Minami Matsui Keng-See Chow *Editors*

The Rubber Tree Genome



Compendium of Plant Genomes

Series Editor

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Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant Arabidopsis thaliana in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

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The Rubber Tree Genome



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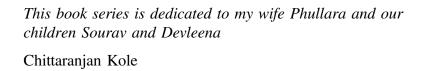
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Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period, a number of new mapping populations beyond F₂ were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

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As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But the development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, the development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes," a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated Web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful to both students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology,

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physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with lifetime experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, particularly Dr. Christina Eckey and Dr. Jutta Lindenborn for the earlier set of volumes and presently Ing. Zuzana Bernhart for all their timely help and support.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

Kalyani, India

Chittaranjan Kole

Preface

Hevea brasiliensis Müll. Arg., also known as the para rubber tree, is the sole species that is cultivated for the commercial production of natural rubber. Natural rubber (or *cis*-polyisoprene) is synthesized in the latex of this tree and harvested by systematic tapping of the tree trunk. The productive life of the rubber tree may extend beyond 20 years from first bark tapping in plantations managed under optimal agronomic practices. The genus Hevea (2n = 36) comprises 10 species, however only the *brasiliensis* species has been commercialized on a wide scale, primarily in Southeast Asian countries. The success of rubber production facilitated the growth of the automobile, medical, aviation, electronics, and building and construction industries, not to mention the manufacturing of a substantial range of rubber products essential to daily human life. Southeast Asian countries generate more than 95% of the global natural rubber production to meet increasing world demand. Through the effort of breeding and selection, the production capacity of modern rubber clones has reached more than four times that of the original parental trees. Nonetheless, the plantation industry is still facing the threat of diseases and the limitations of a narrow gene pool for breeding.

The significance of the rubber tree as a world crop warrants investment into genome-based technologies to deepen the current understanding of tree physiologies, particularly in latex, which affect production capacity. The rubber tree has accumulated a wealth of fundamental biology based on conventional scientific approaches spanning most of the twentieth century. Genomics and biotechnology approaches complement the existing knowledgebase and have great potential in discovering genes, proteins, and other regulatory components that could be engineered to enhance tree productivity. The period 2013–2017 was pivotal for rubber genome sequencing: the first draft genome sequence was published in 2013, followed by the release of three higher quality genome sequences of rubber tree clones using advanced sequencing technologies. Concurrently, genome-based investigations flourished as evidenced by the large number of publications by members of the rubber tree research community, particularly among the molecular biologists, physiologists, geneticists, and breeders.

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In this book, we have compiled recent genome-related research, beginning with the experiences of the first three research groups in sequencing the genomes of rubber clones Reyan 7-33-97, RRIM 600, and BPM 24. The importance of harnessing benefits from genome information is reflected in chapters discussing the development of markers, genetic linkage maps, marker-trait association, and databases to facilitate data sharing and utilization. Applications of transcriptome and proteome analyses of major metabolic pathways involved in rubber biosynthesis and latex yield are also included. This book has drawn on the experience of contributors with a broad range of expertise in rubber tree research and/or background in genomic technologies. Nonetheless, we also recognize the value of parallel developments in other plants which have similarly accumulated sequence resources, namely, the Euphorbiaceae species such as *Manihot esculenta* (cassava), Ricinus communis (castor bean) and Jatropha curcas (physic nut), and alternative rubber-producing species such as Taraxacum kok-saghyz. We hope that this book will serve to record genome sequencing as a milestone in rubber tree research, and also the scientific investigations made during the initial phase of the research community's engagement with genome data.

Genomics is rapidly evolving and so will the directions of rubber tree genomics as more and more cutting-edge technologies become available. In the continuing quest for high-resolution genomes, we can expect to see publications of chromosome-level genome assemblies, high-density genetic maps, and tools for genome selection in the near future. Given the potential of inter- and intra-species genetic diversity for molecular breeding, we may also expect additional genomes of different *Hevea* species and cultivated genotypes to be sequenced. Insights from comparative genomics are likely to promote closer cooperation between genome scientists in the fields of *Hevea* and non-*Hevea* rubber research.

The rubber tree research community is relatively small compared to many major agricultural crops. The International Rubber Research and Development Board (IRRDB) has played an important role in promoting links between genomics and biotechnology researchers from different natural rubber-related organizations. In the course of preparing this book, we have benefited from some of the annual activities and wish to thank the IRRDB for facilitating such useful meetings and discussions. Finally, we also thank Prof. Chittaranjan Kole and the Springer team for their valuable advice and kind assistance.

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Members from three rubber genome groups and the IRRDB Molecular Biology and Physiology Specialist Group gathered at the Sungai Buloh Research Station, Malaysian Rubber Board, Selangor on April 9, 2019.

From left: Azlina Bahari (MRB), Yuko Makita (RIKEN), Han Cheng (CATAS), Minami Matsui (RIKEN), Roslinda Sajari (MRB), Thitaporn Phumichai (RAOT), Keng-See Chow (MRB), Sithichoke Tangphatsornruang (BIOTEC).

MRB: Malaysian Rubber Board, Malaysia. RIKEN: RIkagaku KENkyusho, Japan.

CATAS: Chinese Academy of Tropical Agricultural Sciences, China.

RAOT: Rubber Authority of Thailand, Thailand.

BIOTEC: National Center for Genetic Engineering and Biotechnology, Thailand.

Yokohama, Japan Subang Jaya, Malaysia January 2020 Minami Matsui, D.Sci Keng-See Chow, Ph.D.

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Abbreviations

4DTv Transversion rate at fourfold degenerate sites

ABA Abscisic acid

ACAT Acetyl-CoA acetyltransferase

ACO ACC oxidase

ACS 1-aminocyclopropane-1-carboxylic acid synthase

AFLP Amplified fragment length polymorphism AFSM Amplified fragment SNP and methylation

ATAC-seq Assay for transposase-accessible chromatin using

sequencing

atm Atmosphere

BAC Bacterial artificial chromosome

BLASTP Basic local alignment search tool (protein)

bp Base pairs

bZIP Basic leucine zipper transcription family

CAGE Cap analysis gene expression

CDS Coding sequence

ChIP-seq Chromatin immunoprecipitation sequencing

cM Centimorgan

COP Coat protein complex

COR Coronatine

CPT *cis*-prenyltransferase

CPTL/CPTBP CPT-like/CPT-binding protein

CTC CAGE tag cluster DC Donor clone

DEG Differentially expressed gene
DEP Differentially expressed protein

DNase-seq DNase I hypersensitive sites sequencing

dpi Days post-infection

DXR 1-deoxy-D-xylulose 5-phosphate reductoisomerase

DXS 1-deoxy-D-xylulose 5-phosphate synthase

EIN2 Ethylene insensitive 2
ER Endoplasmic reticulum
EST Expressed sequence tag

ET Ethylene

ETR Ethylene resistance 1

xx Abbreviations

EU Extension unit

FDE Filtered differentially expressed

FL-cDNA Full-length cDNA

FPKM Fragments per kilobase of transcript per million fragments

mapped

FPP Farnesyl pyrophosphate FPPS Farnesyl diphosphate synthase FPS Farnesyl diphosphate synthase

Gb Giga base pairs

GBS Genotyping-by-sequencing

GGPS Geranylgeranyl pyrophosphate synthase

GO Gene Ontology

GPPS Geranyl diphosphate synthase
GPS Geranyl pyrophosphate synthase

GS Genome selection

GWAS Genome-wide association study

HiC High-throughput chromosome conformation capture

HMGR 3-hydroxy-3-methylglutaryl-CoA reductase HMGS 3-hydroxy-3-methylglutaryl-CoA synthase

HMM Hidden Markov model
hpi Hours post-infection
HRBP HRT1-REF bridging protein
IDP Isopentenyl diphosphate
Indel Insertion and deletion

Inr Initiator element

IPP Isopentenyl pyrophosphate

IPPI Isopentenyl diphosphate isomerase

IRRDB International Rubber Research and Development Board

Iso-Seq Isoform sequencing

JA Jasmonate

JAZ Jasmonate ZIM-domain

JC Juvenile clones Kb Kilobase pairs

KEGG Kyoto Encyclopedia of Genes and Genomes

kg/ha/yr Kilograms/hectare/year

K_m IPP-Mg Binding constant for the IPP-magnesium substrate

LA Linolenic acid

LC-MS/MS Liquid chromatography with tandem mass spectrometry

LG Linkage group
LRP Large rubber particle
LTR Long-terminal repeat

LTR-RT Long-terminal repeat retrotransposon

MALDI-TOF Matrix-assisted laser desorption/ionization-time of flight

MAPK Mitogen-activated protein kinase

MAS Marker-assisted selection

Mb Megabase pairs

MEP 2-C-methyl-D-erythritol 4-phosphate

Abbreviations xxi

miRNA Micro ribonucleic acid

MNase-seq Micrococcal nuclease-sequencing mRNA Messenger ribonucleic acid

MVA Mevalonate

MWD Molecular weight distribution

mya Million years ago

NCBI The National Center for Biotechnology Information

ncRNA Non-coding RNA NgBR Nogo-B receptor

NGS Next-generation sequencing NMD Nonsense-mediated decay

Nr Non-redundant

NRS Non-redundant sequence ORF Open reading frame

PAML Phylogenetic analysis by maximum likelihood

PCD Programmed cell death

PDC Pyruvate dehydrogenase complex

PE Paired-end

QTL Quantitative trait locus

RACE Rapid amplification of cDNA ends
RAPD Random amplified polymorphic DNA
RBIP Rubber biosynthesis inhibitor protein
RBSP Rubber biosynthesis stimulator protein

REF Rubber elongation factor

RFLP Restriction fragment length polymorphism

RNA-seq RNA sequencing

ROS Reactive oxygen species

RPKM Reads per kilobase of transcript per million mapped reads

rRNA Ribosomal RNA RT Rubber transferase RT-ase Rubber transferase

SALB South American Leaf Blight
SAMS S-adenosyl-L-methionine synthase
SDR Short-chain dehydrogenase/reductase

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SEM Scanning electron microscope
SMRT Single-molecule real time
spaPNA Small puelled ribonuclaic acc

snoRNA Small nucleolar ribonucleic acid SNP Single nucleotide polymorphism

snRNA Small nuclear RNA
SOD Superoxide dismutase
SRA Sequence read archive
SRPP Small rubber particle protein
SRPRNA Signal recognition particle RNA
SSH Suppression subtractive hybridization

SSR Simple sequence repeat

xxii Abbreviations

TE Transposable element

TEM Transmission electron microscopy

TF Transcription factor

TFBS Transcription factor-binding site

TGN Trans-Golgi network
tmRNA Transfer-messenger RNA
TPD Tapping panel dryness
TPM Tags per million

TPM Tags per million tRNA Transfer RNA Trichostatin A

TSS Transcription start site

uORF Upstream ORF

UPP Ubiquitin proteasome pathway

WGCNA Weighted gene co-expression network analysis

WGS Whole-genome shotgun

WRP Detergent-washed rubber particle