

Ocimum Genome Sequencing—A Futuristic Therapeutic Mine

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Abstract

Next-generation sequencing (NGS) platforms from the past decade are in the continuous efforts of changing the impact of sequencing on our current knowledge about plant genes, genomes, and their regulation. Holy basil (*Ocimum tenuiflorum* L. or *sanctum* L.) genome sequencing has also paved the path for deeper exploration of the medicinal properties of this beneficial herb making it a true ‘elixir of life.’ The draft genome sequence of the holy basil has not only opened the avenues for the drug discovery but has also widened the prospects of the molecular breeding for development of new improved plant varieties.

plants. Among such highly important herbs, *Ocimum* ranks first because of its wide range of health-promoting benefits and sanative properties in traditional as well as in the modern pharmacological system (Bhasin 2012) and hence is regarded as royal herb (Sharma 2010) or a queen of herbs (Kayastha et al. 2014). *Ocimum* is an omnipresent herb which belongs to the family lamiaceae, and this whole genus corresponds to the common name, ‘basil.’ The genus *Ocimum* is highly variable and possesses wide genetic diversity at intra- and inter-species levels (Upadhyay et al. 2015). It includes many aromatic herbs and shrubs having diverse essential oils of tremendous palliative importance which finds its applications in pharmaceutical, modern perfumery, and food processing industry.

10.1 Introduction

Herbs with medicinal and aromatic properties are becoming very popular worldwide. India including many Asian countries stands to be a gallery of many medicinal plant variety resources (Dhawale and Ghyare 2016) because of their ancient, prosperous, and the most distinct cultural backgrounds related to the use of such

Basically, nine species of *Ocimum* are grown worldwide, viz. *O. tenuiflorum* L., *O. gratissimum* L., *O. basilicum* L., *O. micranthum* L., *O. kilimandscharicum*, *O. americanum* L., *O. campechianum* L., *O. citriodorum* L., and *O. minimum* L., three of which *O. americanum* L., *O. citriodorum* L., and *O. minimum* L. All the species of *Ocimum* are economically and medicinally important. These plants have been extensively used in systems of traditional medicine across the world, primarily in Ayurveda and traditional Chinese medicine. Various parts of the plant have been consumed as a home remedy to cure many diseases since ages. According to Ayurveda, basil leaves are very good expectorant

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to relief cough and cold as well as are also used to treat digestive disorder. It is also beneficial in soothing itchy and irritated eyes (Brien 2009). In Chinese traditional medicine, basil leaves are resorted to relief the irritation caused due to insect bites (Brien 2009). These 5000-year-old methods of treatment are not just hearsay but are being supported by modern research. It has been demonstrated by the recent scientific research that constituents of basil essential oil acquire strong anticancer, antioxidant, antimicrobial and antiviral, properties (Bhattacharyya 2013). Hence, basil can be venerated as God's recipe for the elixir of life.

According to recent findings, it is suggested that some very high-value secondary metabolites such as geraniol, linalool, linalyl, camphor, citral, methyl eugenol, eugenol, methyl cinnamate, methyl chavicol, urosolic acid, safrol, thymol, taxol can be obtained from different plant parts which may prove to be a basis for generation of new drugs (Khare 2007). The mentioned metabolites are of great importance in medicine, aroma, and cosmetic trade. Despite its incomparable importance in the traditional system of medicine and huge repository of phytochemicals, our understanding behind the molecular aspect of this medicinal plant is still limiting. Recently, a comparative transcriptome study between

O. basilicum and *O. sanctum* was published (Rastogi et al. 2014) wherein many transcripts of phenylpropanoids in *O. basilicum* and of terpenoid biosynthesis in *O. sanctum* were recorded. Again to decipher the complete metabolic routes and the aspects affecting the regulation and the channeling of these pathways, complete chloroplastic and nuclear genomes were sequenced for the first, after obtaining the sequencing data from three NGS platforms using four libraries (Rastogi et al. 2015). This report was the first step to unveil the secrets of its therapeutic potential with the scientific validation to the traditional claims of its utility in diverse medicinal usage and hence can be served as a milestone work for the identification of unidentified genes involving in the downstream biochemical pathway of medically significant metabolite. Figure 10.1 shows the pathways attributed to the proteins predicted from scaffolds of *O. tenuiflorum* genome as predicted by KAAS server in the study by Rastogi et al. (2015) considering *Oryza sativa* and *Arabidopsis thaliana* as reference organisms. Of the total annotated proteins, only 2.53% belonged to the category 'Biosynthesis of secondary metabolites' while 2.83% to the 'Metabolism of Terpenoids and polyketides.' In order to identify the key genes behind the strong medicinal properties of

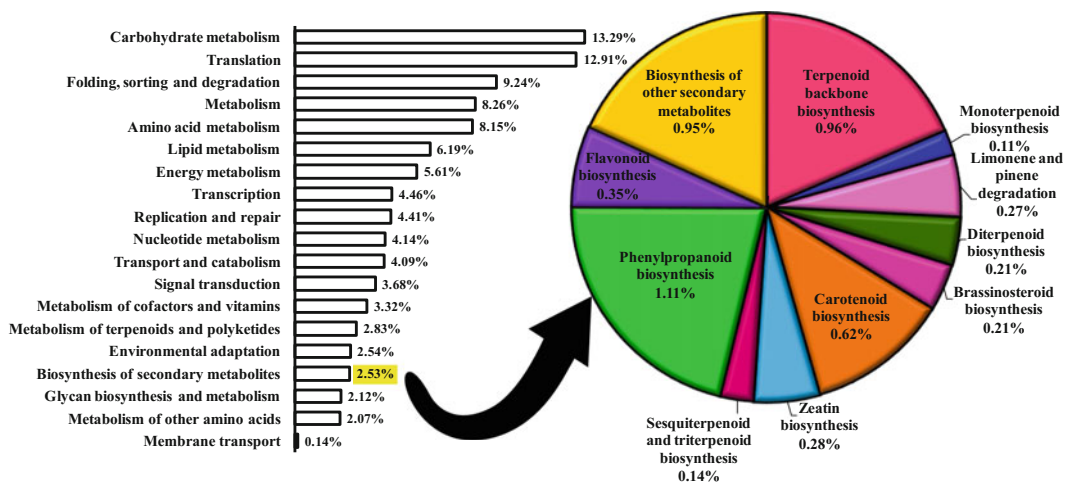


Fig. 10.1 Pathways related to the scaffolds predicted proteins by KAAS server considering *Oryza sativa* and *Arabidopsis thaliana* as reference organisms

this herb, another group (Upadhyay et al. 2015) also reported the draft genome of tulsi using Illumina HiSeq 1000 platform.

Figure 10.2 shows the top ten species that from which the predicted genes were annotated against all Viridiplantae clade genes in the UniProt database. Most annotations were made from the *Genlisea aurea* (order-Lamiales and family-Lentibulariaceae), i.e., 30,983 hits out of the total 53,480 annotated genes in UniProt. *G. aurea* is a carnivorous plant which is reported to be the plant with smallest known genome of size 63.6 Mb among the higher plants (Leushkin et al. 2013).

10.2 *Ocimum sanctum* (Syn. *tenuiflorum*): Whole Genome Sequencing

Modern sequencing techniques and bioinformatics approaches have presented better opportunities for rapid and efficient development of genomic resources for non-model plants such as *O. sanctum*. However, in contrast to the model plants and important crop plants, the projects on medicinal plant genomes are yet lagging behind. A speedy research is needed for the further development of natural medicines and high-yielding medicinal cultivars. In this context,

recently CSIR-CIMAP, Lucknow, has succeeded in sequencing complete whole genome sequence of *O. sanctum*. Genome sequence of about 386 Mb from *O. sanctum* was assembled using whole-genome shotgun sequencing approach by generating short and long paired-end reads. Two libraries of long and short reads were of Illumina HiSeq 2000, one of SOLiD 550 XL and one of 454 GS FLX was combined to generate the draft genome assembly. Complete workflow of the sequencing process is provided in Fig. 10.3.

10.2.1 *O. sanctum* Whole Genome Sequencing: The Chloroplast and Mitochondrial Genome

This report by CSIR-CIMAP also revealed that chloroplastic genome of the *O. sanctum* is the smallest among lamiales with 142,524 bp in length challenging the investigation of Qian et al. (2013) who described the *Salvia miltiorrhiza* chloroplast genome to be the smallest (member of the *Ocimum* family Lamiaceae) with cp genome of length 151,328 bp (Table 10.1). In this analysis, *S. miltiorrhiza* was noticed to be phylogenetically nearest to *O. sanctum* with diploid chromosome no. $2n = 16$ (Rastogi et al. 2014). Both the plants predominantly produce

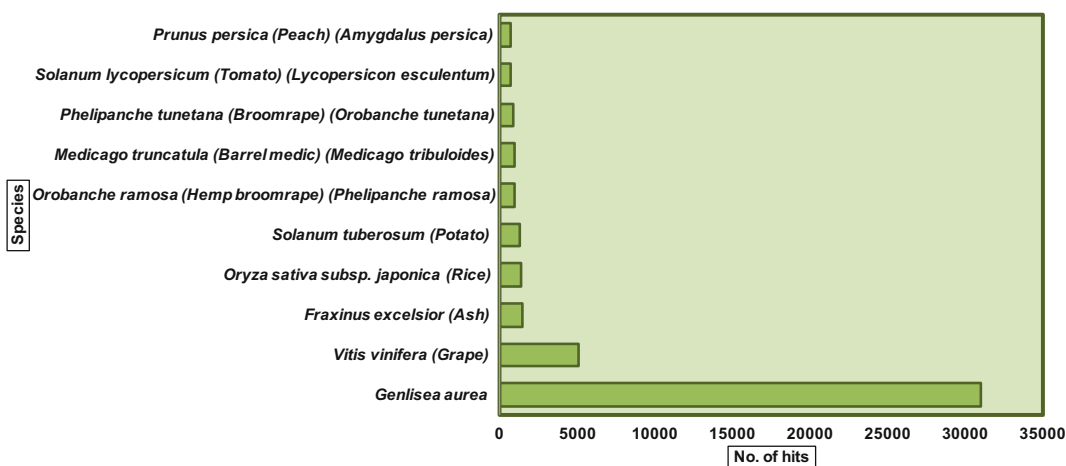


Fig. 10.2 Top 10 species of the predicted proteins from the viridiplantae clade in UniProt annotation table

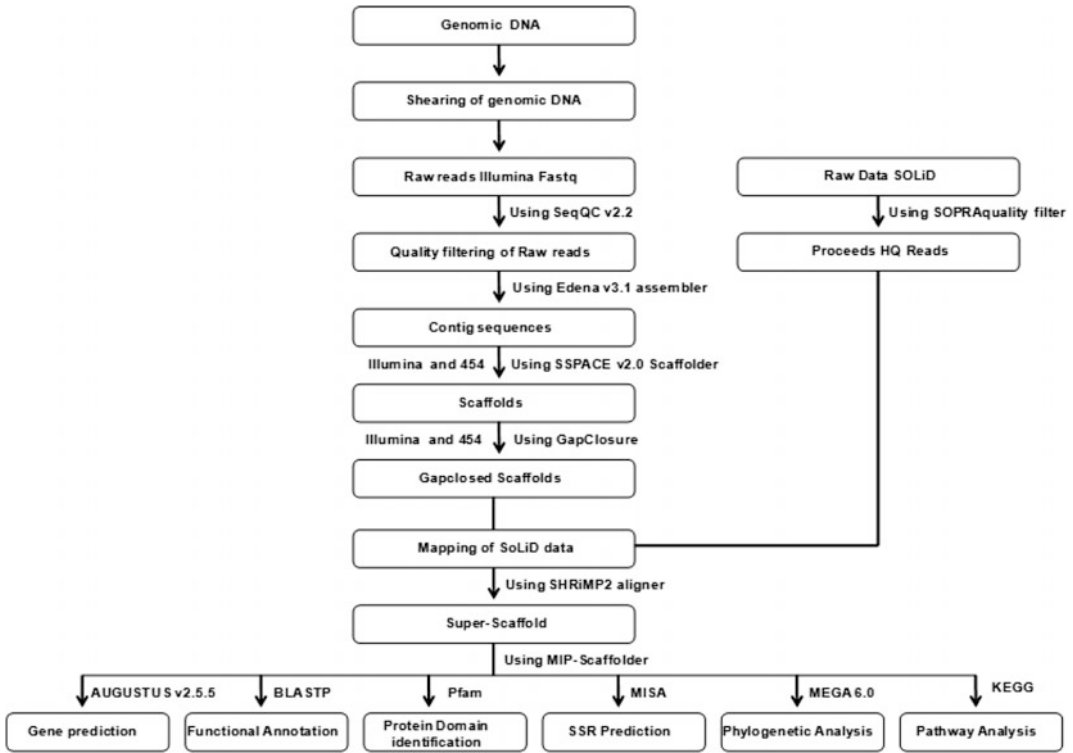


Fig. 10.3 Process workflow of *O. sanctum* whole genome sequencing and assembly

Table 10.1 QC statistics of chloroplast genome de novo assembly at each step

Particulars	Contigs	Scaffolds	Gap-closed	Gap-closed filtered	Draft genome
Contigs generated	140	48	48	2	1
Maximum contig length	25,631	78,214	78,166	78,166	1,42,524
Minimum contig length	61	61	61	64,356	1,42,524
Median contig length	296.5	133.5	70	71,261	1,42,524
Total contigs length	109,671	156,442	156,070	142,522	1,42,524
Total number of non-ATGC characters	55	1373	24	23	25
Percentage of non-ATGC characters	0.05	0.88	0.02	0.02	0.018
Contigs ≥ 100 bp	108	32	32	2	1
Contigs ≥ 200 bp	98	15	15	2	1
Contigs ≥ 500 bp	50	9	9	2	1
Contigs ≥ 1 Kbp	29	5	5	2	1
Contigs ≥ 10 Kbp	1	2	2	2	1
Contigs ≥ 1 Mbp	0	0	0	0	0
N50 value	1846	64,681	78,166	78,166	1,42,524

phenylpropanoids and their derivatives which are used in different traditional as well as in modern medicinal system. Like other asterid cp genomes, the overall GC content of *O. sanctum* was also found to be 36.2%. A total 158 genes from the cp genome of *O. sanctum* were reported that included 43 tRNA (transfer RNA) genes and 4 rRNA (ribosomal RNA) genes. Later in the same year, Upadhyay et al. (2015) published a reported discussing the draft genome of *O. tenuiflorum* L. (subtype *Krishna Tulsi*) using mate-pair and paired-end sequence library resulting 374 Mb draft genome. This report was published along with the comparative study of transcriptome of *O. tenuiflorum* subtype Rama and Krishna to find out the important differentially regulated gene and their role in the medicinally useful metabolite(s) synthesis. Both the reports published by Rastogi et al. (2015) and Upadhyay et al. (2015) are an important achievement in discovering the specialized plant metabolites of immense medicinal interest, glorifying the sacredness of the ‘holy tulsi.’ Chloroplasts have a vital function in supporting life on this planet. At present, there is an access to more than 800 chloroplast genomes which are sequenced from a range of land plants leading to expansion of our knowledge in chloroplast biology, gene transfer within the cells, preservation, diversity, and the basis of inheritance as a result of which chloroplast recombinant genes can be engineered for enhancement of agronomical plant traits or production of high-value farming or bio-scientific products. Since the chloroplast genome or the plastome exhibits a uniparental inheritance which is primarily maternal in angiosperms while paternal in gymnosperms, hence the sequence information coded in the chloroplast DNA loci is used in plant systematic for elucidating intraspecific relatedness via deep divergence (Martin et al. 2002; Kugita et al. 2003; Yamane et al. 2003; Ahmed et al. 2012, 2013). In some plant species, exogenous genes which encode important metabolites have also been transformed into the chloroplast genomes, like the genes for vaccines production effective against human diseases (Lössl and Waheed 2011;

Waheed et al. 2011a, b). Particular loci in chloroplast DNA have also been used as barcodes which are helpful in the identification of plant species (Fazekas et al. 2008; Peter et al. 2009); however, the hypothesis of a universal barcode has natural precincts (Ahmed et al. 2013). Whole chloroplast genome sequence is considered to be a super-barcode (Li et al. 2015), but this notion requires reconsideration for outcrossing plant species that are, or have been occupying the same geographical range without loss of identity from interbreeding (sympatric species).

10.2.1.1 Some of the Biotechnological Applications of Chloroplast Genome Engineering

Production of Biomaterials and Enzymes

There are reports of the chloroplast genome engineering for the synthesis of important biomaterials, enzymes, and biofuels as well as biomass enhancement. Viitanen et al. (2004) for the first time reported the production of poly (*p*-hydroxybenzoic acid (pHBA) polymer at the highest level (25% dry weight) in normal healthy plants regardless of the alteration in the key metabolic intermediate via chloroplast genome engineering. Use of plant-derived enzyme cocktails from lignocellulosic biomass for the producing fermentable sugars was achieved for the first time by Verma et al. (2010). In contrast to the single biofuel enzymes which were formerly expressed in chloroplasts, nine discrete genes from heterologous systems (bacteria or fungi) were expressed in tobacco chloroplasts or *E. coli* by means of a novel method facilitating the introduction of fungal genes with several introns, eradicating the requirement of cDNA library preparation. Enzyme cocktails derived from the chloroplast propose several remarkable benefits like cost effectiveness, enhanced permanence of chloroplast-derived enzymes with no requirement of enzyme purification while the industry-based fermentation systems incorporate high expenditure and low manufacturing capability. Interestingly, β -glucosidase expression

was able to release the hormones from conjugates, ensuing improved phytohormone levels and enhanced biomass (Jin et al. 2012), which was an unpredicted result of enzyme expression.

Improving Nutrition

Maize, soybean, and rapeseed (*Brassica napus*) seed oils are the main nutritional source of vitamin E. They acquire minimal α -tocopherol content but possess γ -tocopherol at fairly high levels. Only some oils from seed like sunflower seed oil (*Helianthus annuus*) are found to have high α -tocopherol content which is a key precursor leading to vitamin E biosynthesis (Schneider 2005). γ -Tocopherol leads to the synthesis of α -tocopherol catalyzed by γ -tocopherol methyl transferase (γ -TMT) and is thus the rate-limiting step (Shintani and DellaPenna 1998). When γ -tmt gene was overexpressed by engineering it into the chloroplast genome, it was observed that manifold layers were formed in the inner chloroplast envelope. In addition, approximately tenfold high production of α -tocopherol in the seed from γ -tocopherol was evident (Jin and Daniell 2014). Similarly, introduction of lycopene β -cyclase genes into the plastid genome of tomato improved the production of provitamin A (β -carotene) from lycopene, with apparent phenotypic modifications (Apel and Bock 2009).

Acquiring Stress Tolerance

For more than a decade, genetic engineering of chloroplast mainly focused on the over-expressing potential genes for increasing tolerance against biotic stress, which is imperative to plant defense and improving the yield. Insect pests are a real threat to the yield loss in many countries. Besides cotton bollworm resistance acquired by over-expressing Bt protein in chloroplasts (De Cosa et al. 2001), there are various other latest and prominent examples of enhanced biotic stress tolerance. Retrocyclin-101 and Protegrin-1 proteins were responsible for defending against tobacco mosaic virus (TMV) and *Erwinia* soft rot accountable to the yield deficit in a number of cultivated plants (Lee et al. 2011). β -glucosidase gene expression leads

to the resistance against whitefly and aphid (Jin et al. 2011), which induces the liberation of insecticidal sugar esters by conjugating with hormones. Multiple defiances hostile to aphids, lepidopteran insects, whiteflies, and viral and bacterial pathogens were accomplished by expression of the *Pinellia ternata* agglutinin (PTA) gene in the genome of chloroplast (Jin et al. 2012). Edible crops like cabbage (*Brassica oleracea*) (Liu et al. 2007), soybean (Dufourmantel et al. 2004, 2005), and eggplant (*Solanum melongena*) (Singh et al. 2010) have been engineered and expressed stably by integrating over 40 transgenes in their chloroplast genomes leading to the improvement in the agronomic traits as well as the acquiring resistance against insects. As per the new strategy, scientists have now started exploring possibilities of downregulating the specific gene(s) of interest. Expressing dsRNAs (double-stranded RNAs) inside the genome of chloroplast to employ RNAi (RNA interference) for acquiring preferred agronomic traits, principally insect resistance in order to check yield loss is one such approach. This is achieved by expression of long or short dsRNAs activating the RNAi leading to the disruption of the target gene(s) in insects, thereby conferring effective protection from insects avoiding the use of harmful pesticide chemicals. This strategy was used by Jin et al. (2015) where they suppressed three vital proteins, namely Chi (lepidopteran chitin synthase), P450 (cytochrome P450 monooxygenase), and V-ATPase required for the survival of the insects by means of introducing dsRNAs in the chloroplast system of tobacco. Each of the double-stranded RNA was discretely expressed in chloroplasts, and leaves were then fed to the insects. The level of transcription in the target genes of *Helicoverpa* insects was found to be tremendously decreased to almost negligible in the midgut, ensuing considerable drop in the net larval weight as well as in the pupation rate (Jin et al. 2015). Transplastomic potato plants which produced β -actin and targeted long dsRNA were proved to be lethal to the larvae of Colorado potato beetle (*Leptinotarsa decemlineata*), demonstrating an additional crop protection method (Zhang et al. 2015).

Phyto-Pharmaceuticals

At this point in time, protein-based drugs are tremendously costly; for instance, insulin (a well-known drug for treating prevalent diabetes disease worldwide) is unaffordable to more than 90 percent of the world population. The excessive price of protein-based drugs is because of their expensive fermentation systems' setup (approximately \$450–700 million based on their capacity) (Grabowski et al. 2006; Spök et al. 2008), exorbitant protein purification cost from the host, the requirement for low temperature storage and transfer, and the brief shelf-life of the ultimate product. Protein-based drugs prepared by plant chloroplasts defeat most of these challenges because there is no requirement of the costly fermentation systems and can easily be synthesized in hydroponic greenhouses approved by FDA (federal drug administration) (Holtz et al. 2015). Lettuce leaves which express protein drugs can be lyophilized and could be put in storage for an indefinite period at surrounding temperature without the loss in its efficacy (Su et al. 2015). Protein drugs encapsulated in the plant cell wall remain protected from the enzymes, and acids secreted by the human stomach as the plant cell wall glycans do not get digested by the human secreted enzymes. On the other hand, the microbes in the human gut have progressed in the breaking down each of the glycosidic bond present in the plant cell wall, consequently liberating the protein drug in the lumen of gut, guiding its transport to the immune system or blood (El Kaoutari et al. 2013; Kwon and Daniell 2015). Mode of oral release of various human curative proteins expressed in chloroplasts is has proven to be an effective medication for treating numerous human ailments, like diabetes, pulmonary hypertension, cardiovascular disease, and Alzheimer's disease. Majority of the proteins for the preliminary testing were expressed in chloroplasts of tobacco followed by their expression in chloroplasts of lettuce for subsequent expansion toward the clinic. Exendin-4 protein regulated the insulin secretion in a glucose-dependent way when delivered orally. Also the drop in glucose levels

due to stimulation of insulin production in diabetic animals was observed, and this response was parallel to that generated by an injectable drug (Kwon et al. 2013). Oral intake of the bioencapsulated angiotensin-converting enzyme 2 (ACE2) and angiotensin (Ang) (1–7) enzymes leads to the better cardiopulmonary functioning, reduction in the high ventricular systolic blood pressure and recovered the blood flow in the animals with artificially generated pulmonary hypertension (Shenoy et al. 2014). Plant cell-encapsulated proteins, ACE2 and Ang (1–7), when delivered orally were also found to be reducing endotoxin induced uveitis (EIU) and significantly reduced retinal vasculitis and cellular infiltration, and additionally prevented folding and damage in induced autoimmune uveoretinitis animals (Kwon and Daniell 2015). Nonetheless, when the protein drugs were orally released to the Alzheimer's brain across the blood–brain barrier, it leads to the removal of plaques (Kohli et al. 2014). Su et al. (2015) for the first time reported the commercial-scale manufacturing of human blood-clotting factor (up to 30,000 doses for a 20-kg pediatric patient) in a 1000 ft² hydroponic cGMP facility. Lyophilized cells of clotting factor made in lettuce were found to last for the span of up to 2 years when stored at normal surrounding temperature, entirely removing the requirement of refrigerated storage. This leads to the advent of industrial level expansion of oral medicine addressing the challenges of high-priced purification, refrigerated storage and shipping, as well as the small shelf-life of the conventional protein drugs. Oral release of wider dose range proved to be efficient in the deterrence of antibody formation in response to clotting factor IX (FIX) injection, therefore making smooth the progress of human clinical studies.

Vaccines to Combat Infectious Diseases

The present iterated use of vaccines via attenuated strains of viruses and bacteria put forth safety against crucial transmittable diseases; however, they also offer major challenges. For instance, the oral polio vaccine which is used

worldwide resulted in acute polio in consequence to the recombination and mutation other viruses (Chan and Daniell 2015). Besides, all existing vaccines demand refrigerated storage and shipping, making supply the major challenge in developing countries. Chloroplasts are the answer to such challenges. One vaccine derived from the chloroplast has been proved to be success granting double immunity against malaria and cholera in animal research (Davoodi-Semiromi et al. 2010). Cholera causes a high mortality rate, though there is only one approved vaccine which is not only high-priced but also has very small shelf-life. As far as malaria is concerned, there is no vaccine till date. In order to address this problem, cholera toxin-B subunit (CTB) of *Vibrio cholerae* was combined with the malarial vaccine antigen apical membrane antigen-1 (AMA1) and merozoite surface protein-1 (MSP1) for expression in lettuce or tobacco chloroplasts. Due to the unavailability of the human malarial model, the developed chloroplast-expressed CTB was tested by immunizing the mice and was found to be highly efficient. In addition, it also provided the highest duration of defense as per the literature (Davoodi-Semiromi et al. 2010). These initial results exhibit that chloroplasts are perfect model for production of inexpensive booster vaccines in counter to numerous contagious diseases (Lakshmi et al. 2013) for which the world is prepared; however, the deficient oral priming strategies yet are the major restraint in this area.

An attempt was also made to get the complete mitochondrial genome map of *O. sanctum* from the sequence assembly by considering *S. miltiorrhiza* as the reference mitochondrial genome instead of *Origanum vulgare* which was the reference chloroplast genome (Fig. 10.4). A total of 48 scaffolds from 140 contigs from cp genome, and 41 scaffolds from 124 contigs from the mitochondrial genome got generated (Table 10.2). Mitochondria play a significant role in the development of plant, maintaining its health as well as in plant reproduction. They are said to be semi-autonomous due to their own genetic system that makes it function for energy production, maintaining cell homeostasis and

metabolism. Similar to other organisms, the plant mitochondrial genome determines a sequence of crucial polypeptides that put together the oxidative phosphorylation chain complexes with nuclear-encoded subunits. However, plant mitochondrial DNAs (mtDNAs) have distinguished characteristics that differentiate them from their fungal and animal counterparts. Particularly, higher plants contain large mtDNAs which greatly vary in sizes and structural make up. Furthermore, mitochondria in many plant species comprise a range of plasmids which are capable of independent replication. In comparison to animal mtDNA sequences, the majority of plant species harbor slow-evolving mtDNA gene sequences with an extremely low rate of point mutations. It is so due to the fact that mitochondrial DNA recombination system is very active and hence permits the mutations copy correction. Certainly, several investigations have demonstrated that mitochondrial genome of plants endures massive and highly frequent homologous recombination (HR). These events increase the rate of recurrence of rearrangements in plant mtDNA. Many characteristics of plant mtDNA genetics make it much complicated than most the other organisms. The ability of integration and/or expansion of intervening non-coding genetic sequences in the genomes of organelles is notable, particularly in comparison to the severely condensed mtDNA of mammals. It may therefore be hypothesized that advantage of sequences is associated to the competency of plant mitochondria for bringing in the DNA (Koulintchenko et al. 2003). Particularly, both the plasmid acquisition and the competency to import are common to mitochondria of fungus (Weber-Lot fi et al. 2009). On the other hand, mammalian organelles too are proficient (Koulintchenko et al. 2006) with stable genome size. Besides, many years later to the breakthrough, the importance of mitochondrial plasmids and their interaction with the core genome of mitochondria are yet obscure. It is not sequence content only, but the physical structure of the plant mitochondrial DNA is an intricate blend that results either from or by holds up an array of maintenance and replication method. Though

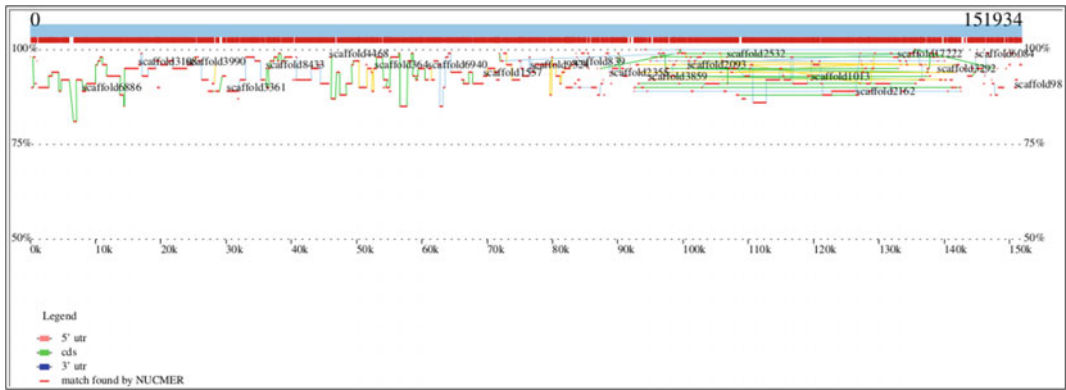


Fig. 10.4 Alignment of *O. sanctum* scaffolds with the chloroplast genome of *Origanum vulgare* using Nucmer

Table 10.2 QC statistics of mitochondrial genome de novo assembly at each step

Particulars	Contigs	Scaffolds	Gap-closed	Gap-closed filtered	Draft genome
Contigs generated	124	41	41	37	37
Maximum contig length	16,768	43,386	43,392	43,392	43,398
Minimum contig length	103	145	145	145	145
Median contig length	600	6261	505	524	20029
Total contigs length	174,923	442,572	442,370	446,661	4,45,881
Total number of non-ATGC characters	0	1616	51	4342	54
Percentage of non-ATGC characters	0	0.37	0.01	0.97	0.012
Contigs ≥ 100 bp	124	41	41	37	37
Contigs ≥ 200 bp	122	40	40	36	36
Contigs ≥ 500 bp	68	38	38	34	34
Contigs ≥ 1 Kbp	45	32	32	29	29
Contigs ≥ 10 Kbp	1	16	16	16	16
Contigs ≥ 1 Mbp	0	0	0	0	0
N50 value	3472	24,958	24,833	25,250	25,315

unusual in organelles of mammals, recombination plays a key role in determining plant mitochondrial genomes, reorganizing sequences, creating polymorphism, compelling evolution, and simultaneously conserving the genetic information. Eventually, challenge that stays behind is to know, that how could the high agility of the plant mitochondrial DNA be amalgamated with gene preservation, coordinating inter-compartment

genome and apposite functional competence of the organelles. In this regard, it could be noticed that, despite reported mutants, mitochondrial DNA maps and restriction profiles have also been replicated persistently for a lot of species and generations, which is indicative of the fact that, even though vibrant and energetic, the genome of plant mitochondria remains constant or stable on an apparent timescale.

10.2.2 *O. sanctum* Whole Genome Sequencing: Re-routing the Traditional Health Practices to Scientific Drug Discovery

Traditional herbs have been used as medicines from the times immemorial, and *O. sanctum* (Tulsi) is one of the most indispensable ingredients of the traditional medicinal systems all round the globe. Despite this, the genetic makeup, the agricultural characteristics, as well as the curative properties of majority of conventional herbs are not properly understood. With speedy developments in the high-throughput sequencing methods and highly cut-down costs, a recent era of ‘herbal genomics’ has turned up. Research is now methodically classifying medicinal plants utilizing the next-generation sequencing approaches for annotation of their genomes for identifying and exploring the functions of their genes. The genomes of a few normally used medicinal herbs by now have been sequenced, like salvia (*Salvia miltiorrhiza*), tulsi (*O. sanctum*), lotus (*Lotus japonicus*), lingzhi (*Ganoderma lucidum*) (Sato et al. 2008; Chen et al. 2012; Rastogi et al. 2015; Xu et al. 2016). These medicinal plants could be presented as efficient model systems expanding the horizons for investigating secondary metabolites biosynthetic pathways in other medicinal plants. Genome data, in addition to the metabolomics, proteomic and transcriptomic information, could thus be helpful in predicting the biosynthetic pathways of secondary metabolites as well as their regulation. This would prove to be a reform in research leading to discovery fulfilling the goal to identify with the metabolic activities and genetics of medicinal plants.

Although, the modern healthcare system is developing day by day, but the emergence of many new diseases and the side effects of modern medicine, it is the call of an hour to integrate the traditional medical system for the ailment of lifestyle related health issues (Shakya 2016). *O. sanctum* is a model herbal plant used in the traditional Ayurvedic and Unani system of herbal medicine since ages (Pattanayak et al. 2010). It

has incomparable health healing properties, and therefore is regarded as a ‘boon’ which provides health and longevity. Within few decades, bioactive compounds isolated from this plant may become the foundation for new pharmaceutical drugs. However, vague and scattered information of the ethnomedicines and unlinked databases to the other biomedical databases are the major obstacles for the development of phytochemicals isolated from *O. sanctum* into pharmaceutical drug with therapeutic efficacies. Therefore, the availability whole genome sequence of *O. sanctum* may help in developing few databases for sorting information concerned with single or multiple aspects of medicinal plants, like biologically active metabolites, ethnobotany, medicinal uses, information based on genome and or transcriptome, and molecular targeting active ingredients. Figure 10.5 shows the applications of *O. sanctum* genome sequence in pharmaceutical research based on traditional system.

10.2.3 *O. sanctum* Whole Genome Sequencing: A Tool for Molecular Identification and Elucidation of Novel Phytomolecules

O. sanctum produces a wide array of specialized metabolites of tremendous therapeutic potentials. Different plant part (leaf, stem, and root) contains a varying amount of many bioactive compounds which can be classified into several important classes on the basis of their synthesis in plants: terpenes (isoprenoids), polyketides, alkaloids, flavonoids, and phenylpropanoids. All of these classes contain many high-value chemicals whose genome sequence information is still unavailable. Identification of the biosynthetic gene involved in the production of such commercially important phytochemicals is often challenging. Prediction of the specific function to individual genes is a long and complex process because of limited genomic resources. For example, Orientin and Vicenin-2 are plant

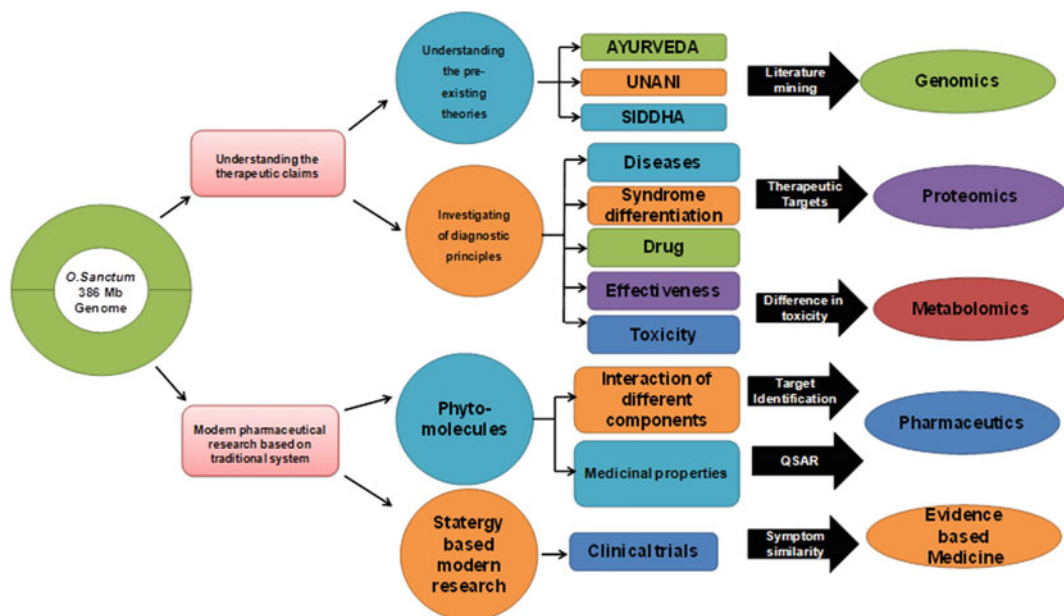


Fig. 10.5 Schematic diagram showing modern pharmaceutical research inspired by traditional medicine and the application of *O. sanctum* genome sequence in pharmaceutical research based on traditional system

flavonoid specifically isolated from the leaf extract of *O. sanctum* extensively studied for its antioxidant, anti-aging, antiviral, anti-inflammation, vasodilation, cardioprotective, radioprotective properties (Lam et al. 2016). Yet the underlying pathways and the mechanism of action of these flavonoid c-glycosides have remained indecisive. Similarly, putative genes and the network involved in the production of many other phytochemicals isolated from *Ocimum* such as ocimumosides, ocimarin, cirsimaritin, circineol are still unknown. Therefore, the availability of massive data in terms of the whole genome sequence of *O. sanctum* can be an efficient method for the investigation of the gene and gene families involved in the production of these phytochemicals. Few examples of such compounds are illustrated in Table 10.3.

Also, data mining and the analysis of whole genome sequencing can be helpful in the identification of transcription factors and the response elements involved in their metabolite synthesis.

10.2.4 Functional Genomics Research in *Ocimum* Species

Though *Ocimum* species have a wide horizon in the food, flavor, and pharmaceutical industries, not much research has been carried out at the molecular genomic level. Table 10.4 reports the significant contribution of *Ocimum* genomics taking account of studies carried out at transcriptome, metabolome, and bioinformatics level leading to an assimilated knowledge of its natural products biosynthesis. But with the advent of draft genome of *Ocimum*, the sequence analysis may help in the identification of the small changes in amino acid at the substrate binding sites of metabolite biosynthesis pathway genes conferring unique therapeutic properties to this medicinal herb.

Beside the genomics, in the recent years, the research on in vitro methods of growing the *Ocimum* by the micropropagation and regeneration via *Agrobacterium*-mediated transformation

Table 10.3 List of some important plant molecules isolated from *O. sanctum*

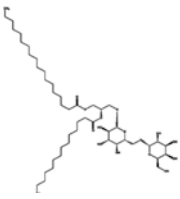
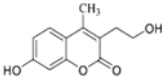
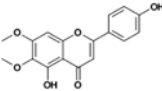
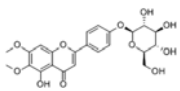
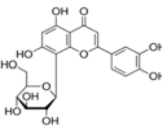
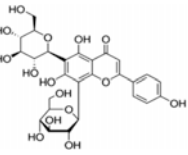
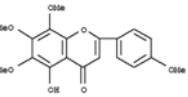
Phytomolecule	Category	Structure	Therapeutic value	References
Ocimumoside A and Ocimumoside B	Glycoglycerolipids		Used as an antistress agent	Ahmad et al. (2012)
Ocimarin	Coumarin		Used as an antistress agent	Gupta et al. (2007)
Cirsimaritin	Flavonoid		Possesses anti-inflammatory activity	Shin et al. (2017)
Cirsimaritin	Flavonoid		Used as a potent antilipogenic flavonoid to treat obesity	Zarrouki et al. (2010)
Orientin	Flavone-C-glycoside		Used as an antioxidant, anti-aging, antiviral, antibacterial, anti-inflammation, vasodilatation and cardioprotective, radiation protective, neuroprotective, antidepressant-like, antiadipogenesis, and antinociceptive agent	Lam et al. (2016)
Vicenin-2	Flavone-C-glycoside		Used as a potential anti-inflammatory constituent	Marrassini et al. (2011)
Gardenin B	Flavone		Possesses antiproliferative activity against the human leukemia cell	Cabrera et al. (2016)

Table 10.4 Functional genomics studies in *Ocimum* species

S.N.	Species	Gene(s)/biosynthetic pathway	Research	References
1.	<i>O. basilicum</i>	Flavonoid O-methyltransferases (flavonoid biosynthetic pathway)	Production of methoxylated flavonoids in yeast using ring A hydroxylases and flavonoid O-methyltransferases from sweet basil	Berim and Gang (2018)
2.	<i>O. kilimandscharicum</i>	HMG-CoA reductase (MVA pathway)	HMG-CoA reductase from camphor tulsi (<i>Ocimum kilimandscharicum</i>) regulated MVA-dependent biosynthesis of diverse terpenoids in homologous and heterologous plant systems	Bansal et al. (2018)
3.	<i>O. americanum</i>	Pb1 (downy mildew resistance gene)	Transfer of downy mildew resistance from wild basil (<i>Ocimum americanum</i>) to sweet basil (<i>O. basilicum</i>)	Ben-naim et al. (2017)
4.	<i>O. basilicum</i>	CYP716A (triterpenoid) (terpene biosynthesis pathway)	Two CYP716A subfamily cytochrome P450 monooxygenases of sweet basil play similar but nonredundant roles in ursane- and oleanane-type pentacyclic triterpene biosynthesis	Misra et al. (2017)
5.	<i>O. basilicum</i>	4-coumarate: CoA (4Cl), p-coumarate 3-hydroxylase (C3H0, caffeic acid O-methyltransferases (COMT), chavicol O-methyltransferase (CVOMT) (phenylpropanoid biosynthesis pathway)	Water-deficit stress fluctuates expression profiles of 4Cl, C3H, COMT, CVOMT, and EOMT genes involved in the biosynthetic pathway of volatile phenylpropanoids alongside accumulation of methyl chavicol and methyl eugenol in different Iranian cultivars of basil	Khakdan et al. (2017)
6.	<i>O. basilicum</i>	MeJA responsive oxidosqualene cyclases (ObAS1 and ObAS2) (pentacyclic triterpene biosynthesis)	Methyl jasmonate-elicited transcriptional responses and pentacyclic triterpene biosynthesis in sweet basil	Misra et al. (2014)
7.	<i>O. basilicum</i>	Biosynthesis of flavonols and carotenoids	De novo assembly and comparative transcriptome analyses of red and green morphs of sweet basil grown in full sunlight	Torre et al. (2016)
8.	<i>O. basilicum</i>	Thaumatococin-like proteins (TLPs) related to secondary metabolism	A thaumatococin-like protein of <i>Ocimum basilicum</i> confers tolerance to fungal pathogen and abiotic stress in transgenic <i>Arabidopsis</i>	Misra et al. (2016)
9.	<i>O. americanum</i>	Targeted mainly on cold responsive genes	De novo assembly and analysis of the transcriptome of <i>Ocimum americanum</i> var. <i>pilosum</i> under cold stress	Zhan et al. (2016)

(continued)

Table 10.4 (continued)

S.N.	Species	Gene(s)/biosynthetic pathway	Research	References
10.	<i>O. gratissimum</i> <i>O. tenuiflorum</i> <i>O. kilimandscharicum</i> <i>Gürke</i> <i>O. americanum</i> <i>O. basilicum</i>	Eugenol synthase (phenylpropanoid biosynthetic pathway)	Comparative functional characterization of eugenol synthase from four different <i>Ocimum</i> species: implications on eugenol accumulation	Anand et al. (2016)
11.	<i>O. kilimandscharicum</i>	Sesquiterpene synthase (terpenoid biosynthesis pathway)	Functional characterization and transient expression manipulation of a new sesquiterpene synthase involved in β -caryophyllene accumulation in <i>Ocimum</i>	Jayaramaiah et al. (2016)
12.	<i>O. sanctum</i>	MEP/MVA pathway; phenylpropanoid biosynthesis pathway	Unraveling the genome of holy basil: an ‘incomparable’ ‘elixir of life’ of traditional Indian medicine	Rastogi et al. (2015)
13.	<i>Ocimum tenuiflorum</i>	Phenylpropanoid biosynthesis pathway/flavonoid biosynthesis pathway; terpenoid biosynthesis pathway	Genome sequencing of herb tulsi (<i>Ocimum tenuiflorum</i>) unravels key genes behind its strong medicinal properties	Upadhyay et al. (2015)
14.	<i>O. basilicum</i> <i>O. sanctum</i>	MEP/MVA pathway; phenylpropanoid biosynthesis pathway	De novo sequencing and comparative analysis of holy and sweet basil transcriptomes	Rastogi et al. (2014)
15.	<i>O. tenuiflorum</i>	Eugenol O-methyltransferase (EOMT) (phenylpropanoid biosynthesis pathway)	Characterization and functional analysis of eugenol O-methyltransferase gene reveal metabolite shifts, chemotype specific differential expression and developmental regulation in <i>Ocimum tenuiflorum</i> L.	Renu et al. (2014)
16.	<i>O. basilicum</i>	Flavone 7-O-demethylase (flavonoid biosynthesis pathway)	Identification of a unique 2-oxoglutarate-dependent flavone 7-O-demethylase completes the elucidation of the lipophilic flavone network in basil	Berim et al. (2014a, b)
17.	<i>O. basilicum</i>	Flavones 8-hydroxylase (F8H) CYP82D33 (flavonoid biosynthesis pathway)	Unexpected roles for ancient proteins: flavone 8-hydroxylase in sweet basil trichomes is a Rieske-type, PAO-family oxygenase	Berim et al. (2014a, b)
18.	<i>O. sanctum</i>	4-coumarate: CoA ligase (4Cl) (phenylpropanoid biosynthesis pathway)	4-coumarate: CoA ligase partitions metabolites for eugenol biosynthesis	Rastogi et al. (2013)
19.	<i>O. basilicum</i>	Flavone-6-hydroxylase (F6H) CYP82D monooxygenases (flavonoid biosynthesis pathway)	The roles of a flavone-6-hydroxylase and 7-O-demethylation in the flavone biosynthetic network of sweet basil	Berim and Gang (2013)
20.	<i>O. basilicum</i>	MEP/terpenoid and shikimate/phenylpropanoid pathways	A systems biology investigation of the MEP/terpenoid and shikimate/phenylpropanoid pathways points to multiple levels of metabolic control in sweet basil glandular trichomes	Xie et al. (2008)

(continued)

Table 10.4 (continued)

S.N.	Species	Gene(s)/biosynthetic pathway	Research	References
21.	<i>O. basilicum</i>	Cinnamate/p-coumarate carboxyl methyltransferases CCMT (phenylpropanoid Biosynthesis pathway)	Evolution of cinnamate/ <i>p</i> -coumarate carboxyl methyltransferases and their role in the biosynthesis of methyl cinnamate	Kapteyn et al. (2007)
22.	<i>O. basilicum</i>	Methyl jasmonate	Effect of methyl jasmonate on secondary metabolites of sweet basil	Kim et al. (2006)
23.	<i>O. basilicum</i>	Chavicol O-methyltransferase (CVOMT) Eugenol O-methyltransferases (EOMT) (phenylpropanoid biosynthesis pathway)	Characterization of phenylpropene <i>O</i> -methyltransferases from sweet basil	Gang et al. (2002a, b)
24.	<i>O. basilicum</i>	<i>meta</i> hydroxylated phenylpropanoids (phenylpropanoid biosynthesis pathway)	Differential production of <i>meta</i> hydroxylated phenylpropanoids in sweet basil peltate glandular trichomes and leaves is controlled by the activities of specific acyltransferases and hydroxylases	Gang et al. (2002a, b)
25.	<i>O. basilicum</i>	Caffeic acid O-methyltransferases (COMT) (phenylpropanoid biosynthesis pathway)	Nucleotide sequences of two cDNAs encoding caffeic acid <i>O</i> -methyltransferases from sweet basil (<i>Ocimum basilicum</i>)	Wang et al. (1999)
26.	<i>O. basilicum</i>	Biosynthesis of phenylpropenes	An investigation of the storage and biosynthesis of phenylpropenes in sweet basil	Gang et al. (2001)

has also taken pace. Table 10.5 shows the record of in vitro studies in *Ocimum* species.

10.2.5 *O. sanctum* Whole Genome Sequencing: An Opportunity of Bringing Medicinal Plant into Cultivation

Today, about 80% of the world's population is dependent upon herbal medicine for their daily health care needs (Vines 2004). Hence, there is a threatening concern for a diminishing population of medicinal plants like *O. sanctum*. *O. sanctum* is one of the major medicinal plants used worldwide for its valuable herbal medicine. Infinite numbers of new complex, rare and bioactive compound are adding day by day to the

list of important plant-based chemicals obtained from *O. sanctum*. There are still many such compounds needed to be explored which could be the foundation of many new pharmaceutical drugs synthesized artificially. There is an increasing demand of *O. sanctum* in pharmaceutical industry and is exploited regularly for its valuable compounds. This imposes a constant repression on the genetic diversity and its germplasm (Bhau 2012). With the advancement of DNA-based different molecular markers, conservation of biodiversity of medicinal plant could be supported. Molecular marker-based approaches can also be used for the medicinal crop improvement and molecular breeding strategies. Molecular markers are the unique DNA sequences used for determining genetic diversity among the individual or a population. These DNA sequences may be helpful in the

Table 10.5 In vitro studies in *Ocimum* species

S.N.	Species	Research	References
1.	<i>Ocimum tenuiflorum</i>	Elicitation of phenylpropanoids and expression analysis of PAL gene in suspension cell culture of <i>Ocimum tenuiflorum</i> L.	Vyas and Mukhopadhyay (2017)
2.	<i>Ocimum basilicum</i>	Efficient adventitious shoot organogenesis on root explants of <i>Ocimum basilicum</i> L.	Fraj et al. (2017)
3.	<i>Ocimum gratissimum</i>	Plant tissue culture: an alternative for production of useful secondary metabolites	Sravanthi et al. (2016)
4.	<i>O. gratissimum</i>	<i>Agrobacterium tumefaciens</i> -mediated genetic transformation of <i>Ocimum gratissimum</i> : a medicinally important crop	Khan et al. (2015)
5.	<i>Ocimum sanctum</i>	Protocol establishment for multiplication and regeneration of 'holy basil' (<i>Ocimum sanctum</i> Linn). An important medicinal plant with high religious value in India	Mishra (2015)
6.	<i>O. basilicum</i>	In vitro plant regeneration of Turkish sweet basil (<i>Ocimum basilicum</i> L.)	Ekmekci and Aasim (2014)
7.	<i>O. basilicum</i>	A calibrated protocol for direct regeneration of multiple shoots from in vitro apical bud of <i>Ocimum basilicum</i> —an important aromatic medicinal plant	Leelavathi et al. (2014)
8.	<i>O. sanctum</i>	An improved plant regeneration system of <i>Ocimum sanctum</i> L.—an important Indian holy basil plant	Sharma et al. (2014)
9.	<i>O. tenuiflorum</i>	Development of a rapid and high-frequency <i>Agrobacterium rhizogenes</i> -mediated transformation protocol for <i>Ocimum tenuiflorum</i>	Vyas and Mukhopadhyay (2014)
10.	<i>O. basilicum</i>	An efficient system for in vitro multiplication of <i>Ocimum basilicum</i> through node culture	Shahzad et al. (2012)
11.	<i>O. basilicum</i>	An efficient method for micropropagation of <i>Ocimum basilicum</i> L.	Saha et al. (2010)
12.	<i>O. sanctum</i>	Protocol establishment for multiplication and regeneration of <i>Ocimum sanctum</i> Linn. An important medicinal plant with high religious value in Bangladesh	Banu and Bari (2007)
13.	<i>O. basilicum</i>	Rapid micropropagation of <i>Ocimum basilicum</i> using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium	Siddique and Anis (2007)
14.	<i>O. gratissimum</i>	In vitro multiplication of <i>Ocimum gratissimum</i> L. through direct regeneration	Gopi et al. (2006)
15.	<i>O. basilicum</i>	In vitro propagation of <i>Ocimum basilicum</i> L. (Lamiaceae)	Dode et al. (2003)
16.	<i>O. basilicum</i> and <i>O. citriodorum</i>	<i>Agrobacterium tumefaciens</i> -mediated transformation of <i>Ocimum basilicum</i> and <i>O. citriodorum</i>	Deschamps and Simon (2002)
17.	<i>O. basilicum</i>	Shoot regeneration of young leaf explants from basil (<i>Ocimum basilicum</i> L.)	Phippen et al. (2002)
18.	<i>O. basilicum</i>	In vitro rapid clonal propagation of <i>Ocimum basilicum</i>	Begum et al. (2002)
19.	<i>O. sanctum</i>	In vitro propagation of <i>Ocimum sanctum</i> L. through nodal explants. Bangladesh	Banu et al. (2001)
20.	<i>O. sanctum</i>	Micropropagation of 'holy basil' (<i>Ocimum sanctum</i> Linn.) from young inflorescences of mature plants	Singh and Sehgal (1999)

(continued)

Table 10.5 (continued)

S.N.	Species	Research	References
21.	<i>O. sanctum</i>	Callus induction and plant regeneration of <i>Ocimum sanctum</i>	Banu et al. (1999)
22.	<i>O. basilicum</i>	In vitro clonal propagation of an aromatic medicinal herb <i>Ocimum basilicum</i> L. (sweet basil) by axillary shoots proliferation	Sahoo et al. (1997)
23.	<i>O. americanum</i> <i>O. sanctum</i>	In vitro propagation of the medicinal herbs <i>Ocimum americanum</i> L. syn. <i>O. canum</i> Sims. (hoary basil) and <i>Ocimum sanctum</i> L. (holy basil)	Pattnaik and Chand (1996)

identification of homologous sequences in another related species (Canter et al. 2005). Whole genome sequencing of *O. sanctum* opens up an opportunity in the designing of new DNA markers from DNA sequences. Development of new DNA markers can be effectively utilized in integrating marker-based technology in conventional breeding.

According to Rastogi et al. (2015), simple sequence repeat (SSR) in the *O. sanctum* whole genome was predicted. A total of 4827 sequences which were greater than 500 bp length were studied for SSR and of total obtained sequences, 2612 possessed SSR repeats while the remaining (2364) sequences had more than one SSR (Fig. 10.6). The SSR markers obtained from *O. sanctum* can be utilized for the quantification of genetic diversity among different *Ocimum* genotype (Zietkiewicz et al. 1994; Dhawan et al.

2016). Prediction of other DNA-based markers from *O. sanctum* whole genome sequencing may bring advancement in breeding strategies as well as in resolving the problems faced during conventional breeding of *Ocimum* species. It could also be used for alteration of various traits among *Ocimum* species, for example; a cold tolerant gene from *O. kilimandscharicum* could be manipulated for the development of a cold tolerant *O. sanctum*. Similarly, many other genes from different biosynthetic pathways could be manipulated for the development of superior traits.

Further, gene editing tools such as CRISPR/Cas9 can also be utilized for simple manipulations and for the selection of an excellent trait of *O. sanctum*. It could find tremendous application in the identification of novel gene function and in pathway engineering of

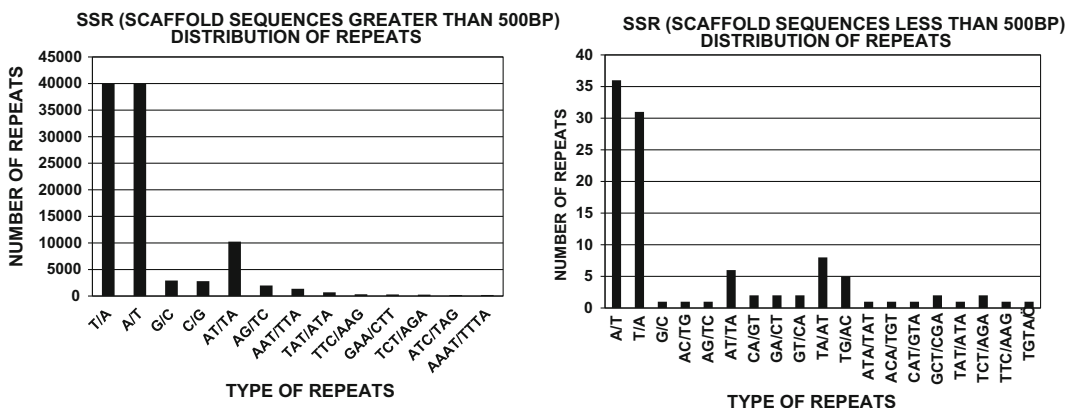


Fig. 10.6 SSRs predicted from the *O. sanctum* whole genome. Scaffold sequences of length >500 bp as well as <500 bp were individually examined for simple sequence

repeats (SSRs) using MISA (<http://pgrc.ipkgatersleben.de/misa/>)

O. sanctum (Liu et al. 2017). However, it has to be done in a controlled manner and on a large scale. And it is only possible by bringing *O. sanctum* into cultivation.

10.3 Conclusion and Future Prospects

As compared to the animals, the genomes of plants are larger and intricate which code for the complex molecular systems those controlling the smooth functioning of the physiology. Due to the sessile nature of plants, their genomes had undergone evolution for generating an organism which adapts to the constant environmental changes. Additionally, in response to the theory of natural selection, the local wild-type crops have been sorted for preferred traits via plant breeding. Up to now, the selection breeding and the plant adaptability have produced plants that constitute several secondary metabolites. Nevertheless, global warming and the simultaneous diminution in the arable land, existing breeding approaches only would be unable in maintaining the endurance capacity or the exquisite metabolic composition of the plants. Hence, getting a comprehensive overview of the molecular networks operational in plants which regulate plant secondary metabolism is becoming essential in order to engineer those, as well as the detection of the novel genome-based markers for selecting such phenotypes would be critical to obtain the plants with specialized metabolic composition.

Availability of the whole genome sequence of *O. sanctum* will definitely accelerate the research of functional genes related to secondary metabolism. Such research can greatly contribute in the development of drug discovery based on natural product and acceptable exploitation of plant pharmaceuticals sources. The genome sequence along with transcriptome sequence of *O. sanctum* can be greatly utilized for the genome re-sequencing which will be helpful in the detection of genetic molecular markers (GMMS), such as InDel, SSR, and SNP. Through the recent advances in NGS technologies, the genome sequencing of many targeted medicinal plant has

now became possible because of reduced cost and lesser time utilized in the sequencing process. Sequencing and assembly of more and more medicinal plant will lead to the development of comparative genomics which would prove a widespread means to study the secondary metabolism of such therapeutic plants. Furthermore, studies on GMMS genetic linkage and the improved character, genotype, and phenotype of curative plant might play a major role in the molecular breeding of medicinal plants as well as in the development of the transgenics of such medicinal plants.

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