International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

ISSN 2347-2677
www.faunajournal.com
IJFBS 2020; 7(6): 53-57
Received: 24-09-2020
Accepted: 28-10-2020

Dr. Ashwini Kumar Sinha
Assistant Professor and Head,
Department of Botany,
SMJ College, Khajedih,
Ladania, Madhubani, Bihar,
India

Dr. TP Singh
University Professor (Retired.),
Department of Botany,
LN Mithila University,
Darbhanga, Bihar, India

Corresponding Author:
Dr. Ashwini Kumar Sinha
Assistant Professor and Head,
Department of Botany,
SMJ College, Khajedih,
Ladania, Madhubani, Bihar,
India

Distribution, cytology, genetics and biotechnology of *Ocimum Basilicum* L. (Lamiaceae) for its commercial exploitation

Dr. Ashwini Kumar Sinha and Dr. TP Singh

DOI: https://doi.org/10.22271/23940522.2020.v7.i6a.811

Abstract
The aromatic genus *Ocimum* L. is commercially and medicinally important in the family Lamiaceae was known by 180 genera and 3500 species spread from tropical to sub-tropical parts of the world, of which *O. basilicum* L. prefers both plains and high altitudes. The species is the most important of all as its various subspecies, varieties and chemotypes contain a number of terpenoids and phenols along with many other compounds of high medicinal and commercial values. It is a natural tetraploid (2n = 4x = 48 chromosomes) with almost normal mitosis and meiosis and belongs to the sub-genus Basilicum with basic chromosome number x =12. Free intervarietal hybridization in the species has resulted in a number of viable and stable chemotypes with methyl chavicol, linalool, eugenol, camphor and methyl cinnamate as main constituents of their essential oils, either occurring singly or in different combinations. This feature of the chemotypes has been suggested to be genic in nature as an alteration in their chromosome number and structure is a rare phenomenon. While α-phenylalanine has been found to be the precursor of terpenoids and phenols boch, monoterpenoid linalool has been considered to be the initial terpenoid in the biogenesis of the two compounds. A semblance in the biogenetic pathways of the said main compounds in the various species of the genus *Ocimum*, including the one understudy, with those of *Mentha* L, looks plausible, which throws light on homogeneity in the family Lamiaceae. Various aspects of the species, such as distribution, cytology, genetics, biogenesis, biotechnology, etc. of the commercially important species have been discussed in detail.

Keywords: cytology, genetics and biotechnology, commercial exploitation

Introduction
The genus *Ocimum* L. belongs to the family Lamiaceae, the mint family. The family has altogether 180 genera (Willis, 1973) of medicinal and aromatic importance. The genus *Ocimum* is one of them and possesses 160 species with a worldwide distribution. Of the various species of *Ocimum*, *O. basilicum* L., also known as French basil or Sweat basil or Common basil is medicinally and commercially very important. Altogether 6 sub-species and varieties of *O. basilicum* have so far been reported to grow in India, namely, *O. basilicum* ssp. Minimum Danert (Syn. *O. minimum* L). *O. basilicum*var, *glabratum* Benth. *O. basilicum*var, majus Benth., (*O. basilicum* var. *pilosum* Benth. (Syn. *O. pilosum* Roxb.), *O. basilicum* var. *purpurascens* Benth. and *O. basilicumvar. thyrsiflorus* Benth. In addition to these, there are several varieties and chemotypes of the species with worldwide distribution.

Distribution
Over160 species of *Ocimum* has been reported to grow in different parts of the world including tropical Asia, Africa, America and sub-tropical regions occurring from sea level to an altitude of 1800m Sharma *et al.* 1987 [46-50, 51, 57]. The sub-species and varieties of *O. basilicum* are either cultivated or grown wild in France, Egypt, Hungary, Indonesia, Morocco, U.S.A., Nigeria, Tanzania Sicily, Italy, Pakistan, Senegal, Samoan Islands, erstwhile USSR, Latin America, Mexico. Seychelles and Greece including various states of India. The species prefers both plains and high altitudes. The herb *Ocimum*, including the species under study, is said to have three main centers of diversity - tropical parts of Africa, South America, possibly Brazil and Asia (Sobti *et al.* 1976) [40-42, 59-62], of which the former seems to be the place of origin as different species have migrated from the place to various parts of the world, evolving

simultaneously during the course of migration. Different varieties and their chemotypes have been shown in Table- I.

Commercial importance

Of the many species of the genus, *O. basilicum* is commercially very important as it contains a number of phytochemical constituents, various terpenoids being the most significant ones. In addition to many other terpenoids, methyl chavicol, linalool, eugenol, camphor and methyl cinnamate are major ones, found in the sub-species and varieties of the plant. Aromatic compounds of the species are used variously as in medicines, perfumes, toiletries, food flavouring, etc. Its commercial importance can be estimated by the fact that the International price of the essential oil produced by the herb is US $90/kg (As of December 1995, Sharma *et al.* 1996) [46-50, 51, 57]. The volatile oils have been known to possess antibacterial and insecticidal properties (Khorana and Vangikar 1950, Sirsi *et al.* 1952, Kurup 1956, Jain and Jain 1972, Lahariya and Rao 1979) [20, 21, 43, 58]. Antitubercular and antimalarial actions of oils are reported by Spencer *et al.* (1947) [63], Ramaswami and Sirsi (1967) [43, 58]. The whole herb is used to treat snake bites and insect stings as reported by Mhaskar and Caius (1931) [34]. Turowka *et al*. (1956) and Bhargava *et al*. (1979) [5]. The species is commonly used in traditional medicine to cure diseases, like bronchitis, chest and lungs complaints (Lubini 1990) [30], rheumatism and inflammation (Girach 1992) [13] and hypertension (Adjanohoun *et al.* 1989) [1]. The species is also used in "Aromatherapy.

Cytology

Chromosome studies made in *Ocimum* spp. by various authors revealed the occurrence of two basic chromosome numbers, such as x=8 and 12 (Darlingion and Wylie 1955) [10]. Findings

Table 1: Varieties of *O. basilicum* and their chemotypes

S. No.	Varieties	Chemo types
1.	*O. basilicum* L. var. *basilicum*	
2.	*O. basilicum* var. *crispa*	MethyIchavicol – Linalool type
3.	*O. basilicum*var Darkopal (var /cultivar)	
4.	*O. basilicum* var. *glabratum* Benth.	
5.	*O. basilicum* var. *glabratum* Benth.	Methyl chavicol type and Camphor type
6.	*O. basilicum* var. *majus* Benth.	
7.	*O. basilicum* var *minimum* Danert.	Eugenol type
8.	*O. basilicum* var. *pilosum* Benth.	Geranyl acetate type
9.	*O. basilicum* var. *purpurascens* Benth.	Methyl cinnamate type, Linol type and Eugenol>Linalool type
10.	*O. basilicum* var. *thyrsiflorus* Benth.	Methyl cinnamate type

by various later workers (Sobti and Pushpangadan 1977, Singh, 1987, 1995) [40-42, 59-62] have established the existence of two sub-genera in *Ocimum*, namely. Sanctum and Basilicum, having two different base numbers, x=8 and 12 chromosomes, respectively. However, lower basic chromosome numbers, such as x=4 and 6, have been suggested for the two sub-genera, respectively, by Stebbins (1971) [65], Singh and Sharma (1981) [46-50, 51, 57] and Sharma and Singh (1981) [46-50, 51, 57]. These two small numbers might have been the progenitors of the aforesaid higher basic numbers (Singh 1995) [46, 48-57]. The species under review belongs to the sub-genera Basilicum.

Somatic chromosome numbers in all the varieties of the species are 48, except a report of 52 in one of the populations by Singh (1987) [46, 48-57]. This indicates the species after attaining autotetraploid level got stabilised and any variations at varietal and chemotype levels are probably due to genic alterations.

Meiosis of all the species of *Ocimum*, including *O. basilicum*, was almost normal with regular bivalent formation, barring a few stray abnormalities. The only exception is *O kilimandscharicum* Guerke which is an aneuploid (2n=6x+4= 76 chromosomes), the x being 12 chromosomes (Singh 1990) [46, 48-57]. Cytological evolution in the Labiatae herbs growing in India, in general, has been reviewed by Saggoo and Bir (1985) [45, 59] and the genus *Ocimum* in particular by Singh and Sharma (1981) [46-50, 51, 57]. Somatic and meiotic chromosome numbers reported for *O. basilicum* have been compiled in Table-2

Genetics

Among the various species of the Lamiaceae family, genetic studies, with special reference to inheritance patterns of various compounds, have been made in some detail in *Mentha* L. only. Though, studies have been made in *Ocimum* spp., a lot needs to be investigated. Outbreeding within the population of a species is commonly observed in the species of *Ocimum*, such as *O. americanum* L. *O. basilicum*, *O. canum* Sims. and *O. sanctum* L. Sobti and Pushpangadan (1982) [40-42, 59-62] studied breeding in the genus and reported frequency of intravarietal hybrids in *O. basilicum* in the range of 5.8-18.5%. Naragund *et al*. (1979) [39] studied inheritance pattern in pigmentation of seedlings and adult plants of this species and valuable gene marker for seedling pigmentation. Genetics of inheritance pattern of different chemical constituents of essential oils from *O. basilicum* was studied by Sobti and Pushpangadan (1982) [40-42, 59-62] and Gupta (1994) [14-16] and reviewed by Sharma *et al*. (1987) [46-50, 51, 57]. Sobti and Pushpangadan (1982) [40-42, 59-62] showed that genes responsible for the synthesis of citral, Linalool, camphor, geraniol (all monoterpenoids) are independent of genes responsible for phenols, such as methyl chavicol and eugenol. Manitto *et al*. (1974, 1975) [31, 32] showed the existence of a dominant gene that inhibits the conversion of cinnamic acid into other components, such as, cugenol, cthyleugenol, chavicol and methyl chavicol. They also showed interference of this gene with the formation of some monoterpenoids of *O. basilicum*, namely, citral, linalool and camphor. They further showed that cinnamic acid is finally methylated to methyl cinnamate by the same gene meant for the methylation of eugenol and chavicol. Gupta (1994) [14-16] on the basis of hybridisation, the experiment carried out among three chemotypes, namely, methyl chavicol, eugenol and camphor, reported the existence of a gene *M* for the bio-synthesis of the aforesaid three major components in *O. basilicum* var. *glabratum.* The gene was suggested to occur in three or even more allelic forms: M_0 responsible for the synthesis of methyl chavicol, $M_1$for eugenol and M_2 for camphor. The gene M_0 was found to be dominant over the M_1 and the $M_2$and the M_1 over the M_2. He suggested some other forms of alleles, such as, M_3, M_4.... as well, which might be responsible for the biosyntheses of methyl cinnamate, methyl eugenol and some other aromatic compounds, like methyl salicylate, isoeugenol, acetyl eugenol, methylisovalyrate, methyl jasmonate, methyl

epijasmonate, trans-jasmone, 2-methoxy-3methyl pyrazene, tetramethylpyrazine, etc. (Hasegawa *et al*. 1997) [17].

Among the three chemotypes discussed, methyl chavicol type segregated into all the three types (methyl chavicol, eugenol and camphor), eugenol type segregated into eugenol itself and camphor, while camphor type did not segregate at all and progenies obtained were of parental type only. It was, therefore, suggested that while methyl chavicol and eugenol type existed in heterozygous forms, camphor type existed in the homozygous state. Pushpangadan and Sobti (1982) [40-42, 59-62] performed hybridization experiments between *O. canum* (a true diploid with 2n= 2x=24 chromosomes) and *O. basilicum* (a natural tetraploid with 2n=4x=48 chromosomes). This resulted in a sterile F_1, hybrids and, upon chromosome doubling, it gave a fertile hexaploid plant having 2n=72 chromosomes. It was phenotypically almost similar to *O. americamum*. The authors also claimed that this synthesized amphidiploid also inherited terpenoid constituents of both the parents. A report by one of the (TPS) the present author, however, regarding the existence of 2n=84 chromosomes in one Indian population from Allahabad from the state of Uttar Pradesh suggested an autopolyploid origin of the species. DNA estimation was carried out by Kundu (1987) [23] in *O. basilicum* (a tetraploid with 2n=48 chromosomes) along with three other species, namely, *O canum* (a true diploid with 2n=24 chromosomes), *O. sanctum* (a tetraploid with 2n=32 chromosomes) and *O. viride* Willd. (a pentaploid with 2n=40 chromosomes). It was concluded that 4C DNA content per nucleus does not have a linear relationship with the diploid chromosome numbers of the species studied, supporting the well-known idea of the C-value paradox.

Cytogenetics

Both auto-and allopolyploidy has played an important role in the speciation and evolution of *Ocimum* (Sobti and Pushpangadan 1982, Singh 1995) [40-42, 59-62] as evidenced by the presence of true diploid (*O. canum*: 2n=2x=24 chromosomes, x=8), tetraploids (*O. sanctum*: 2n=4x=32 chromosomes, x=8), pentaploid (*O. gratissimum*L. and *O. viride*Willd: 2n=5x=40 chromosomes, x=8), hexaploid (*0. americanum:* 2n=6x=72 chromosomes, x=12) and hexaploid accompanied by aneuploidy (*O. kilimandscharicum*: 2n=6x+4=76 chromosomes, x=12) species. The species under discussion is a tetraploid (2n=4x=48 chromosomes, x =12) with the normal bivalent formation and least chromosomal abnormalities during meiosis, except the occasional occurrence of quadrivalents in some samples which comes to 17.34±1.48 in an Indian variety *O. basilicum* var. *purpurascens* (authors, unpublished). Little work has so far been done on induced autopolyploidy in the family Lamiaceae in general and *Ocimum* in particular. Species of the family subjected to chromosome doubling is *O. kilimandscharicum* Gurke (Kumar, Thombre, D'cruz 1957 and Bose and Choudhury 1959) [7, 25, 48]. The present authors carried out chromosome doubling work in *O. basilicum var. purpurascens* which showed significant improvement in relation to total herbage, total seed output and germination and terpenoid contents in the third generation autopolyploid (2n=8x= 96 chromosomes). The contents of eugenol in leaves and linalool in inflorescences rose to 44.60% and 36.85%, respectively.

Table 2: Meiotic and somatic chromosome numbers in *O. basilicum*

S. No.	Species/Varieties	Chromosome number		Reference*
		n	2n	
1.	*O. basilicum* I…..		48	VAARAMA1947
			16	Sz-BORSOS1970
			24	Mehra and Gill 1972 [33]
			48, 52	Singh and Sharma 1983 [46-50, 51, 57]
		24		Khoshla 1995
2.	*O. basilicum* var. glabratum Benth.		48	Thoppil and Jose 1994 [67]
			52	Singh 1987 [46, 48-57]
3.	*O. basilicum* var. pilosum Benth		48	Thoppil and Jose 1994 [67]
4.	O. basilicumvar. Pilosum (Wild) Benth.		48	Singh 1987 [46, 48-57]
5.	*O. basilicum* var. purpurascens Benth		48	Thoppil and Jose 1994 [67], Authors
6.	*O. basilicum* var. thyrsiflorus Benth.		48	Thoppil and Jose 1994 [67]

*Available first report only considered

Biogenesis

Biogenetic pathways of terpenoids are known to a greater extent only in Mentha of the family. In *Ocimum*, no acceptable scheme has so far been proposed by any author. However, scattered reports are available on the biosynthetic pathways of its terpenoids (Dro and Hefendehl 1973; Manitto *et al*. 1974, 1975; Sobti 1976; Sharma *et al.*, 1987; Gupta 1994) [11, 31, 32. 40-50, 51, 52]. Dro and Hefendehl (1973) [11], though in *O. gratissimum*, belonging to the Sanctum sub-genus with x = 8 chromosomes, suggested biosyntheses of the terpenoids and phenols separately. However, Sobti (1976) [40-42, 59-62] suggested the two pathways have originated after the formation of phenyl-alanine from which the aromatic ring of phenolics is considered to have derived. Biogenetic studies have been made in two varieties of commercially important species *O. basilicum-O. basilicum* var. *glabratum* and *O. busilicum* var. *purpuruscens* (Sobti 1976) [40-42, 59-62]. Manitto *et al*. (1974, 1975) [31, 32] showed that L-phenyl-alanine is the precursor of terpenoids and phenols. The latter is synthesized by the loss of carboxylic C-atom and the introduction of an extra carbon atom without any skeletal rearrangement. Cinnamic acid and ferulic acid were intermediates in the syntheses of the compounds as labelled phenylalanine, cinnamic acid and ferulic acid were found incorporated.

Taking into consideration works carried out on the biogenetic pathways of terpenoids in *Mentha* spp. (Murray and Lincoln 1970, Lincoln *et al*. 1971, Murray *et al*. 1971, Hefendehl and Murray 1972, Murray and Hefendehl 1972, Murray *et al*. 1972. Murray and Hefendehl 1973, Singh and Sharma 1986) [37, 38, 46-50, 51, 57] and the frequent occurrence of acyclic monoterpenoid linalool, high or low in *Ocimum* spp. as well, Sobti (1976) and Thoppil (1996) [40-42, 59-62] suggested that the compound might be the initial one from which various other terpenoids and phenols might have been derived.

Biotechnology

Considering the commercial importance of essential oils of *O. basilicum*, Lange and Hoerster (1977) [27] studied the production and accumulation of the oil in its cell culture. They found free monoterpenoids and phenylpropanoid components and their glycosides in differentiated callus and suspension culture both. The chief glycoside components were linalool, borneol, eugenol and thymol glycosides and high content of monoterpenoid glycosides as well.

Essential oil-bearing plants possess a good number of chemotypes and even minor alterations in their genetic make-up is expected to result in a considerable impact on production and accumulation of secondary metabolites, chemical characteristics of essential oils are genetically determined and controlled (Erdtman 1962, Hefendehl and Murray 1976, Thoppil and Jose 1994) [11, 12, 18, 19, 35, 36]. Exploiting this rich biological behaviour of the herbs, Ahuja *et al.* (1982) [2] studied clonal propagation of some *Ocimum* species, including *0 basilicum*, and showed that after an initial lag- phase of 15-20 days, a uniform increase in the number of shoots per explant up to 40-45 days took place.

Chlorophyll production and photosynthetic development in *0. basilicum* were studied by Dalton (1983, 1984**)** [8, 9]. Its cell-suspension were cultured in the glucose limiting conditions batch cultures and glucose- excess conditions of both cultures. Of the two, the former had a higher production rate of total chlorophyll and also a higher photosynthetic rate.

However, a later report by Banthorpe *et al.* (1986) [4] showed callus culture of *O. basilicum* maintained under different regimes of media, temperature and illumination, not have any detectable accumulation of monoterpenoids in the callus or in the media. On the other hand, it's culture yielded cell-free extracts having prenyltransferase and isomerising system with activities nearly 400 – folds greater than those extracted from the parent matured plants. Similar findings were made in essential oils bearing Labiataeherbs, *Rosmarinus officinalis* L. and *Lavanula angustifolia* L. Mill as well.

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