The Lychee Biotechnology

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Editors

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Preface

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

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

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

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

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

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

Chapter 2. As for plant management, by means of studying the biology of flower and fruit development, researchers and growers have developed several special cultural technologies to apply for the commercial production of litchi. The current status of breeding, biology of flower and fruit development and cultural research in Taiwan are discussed in this review.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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