seeds macerated in alcohol are utilized to treat intestinal problems in Indian and Chinese traditions. The seed blend is in use as cough therapy in Palau, and the Malays use root decoction for treating fever and the bark for severe tongue diseases (Perry and Metzger 1980). The fatty acids of litchi seed have potential significance in the industry of lubricants, inks, detergents, and cosmetics. The litchi fruit is also

processed into healthy juice, ice cream, added yogurt, pickles, and colored wine in South Asian continents (Gontier et al. 2000).

13.4 Biological Activities of Litchi

13.4.1 Anti-cancer Activities

It is now well established that litchi-medicated serum (water extract and granules) can considerably restrain the cell augmentation of S180 sarcoma and EAC of mice in vivo and in vitro, as well as the HepG2 human liver cancer, inducing cell apoptosis (Lin et al., 2008). It was also reported by Hsu et al. (2012) that the polyphenol-rich litchi extract can considerably persuade apoptotic cell death in a dose-dependent mode and seize cell cycle in G2/M in colorectal carcinoma (SW480 and Colo320DM) cells performing as latent chemoprotective compound for colorectal malignancy. Lin et al. (2013) published data on the use of polyphenol-rich litchi extract on in vitro cytotoxic actions toward A549, Colo 320DM, C33-A, ES-2, MDA-MB-231, NCI-H661 SW480, and SCC-25 with IC50 values of 22.49, 23.91, 24.45, 26.33, 36.80, 43.70, 45.46, and 52.47 μg/mL, respectively. It was also recognized that treatment with polyphenol-rich litchi extract could control proliferation in diverse cancerous cells and stimulate cell cycle arrest with apoptosis in CRC cells. Zhang et al. (2012) reported that water extract of litchi seeds could inhibit the effect of malignancy in CNE2 under in vitro conditions. This inhibition ratio was found to be 89.03% at 50 μg/mL and 98.54% at 100 μg/mL after 48 h incubation under the in vitro condition at a constant temperature.

Wang et al. (2006) reported that litchi fruit pericarp (LFP) water-soluble crude ethanolic extract (CEE) had controlled dose- and time-dependent anti-cancer activity against MCF-7 (human breast adenocarcinoma) and MDA-MB-231(human MDA-MB-231 breast carcinoma) cell lines with IC50 value of 80 μg/mL. However, they considerably repressed colony formation and BrdU (5- bromo-2-deoxyuridine) inclusion of human breast cancer cells as seen under in vitro condition using MTT cell survival assay. The inhibitory effect of water-soluble crude ethanolic extract on MCF-7 cells was again established using BrdU inclusion into the unprocessed and treated (100 μg/ml) breast cancer cells under in vitro conditions. BrdU-labeled cells in water-soluble CEE-treated cells were 15.20 ± 1.30%, and lower than that in untreated controls (33.50 ± 2.18%), a statistical significance was found ($P < 0.05$). They also investigated the anti-cancer system of LFP extract by oligonucleotide microarray and found that nontoxic dose of LFP water-soluble CEE altered the gene expression profile of cancer cells by upregulation of 41 genes (1.22%) and downregulation of 129 genes (3.84%), implicated in a variety of biological functions together with cell cycle regulation and cell proliferation, apoptosis, signal transduction, and transcriptional regulation. Diversity of fold regulation varied broadly with ADPRTL1 exhibiting the utmost upregulation up to 29.25-fold and RHAMM exhibiting the utmost downregulation up to 15.48-folds.

In vitro cytotoxic activity determined by MTT colorimetric assay was reported by Xu et al., (2010b) using the bioactive compounds of litchi such as litchioside A, litchioside B, pumilaside A, and funingensin A on some of the special cell lines such as A549 (human lung cancer), LAC (human pulmonary carcinoma cell), HeLa (human cervical carcinoma), and HepG2 (human hepatoma) cell lines. Pumilaside A exhibited notable activity toward all the tested cell lines with IC50 values ranging from 0.012 to 6.29 μM, which were more effective than admycin (IC50 15.2–79.5 μM). Furthermore, funingensin A exhibited modest activity toward HepG2 cells with IC50 value of 39.3 μM. However, litchiosides A and B were inactive against all the tested cell lines (IC50 > 100 μM). The cytotoxic activities of (−)-epicatechin, proanthocyanidin B2, proanthocyanidin B4, and the ethyl acetate fraction were also evaluated with MCF-7 and HELF (human embryonic lung fibroblast) cancer cell lines. Proanthocyanidin B4 and ethyl acetate fraction showed stronger inhibitory effects on HELF than MCF-7. The epicatechin and proanthocyanidin B2 had inferior cytotoxicities to MCF-7 and HELF than paclitaxel (Zhao et al. 2007). Epicatechin, proanthocyanidin B2, proanthocyanidin B4, and the ethyl acetate fraction from litchi pericarp tissues might play a protective role in preventing breast cancer.

Another important bioactive compound of litchi named kaempferol also showed noteworthy cytotoxic activity toward A549, LAC, HepG2, and HeLa cell lines with IC50 values of 0.53, 7.93, 0.020, and 0.051 μM, respectively (Xu et al. 2011b). Procyanidin A2 exhibited powerful anti-cancer activities against HepG2 and HeLa with stronger inhibition reaching 81.57 and 82.77% at 200 μg/mL, respectively. However, it had poor activities toward A549 and MCF-7 cancer cells (Wen et al. 2014a). Just only in 2015, the same group (Wen et al.) again published data showing bioactive compound cinnamtannin B1 with strong antiproliferative effects against HepG2 and SiHa cell lines. In the case of the HepG2 cell line, cell cycle arrest and apoptosis induction were the underlying anti-cancer mechanisms of cinnamtannin B1. The laboratory of Prasad et al. (2009) also reported litchi seed extracts showing inhibitory activity of tyrosinase in a dose-dependent manner. The ethanol extract (50%) showed the highest anti-tyrosinase activity at 100 μg/mL compared with the other extracts. In addition, epicatechin, epicatechin-3-gallate, proanthocyanidin B2, and gallic acid identified in litchi seed extracts proved to be efficient inhibitors of tyrosinase action.

Huang et al. (2014b) carried out experiments with the polysaccharides of fresh litchi pulp and polysaccharides of dried litchi pulp for the inhibition of in vitro proliferation of HepG2, HeLa, and A549 cancer cell lines in dose-reliance method. They reported that both kinds of pulp exhibited maximum antiproliferative actions toward HepG2, HeLa, and A549 cells with % inhibition toward the three tested cell lines ranged from 3.11 to 41.37, 4.61 to 28.04, and 2.56 to 27.17%, respectively. The experiential superior action of pulp extract could be credited to its higher percentage of galactomannan and uronic acid. The anti-tumor cell line activity of crude polysaccharides with free protein was also determined. The results showed that polysaccharides without free protein showed better anti-tumor cell line activities of litchi pulp, which implied that the potential active component was the

polysaccharides. The bioactivity of polysaccharides is closely related with their chemical composition and structure. Litchi pulp, which had higher uronic acid, showed stronger anti-tumor cell line activity in vitro than litchi pulp fraction in this study. It was previously reported that polysaccharides rich in uronic acid exhibited high biological activities because uronic acid residues may alter the properties of polysaccharides and modify their solubility.

13.4.2 Anti-oxidant Activities

The reactive oxygen species (ROS) signify direct or indirect aspect for expansion and succession of several human diseases, including physiological, cardiovascular disease, neurodegenerative, and different types of cancer (Kasote et al. 2013, 2015). Nutritional supplements of anti-oxidants or enhancing the endogenous anti-oxidant defenses of the body is a potential way of combating the adverse effects of ROS-induced oxidative damage. Natural anti-oxidants have achieved a broad acceptance among the huge population due to their accessibility and secure edible confines. ROS-induced DNA damage leads to embryonic, somatic mutations and organ malignancies. Microelement and macromolecule binding sites in the cells and tissues provide fundamental sites for the free radical synthesis at the cellular level. The metal ion has capacity to inhibit the free radicals by chelation reaction with the help of anti-oxidants such as flavonoids, tannins, phenolic acids, and many other organic elements taken in due course of nutrition (Chevion 1988; Sies 1997).

Prasad et al. (2009) also reported the anti-oxidant capacity of litchi seed extract by the phosphomolybdenum technique. High absorbance value of the sample indicates high anti-oxidant activity. In view of these results, all the extracts, except water extract, showed a significant total anti-oxidant activity and were concentration dependent. Total anti-oxidant activity of 50% EE, at 100 g/ml, was 0.82 ± 0.02, while that of BHT was 0.76. The anti-oxidant activities of all the extracts, except WE, were higher than those of BHT at 75–100 g/ml. This anti-oxidant capacity of extracts from the litchi seeds may be credited to their chemical composition and phenolic acid content. Lipid peroxidation inhibitory activity is an oxidative alteration of polyunsaturated fatty acids (in the cellular membranes) which generates a number of free radicals. The level of TBA-reactive substances and products of lipid peroxidation is often used to assess the extent of oxidation occurring in biological system. TBA reacts with malondialdehyde to form a pink chromogen which can be detected spectrophotometrically. The degree of discoloration indicates the inhibitory activity of the anti-oxidants which could be determined by measuring a decrease in absorbance at 532 nm. To assess the anti-oxidant activity of litchi seed extracts against lipid peroxidation, a liposome model system was used where the inhibitory activity of lipid peroxidation by all the extracts from litchi seed, except water extract, was remarkable and comparable to that of BHT. The activities of all the extracts were dose dependent. At 100 g/ml, the inhibitory activities of lipid peroxidation were 61.7 ± 0.4, 61.7 ± 2, 60 ± 0.8, 52 ± 0.7, 50 ± 2.9, and 57.6 ± 1.4 for ethanol extract, 50% ethanol + methanol extract, 50% methanol + water extract,

respectively. However, no significant differences (P < 0.05) were observed in any extracts tested excluding water extract.

Analyses of reducing power and scavenging behavior of DPPH (1,1-diphenyl-2-picrylhydrazyl), hydroxyl (•OH), and superoxide radicals ($O2^{\bullet-}$) proved that epicatechin extracted from litchi showed stronger reducing power and radical scavenging activities than procyanidin A2 (a bioactive compound of litchi) compared to vitamin C which was used as positive control in the experiment (Sun et al. 2010). The water-soluble polysaccharide of litchi fruit pericarp fractions extracted from pulp of litchi was also evaluated for their anti-oxidant activities. The results indicated that litchi fruit pericarp fractions possessed better scavenging effect of superoxide and hydroxyl radicals and also enhanced reducing power. (Kong et al. 2010). These results also showed that the litchi pericarp pulp has the better portion of several anti-oxidants. The litchi fruit was found to exhibit noteworthy anti-oxidant and radioprotective role as the litchi juice induced significant protection to pBR322 plasmid DNA and *Escherichia coli* cells from gamma radiation-induced damage as reported by Saxena et al. (2011). They also elucidated the anti-oxidant and radioprotective properties of the litchi fruit var. "Shahi" and "China" from India and the effect of radiation processing on these properties. They found that in nonirradiated "China" fruit juice (effective dilution in the reaction mixture, 1:20), the DPPH radical scavenging activity was found to be 85% on day zero which remained almost constant until 10 days of storage. This was found to be equivalent to 2 mM of BHT (butylated hydroxytoluene). For the "China" variety, the fruits treated with 0.5 kG dose of gamma radiation, the activities were found to be 85% on day zero. An almost constant radical scavenging activity was observed until 20 days of storage. A significant decrease to 73% was observed in the 28 days stored sample. Concomitant with "Shahi," the radiation treatment was not found to alter the anti-oxidant property in "China" fruits as well. The difference in the DPPH radical scavenging activity (%) shown by both of these varieties "Shahi" and "China" was found to be statistically significant (P 6 0.05). Interestingly the "Shahi" fruits were found to have comparatively high DPPH scavenging activity than "China," whereas the vitamin C content was significantly lesser than "China." The findings indicate that the vitamin C level in a fruit is not only the factor responsible for providing anti-oxidant potential but other phytochemicals also contributes significantly. The two bioactive compounds isolated from litchi flower named epicatechin and procyanidin A2 had extraordinary activities in the inhibition of Cu^{2+}-induced human LDL oxidation with Δt lag values 138.52 and 94.73 min, respectively. Moreover, the epicatechin showed better capability for delaying Cu^{2+}-induced human LDL oxidation than the procyanidin A2.

Xu et al. (2010a) have reported the anti-oxidant activity of the different litchi extract containing epicatechin, litchitannin A1, litchitannin A2, procyanidin A2, and procyanidin A6 and reported strong anti-oxidant activity than *L*-ascorbic acid with FRAP (ferric reducing anti-oxidant power) values of 3.71–24.18 mmol/g and IC50 values of 5.25–20.07 μM toward DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals. The epicatechin and proanthocyanidin A6 exhibited better free radical scavenging effects with IC50 values of 1.75 and 1.65 μg/mL, respectively. Lv et al. (2014) reported extraction of litchi fruit from three different litchi cultivars, viz.,

Hemaoli, Feizixiao, and Lanzhu, and analyzed the anti-oxidant activity using DPPH and ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) free radical scavenging assays. The pulp extract of Hemaoli showed the highest anti-oxidant activity based on both DPPH (IC50 2.26 μg/mL) and ABTS (IC50 2.22 μg/mL) radical scavenging data, followed by Feizixiao cultivar (IC50 3.98 μg/mL for DPPH assay and 4.38 μg/mL for ABTS assay), while Lanzhu cultivar showed fairly lower activity. The EtOAc portion of fruit pericarp showed better activity than ascorbic acid, as assessed by ABTS (IC50 7.137 μg/mL), DPPH (IC50 2.288 μg/mL), and FRAP (EC1mMFeSO4 8013.183 μg/mL) assays (Kanlayavattanakul et al. 2012). Anti-oxidant activities of compounds 2*α*,3*α*-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4*β*-8-catechin),2*α*,3*α*-epoxy-5,7,3′,4′- tetrahydroxyflavan-(4*β*-8-epicatechin), 2*β*,3*β*-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4*α*-8-epicatechin), narirutin, naringin, protocatechuic acid, coumaric acid, scopoletin, pterodontriol-D-6-*O*-*β*-D glucopyranoside, 2,5-dihydroxy-hexanoic acid, litchiol A, and litchiol B were determined by DPPH and TEAC assays. 2*α*,3*α*-Epoxy-5,7,3′,4′-tetrahydroxyflavan-(4*β*-8-catechin) and 2*α*,3*α*-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4*β*-8-epicatechin) showed high anti-oxidant capacities with TEAC values 2.64 and 4.16 μM Trolox/*μ*M, respectively, while, 2*β*,3*β*-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4*α*-8-epicatechin), dihydrocharcone-4′-*O*-*β*-D-glucopyranoside, protocatechuic acid, coumaric acid, and scopoletin showed moderate capacities with TEAC (trolox equivalent anti-oxidant capacity) values ranging from 0.26 to 1.16 μM Trolox/μM. The rest of compounds showed weak or even no anti-oxidant capacities (TEAC values 0.15 *μ*M Trolox/μM) (Wang et al. 2011). Free radical scavenging activities of kaempferol, methyl-3,4-dihydroxybenzoate, 2-(2-hydroxy-5-(methoxycarbonyl)phenoxy)benzoic acid, isolariciresinol, and stigmasterol isolated from litchi pericarp were evaluated in comparison with BHT (butylated hydroxytoluene) using a DPPH assay. Methyl-3,4-dihydroxybenzoate and 2-(2-hydroxy-5-(methoxycarbonyl)phenoxy) benzoic acid showed free radical scavenging effects better than BHT (Jiang et al. 2013). Moreover, epicatechin, proanthocyanidin A6, luteolin, and quercetin-3-*O*-rutinoside showed stronger anti-oxidant activities than BHT (Wen et al. 2014b). Schizandriside, litchiol B, sesquipinsapol B, and sesquimarocanol B isolated from litchi leaf possessed stronger oxygen radical absorbance capacity (ORAC) than quercetin (ORAC values 29.79 μM Trolox/μM) with their ORAC values ranging from 11.25 to 15.36 μM Trolox/μM. In addition, other compounds exhibited remarkably stronger DPPH radical scavenging activity than BHT (IC50 value 38.66 μM) with their IC50 values ranging from 13.21 to 29.68 μM. Intercellular and intracellular anti-oxidant activities of cinnamtannin B1, secoisolariciresinol-9`-*O*-*β*-D-xyloside, and 4,7,7′,8′,9,9′-hexahydroxy-3,3′- dimethoxy-8,4′-oxyneolignan were also evaluated and found that the cinnamtannin B1 showed stronger intercellular and intracellular anti-oxidant activities than secoisolariciresinol-9′-*O*-*β*-D-xyloside and 4,7,7′,8′,9,9′-hexahydroxy-3,3′-dimethoxy-8,4′-oxyneolignan. Recently, Wen et al. (2015) reported that the intracellular activity of cinnamtannin B1 was also linked to the upregulation of major endogenous anti-oxidant enzyme activities such as superoxide dismutase, catalase, and glutathione peroxidase, and hence the

inhibition of ROS generation was also observed as confirmed by spectrophotometric assay.

13.4.3 Antimicrobial and Anti-viral Activities

Antimicrobial activities of the litchi extract aqueous solution containing higher amount of protein were done to analyze the moderate growth inhibition against five microbial strains, viz., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacilllus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Bhat and Al-daihan, 2014). Four of the five selected strains were found sensitive to the protein extracts of *L. chinensis* and *N. lappaceum* seeds. The seed extracts inhibited the growth of gram-positive bacteria better than gram-negative strains. Among gram (+) bacteria, the strongest activity of *L. chinensis* was observed against *S. pyogenes* (15 ± 0.55) followed by *B. subtilis* and *S. aureus* and *N. lappaceum* seed extract shows highest activity against *S. aureus*. However, *P. aeruginosa* and *E. coli* exhibited moderate to weak activities, respectively, for both the test extracts. Gram (−) bacteria are surrounded with the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. The results also confirmed that that the maximum inhibitory activity of *L. chinensis* (MIC 15 ± 0.55 mg/mL) was toward *S. pyogenes.*

The EtOAc-soluble extract of litchi leaves was analyzed to possess the bioactive compounds such as quercetin-3-*O*-rutinoside, procyanidin A2, luteolin, and epicatechin. The luteolin showed strong antimicrobial activity toward *S. aureus*, *E. coli*, *S. dysenteriae*, *Salmonella*, and *B. thuringiensis*. However, quercetin-3-*O*-rutinoside, procyanidin A2, and epicatechin had comparatively weaker antimicrobial activities with MIC values of 62.5 μg/mL (Wen et al. 2014a). Xu et al. (2010a) reported the in vitro anti-viral activity, with the ethanolic extract of litchi seeds, against CVB3 and HSV-1 using the cytopathic effect (CPE) inhibitory assay and the plaque reduction assay. Litchitannin A2 was found to display in vitro anti-CVB3 activity with IC50 and TI (CC50/IC50) values of 35.2 μg/mL and 3.2. Meanwhile, aesculitannin A and proanthocyanidin A2 also exhibited anti-HSV-1 activity with IC50 values of 27.1 and 18.9 μg/mL and TI values of 2.0 and 3.0, respectively. However, the other tested compounds with the ethanolic extract of litchi seeds did not show in vitro anti-viral activity against CVB3 and HSV-1. Nimmanpipug et al. (2009) reported that the 3-oxotrirucalla-7,24-dien-21-oic acid, a terpenoid isolated from the *L. chinensis* seeds extract, also exhibited anti-viral activity toward HIV-1PR with an IC50 value of 20 mg/L (42.9 μM).

13.4.4 Anti-diabetic Activities

Litchi has been reported to have anti-diabetic activity as reported by several laboratories throughout the world. The litchi seed extract or its components could repress blood sugar and liver glycogen in mouse non-insulin diabetes mellitus model. The

water extract of litchi seed was found to reduce the concentrations of fasting blood glucose, triglyceride, leptin, and tumor necrosis factor. There are reports from China where scientists have studied a positive effect of litchi seeds as anti-diabetic pills (each pill equivalent to about 7.5 g of crude fraction) in 45 cases of diabetes mellitus human patient. The litchi pills result in a dose-dependent improvement of the noninsulin-dependent diabetes in 80% of the studied cases. Surprisingly, no change in the insulin was observed, but better use of glucose by tissues was the actual reason to control the diabetes. The analysis of Queiroz et al. (2015) with the fresh and dried fruit pulp, peel, and seed extracts exhibited inhibitory effects on α-amylase. Lee et al. (2009) reported that MeOH extract and EtOAc fraction of litchi fruits showed potent inhibitory activities of rat lens aldose reductase (RLAR) under in vitro condition with IC50 values of 3.6 and 0.3 μg/mL, respectively. Furthermore, (2*R*)-naringenin-7-O-(3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) and narirutin isolated from *L. chinensis* seeds showed dose-dependent α-glucosidase inhibitory activities. Ren et al. (2011) identified several compounds in 50% aqueous ethanol extract of litchi seeds including quercetin, narcissin, narirutin, onychin, (2*S*)-pinocembrin-7-*O*-(6″-*O*-α-L-arabinosyl-β-*D*-glucopyranoside), pinocembrin-7-*O*-[(2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside], and gentisic acid, and these were evaluated for their α-glucosidase inhibitory effects. It is noteworthy that quercetin and narirutin showed better effects at a concentration of 1 mg/mL. The pinocembrin-7-*O*- -β-D-glucopyranoside showed lesser effect among the tested compounds. The fruit pulp extracts of two Chinese litchi cultivars (Hemaoli and Feizixiao) significantly increased glucose consumption in HepG2 cells at concentrations of 1 and 5 μg/mL after 24 h incubation compared to metformin under laboratory conditions (Lv et al. 2014). However, another Chinese cultivar, Lanzhu, did not illustrate any noteworthy effect on glucose use in these cells. The epicatechin also exhibited a major enhancement of glucose consumption in HepG2 cells at concentrations from 0.2 to 25 mg/mL. They also showed that the glucose consumption activity is significantly associated with the anti-oxidant activity. Wu et al. (2015) indicated that litchi pulp extract exhibited a dose-dependent inhibitory activity against α-glycosidase. The anti-diabetic effects were evaluated by comparing the inhibitory properties of α-glycosidase, aldose reductase, and anti-oxidant activity. The results indicated that litchi extract exhibited the best dose-dependent inhibitory activity against α-glycosidase with IC_{50} of 10.4 mg/mL, and lemon peel extract exhibited aldose reductase inhibitory potential with IC_{50} value at 3.63 mg/mL. It was also concluded that oligonol decreased body weight, abdominal circumference, and visceral fat volume. In addition, it elevated serum adiponectin levels, thus improving insulin resistance (Chang et al. 2013).

13.4.5 Immuno-Modulation Activities

Zhao et al. (2007) reported the identification and immunomodulatory analysis with the litchi pericarp extract using the fractions of hexane, ethyl acetate, and water. Epicatechin, proanthocyanidin B2, and proanthocyanidin B4 were isolated and

purified from the ethyl acetate fraction by reverse-phase high-performance liquid chromatography. The immunomodulatory activities of epicatechin, proanthocyanidin B2, proanthocyanidin B4, and the ethyl acetate fraction were examined using proliferation of mouse splenocytes. The results showed all these samples had striking stimulatory effects on splenocyte proliferation than that of the reference, rutin. Epicatechin and the ethyl acetate fraction showed a noteworthy (Pb0.05) stimulatory result when the concentration increased up to 12.5 μg/ml.

Huang et al. (2014a) used dried litchi pulps containing the polysaccharides which were evaluated for their immunomodulatory activities. It was observed that dried litchi pulps exhibited enhanced stimulatory effect on spleen lymphocyte proliferation at 200 *m*g/mL and triggered higher NO, TNF-α, and IL-6 secretion from RAW264.7 macrophages than PLP-VM and PLP-VF. Therefore, drying pulp proved to be the best process for preparing litchi pulp with enhanced immunomodulatory properties. The same research group also reported that the dried pulp exhibited stronger in vitro stimulatory activities of spleen lymphocyte proliferation, NK cell cytotoxicity, and macrophage phagocytosis at concentration 50–400 μg/mL than PLPF (polysaccharides of fresh litchi pulp) compared with LPS (lipopolysaccharide) (10 μg/mL, positive control).

13.4.6 Cardiovascular and Hepato-Protective Activities

Yang et al. (2010) reported that high-fat/cholesterol diet-fed hamsters showed decreased serum and hepatic lipids (cholesterol and triglyceride) when they were given polyphenol-rich litchi flower solution to drink. It was also found in their experiments that drinking LFWE (litchi-flower-water-extract) lowered ($p < 0.05$) serum malondialdehyde (MDA) contents in high-fat/cholesterol-dietary hamsters and even showed the same ($p > 0.05$) serum MDA contents as the LFCD/NDW group which could be due to augmented ($p < 0.05$) serum trolox equivalent antioxidant capacity (TEAC). Therefore, this study indicated that LFWE definitely characterizes a defensive result on cardiovascular health. In general, LFWE showed a superior defensive effect on cardiovascular health, and it is significant in developing healthy food in markets. Basu et al. (2012) reported that the $CHCl_3$ and MeOH extracts of *litchi* leaf showed hepato-protective effects on paracetamol-induced liver damage in Wistar albino rats. Remarkably the MeOH extract was established to be more effectual than $CHCl_3$ extract compared to silymarin. Bhoopat et al. (2011) reported that the aqueous extracts of the Gimjeng and Chakapat litchi cultivar extracts were rich in vitamin C and phenolics like trans-cinnamic acid and pelargonidin-3-O-glucoside. The Gimjeng as compared to the Chakapat confirmed an improved anti-oxidant activity as revealed by anti-lipid peroxidation activity with the TEAC values.

13.5 Conclusions

Phytochemical investigations indicated that phenolics were the major bioactive components of *L. chinensis* with potential pharmacological activities. The ethnopharmacological relevance of *L. chinensis* is completely acceptable by the current findings representing it as medicinal and nutritional agent for management of a broad range of human disorders and aliments. Detailed research is prerequisite to completely know the mode of action of the active constituents and to fully exploit its preventive and therapeutic potentials.

Bibliography

Ahmad SA, Sharma SCS (2001) Fruit & vegetable juice therapy. Pustak Mahal, New Delhi

Ahmad J, Lobo MG, Ozadali F (2012) Tropical and subtropical fruits postharvest physiology processing and packaging. Wiley, Oxford, pp 251–252

Alves JA, de Oliveira LLC, Nunes CA, Dias DR, Schwan RF (2011) Chemical, physical-chemical, and sensory characteristics of lychee (*Litchi chinensis* Sonn) wines. J Food Sci 76:330–336

Basu S, Haldar N, Bhattacharya S, Biswas S, Biswas M (2012) Hepatoprotective activity of *Litchi chinensis* leaves against paracetamol-induced liver damage in rats. Am Eurasian J Sci Res 7:77–81

Bhalla-Sarin N, Prasad US, Kantharajah AS, Jain SM (2003) Micropropagation of litchi (*Litchi chinensis* Sonn.). In: Micropropagation of woody trees and fruits, vol 75. Springer, Dordrecht, pp 721–731

Bhat RS, Al-daihan S (2014) Antimicrobial activity of *Litchi chinensis* and *Nephelium lappaceum* aqueous seed extracts against some pathogenic bacterial strains. J King Saud Univ Sci 26:79–82

Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T (2011) Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): a combination of anti-oxidant and anti-apoptotic activities. J Ethnopharmacol 136:55–66

Castellain RCL, Gesser M, Tonini F, Schulte RV et al (2014) Chemical composition, anti-oxidant and antinociceptive properties of *Litchi chinensis* leaves. J Pharm Pharmacol 66:1796–1807

Chang Y, Yang D, Chiu C, Lin Y, Chen J, Chen Y (2013) Anti-oxidative and anti-inflammatory effects of polyphenol-rich litchi (*Litchi chinensis* Sonn.)-Flower-water extract on livers of high-fat-diet fed hamsters. J Funct Foods 5:44–52

Chen YB, Wu KS, Gu Y, Chen JZ (2007) Research progress in the chemical constituents and pharmacological effects of lychee seeds. Chin J Infect Tradit Chin Med 14:97–98

Chevion M (1988) A site-specific mechanism for free radical induced biological damage: the essential role of redox-active transition metals. Free Radic Biol Med 5:2737

Chyau CC, Ko PT, Chang CH, Mau JL (2003) Free and glycosidically bound aroma compounds in lychee (*Litchi chinensis* Sonn.). Food Chem 80:387–392

Cohen A, Dubois LJ (2010) Raw food for everyone: essential techniques and 300 simple to-sophisticated recipes. Penguin group (USA) Inc., New York

Gaydou EM, Ralaimanarivo A, Bianchini JP (1993) Cyclopropanoic fatty acids of litchi (*Litchi chinensis*) seed oil: a reinvestigation. J Agric Food Chem 41:886–890

Gontier EN, Terrasse BC, Jannoyer M, Menard M, Thomasset B, Bourgaud F (2000) *Litchi chinensis* fatty acid diversity: occurrence of the unusual cyclopropanoic fatty acids. Biochem Soc Trans 28:578–580

Hsu CP, Lin CC, Huang CC (2012) Induction of apoptosis and cell cycle arrest in human colorectal carcinoma by litchi seed extract. J Biomed Biotechnol 34:1479–1485

Huang F, Guo Y, Zhang R, Yi Y, Deng Y, Su D, Zhang M (2014a) Effects of drying methods on physicochemical and immunomodulatory properties of polysaccharide-protein complexes from litchi pulp. Molecules 19:12760–12776

Huang F, Zhang R, Yi Y, Tang X et al (2014b) Comparison of physicochemical properties and immunomodulatory activity of polysaccharides from fresh and dried litchi pulp. Molecules 19:3909–3925

Ibrahim SRM, Mohamed GA (2015) *Litchi chinensis:* medicinal uses, phytochemistry, and pharmacology. J Ethnopharmacol. http://dx.doi.org/10.1016/j.jep.2015.08.054

Jiang YM, Duan XW, Joyce D, Zhang ZQ, Li JR (2004) Advances in understanding of enzymatic browning in harvested litchi fruit. Food Chem 88:443–446

Jiang G, Lin S, We L, Jiang Y, Zhao M, Chen F, Prasad K, Duan X, Yang B (2013) Identification of a novel phenolic compound in litchi (*Litchi chinensis* Sonn.) pericarp and bioactivity evaluation. Food Chem 136:563–568

Jing Y, Huang L, Lv W, Tong H, Song L, Hu X, Yu R (2014) Structural characterization of a novel polysaccharide from pulp tissues of *Litchi chinensis* and its immunomodulatory activity. J Agric Food Chem 62:902–911

Kang SW, Hahn S, Kim JK, Yang SM, Park BJ, Chul LS (2012) Oligomerized lychee fruit extract (OLFE) and a mixture of vitamin C and vitamin E for endurance capacity in a double blind randomized controlled trial. J Clin Biochem Nutr 50:106–113

Kanlayavattanakul M, Ospondpant D, Ruktanonchai U, Lourith N (2012) Biological activity assessment and phenolic compounds characterization from the fruit pericarp of Litchi chinensis for cosmetic applications. Pharm Biol 50:1384–1390

Kasote DM, Hegde MV, Katyare SS (2013) Mitochondrial dysfunction in psychiatric and neurological diseases: cause(s), consequence(s), and implications of anti-oxidant therapy. Biofactors 39:392–406

Kasote DM, Katyare SS, Hegde MV, Bae H (2015) Significance of anti-oxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 11:982–991

Kong F, Zhang M, Kuang R, Yu S, Chi J, Wei Z (2010) Anti-oxidant activities of different fractions of polysaccharide purified from pulp tissue of litchi (*Litchi chinensis* Sonn.). Carbohydr Polym 81:612–616

Lee GH, Shin Y, Oh MJ (2008) Aroma-active components of Lycii fructus (kukija). J Food Sci 73:500–505

Lee S, Park W, Park S, Moon H (2009) Aldose reductase inhibitors from *Litchi chinensis* Sonn. J Enzyme Inhib Med Chem 24:957–959

Lee JB, Shin YO, Min YK, Yang HM (2010) The effect of oligonol intake on cortisol and related cytokines in healthy young men. Nutr Res Pract 4:203–207

Li TSC (2009) Chinese and related North American herbs phytopharmacology and therapeutic values, 2nd edn. CRC Press Taylor & Francis Group, Boca Raton/London/New York, p 88

Li J, Jiang Y (2007) Litchi flavonoids: isolation, identification and biological activity. Molecules 12:745–758

Li W, Liang H, Zhang M et al (2012) Phenolic profiles and anti-oxidant activity of Litchi (*Litchi chinensis* Sonn.) fruit pericarp from different commercially available cultivars. Molecules 17:4954–4967

Lim TK (2013) Edible medicinal and non-medicinal plants: Volume 6, Fruits. Springer, Dordrecht, pp 45–58

Lin N, Xiao LY, Pan JQ (2008) Effects of semen litchi on the expressions of S180 and EAC tumor cells and Bax and Bcl-2 proteins in rats. Chin Pharm 19:1138–1140

Lin C, Chung Y, Hsu C (2013) Anti-cancer potential of litchi seed extract. World J Exp Med 3:56–61

Lin Y, Chang J, Cheng S et al (2015) New bioactive chromanes from *Litchi chinensis*. J Agric Food Chem 63:2472–2478

Liu L, Xie B, Cao S, Yang E, Xu X, Guo S (2007) A-type procyanidins from *Litchi chinensis* pericarp with anti-oxidant activity. Food Chem 105:1446–1451

Lv Q, Si M, Yan Y, Luo F, Hu G, Wu H, Sun C, Li X, Chen K (2014) Effects of phenolic-rich litchi (*Litchi chinensis* Sonn.) pulp extracts on glucose consumption in human HepG2 cells. J Funct Foods 7:621–629

Lv Q, Luo F, Zhao X, Liu Y, Hu G, Sun C, Li X, Chen K (2015) Identification of proanthocyanidins from Litchi (*Litchi chinensis* Sonn.) pulp by LC-ESI-QTOF-MS and their anti-oxidant activity. PLoS One 10:120–148

Mahattanatawee K, Perez-Cacho PR, Davenport T, Rouseff R (2007) Comparison of three lychee cultivar odor profiles using gas chromatography-olfactometry and gas chromatography-sulfur detection. J Agric Food Chem 55:1939–1944

Maity SC, Mitra SK (1990) Litchi. In: Bose TK, Mitra SK (eds) Fruits: tropical and subtropical. Naya Prakasak, Calcutta, pp 420–468

Malik I, Ahmad VU, Anjum S, Basha FZ (2010) A pentacyclic triterpene from Litchi chinensis. Nat Prod Commun 5:529–530

Menzel CM (2002) The lychee crop in Asia and the Pacific. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific Bangkok, Bangkok, pp 8–13

Menzel C, Waite G (2005) Litchi and longan: botany, production, and uses. CABI Publishing/CAB International, Wallingford/Oxford, pp 24–27

Miller L (2011) Ayurvedic curative cuisine for everyone. Lotus Press, Twin Lakes, pp 79–82

Morton J (1987) Lychee. In: Fruits of warm climates. Julia F. Morton, Miami, pp 249–259

Nacif SR, Paoli AS, Salomão LCC (2001) Morphological and anatomical development of the litchi fruit (*Litchi chinensis* Sonn. cv. Brewster). Fruits 56:225–233

Nimmanpipug P, Lee VS, Wolschann P, Hannongbua S (2009) *Litchi chinensis*-derived terpenoid as anti-HIV-1 protease agent: structural design from molecular dynamics simulations. Mol Simul 35:673–680

Nishizawa M, Hara T, Miura T, Fujita S, Yoshigai E, Ue H, Hayashi Y, Kwon AH, Okumura T, Isaka T (2011) Supplementation with a flavanol-rich lychee fruit extract influences the inflammatory status of young athletes. Phytother Res 25:1486–1493

Obrosova IG, Chung SS, Kador PF (2010) Diabetic cataracts: mechanisms and management. Diabetes/Metabolism. Res Rev 26:172–180

Ogasawara J, Kitadate K, Nishioka H et al (2009) Oligonol, a new lychee fruit-derived low-molecular form of polyphenol, enhances lipolysis in primary rat adipocytes through activation of the ERK1/2 pathway. Phytother Res 23:1626–1633

Ong PKC, Acree TE (1998) Gas chromatography/olfactory analysis of Lychee (*Litchi chinesis* Sonn.). J Agric Food Chem 46:2282–2286

Pandey RM, Sharma HC (1989) The litchi. ICAR, New Delhi, pp 1–79

Perry LM, Metzger J (1980) Medicinal plants of East and Southeast Asia: attributed properties and uses. MIT Press, Cambridge, MA/London, p 374

Prasad KN, Yang B, Yang S et al (2009) Identification of phenolic compounds and appraisal of anti-oxidant and tityrosinase activities from litchi (*Litchi sinensis* Sonn.) seeds. Food Chem 116:1–7

Queiroz E, de Abreu CMP, Rocha DA, Simao AA et al (2015) Anti-nutritional compounds in fresh and dried lychee fraction (*Litchi chinensis* sonn.). Afr J Agric Res 10:499–504

Ren S, Xu D, Pan Z, Gao Y, Jiang Z, Gao Q (2011) Two flavanone compounds from litchi (*Litchi chinensis* Sonn.) seeds, one previously unreported, and appraisal of their αglucosidase inhibitory activities. Food Chem 127:760–1763

Roux LE, Doco T, Sarni-Manchado P, Lozano Y, Cheynier V (1998) A-type proanthocyanidins from pericarp of *Litchi chinensis*. Phytochemistry 48:1251–1258

Sakurai T, Nishioka H, Fujii H, Nakano N, Kizaki T, Radak Z, Izawa T, Haga S, Ohno H (2008) Antioxidative effects of a new Lychee fruit-derived polyphenol mixture, oligonol, converted into a low-molecular form in adipocytes. Biosci Biotechnol Biochem 72:463–476

Saxena S, Hajare N, More V, Kumar S et al (2011) Anti-oxidant and radioprotective properties of commercially grown litchi (*Litchi chinensis*) from India. Food Chem 126:39–45

Sayre JK (2001) Ancient herb and modern herbs: a comprehensive reference guide to medicinal herbs, human ailments, and possible herbal remedies. Bottlebrush Press, Auburn/Washington, DC, p 156

Sies H (1997) Physiological society symposium: impaired endothelial and smooth muscle cell function in oxidative stress oxidative stress: oxidants and anti-oxidants. Exp Physiol 82:291–295

Sivakumar D, Naudé Y, Rohwer E, Korsten L (2008) Volatile compounds, quality attributes, mineral composition and pericarp structure of South African litchi export 74 cultivars Mauritius and McLean's Red. J Sci Food Agric 88:1074–1081

Stuart LJ, Buist PH (2004) The absolute configuration of methyl dihydrosterculate: an unusual phytofatty acid isolated from the seed oil of *Litchi chinensis*. Tetrahedron Asymmetry 15:401–403

Su D, Ti H, Zhang R, Zhang M, Wei Z, Deng Y, Guo J (2014) Structural elucidation and cellular anti-oxidant activity evaluation of major anti-oxidant phenolics in lychee pulp. Food Chem 158:385–391

Sun J, Jiang Y, Wei X, Shi J, You Y, Liu H, Kakuda Y, Zhao M (2006) Identification of (−)-epicatechin as the direct substrate for polyphenol oxidase isolated from litchi pericarp. Food Res Int 39:864–870

Sun J, Jiang Y, Shi J, Wei X, Xue SJ, Shi J, Yi C (2010) Anti-oxidant activities and contents of polyphenol oxidase substrates from pericarp tissues of litchi fruit. Food Chem 119:753–757

U.S. Department of Agriculture, Agricultural Research Service (2012) USDA national nutrient database for standard reference. http://www.ars.usda.gov/ba/bhnrc/ndl

Vardhana V (2008) Direct uses of medicinal plants and their identification, 1st edn. Prabhat Kumar Sharma for Sarup & Sons, Delhi, p 211

Wall M (2006) Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. J Food Compos Anal 9:655–663

Wang X, Yuan S, Wang J, Lin P, Liu G et al (2006) Anti-cancer activity of litchi fruit pericarp extract against human breast cancer in vitro and in vivo. Toxicol Appl Pharmacol 215:168–178

Wang L, Lou G, Ma Z, Liu X (2011) Chemical constituents with anti-oxidant activities from litchi (*Litchi chinensis* Sonn.) seeds. Food Chem 126:1081–1087

Wang YJ, Zhao DX, Gao JL, Peng ZQ (2013) Volatile components of *Litchi chinensis* Sonn. leaf oil extracts obtained by simultaneous distillation extraction. J Essent Oil Bear Plants 6:161–165

Wen L, Wu D, Jiang Y et al (2014a) Identification of flavonoids in litchi (*Litchi chinensis* Sonn.) leaf and evaluation of anti-cancer activities. J Funct Foods 6:555–563

Wen L, He J, Wu D, Jiang Y et al (2014b) Identification of sesquilignans in litchi (*Litchi chinensis* Sonn.) leaf and their anti-cancer activities. J Funct Foods 8:26–34

Wen L, You L, Yang X et al (2015) Identification of phenolics in litchi and evaluation of anti-cancer cell proliferation activity and intracellular anti-oxidant activity. Free Radic Biol Med 84:171–184

Wiart C (2006) Medicinal plants of Asia and the Pacific. CRC Press/Taylor & Francis Group, Boca Raton, p 171

Wills RBH, Lim JSK, Greenfield H (1986) Composition of Australian foods. 31. Tropical and subtropical fruit. Food Technol Aust 38:118–123

Wu Y, Pan Q, Qu W, Duan C (2009) Comparison of volatile profiles of nine Litchi (*Litchi chinensis* Sonn.) cultivars from Southern China. J Agric Food Chem 57:9676–9681

Wu T, Luo J, Xu B (2015) In vitro anti-diabetic effects of selected fruits and vegetables against glycosidase and aldose reductase. Food Sci Nutr 3(6):495–505

Xu X, Xie H, Wang Y, Wei X (2010a) A-type proanthocyanidins from lychee seeds and their anti-oxidant and anti-viral activities. J Agric Food Chem 58:11667–11672

Xu X, Xie H, Hao J, Jiang Y, Wei X (2010b) Eudesmane sesquiterpene glucosides from lychee seed and their cytotoxic activity. Food Chem 123:1123–1126

Xu X, Xie H, Xu L, Wei X (2011a) A novel cyclopropyl-containing fatty acid glucoside from the seeds of *Litchi chinensis*. Fitoterapia 82:485–488

Xu X, Xie H, Hao J, Jiang Y, Wei X (2011b) Flavonoid glycosides from the seeds of *Litchi chinensis*. J Agric Food Chem 59:1205–1209

Yamanishi R, Yoshigai E, Okuyama T, Mori M, Murase H et al (2014) The anti-inflammatory effects of flavanol-rich lychee fruit extract in rat hepatocytes. PLoS One 9:793–818

Yan X, Zhou J, Xie G, Milne GWA (1999) Traditional Chinese medicines: molecular structures, natural sources, and applications. Ashgate Publishing Company, Hampshire, p 730

Yang DJ, Chang YY, Hsu CL, Liu CW, Wang Y, Chen YC (2010) Protective effect of a litchi (*Litchi chinensis* Sonn.)-flower-water-extract on cardiovascular health in a high-fat/cholesterol-dietary hamsters. Food Chem 119:1457–1464

Yang D, Chang Y, Lin H, Chen Y, Hsu S, Lin J (2014) Inhibitory effect of Litchi (*Litchi chinensis* Sonn.) flower on lipopolysaccharide-induced expression of proinflammatory mediators in RAW264.7 cells through NF-κB, ERK, and JAK2/STAT3 inactivation. J Agric Food Chem 62:3458–3465

Zhang N, Zhou Z, Feng X (2012) Comparison and elevation on anti-tumor activity in vitro of the litchi seeds and longan seeds water extract. Hunan J Tradit Chin Med 28:133–135

Zhang R, Zeng Q, Deng Y, Zhang M, Wei Z, Zhang Y, Tang X (2013) Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. Food Chem 136:1169–1176

Zhao M, Yang B, Wang J, Liu Y, Yu L, Jiang Y (2007) Immunomodulatory and anti-cancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. Int Immunopharmacol 7:162–166

Zhou H, Lin Y, Li Y, Li M, Wei S, Chai W, Tam NF (2011a) Anti-oxidant properties of polymeric proanthocyanidins from fruit stones and pericarps of *Litchi chinensis* Sonn. Food Res Int 44:613–620

Zhou Y, Wang H, Yang R, Huang H, Sun YM, Shen YD, Lei HT, Gao H (2011b) Effects of *Litchi chinensis* fruit isolates on prostaglandin E2 and nitric oxide production in J774 murine macrophage cells. Afr J Biotechnol 10:13180–13188

Advances in Litchi Biotechnology: Achievements and Opportunities

14

Arun Vincent Kisku, Subhash Chandra Prasad, Aayushi Agrawal, and Deepak Kumar

Abstract

Trees are an important and vital part of human survival and an essential part of biodiversity. The forest trees are renewable sources of wood, timber, food, fodder, fuel, and other valuable non-timber products, and due to increase in population and the ardent desire of human being for survival, there has been a constant reduction in forest from the earth's surface. The current scenario of global climate change threatens to hamper the agro-ecosystems by drastically affecting the key components of environment such as temperature, pattern of rainfall, drought, and water logging. These changes not only affect productivity but are also responsible for decline in the quality of fruits, and litchi (*Litchi chinensis* Sonn.) is one of them. Several conventional approaches have been exploited in the past for the promulgation and improvement of litchi. However, such efforts are confronted with several natural drawbacks and need extensive research to solve the challenges. The application of biotechnological tools for in vitro regeneration, micropropagation, and genetic engineering in litchi species has been applied with success, especially in the last decade, and by the help of genetic engineering technology, it has become possible to introduce desired genes in a much simpler way in litchi. This chapter reviews some of the basic aspects and advancement made in litchi propagation and genetic transformation techniques for further improvement.

A.V. Kisku • S.C. Prasad
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India
e-mail: arunkisku@gmail.com; chandra.subhash63@gmail.com

A. Agrawal
Department of Biotechnology, Sharda University, Greater Noida 201306, Uttar Pradesh, India
e-mail: aayushi.agrawal@outlook.com

D. Kumar (✉)
Department of Plant Science, Central University of Jammu, Jammu 180011, India
e-mail: deepakinjnu@gmail.com

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14.1 Introduction

Litchi is an evergreen tree distributed subtropically and well known for its delicious, juicy aril, and refreshing fruit. The ripe fruits are consumed fresh and also processed into value-added products. The fruit is also exported as pulp, canned aril, dried fruits, and "litchi nuts." The aril of litchi is dried and eaten like raisins or used as tea sweetener by the Chinese. Litchi fruits are also spiced or pickled or made into sauce, preserves, or wine. Litchi seeds are also used as medicine in neuralgic disorders and bronchitis. Litchi cultivation is restricted to very few countries in the world, India being one among them. Together India and China account for 91% of the world litchi production and at the same times form the major market for fresh and processed fruits. On an average 585,300 metric tonnes of litchi is produced annually from 84,170 ha in India of which 40% production comes from Bihar. Litchi, being exacting in climatic requirement, is confined to a few states in India. In Bihar, litchi is the source of livelihood for millions of people as it provides both on-farm and off-farm employment and adds up to the income of small and marginal farmers from litchi plants in their homesteads.

Litchi is an attractive as well as uncommon subtropical fruit tree which contains a delicious red fruit. The litchi tree is known over 2000 years back first within the north exotic rain forests as well as mountain forests of Southern China, exactly where it is really a dominating tree varieties. The other location where wild litchi trees were reported was at the southern sub-exotic lower elevations of Southern China within the states of Guangdong/Kwangtung as well as Fujian/Fukien mainly alongside rivers as well as close to the seacoast. The litchi population types in such lowlands are likely a direct result of fruit/seeds which were cleaned down by rivers from the mountains or were enthused simply by birds and after that were transferred by man in excellent circumstances for litchi development. Litchi is mainly cultivated in the northern tropical as well as southern subtropical climate zones in between 19 degrees and 24 degrees north latitude in China. In the north the exotic environment consists of southwestern Guangdong, Hainan, Southern Taiwan, as well as Leizhou Peninsula, whereas the southern subtropical climate zone consists of central as well as southern Guangdong and Guangxi, southeastern coastal Fujian, and central, southern, and also western coastal Taiwan (Chaikiattiyos et al. 1994). Table 14.1 depicts some of the major production and distribution of litchi, from indigenous China to neighboring regions of southeastern Asia as well as offshore islands, India, the West Indies, South Africa, Madagascar, then to France, and England. Litchi cultivation has also gained pace in Australia in recent years and has turned into a main manufacturer of Litchi within Queensland as well as New South Wales. Litchi trees were introduced in Australia during the 1850s by Chinese gold

Table 14.1 Major litchi production around the world

Country	Production(tones)	Main growing areas
China	200,000	Guangdong, Fujian
Taiwan	1,310,000	Taichung
Thailand	10,000	Chiang Mai, Lamphun, and Fang
India	90,000	Bihar
Madagascar	5000	Eastern coastal belt
South Africa	8000	Transvaal lowveld region
Mauritius	1000	Hawaii, Florida
Australia	2000	Eastern coastal strip

Fig. 14.1 Litchi fruit (**a**), aril (**b**), and seeds (**c**)

miner employees. Reverend W.M. Brewster imported Chen Tze or Royal Chen Purple litchi trees to South and Central Florida from the northernmost growing region of Fukien in the years between 1903 and 1906; later on this particular litchi variety/cultivar was renamed Brewster. Today we can find an even part of an authentic "Brewster" grove in Davie, Florida. In the present book chapter, we intend to provide an up-to-date comprehensive insight into the progress toward the improvement of litchi through tissue culture propagation and using genetic engineering approach for sustainable agriculture and food security.

14.2 Botanical Characterization

Morphologically litchi is an evergreen, medium-sized round-topped tree with a smooth, gray trunk and limbs. Sometimes it may reach a height of 10–15 m, but is usually much smaller. Leaves are leathery; pinnate; divided into 4–8 pairs of elliptic or lanceolate, acuminate, glabrous leaflets; 5–7 cm long; reddish when young; becoming shiny; and bright green. Inflorescence has a many-branched panicle, 5–30 cm long. Flowers are small, yellowish white, and functionally male or female, with tetramerous calyx and absent corolla. Fruits are characterized by a rough leathery covering or pericarp, pink to strawberry red. Fruits are oval, heart shaped, or nearly round, 2.5 cm or more in diameter (Fig. 14.1). The edible portion or aril is white, translucent, firm, juicy, and sweet in flavor, with distinct fragrance. Inside the

aril is a seed that varies considerably in size between 1 and 2 cm in length, globose or oblong, egg shaped, and have a smooth and glossy surface with brown or reddish color (Nacif et al. 2001).

14.3 Types of Litchi

There are numerous types and varieties of litchi. The greatest and mouth-watering litchi variety is "No Mai Tze" from China which is known to bear fruits every 4–5 years. While the majority of litchi varieties provide you with fruits year to year, the younger trees usually are not as dependable in productivity.

14.3.1 The Emperor Litchi

The "emperor" variety of litchi is the biggest of the litchi fruits known. It attains a golf ball size and also usually generates aborted "chicken tongue" seeds or no seeds (Fig. 14.2a). The tree is a sluggish and compact grower that will not exceed heights of 10 feet, so the cultivating possibilities are limitless where one can choose to locate it; however it is not really a well-known industrial selection. The litchi tree is good looking, thick, round topped, slow growing, 30–100 ft. (9–30 m) higher, as well as equally extensive.

Fig. 14.2 Different types of litchi. (**a**) Emperor litchi (**b**) Brewster litchi (**c**) Ohia pink litchi (**d**) Kwai Mai pink litchi (**e**) The sweet cliff

14.3.2 The Brewster Litchi

Its fruit is actually moderate or even big, fairly sweet, and also succulent. At peak ripeness the Brewster is actually scrumptious as well as sweet in flavor, typically testing against other types regularly outranking the others. This variety is probably the most thoroughly grown industrial types in Florida. The fruit is big and deep red in color, and it is also of fine consuming quality (Fig. 14.2b) (Shukla et al. 1974).

14.3.3 The Ohia Litchi

Ohia is a delicious litchi which is bigger in size with a relatively smaller seed (Fig. 14.2c). The tree is distinct with unusual fruiting every two out of 3 years. The production of this is restricted in Southeast Asia, and this is comparatively unfamiliar within the United States (Stern and Gazit 1997).

14.3.4 Kwai Mai Pink Litchi

This is also referred to as Bosworth 3. The fruit ranges from fine to superior quality, and it is not an industrial cultivar because the fruit is comparatively smaller in size. In spite of the deficiency of less industrial producers, it is a favorite alternative for the home garden (Fig. 14.2d).

14.3.5 The Sweet Cliff

Its fruit is usually small compared to its well-known cousins, the Brewster as well as Mauritius varieties. Its fruit has a good flavor but relatively slow-developing trees having a smaller size. "Sweet cliff" is a little pink in color which has a pebbly covering (Fig. 14.2e). The fruit is of excellent eating quality; however the tree is an additional alternate bearer. It is a commonly well-known cultivar as it is not grown commonly because of the accessibility to exceptional types (Yuan and Huang 1988).

14.4 Litchi Production and Distribution in India

Litchi arrived in India through Burma at the end of the seventeenth century, and India is now producing as much litchi as China, and since then, it has been spread to several regions of India. Northern Bihar is attributed for more than 70% of the litchi production, while other litchi-growing states include West Bengal (15%) and Uttar Pradesh (6%). Most of the litchi cultivars in India are the outcome of breeding local seedlings and their careful selection from the Chinese origin varieties. Although a large number of litchi cultivars are grown out of them, most are not widely accepted for commercial plantation. There is ambiguity in the names of

litchi varieties as the same cultivar is known under several different names in different places. Nevertheless, few of the Indian cultivars have been renamed as Chinese cultivars as has happened in Thailand, Hawaii, and Australia. Hot and desiccating winds are the main factor restraining litchi cultivation in India; hence cultivars which can reputedly set and carry fruit under these adverse conditions have been selected over the time. Out of the ten commercial cultivars growing, Shahi (Muzaffarpur, Bihar), rose scented, and China are the most popular, due to their excellent quality and larger fruit size. Some of the other important popular cultivars are Deshi, Kasba, Purbi, and early and late Bendana (Oosthuizen 1991).

Litchi plant being exacting in climatic and soil requirements has limited distribution throughout. It is grown in the states of Bihar, West Bengal, Uttar Pradesh, Punjab, Haryana, and Tripura. Out of the total production of litchi in India, Bihar itself contributes approximately 74%. Other than Bihar, the second largest producer is West Bengal followed by Tripura and Assam. An interesting feature of distribution of Litchi in India is that maturity commences first in Tripura, followed by West Bengal and Bihar. The first and second week of May is the time for harvest in the eastern region, while litchi of Bihar matures in the third to fourth week and continues up to the first week of June. The litchi growing in Uttar Pradesh and Punjab is available for harvest during the second to third week of June. In Himachal Pradesh, litchi of the same cultivar is ready for harvest in the last week of June (Fivas 1994).

14.5 Methods of Propagation for Improvement in Litchi

Till now only few new improved cultivars of litchi and longan have been developed by conventional breeding methods. The drawbacks are long juvenile period of the species, the apparent lack of genetic variability in the existing germplasm (Menzel 1992), and the excessive expenditure that is required in terms of land, time, and money. Genetic engineering, genetic transformation, and induced mutations in vitro, followed by selection, can potentially be used to alter one or more horticulturally important traits. Thus modern biotechnological tools combined with breeding can pave the pathway for improvement of vegetatively propagated fruit trees such as litchi and longan. The serious problems affecting production or quality can thus be resolved without altering the overall phenotype of the cultivar. Large-scale breeding programs for litchi and longan have so far been not tried. Most of the superior varieties of litchi and longan that are vegetatively propagated have been derived from open-pollinated seedlings by selections. McConchie et al. (1994) reported that litchi and longan are partially sexually compatible and can be bred to recover hybrids, but only if litchi is the maternal parent. Among their progeny, some of the plants produced seedless fruit, which could have interesting implications for selections of superior, seedless fruit. Litchi is propagated by sexual (from seed) as well as asexual (from vegetative parts) method.

14.5.1 Propagation from Seeds

Though seedling propagation is a rich source of genetic variability and provides events for selection of new variety, it is not practiced much and is discouraged. The new seedlings do not maintain a uniform crop containing chosen desirable characteristics and are an extreme disadvantage for a nursery operator to search for a plant with improved or different characteristics. However, crossbreeding two selected parents in a planned breeding program results in valuable seeds with desirable variation in fruit quality and yield. Another disadvantage is that the seed of litchi is recalcitrant in nature and, therefore, loses its viability if exposed to air in the shade under normal humid conditions. The seed shrivels and is unable to germinate if it is kept beyond 5 days. Litchi seeds can be preserved for up to 8 weeks by keeping them in between 2–2.5 cm thick layers of wet sphagnum moss or for shorter periods, if wrapped in peat in a refrigerator or by storing them for at least a month in the shade or in closed petri dishes dusted with captan at around temperatures of 150–250 °C. Alternately the seeds can be left in the fruit; this prevents them from drying and preserves their viability for at least more than 3–4 weeks. Litchi seeds are sown horizontally to a depth of 1–2.5 cm in a well-drained sowing medium in partly shady, well-irrigated location. Soil incorporation from old plantations into the sowing medium usually induces germination due to mycorrhizal presence, but this may also prove disadvantageous as the soil may also be contaminated with other pathogens. However, the propagation of litchi from the seed is usually not much practiced, as the varieties do not reproduce true-to-type plant and take longer time of more than 10 years to come into bearing.

14.5.2 Asexual or Vegetative Propagation

The cuttings are prepared from young and vigorous trees with semihard to hard wood cuttings with two leaves, measuring approximately 13–20 cm in length and 15 mm in diameter. Combinations of different growth regulators are used for promoting rooting. Approximately 100–200 ppm IBA, 2000–5000 ppm of indolebutyric acid (IBA), indoleacetic acid (IAA), and nephtalenacetic acid (NAA) is used for dipping the basal part of cuttings for 2 s (quick dip). Then the cuttings are placed in a propagation bed, under mist. The medium is kept loose as possible, and preferably, the temperature maintained is between 30 and 320 °C. The cuttings are then left in the rooting bed for 3–4 months for the root to develop. The rooting is extremely slow, and it takes 4 months to develop proper root; then on they can be transferred to bags. The potted plants are then grown in glasshouse for 15–16 months and then further planted in the field condition. Because of the slow development of callus tissue, the slow-growing cultivars do not root well. The success also depends on selecting the correct type of wood, misting or fogging, and proper temperature control in the propagation house. The rooting media additionally also require free drainage. The young plants of approximately 50–60 cm in height are recommended for planting because smaller plants often die in the field. Soft

terminal cuttings are unsuccessful and, therefore, should be avoided for cuttings. Better results are obtained if the shoots are girdled a few months before taking the cuttings because of accumulated carbohydrate content in the above-girdle portion. The disadvantage of litchi propagation by cuttings is sometimes it fails to have the desired results. The method is also costly and complex, and the plants produced are weaker, and root systems are usually less developed than those obtained by layering.

14.5.3 Air Layering

Air layering or commonly known as "gootee" is the most preferred and accepted method of litchi propagation. The major advantages of it are it is simple to use and genetically identical plants can be produced. Air layering can be done throughout years as long as there is sufficient moisture, but best results are obtained in rainy and spring season. The best result of air layering can be obtained with branches of 10–25 mm diameter and 46–60 cm length. These shoots produce rooting rate above 90%, and damage to the mother plant is also minimal. The branches selected for air layering should be ideally on the periphery of the trees, so that they can easily be worked on. Only a single stem is selected for air layering and other stems in the branch are removed. The preference to plant propagation is given to branches that are erect and in satisfactory physiological conditions, i.e., the last vegetative flush must be well advanced. There is also some serious drawback of air layering method; they may cause damage to the parent plant if a large number of layers are prepared and poor survival of plant in the nursery after shifting of layers. At the same time, the plants prepared by this method are delicate and difficult to transport. Traditionally litchi trees have been vegetatively propagated in Mauritius by air layering. Although this method of propagation has been improved by the use of younger branches, small earth balls, and 1, 4-indole-3-butyric acid (IBA), the process is still slow and inefficient.

14.6 Role of Tissue Culture in Improvement of Litchi

The tissue culture technique is not only used for mass multiplication of litchi but also plays a vital role in crop improvement through the production of desirable traits.

There are several reports where scientists have used different types of explants for the tissue culture of litchi with their respective merits and demerits. A well-established in vitro regeneration system is a prerequisite for the improvement of litchi by genetic engineering. There are several well-established regeneration systems of litchi which have been reported in literature as further discussed in this chapter.

14.6.1 Somatic Embryogenesis

The first report on somatic embryogenesis in litchi was by Amin and Razzaque (1995) where they managed to induce somatic embryogenesis in the cultures of zygotic embryos of litchi using BA (5 mgL^{-1}) and activated charcoal (1 gL^{-1}) but failed to obtain regenerated plantlets, in spite of obtaining about 40% of the in vitro formed matured embryos. Callus induction, somatic embryogenesis, and plant regeneration from immature zygotic embryos and anthers have been reported by (Yu and Chen 1997; Zhou et al. 1996; Kantharajah et al. 1992; Fu and Tang 1983). Previously, embryogenic litchi cultures have been induced from immature zygotic embryos of "Xiafanzhi" and "Chen Zi" (also known as "Brewster") (Yu and Chen 1997; Yu et al. 2000; Zhou et al. 1996); however, the regeneration of plants from mature-phase trees has not been reported. The induction of embryogenic cultures has been reported from leaves of the related fruit tree species longan *Dimocarpus longan* (Litz 1988; Matsumoto et al. 2004).

14.6.2 Clonal Propagation by Tissue Culture

Plants propagated from a single plant asexually are called "clones." Clones can exist as a species adaptation in nature where vegetative expansion occurs by adopting asexual propagation. Most asexual propagation techniques ensure that plants being propagated will have the same characteristics as the parent plants. Although the advantages of clonal propagation are numerous, like it requires very small amounts of propagating material and has the potential of providing very large numbers of cloned plants, one major disadvantage is the lack of genetic variability. In a monoculture production of a single crop with limited genetic variability, there is more threat of rapid spread of disease and insect infestations. Nevertheless, several superior clones of litchi have been selected in almost all litchi-growing areas. Production of plants by culturing embryos prior to abortion would be expected to yield progeny with high proportion showing the chicken tongue character (Anon 1991).

14.6.3 In Vitro Cultures for Litchi Propagation

Improved cultivars play a crucial role in improving the production of crop. They possess built-in production stability factors and aid in ensuring high production and best quality. As the crop yield is an interactive and integrative interplay of several factors involved in all aspects of cultivation, the yielding capacity of a cultivar depends not only on its genetic makeup but also on how it is handled in the field condition.

At present, no any commercial varieties of litchi has all these characteristic features added to one. Since, all these characters may not be available in a single variety or if at all it is available, the variety may not perform well in all the litchi-growing areas. Therefore, it is prerequisite to choose a wider acceptable variety, because the

success of any litchi plantation depends on tree health and its management procedures. The adaptable variety and its compatible environment are the foundation on which tree management systems are built up. Fu and Tang (1983) reported that they obtained 24 plantlets via organogenesis from pollen callus. Subsequently the reports were made available on somatic embryogenesis and plantlet regeneration in litchi by culturing the anthers (Deng 2005; Xie et al. 2006; Wang et al. 2013), immature embryos (Zhou et al. 1993, 1996; Kuang et al. 1997), or protoplasts (Yu et al. 1996). Das et al. (1999) reported a high proliferation rate from mature seeds of litchi utilizing two methods. Yu et al. (2000) achieved somatic embryogenesis and plantlets from "Xiafanzhi" litchi protoplasts isolated from embryogenic suspensions. Puchooa (2004) used young unvaccinated "Tai So" leaves as explants to study the effect of various factors on the regeneration of the leaf blade and eventually obtained the regenerated plants. By "Heli" anther culture, Guo et al. (2014) obtained somatic embryos with roots without sprout. All the above results depict that the regeneration of different litchi varieties is inconsistent with their type of medium. The same culture medium plays different roles on the regeneration of different litchi cultivars. Therefore, different genetic backgrounds of litchi varieties have a remarkable effect on in vitro regeneration capacity. To date, only a few cultivars, like "Nuomici" (Kuang et al. 1997), "Xiafanzhi" (Lai and Sang 2003), and "Hushanjiaohe" (Fu and Tang 1983), have been successfully regenerated in vitro. "Feizixiao" is an early matured variety with a tender, juicy, sweet aril with high and stable yield. Guo et al. (2014) tried the following PGRs combination for induction of embryonic callus. They found medium L9 (2.22 μM BA, 2.69 μM NAA, and 13.57 μM 2,4-D), along with medium L8 (0.89 μM BA, 9.29 μM KT, 2.69 μM NAA, and 4.52 μM 2,4-D) and medium L3 (4.65 μM KT, 2.69 μM NAA, and 9.05 μM 2,4-D), which resulted higher number of callus and more friable embryonic callus from anther explant.

14.6.4 In Vitro Mutagenesis

Genetic improvement in litchi is prerequisite for increasing fruit quality and production. The major problems facing litchi breeding are long juvenile periods, unavailability of suitable germplasm, and large tree size. In litchi, breeding by controlled crosses is hampered due to delayed flowering, or unsuccessful fruit setting due to abortive embryos or massive fruit drop. The exploitation of genetic variation, natural or induced, assists greatly in the genetic improvement of fruit trees. Mutations can be induced either by physical or chemical mutagens. Induced mutations are highly effective in enhancing natural genetic resources and have significantly assisted in developing improved fruit cultivars. Advances in plant tissue culture and plant molecular biology can be integrated with conventional techniques in generating new mutations. Thus nuclear technology has a great potential in genetic improvement of litchi. It is reported that more than 2300 mutant varieties have officially been released in many countries. The gamma rays and ethyl methanesulfonate (EMS) are commonly used for mutation induction agents, and fine embryogenic cell suspension cultures are most suitable for inducing mutations. The embryogenic

cell suspension cultures are first transferred to the filter paper and then plated on the agar-solidified culture medium for gamma irradiation. LD_{50}, which is the optimum dose for inducing mutation, is determined first, which is then used for mutation induction. Irradiated cells are further cultured to the fresh medium for the development, maturation, and germination of mutated somatic embryos. This approach provides mutated somatic seedlings in a short period and also prevents chimera problem which otherwise requires to multiply plants up to M1V4 generation for chimera dissociation. Some workers (Vos et al. 2009) have also used shoot tip or budwood for inducing mutation and to multiply plants up to M1V4 generation for producing pure mutants by dissociation of chimeras. LD_{50} of 20 and 30 gray was used for irradiation of litchi budwood and shoot tips. Exposure to mutagens breaks the nuclear DNA, and during the process of DNA repair mechanism, new heritable mutations are induced randomly. The mutational changes can occur in cytoplasmic organelles as well as nuclear genome, some resulting in desirable mutations and phenotypes enabling plant breeders to select useful mutants such as flower color, flower shape, disease resistance, and early flowering types (Jain and Maluszynski 2004).

14.7 Selection of Litchi Cultivars Using Genetic Markers

A genetic marker is a trait that is readily identified and is linked to the other trait. Breeders seek to select the desirable trait in an improved variety. When the marker is observed, the other trait, which is usually difficult to observe or evaluate, is assumed to be present. After crossing, the breeding program proceeds with a series of selections (genetic discrimination) of desirable recombinants. Thus molecular genetic marker technology provides the most direct means for cultivar identification and genetic relationship analysis. Recent reports have focused on using DNA-based markers, particularly random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers, to measure the genetic diversity in numerous fruit species. A number of systems have been studied for litchi germplasm in China, including random amplified polymorphic DNA (RAPD) (Ding et al. 2000; Chen et al. 2004; Liu and Mei 2005; Wang et al. 2006), amplified fragment length polymorphism (AFLP) (Yi et al. 2003; Peng et al. 2006), sequence-related amplified polymorphism (SRAP) (Zan et al. 2009), inter-simple sequence repeat (ISSR) (Wei et al. 2006), and simple sequence repeat polymorphism (SSR) (Yao et al. 2009; Xiang et al. 2010). However, these studies were not based on broad genomic resource, and the numbers of markers used are limited. Recently, single nucleotide polymorphisms (SNPs) have become interesting and most popular molecular genetic marker in plants. Compared to previous marker systems, SNPs have several advantages. Firstly, SNPs are the most abundant sequence variation class in plant genomes (Rafalski 2002). Secondly, SNP markers display a low rate of new mutations (Brumfield et al. 2003). Thirdly, SNPs are mostly biallelic and amenable to high-throughput genotyping and automation (Chagné et al. 2007). Fourthly, different SNP alleles are distinguished on the basis of the nucleotides present at a given position, avoiding any allele binning and allowing standardization and direct

comparison of data arising from different laboratories. In 2010, the litchi genome sequencing and resequencing project was initiated by researchers from the institutes of China. Litchi and Longan Industry Technology Research System (Project No. CARS-33) and many genomic resources have been developed for litchi including validated SNP sequences and high-density linkage maps. Deployment of available genomic resources and high-throughput tools will expedite cultivar identification standardization and the genetic relationship assessment of the litchi collection. Isozymes have also been reported to be used as a convenient and reliable genetic marker because they exhibit codominant expression and do not show environmental effects (Torres and Bergh 1980). Recently, Stern et al. (1993) reported polymorphism of phosphoglucose isomerase (PGI) isozyme system and demonstrated its use for the unequivocal identification of selfed or outcrossed litchi fruits produced in adjacent blocks of "Mauritius" and "Floridian." The study extends the number of useful isozyme systems in litchi and helps to identify litchi cultivars in germplasm collection. Degani et al. 1995 have shown isozyme polymorphism in aconitase, aspartate aminotransferase, isocitrate dehydrogenase, phosphoglucomutase, shikimate dehydrogenase, superoxide dismutase, and triosephosphate isomerise.

14.8 Cytogenetics in Litchi

The cytogenetics study of litchi has not received much attention, and there are very few reports like Liu's (1954) and Chapman's (1984). Thus this approach needs proper attention and extensive exploration to identify economically important cultivars and evolve new cultivars. The new species probably originated and evolved through hybridizations of wild progenitor varieties. Haploid chromosome numbers of 14, 15, 16, and rarely 17 were reported, and variable chromosome number pointed to multiple progenitor origin. Liu (1954) studied the "mountain litchi" and reported them to be of inferior fruit quality clearly distinct from other elite varieties in tolerating frost.

14.9 Breeding Strategy for Improvement of Litchi

Litchi seedlings from a cultivar generally resemble the parent tree, but few bear regularly and maintain the genomic set and fruit quality at next generation. Breeding objectives include regular high yields, good tree structure, large fruit, bright red skin, small seed or seed abortion, better flavor and texture, and early or late fruit maturity. Resistance to pests and diseases and extremes of environment, acceptable fruit ripening pattern, and acceptable shelf life have received less attention. In addition to these, other characters like precocity, dwarfness, and regularity of bearing diversify litchi cultivation and lead to wider adaptability of tree characters and resistance to physiological disorders. In nature variations arise through sexual reproduction between different varieties, providing the basis for new selections with new genotypes. Thus sexual reproduction can be exploited and regulated to meet the

urgent need for raising new varieties through breeding. There are few literary reports on litchi breeding to raise new varieties and their selections, for which programs were initiated sporadically at Hawaii (Storey et al. 1953), Queensland, Australia (Cull 1977), and Saharanpur, India (Lal and Nirwan 1980). The outcome of this program was important cultivars like "Groff" and "Brewster." Undertaking a breeding program on a large scale needs a comprehensive survey of various genotypes and their inheritance pattern because of obvious difficulties in litchi breeding (Hamilton and Yee 1970; Menzel 1985).

Several cultivars have been developed in the past 60 years which includes "Salathiel" in Australia; "Sah Keng" and "Haak Yip" in Taiwan province of China; "Saharanpur Selection," "Swarna Roopa," and "Sabour Bedana" in India; and several new cultivars from different litchi-growing areas of China.

New cultivars have mainly been developed from the selection of open-pollinated seedlings from existing cultivars in different countries. Most of the modern cultivars have been developed in China and India, with new cultivars still being released in Guangdong. Some of the industries elsewhere in Asia are based on seedlings of cultivars imported from China. Breeding programs have generally been limited to clonal selection or selection from open-pollinated seedling population.

14.10 Genetic Engineering Approaches for Litchi Improvement

The conventional method like breeding has produced few new cultivar of litchi because the species has a long juvenile period, the available germplasm exhibited apparent lack of genetic variability, and also it costs enormous amount of money, time, and land (Menzel 1992). Genetic engineering, including genetic transformation and induced mutations in vitro, provides the means for modifying important horticultural traits in perennial plant cultivars. Genetic engineering, induced mutations in vitro combined with tissue culture, followed by selection, can potentially be used to produce one or more horticulturally important traits in litchi.

Puchooa (2004) have reported the genetic transformation of leaf tissue of litchi using *Agrobacterium*. The in vitro grown leaf explants were infected with *Agrobacterium* (strain LBA 4404), carrying the modified green fluorescent protein (mGFP4) driven by 35S promoter, in petri dish containing *Agrobacterium* transformation medium that consisted of MS salts and vitamins supplemented with 225 mg L^{-1} of each ascorbic and citric acid, 30 g L^{-1} sucrose, 2,4-D (4.52 mM), BAP (2.22 mM), and 1 ml of bacterial suspension. The plates were incubated for 20 min at room temperature with gentle shaking to achieve the *Agrobacterium* infection. Further, the leaf explants were cocultivated for 2 days on solid medium having the same composition but supplemented with 0.25% Phytagel. The cocultivation was followed by washing with liquid culture medium to remove the bacteria. The washed leaves were then transferred to regeneration medium comprised of MS salts and vitamins, 225 mgL^{-1} of each ascorbic and citric acid, 30 g L^{-1} sucrose, IAA (3.42 mM), and BAP (13.31 mM) solidified with 0.25% Phytagel, containing 50 mg L^{-1} kanamycin and

300 mg L^{-1} vancomycin. The explant plates were kept under white fluorescent light at a photon flux of 27 μE m^{-2} s^{-1} at 16 h light/8 h dark period at a temperature of 25 ± 2 °C. Expression of mGFP gene was confirmed by fluorescent microscopy in kanamycin-resistant regenerated callus. Most of the kanamycin-resistant regenerants showed glowing cells surrounded with tissue. The results demonstrated that leaf tissue can be used for transiently or stable transformation of gene of interest.

Litchi has been transformed with rice chitinase gene which showed enhanced tolerance to fungal pathogen (Das 2012). Zygotic embryos of litchi were incubated with *Agrobacterium* (strain LBA4404) for 2 days. *Agrobacterium tumefaciens* were transformed with gene construct: pGL2 vector containing selectable marker, hygromycin phosphotransferase (hpt) driven by 35S promoter, and RCC11 gene (chitinase-coding region, GenBank Acc. No. X54367) under the control of constitutive maize ubiquitin promoter. The presence of foreign gene in regenerated plantlets was confirmed by PCR and southern hybridization. Further, expression of RCC11 gene mRNA and protein in putative transgenic plantlets was analyzed by RT-PCR and Western blot, respectively. In gel analysis the presence of a number of chitinase isoform in transformed and untransformed plantlets was exhibited, but an additional isoform was observed only in transformed lines corresponding to the expected size of foreign gene. The regenerated transformed lines displayed differential chitinase enzyme activity in chitinase enzyme assay; some of the transgenic lines had two- to fourfold increased chitinase activity in comparison to non-transformed plants. To check the resistance to fungus, detached transgenic leaves as well as non-transformed leaves were infected with phytopathogenic fungus, *Phomopsis sp*. The transgenic lines demonstrated approximately three times decreased disease rating score in addition to delay in the onset of necrosis and spread of lesion areas from the disease in comparison to non-transformed control.

Two different types of seedless litchi fruit occur: aborted-seeded fruit and parthenocarpic fruit. Aborted-seeded fruits, known as "chicken tongue," arise from abnormal ovules or as a result of incomplete fertilization, whereas parthenocarpic seedless fruits develop when the ovary has not been fertilized. Padilla et al. (2013) have taken an approach to develop parthenocarpic litchi fruits. In accordance, they have transformed embryogenic cultures of "Brewster" ("Chen Tze") litchi with an *Arabidopsis thaliana* floral regulatory gene, *PISTILLATA* (*PI*), in the antisense orientation, expected to suppress the homologous gene (*PI-Lch*) in litchi and ultimately to get parthenocarpic fruits. The embryonic culture of Brewster was cocultured with EHA105/pCAMBIA3301/BPISTILLATA continuously for 3 months along with selection agent, phosphinothricin (2 mg L^{-1}). The transformants were verified by histochemical staining to check β-glucuronidase activity. Further, the presence of foreign gene and its copy number were confirmed by PCR and qPCR, respectively. It was found that the expression level homologous gene in transformed litchi was successfully suppressed, even up to sevenfold in one of the lines. This result suggests that post-transcriptional silencing of the litchi *PISTILLATA* (*PI*) homolog induced by an antisense-oriented transgene could be a successful strategy to produce parthenocarpic fruit; however, it can only be confirmed when the author will mature plants after several years.

14.11 Conclusion and Future Perspective

Due to its increasing demand, the area under litchi cultivation has increased rapidly. However, there is need for improving productivity by adoption of suitable cultivars for various climatic conditions. It is also essential to develop promising lines/hybrids, which have larger fruit size, small/chicken-tongued seeds, and tolerance to pericarp splitting, and having various maturity groupings. This can only be achieved only if current progress in tissue culture and genetic transformation is combined with biotechnological applications. The basic in vitro protocol for manipulating elite longan selections is available and could be utilized to address litchi plant-breeding objectives. Defined protocol for de novo regeneration pathway from elite litchi selections should be developed, and comparable studies must be made with other species. From the literature it is evident that genetic transformation of litchi, and longan, is feasible and requires further research to develop an easy and less economical reproducible method. Longan, because it can be regenerated from embryogenic cultures of elite selections with a high rate of conversion, could be transformed with genes that encode for resistance to fungal diseases such as *Anthracnose*. Further work on in vitro mutation induction and selection procedures needs to be addressed to develop fungal-resistant litchi and longan cultivars. Genetic transformation could possibly be utilized to develop the preferred "chicken tongue" seeds, using the pistillate gene from *Arabidopsis thaliana*, which mediates seedlessness. Protoplast technology could be harnessed to produce somatic hybrids between haploids and diploids, as a way of producing seedless triploids. The resolution of problems associated with rapid deterioration of fruit after harvest is more difficult to address at this time, since litchi and longan are non-climacteric. The successful regeneration of mature longan and soap nut and related species from embryogenic cultures should be undertaken in litchi. The fast developing tissue culture approach and genetic engineering tools in future may witness super tree species tailored for special agronomic and economic characteristics.

References

Amin MN, Razzaque MA (1995) Induction of somatic embryogenesis in the cultures of zygotic embryos of lychee. Bangladesh J Bot 24:25–29

Anon (1991) Tissue culture of lychee (*Litchi chinensis* Sonn.). CSFRI Inf Bull 229:1–2

Brumfield RT, Beerli P, Nickerson DA, Edwards SV (2003) The utility of single nucleotide polymorphisms in inferences of population history. Trends Ecol Evol 18:249–256

Chagné D, Batley J, Edwards D, Forster J (2007) Single nucleotide polymorphism genotyping in plants. In: Oraguzie N, Rikkerink EA, Gardiner S, De Silva HN (eds) Association mapping in plants. New York, Springer, pp 77–94

Chaikiattiyos S, Menzel CM, Rasmussen TS (1994) Floral induction in tropical fruit trees: effects of temperature and water supply. J Hort Sci 69:397–415

Chapman KR (1984) Sapindaceae. In: Page PE (ed) Tropical tree fruits for Australia. Queensland Dept. of Primary Industries, Queensland, pp 179–191

Chen YY, Deng SS, Zhang X, Wei SX, Gao AP, Wang JB (2004) RAPD analysis of genetic relationship among partial litchi germplasms in Hainan Island. Acta Hort Sinica 31:224–226

Cull BW (1977) Report on overseas study tour, Queensland Department of Primary Industries, pp 1–92
Das DK (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell Tissue Organ Cult 109:315–325
Das DK, Shiva Prakash N, Bhalla-Sarin NB (1999) Multiple shoot induction and plant regeneration in litchi (*Litchi Chinensis* Sonn.). Plant Cell Rep 18:691–695
Degani C, Beiles A, El-Batsri R, Goren M, Gazit S (1995) Identifying lychee cultivars by isozyme analysis. J Amer Soc Hort Sci 120(2):307–312
Deng CJ (2005). Studies on high frequency somatic embryogenesis and regeneration culture in litchi. Master's thesis of Hunan Agricultural University, Changsha, Hunan, China.
Ding XD, Lv LX, Chen XJ, Guan X (2000) Identifying litchi cultivars and evaluating their genetic relationships by RAPD markers. J Trop Subtrop Bot 8:49–54
Fivas J (1994) Litchi production in Israel. Yearbook of the South African Litchi Growers' Association 6:39–41
Fu L, Tang D (1983) Induction pollen plants of litchi tree (*Litchi chinensis* Sonn.). Acta Genet Sinorama 10(5):369–374
Guo SY, Peng HX, He XH, Ding F, Li HL, Qin XQ, Xu N (2014) Callus induction from different explants of litchi. Southwest China J Agric Sci 27(2):748–753
Hamilton RA, Yee W (1970) Lychee cultivars in Hawaii. ProcTrop Am Soc Hortic Sci 14:7–12
Jain SM, Maluszynski M (2004) Induced mutations and biotechnology on improving crops. In: Mujib A, Cho M, Predieri S, Banerjee S (eds) In vitro applications in crop improvement: recent progress. IBH-Oxford, New Delhi, pp 169–202
Kantharajah AS, McConchie CA, Dodd WA (1992) In vitro embryo culture and induction of multiple shoots in lychee (*Litchi chinensis* Sonn.). Ann Bot 70:153–156
Kuang ZS, Zhou LN, Ma XJ, Chen JQ, Cai J (1997) Plant regeneration via somatic embryogenesis in litchi. Guangdong Agric Sci 1:15–17
Lai ZX, Sang QL (2003) Optimization of somatic embryogenesis system from embryogenic callus and plant regeneration from transformed *hpt*-resistant calli of litchi (*Litchi chinensis* Sonn.). Chin J Appl Environ. Australas Biotechnol 9(2):131–136
Lal BS, Nirwan RS (1980) Saharanpur selection a new litchi cultivar. Progress Hortic 12:75–77
Litz RE (1988) Somatic embryogenesis from cultured leaf explants of the tropical tree Euphoria longan Stend. J Plant Physiol 132:190–193
Liu CM, Mei MT (2005) Classification of lychee cultivars with RAPD analysis. Acta Hortic 665:149–159
Liu SY (1954) Stuidies on (*Litchi chinensis* Sonn.). Diss Abstr 14:1018–1019
Matsumoto K, Raharjo SHT, Dhekney S, Moon PA, Litz RE (2004) Criopreservação e embriogênese somática de calos de *Dimocarpus longan* (Cryopreservation and somatic embryogenesis from *Dimocarpus longan calli*). Pesq Agropec Brasil Brasília 39:1261–1263
McConchie CA, Vithanage V, Batten DJ (1994) Intergeneric hybridization between litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan Lour.*). Ann Bot 74:111–118
Menzel CM (1985) Propagation of lychee: a review. Sci Hortic 25:31–48
Menzel CM (1992) *Litchi chinensis* Sonn. In: Verheij EWM, Coronel RE (eds) Plant resources of South-East Asia No. 2: edible fruits and nuts. Pudoc, Wageningen, pp 191–195
Nacif SR, Paoli AAS, Salomao LCC (2001) Morphological and anatomical development of the litchi fruit (*Litchi chinensis* Sonn. cv. Brewster). Fruits 54:225–233
Oosthuizen JH (1991) Lychee cultivation in South Africa. Year book of the Australian Lychee Growers' Association 1:51–55
Padilla G, Pérez JA, Arango IP, Moon PA, Gómez-Lim AM, Borges AA, Rodríguez ME (2012) Richard Agrobacterium tumefaciens-mediated transformation of 'Brewster' ('Chen Tze') litchi (*Litchi chinensis* Sonn.) with the PISTILLATA cDNA in antisense. In Vitro Cell Dev Biol-Plant 49:510–519
Peng HX, Cao HQ, Zhu JH, Liao HH, Huang FZ, Li JZ et al (2006) Studies on the application of AFLP molecular markers on genetic diversity and classification of good and rare litchi resources in Guangxi. Southwest China J Agr Sci 19:108–111

Puchooa D (2004) *In vitro* regeneration of lychee (*Litchi chinensis* Sonn.). Afr J Australas Biotechnol 3(11):576–584
Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94–100
Shukla RK, Bajpai PN (1974) Blossom bud differentiation and ontogeny in litchi (*Litchi chinensis* Sonn.). Indian J Hortic 31:224–228
Stern RA, Gazit S (1997) Effect of 3, 5, 6-trichloro-2-pyridyl-oxyacetic acid on fruitlet abscission and yield of 'Mauritius' litchi (*Litchi chinensis* Sonn.). J Hortic Sci Biotech 72:659–663
Stern RA, Gazit S, El-Batsri R, Degani C (1993) Pollen parent effect on outcrossing rate, yield and fruit characteristics of 'Floridian' and 'Mauritius' lychee. J Am Soc Hortc Sci 118:109–114
Storey WB, Hamilton RA, Nakasone HY (1953) Groff – a new variety of lychee. Circ Hawaii Agric Exp Stn 39:1–8
Torres AM, Bergh BO (1980) Fruit and leaf Isozymes as genetic markers in avacado. J Amer Soc Hort Sci 105:614–619
Vos JE, Du Preez RJ, Froneman I, Hannweg K, Husselman J, Rheeder S (2009) Induced mutation in tropical fruit trees. IAEA, Vienna, pp 123–126
Wang JB, Deng SS, Liu ZY, Liu LZ, Du ZJ, Xu BY et al (2006) RAPD analysis on main cultivars of litchi (*Litchi chinensis* Sonn.) in Hainan. J Agr Biotechnol 14:391–396
Wang G, Li HL, Wang JB (2013) Primary study on the callus induction from anther of two litchi (*Litchi chinensis* Sonn.) cultivars. Chin J Trop Crop 34(4):669–674
Wei SX, Chen YY, Xie ZS, Zhu M, Luo SR (2006) ISSR analysis of parts of lychee germplasm in Hainan province. Chin J Trop Crops 27:51–55
Xiang X, Ou LX, Chen HB, Sun QM, Chen JZ, Cai CH et al (2010) EST-SSR analysis of genetic diversity in 96 litchi (*Litchi chinensis* Sonn.) germplasm resources in China. Genomics Appl Biol 29:1082–1092
Xie YM, Yi GJ, Zhang QM, Zeng JW (2006) Somatic embryogenesis and plantlet regeneration from anther of Feizixiao litchi. Chin J Trop Crops 27(1):68–72
Yao QR, Zhao CZ, Wang WQ (2009) Analysis of genetic relationship of Hainan litchi germplasm resources by SSR marker. B Bot Res 29:628–632
Yi GJ, Huo HQ, Chen DC, Huang ZR, Cai CH, Qiu YP (2003) Studies on genetic relationship among litchi varieties by using AFLP. Acta Hort Sinica. 30:399–403
Yuan RC, Huang HB (1988) Litchi fruit abscission its pattern, effect of shading and relation to endogenous abscisic acid. Sci Hortic 36:281–292
Yu CH, Chen Z (1997) Induction of litchi embryogenic calli by immature embryos and anthers culture *in vitro*. J Fujian Agric Univ 26:168–172
Yu CH, Chen ZG, Lu LX (1996) Regeneration of plantlets from protoplasts culture of litchi
Yu CH, Chen ZG, Lu LX, Lin JW (2000) Somatic embryogenesis and plantlets from Xiafanzhi litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Organ Cult 61:51–58
Zan FG, Wu ZD, Zeng Q, Zhang HY, Li MF, Zheng XQ (2009) Genetic diversity analysis of litchi germplasm by SRAP markers. Mol Plant Breeding 7:562–568
Zhou LN, Kuang ZS, Ma XJ, Cheu JQ, Cao J, Tang XJ, Ling X (1993) Preliminary studies on culture of immature embryos and embryogenesis of somatic cells of litchi (*Litchi chinensis* Sonn) Guangdong Agric. Science 5:14–15
Zhou LN, Kuang ZS, Ma XJ, Cheu JQ, Cao J (1996) The study of factors affecting somatic embryogenesis in young embryo culture of *Litchi chinensis*. J Agric Biotechnol 4(2):161–165

Ex Situ Conservation Strategies in Litchi Germplasm

15

Aparajita Das, Ajit Varma, Ruchira Pandey, and Rekha Chaudhury

Abstract

The delicious fruit *Litchi chinensis* is a commercially important heterozygous cross-pollinated fruit species of the world. To increase its production of this fruit, plant breeding is required. Henceforth, the conservation of its germplasm resources is an important step toward improvement of new cultivars resistant to abiotic and biotic stresses through selection and breeding technologies. 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.

Keywords

Cryopreservation • Litchi • Germplasm • In situ and ex situ conservation • In vitro • Seed gene bank • Recalcitrant seeds

A. Das (✉) • A. Varma
Amity Institute and Microbial Technology, Amity University, Noida, Uttar Pradesh, India
e-mail: aparajitadas11@gmail.com

R. Pandey • R. Chaudhury
Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources, Pusa campus, New Delhi 110012, India

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15.1 Introduction

Litchi chinensis commonly called Litchi (2n = 8–32) is an economically important heterozygous cross-pollinated fruit crop of the world. This delicious fruit has its origin in southern China and adjoin areas. The plant species belonging to family Sapindaceae has a tremendous domestic market as well as export potential. It has highly specific climatic requirements, and probably due to this reason, its cultivation is restricted to few countries in the world. This important tropical and subtropical fruit species is grown in South Africa, Madagascar, Japan, China, Taiwan, India, Jamaica, Thailand, Kenya, the USA (Florida), Australia, Brazil, and New Zealand. China is the leading producer of litchi followed by India. In India, it is grown in the states of Bihar (North Muzaffarpur and Darbhanga districts), West Bengal, Tripura, Punjab, Uttar Pradesh, and Haryana (Karihaloo et al. 2005).

Litchi is a seasonal and non-climacteric fruit that grows in loose bunches of 2–20 (Fig. 15.1). Fruits are oval or round in shape with a diameter of about 3–4 cm and are very attractive, with bright red skin covered by angular or conical protuberances. Edible portion of litchi is aril (fleshy appendage or covering of seeds), which is firm and sweet and has juicy pulp (Fig. 15.2). In general, fruit consists of 60% juice, 8% rag, 19% seed, and 13% skin varying upon different genotypes and climatic conditions. In a good cultivar, translucent white, juicy, and sweet aril may comprise 80% of fruit weight. The aril surrounds a single dark brown seed 6–12 mm wide and 10–23 mm long (http://www.nrclitchi.org/ dated 20.5.2016). Seeds of litchi are large and recalcitrant in seed storage behavior. In some varieties seeds are partly developed as a result of failure of pollination and these are known as "chicken-tongue" seeds. The tree varieties with small seeded fruits are of greater value due to larger pulp content.

Litchi is a good source of vitamin C (40-90 mg/100 g). Vitamin C is an effective anti-oxidant that helps the body to fight diseases and promotes healthy bones and skin. This juicy delightful fruit is also rich in B vitamins that play vital roles in metabolism. It is also good source of copper, which is needed for red blood cell production, and potassium, which helps regulate electrolyte levels, blood pressure, and heart rate in the body. But litchi fruit has minor amount of protein (0.8–0.9%), fat (0.3%), pectin (0.43%), and minerals especially calcium, phosphorus, and iron (0.7%) (Monshi et al. 2015; http://www.fruitipedia.com/ dated 20.5.2016).

To improve the production of this commercially important fruit species, plant breeding is required. Genetic diversity in litchi germplasm comprises large number of cultivars that are grown in India and China which offers options for development of new cultivars resistant to abiotic and biotic stresses through selection and breeding technologies. For litchi breeding projects, it is decisive to understand the genetic relationships and population structures of litchi germplasm as it aids in the selection of optimal parental combinations and avoidance of genetic redundancy (Liu et al. 2015). In India, Litchi has a narrow genetic base. Under given climatic conditions in India, fruits are available only for 3–4 weeks. (http://www.fao.org/.dated 20.5.2016; Bajpai et al. (2016); http://tmnehs.gov.in/ dated 20.5.2016). Germplasm collection and their appropriate conservation is henceforth required for good

Fig. 15.1 (**a**) Mature litchi fruits. (**b**): Litchi tree with fruits

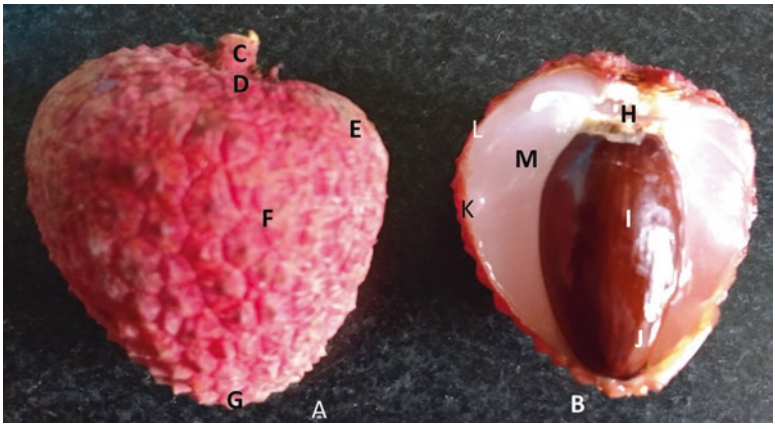

Fig. 15.2 External (**a**) and internal (**b**) structure of a litchi fruit; *C*: Stalk; *D*: Base; *E*: Shoulder; *F*: Skin segment; *G*: Apex; *H*: Seed Stalk; *I*: Seed; *J*: Testa (Seed coat); *K*: Exocarp (outer seed coat); *L*: Endocarp (Inner fruit skin); *M*: Aril

breeding program. There are two major strategies for conserving plant biodiversity, namely, in situ and ex situ conservation. Both conservation are equally significant and should be regarded as complementary (Dar et al. 2015; Thormann et al. 2006; Engelmann and Engels 2002). This chapter highlights the different ex situ germplasm conservation strategies for litchi.

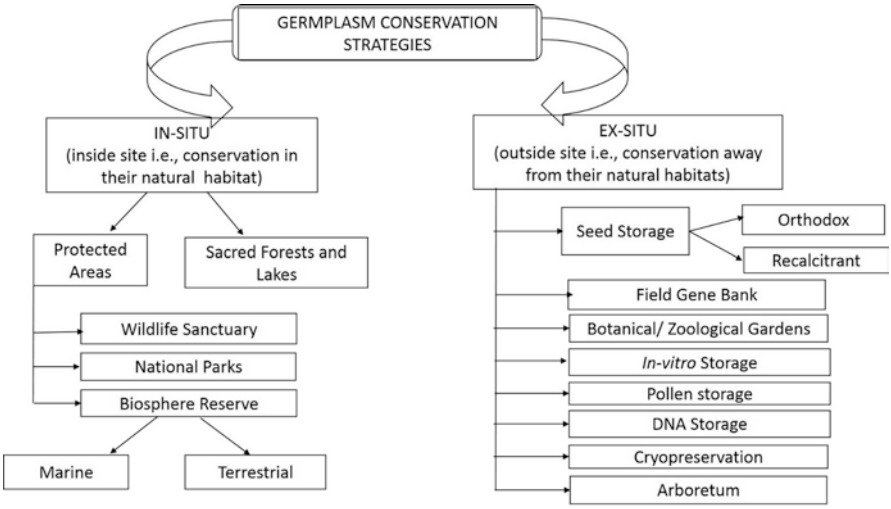

Fig. 15.3 Major approaches for germplasm conservation

15.2 Major Approaches for Germplasm Conservation

Two major approaches used for conservation of plant genetic resources are in situ and ex situ (Fig. 15.3). Conservation approach involving maintenance of genetic resources in their natural habitats or in man-made ecosystem where they occur is known as in situ conservation. Conservation of genetic resources outside their natural habitats by perpetuating sample populations in an artificially created environment is called ex situ conservation (Dar et al. 2015).

15.2.1 In Situ Germplasm Conversation

In situ (on-site, in place) conservation is the most suitable way of conserving germplasm in its natural habitat or in man-made ecosystem. In situ conservation sites maintain many potentially useful and valuable genes and thus can provide important knowledge about a species' development and evolutionary processes. This method of conservation has its own disadvantages also, like risk of losing all germplasm because of sudden environmental hazards as well as other issues like cost, size and maintenance, political, and social issues. Different types of in situ conservation are biosphere reserves, national parks, and wildlife sanctuaries (Fig. 15.3). For in situ conservation, preference is for the perennials that are vegetative propagated (Hawkes 1975), and species whose seeds cannot survive cold storage are generally preferred for conservation (King and Roberts 1979; Hawkes 1982).

15.2.2 Ex Situ Germplasm Conservation

Ex situ (off-site, outside of place) conservation is used for preserving genetic resources away from its natural habitat. Its key objective is to ensure the survivability of endangered species and the conservation of associated genetic diversity. Different approaches used for ex situ conservation include seed, tissue and pollen storage Franchi et al. (2011), field gene banks, in vitro storage, DNA storage, and botanical gardens (Fig. 15.3). Different techniques like slow growth and cryopreservation are applied during ex situ conservation and are useful for declining population of species. Through this strategy the vulnerable species can be multiplied in captivity and then released in their natural habitats. This method of conservation also has its own advantages and disadvantages.

15.3 Ex Situ Germplasm Conservation Strategies in Litchi

15.3.1 Germplasm Conservation Through Seed

The most useful and convenient means of ex situ conservation of plant germplasm is by storage of seeds. According to Roberts (1973), seeds can be categorized into two groups based on their storage characteristics: orthodox and recalcitrant (Table 15.1).

A third group comprises intermediate seeds that show the drying tolerance characteristic of the orthodox seeds but lose their viability when exposed to low temperature storage like the recalcitrant seeds (Ellis et al. 1990a). Examples include seeds of *Carica papaya* and *Citrus limon*, etc. According to Bonner (1990) seeds are grouped into four types: (1) true orthodox, (2) sub-orthodox, (3) temperate recalcitrant, and (4) tropical recalcitrant (Table 15.2).

There are numerous limitations regarding storage of seeds for germplasm conservation which includes seed dormancy; non-applicability to vegetative-propagated plant species like sweet potato, ginger, and others; non-applicability to recalcitrant seeds like jackfruits, litchi, etc.; and high inputs of labor and cost of maintenance. Seeds of litchi are recalcitrant and lack desiccation tolerance presenting a big

Table 15.1 Difference between orthodox and recalcitrant seeds

Orthodox	Recalcitrant
Seeds which can be dried down to a low moisture content of around 5% (wet basis) without any damage and hence successfully stored at low or subfreezing temperatures for long periods	Seeds that cannot tolerate drying below a critical moisture level which range from 20–50% wet basis and hence cannot withstand low temperatures for storage
Desiccation-tolerant seeds	Desiccation-sensitive seeds
Seeds undergo maturation drying during their development	Seeds do not undergo period of maturation drying during their development
Examples: wheat, rice, etc.	Examples: *Litchi chinensis*, mango, etc.

Table 15.2 Difference between four types of seeds according to Bonner (1990)

True orthodox	Sub-orthodox	Temperate recalcitrant	Tropical recalcitrant
These seeds can tolerate desiccation and can be stored for long periods at subfreezing temperature, if their moisture contents are reduced to about 5–10% (wet weight basis)	Sub-orthodox seeds can be stored under the same conditions as true orthodox seeds but for much shorter periods	Temperate-recalcitrant seeds cannot be desiccated but can be stored at or slightly below temperature	These seeds are desiccation sensitive and are also sensitive to low temperatures. Viability is lost even short periods of exposure to temperatures below 10–15 °C
Examples: *Betula*, *Abies*	Examples: *Juglans*, *Fagus*	Examples: *Castanea*, *Quercus*	Examples: *Theobroma cacao*, *Araucaria*

challenge for its germplasm conservation through seeds (Chaudhury et al. 1996; Fu et al. 1990, Karihaloo et al. 2005). According to Ray and Sharma (1987) and Chaudhury and Malik (1999), recalcitrant litchi seeds have short viability with moisture content of seed around 28.5% (wet weight basis) at fruit maturity; there was 100% germination. When stored under ambient temperature (29–33 °C) for one week, the moisture content came down to 19%, and the seeds completely lost their viability after six days. Seeds lost their viability when the moisture content was below the critical level of 20%. For long-term germplasm conservation of both true and sub-orthodox seeds, it has been recommended that moisture content be reduced to 5 ± 1 °C (IBPGR 1976). Litchi being a recalcitrant species, efforts were made to store litchi seeds at 5 °C wherein about 36% viability could be maintained for 100 days (Fu et al. 1990). Limited success was obtained when attempts were made to desiccate litchi seeds and conserve them at sub-zero temperatures (Karihaloo et al. 2005) and when vitrification was used (Malik and Chaudhury 2003).

15.3.2 Field Gene Bank

Plant species which produce either recalcitrant seeds, little or no seeds, or reproduce by vegetative means are grown and maintained as live plants in fields known as field gene banks. Field gene banks offer a ready and easy access to the plant germplasm, for characterization, evaluation, or utilization in contrast to other methods of ex situ conservation like seed storage or cryopreservation wherein plants/explants are required to grow. At the same time, field gene banks are generally more costly to maintain and require more inputs, labor, and space (land) than other methods of conservation. There is also potential risk of losing germplasm due to natural disasters and adverse environmental conditions like floods, drought, or losses due to plant diseases (http://cropgenebank.sgrp.cgiar.org/ dated 27.5.16; Hawkes et al. 2000). Field gene banks are commonly used in the conservation of plant species like coconut, rubber, banana, cassava, etc.

Fig. 15.4 View of National Bureau of Plant Genetic Resources (NBPGR) in vitro gene bank

Litchi germplasm is currently conserved in field gene banks at various locations in India including Bihar Agricultural University, Sabour, Bihar (21 accessions); Agricultural Research Institute, Pusa, Bihar (15 accessions); ICAR Research Complex for Eastern Region, Ranchi, Jharkhand (51 accessions); Bidhan Chandra Krishi Vishwavidhalaya, Kalyani, West Bengal (15 accessions); and Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (20 accessions) (Singh 1998, Karihaloo et al. 2005). Intensive research is being carried out at ICAR-National Research Centre on Litchi, Muzaffarpur, Bihar.

15.3.3 In Vitro Storage

Biotechnological techniques are used effectively to conserve the economically important germplasm for plants which have difficult-to-store seeds through seed gene bank (Engelmann and Engels 2002). In vitro storage techniques are good options for plant species with recalcitrant seeds (Fig. 15.4). Plant species that do not produce fertile seeds or reproduce only by means of vegetative propagation are generally preferred to be conserved through in vitro techniques. Hence, in vitro techniques have pronounced potential for collection, exchange, and conservation of

plant germplasm although it has several advantages and some disadvantages as well (Bhojwani and Razdan 1996). Pathogen-free plants on aseptic medium can be maintained and is a useful alternative for providing backup to field collections (Monshi et al. 2015).

Advantages of Conservation of Plants Through In Vitro Techniques

1. Comparatively less space is required for maintaining of large numbers of clonally multiplied plants that are free from pests, pathogens, etc.
2. Under in vitro storage conditions, the plants do not require splitting and pruning and are protected from natural hazards.
3. Whenever required conserved germplasm can be multiplied in large numbers.
4. Enables conservation of endangered and exclusive economically important plants.
5. Plant species that either do not produce seeds or produce sterile seeds can be maintained via this technique.
6. In vitro plants can be easily transported and have less quarantine restrictions with respect to germplasm exchange.

Disadvantages of Conservation of Plants Through In Vitro Techniques

1. May lead to soma clonal variation due to repeated subculture.
2. Maintenance is difficult as it requires numerous serial subcultures, and germplasm may be lost because of contamination from frequent serial subculture.

The technique of in vitro culture is based on standardizing media for each genotype to get the optimal response in terms of growth of the explants. Conversely, when this technique is employed for conservation of germplasm, the aim is to develop a medium that would reduce the growth of explants, thus reducing the subculture intervals (Engelmann and Drew 1998; Sarkar and Naik 1999 and Ogbu et al. 2010; Ramya et al. 2014). Various approaches to accomplish this includes use of growth retardants like abscisic acid and use of osmotic regulators like sorbitol, mannitol, etc., size and type of culture vessels, type of enclosures like plastic caps or cotton plugs, type of explant like apical/auxiliary buds, reduction in oxygen concentration, maintenance under reduced light intensity and temperature, and combination of more than one treatment. Explants used for in vitro conservation must be of right size and physiological stage. In terms of genetic stability of germplasm, organized culture systems are preferred (Mandal 2003; Reed et al. 2004; Chaudhury and Vasil 1993; Kameswara Rao 2004; Ogbu et al. 2010; Ramya et al. 2014).

Litchi being a cross-pollinated species is highly heterozygous, and thus the progeny is not true to its parental type. As a result litchi plant propagation via seeds is not desirable, and asexual means of propagation is preferred for plant multiplication. To raise plantlets of elite germplasm of litchi through in vitro techniques has plenty of potential. However, it is a challenging task to propagate litchi by employing in vitro techniques due to various reasons (Figs. 15.5 and 15.6). One of the problem is the secretion of polyphenols into the medium by its implanted explants.

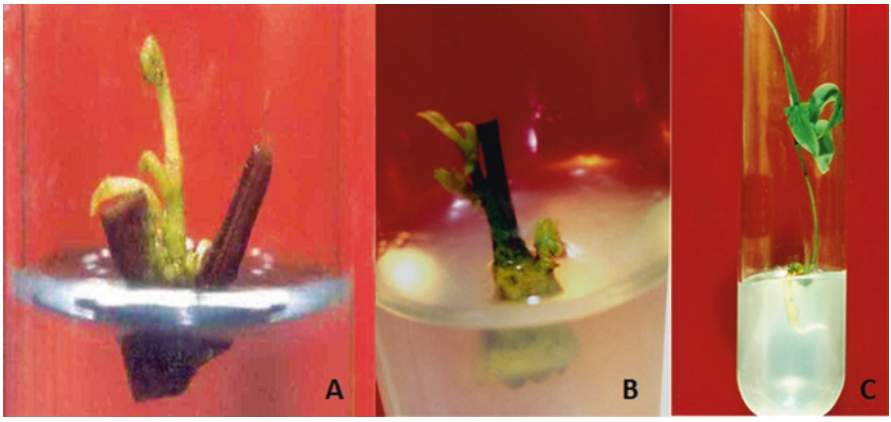

Fig. 15.5 (**a**), (**b**), (**c**) Different stages of in vitro propagation of litchi through nodal cutting (Source: Ph.D thesis, Manoj Kumar-2006)

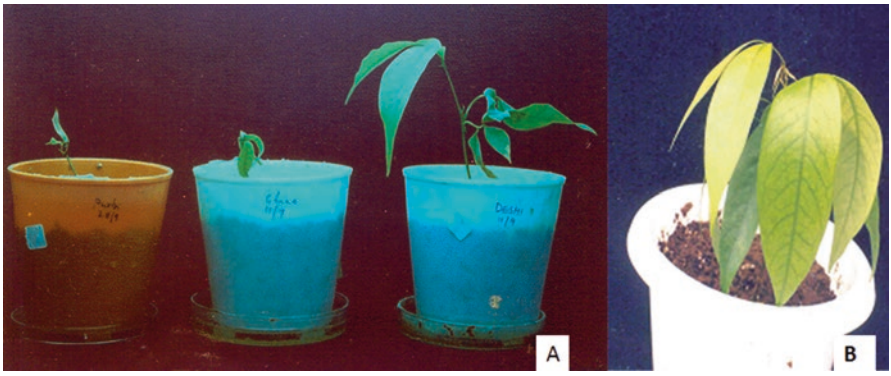

Fig. 15.6 (**a**) and (**b**) In vitro regenerated plants (derived from nodal explants) under acclimatization under greenhouse condition (Source: Ph.D thesis, Manoj Kumar-2006)

Polyphenols undergo oxidation to produce substances that kill the tissues (Kantharajah et al. 1992; Kumar et al. 2004). Another difficulty is the high degree of contamination in explants obtained from field-maintained litchi plants which is difficult to remove prior to culture (Kumar et al. 2004). Multiple shoot formation in five genotypes of *Litchi chinensis* Sonn. was induced by direct germination of litchi seeds in MS liquid medium supplemented with benzyl adenine. Somatic embryogenesis and plant regeneration were achieved from litchi protoplasts isolated from immature zygotic embryos (Yu et al. 2000) and from leaves of mature phase trees (Raharjo and Litz 2007).

Fig. 15.7 (**a**) View of National Bureau of Plant Genetic Resources (NBPGR) in vitro gene bank. (**b**) Cryo-banking of germplasm

15.3.4 Cryopreservation

Cryopreservation is a worthwhile technique for long-term conservation of germplasm of biological tissues. In this method living tissues are preserved at ultralow temperatures (−196 °C) of liquid nitrogen (LN) or in the vapor phase (−150 °C) wherein mitotic and metabolic activities are arrested and hence need for subculture, and threat of soma clonal variation are also reduced (Thormann et al. 2006). Cryopreserved samples require less space for storage although constant supply of liquid nitrogen is required (Engelmann 2000) (Fig. 15.7). Various techniques of cryopreservation have been advocated as the most promising approach to preserve germplasm of recalcitrant seeds like litchi, jackfruit, etc. (Malik et al. 2003; Chaudhury et al. 2010a, b; Malik et al. 2010; Ramya et al. 2014).

Litchi seeds remain viable for 4 weeks when they are intact in fruits. Seeds lose viability within a day of removal from fruits since it is a highly recalcitrant species. Depending on the relative humidity of storage environment, the litchi seeds become nonviable within 4–15 days. Hence, long-term storage of litchi germplasm via cryopreservation techniques using embryonic axis or pollens is an encouraging option (Chaudhury et al. 2010a, b; Malik et al. 2010; Berjak and Pammenter 2013; Dar et al. 2015). Scientists have reported successful cryopreservation of embryonic axes of litchi varieties Calcuttia and Rose scented with a recovery of 40–70% viability (Karihaloo et al. 2005).

In litchi plants, out of three different types of flowers, i.e., male, pseudohermaphrodite, and female, only the female flowers set fruit. Long-term storage of viable pollen of litchi cultivars for breeding and exchange of germplasm programs are highly desirable. Cryopreserved pollen can be simply stored in large quantities in a reasonably small space (Dar et al. 2015; King 1965). Scientists at National Bureau of Plant Genetic Resources (NBPGR), India, have developed an improved

method for pollen collection from freshly dehiscing dehisced anthers of litchi using cyclohexane. Using this technology pollen of 19 cultivars of litchi has been stored in the cryogene bank thus facilitating breeding programs over the long-term. With the help of this technique, cryopreservation was effectively applied to obtain long-term storage of pollen over a period of up to 4 years (Chaudhury et al. 2010a, b).

15.4 Conclusion

In this book chapter, various methods of ex situ conservation of litchi germplasm are discussed. Litchi being an important fruit plant species has a remarkable domestic and international market potential. Conservation of litchi germplasm via various ex situ approaches to ensure the survivability of litchi genetic diversity is an important step toward sustainable agriculture. Litchi being a recalcitrant plant species is difficult to store its germplasm through its seeds in seed gene bank. To maintain litchi germplasm in the field gene bank and in the in vitro gene bank is a challenging task owing to various biotic and abiotic factors. Hence, cryopreservation of litchi germplasm using its embryonic axis or pollens is a promising option for conservation of germplasm. Development of an effective and sustainable conservation, ex situ conservation strategies for litchi germplasm should be a priority for human welfare.

References

Bajpai A, Muthukumar M, Singh A, Nath V, Ravishankar H (2016) Narrow genetic base of Indian litchi (*Litchi chinensis*) cultivars based on molecular markers. The Indian Journal of Agricultural Sciences. http://epubs.icar.org.in/ejournal/index.php/IJAgS/article/view/57435

Berjak P, Pammenter NW (2013) Implications of the lack of desiccation tolerance in recalcitrant seeds. Front Plant Sci 4: http://dx.doi.org/10.3389/fpls.2013.00478

Bhojwani SS, Razdan MK (1996) Germplasm storage. In: Bhojwani SS, Razdan MK (eds) Plant tissue culture: theory and practice. Elsevier, Amsterdam, pp 563–586

Bonner FT (1990) Storage of seeds: Potential and limitations for germplasm conservation. Conserv Div For Ecosys 35:35–43

Chaudhury MKU, Vasil IK (1993) Molecular analysis of plant regenerated from embryogenic cultures of apple. Genetics 86:181–188

Chaudhury R, Malik SJ (1999) Cryopreservation and *in vitro* technology for conservation of recalcitrant seeds of some tree species. In: Marzalina M, Khoo KC, Jayanthi N, Tsan FY, Krishanapillay B (eds) Proc. IUFRO seed symposium 1998 'Recalcitrant seeds', held at FRIM, Kuala Lumpur, Malaysia, pp 119–131

Chaudhury R, Chandel KPS, Malik SK (1996) Development of cryopreservation techniques for conservation of germplasm of jackfruit, litchi and cocoa: biochemical, biophysical and ultrastructural approach. In: Proc. 33rd Annual meeting of the Society for Cryobiology, held at Indianapolis, Indiana, USA. Aug 17–21, 1996

Chaudhury R, Malik S, Rajan S (2010a) An improved pollen collection and cryopreservation method for highly recalcitrant tropical fruit species of Mango (*Mangifera indica* L.) and Litchi (*Litchi chinensis* Sonn.). CryoLetters 31:268–278

Chaudhury R, Malik SK, Joshi J, Shivanna KR (2010b) Cryoconservation of pollen for germplasm storage and for utilization in breeding programmes. J Palynol 46:205–218

Dar RA, Ahmad M, Kumar S, Reshi M (2015) Agriculture germplasm resources: a tool of conserving diversity. Sci Res Essays 10:326–333

Ellis RH, Hong TD, Roberts EH (1990a) An intermediate category of seed storage behaviour? I. Coffee. J Exp Bot 41:1167–1174

Engelmann F (2000) Importance of cryopreservation for the conservation of plant genetic resources. In: Engelmann F, Takagi H (eds) Cryopreservation of tropical plant germplasm, Current research progress and application. IPGRI, Rome, pp 8–20

Engelmann F, Drew RA (1998) *In vitro* germplasm conservation. Acta Hortic 461:41–47

Engelmann F, Engels JMM (2002) Technologies and strategies for *ex situ* conservation. In: Engels JHH, Rao VR, Brown AHD, Jackson MT (eds) Managing plant genetic diversity. CABI, Wallingford; IPGRI, Rome, pp 89–104

Franchi GG, Piotto B, Nepi M, Baskin CC, Baskin JM, Pacini E (2011) Pollen and seed desiccation tolerance in relation to degree of developmental arrest, dispersal, and survival. J Exp Bot:1–15. doi:10.1093/jxb/err154

Fu JR, Zhang BZ, Wang XP, Qiao YZ, Huang XL (1990) Physiological studies on desiccation, wet storage and cryo- preservation of recalcitrant seeds of three fruit species and their excised embryonic axes. Seed Sci Technol 18:743–754

Hawkes JG (1975) Vegetative propagated crops. In: Frankel OH, Hawkes JG (eds) Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, pp 117–121

Hawkes JG (1982) Genetic conservation of recalcitrant species – an overview. In: Withers LA, Williams JT (eds) Crop genetic resources – The conservation of difficult material. Proc. Int. Workshop, Univ. of Reading, U.K., 1980. Paris, International Union of Biological Sciences, Series B 42. pp. 117–121

Hawkes JG, Maxted N, Lloyd F (2000) Field gene banks, botanic gardens, *In vitro*, DNA and pollen conservation. In: Hawkes JG, Maxted N, Lloyd F (eds) The ex situ conservation of plant genetic resources. Springer, Dordrecht, pp 92–107. doi:10.1007/978-94-011-4136-9_7

IBPGR (1976) Report of IBPGR working group of engineering, design and cost aspects of long term seed storage facilities. International Board for Plant Genetic Resources Secretariat, Rome

Kameswara Rao N (2004) Plant genetic resources: Advancing conservation and use through biotechnology. Afr J Biotechnol 3:136–145

Kantharajah AS, Dodd WA, McConchie CA (1992) In vitro embryo culture and induction of multiple shoots in litchi (*Litchi chinensis* Sonn.). Ann Bot 70:153–156

Karihaloo JL, Malik SK, Rajan S, Pathak RK, Gangopadhyay KK (2005) Tropical fruits In: Dhillon BS, Tyagi, RK, Saxena S, Randhawa GJ (eds) Plant genetic resources: horticultural crops. Narosa Publishing House, New Delhi, pp135–138

King JR (1965) The storage of pollen-particularly by freeze drying method. Bull Torrey Bot Club 92:270–287

King MW, Roberts EH (1979) The storage of recalcitrant seeds-achievements and possible approaches. IBPGR, Rome

Kumar M, Prakash NS, Muthusamy A, Prasad US, Sarin NB (2004) Problems and perspectives of mass scale production of litchi (*Litchi chinensis* Sonn) using in vitro cultures. Prac. National Seminar on recent advances in Production and post-harvest Technology of litchi for export BCKV West Bengal. pp 12–17

Liu W, Xiao Z, Bao X, Yang X, Fang J, Xiang X (2015) Identifying Litchi (*Litchi chinensis* Sonn.) cultivars and Their Genetic Relationships Using Single Nucleotide Polymorphism (SNP) Markers. PLoS One 10(8):e0135390

Malik SK, Chaudhury R, Kalia RK (2003) Seed storage behaviour and cryopreservation of tropical fruit species. In: *In Vitro* conservation and cryopreservation of tropical fruit species. IPGRI Office for South Asia, New Delhi/NBPGR, New Delhi. pp 175–190

Malik SK**,** Chaudhury R (2003) Cryopreservation of desiccation sensitive seeds: Prospects and limitations. In: Mandal et al. (eds) Conservation Biotechnology of Plant Germplasm NBPGR, New Delhi/IPGRI, Rome/ FAO, Rome. pp 209–222

Malik SK, Chaudhury R, Patil PD (2010). Ultrastructural studies on embryonic axes of litchi (*Litchi chinensis* Sonn.) after desiccation and freezing. In: International Conference on Advances in Electron Microscopy and Related Techniques & XXXI Annual Meeting of EMSI, Mumbai, March 8–10, 2010. Organised by Electron Microscope Society of India &Bhabha Atomic Research Centre

Mandal BB (2003) Cryopreservation Techniques for plant germplasm conservation. In: Mandal BB, Chaudhury R, Engelmann F, Mal B, Tao KL, Dhillon BS (eds.) Biotechnology of plant germplasm NBPGR, New Delhi/IPGRI, Rome, pp 209–222

Monshi FI, Bhuiyan MSU, Tabassum R (2015) Adaptability of Litchi Germplasm in Hilly Areas of Sylhet Agricultural University and Screening their Genetic Variation by Using RAPD Markers. Int J Plant Breed Genet 9:218–227

Ogbu JU, Essien BA, Essien JB, Anaele MU (2010) Conservation and management of genetic resources of horticultural crops in Nigeria: Issues and biotechnological strategies. J Hortic For 2:214–222

Raharjo SHT, Litz RE (2007) Somatic embryogenesis and plant regeneration of litchi (*Litchi chinensis* Sonn.) from leaves of mature phase trees. Plant Cell Tissue Organ Cult 89:113–119

Ramya AR, Rajesh V, Jyothi P, Swathi G (2014) A review on in situ and ex situ conservation strategies for crop. Int J Appl Biol Pharm Technol 15:267–273

Ray PK, Sharma SB (1987) Growth, maturity, germination and storage of litchi seeds. Sci Hortic 33:213–221

Reed BM, Engelmann F, Dulloo ME, Engels JMM (2004) Technical guidelines for the management of field and in vitro germplasm collections (IPGRI Handbooks for Genebanks No.7). International Plant Genetic resources Institute, Rome

Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Technol 1:499–514

Sarkar D, Naik PS (1999) Factors effecting minimal growth conservation of potato microplant *in vitro*. Euphytica 102:275–280

Singh HP (1998) Tropical fruits in Asia, diversity, maintenance, conservation and use. In: RK Arora & V Ramanatha Rao (eds) IPGRI, pp 185–195

Thormann I, Dulloo ME, Engels JMM (2006) Techniques of ex situ plant conservation, 7–36. In: Robert Henry (ed) Plant conservation genetics. Centre for Plant Conservation Genetics, Southern Cross University, Lismore, Australia. The Haworth Press inc.

Yu C, Chen Z, Lu L, Lin J (2000) Somatic embryogenesis and plant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Org Cult 61:51–58

Isolation, Identification, and Pharmacological Activity of Phytochemicals Present in *Litchi chinensis*

16

Sunita J. Varjani and Pooja Patel

Abstract

Litchi fruit pericarp (LFP) tissues constitute about 15% of total weight of the entire fresh fruit and have tremendous quantities of flavonoids, especially flavonols and anthocyanins. The major flavonols in LFP are procyanidin B4, procyanidin B2 and epicatechin, while cyanindin-3-rutinoside, cyanidin-3-glucoside, quercetin-3-rutinoside and quercetin-3-glucoside have been identified as important anthocyanins. A few genes are responsible for anthocyanin accumulation in LFP. Litchi flavonoids exhibit good anti-oxidant activity. Furthermore, LFP extract displays a dose- and time-dependent inhibitory effect on human breast cancers, which may be attributed, in part, to its inhibition of proliferation and induction of cancer cell apoptosis through up- and down regulation of more than one gene. It is suggested that flavonoids from LFP play a crucial role as additives for functional foods and anti-breast-cancer drugs. This review discusses the current knowledge of phytochemicals present in LFP and the medicinal benefits of litchi seed extract.

Keywords

Anthocyanins • Flavonoids • *Litchi chinensis* • Litchi fruit pericarp (LFP) • LC-MS • NMR

S.J. Varjani (✉) • P. Patel
School of Biological Sciences and Biotechnology, University and Institute of Advanced Research, Gandhinagar 382007, Gujarat, India
e-mail: drsvs18@gmail.com

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16.1 Introduction

Litchi (*Litchi chinensis*) is a tropical to subtropical crop, which originated from South-East Asia. As litchi has been consumed for its scrumptious flavor and crimson skin, its production has increased in the last few years (Ghosh 2001; Jiang et al. 2006). Litchi fruit pericarp (LFP) accounts for about 15% of the total weight of the entire fresh fruit and has significant amounts of phenolics such as anthocyanins, which are the foremost polyphenols (Jiang et al. 2006). LFP tissues are a critical source of dietary flavonoids such as anthocyanins, which are powerful free radical scavengers with strong anti-oxidant activity in lipid environments such as emulsified methyl linoleate, human low-density lipoprotein, and liposomes (Duan et al. 2007; Zhao et al. 2006).

Queiroz et al. (2012) performed research to assess the nutritional potential of products from litchi fruit, with initial results indicating that the peel and seed have high energy and nutritional content and are rich in anti-oxidants, including ascorbic acid, phenolic compounds such as gallic acid, flavonoids, and anthocyanins. Pharmacological studies have indicated that litchi by-products have anti-inflammatory, antihyperlipidemic, antihyperglycemic, hepatic, cardioprotective, and anti-oxidant effects (Bhoopat 2011; Xu et al. 2011; Jiang 2013). As a consequence, LFP tissues can be used as an available source of natural anti-oxidants or as a supplement in the food and pharmaceutical industries. To augment the use of LFP tissues, this paper reviews the advances in isolation, identification, and extraction of phenolics from LFP and promotes increased use of anthocyanins extracted from litchi as anti-oxidants. This paper also focuses on the major phenolic compounds present in LFP, *viz.*, flavonoids and anthocyanins, with attention to their pharmacological properties.

16.2 Gene Responsible for Anthocyanin Accumulation in LFP

The red coloration of litchi fruit relies on the accretion of anthocyanins, which varies widely depending on cultivars, developmental stages, and environmental stimuli. Previous research on diverse plant species confirmed that anthocyanin biosynthesis is controlled at the transcriptional level. Biao et al. (2014) identified a litchi R2R3-MYB transcription factor gene (LcMYB1) that demonstrates a sequence similar to those of other recognized anthocyanin regulators. The transcription levels of LcMYB1 and anthocyanin biosynthetic genes were investigated in samples with specific anthocyanin levels. The expression of LcMYB1 is strongly related to anthocyanin content in tissues. LcMYB1 transcripts were most effectively detected in anthocyanin-accumulating tissues and were positively correlated with anthocyanin accumulation in pericarps of 12 genotypes. Wei et al. (2011) cloned six structural genes of anthocyanin biosynthetic enzymes—CHS, CHI, F3H, DFR, ANS, and UFGT—and studied the expression of these genes in cultivars of three exclusive coloration sorts.

16.3 Extraction of Anthocyanins

Phenolic compounds exist in free and bound forms in plant cells. Free phenolic compounds are solvent extractable. In evaluation, bound phenolic compounds cannot be extracted into aqueous or natural solvent combinations (Perez-Jimenez and Torres 2011). However, the general phenolic content and anti-oxidant activity of litchi pericarp have been previously reported (Bhoopat 2011; Saxena et al. 2011; Luximon-Ramman et al. 2003).

Extraction is the most vital step in the recovery and purification of active components from raw plant materials. Some traditional techniques, including cold extraction and heat reflux, are more often than not based on appropriate selection of solvents, input of energy, and agitation to increase the chemical solubility and rate of mass transfer. Commonly these strategies require long extraction times with a low yield and are highly laborious (Xiao et al. 2004). The majority of the pigments found in ripe LFP tissues are soluble in polar solvents, which can be extracted using methanol containing small amounts of hydrochloric or formic acid. Ethyl acetate is used to extract most of the flavonoids using reverse-phase high-performance liquid chromatography (HPLC) (Zhang et al. 2000; Zhao et al. 2006). Litchi has been examined for its nonvolatile acid (Chan and Jr Kwok 1974), amino acid (Faroogi and Kaul 1964), sugar (Mathew and Pushpa 1964), and mineral (Singh 1952) content and the effect of γ irradiation upon it (Beyers et al. 1979). Judy et al. (1980) analyzed the volatile components of litchi fruit.

High pressure can enhance the mass transfer rate and increase solvent permeability in cells and secondary metabolite diffusion (Dornenburg and Knorr 1993; Ahmed and Ramaswamy 2006). Studies have confirmed that the high pressure extraction (HPE) approach could significantly shorten processing times and obtain better yields (Zhang et al., 2004, b; Corrales et al. 2008). Prasad et al. (2009) executed this method for the first time for extraction of phenolics from litchi.

16.4 Medicinal Benefits of Litchi Seed Extract

In China, litchi seeds are used to release stagnant humors and get rid of chills, and the seeds function as an analgesic agent, which may relieve symptoms of coughing, gastralgia, neuralgia, and testicular swelling. Pharmacological studies have discovered that litchi seeds exert antihyperlipidemic, hypoglycemic, and anti-tumor effects (Xiao et al. 2004; Chen et al. 2007; Xiong et al. 2008). In India, the seeds are powdered as a natural medicinal drug as a result of their astringency, and after oral consumption, they have been credited with relieving neuralgic pains (Li and Jiang 2007). Polyphenol compounds from litchi seeds have been identified and are composed of a combination of proanthocyanidins and flavonoid glycoside (Xu et al. 2010, 2011). Some of these compounds seem to showcase antineoplastic activities in lung cancer, cervical cancer, and hepatocellular carcinoma cells (Xu et al. 2011).

Table 16.1 Flavonols and anthocyanins present in litchi fruit pericarp (LFP)

Phytochemical	References
Epicatechin	Zhang et al. (2004a, b) and Li and Jiang (2007)
Procyanidin A2	Zhang et al. (2004a, b), and Li and Jiang (2007)
Cyanidin 3-rutinoside	Xu et al. (2010, 2011)
Cyanidin glucoside	Xu et al. (2010)
Quercetin 3-rutinoside	Xu et al. (2010, 2011)
Quercetin glucoside	Xu et al. (2011)

16.5 Effects of Various Temperatures and pH Values on Yield of Phenolics

Several anthocyanins such as epicatechin, proanthocyanidin B4, and proanthocyanidin B2 have been widely found in LFP (Zhang et al. 2004a; Li and Jiang 2007). Various anthocyanins present in LFP are listed in Table 16.1. Anthocyanins illustrate good anti-oxidant ability (Duan et al. 2007), but they are relatively unstable (Zhang et al. 2000). The stability of anthocyanins present in litchi is dependent on various factors such as pH value and temperature (Zhang et al. 2001). The application of pH 4.0 exhibited the most efficient extraction, while an extraction temperature of 60 °C could be used in terms of the combined effects of the phenolic extraction yield and the stability of the extracted litchi anthocyanin (Neungnapa et al. 2008).

16.6 Purification of Anthocyanins

Anthocyanins are strongly bound to chromatographic adsorbents via their unsubstituted hydroxyl groups and may be separated from unrelated compounds with the usage of solvents of increasing polarity (Harborne 1988; Revilla et al. 1998; Kong et al. 2003). Zhang et al. (2001) performed a comparative evaluation of extraction of litchi anthocyanins with the aid of 0.5 M hydrochloric acid. Amberlite XAD-7 column or Sephadex LH-20 column chromatography has been normally used for preliminary purification of crude anthocyanin extracts. Zhang et al. (2004a; b) reported that litchi anthocyanins can be easily purified by using an Amberlite XAD-7 column, observed with the aid of a Sephadex LH20 column, and a major anthocyanin from LFP tissues, accounting for 94.3% overall, was obtained after purification. However, strategies for quick purification of anthocyanins from LFP tissues need to be progressed to fulfill the requirements for high mass sensitivity and resolution with low sample consumption and minimum generation of solvent

wastes. The volatile components were separated on a Varian Aerograph collection 1200 gas chromatograph geared up with a flame ionization detector and a 3 m × 2 mm silanized glass column full of 5% Carbowax 20 M on 100/120 mesh Chromosorb W AW DMCS.

16.7 Identification of Phytochemicals

The identification of plant flavonoids generally includes separation of every compound and then evaluation through mass spectroscopy (MS) and nuclear magnetic resonance (NMR) spectroscopy (Ma et al. 2004). A liquid chromatography–mass spectrometry (LC-MS) approach is used for separation and structural evaluation, which combines the separation power of LC with the high selectivity and sensitivity of MS detection, allowing identification and quantification of specific compounds in a flavonoid mixture. For identification of individual anthocyanins in plant tissues, liquid chromatography–electrospray ionization mass spectrometry (LC ESI-MS) techniques were used by Glassgen et al. (1992). Zhao et al. (2006) obtained three fundamental flavonoids with the aid of reverse-section HPLC and similarly identified them as procyanidin B4, procyanidin B2, and epicatechin via combined MS and NMR strategies. The molecular structures of selected flavonols found in *Litchi chinensis* are represented in Fig. 16.1.

16.8 Stability of Flavonoids

The majority of flavonoids found in plant tissues are comparatively unstable. LFP flavonoids, in particular, involve anthocyanins. Anthocyanins might also go through reactions that alter their structures because of electronic deficiency of their flavylium nuclei (Harborne and Williams 2001). The stability of litchi anthocyanins increases with the range of methoxyls in the B-ring and reduces as the range of hydroxyls increases (Zhang et al. 2001; Kong et al. 2003). Glycosylation and acylation of sugars also increase stability; therefore, diglycosides are more stable than their corresponding monoglycosides (Tomás-Barberán and Clifford 2000; Harborne and Williams 2001). Of the most common anthocyanidins, the most stable is malvidin, followed by peonidin, petunidin, cyanidin, and delphinidin (Kong et al. 2003). The stability of the flavylium cation, as judged by UV-VIS spectrophotometry, is achieved by increasing the pH to 8 or 9.

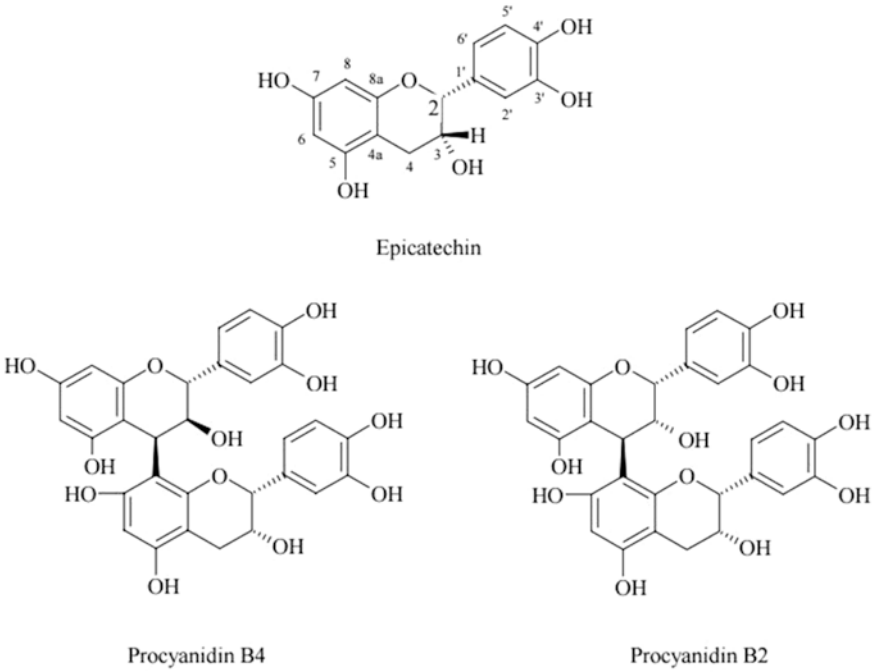

Fig. 16.1 Molecular structures of selected flavonols present in *Litchi chinensis* (Zhao et al. 2006)

16.9 Pharmacological Properties of Flavonoids

16.9.1 Anti-oxidant Activity

LFP extract contains massive quantities of polyphenolic compounds. Anthocyanins and flavonols can prevent oxidation of ascorbic acid caused by metallic ions via chelation of steel ions and formation of ascorbic (copigment)–metallic–anthocyanin complexes (Lottto and Frei 2006). Anthocyanins also can scavenge superoxide anions (Harborne and Williams 2001). Anthocyanins from LFP tissues have also been found to have a high-quality reducing capacity. The measured reducing powers of litchi anthocyanins, ascorbic acid, and butylated hydroxyl toluene at 100 μg/ml are 3.70, 0.427, and 0.148, respectively, indicating that LFP tissues have a strong electron-donating potential (Cook and Samman 1996).

16.9.2 Anti-cancer Activity

Flavonoids are known to have pharmacological properties and are used by humans for therapeutic purposes (Middleton and Kandaswami 1992; Yochum et al. 2000; Mukherjee et al. 2001; Cai et al. 2004). LFP extract demonstrated a dose- and

time-based inhibitory effect on most cancer cell proliferation (Zhao et al. 2006). Wang et al. (2006) described the anti-cancer effect of LFP extract on DNA damage and induction of cancer cell apoptosis through up- and down regulation of a couple of genes involved in cell cycle regulation and cell proliferation. Zhao et al. (2006) tested epicatechin, procyanidin B2, procyanidin B4, paclitaxel and the ethyl acetate fraction from LFP in breast cell lines and concluded that epicatechin and procyanidin B2 can be employed as components of anti-breast-cancer drugs. They reported a stronger inhibitory effect on HELF than on MCF-7 with procyanidin B4 and the ethyl acetate fraction. In addition, they reported lower cytotoxic effects on MCF-7 and HELF with epicatechin and procyanidin B2 than with paclitaxel.

16.10 Conclusion and Future Development

The prospect of growing production of litchi fruit raises expectancies for elevated processing opportunities for this crop. As LFP tissues account about 15% of the total weight of fresh fruit, extra interest to unitization of ingredients from LFP tissues has been paid via litchi enterprise in generating functional foods for consumers with regards to their useful consequences against gastralgia, tumors and gland enlargement. In vitro anti-oxidant and anti-cancer efficacy of flavanols and anthocyanins present in LFP tissues were less very well studied, likely due to constrained expertise in pharmacokinetics. Based on recent take a look at and excessive quantity of flavonoids in LFP tissues, additives from LFP extract might be taken into consideration as a strong novel anti-tumour agent. More research has to focus on functional characterization of genes intricate in metabolic pathways. Molecular genetics approach provides great cultivar variability in phrases of flavonoid contents. Selective breeding of new litchi cultivars is a drastically a thrust area of research. Capability of genetic manipulation to provide a critical supply of flavonoids found in LFP as compositions of anti-breast cancer tablets should be explored. Excessive fruit drop through fruit development is a first-rate hassle inflicting extreme financial loss. Further observe related to gene expression profile for fruit abscission is vital. It's far recommended that litchi industry need to retain to expand new method for better usage of LFP tissues to boom cost-efficient benefit.

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References

Ahmed J, Ramaswamy HS (2006) High pressure processing of fruits and vegetables. Stewart Postharvest Rev 1:1–10

Beyers M, Thomas AC, Van Tonder AJ (1979) Gamma irradiation of subtropical fruits. I compositional tables of mango, papaya, strawberry, and litchi fruits at the edible-ripe stage. J Agric Food Chem 27(1):37–42

Bhoopat L (2011) Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): a combination of anti-oxidant and anti-apoptotic activities. J Ethnopharmacol 136(1):55–66
Biao L, Xiao JL, Gui BH (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS One 9(1):86293
Cai Y, Luo Q, Sun M, Corke H (2004) Anti-oxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anti-cancer. Life Sci 74:2157–2184
Chan HT, Jr Kwok SCM (1974) Non-volatile acid in lychee. J Food Sci Technol 39:792–793
Chen YB, Wu KS, Gu Y, Chen JZ (2007) Research progress in the chemical constituents and pharmacological effects of lychee seeds. Chin J Infect 14:97–98
Cook NC, Samman S (1996) Review: flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 7:66–76
Corrales M, Toepfl S, Butz P, Knorr D, Tauscher B (2008) Extraction of anthocyanins from grape by-products assisted by ultrasonic, high hydrostatic pressure or pulsed electric fields: a comparison. Innovative Food Sci Emerg Technol 9:85–91
Dornenburg H, Knorr D (1993) Cellular permeability of cultured plant tissues by high electric field pulses or ultra high pressure for the recovery of secondary metabolites. Food Biotechnol 7:35–48
Duan XW, Jiang YM, Su XG, Zhang ZQ, Shi J (2007) Anti-oxidant property of anthocyanins extracted from litchi (*Litchi chinenesis* Sonn.) fruit pericarp tissues in relation to their role in the pericarp browning. Food Chem 101:1382–1388
Faroogi MIH, Kaul KN (1964) Qualitative studies of sugars and amino-acids from the aril of litchi fruit. Curr Sci 33(6):183
Ghosh SP (2001) World trade in litchi: past, present and future. Acta Hortic 558:23–30
Glassgen WE, Wray SD, Metzger JW, Seitz HU (1992) Anthocyanins from cell suspension cultures of *Daucus carota*. Phytochemistry 31:1593–1601
Harborne JB (1988) The flavonoids: advances in research since 1980. Chapman and Hall, London
Harborne JB, Williams CA (2001) Anthocyanins and other flavonoids. Nat Prod Rep 2001(18):310–333
Jiang G (2013) Identification of a novel phenolic compound in litchi (*Litchi chinensis Sonn*.) pericarp and bioactivity evaluation. Food Chem 136(2):563–568
Jiang YM, Wang Y, Song LL, Liu H, Lichter A, Kerdchoechuen O, Joyce DC, Shi J (2006) Production and postharvest characteristics and technology of litchi fruit: an overview. Aust J Exp Agric 46:1541–1556
Judy CJ, Ronald CW, Hunter GLK (1980) Volatile constituents of litchi (*Litchi chinesis Sonn.*). J Agric Food Chem 28:859–861
Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003) Analysis and biological activities of anthocyanins. Phys Ther 64:923–933
Li J, Jiang Y (2007) Litchi flavonoids: isolation, identification and biological activity. Molecules 12(4):745–758
Lottto SB, Frei B (2006) Consumption of flavonoid-rich foods and increased plasma anti-oxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med 41:1727–1746
Luximon-Ramman A, Bahorun T, Crozier A (2003) Anti-oxidant actions and phenolicand vitamin C contents of common Mauritian exotic fruits. J Sci Food Agric 83(5):496–502
Ma TE, Celestino S, Julián CR (2004) Anthocyanins in cereals. J Chromatogr 1054:129141
Mathew AB, Pushpa MC (1964) Organic acid and carbohydrates of litchi. J. Food Sci Technol 1(4):71
Middleton E, Kandaswami C (1992) Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol 43:1167–1179
Mukherjee AK, Basu S, Sarkar N, Ghosh AC (2001) Advances in cancer therapy with plant based natural products. Curr Med Chem 8:1467–1486

Neungnapa R, Jia Z, Xuewu D, Bao Y, Jianrong L, Yueming J (2008) Effects of various temperatures and pH values on the extraction yield of phenolics from litchi fruit pericarp tissue and the anti-oxidant activity of the extracted anthocyanins. Int J Mol Sci 9:1333–1341

Perez-Jimenez TJL (2011) Analysis of nonextraxtable phenolic compounds in foods: the current state of the art. J Agric Food Chem 59:12713–12724

Prasad KN, Yang B, Zhao MM, Ruenroengklin N, Jiang YM (2009) Application of ultrasonication or high-pressure extraction of flavonoids from litchi fruit pericarp. J Food Process Eng 32:828–843

Queiroz ER, Abreu CMP, Oliveira KS (2012) Constituintes químicos das frações de lichiain natura e submetidas à secagem: potencial nutricional dos subprodutos. Rev Bras Frutic 34(4):1174–1179

Revilla E, Ryan JM, Martín-Ortega G (1998) Comparison of several procedures used for the extraction of anthocyanins from red grapes. J Agric Food Chem 46:4592–4597

Saxena S, Hajare SN, More V, Kumar S, Wadhawan S, Mishr BB, Parte MN, Gautam S, Sharmz A (2011) Anti-oxidant and radio protective properties of commercially grown litchi (*Litchi chinensis*) from India. Food Chem 126(1):39–45

Singh MP (1952) Mineral composition of fruits of the litchi (*Litchi chinensis Sonn.*) and loquat (*Eriobotrya japonica Lindl.*). Indian J Horticulture 9(4):53–58

Tomás-Barberán FA, Clifford MN (2000) Review: flavanones, chalcones and dihydrochalcones—nature, occurrence and dietary burden. J Sci Food Agric 80:1073–1080

Wang X, Yuan S, Wang J, Lin P, Liu G, Lu Y, Zhang J, Wang W, Wei Y (2006) Anti-cancer activity of litchi fruit pericarp extract against human breast cancer *in vitro* and *in vivo*. Toxicol Appl Pharmacol 215:168–178

Wei YZ, Hu FC, Hu GB, Li XJ, Huang XM et al (2011) Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PLoS One 6:e19455

Xiao LY, Zhang D, Feng ZM, Chen YW, Zhang H, Lin PY (2004) Studies on the anti-tumor effect of lychee seeds in mice. J Chin Med Mater 27:517–518

Xiong AH, Shen WJ, Xiao LY, Lv JH (2008) Effect of semen litchi containing serum on proliferation and apoptosis of HepG2 cells. Zhongyaocai 31(10):1533–1536

Xu X, Xie H, Wang Y, Wei X (2010) A-type proanthocyanidins from lychee seeds and their antioxidant and anti-viral activities. Agric Food Chem 58(22):11667–11672

Xu X, Xie H, Hao J, Jiang Y, Wei X (2011) Flavonoid glycosides from the seeds of *Litchi chinensis*. Agric and Food Chem 59(4):1205–1209

Yochum LA, Kushi LH, Meyer K, Folsom AR (2000) Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Amer J Epidemiol 149:943–949

Zhang DL, Grigor JM, Quantick PC (2000) Changes in phenolic compounds in litchi (*Litchi chinensis Sonn.*) fruit during postharvest storage. Postharvest Biol Technol 19:165–172

Zhang ZQ, Pang XQ, Ji ZL, Jiang YM (2001) Role of anthocyanin degradation in litchi pericarp browning. Food Chem 75:217–221

Zhang ZQ, Pang XQ, Yang C, Ji ZL, Jiang YM (2004a) Purification and structural analysis of anthocyanins from litchi pericarp. Food Chem 84:601–604

Zhang S, Junjie Z, Changzhen W (2004b) Novel high pressure extraction technology. Int J Pharm 78:471–474

Zhao M, Yang B, Wang JS, Li BZ, Jiang YM (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their anti-oxidant activities. Food Chem 98:737–742

Litchi chinensis (Litchi): A General Account with Special Reference to Propagation Practices

17

Prerna Chaudhary and Neeti Dhaka

Abstract

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. Here we address the postharvest technology and its implication in litchi biotechnology.

Keywords

Litchi • Climate requirement • Soil factors • Postharvest technology

17.1 Introduction

The lychee (*Litchi chinensis Sonnnerat)* is an evergreen, medium-sized tree of family Sapindaceae that has over 2000 species and 150 genera. Longan, pulasan, pitomba, korlan, rambutan, and ackee are the other fruits from the same species. Litchi has its origin in Southeast Asia and is known by several other names like litchi, lychee, licy, le-ci, lichee, and many others. The major litchi-producing countries of the world are China, India, Vietnam, Thailand, Bangladesh, South Africa, and Nepal. It is also grown in Hawaii, Israel, Mexico, Australia, and South Africa as a commercial crop.

P. Chaudhary • N. Dhaka (✉)
Department of Botany, Sri Venkateswara College, University of Delhi, New Delhi, India
e-mail: neetidhaka@yahoo.com

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Litchi is an evergreen long-lived tree growing new leaves, flowers, and fruit on terminal shoots with many panicles. Inflorescence in litchi is compound raceme, developed from both terminal and axillary buds. Pollination in litchi is done by insects including honeybees and flies. The fruit is a one-seeded nut and grows in bunches in various shapes and sizes. The fruits are translucent, having sweet juicy aril surrounded by red-colored pericarp. The fruit can be eaten directly as well as used to manufacture juice, vinegar, jelly, and wine. Dried litchi, commonly known as "litchi nuts," are very popular among Chinese people. The fruits containing aborted type of seeds also known as "chicken tongue" are preferred over normal seeds as they have more flesh to seed ratio. Litchi fruits are rich source of flavonoids with high nutritional and medicinal values (Sabrin and Ibrahim 2015). Recently it has been reported that *L. chinensis* fruit and seeds have anti-cancer properties and are very effective against breast cancer.

Various types of cultivars of litchi are grown all around the world depending on the climate (Sachin et al. 2010). Litchi plantation requires a highly specific tropical or subtropical climate. Sometimes the same cultivar grows differently in different climatic and soil conditions. Litchi is a cross-pollinated and highly heterozygous plant and does not produce true parental progeny. New litchi cultivars are difficult to create due to its narrow genetic base. It can be grown through seeds as well as vegetative propagation, but seed-grown litchi plants produce inferior quality of fruits. Air layering is the most popular technique to propagate litchi plants, but it is a very slow and inefficient process (Menzel 1985, 1987; Cristina et al. 2015; Khan and Ahmad 2005). Several in vitro techniques have been used to propagate litchi from the embryo culture, anther, or pollen culture and protoplast culture. The genetic improvement of litchi has been carried out all over the world by the use of modern biotechnological techniques, including mainly genetic engineering, molecular marker, and new germplasm construction.

17.2 Origin and Geographical Distribution

Litchi is a subtropical fruit tree of *Sapindaceae* family; the two species of litchi are *Litchi philippinensis* and *Litchi chinensis* (*Nephelium litchi* Camb). The former is a wild plant grown in the Philippines and is being used as a rootstock. On the basis of twig thickness, inflorescence, stamen number, and fruit characteristics, three well-defined subspecies of litchi are available (Menzel 1991). Litchi is indigenous to China particularly provinces of Kwangtung and Fukien, where it has been cultivated for over 3000 years as main fruit crop but now widely cultivated as an economical crop in tropical and subtropical areas of the world (Julia et al. 1987).

From China, litchi was introduced to India, Burma, and other Jamaican countries in eighteenth century (Peng et al. 2003). In China, litchi has been contributing to its economy and providing livelihood of more than a million people (Liu et al. 2015a, b). Popular cultivars of China include Sanyuehong, Baitangying, Baila, Shuidong, Feizixiao, Dazou, Heiye, Nuomici, Guiwei, Huaizhi, Lanzhu, and Chenzi. After China, major litchi-producing countries are India, Israel, Australia, Thailand,

Taiwan, and Vietnam. Minor producing countries include the United States, Mexico, and Central and South America. Commercialization of many litchi species around the world has been slow due to the different climatic conditions in many areas, as well as the short life of the seeds.

World production of litchi is estimated to be around 2.11 million tons, with more than 95% of the area and production share of Asia. Over the years, production of litchi in India is increasing rapidly; as of now, the production of litchi in India is the same as in China; and about one-fifth of the global production of litchi is contributed by India (Jahiel et al. 2014). Northern Bihar is the main producer (70%) of litchi in India, followed by West Bengal, Punjab, Jharkhand, and Uttar Pradesh. The main commercial cultivars of litchi in Bihar are Shahi (Muzaffarpur), Rose Scented, and China, having large fruit size and excellent taste. Other important cultivars are Deshi, Kasba, Purbi, and Early and Late Bedana. In Saharanpur and Dehradun, Early Large Red, Shahi, Seedless Late (Late Seedless or Late Bedana), and Rose Scented are the popular varieties of litchi.

New cultivars of litchi are being produced in many countries by using traditional breeding and biotechnological methods. Some new cultivars that have been developed in China recently include Donguan Seedless and Hexiachuan that produce seedless/small-seeded fruits and Maguili that crops late in the season (Bose 2001). Most of the litchi cultivars in India have been developed locally from seedlings from Chinese cultivars.

A number of molecular genetic marker techniques like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR), and simple sequence repeat polymorphism (SSR) have been used to identify the genetic variability among different cultivars of litchi. Recently, Liu et al. have used single nucleotide polymorphisms (SNPs) for the identification of 96 representative litchi accessions and their genetic relationships (Liu et al. 2015a, b).

17.3 Climatic Conditions

Litchi cultivation is highly specific to its climatic requirements as different temperature and humidity conditions are required for flowering and fruit development (Rui et al. 2012). Size and quality of the fruit is also highly dependent on the climatic conditions. The plant is very well adapted in the subtropics where summer months are hot and wet and winter months are dry and cool. For healthy growth of plant and fruits, hot summers without hot wind and winters free from frost are essentially required. A seasonal variation in temperature and humidity is prerequisite for flower bud formation, flowering, and fruit production. Litchi requires a relatively low temperature for initiation of flowering (Cui et al. 2013); the areas where the minimum temperature is about 10 °C in winters and 38 °C in summers are ideal for the cultivation of litchi (Peng et al. 2003). In India, temperature of most of the litchi-growing areas ranges from 21 to 38 °C during flowering and fruiting. Litchi can also be grown from subtropical to mild temperate climate of Himalayan valleys where time

of flowering and fruiting is determined by the rise in the temperature after winter season. In tropical areas, litchi plants grow without any fruit formation except for the hilly areas of southern part where crop is harvested in November–December. During the flowering period, heavy rain, fog, hot, dry, and strong winds are very harmful, as they cause shedding of flowers and splitting of the fruit skin. Splitting of the fruit can be reduced by spraying with ethephon at 10 ppm concentration (Morton 1987).

17.4 Soil Conditions

Litchi can be grown successfully on a wide range of soil types, including sandy loams, laterite, alluvial sand, and calcareous soil, but cannot grow under saline soil. The best soil for the litchi cultivation is alluvial loam with good drainage and access to the water table. Though litchi has a high water requirement, it cannot stand water-logging. The plants do not grow well on clay soil due to the poor drainage and display significant stunting of their growth. Litchi can also be grown well even in calcareous soil with added lime to the soil. As described by Pandey and Sharma (1989), litchi plants should be grown in the presence of soil taken from the old orchards which is a good source of mycorrhiza for the growth and production of good quality of fruits.

Soil pH plays an important role in the nutritional health of a tree particularly with respect to the trees' ability to absorb minor elements such as iron. Generally, the best soil for litchi cultivation is acidic, but it can be grown in the soil with a little higher pH. In India, the pH of soils in North Bihar ranges from 7.5 to 8, while in Jharkhand 6 to 6.5, but in both areas production of litchi is very high.

17.5 Propagation of Litchi

The high demand of the litchi in the international market has generated interest in its cultivation by many countries. Litchi can be propagated by seeds as well as vegetatively; however, the seeds are recalcitrant in nature, and they lose their viability in a very short time. The plants produced through sexual methods grow slowly with a long juvenile period and bear the fruit which is not true type. Litchi is generally multiplied by vegetative propagation methods like air layering, cutting, grafting, and budding; however, sometimes these methods are not very successful even with the use of rooting hormones (Abutiate and Nakasone 1972).

17.5.1 Sexual Propagation

The initial introduction of litchi to the different part of the world was possibly through seeds, which helped in the selection of cultivars with superior traits. Propagation of litchi using seeds is generally not recommended due to the presence

of long juvenile years of 7–12, and the fruit produced is of inferior quality. Usually, the seeds are germinated to raise the rootstock. A lot of care is needed to be taken for seed to germinate as they start to lose viability within a day after removal from the fruit. To get 100% germination rate, seed should not be allowed to dry and should be sown fresh immediately after taking out from the fruit. Litchi seeds lost their viability in the fruits after 3–4 weeks if they are not kept under moist conditions. Water-soaked seeds remain viable for a longer period of time. The treatment of seed with growth regulators like GA3, IBA, and ethephon increases the germination rate (Shirzad et al. 2012).

17.5.2 Vegetative Propagation

Propagation of litchi through vegetative means is the most common and easiest method in the absence of a successful sexual route. Air layering, cuttings, grafting, and budding are the most common methods to propagate the litchi.

17.5.2.1 Air Layering

Air layering or pot layering is the most common method for the propagation of litchi due to high percentage of rooting and shorter juvenile period with higher fruit production. In China, air layering is called "marcottage," while in India it is known as "goottee," and it is the main method for the large-scale multiplication of the plants. This method is very old and has not been documented anywhere, with the course of development; it has been gone through several modifications and transformations. In the early practices of layering, clay soil was used with the sufficient watering, but these days air layering is practiced with the use of nutrient media mixed with peat moss or coir along with the growth hormone and nutrient (Cristina et al. 2015; Ali et al. 2016).

The air layer is prepared from the healthy terminal branch which is exposed to sufficient sunlight. On a 1.5–2.0 cm thick branch below the apical growth, a ring is made by removing the cambium layer. For the formation of roots, IBA is used in the form of either paste or powder along with the other organic nutrients. For the development of root and to maintain moisture around this area, either moist moss or coir pith is wrapped around with the polythene sheet at both ends. Depending upon the temperature and humidity, it takes at least 2 months for the development of proper rooting system. The layer is removed by cutting from the lower end as the roots start to develop from the upper part of the ring. The detached layers are planted in the partial shade. Peak summers of June are considered to be the best time for air layering as the temperature in this time suits for the development of healthy roots. Regular irrigation and time to time removal of weeds are required to facilitate better establishment and growth of the plants. Development of layers in the controlled greenhouse conditions has been found more successful. Within 4–5 months, layers are now ready to be transferred to the field, and once the plantlets start to grow, fertilizer can be applied for better growth and development of the plants (Menzel 1985).

In pot layering, the lower branch of mature plant is cut and the circumcised surface is buried in a pot supplemented with rooting medium. The roots start to develop from the cut surface of the branch after almost 2 months; later the branch is detached from the main plant to transfer it to the field.

17.5.2.2 Cuttings

Stem cutting is another way to cultivate litchi plants; however, this method has been adopted commercially. A successful cutting method depends on the type of the stem, misting, and appropriate temperature of the greenhouse. With the use of rooting hormone and moist conditions, a good number of roots can be obtained.

In some reports, it has been indicated that the cuttings grown under partial shade with intermittent misting form better rooting. April–May are the best month for the propagation as air temperatures of 20–25 °C and 30–32 °C for roots are recommended for successful cuttings. The cuttings can be grown in sand with a little peat or vermiculite, but the pot must be well drained. Some workers also recommend the use of fungicides on the cuttings. Semi hardwood cuttings are used rather than the soft terminal cuttings as the latter gives unsuccessful results. The use of rooting hormone (Auxin) improves rooting in the cuttings; however, cuttings take 4 months to root and another 18 months in the nursery before they can be planted out (Menzel 1991).

17.5.2.3 Grafting and Budding

Propagation of litchi through grafting is also another popular method. Grafting is a method of joining two different plant parts, i.e., rootstock and scion. The upper part of the plant is called the scion, while the lower part, the stock, makes the root system. The success of grafting mainly depends on the cambium activity near the graft areas called "the matrix." In litchi grafting is difficult as the cambium layer is not continuous although grafting is used to change the scion cultivar or the unproductive and old plants by top working. Sometimes, sour litchi seedling rootstocks and scions from well-selected mother trees are used for grafting.

Many different approaches like, apical, side, splice, and tongue grafting have been practiced for the cultivation of litchi. Apical grafts are not very successful for large-scale multiplication of litchi, whereas softwood grafting has been found to be successful in many countries.

Propagation through grafting often fails due to the incompatibility between scion and stock, poor grafting techniques, and wrong physiological conditions. With the selection of compatible scion and stock, the protection of graft from direct heat and sunlight and the use of girdling can produce better results.

Propagation of litchi through budding is not commonly accepted, but in some countries like India, Hawaii, the Philippines, and South Africa, this method is being used with a little success. Different methods of budding include chip budding, shield budding, and T budding. Shield budding is a common and more successful method than chip budding. With the proper selection of bud wood, the buds grow rapidly and are ready for field planting after 18 months. A lot of work needs to be done to bring these methods in regular practices (Rajan 2007).

17.6 Micropropagation of Litchi

Conventional breeding is particularly difficult and time taking in litchi due to its long reproductive cycle and its highly heterozygous genetic background. Several in vitro techniques like somatic hybridization, direct DNA uptake, transformation, and mutation and selection have been used for the propagation of litchi. Other genetic tools like gene cloning and the use of DNA marker are continuously being used for the production and improvement of new varieties of litchi. Lai et al. (1997, 2008), Zhang et al. (2001), and Sarin and Prasad (2003) have already described somatic embryogenesis and multiple shoot formation in litchi (Simon et al. 2007). These biotechnological methods have opened new vistas for the cultivation of litchi (Guo et al. 2016).

The process of initiation and development of embryos from vegetative or non-gametic cells (callus or suspension culture) is known as somatic embryogenesis. The mass production of adventitious embryos in cell culture is still regarded by many as the ideal propagation system. The adventitious embryo is a bipolar structure having a preformed radicle; hence there is no need of root induction. In litchi, several reports have been published where complete plant has been raised by somatic embryogenesis.

In one of the earlier reports, Zhou et al. have described the formation of somatic embryos from immature embryos in different varieties of litchi. The embryogenic calli were obtained by culturing on MS medium supplemented with 2.0 mg/l 2,4D, 0.2 mg/l BA, and 0.1 mg/l NAA. Complete plantlets from somatic embryos were obtained on MS medium with low concentration of NAA and BAP (Zhou et al. 1993).

In Yuherbau cultivar of litchi, somatic embryogenesis and plant regeneration were investigated by Liao and Ma (1998). They observed the development of a large number of secondary embryos from immature primary embryos when grown on a medium containing 0.05 mg/l NAA, 0.05 mg/l 2ip (isopentenyl adenosine), and 0.2 mg/l ABA with an increased amount of sucrose (4.5%). The immature somatic embryos gave rise to more number of secondary embryos in comparison to mature somatic embryos. The secondary embryos developed into plantlets were transferred to pots for maturation under glass house conditions (Jain and Ishii 2003).

Kantharajah et al. (1992) carried out a thorough investigation on multiple shoot induction in litchi and effect of different media on the shoot induction. In this study, they cultured the immature embryos of different varieties of litchi on several different kinds of media. The embryos taken were of different ages and sizes. Further, in their study they found out that MS medium supplemented 2% sucrose and 150 ml/l coconut water was most effective in stimulating the germination of immature litchi embryos. They tried pretreatment of various concentration of BAP to induce adventitious buds from embryogenic shoots and concluded that the induction with 100 mg/l BAP was most effective. The adventitious shoots were transferred on MS medium containing 0.5 mg/l NAA and activated charcoal for the development of roots. The complete plantlets were later transferred to the green house maturation.

In this study, they concluded the formation of multiple shoots from the litchi embryos using different nutrient media (Kantharajah et al. 1992).

Zhou et al. (1996) studied various factors that affect the somatic embryogenesis in litchi. They grow young embryos to obtain embryogenic callus, the callus was further grown for 7–10 days on MS medium in the presence of 2,4-D (8.0 mg/l), NAA (0.2 mg/l), and 5% sucrose, and as a result, the induction frequency was 64.2%. The embryogenic callus was subcultured at regular interval and was maintained on MS medium supplemented with 2,4-D (1.0 mg/l), and it was observed that the frequency of embryogenesis was affected by the presence of 2,4-D. Plantlets were obtained by germinating the mature embryoids on 1/2MS medium supplemented with NAA (0.2 mg/l), IBA (1.0 mg/l), and sucrose 2%.

Puchooa (2004) described the in vitro regeneration of main variety of litchi in Mauritius (*Litchi chinensis* Sonn. cv Tai So). They obtained litchi plantlets by inducing callus on MS (Murashige and Skoog) medium supplemented with Auxin, using young leaves as explant. In their experiment they tried different auxins (2,4-D, NAA, and IAA) in combination with cytokinin, and it was observed that 2,4-D (1.5 mg/l) in combination with BAP (1.0 mg/l) and 2,4-D (1.5 mg/l) with kinetin (1.0 mg/l) showed the maximum callusing response. The calli were further transferred on MS medium supplemented with IAA (3.0 mg/l) and BAP (2.0 mg/l) for the regeneration of shoots and were later transferred onto the MS medium containing IBA (2.0 mg/l) for the formation of roots. Regeneration of plantlets was basically through organogenesis, and somatic embryogenesis could be observed when callus was grown on MS medium supplemented with 2,4-D (1.5 mg/l).

In another report, leaf callus was induced along with the establishment of suspension culture using the leaf explant of litchi (*Litchi chinensis* Sonn. cv Huaizhi). In their study, Ma et al. (2009) established a protocol for the induction of callus using leaf explants of different age. The 12-day-old leaf explants showed the optimum callus induction when cultured on callus inducing medium containing MS Basal medium supplemented with 2,4-D (2 mg/l), NAA (0.5 mg/l), kinetin (2 mg/l), and activated charcoal (200 mg/l). In their experiments, they also described the effect of position and orientation of leaf explant on callus induction medium for the induction of callus. Photoperiod was also found to be a major factor for the induction of callus as explants under the 16/8 h photoperiod could produce more number of calli in comparison to complete dark condition.

The callus obtained from leaf explants had embryogenic characteristics, and globular embryos were induced from callus when grown on callus induction medium in the absence of 2,4-D.

For the establishment of suspension culture, the callus obtained from leaf explants was transferred to MS medium supplemented with IAA (3 mg/l) and BAP (2 mg/l) with continuous subculturing. After several subcultures, the friable calli were transferred to the liquid callus inducing medium without agar and activated charcoal. The suspension cultures were maintained in the dark conditions with regular subcultures.

Sarin and group have also reported multiple shoot induction and regeneration of plants from the cotyledonary nodes. The nodal explants were pre-cultured in a

liquid woody plant medium supplemented with polyvinylpyrrolidone (PVP, 0.2%) and fungicide bavistin by using filter paper bridge technique (Das et al. 1999). The cultures were incubated at 25 ± 2 °C under light at a photon fluence density of 50 μmol m^{-2} s^{-1} for 16 h per day. After 15 days, the explants were transferred to modified liquid woody plant medium supplemented with BAP (11 μM), Kn (2.30 μM), GA3 (0.60 μM), bavistin (30 μg/l), and PVP (0.2%) without any sucrose.

For multiple shoot formation, the explants were transferred on semisolid woody plant medium supplemented with added sucrose (3% w/v), deproteinized coconut water (CW, 15% w/v), and casein hydrolysate (CH, 400 mg/l). The medium was supplemented with the same plant growth regulators as described above; however, the concentration of PVP and bavistin was reduced. After several subcultures, the small shoots obtained were transferred on a fresh semisolid MS medium supplemented with BAP (6.6 μM), GA3 (0.15 μM), silver nitrate (SN 30 μM), and CH (300 mg/l) for further multiplication and elongation. The elongated shoots were excised from the clumps of multiple shoots, and after a brief treatment with IBA, the shoots were later transferred on rooting medium supplemented with IBA or NAA. The rooted plantlets were transferred to pots containing vermiculite and litchi seed powder (3:1) and maintained at 25 ± 2 °C, 16 h photoperiod at 200 μmol m-2 s-$^{-1}$ light intensity, and 80% RH for further maturation (Kumar et al. 2006).

In another report, Raharjo and coworkers showed somatic embryogenesis and plant regeneration from leaves of mature litchi plant. They induced the embryogenic calli from the leaves by using B5 medium supplemented with 400 mg/l glutamine, 200 mg/l casein hydrolysate, 30 g/l sucrose, 4.52 mM 2,4-D, 9.30 mM kinetin, and 3 g/l gellan gum under dark conditions. The embryogenic cultures thus obtained were maintained on both semisolid MS medium supplemented with 4.52 mM 2,4-D and 0.91 mM zeatin and embryogenic suspension cultures in liquid medium of the same composition. Mature somatic embryos were obtained by transferring them onto the semisolid MS medium, supplemented with 5–20% (v/v) filter-sterilized coconut water again under dark conditions. Later, the mature white-opaque somatic embryos were transferred on plant recovery medium containing 14.4 mM GA3 on half-strength MS medium with 0.2 g/l activated charcoal under a 16 h photoperiod provided by cool white fluorescent lights (60–80 mmol s^{-1} m^{-2}). After culturing for 6–10 weeks, the plantlets with well-developed root and shoot systems were transplanted to pots in a greenhouse to acclimatize further (Raharjo et al. 2007).

17.7 Anther Culture

Fu and coworkers (1983) developed the litchi plants through the anther culture. In their experiment, the anthers were inoculated on the MS medium supplemented with 2 mg/l kinetin, 2 mg/l 2,4-D, 0.5 mg/l NAA, and 3% sucrose. After 3 months of culture, the calli obtained from anthers were transferred onto modified B5 medium containing 0.5 mg/l kinetin, 0.1 mg/l NAA, 400 mg/l bee royal jelly, 500 mg/l HL, and 3% sucrose for the differentiation of the embryoids. The calli were grown on this medium until the formation of embryoids. Later, the embryoids were

transferred to another B5 medium supplemented with 400 mg/l, 500 mg/l L-glutamine, and 3% sucrose to get the shootlets. For the further development, the shootlets were transferred to the 1/2 modified B5 medium or white medium containing 0.1 mg/l kinetin, 0.01 mg/l IAA, 500 mg/l LH, 1600 mg/l L-glutamine, and 1% sucrose. The plantlets were grown further to get root, stem, and shoots. Cytological studies showed that the plant produced by anther culture was haploid (Bajaj 2012; Tang Daoyi 1983).

Amin and Razzaque (1995) tried to regenerate litchi plantlets from anther cultures. In this study, zygotic embryos of different developmental stages were cultured on half strength major salt MS medium as well as on more effective blueberry medium containing major salts (480 mg/l KNO_3, 400 mg/l NH_4NO_3, 41.3 mg/l $FeSO_4$, and 55.7 mg/l Na-EDTA) with activated charcoal. Somatic embryos were obtained by culturing on medium containing BA (5.0 mg/l) and activated charcoal (1.0 g/l) with visible globular and heart-shaped stages. The embryogenic cultures were maintained on the blueberry medium supplemented with BA and activated charcoal, but viable plantlets could not be obtained.

Wang et al. have also reported in vitro regeneration of litchi via anther culture. In their report, they cultured anthers on MS media, supplemented with several plant growth regulators like auxins naphthalene acetic acid [NAA] and 2,4-dichloriphenoxyacetic acid (2,4-D) and cytokines (kinetin [KT] and 6-benzyladenine [BA]) for callus induction. The callus obtained after 8 weeks of anther culture was further subcultured on MS medium in the presence of 2,4-D, to obtain synchronized friable embryogenic callus. The 18-day-old embryonic calli were grown on MS medium supplemented with 6% (w/v) sucrose, 0.4 g/L LH, 0.56 μM inositol, and 10% (w/v) coconut water, in combination with auxin NAA (0–0.54 μM) and cytokines KT (13.94, 23.23, and 32.53 μM), ZT (13.68, 22.81, and 31.93 μM), and TDZ (13.62, 22.71, and 31.79 μM). The embryos were grown to different developmental stages and later were transferred on different MS media for plant regeneration, where, after 9 weeks of culture, regenerated plants were obtained (Wang et al. 2016).

17.8 Protoplast Culture in Litchi

DNA polymorphism among the litchi cultivars is limited; as a result, to develop new litchi cultivars is very difficult. Currently, new cultivars of litchi are being produced by mutation and selection. In their report, Yu et al. 2000 described the isolation and culture of protoplast from embryogenic suspensions of litchi. The somatic embryos were raised from the fruits of the "Xiafanzhi" cultivar of litchi. The zygotic embryos were removed from the fruits and were subcultured onto the B5 medium supplemented with 2 mg/l 2,4-D, 50 g/l sucrose, and 8g/l agar. The cultures were maintained in the dark for about 6–8 weeks to get the embryogenic cultures of litchi. These embryogenic cultures were later transferred on MS2 liquid medium to get the suspension cultures. These embryogenic cultures were maintained on an alternative

solid-liquid medium for almost 2–9 months. Protoplasts were isolated from these cultures using the filter-sterilized enzyme solution containing 0.8% (w/v) Cellulase "Onozuka" RS, 0.4% (w/v) Macerozyme R-10, 11% (w/v) mannitol, and CPW salts at pH 5.8. Protoplasts were further cultured in liquid shallow layers and on agarose beads or calcium alginate beads with or without nurse cells in the dark conditions.

After 6 weeks culture in Ca-alginate beads, culture medium including free proembryos was removed and replaced with a fresh liberating medium (MS with 8% (w/v) sucrose and 0.1 M citric acid at pH 5.8). Liberated protoplast-derived colonies were collected by centrifugation and then transferred to B3MS containing MS salts and B5 vitamins with 1 mg/l kinetin, 0.1 mg/l NAA, 500 mg/l glutamine, 8% sucrose, and 15 g/l agar to initiate somatic embryos.

Somatic embryos were transferred to maturation medium (B4MS) supplemented with MS salts and B5 vitamins with 500 mg/l glutamine, 50 ml/l coconut water, 5% (w/v) sucrose, and 9 g/l agar and maintained in dark condition. Somatic embryos were transferred and germinated on B5MS medium containing MS salts and B5 vitamins with 1 mg/l kinetin, 5 mg/l GA, 50 ml/l coconut water, 3% (w/v) sucrose, and 7 g/l agar with a photoperiod of 16 h (80 μmol. s^{-1}. m^{-2}). The plantlets obtained were transferred to soil for maturation in greenhouse conditions (Yu et al. 2000).

17.9 Methods to Increase the Production of Litchi

Recently, the application of biotechnological methods has effectively contributed to enhance the quality and production of litchi fruits. Several methods have been implemented to raise the fruit quality of litchi; one of them is to develop parthenocarpic fruits. In litchi, the fruit is drupe, where edible part is translucent and fleshy aril. Along with the seed and pericarp, the total flesh contentment within the fruit is reduced by 50–70%. Several external and internal factors affect the litchi fruit size, the internal factors including genetic makeup and cytology and external factors including climate, water level, supplemented plant regulators and nutrient, regular pruning, and girdling. Immature fruit dropping, pericarp browning, and fungal diseases are the main cause of crop losses in litchi. Several attempts have been made to control these losses.

Parthenocarpic fruits of litchi were produced by Padilla and group by transforming the Brewster cultivar of litchi (*Litchi chinensis* Sonn.) with the *PISTILLATA (PI)* cDNA in antisense direction via *agrobacterium*-mediated transformation. The leaflet-induced embryogenic cultures were co-cultured with *agrobacterium* EHA105/pCAMBIA3301/B-PISTILLATA for 3 days at 27 °C. After three months of co-cultivation of embryogenic cultures, the presence of PISTILLATA sequence in the transgenic plants was confirmed by PCR, whereas the expression was estimated by qPCR and GUS assay. All these results suggested that the posttranscriptional silencing of the litchi PI homolog induced by an antisense-oriented transgene might produce the parthenocarpic fruits in mature plants (Padilla et al. 2013).

Studies conducted by Xia Rui and coworkers describe the role of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) gene in the determination of the fruit size of the litchi by regulating the early cell division. This report demonstrated that the LcHMG1 gene is involved in the early cell division and fruit size determination in litchi, whereas the gene LcHMG2 expressed in the late stage of the fruit development is associated with biosynthesis of isoprenoid compounds required for later cell enlargement. These findings suggested the role of HMGR genes during fruit development (Rui et al. 2012).

17.10 Postharvest Technology

17.10.1 Pericarp Browning

Pericarp browning and pathogens are the major postharvest difficulties; Litchi fruits deteriorate very rapidly if proper techniques are not employed after harvesting (Singha et al. 2014). Peroxidase activity along with ascorbic acid oxidation elevates anthocyanin degradation. In their report, Zhang et al. have described the presence of an unknown anthocyanin degradation enzyme (ADE) in the litchi pericarp and suggested the involvement of multienzyme system in the browning of litchi pericarp (Zhang et al. 2001).

With the storage, the polyphenol oxidase (PPO) degrades the anthocyanin in the pericarp of litchi; as a result, the color of the pericarp changes to brown from bright red (Jiang et al. 2003). Storing at low temperature can reduce the pathological decay, but browning of the pericarp is uncontrollable after few days. Treatment with SO_2 fumes and acid dip is also another way to control the pericarp browning, but the use of chemicals is not recommended due to health issues (Jiang et al. 2003). According to Jiang and coworkers, a coating of chitosan on the fruits may increase the shelf life of the fruits more than 20 days; it also reduces the development of disease on the fruit surface. Chitosan-treated fruits showed reduced activity of PPO; as a result, there was delay in the skin browning of the litchi fruit.

In another report, Singh et al. have described the use of ABA in the accumulation of anthocyanin in pericarp of litchi. In this work, they have emphasize the role of plant growth hormones like abscisic acid (ABA) and ethephon (2-chloroethylphosphonic acid) in the development of peel color of litchi during maturation and ripening. In litchi pericarp, an increase in the level of ABA at the color-break stage is associated with an increased activity of chlorophyllase enzyme leading to chlorophyll degradation and enhanced accumulation of anthocyanins. Ethephon is known to be involved in the up-regulation of some important enzymes of anthocyanin synthesis pathway, resulting in the increased accumulation of anthocyanin in litchi pericarp. These studies have demonstrated that the exogenous applications of ABA alone or in combination with ethephon have great potential to improve pericarp coloration of litchi fruit (Singh et al. 2014).

17.11 Disease Control in Litchi

Litchi is susceptible to a number of preharvest and postharvest fungal pathogens like fruit rot (*Colletotrichum gloeosporioides*), powdery mildew (*Odium* spp.), leaf rust (*Cephaleuros virescens* Kunze.), and downy blight (*Peronophythora litchi*). These fungal pathogens have become major blockade in the production of litchi as they infect shoots, leaves, flower panicles, and fruits, in turn reducing the quantity and quality of fruits. These pathogens are generally controlled by the application of agrochemicals including traditional fungicides in regular practice, such as mancozeb, cymoxanil, and metalaxyl, and the recently introduced QoI fungicide azoxystrobin (Yuxin Zhou et al. 2015). All these applications have a number of limitations as they are costly and harmful to the environment as well as the pathogens are becoming mutant to these chemicals.

Several attempts have been made by scientists from all over the world for the development of disease resistant litchi. In one study, have developed transgenic litchi expressing rice *chitinase* gene to make it fungal resistant. In this study, they have successfully transferred the rice *chitinase* gene under the control of a maize ubiquitin promoter into the zygotic embryos of the litchi through *agrobacterium*-mediated transformation. The zygotic embryos were cultured for almost 14 months to get the transgenic plants. The molecular analysis of the plants confirmed the higher chitinase activities in these plants with delayed commencement of the disease in comparison to the untransformed plants (Das and Rahman 2012).

References

Abutiate WS, Nakasone NY (1972) Studies of vegetative propagation of the lychee (*Litchi chinensis* Sonn.) with special reference to graftage. Ghana J Agric Sci 5:201–211

Ali M, Rab A, Ahmad H, Hayat S, Wali K, Ali J (2016) The influence of different media on rooting of litchi plant through air layering. New Media Mass Commun 47:12–15

Amin MN, Razzaque MA (1995) Induction of somatic embryogenesis in the cultures of zygotic embryos of lychee. Bangladesh J Bot 24(1):25–29

Bajaj YPS (2012) Haploids in crop improvements. Biotechnology in agriculture and forestry 12. Springer, Berlin. ISBN-13:978-3-642-648-564

Bose TK (2001) Fruit: tropical and subtropical, 1. Naya Yug, p 721

Cristina RL, Lins D, Carlos L, Salomão C, Paulo RC, Dalmo LDS (2015) The Lychee tree propagation by layering. Rev Bras Frutic Jaboticabal SP 37(2):480–487

Cui Z, Zhou B, Zhang Z, Hu Z (2013) Abscisic acid promotes flowering and enhances LcAP1 expression in *Litchi chinensis* Sonn. S Afr J Bot 88:76–79

Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell Tiss Organ Cult 109:315–325

Das DK, Shiva Prakash N, Bhalla-Sarvin N (1999) Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.). Plant Cell Rep 18:691–695

Fu L, Tang D (1983) Induction pollen plants of litchi tree (*Litchi chinensis* Sonn.). J Genet Genomics 10(5): 369–374

Guo W, Huanling L, Shujun W, Jinhua S, Xinchun Z, Jiabao W (2016) *In vitro* regeneration of 'Feizixiao' litchi (*Litchi chinensis* Sonn.). Afr J Biotechnol 15(22):1026–1034
Jahiel M, Andreas C, Penot E (2014) Experience from fifteen years of Malagasy lychee export campaigns. Fruits 69:1–18
Jain SM, Ishii K (2003) Micropropagation of woody trees and fruits. Springer, Dordrecht. ISBN 978-94-010-0125-0
Jiang Y, Yao L, Amon L, Jianrong L (2003) Postharvest biology and technology of litchi fruit Food. Agric Environ 1(2):76–81
Julia FM, Miami FL, Morton J (1987) Litchi. In: Fruits of warm climates. pp 249–259
Kantharajah AS, Mcconchie CA, Dodd WA (1992) *In Vitro* embryo culture and induction of multiple shoots in Lychee (*Litchi chinensis* Sonn.). Ann Bot 70(2):153–156
Khan AM, Ahmad I (2005) Multiple shoot induction and plant regeneration in Litchi (*Litchi chinensis* Sonn.) Int J Agric Biol 7(3)
Kumar MN, Prakash S, Prasad US, Sarin NB (2006) A novel approach of regeneration from Nodal explants of field-grown Litchi (*Litchi chinensis* Sonn.) fruit trees. J Plant Sci 1:240–246
Lai ZX, Pan LZ, Zhen ZG (1997) Establishment and maintenance of longan embryogenic cell lines. J Fujian Agric Univ 26(2):160–167
Lai CC, Yu YB, Lai ZX, Xie HG, Wang Q, Chen Y (2008) Induction of the calli for the embryos of „Premier" honey peach and their subculture and maintenance. Chin Agric Sci Bull 24(11):54–59
Liao YW, Ma SS (1998) Adventitious embryogenesis of Litchi chinensis. J Chin Soc Hortic Sci 44(1): 29–40
Liu W, Xiao Z, Xiuli B, Yang X, Fang J, Xiang X (2015a) Identifying Litchi (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using single nucleotide polymorphism (SNP) markers. PLoS ONE 10(8):1–15
Liu W, Xiao Z, Bao X, Yang X, Fang J, Xiang X (2015b) Identifying Litchi (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using single nucleotide polymorphism (SNP) markers. PLoS ONE 10(8):1–15
Ma X-Y, Yi G-J, Huang X-L, Zeng J-W (2009) Leaf callus induction and suspension culture establishment in lychee (*Litchi chinensis* Sonn.) cv. Huaizhi Acta Physiol Plant 31:401–405
Menzel CM (1985) Propagation of lychee: a review. Sci Hortic 25:31–48
Menzel CM (1991) Litchi. In: EWM Verheij, RE Coronel (eds) Plant resources of South-East Asia vol 2. Edible fruit and nuts. Pudoc, Wageningen, pp 191–195
Padilla G, Pérez JA, Irene P-A, Pamela A (2013) *Agrobacterium tumefaciens*-mediated transformation of 'Brewster' ('Chen Tze') litchi (*Litchi chinensis* Sonn.) with the PISTILLATA cDNA in antisense. In Vitro Cell Dev Biol Plant 49:510–519
Pandey RM, Sharma HC (1989) The litchi. ICAR, New Delhi, pp 1–79
Peng H, Liu R, Liu J (2003) The variety of resources and cultivation of Lychee in China. Proc Fla State Hort Soc 116:1–3
Puchooa D (2004) In vitro regeneration of lychee (*Litchi chinensis* Sonn.). Afr J Biotechnol 3(11):576–584
Rajan S (2007) Propagation of horticultural crops. New India Publishing Agency, New Delhi. ISBN 81-8942248-0
Rui X, Caiqin L, Wangjin L, Juan D, Zehuai W, Li (2012) Jianguo 3-Hydroxy-3-methylglutaryl coenzyme A reductase 1 (HMG1) is highly associated with the cell division during the early stage of fruit development which determines the final fruit size in *Litchi chinensis*. Gene 498:28–35
Sabrin RM, Ibrahim GA (2015) *Litchi chinensis*: medicinal uses, phytochemistry, and pharmacology. J Ethnopharmacol 174:492–513
Sachin NH, Saxena S, Kumar S, Wadhawan S, More V, Mishra BB, Narayan PM, Gautam S, Sharma A (2010) Quality profile of litchi (*Litchi chinensis*) cultivars from India and effect of radiation processing. Radiat Phys Chem 79:994–1004

Sarin NB, Prasad US (2003) In: Jaiwal PK, Singh RP (eds) Plant genetic engineering, improvement of fruits, vol 6. In vitro regeneration and transformation of litchi (*Litchi chinensis Sonn.*). Sci Tech publishing LLC, USA, pp 211–222

Sarin NB, Prasad US, Kantharajah AS, Jain SM (2003) Micropropagation of woody trees and fruits, Volume 75 of the series Forestry Sciences. pp 721–731

Simon HT, Raharjo R, Litz E (2007) Somatic embryogenesis and plant regeneration of litchi (*Litchi chinensis* Sonn.) from leaves of mature phase trees. Plant Cell Tissue Organ Cult 89:113–119

Singh SP, Saini MK, Singh J, Pongener A, Sidhu GS (2014) Preharvest application of abscisic acid promotes anthocyanins accumulation in pericarp of litchi fruit without adversely affecting postharvest quality. Postharvest Biol Tec 96:14–22

Singha SP, Sainia MK, Singha J, Pongenera A, Sidhu GS (2014) Preharvest application of abscisic acid promotes anthocyanins accumulation in pericarp of litchi fruit without adversely affecting postharvest quality. Postharvest Biol Technol 96:14–22

Yu C, Chen Z, Lu L, Lin J (2000) Somatic embryogenesis and plant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Org Cult 61:51–58

Zhang ZQ, Pang XQ, Ji ZL, Jiang YM (2001) Roles of anthocyanin degradation in litchi pericarp browning. Food Chem 75:217–221

Zhou L, Kuang Z, Ma X, Chen J, Cao J (1996) The study of factors affecting somatic embryogenesis in young embryo culture of litchi chinesis. J Agric Biotechnol 4(2):161–165

Zhou Y, Chen L, Hu J, Duan H, Lin D, Liu P, Meng Q, Li B, Si N, Liu C, Liu X (2015) Resistance mechanisms and molecular docking studies of four novel QoI fungicides in peronophythora litchi. Sci Rep 5:1–10

Advances in Genetic Transformation of Litchi

18

Kalpana Dalei, Binod Bihari Sahu, Maya Kumari, Ravi Mani Tripathi, and Ramesh N. Pudake

Abstract

Litchi (*Litchi chinensis* Sonn.) is one of the most delicious fruits fetching high values in the market, and the area under its cultivation has increased manyfolds. It is generally multiplied by vegetative propagation method, and breeding is being done by conventional and molecular marker-assisted methods to achieve the quality improvement. There are various hybrids and cultivars developed conventionally by plant breeders in litchi. But due to laborious process, linkage drag, low fertility, longer flowering and fruiting time and high levels of heterozygosity, these conventional methods haven't used to its potential in litchi. Plant genetic transformation can be a great tool in the modern molecular breeding of crops. It helps in transfer genes between unrelated plants resulting in genetically modified crop species with better agronomical traits, better nutritional values, disease resistance, insect tolerance and other desirable characteristics. 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 technique can be used to introduce new traits in to popular genotypes, which can result into new cultivars with desirable traits. In this chapter we review the transformation methods which are being used or can be used for genetic improvement in litchi.

K. Dalei • B.B. Sahu
Department of Life Science, NIT Rourkela, Rourkela, Odisha 769008, India

M. Kumari
Defence Research and Development Organization (DRDO), New Delhi 110011, India

R.M. Tripathi • R.N. Pudake (✉)
Amity Institute of Nanotechnology, Amity University, Noida, Uttar Pradesh 201313, India
e-mail: rnpudake@amity.edu

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18.1 Introduction

In the economy of developed and developing countries, fruit crops play a major role both commercially and nutritionally. Fruits are the major source of nutrients, fibre and anti-oxidants, which were essential for healthy diet. Amongst various fruits produced all over the world, litchi (*Litchi chinensis* Sonn.) is one of the most delicious fruits fetching high values in the market. Litchi is a tropical and subtropical fruit tree indigenous to parts of Southern China and belongs to Sapindaceae family. It is commercially grown in Thailand, China and Taiwan with major markets in Hong Kong, Singapore and Japan (Ghosh 2000; Menzel 2002). The other producers of litchi are Brazil, Malaysia, Myanmar, South Africa, Mauritius, New Zealand, Australia, Madagascar and Taiwan. China has the largest industry of litchi in the world, where it has been cultivated for more than two thousand years back (Li et al. 2013), but is relatively new to the rest of Asia and the Pacific region. The production of litchi per year is highest in China which is about 1.3 million tonnes and followed by India, i.e. 0.43 million tonnes.

In India, it was introduced in the eighteenth century and has well adapted to the climate of Eastern Indian states, viz. Jharkhand, Bihar, Tripura, West Bengal, Uttar Pradesh, Chhattisgarh, Punjab, Uttarakhand and Himachal Pradesh (Singh and Babita 2002). Because of the increasing demand, the area under its cultivation has increased manyfolds. There are numerous litchi cultivars grown in different climates producing different fruit qualities. India grows more than a dozen different cultivars, and during the season, the fruit remains in great demand and also fetches high premium in the market.

The fruit of litchi (white flesh called aril) consists of about 60% juice, 19% seed, 8% rag and 13% skin. The percentage of each constituent varies depending upon the variety of litchi and the climatic conditions under which it is grown. The principal constituents of litchi fruit are carbohydrates, proteins, fats, vitamins, minerals, pigments and organic acids. It is rich in sugar content, and the range of sugar in various Indian varieties varies from 6.74 to 18.0% with an average value of 11.85% (Abrol 2015). The dried form of litchi fruit is known as 'litchi nut' which tastes like raisin and can be also preserved in the form of canned fruit in syrup, squash and jelly (Sidhu 2012). The other parts of the plant like bark, leaves and roots can also be used for various medicinal purposes.

18.2 Litchi Breeding and Its Limitations

Litchi plant is generally multiplied by vegetative propagation method because through seed it grows slowly, has long juvenile period and also leads to genetically different progenies because of genetic segregation. The commonly used methods of vegetative propagation are cutting, air layering, grafting and budding (Menzel 1985). Litchi breeding is being done by conventional and molecular marker-assisted methods to achieve the quality improvement, and this aspect has been reviewed earlier (Sarin et al. 2009). There are various hybrids and cultivars developed conventionally by plant breeders in litchi. In China natural intergeneric hybrid – lungly – has been reported to be developed by crossing litchi (*Litchi chinensis* Sonn.) as female and longan (*Dimocarpus longan* Lour.) as male parent, and the hybrid produced was similar to the maternal parent except for the leaves which were comparatively smaller in size (McConchie et al. 1994).

Conventional breeding methods, like repeated backcrossing, multiline breeding and composite crossing used for crop improvement, are not commonly used in litchi breeding due to its several limitations. Due to more efforts and labour requirement, transfer of non-desirable genes, low fertility, long juvenile period (7–8 years), the length of time before horticultural traits can be evaluated, longer flowering and fruiting time and high levels of heterozygosity, the conventional breeding methods are not commonly used in fruit crops (Gómez-Lim and Litz 2004). Cross-breeding is not commonly used in litchi breeding because the genetic constituents of most litchi germplasm is unclear (Wu et al. 2007). This led to the advancement of many modern methods of breeding which uses the techniques of molecular biology like mutation breeding and genetic transformation. Fingerprinting and analysis of genetic diversity of litchi accessions has been studied by using microsatellite markers, AFLP markers and partial *rbcL* gene sequences (Lin et al. 2005; Madhou et al. 2013; Viruel and Hormaza 2004). In this chapter we review the transformation methods which are being used or can be used for genetic improvement in litchi.

18.3 The Need for Genetic Transformation of Litchi

The agricultural practices have improved a lot through the applications of modern technology, augmenting conventional breeding methods to improve quality and yield. However, there is demand for further improvements in fruit crops because of growing demand owing to rapid population growth, ecological considerations, environmental stress and renewable energy source. Plant genetic engineering offers new thoroughfare in this regard and has become the most important molecular tool in the modern molecular breeding of crops (Job 2002; Liu et al. 2013). Genetic engineering advancements in plants opened an avenue to transfer genes between unrelated plants resulting in genetically modified crop species with better agronomical traits, better nutritional values, disease resistance, insect tolerance and other desirable characteristics (Liu et al. 2013; Vain 2007). Genetic transformation in plants is considered as continuation of conventional plant breeding techniques (Visarada et al.

2009) by advanced technologies. It offers introduction of novel genes irrespective of species barrier and creates phenotypes with desired characters which are not available in the gene pool of original crop plant. Over the last decade, some remarkable achievements have been made in the field of development of efficient transformation methods in field crops (Mittler and Blumwald 2010).

Currently in case of fruit crops, the focus has also shifted to improve the quality of these crops using both conventional and genetic engineering (Kanchiswamy et al. 2015) techniques. Presently, several problems faced during the conventional and molecular breeding methods could not achieve the required potential genetic improvement in fruit crops including litchi. These methods made a relatively small contribution to global litchi supplies; therefore, reliable and speedy methods are needed to meet the future market requirement. To accelerate the process of genetic improvement in perennial fruit crops like litchi, the recently developed techniques can be used for integrating cross species DNA in plant genome resorting to different transformation tools. The main objective of transformation is creating phenotypes with desired traits that are not available in the germplasm pool of crop plants. During the last two decades, many genetically modified plants were generated with DNA that could not have been introgressed in its genome through any conventional breeding method. As a result, the number of genes for agronomically important traits such as herbicide tolerance, insect and pathogen resistance are being transformed in plants. Also in litchi genetic engineering technique can be used to introduce new traits into popular genotypes, which can result into new cultivars with desirable traits such as pest and disease resistance, herbicide resistance, drought and frost tolerance and improved fruit quality.

The recent developments in next-generation sequencing (NGS) technology have made it possible to produce transcriptomics and expressed sequence tag (EST) data from various tissues and cultivars of litchi (Li et al. 2013, 2014, 2015a; Lu et al. 2014, 2015; Wang et al. 2014, 2015; Zhang et al. 2014). This vast resource has opened up new possibilities in the area of litchi functional genomics. To achieve the potential, key requirement is the availability of efficient transformation system for executing the functional genomics strategies like gene overexpression and gene mutation. Despite significant advances over the last 20 years in genetic transformation of fruit plant, very limited studies have been conducted in litchi. The objective of this chapter is to provide an update on litchi genetic transformation and insight on unexplored venues of genetic improvement in it.

18.4 Transformation Systems for Fruit Trees and Their Use in Litchi

The gene transformation methods for delivering exogenous DNA into plant cells can be broadly divided into two main categories: indirect and direct DNA deliveries. In indirect gene transfer approach, the gene of interest is introduced into the target cell *via* soil bacteria, e.g. *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*

(Tzfira and Citovsky 2006) which naturally infects the plants. In contrast, direct gene transfer does not use bacterial cells, instead use direct DNA delivery methods like microinjection, electroporation and particle bombardment.

18.4.1 Direct DNA Delivery

18.4.1.1 Electroporation

Electroporation is a direct gene transfer technique used to introduce substances like drugs and piece of coding DNA into cell. In this process the permeability of plasma membrane is increased significantly by application of external electric field. Upon application of high voltage pulse for microseconds to milliseconds, aqueous pores are formed in the lipid bilayer through which different molecules like drugs, ions, tracers, dyes, antibodies, oligonucleotides, DNA and RNA pass inside cell (Faurie et al. 2005). First plant protoplast electroporation was reported in 1985 (Fromm et al. 1985), where the expression of DNA in protoplast of both dicots (carrots and tobacco) and monocots (maize) was achieved. The use of low voltage, typically 10–1000 V for 30–50 milliseconds, provides efficient transfection. Depending upon the cell type to be transformed, the optimal voltage strength, the pulse length and the number of pulse vary. Besides electric pulse parameters, other factors, such as electroporation medium composition and osmotic pressure, play significant roles in electroporation effectiveness (Fig. 18.1).

Electroporation technique needs viable protoplasts to transfect gene into it, and current advancement of protocols for efficient protoplast isolation and maintenance has made this simple and low-cost technique of interest to many researchers. In majority of the cases, only single copy of transgene is inserted in genome through protoplast transformation. This is one of the major advantages of electroporation over particle bombardment (Bates 1999), where it tends to insert multiple copies of transgene in the genome. High viability of cells after application of the electric pulse (up to 50% of the treated cells survive the treatment) is the another advantage of electroporation. It is also reported that it has higher DNA delivery rate (40–60% of the cell population received DNA under optimal electroporation conditions) (Sorokin et al. 2000). Single cells and cell clusters can be used efficiently in electroporation, which are susceptible to damage by other techniques. Also during the transfection, same culture condition is maintained which results in increasing the efficiency of selection. This is unlike the particle bombardment where the targeted cells require time to recover from particle damage.

Many studies have reported the successful transfer of DNA in plant tissues, cells or organelles with the help of electroporation. Studies with three different plant species – tobacco, soybean and alfalfa – and three different tissues, protoplasts, suspension cell culture and germinating pollen, have indicated that requirement of optimal field strength for each of these cells differs. Whereas protoplasts needed the lowest optimal pulse field strength, followed by suspension cells and finally germinating pollen requiring the strongest electroporation pulse (Saunders et al. 1995). In brief,

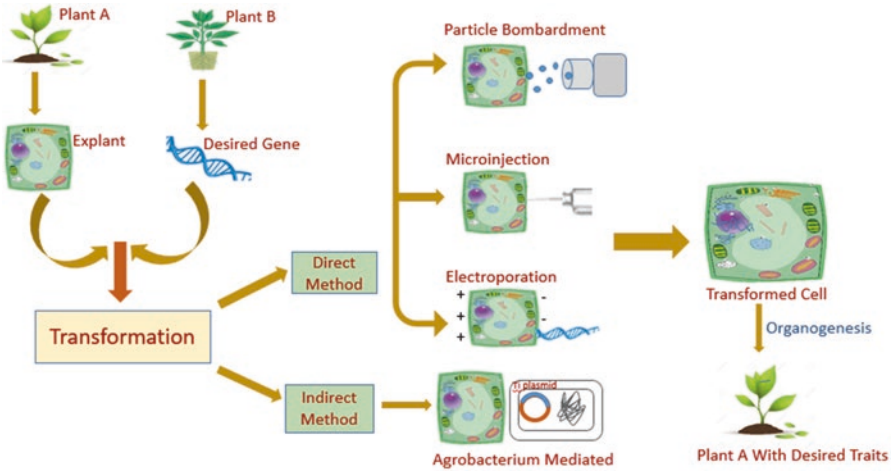

Fig. 18.1 Schematic representation of methods for transfer foreign genes to plant genome

electroporation is a useful technique that can be used to directly transfer desired gene into the litchi plant to produce cultivar with superior traits.

18.4.1.2 Particle Bombardment for Transformation

Particle bombardment with the help of gene gun is another gene transfer technique which utilizes high-velocity micro-projectiles to inject external DNA molecules into cells and tissues. In this method the DNA is precipitated on micron-sized gold or tungsten particles with spermidine and calcium chloride, and these DNA-coated particles are loaded into particle/gene gun and accelerated to high speed using pressurized helium gas to penetrate the plant cell walls and membranes. After the entry of micro-projectile, it releases the transgene from its surface, and it may transiently get expressed in cell or stably get incorporated into the plant's chromosomal DNA (Fig. 18.1).

The particle bombardment process was developed by Sanford and colleagues at Cornel University, and they termed it as "biolistics" gene gun (Sanford et al. 1987). This method is physical in nature and has simple methodology so can be used to transfer genetic material into a wide range of cells from a diverse group of organisms. An important feature of particle bombardment is the flexibility by which co-infection of various genomic components can be achieved. The ability of particle bombardment to transform a wide range of cell types without any biological limitation facilitates a wide range of applications which are difficult to achieve in other transformation methods.

Particle bombardment system is low cost and was successfully used in the transformation of many fruit plants like cherry (Li et al. 2015b) to slow down fruit ripening and drought tolerance and in avocado for transient expression (Chaparro-Pulido et al. 2014). This method was also used in American cranberry (*Vaccinium macrocarpon*) to transfer the *cry1* gene of *Bacillus thuringiensis* to increase the pest

resistance (Serres et al. 1992) and later on transformed by *bar* gene that confers tolerance to glufosinate herbicide (Zeldin et al. 2002). Until now, there are no reports of using this technique for transformation of litchi. However, this technique can be effectively used for litchi transformation and to deliver transgenes to a variety of cell types without any virus-based vectors or toxic chemicals. This is its distinctive advantage over other delivery methods.

18.4.1.3 Indirect DNA Delivery by *Agrobacterium tumefaciens*

A. tumefaciens is a pathogenic bacterium that lives in soil which causes crown gall disease characterized by a tumour in dicot plants. *A. tumefaciens*-mediated plant transformation (ATMT) is the most widely used method for introducing foreign genes into plant for making transgenic plants (Fig. 18.1). The first evidence that indicates this bacterium as causative agent of crown gall tumour goes back to 90 years or more (Smith and Townsend 1907). It is reported to induce tumours at wound sites on root, stem and crown of in about hundreds of dicot plants and some of the monocots and gymnosperms (De Cleene and De Ley 1976). This bacterium has the ability to naturally transfer DNA segment called T-DNA of Ti plasmid which after integration into host genome gets transcribed and produce crown gall disease (Binns and Thomashow 1988; Nester et al. 1984).

The T-DNA carries two types of genes: oncogenic genes and opine genes. Oncogenic genes encode enzymes which help in the synthesis of growth hormones-auxins and cytokinins that produce tumour; and opine genes help in the synthesis of opines which are consumed by *A. tumefaciens* as source of carbon and nitrogen. In Ti plasmid, outside the T-DNA opine catabolism genes, the genes help in T-DNA transfer from bacterium to plant cells, and genes involved in conjugative transfer of bacterial plasmids were present (Hooykaas and Schilperoort 1992; Zupan and Zambryski 1995).

The advanced molecular biology techniques have empowered the development of Ti binary vectors which are compatible with both *Agrobacterium* and *Escherichia coli*. The binary vectors are developed by placing virulence genes in one plasmid (large Ti plasmid) and gene to be transformed on a separate plasmid vector (small binary vector) (Hoekema et al. 1983). Binary vectors are designed in such a way that it can replicate both in *E. coli* and *Agrobacterium*. Recently the advancement in cloning techniques has led to the development of binary bacterial artificial chromosome (BIBAC) vectors that can transfer large-sized DNA into the host (Hamilton 1997; Rui-Feng et al. 2006).

The T-DNA fragment is flanked by 25-bp direct repeats, which act as a *cis* element signal for the transfer apparatus. The protein coded by the virulence region (*Vir* genes) of Ti plasmid and genes of bacterial chromosome cooperatively helps in the process of transfer of T-DNA. The virulence region is 30 kb in size and organized in six operons (*virA*, *virB*, *virC*, *virD*, *virE* and *virG*), all helps in the transfer of T-DNA (Hooykaas and Schilperoort 1992; Zupan and Zambryski 1995). The T-DNA of bacterium is genetically engineered to produce transgenic Ti plasmid

with desired characteristic of litchi and allowed to infect the plant and to express.*ATMT* has some advantages over direct transformation methods. It has reduced problems with transgene instability and cosupression because of less copy number of transgene (Hansen et al. 1997; Koncz et al. 1994). It is also a single-cell transformation process that does not form mosaic plants which are more frequent in direct gene transformation methods (Enríquez-Obregón et al. 1998; Enriquez-Obregon et al. 1997). *Agrobacterium*-mediated gene transfer method is widely used for transformation of a large number of fruit crops including almond, apple, banana, grapevine, orange, melon and litchi (Rao et al. 2009).

Very few reports on genetic transformation of litchi are available in literature, and all of them have used *Agrobacterium* as a tool for indirect gene transfer. In one study with the green fluorescent protein (GFP) (Puchooa 2004), the expression was achieved in the leaf tissues of litchi by ATMT. The GFP was observed in leaves and callus during fluorescent microscopy after 4 weeks of culture indicating successful transformation. This is one of the pioneer studies in litchi transformation, which opened the new venue of genetic transformation in this important fruit crop.

As we know, fungal diseases are one of the major causes of loss in the quality and yield of litchi worldwide (Crane et al. 1997). The most economically important fungal diseases of litchi in India and other countries are leaf spots and dieback caused by *Phomopsis* sp. and leaf blight caused by *Gloeosporium* sp. Transgenic plant resistant to *Phomopsis* sp. pathogen was developed by transferring bacterial chitinase (ChiB) gene into litchi cultivar *Bedana* by *Agrobacterium*-mediated transfer method (Das and Rahman 2010). Plants also produce pathogenesis-related (PR) proteins like chitinases which help in the plant defence system against pathogens (Legrand et al. 1987; Nishizawa and Hibi 1991). These pathogenesis-related proteins have been reported to provide resistance against many fungal diseases (Jayaraj et al. 2004; Punja 2006). Chitinase gene (Chi11) of rice has been reported to show resistance against many fungal pathogens (Das and Rahman 2012), so rice chitinase gene (RCC11) was transformed into *L. chinensis* cv. Bedana through ATMT. The resultant transgenic exhibited increased resistance to *Phomopsis* sp.

PISTILLATA (PI) gene of *Arabidopsis thaliana* helps in the floral organ development, and its mutation converts stamen to carpel and petal to sepal (Bowman et al. 1991; Goto and Meyerowitz 1994). Recently antisense strategy has been one of the important approaches for silencing gene for the study of its function and in assisting plant breeding. One cultivar of litchi, 'Brewster', was transformed with *Arabidopsis* antisense gene Pistillata cDNA by *Agrobacterium* method to induce parthenogenesis (Padilla et al. 2013). This interferes with the development of stamen and forces fruit production without pollination, and results have indicated that genetic transformation can be used to generate parthenocarpy in litchi.

Several factors affecting *Agrobacterium tumefaciens*-mediated gene transfer efficiency of litchi were studied by using beta-glucuronidase (GUS) gene. When three strains of *Agrobacterium* – LBA4404, AGL-1 and EHA105 – were compared for their virulence, it was found that EHA105 strain had the strongest virulence to litchi amongst them. Coculture for 2 days only gave higher GUS transient expression (Lihui and Liuxin 2003). The bacterial concentration of 0.5×10^8 cells/mL was found

to be enough for achieving best results. The embryogenic calli was found to be suitable starting material for transformation. Using these optimized inoculating conditions, callus with stable GUS expression was obtained (Lihui and Liuxin 2003).

We know that ATMT is generally dependent on host specificity and also the penetration of bacterium to proper cells in targeted tissues of plants. In order to increase transformation frequency in citrus, some modified methods like sonication-assisted *Agrobacterium*-mediated transformation (SAAT), vacuum infiltration and a combination of these two procedures were compared with conventional *Agrobacterium*-mediated inoculation method ('dipping' method) (de Oliveira et al. 2008). Sonication-assisted *Agrobacterium*-mediated transformation (SAAT) is a technique which uses a brief periods of ultrasound wave to the plant tissue in presence of *Agrobacterium*. The microscopic study has revealed that sonication produces small and uniform channel in cell wall allowing the bacterium easy access to internal plant tissues. Unlike other transformation methods, this system has the potential to transform meristematic tissue buried under several cell layers. This technique is being widely used in many crops and tissues like leaf, roots, shoot tips, immature cotyledons, embryogenic callus and also whole small seedlings (Trick and Finer 1997). The time of exposure to sonication along with vacuum may affect the transformation efficiency and regeneration capacity of tissues. Generally it is found that the combination of SAAT and vacuum infiltration treatments significantly increase the transformation efficiency.

18.4.1.4 Selection of Transformants

Now it is possible to introduce foreign DNA molecule into any plant cell or tissue using either direct gene transfer or *Agrobacterium*-mediated gene transfer method. In all the transformation methods, only a small fraction of cells become transgenic, while majority of cells remain untransformed. Therefore, it is necessary to select the transformed cells from the untransformed cells. So there was a need of selectable marker genes, which became an important tool in genetic engineering. The gene of interest to be transformed is introduced along with the selectable marker gene, and the transformed cells only will survive under the selection pressure imposed on them. The regenerated plants from surviving cells will contain the selectable marker gene along with the gene of interest. The genes for herbicide or anti-biotic resistance are being popularly used to make transgenic plant and selected on the basis of their growth on the media containing the corresponding toxic substances. The most commonly used selectable marker gene is neomycin phosphotransferase II gene (Fraley et al. 1986) that provides resistance to aminoglycoside anti-biotics neomycin, kanamycin, paromomycin and G-418 (Bevan et al. 1983; Guerche et al. 1987). There are some more selectable marker genes developed which show resistance to anti-biotics like bleomycin (Hille et al. 1986), bromoxynil (Stalker et al. 1988), glyphosate (Franz et al. 1997), hygromycin (Waldron et al. 1985), chloramphenicol (Fraley et al. 1983), 2,4- dichlorophenoxy acetic acid or phosphinothricin (De Block et al. 1987).

The reporter genes are like selection markers, which help in labelling and screening of transformed cells and also useful to investigate the transcriptional regulation

of gene expression. Expressions of reporter genes confirm the genetic modifications in transformed cells. They are generally fused with the coding sequence of gene of interest or under the control of regulating sequence like promoter of gene of interest. A wide range of reporter genes have been identified, and most of them are enzymes and can be a good resource for selection of a marker that is most suitable for the plant species or tissues to be transformed. Commonly used reporters include gene encoding neomycin phosphotransferase (NPT-II), chloramphenicol acetyl transferase (CAT), luciferase (LUC), ß-glucuronidase (GUS) and protein involved in the regulation of anthocyanin biosynthesis.

The enzyme glucuronidase (GUS)-encoding gene from *E. coli* is an important reporter system for screening the transformed plants (Jefferson et al. 1986; Vancanneyt et al. 1990). Being a hydrolytic enzyme, it catalytically cleaves a number of glucuronides which are commercially available as fluorometric, spectrophotometric and histochemical substrates. The luciferase enzyme-encoding gene is also a highly effective reporter gene because the enzyme assay is extremely rapid, easy to perform, sensitive and inexpensive (Ow et al. 1986). The enzyme produces light with highest quantum efficiency than the chemiluminescent reactions. Another benefit is that luciferase is a monomeric protein which does not require post-translational modification for enzymatic activity (De Wet et al. 1985).

The green fluorescent protein (GFP) isolated from coelenterate group like jellyfish *Aequorea victoria* can also be used as reporter molecule for monitoring the expression of gene in plants. The GFP emits green fluorescent light upon excitation with UV light. The molecular cloning of GFP encoding gene with the transgene of interest can be used to select the transformant. In this method there is direct fluorescent imaging of gene product which does not need other histochemical staining methods which may be lethal to the living cells. So this method can be used to select the genetic transformants in litchi.

18.5 Futuristic Methods for Transgenic Development in Litchi

The recently developed techniques like Zinc-finger endonucleases (ZFNs), transcription activator-like effector nucleases (TALENs), CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) system can also be used in litchi breeding. The ZNFs are also called as molecular scissors which can be used to target specific genes in plant systems. These currently used engineered proteins have highly specific Zinc Finger domains (usually three) along with the sequence-independent nuclease domain of the Fok I restriction enzyme. After transformation, ZNFs introduce a targeted specific double-strand break in the genomic DNA of organism, which result in recombination at the genomic site of interest. ZFN-mediated gene targeting can be a powerful tool in inducing specific gene mutations (Townsend et al. 2009). But this promising tool can also be useful for homologous recombination of the transferred gene at site of double-strand DNA

break, help in transgene integration and successfully be demonstrated in tobacco. Based on this finding, the ZNFs can be useful tool in gene characterization and transgene integration in litchi in the future.

Transcription activator-like effector nucleases (TALENs) are recently developed techniques for gene editing and can be considered as improved alternative to ZNFs (Joung and Sander 2013). The concept of TALEN was developed after the identification and understanding of the working principles of the type III transcription activator-like (TAL) effectors secreted by *Xanthomonas* – a plant pathogenic bacteria (Boch and Bonas 2010). After entry into plant cells, the TAL effectors cross the nuclear membrane and bind to specific sequence in promoter of host gene and initiate its transcription (Moscou and Bogdanove 2009). The recognition of specific DNA sequence by TAL effectors is governed by tandem amino acid repeats (34 aa). Two repeat-variable di-residues (RVDs) that are located in 12th and 13th position in each of the 34 aa repeat are the key for binding specificity of these effectors. Similar to ZNF, the TALEN is generally engineered with two flanking domains – one with the specific DNA-binding domain at the N-terminal and a nonspecific *Fok I* nuclease domain at the C-terminal. Due to its simpler manipulation technique, TALEN has been successfully used for gene mutation, gene correction and transgene insertion in many crops (Xiong et al. 2015) and can also be applied in litchi breeding.

Most recently, a new revolutionary technique for genome editing is developed, and many laboratories are using it in many organism. The CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) system is based on the principle of the CRISPR/Cas system which was derived from a type II prokaryotic organism adaptive immune system (Jinek et al. 2012). The repeat-spacer-repeat (29-32-29 bp) pattern of CRISPRs functions through an RNAi-like mechanism, in which the flanking sequences used to recognize the specific sequences and induce cleave in DNA. Since 2013, this technique has been widely applied in gene modification in plants to modify the traits. All these three genome technologies can produce transgene expression in litchi, but the CRISPR/Cas method is the most promising because of its efficiency, low cost and user-friendly characteristics.

18.6 Conclusion

Litchi is an important tropical and subtropical fruit crop that is rich in nutrients, and the major constituents are carbohydrates, proteins, fats, vitamins, pigments and organic acids. The major breeding objective to increase yield and quality of fruit led to the development of various breeding methods and gene transfer technologies to produce high yielding and disease-resistance varieties of litchi. In India, various conventional breeding methods used for litchi breeding are backcross breeding, multiline breeding, hybridisation and composite breeding. But there are several limitations associated with conventional breeding methods, *viz*. it takes longer time to generate breeding lines, more resources and labour requirement, low fertility, transfer of non-desirable genes (genetic load), high levels of heterozygosity and

long juvenile period of about 7–8 years. This led to the development of new breeding approaches like mutation breeding and genetic transformation technologies which uses the principles of molecular biology. The gene transfer techniques for delivering genes into target cells are broadly divided into two categories: direct and indirect methods. The direct DNA delivery method includes electroporation, microinjection and particle bombardment in which the foreign genes are directly targeted into the cells. In indirect gene transfer method, the gene of interest is introduced into the target cell via bacterium *Agrobacterium tumefaciens* or *A. rhizogenes* and naturally infects the plants for inducing tumour. *Agrobacterium*-mediated gene transfer method has more advantage over direct gene transfer because of reduced transgene instability and cosupression; and also does not form mosaic plants which are frequent in direct transfer. So these genetic transformation approaches can be used to produce transgenic litchi varieties with better quality, yield and resistance to disease. After the genes are successfully transformed into the plant, cells need to be selected for transformation. The litchi transformants are selected by using a wide range of selectable marker and reporter genes. The genes of interest are transformed along with the selectable marker gene, and the transformed cells will only survive under the selective pressure. A wide range of reporter genes are also available which can be used for screening transformants. These gene transfer technologies and selection techniques can be future perspectives to produce litchi varieties with desired characteristics.

References

Abrol DP (2015) Subtropical fruits. In: Pollination biology, vol 1. Springer, pp 347–397

Bates GW (1999) Plant transformation via protoplast electroporation. Methods Mol Biol 111:359–366

Bevan MW, Flavell R, Chilton MD (1983) A chimeric anti-biotic resistance gene as a selectable marker for plant cell transformation

Binns AN, Thomashow MF (1988) Cell biology of Agrobacterium infection and transformation of plants. Annu Rev Microbiol 42:575–606

Boch J, Bonas U (2010) Xanthomonas AvrBs3 family-type III effectors: discovery and function. Phytopathology 48:419

Bowman JL, Smyth DR, Meyerowitz EM (1991) Genetic interactions among floral homeotic genes of Arabidopsis. Development 112:1–20

Chaparro-Pulido CA, Montiel MM, Palomo-Ríos E, Mercado JA, Pliego-Alfaro F (2014) Development of an efficient transient transformation protocol for avocado (*Persea americana* Mill.) embryogenic callus. In Vitro Cell Dev Biol Plant 50:292–298

Crane JH, Sanford RE, McMillan Jr RT (1997) Control of lychee anthracnose by foliar applications of tebuconazole, mancozeb, and copper hydroxide on 'Mauritius' lychee fruit under south Florida conditions. Paper presented at: Proceedings of the Florida State Horticultural Society

Das D, Rahman A (2010) Expression of a bacterial chitinase (ChiB) gene enhances antifungal potential in transgenic Litchi chinensis Sonn. (cv. Bedana). Curr Trends Biotechnol Pharm 4:820–833

Das D, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell Tissue Organ Cult 109:315–325

De Block M, Botterman J, Vandewiele M, Dockx J, Thoen C, Gossele V, Movva NR, Thompson C, Van Montagu M, Leemans J (1987) Engineering herbicide resistance in plants by expression of a detoxifying enzyme. EMBO J 6:2513

De Cleene M, De Ley J (1976) The host range of crown gall. Bot Rev 42:389–466

de Oliveira MLP, Febres VJ, Costa MGC, Moore GA, Otoni WC (2008) High-efficiency Agrobacterium-mediated transformation of citrus via sonication and vacuum infiltration. Plant Cell Rep 28:387–395

De Wet JR, Wood KV, Helinski DR, DeLuca M (1985) Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*. Proc Natl Acad Sci 82:7870–7873

Enríquez-Obregón GA, Vazquez-Padron RI, Prieto-Samsonov DL, Perez M, Selman-Housein G (1997) Genetic transformation of sugarcane by *Agrobacterium tumefaciens* using anti-oxidant compounds. Biotecnol Apl 14:169–174

Enríquez-Obregón GA, Vázquez-Padrón RI, Prieto-Samsonov DL, Gustavo A, Selman-Housein G (1998) Herbicide-resistant sugarcane (*Saccharum officinarum* L.) plants by Agrobacterium-mediated transformation. Planta 206:20–27

Faurie C, Golzio M, Phez E, Teissie J, Rols MP (2005) Electric field-induced cell membrane permeabilization and gene transfer: theory and experiments. Eng Life Sci 5:179–186

Fraley RT, Rogers SG, Horsch RB, Sanders PR, Flick JS, Adams SP, Bittner ML, Brand LA, Fink CL, Fry JS (1983) Expression of bacterial genes in plant cells. Proc Natl Acad Sci 80:4803–4807

Fraley RT, Rogers SG, Horsch RB, Gelvin SB (1986) Genetic transformation in higher plants. Crit Rev Plant Sci 4:1–46

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide (American Chemical Society)

Fromm M, Taylor LP, Walbot V (1985) Expression of genes transferred into monocot and dicot plant cells by electroporation. Proc Natl Acad Sci 82:5824–5828

Ghosh S (2000) World trade in litchi: past, present and future. In: Paper presented at: I International Symposium on Litchi and Longan 558

Gómez-Lim MA, Litz RE (2004) Genetic transformation of perennial tropical fruits. In Vitro Cell Dev Biol Plant 40:442–449

Goto K, Meyerowitz EM (1994) Function and regulation of the Arabidopsis floral homeotic gene PISTILLATA. Genes Dev 8:1548–1560

Guerche P, Bellini C, Le Moullec J-M, Caboche M (1987) Use of a transient expression assay for the optimization of direct gene transfer into tobacco mesophyll protoplasts by electroporation. Biochimie 69:621–628

Hamilton CM (1997) A binary-BAC system for plant transformation with high-molecular-weight DNA. Gene 200:107–116

Hansen G, Shillito RD, Chilton MD (1997) T-strand integration in maize protoplasts after codelivery of a T-DNA substrate and virulence genes. Proc Natl Acad Sci 94:11726–11730

Hille J, Verheggen F, Roelvink P, Franssen H, Van Kammen A, Zabel P (1986) Bleomycin resistance: a new dominant selectable marker for plant cell transformation. Plant Mol Biol 7:171–176

Hoekema A, Hirsch P, Hooykaas P, Schilperoort R (1983) A binary plant vector strategy based on separation of vir-and T-region of the Agrobacterium tumefaciens Ti-plasmid. Nature 303:179–180

Hooykaas PJ, Schilperoort RA (1992) Agrobacterium and plant genetic engineering. In: 10 years plant molecular biology. Springer, pp 15–38

Jayaraj J, Anand A, Muthukrishnan S, Punja Z (2004) Pathogenesis-related proteins and their roles in resistance to fungal pathogens. In: Fungal disease resistance in plants: biochemistry, molecular biology, and genetic engineering, pp 139–177

Jefferson RA, Burgess SM, Hirsh D (1986) beta-Glucuronidase from *Escherichia coli* as a gene-fusion marker. Proc Natl Acad Sci 83:8447–8451

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821
Job D (2002) Plant biotechnology in agriculture. Biochimie 84:1105–1110
Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14:49–55
Kanchiswamy CN, Sargent DJ, Velasco R, Maffei ME, Malnoy M (2015) Looking forward to genetically edited fruit crops. Trends Biotechnol 33:62–64
Koncz C, Németh K, Rédei GP, Schell J (1994) Homology recognition during T-DNA integration into the plant genome. In: Homologous recombination and gene silencing in plants. Springer, pp 167–189
Legrand M, Kauffmann S, Geoffroy P, Fritig B (1987) Biological function of pathogenesis-related proteins: four tobacco pathogenesis-related proteins are chitinases. Proc Natl Acad Sci 84:6750–6754
Li C, Wang Y, Huang X, Li J, Wang H, Li J (2013) De novo assembly and characterization of fruit transcriptome in *Litchi chinensis* Sonn and analysis of differentially regulated genes in fruit in response to shading. BMC Genomics 14(1): 552
Li WC, Wu JY, Zhang HN, Shi SY, Liu LQ, Shu B, Liang QZ, Xie JH, Wei YZ (2014) De novo assembly and characterization of pericarp transcriptome and identification of candidate genes mediating fruit cracking in *Litchi chinensis* Sonn. Int J Mol Sci 15:17667–17685
Li C, Wang Y, Huang X, Li J, Wang H, Li J (2015a) An improved fruit transcriptome and the identification of the candidate genes involved in fruit abscission induced by carbohydrate stress in litchi. Frontiers in Plant Science 6
Li Q, Chen P, Dai S, Sun Y, Yuan B, Kai W, Pei Y, He S, Liang B, Zhang Y (2015b) PacCYP707A2 negatively regulates cherry fruit ripening while PacCYP707A1 mediates drought tolerance. J Exp Bot 66:3765–3774
Lihui Z, Liuxin L (2003) A preliminary report on *Agrobacterium tumefaciens* mediated genetic transformation of Litchi. J Fruit Sci 4:011
Lin T, Lin Y, Ishiki K (2005) Genetic diversity of *Dimocarpus longan* in China revealed by AFLP markers and partial rbcL gene sequences. Sci Hortic 103:489–498
Liu W, Yuan JS, Stewart CN Jr (2013) Advanced genetic tools for plant biotechnology. Nat Rev Genet 14:781–793
Liu W, Xiao Z, Bao X, Yang X, Fang J, Xiang X (2015) Identifying litchi (*Litchi chinensis* Sonn.) cultivars and their genetic relationships using single nucleotide polymorphism (SNP) markers. PLoS One 10:e0135390
Lu X, Kim H, Zhong S, Chen H, Hu Z, Zhou B (2014) De novo transcriptome assembly for rudimentary leaves in *Litchi chinensis* Sonn. and identification of differentially expressed genes in response to reactive oxygen species. BMC Genom 15:1
Madhou M, Normand F, Bahorun T, Hormaza J (2013) Fingerprinting and analysis of genetic diversity of litchi (*Litchi chinensis* Sonn.) accessions from different germplasm collections using microsatellite markers. Tree Genet Genomes 9:387–396
McConchie C, Vithanage V, Batten D (1994) Intergeneric hybridisation between litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour.). Ann Bot 74:111–118
Menzel C (1985) Propagation of lychee: a review. Sci Hortic 25:31–48
Menzel C (2002) The lychee crop in Asia and the Pacific. Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific, Bangkok, Thailand
Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Annu Rev Plant Biol 61:443–462
Moscou MJ, Bogdanove AJ (2009) A simple cipher governs DNA recognition by TAL effectors. Science 326:1501–1501
Nester EW, Gordon MP, Amasino RM, Yanofsky MF (1984) Crown gall: a molecular and physiological analysis. Annu Rev Plant Physiol 35:387–413
Nishizawa Y, Hibi T (1991) Rice chitinase gene: cDNA cloning and stress-induced expression. Plant Sci 76:211–218

Ow DW, De Wet JR, Helinski DR, Howel SH, Wood KV, Deluca M (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science 234:856–859

Padilla G, Pérez JA, Perea-Arango I, Moon PA, Gómez-Lim MA, Borges AA, Expósito-Rodríguez M, Litz RE (2013) *Agrobacterium tumefaciens*-mediated transformation of 'Brewster' ('Chen Tze') litchi (*Litchi chinensis* Sonn.) with the PISTILLATA cDNA in antisense. In Vitro Cell Dev Biol Plant 49:510–519

Puchooa D (2004) Expression of green fluorescent protein gene in litchi (*Litchi chinensis* Sonn.) tissues. J Appl Hortic 6:11–15

Punja ZK (2006) Recent developments toward achieving fungal disease resistance in transgenic plants. Can J Plant Pathol 28:S298–S308

Rao AQ, Bakhsh A, Kiani S, Shahzad K, Shahid AA, Husnain T, Riazuddin S (2009) The myth of plant transformation. Biotechnol Adv 27:753–763

Rui-Feng H, Yuan-Yuan W, Bo D, Ming T, Ai-Qing Y, Li-Li Z, Guang-Cun H (2006) Development of transformation system of rice based on binary bacterial artificial chromosome (BIBAC) vector. Acta Genet Sin 33:269–276

Sanford JC, Klein TM, Wolf ED, Allen N (1987) Delivery of substances into cells and tissues using a particle bombardment process. Part Sci Technol 5:27–37

Sarin N, Prasad U, Kumar M, Jain SM (2009) Litchi breeding for genetic improvement. In: Breeding plantation tree crops: tropical species. Springer, pp 217–245

Saunders JA, Lin CH, Hou BH, Cheng J, Tsengwa N, Lin JJ, Smith CR, McIntosh MS, Van Wert S (1995) Rapid optimization of electroporation conditions for plant cells, protoplasts, and pollen. Mol Biotechnol 3:181–190

Serres R, Stang E, McCabe D, Russell D, Mahr D, McCown B (1992) Gene transfer using electric discharge particle bombardment and recovery of transformed cranberry plants. J Am Soc Hortic Sci 117:174–180

Sidhu JS (2012) Tropical fruit II: production, processing and quality of guava, lychee, and papaya. In: Handbook of fruits and fruit processing, 2nd edn, pp 591–628

Singh H, Babita S (2002) Lychee production in India. Lychee Production in the Asia-Pacific Region Food and Agricultural Organization the United Nations, Bangkok, Thailand, pp 55–67

Smith EF, Townsend CO (1907) A plant-tumor of bacterial origin. Science:671–673

Sorokin AP, Ke X-Y, Chen D-F, Elliott MC (2000) Production of fertile transgenic wheat plants via tissue electroporation. Plant Sci 156:227–233

Stalker DM, McBride KE, Malyj LD (1988) Herbicide resistance in transgenic plants expressing a bacterial detoxification gene. Science 242:419–423

Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, Voytas DF (2009) High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 459:442–445

Trick HN, Finer JJ (1997) SAAT: sonication-assisted Agrobacterium-mediated transformation. Transgenic Res 6:329–336

Tzfira T, Citovsky V (2006) Agrobacterium-mediated genetic transformation of plants: biology and biotechnology. Curr Opin Biotechnol 17:147–154

Vain P (2007) Thirty years of plant transformation technology development. Plant Biotechnol J 5:221–229

Vancanneyt G, Schmidt R, O'Connor-Sanchez A, Willmitzer L, Rocha-Sosa M (1990) Construction of an intron-containing marker gene: splicing of the intron in transgenic plants and its use in monitoring early events in Agrobacterium-mediated plant transformation. Mol Gen Genet 220:245–250

Viruel M, Hormaza J (2004) Development, characterization and variability analysis of microsatellites in lychee (*Litchi chinensis* Sonn., Sapindaceae). Theor Appl Genet 108:896–902

Visarada K, Meena K, Aruna C, Srujana S, Saikishore N, Seetharama N (2009) Transgenic breeding: perspectives and prospects. Crop Sci 49:1555–1563

Waldron C, Murphy E, Roberts J, Gustafson G, Armour S, Malcolm S (1985) Resistance to hygromycin B. Plant Mol Biol 5:103–108

Wang J, Liu B, Xiao Q, Li H, Sun J (2014) Cloning and expression analysis of litchi (*Litchi chinensis* Sonn.) polyphenol oxidase gene and relationship with postharvest pericarp browning. PLoS One 9:e93982

Wang B, Tan H-W, Fang W, Meinhardt LW, Mischke S, Matsumoto T, Zhang D (2015) Developing single nucleotide polymorphism (SNP) markers from transcriptome sequences for identification of longan (*Dimocarpus longan*) germplasm. Hortic Res 2:14065

Wu Y, Yi G, Zhou B, Zeng J, Huang Y (2007) The advancement of research on litchi and longan germplasm resources in China. Sci Hortic 114:143–150

Xiong J-S, Ding J, Li Y (2015) Genome-editing technologies and their potential application in horticultural crop breeding. Hortic Res 2:15019

Zeldin EL, Jury TP, Serres RA, McCown BH (2002) Tolerance to the herbicide glufosinate in transgenic cranberry (*Vaccinium macrocarpon* Ait.) and enhancement of tolerance in progeny. J Am Soc Hortic Sci 127:502–507

Zhang H-N, Wei Y-Z, Shen J-Y, Lai B, Huang X-M, Ding F, Su Z-X, Chen H-B (2014) Transcriptomic analysis of floral initiation in litchi (*Litchi chinensis* Sonn.) based on de novo RNA sequencing. Plant Cell Rep 33:1723–1735

Zupan JR, Zambryski P (1995) Transfer of T-DNA from Agrobacterium to the plant cell. Plant Physiol 107:1041

Conventional Versus Modern Approaches in Litchi Tissue Culture

19

Aparna Pareek

Abstract

The litchi (*Litchi chinensis* Sonn) is placed among the topmost subtropical evergreen fruit crops, has its origination from China, and then routed to India with ancient and holistic approaches. In India, litchi holds the ranking of seventh in area and ninth in production among fruit crops, but in value forms, it ranks sixth. With the advent of farming and scientific amalgamations litchi production and productivity is constantly being increased in the country. Due to natural variants among litchi genotypes, it offers significant roles in socioeconomics (i.e., nutrition, taste, medicine, etc.). Litchi cultivation is still based on conventional approaches, viz., grafting, air layering, etc., which have wearing and tearing approaches. In current scenario litchi biotechnology is still in scarcity which needs to be enhanced with modern approaches. Here, I propose the potential ways (micropropagation, germplasm culture, anther culture, etc.) to propagate litchi trees with tissue culture approach.

Keywords

Litchi • Fruit crop • Genotype • Nutrition • Micropropagation • Anther culture

A. Pareek (✉)
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India
e-mail: aparna992000@yahoo.com

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19.1 Introduction

The litchi (*Litchi chinensis* Sonn) is an important subtropical evergreen fruit crop belonging to family *Sapindaceae* and is originated from China, where it grows in southern Guangdong State. It is highly specific to climatic conditions, and probably for this reason, its cultivation is restricted to only a few countries in the world. In India, litchi was introduced in the eighteenth century via Burma, and from there later it spread to many countries. India and China account for about 91% of the world litchi production, but it is solely marketed locally. In India, 428,900 metric tons of litchi is produced annually from 56,200 hectares of land. Litchi being grown in specific climatic requirements is confined only to a few states of India with about 74% of production in Bihar. In Bihar litchi is the source of livelihood for millions of people as it provides both on-farm and off-farm employment to many. Small and marginal farmers get additional income from litchi plants. Thus, litchi cultivation is the livelihood security for a large population, especially in the state of Bihar.

19.2 About the Tree

The litchi tree is a very dense tree having a round top and grows slowly with evergreen leaves having 6–9 elliptic oblong and lanceolate abruptly pointed leaves. Greenish white or yellowish flowers are mostly borne in groups. Fruits are round or heart shaped having thin, leathery skin. The color of fruits often varies with cultivar and is red or rose or pinkish. The edible portion or fruit is the aril, which is immediately found beneath the skin. Seeds are bold, but in certain cultivars, seeds are partially developed, due to failure of pollination, referred to as "chicken tongue" seed.

The growing of litchi in different states of the country under varied climatic conditions has major advantages in terms of its earliness and extended harvest. It has a very narrow genetic base; therefore, under certain given climatic conditions, the fruits are available only for 3–4 weeks. However, due to the spread of cultivation over a wide range of climatic conditions, there is a possibility for extending the cropping period from the first week of May to the first week of July. With an expanding market, there is ample potential for increasing area and production with improved production technology and its efficient post-harvest measures to be taken care of. In the present scenario, looking at the importance of the fruit, an effort has been made to summarize the current status and identification of the conventional versus modern approaches in litchi tissue culture. (www.nansproducts.co).

19.3 Present Scenario of Litchi Cultivation

19.3.1 Area and Production

In India, litchi holds the seventh position in area and ninth in the production among various fruit crops, but in value-related terms, it ranks sixth in position. At the national level, fruits, viz., banana and mango, are the most important fruits, but in Bihar state, litchi fruit is considered to be the most potent fruit as it contributes significantly to its total fruit production. There has been a substantial increase in the area and production of litchi in the last 50 years. Area has increased from 9400 hectares in 1949–1950 to about 56,000 hectares in 1998–1999. The contribution of litchi to total area under fruit has increased from 0.75% to 1.5%. An increase in area between 1991–1992 and 1998–1999 (7 years) has been 14.28%, while production increase during the same period is to the tune of 75%. Productivity also enhanced to an increase of 52.91% during the same period. Evidently, production and productivity of litchi are constantly in an increasing graph (www.fao.org).

19.3.2 Varieties of Litchi in the Country

Litchi varieties grown in India are variable under various climatic and soil conditions. Singh (1954) described 33 varieties and further classified them into 15 groups; varieties of litchi grown in India have also been subsequently described (Singh 1998). Indian cultivars differ greatly in the vegetative flushing pattern, flush color, and flowering ability. Based on these characteristics, cultivars were classified in five groups (Singh 1998). Group A, which has seven cultivars is the early group, B and C groups are mid-season, and group D is the late group. Only one cultivar is there, which is very late, and is under group E, and its cultivation is restricted to Muzaffarpur.

19.3.3 Litchi Cultivation

The litchi cultivation is highly successful in areas having minimum temperature of 10 °C from December to February and 38 °C from April to June. However, a temperature of 32 °C during these months is considered to be optimum for its cultivation. It is highly specific to climatic requirement for its establishment, plant growth, and fruiting. A moist atmosphere, occasional rainfall, cool dry winter free from frost, and hot winds are ideal for its cultivation. In India, litchi is grown successfully on a wide variety of soil types, which includes sandy loams, laterite, alluvial sand, and calcareous soil, but the best litchi orchards can be seen in alluvial sandy loam soils with good drainage and access to the water table.

19.3.4 Production of the Planting Material

Litchi is mostly multiplied by vegetative methods of propagation as plants raised through sexual means, i.e., by means of seeds, grow very slowly and have a very long juvenile period and further do not produce fruits which are true to type. However, its introduction earlier in certain parts of the country was through the seeds, which enabled its selection of all superior varieties and perpetuating the cultivar through vegetative methods. The most common practiced method of vegetative propagation is air layering, through cutting; grafting and budding have also been found to be successful techniques for its multiplication.

19.3.5 Potential for Litchi Production Development

Due to the increased market base, there is an ample opportunity for increasing the area for litchi production as the prevailing agroclimatic conditions have not been fully exploited in such cases. The area under various situations could be exploited for extended harvest. Based on the fruiting behavior, quality development, and area under cultivation of the fruit concerned, the litchi-growing areas can be placed in a manner to take full advantage of climatic variability. However, to increase production and productivity, concrete methods would be required for obtaining technological support and development of infrastructure

19.3.6 Constraints in Litchi Production Development

Despite the truth that litchi is one of the finest fruits and has a growing demand in the national and international market, productivity still continues to be very less, and a lacuna exists between its potential and present existing yield. The ratio in the yield between the best managed orchards and national productivity ranges two to four times at different locations. The probable reasons for the lesser yield of the crop plants are the narrow genetic base of the crop, nonavailability of suitable superior cultivars, traditional production systems, poor technological support system, and incidence of various insect pests, coupled with the poor post-harvest management techniques employed. The shortage of appropriate planting material in addition to the long juvenile period of litchi fruit is also the constraints in its production rate. Suitable agro-techniques particularly for source and sink management, micronutrients, post-harvest technology, and effective marketing need due attention in time to come. In this context exchange of information among countries would be a beneficial step in the present scenario. The following points need due consideration in time to come.

1. Target-oriented programs for high and better yield must be launched so that germplasm can be conserved.
2. A systematic and potential approach for the description of cultivars is required.

3. A litchi descriptor needs to be developed for future generations.
4. Faster multiplication techniques must be employed for the production of quality planting material.
5. There is an urgent need to develop propagation technology for faster multiplication of quality plants.
6. The development of nutrition management systems to maintain tree health and encourage successful flowering, fruiting, and quality in sustainable manner requires attention.
7. Monitoring of the nutritional factors in litchi through leaf analysis would be an important approach for efficient fertilizer use.
8. Integrated management of nutrient and water ratio with efficient monitoring mechanisms would improve input use efficiency of the fruit.
9. Through the effective recycling of the residues with organic manure, it is possible to improve the soil health.
10. Integrated management of insect pests and diseases is required to improve production.
11. Infrastructure for post-harvest management requires further emphasis to reduce risk areas.
12. The litchi production range is to be widened for effective utilization of the fruit.
13. There must be cooperation among litchi-growing countries for the exchange of information, and cultivars are vital.
14. Starting of a network program on litchi would enhance the production and ensure livelihood security of the people (www.fao.org).

19.4 Biotechnology of Litchi

Biotechnological approaches for litchi production have been under research for the last more than two decades. Firstly, cell engineering based on tissue culture techniques was emphasized including (1) maintaining the cultures with young and small embryos, (2) anther or pollen cultures are raised to obtain haploid plants, and (3) basic research is focused on protoplast culture studies. Subsequently, with the rapid development of biotechnological approaches, research was reoriented toward genetic engineering, including mainly molecular marker techniques and new germplasm construction by protoplast fusion and transformation.

Litchi (*Litchi chinensis* Sonn), also known as "the queen of fruit" (Menzel and Waite 2005), is a potential fruit tree in the tropical and subtropical regions of the world (Menzel 1983). The plant if given long reproductive cycles and a very highly heterozygous genetic background (Litz 1988; Raharjo and Litz 2005) proves the fact that new litchi cultivars are difficult to be produced via the conventional breeding methods. Modern breeding techniques such as gene manipulation techniques have the advantages of high efficiency and directional improvement of specific traits in the plant providing a new way for the improvement of litchi cultivars (Das and Rahman 2012). Though many efforts have been made regarding the subject, only a few successful results have been published. Fu and Tang (1983) reported that they

obtained 24 plantlets via organogenesis from pollen callus. Subsequent reports were also made on somatic embryogenesis and plantlet regeneration in litchi via the culture of anthers (Deng 2005; Xie et al. 2006; Wang et al. 2013), immature embryos (Zhou et al. 1996; Kuang et al. 1997), or protoplasts (Yu et al. 1996). Das et al. (1999) reported a high proliferation rate from mature seeds using two kinds of methods. Yu et al. (2000) achieved somatic embryogenesis and plantlets from "Xiafanzhi" litchi protoplasts isolated from embryogenic suspensions. Puchooa (2004) used young unvaccinated "Tai So" leaves as explants to study various factors on the regeneration of the leaf blade and eventually obtained regenerated plants. By "Heli" anther culture, Guo et al. (2014) obtained somatic embryos with root and no sprout. However, Huang and You (1990) and Yu (1991) and Guo et al. (2014) reported that the stem sprout buds failed to produce (Huang and You 1990; Yu 1991). All the above results showed that the regeneration of different litchi varieties is inconsistent with their medium. The same culture medium plays different roles on the regeneration of different litchi varieties. Therefore, different genetic backgrounds of litchi varieties have a remarkable effect on in vitro regeneration ability (academicjournals.org).

Various methods have been employed in the biotechnology of the plant. Some of the methods are described.

19.4.1 Micropropagation

Superior selections and hybrids developed at various research centers failed to reach the orchardists due to the lack of sufficient planting material. It leads to nonrealization of the potential of improved cultivars, thus making the efforts of fruit improvement programmer unfruitful. In this case, micropropagation can be a powerful tool for large-scale propagation of fruit crops. This is an ideal system for production of disease-free plants. Among the fruits, micropropagation has been most successful in banana, papaya, and date palm multiplication.

19.4.2 Germplasm Conservation

The potential importance of natural gene pool to meet the future needs of crop improvement by introducing specific characters such as abiotic stress resistance cannot be underestimated. However, the number of wild species and their natural habitats is disappearing rapidly, as a result of the introduction of highly bred modern varieties, growing urbanization, and an increased pressure on land for cultivation. This leads to the erosion of the natural germplasm to such extent that there is a general fear that potentially valuable germplasm is being lost irretrievable. In plant improvement, it is necessary to facilitate the assimilation of germplasm collection in working for the use of the breeders.

National Bureau of Plant Genetic Resources, New Delhi, is actively engaged in maintaining the large in vitro germplasm collection of certain fruits like banana,

phalsa, bael, jackfruit, pomegranate, etc. There are two basic pathways which are followed for maintaining the germplasm in vitro. The conservation of germplasm through biotechnological approaches is a more efficient technique for short- and medium-term storage. It can be achieved by reducing the temperature and light concentrations. The advantage of this approach is that cultures can be readily brought back to normal culture conditions to produce plants on demand, but the disadvantage is that culture may be subjected to contamination and somaclonal variations. Cryopreservation applied at ultralow temperature of liquid nitrogen at −190 °C offers the potential for long-term storage with maximum phenotypic and genotypic stability.

19.4.3 Anther Culture

In vitro androgenesis holds a myriad of possibilities for improvement of horticultural crops. This technology has been extended for a number of horticultural crops. The purpose of anther and pollen culture is to produce haploid plants by the induction of embryogenesis from repeated divisions of monoploid spores, either microspore or immature pollen grains. The major interest in haploids is based upon the production of homozygous plants as an alternative for repeated cycles of inbreeding in self-pollinated crops. In cross-pollinated species, double haploids are more to be used as parents in the production of single or double cross hybrids which are as follows. As a result of haploid induction, chromosome homozygosity is attained in a very short time. This is particularly useful in heterozygous and self-incompatible crops like mango, sunflower, potato etc. With the use of homozygous parents in crossing program, the production of pure F1 hybrids becomes possible. Haploid cell lines have great advantages in studies on mutant selection in vitro.

19.4.4 Overcoming Crossing Barriers Embryo Culture

This technique pertains to the cultivation of excised plant embryo in artificial medium. Embryo culture technique has found its application both in the applied and basic research. In the conventional plant breeding program, breeder often faces problem in transferring resistance from wild species to the cultivated species and getting the desirable interspecific hybrids. The application of embryo rescue can overcome some of the pre- and post-fertilization barriers in fruit crops. Further, most useful and popular application of zygotic embryo culture has been used in raising hybrids.

19.4.5 Somaclonal Variation

Somaclonal variation explores the naturally occurring or in vitro-induced variability of somatic cells following plant regeneration. Somaclonal variation is an excellent

method for shortening breeding programs. Somaclonal variation may be due to changes in chromosome number and structural variation of chromosomes due to deletions, duplication, translocation, genetic and cytoplasmic mutation, etc. Hwang and Ko (1987) identified Somaclonal variation in the cultivars Giant Cavendish with putative field resistance to Fusarium wilt (race 4) but inferior in agronomic characters.

19.4.5.1 Somatic Hybridization

It is an approach of immense value in the area of fruit breeding. Somatic hybridization provides a method where sexual incompatibility in the plants can be bypassed. Protoplast culture includes a series of operation such as an isolation of the protoplasts from cells, culturing them in a suitable medium, inducing them to divide, and then regenerating plantlets from them. The fusion of protoplasts may occur spontaneously or they may be induced to fuse in the presence of fusogenic agent.

19.5 Molecular Markers

The various morphological characters, chemical composition concerned, and cytological details have been used over the years for the categorization of plants. However, these techniques have certain limitation as they can be influenced by certain environmental and developmental factors. The molecular markers are now being increasingly used in the areas of plant classification and breeding. Polygenic characters which are very difficult to analyze using traditional plant breeding methods can be easily analyzed using molecular markers.

19.6 Genetic Engineering

The advent of recombinant DNA technology has opened tremendous possibilities for transforming almost any plant by transferring any gene from any organism across, taxonomic barriers. The process of genetic drift necessitates measure that germplasm must be conserved in such a manner that there should be minimal losses of genetic variability of a population. The conventional methods of germplasm conservation may be vulnerable to losses due to:

(i) Attack by pest and pathogens.
(ii) Climatic disorders.
(iii) Natural disasters.
(iv) Political and economic causes. Besides this, the seeds of many important fruit plants, such as mango, litchi, etc., may lose their viability in a short time under conventional storage system.

Mutagenesis approach would be an ideal process for developing new mutant lines and also for enhancing the germplasm of litchi. International Atomic energy Agency (IAEA) maintains mutant variety database (www.iaea.org) which includes

over 2300 officially released mutant varieties of various crops in different countries. A wide range of mutants would be of potential use in the molecular characterization of the fruit and further identify the trait-specific molecular markers in study. These markers will be very much helpful in the molecular marker-assisted selection and breeding programs. Plant regeneration of litchi through various micropropagation techniques on a mass scale would require extensive research as this area is still in its infancy state (Sarin 2009).

Vegetative means of propagation of litchi fruit maintains the "true-to-type" nature of cultivars, as propagation through seed often produces inferior cultivars in terms of fruit quality, size, and maturity period. However, the diversification of litchi cultivation also requires some other traits like precocities, dwarfness, regularity in bearing wide adaptability, and resistance to various disorders. Asexual means of propagation techniques cannot provide variations required for such purposes, whereas sexual methods produce a large number of variants. Thus there is an urgent need for raising plantlets through seeds because new variants would provide better selections (Breeding of fruit and plantation crops-Biotechnological approaches).

19.7 Major Problems of Litchi Production

- Narrow genetic base and lack of natural biodiversity
- Less time availability of the fruits with very high post-harvest losses
- Lack of elite planting materials along with scientific knowledge in fast multiplication of the fruit
- Lack of complete knowledge of scientific basis for shoot maturity and flowering
- Lack of quantified role of microorganisms in plant health and the effect on yield
- Short shelf life and lack of information on proper storage environment
- Lack of proper institutional support, infrastructure and various human resource development programs running presently (icar.org.in)

19.7.1 Indian Council of Agricultural Research Constraints in Litchi Development

Various factors are as follows:

- There is a short production season of the fruit at a specific place.
- Absence of post-harvest infrastructure in the specified places.
- There is total absence of an efficient and nationwide marketing system.
- Poor and unorganized production by individual farmers.
- There is lack of trained manpower and entrepreneurs.
- Climatic constraints are also important factors (viz., absence of sufficient chilling temperature during winter, hot winds and hailstorm during summer months, and frost/ low temperature during winter) (icar.org.in).

19.8 Constraints for Litchi Production

19.8.1 Major Constraints or Factors Governing Constraints

Litchi production faces some constraints which include:

1. There is a limited overseas market for litchi production.
2. Limited consuming population of this fruit.
3. Limited transportation capacity.
4. High labor cost.
5. Low yield.
6. Low price of the fruit.
7. Lack of infrastructures.
8. Irrigation and fertilizer system.
9. Cold chain especially cold room.
10. Processing facilities and consumer preferences (icar.org.in).

19.8.2 Government Policies and Plans for Development of Research in Litchi

The overall research program for the varietal species and production technology improvement is given through the All India Coordinated Research Project on Subtropical Fruits, which has four centers located in litchi-growing regions. The Central Horticultural Experimental Station (CHES), Ranchi, Jharkhand, RAU, Pusa, Samastipur, Bihar, G.B. Pant University of Agriculture and Technology, Pantnagar, Udhamsingh Nagar, Uttaranchal, and BCKVV, Mohitnagar, Nadia, West Bengal are engaged in research (www.fao.org). The main thrust of research program is specially designed on the augmentation of germplasm, varietal evaluation, orchard management, propagation studies, and development of fruit production technologies for the higher yield and improved shelf life. A network project for improving productivity of litchi has also been initiated. A National Research Centre on Litchi has also been started for strategic and basic research on litchi.

19.9 Conclusions

The basic in vitro protocol for manipulating elite litchi selections is available and further could be utilized to address various plant breeding objectives. Comparable studies are very necessary in order to define a de novo regeneration pathway from elite litchi selections. Genetic transformation of litchi is feasible. It should also be possible to apply in vitro mutation induction and selection procedures to address certain fungal diseases that affect specific litchi cultivars. Genetic transformation could possibly be utilized to develop the preferred “chicken tongue” seeds, using

the pistillate gene from *Arabidopsis thaliana*, which mediates seedlessness. Protoplast technology could be harnessed to produce somatic hybrids between haploids and diploids, as a way of producing seedless triploids. The resolution of problems associated with rapid deterioration of fruit after harvest are more difficult to address at this time, since litchi is non-climacteric.

References

Das DK, Rahman A (2012) Expression of a rice chitinase gene enhances antifungal response in transgenic litchi (cv. Bedana). Plant Cell Tissue Organ Cult 109:315–325

Das DK, Shiva Prakash N, Bhalla-Sarin NB (1999) Multiple shoot induction and plant regeneration in litchi (*Litchi chinensis* Sonn.). Plant Cell Rep 18:691–695

Deng CJ (2005). Studies on high frequency somatic embryogenesis and regeneration culture in litchi. Master's thesis of Hunan Agricultural University, Changsha, Hunan, China

Fu L, Tang D (1983) Induction pollen plants of litchi tree (*Litchi chinensis* Sonn.). Acta Genet Sin 10(5):369–374

Guo SY, Peng HX, He XH, Ding F, Li HL, Qin XQ, Xu N (2014) Callus induction from different explants of litchi. Southwest China J Agric Sci 27(2):748–753

Huang ZZ, You KZ (1990) Primary study on the litchi shoots induction. Guangdong Agric Sci 3:27–29

Hwang SC, Ko WH (1987) Somaclonal variation of banana and screening for resistance of Fusarium wilt. In: Persley GJ, De Langhe EA (eds) Banana and plantain breeding strategies. ACIAR proceedings no. 21. ACIAR, Canberra, pp 157–160

Kuang ZS, Zhou LN, Ma XJ, Chen JQ, Cai J (1997) Plant regeneration via somatic embryogenesis in litchi. Guangdong Agric Sci 1:15–17

Litz RE (1988) Somatic embryogenesis from cultured leaf explants of the tropical tree *Euphoria longan* stend. J Plant Physiol 132:190–193

Litz RE, Raharjo S (2005) *Dimocarpus longan* and *Litchi chinensis*. In: Litz RE (ed) Biotechnology of fruit and nut crops. CABI, Wallingford, pp 628–636

Menzel CM (1983) The control of floral initiation of lychee: a review. Sci Hortic 21:201–215

Menzel CM, Waite GK (2005) Litchi and longan: botany, production and uses. CABI, Wallingford, p 305

Sarin NB (2009) Litchi breeding for genetic improvement. Breeding plantation tree crpos. Tropical species

Puchooa (2004) In vitro regeneration of lychee (*Litchi chinensis* Sonn.). Afr J Biotechnol 3(11):576–584

Singh HP (1998) Genetic diversity, breeding and utilisation of the Genepool of Litchi. Tropical Fruits in Asia (Arora and Rao Eds) IPGRI, pp 171–184

Singh HP, Yadav IS (1988) Physico-chemical changes during fruit development in litchi cultivar. India J Hortic 45:212–218

Singh LB, Singh OP (1954) The litchi. Superintendent Printing Press, Allahabad, 110p

Wang G, Li HL, Wang JB (2013) Primary study on the callus induction from anther of two litchi (*Litchi chinensis* Sonn.) cultivars. Chin J Trop Crops 34(4):669–674

Xie YM, Yi GJ, Zhang QM, Zeng JW (2006) Somatic embryogenesis and plantlet regeneration from anther of Feizixiao litchi. Chin J Trop Crops 27(1):68–72

Yu YB (1991) Study on some factors in tissue culture of lychee (*Litchi chinensis*). Fujian Agric Sci Technol 5:17–18

Yu CH, Chen ZG, Lu LX (1996) Regeneration of plantlets from protoplasts culture of litchi. J Fujian Agric Univ 25(3):386

Yu CH, Chen ZG, Lu LX, Lin JW (2000) Somatic embryogenesis and plantlets from "Xiafanzhi" litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tissue Organ Cult 61:51–58

Zhou LN, Kuang ZS, Ma XJ, Cheu JQ, Cao J (1996) The study of factors affecting somatic embryogenesis in young embryo culture of *Litchi chinensis*. J Agric Biotechnol 4(2):161–165