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The Holy Basil (*Ocimum sanctum* L.) and its Genome

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Abstract

Ocimum sanctum L. (*O. tenuiflorum*) is popular in India as "Holy basil". It is not only designated as sacred basil but is also used as an important ingredient in Indian and some international traditional medicinal systems. There are many theories about the origin of "Tulsi" in ancient Hindi scripts. This herb is a repository of medicinal compounds and thus is also used in treatment of various diseases and disorders, but the validation of phytomolecules with differential therapeutic activities is still a challenge. Approximately, 80% of the patents on *Ocimum sp*. are on the chemical extracts or the utility of plant parts with main focus on essential oil components. Thus, an effort towards recognition of full metabolic potential of this sacred herb, sequencing of the whole nuclear and chloroplast genomes was performed. The data generated was obtained by combining the sequence reads from 4 libraries of three NGS platforms. This data resulted in a saturated genome draft assembly of about 386 Mb. Pathway analysis from the data indicated the dominance of phenylpropanoids in *O. sanctum*. Comparison of the available genes and the chemical compounds in *O. sanctum* and its nearest neighbor *Salvia miltiorrhiza,* revealed the prospects of discovering new active molecules. This information generated will help in providing new insights relating to the medicinal nature of the metabolites synthesized in the plant.

Key words: Chloroplast Genome, Next Generation Sequencing, Ocimum tenuiflorum, Phenylpropanoids, Terpenoids, Whole Genome

1. Introduction

O. sanctum or *O. tenuiflorum*, is one of the highly respected medicinal plant in India and thus popular as 'holy basil'. This member of family lamiaceae is called *Thulasi* (or *Viṣṇupriya*) in Sanskrit, *Thulasi* in Tamil and *Tulasi* in Hindi. This plant is mentioned in Āyurveda as life saving herb- 'the elixir of life'. It has been described to be the native of Indian subcontinent (Staples and Kristiansen, 1999) and more specifically to North Central India by studying the phylogeography of this species using *trnL-trnF* intergenic spacer of plastid genome as the DNA barcode (Bast et al, 2014). It is also found in the parts of northern and eastern Africa, Taiwan, and Hainan Island, and wildly grows all over India and up to an altitude of 5,900 feet (1,800 meters) in the Himalayas (WHO, 2002; Anonymous, 1991; Gupta *et al.*, 2008). In China, it occurs in dry, sandy areas of Hainan and Sichuan, as well as in Cambodia, Malaysia, Laos, Myanmar, Indonesia, the Philippines, Thailand, and Vietnam (Li and Hedge, 1994). Holy basil is cultivated in Southeast Asia but is also abundantly grown in Australia, West Africa, and some Arab countries (Mondal *et al*., 2009; Parotta, 2001). The Plant List database (http://www.theplantlist.org/1.1/browse/A/Lamiaceae/Ocimum/) includes 327 scientific plant names of species rank for the genus *Ocimum.* Of these 66 are accepted species names (Fig.1).

In India, the dried leaf, seed, and whole plant are used separately in the conventional medicinal systems of Unani, Ayurveda, and Siddha (Ved and Goraya, 2008). Two types of *Ocimum*

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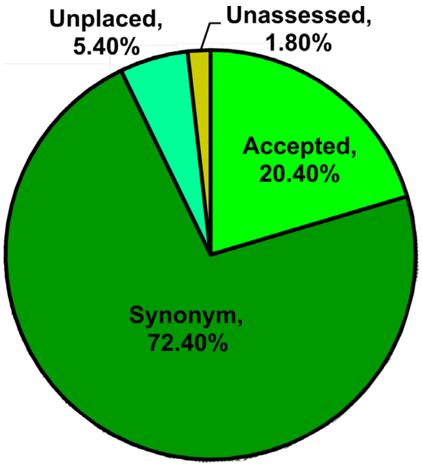

Fig. 1. The status of the 333 species names for the genus *Ocimum* recorded in *The Plant List* (http://www.theplantlist.org/1.1/browse/A/Lamiaceae/Ocimum/).

sanctum L. are found to be cultivated: (i) Green leaved tulsi plants known as Sri/ *Rāma Tulsi* & (ii) Purple leaved *tulsi* plant known as Kṛṣṇa/ *Śyāma Tulsi* (Anonymous, 1991).

2. Holy basil in religion

Basil is a part of religious belief not only in India but around the world including Christianity to Hindu. Although there is no mention of basil in the *Bible* (Darrah, 1980), the plant is said to have grown at the site of Christ's crucifixion (Meyers, 2003). Holy basil, *O. tenuiflorum*, is mainly sanctified in Hindu folklore. It is considered to be the manifestation of the goddess, *Tulasi*, and to have raised from her embers. There are numerous stories regarding the legend, but according to a widely known one comes from *Śiva Purāṇa*. According to it, when Indra and Bṛhaspati once went to meet lord Śiva at Mount Kailash and their way was blocked by a sage with tangled hair and a resplendent face. The sage was Śiva himself, who appeared in that form to test Indra and Bṛhaspati. Indra did not identify the *yogi* and got furious that the man was not moving out of their way. In order to move him out of his way Indra threatened him with his thunderbolt. Śiva became infuriated upon this deed of Indra and his eyes got red, startling Indra. Due to his anger his third eye got opened to kill Indra but at the same time Bṛhaspati recognized Śiva and requested him to pardon Indra. Convinced by Bṛhaspati, Śiva propelled the fire from his eye towards the ocean and in contact with the ocean it took the form of a boy who was named Jalandhara (http://hindumythologybynarin.blogspot.ca/2013/03/jalandhar-demon-son-of-lord-shiva.html). Jalandhara grew up to be exceedingly powerful and was made the king of demons by Śukra, their teacher. Jalandhara got married to Vrinda, who was the daughter of the demon Kālanemi. Jalandhara ruled with justice and nobility but narrations of sage Bhṛgu made him against lord Viṣṇu as well as other gods. A fierce battle occurred between Jalandhara and Viṣṇu which remained inconclusive. Influenced by the bravery of Jalandhara he asked him to claim any boon of his desire. Jalandhara asked Viṣṇu to reside in Kṣīra Sāgara and as accepted by Viṣṇu, in his absence all gods got defeated by demons and Jalandhara became the emperor of the three worlds (heaven, earth and hell) (http://hinduonline.co/Scriptures/Puranas/ŚivaMahaPurana.html; http://hindumythologybynarin.blogspot.ca/2013/03/jalandhar-demon-son-of-lord-shiva.html). Despite being the son of lord Śiva, Devas did not want to be governed by Jalandhara and thus got depressed on their defeat. The sage Nārada in consultation with the Devas went to meet Jalandhara. On being asked by Jalandhara the intention of his visit, he illustrated the beauty of Kailāśa. Nārada then continued to describe Śiva's residence and also described to him about Pārvatī's beauty (Stella 1992). Listening the description of Kailāśa and Pārvatī, Jalandhara disguised himself as Śiva and went to Pārvatī in order to trick her. Pārvatī identified him and became very annoyed. She endeavored to strike him but Jalandhara ran away realizing he could not match for her anger. Pārvatī, then went to Viṣṇu and requested him to trick Vrinda just like Jalandhara tried to trick her (Stella 1992; http://www.sacred-texts.com/hin/hmvp/hmvp43.htm).

Jalandhara's wife Vrinda was very virtuous. Viṣṇu first created an illusion of Jalandhara's destruction by Śiva and then restoration to life by him. Hearing this, Vrinda embraced Jalandhara who is in reality Viṣṇu, in disguise. Upon realization of this fact, Vrinda cursed him that soon somebody would seize his own wife (which happened as abduction of Sītā by Rāvaṇa) and also to become a stone due to this stone hearted conduct. Honoring his real devotee, Lord Viṣṇu accepted the curse and appears as the Sāligrāma *śilā* in the Gandika river (now in Nepal). After this Vrinda stepped in the fire to immolate herself. When Jalandhara came to know about his wife's deception and death, he became mad with anger and left Mount Kailāśa, to return to the battlefield. Short term break in Vrinda's sanctity also broke the shield of Jalandhara's protection. Hence, Jalandhara was killed by Śiva in battle. Upon his death his soul merged with Śiva just like Vrinda's soul had merged with Pārvatī (Stella 1992). In her torment, Tulasi ended up her life, and Viṣṇu affirmed that she would be "worshipped by women for her faithfulness" and would protect women befalling widows (Gupta, 1971). Thus, holy basil, which also goes by the common name *tulsi*, an obvious allusion to the deity, happened to be a symbolic of love, eternal life, purification and protection in Hinduism (Gupta, 1971; Dyer, 1889). In addition to basil's role in the death of Tulasi in the Hindu legend, basil has played a role in burial ceremony and has been nurtured near burial places in many countries (Darrah, 1980).

3. Taxonomic History of *Ocimum*

The circumscription of *Ocimum* itself is also tricky. Estimation of species number revealed 30–160 annual and perennial herbs and shrubs indigenous to the tropics as well as subtropical areas of Asia, Africa, and Central and South America (Darrah, 1980; Paton *et al*., 1999). Different species and forms of *Ocimum* spp. vary in growth habit, color, and aromatic composition, making the true botanical identity of basil difficult.

Because of their economical and medical importance, the most widely cultivated species worldwide are *O. africanum* Lour. (syn. *O.* x *citriodorum* Vis.), *O. americanum* L. (syn. *O. canum* Sims.), *O. basilicum* L., *O. minimum* L., *O. gratissimum* L., and *O. tenuiflorum* L. (syn. *O. sanctum* L.) (Carovic-Stanko *et al.,* 2010). *Ocimum* was described in 1753 by Linneaus, who listed five species. According to Paton *et al* (1999), Bentham (1832) acknowledged approximately 40 species in *Ocimum* genus and clustered them into three sections: *Ocimum* (*Ocymodon* Benth.), *Gymnocymum* Benth, and *Hierocymum* Benth. Based on the calyx morphology, section *Ocimum* was subdivided by Bentham into three subsections: *Ocimum, Hiantia* Benth and *Gratissima*. He also incorporated a novel section, *Hemizgya* Benth., which Briquet (1897) regarded as a separate genus due to the fusion of anterior stamens. Paton (1992), reviewed African species of *Ocimum* and identified around 30 species and drawed on infrageneric classification of *Ocimum* given by Bentham (1848) in which sect. *Hemizygia* and subsect. *Hiantia* were uninvolved, favoring the second as the distinct genus *Becium* (Paton *et al.,* 1999). Pushpangadan (1974; as described in Paton *et al.,* 1999) devised a separate infrageneric classification. Basil taxa was divided into two groups by him: the *Basilicum* group, including annual herbs or at times perennials having ellipsoid, black, intensely mucilaginous seeds with a basic chromosome number x = 12, and the

Sanctum group, consisting of perennial shrubs with brown globose, non-mucilaginous or weakly mucilaginous seeds with a basic chromosome number of x = 8. As per Pushpangadan's categorization, the *Basilicum* group has only section *Ocimum* subsection *Ocimum*. Six major cultivars and varieties that are frequently found in the market worldwide (var. *basilicum* cv. Genovese, var. *basilicum* cv. Sweet Basil, var. *difforme* Benth., var. *purpurascens* Benth., cv. Dark Opal, and var. *thyrsiflorum*/L./Benth.) were kept in *Basilicum* group, section *Ocimum* (=*O. basilicum* group) by Pushpangadan. However, there are numerous issues pertaining to his infrageneric classification, and also not complies with the International Code of Botanical Nomenclature. Elevated levels of both chemical and morphological inconsistency exist within the genus due to polyploidy, interspecific hybridization, and the occurrence of chemotypes which do not considerably vary in morphology (Simon *et al.,* 1990). To get a better overview into inter- and intraspecific taxonomy, an array of various methods based on morphology, geographic origin, karyotype, crossability and chemical composition have been used (Khosla, 1995; Grayer *et al.,* 1996; Marotti *et al.,* 1996; Paton and Putievsky, 1996; Ravid *et al.,* 1997; Putievsky *et al.,* 1999; Martins *et al.,* 1999). Despite these efforts, taxonomy and phylogenetic relationships within *Ocimum* genus are still in question.

4. Metabolites and Medicinal potentialities

Holy basil owes a stronger, somewhat pungent taste than other basils due to *beta* caryophyllene, a sesquiterpenoid, and eugenol which is a phenylpropanoid which is present in the leaf essential oil obtained after hydro-distillation. The oil is applied to reduce joint pains, inflammation and body rashes (Bhasin, 2012). Various investigations performed on the essential oils of a number of species of *Ocimum* suggest the biological activities possessed by the oils. Of these, antibacterial, antimicrobial, antifungal properties are of great importance (Kalita and Khan, 2013). High quality essential oil isolate, Eugenol, was the main component of the oil, which is of great value in flavouring of all kinds of food products in industrial food processing. Also eugenol is used in the synthesis of vanillin; the World's mostly used flavour of all kinds of food products. Eugenol has great importance in pharmaceutical industry and usually extracted from clove buds (70–85%) as well as leaves and barks of *Cinnamomum* (20–50%) (Mukherji, 1987). Although these plant sources are rich in eugenol but because of their higher prices the commercial extraction of eugenol from them is costly. In contrast to these sources *Ocimum sanctum L.* (*Tulsi*) is cheaper source for commercial extraction of eugenol (Mukherji, 1987). The leaf volatile oil also contains α-pienene, β-pienene, carvacrol, linalool, limonene, camphene, sabinene, β-caryophyllene, β-elemene, cineole, germacrene D, methyl carvicol and urosolic acid etc. (Pattanayak *et al.* 2010; Lal *et al.* 2003). Quantitative variations of eugenol have been seen in the composition of essential oils of *Ocimum sanctum L.* growing in different parts of India (Rajeshwari, 1992; Mukherji, 1987). The percent of eugenol in essential oil of *Ocimum sanctum L.* varies in the range between 40% and 70%. During the rains in Indian agroclimatic condition, there is a drastic decrease in the eugenol content of the leaf especially in the case of *Ocimum sanctum* genotypes.

Traditional medical practitioners most often and widely use *O. sanctum* to cure diseases like malaria, bronchitis, bronchial asthma, dysentery, diarrhea, arthritis, skin diseases, painful eye diseases, chronic fever *etc*. It has also been demonstrated to have anticancer, antifertility, antifungal, antidiabetic, antimicrobial, cardioprotective, hepatoprotective, analgesic, antiemetic, antispasmodic, adaptogenic and diaphoretic actions (Prakash and Gupta, 2005). Mixed with mustard oil, the dried powdered leaves in form of a paste is useful cure to pyorrhea, foul smell and other tooth troubles. The seeds are used in genito-urinary system disorders (Bhasin, 2012).

5. CSIR- CIMAP contributing *O. sanctum* varieties

In order to combat the situation of low eugenol yield during the rainy season CSIR-CIMAP, Lucknow developed a new *Ocimum sanctum* cultivar named "CIM-AYU". The said plant produced a high amount of eugenol (83% of oil) with high essential oil and herbage yield. It also produces eugenol in the essential oil even during rainy season (at least 47%) compared to 5.0% in other strains (Lal *et al*., 2003). Other varieties of *O. sanctum* released by CSIR-CIMAP are: "CIM- Angna" and "CIM- Kanchan" (Lal *et al*., 2008 and Kothari *et al*., 2004). "CIM- Angna" was a high yielding dark purple pigmented variety with high eugenol (40%) and better germacrene-D (16%), β-elemene (14%), β-caryophyllene (9%) while "CIM-Kanchan" with 70% methyl eugenol, 15% β-caryophyllene and 7.6% β-elemene in combination in the essential oil compared to the control varieties.

6. Genome of Holy basil

This herb is a repository of medicinal compounds and despite of its religious sanctity and its use in treatment of various diseases and disorders, the validation of phytomolecules with differential therapeutic activities is still a challenge. The chromosome number of this plant was analyzed to be 2n=16 (Rastogi et al, 2014). Approximately, 80% of the patents on *Ocimum sp*. are on the chemical extracts or the utility of plant parts with main focus on essential oil components. Thus, in an effort towards recognition of full metabolic potential of this sacred herb, sequencing of the whole nuclear and chloroplast genomes was carried out (Rastogi *et al*. 2015). This is the first medicinal plant genome sequenced and analyzed completely. The data that got generated was obtained by combining the sequence reads from 4 libraries of three NGS platforms. Out of the 4 libraries, two libraries of long and short reads were of Illumina HiSeq2000, one was of 454 GS FLX and one was the mate-pair library of SOLiD 5500XL. This data resulted in a saturated genome draft assembly of about 386 Mb, with a plastid genome of 142,245 bp, which turned out to be the smallest among the Lamiaceae family members. A total of 107,785 contigs resulted into 22,776 scaffolds and finally 9,059 super-scaffolds. Pathway analysis from the data indicated the dominance of phenylpropanoids in *O. sanctum*. 136 proteins homologous to five important plant genomes were also identified in addition to SSR markers. *Salvia miltiorrhiza* was positioned as the nearest neighbor in the phylogenetic analysis of the chloroplast proteome. Comparison of the available genes and the chemical compounds in *O. sanctum* and *S. miltiorrhiza,* revealed the prospects of discovering new active molecules. Thereafter, another group Upadhyay et al. (2015) also reported draft genome of *O. tenuiflurum* L (*Kṛṣṇa Tulsi*) using paired-end and mate-pair sequence libraries and the assembly resulted in a draft genome of 374 Mb. Both the publications indicate the compact genome in relation to the gene organization and the number of secondary metabolites. The preventive and therapeutic potential of this plant has also being explained in terms of already described phenylpropanoids and terpenoids as well as their biosynthesis described in literature. This valuable information thus generated could prove highly beneficial in identification of explicit metabolites pathway mining in the related species as well. The annotation of the gene sequences will help in providing new insights relating to the medicinal nature of the metabolites and the function of genes as well as their synthesis in the plant.

7. Conclusion

Tulsi (basil) plants are increasingly being recognized as an important source of significant livelihood opportunities for poor rural population and an income source for Government also. The collection and cultivation of *Tulsi* (basil) provides a main source of ready money to many rustic communities specially women, primitive forest dependent tribes, poor and marginalized farmers (GOI, 2000). According to trade portal of India, the need for plants as well as plant-based drugs of proven medicinal importance mentioned within traditional medical systems such as Ayurveda, Chinese, and Unani Tibb medicine has never been much. But the present scenario indicates that many countries, including India, conventional medicine are becoming more widely valued within all sections of society. Rapid increase in population and mounting urbanization are the major reasons of fetching high demand for plant-based drugs. Globally, the increased recognition of "complementary" or "alternative" medicine has created a speedily expanding market for both crude drugs as well as refined compound preparations. The worldwide annual business in herbal drugs has lately been anticipated at US $14 billion to over US $20 billion with the biggest markets found in Europe, Asia, and North America (http://www.tradeportalofindia.com/usrdata/webadmin/Section3.9/HS12_MedicinalAromatic Plants_ITPOFft.html#1).The international demand of curative plants has been expected at 60 to 62 billion US $ which is increasing at a rate of 7-10 % annually (GOI, 2000).

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