

Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Ajit Kumar Shasany · Chittaranjan Kole *Editors*

The *Ocimum* 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 70 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 *Ocimum* Genome

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*This book series is dedicated to
my wife Phullara, and our children
Sourav, and Devleena*

Chittaranjan Kole

Foreword

Members of the genus *Ocimum* are endowed with a wide array of aroma and healing properties. Commonly known as “Tulasi,” the “holy basil” (*Ocimum tenuiflorum*) is revered and worshiped throughout India. It is one of the most important plants of the traditional systems of Indian medicine, which has been used extensively in various Ayurvedic and Yunani formulations for the treatment of various health problems. Due to its medicinal and religious importance, it has also been described as “Mother Medicine of Nature,” “The Incomparable One,” and “The Queen of Herbs” by different authors. Tulasi acts as a stimulant for physical, intellectual, and spiritual activities, which addresses many modern-day health issues, and is an integral part of the Ayurveda’s holistic lifestyle approach to health. It is believed that daily intake of Tulasi supports immunity, averts infections and diseases, promotes general health, longevity, and well-being, and also helps in dealing with the stresses of everyday life. Despite being such an important plant, the molecular studies defining the pathways and plant metabolites responsible for its diverse aroma and therapeutic activities started very late. The next-generation sequencing of the whole genome of *Ocimum* has introduced this wonder herb to the modern science and opened up the doors for unraveling its medicinal secrets, which will pave the way to harness the therapeutic potential of this holy herb.

The essential oils of the genus *Ocimum* find various applications in fragrance and cosmetic industry as well as local medicinal systems of different countries of the world. With its ever-increasing demand from aromatic industry, the concerns over improving productivity and quality of its raw materials are also increasing. Probing the transcriptome and the whole-genome sequence of *Ocimum* can help in identifying molecular markers for plant breeding to facilitate the development of improved varieties with the desired oil composition. The genus *Ocimum* like its usefulness is very wide and diverse. Chemo-profiling and pharmacognostic research followed by in vivo validation of medicinal properties have been the major focus of interest in *Ocimum* research for long, but with the advent of next-generation sequencing “*Ocimum* genomics” is fast emerging and progressing. The book *The Ocimum Genome* has been compiled with an aim to portray some of the most recent developments in *Ocimum* breeding and genomics. I highly appreciate the hard work of the editors in organizing the

diverse information and perception of the distinguished groups of scientists in a concise manner within ten chapters.

I am delighted to compliment the editors, Prof. Chittaranjan Kole, an internationally renowned agricultural scientist with original contributions on crop genomics, and Dr. Ajit Kumar Shasany, Chief Scientist at CSIR-CIMAP, an eminent scientist in the area of medicinal and aromatic plant research, and the anchor person behind the “*Ocimum* genome story” for bringing out a much desired book *The Ocimum Genome*. I am confident that this book would be a very useful resource of knowledge to the scholars and scientists, not only working on *Ocimum* but also on other medicinal or aromatic plants. Besides, this book is also expected to serve science managers and policy makers.

I sincerely hope that the present attempts to understand the hidden potential of *Ocimum* with modern approaches and outlook will open many new opportunities.

Lucknow, India

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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 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 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.

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 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, 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. 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, 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 is 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 both to 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,

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, 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, Dr. Christina Eckey and Dr. Jutta Lindenborn in particular, for all their constant and cordial support right from the inception of the idea.

I always had to set aside additional hours to edit books besides 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

The genus *Ocimum* L. is a member of family Lamiaceae, collectively called basil. The distinctive characteristics in this family are a square stem with leaves arranged in opposite decussate manner having many gland dots. The flowers have two distinct lips and are strongly zygomorphic. Many researchers have observed complexity in their chromosome numbers and ploidy levels. As per the chromosome count database (CCDB), the chromosome count of the genus ranges from a minimum of $(n) = 6$ to the maximum of $(n) = 64$. The name basil is derived from the Greek word *basilikos*, which means royal or the king. There are around 35–150 species included in the genus *Ocimum*, which are annual and perennial herbs/shrubs indigenous to Africa, Asia, Central, and South America, but extensively disseminated worldwide. The finest known strongly aromatic species are the herbs *Ocimum gratissimum* (African basil) and *Ocimum basilicum* (Thai basil) which have a pleasant odor due to the essential oils consisting of monoterpenes, sesquiterpenes, and phenylpropanoids. But the genus is also popular for its medicinal properties and spiritual sanctity which are mainly acquired by the herb *Ocimum sanctum*, also called *Ocimum tenuiflorum* (holy basil or *tulsi* in Hindi). Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal, nematocidal, and fungistatic. Such properties are often attributed to dominating essential oil constituents such as eugenol, methyl chavicol, camphor, linalool, and methyl cinnamate. Three main forms of *Ocimum tenuiflorum* are generally popular, one is *Rama tulsi* with green stems and leaves, other is *Krishna tulsi* with purple colored stems and leaves, and the third one is *Vana tulsi* which is the basic wild form. Though *Ocimum tenuiflorum* is one of the most revered plants in India having the miraculous medicinal properties, there is very less work carried out to know about the biochemical pathways of the miracle compounds which are undoubtedly the secondary metabolites present in the plant. However, in the past 5 years there had been a great pace in research on the transcriptomics and the functional genomics of *Ocimum* sp. in addition to the whole-genome sequencing of the holy basil (*Ocimum tenuiflorum*). This would help enabling the discovery of genes and the molecular markers for plant breeding to develop new improved varieties.

This book covers major aspects in the *Ocimum* spp. research in ten chapters in an attempt to integrate contemporary research efforts and

highlighting some of the most exhilarating advances in *Ocimum* breeding and genomics.

Chapter 1 focuses upon the agro-technological practices and field requirements for growing the basil plant and its economics covering the cost input to output ratio. This chapter not only describes the techniques of successful cultivation of basil, but also gives an overview of earning by farmers by growing the basil crop.

Chapter 2 highlights the medicinal and therapeutic potential of the various *Ocimum* species with the main emphasis on the antioxidant and antiaging potential of phytomolecules and extracts derived from this genus. Since basil is the rich repository of many bioactive molecules such as terpenes, phenylpropanes, phenolic acids, and flavonoids, therefore the longevity-promoting potential of this herb is highlighted in the present chapter. Furthermore, special focus is given to the use of *C. elegans* model system for screening various phytochemicals isolated from the *Ocimum* spp.

Chapter 3 discusses the profound effect of *Ocimum* on treatment and prevention of cardiovascular diseases by means of lowering blood lipid content, suppressing ischemia and stroke, reducing hypertension, and also its higher antioxidant properties. These cardioprotective properties prove that *Ocimum* may be treated as a good remedy against prevention and treatment of cardiovascular diseases.

Chapter 4 demonstrates the evolutionary analysis of few protein super-families in *Ocimum tenuiflorum*. This chapter describes a computational pipeline for identification, validation, and analysis of the key components involved in the synthesis of terpenoids and a less studied class of proteases called rhomboids. This kind of study will have wider implication not only as a tool to understand sequence and structure–function relationships of some of the well-studied metabolites and enzymes, to aid protein engineering for biotechnological utilization of these highly commercially valuable molecules.

Chapter 5 presents the detailed taxonomic description and phylogenetic relationships among the Indian *Ocimum* species. Chapter 6 highlights the genetics, cytogenetics, and diversity in the genus *Ocimum* and the ambiguities in classification of the genus. It elaborates that how the revision in nuclear DNA content has divided the section *Ocimum* into two clades. The first consisting of *O. basilicum* and *O. minimum*, whereas the second consisting of *O. americanum*, *O. africanum*, and two *O. basilicum* var. *purpurascens* accessions. Out of all, *O. tenuiflorum* was found to be the most divergent species.

Chapter 7 emphasizes on the traditional plant breeding carried out in developing new improved *Ocimum* varieties like varieties CIM Ayu, CIM Angana, CIM Saumya, CIM Kanchan, Vikarsudha, CIM Jyoti, CIM Sharada, CIM Surabhi, and CIM Snigdha developed at the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

Chapter 8 describes all the available genomic resources of the *Ocimum* species worldwide and illustrates the medicinal potentialities, uses, and essential oil components of some of the widely used *Ocimum* species.

Chapter 9 discusses the triterpene functional genomics in *Ocimum* via utilization of high-throughput sequencing of genomes and transcriptomes

providing a prospect leading to the understanding of the molecular and biochemical basis for the biosynthesis of diverse triterpenes and other phytochemicals in *Ocimum* species.

Chapter 10 gives an overview of the genome sequencing of the holy basil and the future prospects of utilizing the genome sequencing data.

We consciously tried to present these ten chapters as more or less stand-alone deliberations, and hence, one would find obviously some apparent redundancy while introducing the plant.

We hope that this book would be a great help to the senior and the young *Ocimum* researchers and enthusiasts in providing an insight about the present status of genomic, pharmacognostic, agronomic, and the evolutionary studies. This compilation not only projects massive prospective for plant improvement but also unveils the new avenues which were obscured from long. We are really grateful to each of the authors for sparing their precious time and effort in order to contribute their best. We would also like to thank the entire editorial team of Springer for their assistance.

Lucknow, India
Kalyani, India

Ajit Kumar Shasany
Chittaranjan Kole

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Ocimum as a Promising Commercial Crop

1

R. K. Srivastava, Sanjay Kumar and R. S. Sharma

Abstract

Basil, *Ocimum* Spp, (Family Labiatae), is a herbaceous, erect, annual important aromatic plant, which attains the height of about 80–100 cm. The leaves of the plant are dark green or yellowish green in colour. Flowering tops and leaves of plant yields essential oils, which are used in perfumery and pharmaceutical industries. Stems of the plant are often branched and bear leaves. Many species of basil are available in nature including *Ocimum basilicum*, *Ocimum canum*, *Ocimum gratissimum* and *Ocimum sanctum* (Sharma et al. in *J Med Arom Plant Sci* 18:512–522, 1996). Basil can be grown in wide ranges of soil like light loam and medium loam having good water holding capacity with a pH range of 5.0–8.30. The best crop rotation of basil is basil–chamomile–mint or basil–mustard–mint or basil–potato–mint in the subtropical region. It prefers mild climate with moderate temperature of about 27 °C for successful growth. Basil is propagated through seeds. The nursery is raised in the month of May, and the seedlings are transplanted in the main field in the month of June/July. Nursery-raised seed-

lings of 30 days' age are planted with the spacing of 30–35 cm plant to plant and 45–50 cm row to row depending upon soil fertility. After planting of seedlings, irrigation is necessary. During the whole period of life, 2–3 weedings are required to minimize weed competition. In average fertile soil, 50 kg nitrogen, 40 kg phosphorus and 40 kg potash per ha are sufficient. Nitrogen is applied in three equal doses during the growth period of the plant. It takes about 85–90 days for maturity, when lower leaves start turning yellow and full blooming condition appears. Harvesting is done by sharp sickle. After harvesting and distillation, about 110 kg of oil is received from per hectare area. The present market rate of basil oil is Rs. 650 per kg (Essential oil Market Report 2014), and cost of cultivation is about Rs. 23,546 to per ha. A farmer can earn Rs. 47,954 per ha within a period of 100 days.

1.1 Cultivation Technology

Basil is known for its leaves, roots, stem and essential oils for fragrances, flavours, medicine and sanctity in Hindu mythology. A number of species are available in the world, but there are two common species that are more popular among the farmers, i.e. *Ocimum sanctum* (holy basil) and *Ocimum basilicum* (sweet basil).

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The leaves of this plant or whole plant are used for extraction of its essential oils. The oil contains mainly phenols, aldehydes, tannins, saponin and fats (Smitha et al. 2014). About 150 species of *Ocimum* have been found in the world, out of which some are wildy grown in India. A few are commercially cultivated for their important essential oil and crude drug purpose, but the sweet basil or French basil is cultivated for its essential oil.

Apart from the sweet basil, some of the holy basil is also cultivated for their chemical constituents but the sweet basil is widely cultivated in India and in other parts of the world. The sweet basil is native of India, but it is also cultivated in France, Italy, Bulgaria, Egypt, Hungary, South Africa and USA. The oil of this species contains methyl chavicol, linalool and some other minor constituents (Srivastava et al. 2009; Fig. 1.1).

O. basilicum is erect, herbaceous, strongly aromatic, annual plant having a height of 75–95 cm. Stem often branched from the base, purplish and subglabrous in lower part, four angular, intensely hairy higher up. Leaves are 3–6 cm long and 1.5–3.5 cm in diameter, ovate, lanceolate, with acuminate or rounded base, acute or subobtuse, subentire to serrate, thinly hairy and gland pinnate. Flowers are born in long terminal racemose inflorescences. Pedicels densely hairy, calyx 0.3–0.4 cm long at first,

afterwards 0.5–0.6 cm long hairy outside, glabrous inside, at base with dense whorl of long hairs above it, glandulose upper tip ovate to round. Corolla white or pale purple tube, stamens exert, posterior filaments short, white hairy and transverse (Srivastava et al. 2009).

O. basilicum and *O. sanctum* are cultivated on large scale in Indian states. Mostly, they are cultivated for important essential oil in the states of Uttar Pradesh, Punjab, Haryana, Madhya Pradesh and some other parts of India.

1.2 Common Varieties

Ocimum basilicum

CIM-Saumya: This is a short duration crop of 90 days and has the potential to produce about 85–110 kg/ha essential oil and rich in methyl chavicol (62%) and linalool (25%) (Bahl et al. 2018).

CIM-Snigdha: The variety matures in 80–90 days and yields 75–110 kg per ha essential oil and also rich in methyl cinnamate content (78–80%) (Bahl et al. 2018).

CIM-Surabhi: This is high oil-yielding variety with unique chemical composition having 70–75% (–) linalool with 99% purity. This



Classification:

Kingdom: Plantae

Class: Angiosperm

Oder: Lamiales

Family: Lamiaceae

Genus: *Ocimum*

Species: *basilicum* L.

Fig. 1.1 A field view of sweet basil