Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(2); 252-262

ISSN: 0975-4873

Review Article

Diversity Analyses in *Ocimum* Species: Why and How?

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Available Online:21st January, 2016

ABSTRACT
Basil is an important part of Lamiaceace family. Besides having various economical importance it also has diverse uses in the traditional medicines. It is rich in camphor, geraniol, linalool and linalyl acetate. The genus is highly variable and possesses wide range of diversity at the level of morphology, chemical and genetic make-up. For analyzing the variation existing in the genus *Ocimum*, the chemical analyses of its essential oils and DNA fingerprinting were prominently utilized. This is utmost helpful in plant improvement programs as well as developing a well-organized way to conserve the genetic wealth of the genus. In this paper, we have thrown light on how the combinatorial approach of studying the morphological traits, essential oil composition and DNA markers is useful in verifying the taxonomy of *Ocimum.*

Keywords: Plant Conservation, Genetic diversity, *Ocimum* spp., Authentication, Molecular Markers, DNA Fingerprinting

INTRODUCTION

Genus *Ocimum* contains more than 150 species, collectively called as 'Basil'. It comprises annual and perennial herbs and shrubs of Asia, Africa, Central and South America[1]. In India, its considered as a sacred plant and its leaves are routinely used in worship. Here, three main forms of 'Tulsi' are: 'Rama' or green tulsi, 'Krishna' or purple tulsi and 'Vana' tulsi. Besides being a rich source of essential oils and aromatic compounds[2], it is also valued as a culinary herb and an attractive and fragrant ornamental plant[3]. It is inhabitant of moist soils throughout the world[4]. Depending on the variations in the soil type and rainfall the size and form of the plants may vary and correspondingly their medicinal strength and efficacy.

American basil is a high quality sweet basil with violet leaves of uniform size. While, Egyptian and African Basil have different taste but have lower value in comparison to French and American Basil[5]. Lemon basil is grown as fresh culinary herb and dried in floral arrangements and is a most widely used variety in drug, perfume and cosmetic industry[6]. The other species of *Ocimum* like *O. africanum Lour.(syn. O. 9 citriodorum Vis.), O. americanum L. (syn. O. canum Sims.),O. basilicum L.,O. gratissimum L.,O. minimum L.,* and *O. tenuiflorum L. (syn. O. sanctum L.)* are also preferred to be grown due to their high medicinal and economic importance.

O. kilimandscharicum Baker ex Gu¨rke is known for camphor production, *O. gratissimum* L of essential oil, *O. tenuiflorum* L. and *O. campechianum* Mill. for ornamental and medicinal importance[1]. Table 1 summarizes different species of *Ocimum* along with their medicinal value, secondary metabolites and geographical regions.

Essential Oil Contents of Ocimum; its Economic Importance

Several chemotypes of *Ocimum* has been known, based on the composition of their essential oil[2]. It is the genetic make-up of the plant which decides its chemical composition and in turn determines its aroma[7,8]. The oil consist of eugenol, eugenal, carvaled, methyl chavicol, limatrol and caryophylline and a number of sesquiterpenes. and monoterpenes *viz.*, barnyl acetate, β-elemense, methylengenol, neral, β-pinene, comphene, α-pinene etc. They have been used as antifungal[9,10], antioxidant[11,12], antinociceptive[13], anticonvulsant[14], germicidal[15,16] and antimalarial[17].

Problem of Adulteration and Substitution of the Herbal Drugs

The herbal drug industry is facing a major problem of adulteration and substitution of herbal drugs. Thus, it's a matter of concern while dealing with research on commercial natural products[18]. The factors responsible for adulteration are lack of knowledge of authentic sources, similarity in colour and morphology, careless collection by herbal collectors and suppliers, non availability of native drug and sometimes high cultivation cost of these drugs in wild[19].

In the case of *Ocimum sanctum*, its often found adulterated with its morphologically close relative *Vitex negundo*[19]. The latter plant has same morphological characters like leaf and flower colour, size and shape (Figure 1). To resolve this problem of misidentification and adulteration various techniques have been utilized since time immemorial, which are described in this review along with their advantages and disadvantages.

Classification and Taxonomy of Ocimum

Ocimum species have high levels of phenomic, genetic and metabolic diversity. The cause of this high level of morphological and chemical variability is attributed to inter-specific and intra-specific hybridization, polyploidy

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and evolutionary selection. Humans have influenced the selection, cultivation, and hybridization of the genus, making it difficult for the taxonomists to work upon.

Classification of Ocimum Based on Cytology

Ocimum possess two basic chromosome numbers X = 8 and X =12 (20, 21). Species belonging to "sanctum" group have chromosome number X = 8. The chromosomes of *Ocimum* can be divided into three groups:

Group A: Long; 300 microns

Group B: Medium; between 2.0 to 2.5 microns

Group C: Short; 1.5 microns and below

It is assumed that the evolution of closely related species of *Ocimum* has been through structural differences and re-patterning of chromosomes. There appears to be a general reduction in total length in chromatin during evolution[22,23].

Classification of *Ocimum* Based on Morphological Characters

The taxonomy of Basil genome is considered to be vast and complex. More than 150 species were recognized in the genus[24]. Actually, most of the taxa described by them was based on the morphology and colour of leaves, which frequently depends on environmental conditions leading to ambiguity in the classification within the genus. There's enormous variation in the shape and colour of the leaves within *Ocimum basilicum* and its close relatives. The shape varies from small and liniform to large and round and colour varies from yellow-green to grey-green, to red or to almost black. In view of this, it has been proposed that only 65 species of the genus *Ocimum* should be considered as true species, and the other should be discarded as their synonyms or false attribution[1].

Ibrahim et al.[4] carried out study on seeds of three varieties i.e, French, Purple and Lemon basils, during two growing seasons of 2010 and 2011 at South Tahrir Agricultural Company, Beheira Governorate, Egypt. The results of the study revealed that phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (HB) and expected genetic advance (GA %) had the highest values in case of herb dry weight, stem dry weight, leaf dry weight and linear growth, respectively. The lowest values of these items were essential oil percent and number of primary branches.

In another study done by Carovic Stanko et al.[25] on the morphological characters of *Ocimum* during the year 2006 in the field trial, exhibit twenty-three morphological traits to be polymorphic out of the twenty-seven traits studied among all the accessions. The monomorphic traits observed were: colour of style, number of flowering shoots, serration of leaf blade margin and hairiness of bracts. Average phenotypic dissimilarity was 0.551 between all pairs of accessions.

Classification of Ocimum Based on Biochemical Characters

Further advancement in the classification of *Ocimum* was based on the volatile oil composition[26]. It uses the most prevalent aromatic compounds for classifying the different basil chemotypes. The components which are more than 20%, are taken into consideration. Essential oils varies with the cultivar type, the prevalent components are monotherpenes and phenylpropanoids. Many *Ocimum* species contain primarily monoterpene derivatives such as limonene, camphor, 1,8-cineole, linalool and geraniol. Others, including *O. basilicum*, contain primarily phenoyl derivatives, such as eugenol, methyleugenol, chavicol, estragole, methyl-cinnamate, often combined with various amounts of linalool.

Labra et al.[27] studied nine *O. basilicum* accessions most commonly utilized in the Mediterranean area for culinary and ornamental use for chemical analysis. As expected, the results showed environmental influence on the morphological and chemical traits. Previous studies[28,29] on these cultivars showed the influence of growth stage in the composition of aromatic compounds. Johnson et al.[30] have shown how the light regime can also influence the essential oil compositions within the same genotype. Miele et al.[28] have reported that some chemical constituents are purely dependent on external characters of plants; e.g. eugenol and methyleugenol content is strictly related with plant height: methyleugenol is predominant in plants up to 10 cm height, while eugenol is prevalent in taller plants.

Labra et al.[27] analysed nine *O. basilicum* accessions through GC/MS analysis and found substantial variation in their chemical composition. Among them, the most prominent constituent was linalool, ranged between 19 and 38%. Other components reported to be present in all the cultivars were eugenol, cineole, terpineol and farnesene Terpineol and farnesene were detected, although at a lower concentration.

On the other hand, when De Masi et al.[7] analyzed 12 different accessions corresponding to nine different cultivars of *O. basilicum*, they found the prevalent chemical components as phenylpropanoids (estragole, eugenol, methyl-eugenol and methyl-cinnamate) and monoterpenes (linalool, geranial, neral and eucalyptol) in all 40 different components of essential oils. Based on the study De Masi et al.[7] grouped these accessions into five cultivar types**.**

In this way, European or sweet basil oil, from Bulgaria, Egypt, France, Hungary, Italy, South Africa and occasionally in the USA, matches with types I and II, because it contains almost identical amounts of estragole (20–43%) and linalool (37–55%). Exotic or R´eunion basil oil, comprising high estragole content (75–85%, estragole type), does not correspond to the accessions in study. Other two distinct types of oils are linalool type (linalool eugenol) and methyl-cinnamate type (methyl-cinnamate linalool), which are related to types III and IV, respectively. The recent studies by Javanmardi et al.[29] and Rady and Nazif[31] revealed that rosmarinic acid is the predominant phenolic acid present in both flower and leaf tissues of *Ocimum*.

Hence, the conclusion drawn from these studies is that the chemotype classification based on just one major volatile oil is erroneous, because, one plant may contain two or more chemical compounds in nearly equal amounts. The overall oil profile of major constituents above a fixed threshold level (e.g. 20% of total essential oil content) should be taken into account.

Classification of Ocimum based on Molecular Markers

Table 1. Diversity in the Secondary Metabolite content and Medicinal Importance of *Ocimum* spp.

S.No.	Species and cultivars	Medicinal Importance	Secondary Metabolite	Geographical location	Reference
	Ocimum sanctum	antispasmodic, antiasthamatic, expectorant, hepato- protective, antipyretic	Eugenol, ursolic acid, carvacol and rosmarinic acid	All regions of India	54
	Ocimum basilicum	stimulant, antispasmodic, diuretic, demulcent	Phenolic acid and rosmarinic acid	Cultivated throughout India mainly in Punjab	55
	Ocimum basilicum Var. *Thai Basilikum*	stimulant, antispasmodic, diuretic, demulcent	estragol	Cultivated throughout India mainly in Punjab	8
	Ocimum gratissimum	Neuralgia, cephalalgia and antifertility	Eugenol Flavon-Cirsimaritin	Northern India, Brazil, China, Eastern Nepal and Southern India	56, 57
	Ocimum africanum		Estragol, geranial, neral, thymol	Tropical parts of Africa	8
	Ocimum tenuiflorum	expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic	1,8 cineole, beta bisabolene	Northern India	8
	Ocimum americanum	Febrifuge, hypoglycaemic, leucorrhea and antifungal	Geraniol, neral, Rosmarinic acid	Lower hills of India	31
	Ocimum killimandscharicum	Insecticidal, mosquito repellent, spasmolytic and antibacterial	Camphor, thymol/p–cimene	Himalayan region	8
	Ocimum macrophyllum	Antibacterial, antifertility	Thymol Flavon-Xantomicrol	Northern India, Brazil, China, Eastern Nepal and Southern India	57

Molecular, or DNA-based markers have been utilized for medicinal plant studies since time immemorial and have advantages over other conventional methods like HPLC, GC-MS, Gas Chromatography, TLC etc[18,32]. They are the most recent to be developed. The primary advantages of using molecular markers are: (1) availability of unlimited number of markers; and (2) generally unaffected by developmental differences or environmental influences.

The two general classes of molecular markers used for such studies are DNA-DNA hybridization and polymerase chain reaction (PCR) based. Those based on the Restriction fragment length polymorphism (RFLP) markers depend on DNA-DNA hybridization and have been used for organelle genetic analysis and genetic linkage mapping in forest trees.

These DNA based markers have been utilised extensively for studying genetic relationship in different species of *Ocimum,* as summarised in Table 2. They are also proved useful in differentiating various cultivars or varieties of a particular spp. e.g. *O. basilicum* (Table 3). Some of these examples are explained in the forthcoming sections.

Molecular Biological Analyses of Genus Ocimum

The Molecular Biological studies on the genus *Ocimumare* summarized here.

RAPD Analyses of Ocimum species

Randomly amplified polymorphic DNA (RAPD) markers are developed by PCR of anonymous DNA sequences from random primers (generally decamers)[33,34,35]. These are dominant markers, i.e. The heterozygous (1 copy) and homozygous (2 copies) bands are indistinguishable. Inspite of limitations, the RAPD analysis has emerged as a powerful technique for detecting DNA polymorphisms among cultivars or clones belonging to plant kingdom[28,32,36]. Some of the drawbacks of RAPD technology can be overcomed by careful planning of experiments. Different workers have given different views on the co-migrating RAPD bands. Some said they might not be allelic i.e. composed of similar sequences[37], while some others demonstrated the homology of these comigrating RAPD bands[36,38].

The use of large number of polymorphic markers minimize the skewing of results due to non-allelism[39]. Another problem often encountered and questioned regarding RAPD analysis is the reproducibility of banding pattern. This problem can be resolved by thoroughly optimising the PCR reaction conditions and following the same protocol each time. For more accurate analysis, reaction should be performed twice or thrice, scoring only those bands that are reproducible in each reaction[32,35].

Singh et al.[40] have utilized RAPD markers and found it to be a robust and reliable method to detect intra- and inter-specific genetic diversity. They have studied phylogenetic relationship in the 32 accessions belonging to five *Ocimum* species and detected high degree of polymorphism (98.28%). The dendrogram constructed grouped three species, namely, *O. basilicum, O. kilimandscharicum* and *O. americanum* in Section A and the other two species *O. tenuiflorum* and *O. gratissimum* in Section B. Accessions from one species were clustered together within the Intra-cluster as expected. The study proved RAPD technique to be sensitive, precise and efficient for phylogenetic studies in the genus *Ocimum.*

Table 2 : Molecular Markers Used for Analyses of Polymorphism in *Ocimum* spps:

S. No.	Name of species of *Ocimum* and its cultivars	Place of collection	Type of Marker used	%polymorphism or % similarity	Purpose of the Study	References
1.	*O. sanctum*	New Vallabh Vidyanagar, Anand, Gujarat	RAPD , ISSR and SSR	100% polymorphic bands	Genetic diversity	47
		Bangalore, India	RAPD	13.84 to 3.07% polymorphism	Genetic diversity	23
		Chitrakoot and Bhopal., Madhya Pradesh, India	RAPD	92.31% polymorphism	Interspecies association	58
2.	*O. basilicum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Genetic diversity	8
		Anand, Gujarat, India	RAPD , ISSR, SSR	100% polymorphism	Genetic diversity	47
		Taiwan	ISSR, RAPD, SRAP	97% polymorphism	Genetic diversity	51
		CIMAP, Lucknow, India	RAPD	98.20% Polymorphism	Interspecific diversity	41
			RAPD	13.84 to 3.07% polymorphism	Genetic diversity	23
			RAPD	92.31% polymorphism	Interspecies association	58
3.	*O. viride*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8
		Anand, Gujarat India	RAPD , ISSR, SSR	100% polymorphism	Genetic diversity	47
4.	*O. polystachyon*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8
		Anand Agricultural University (AAU), Anand, Gujarat	RAPD , ISSR, SSR	100% polymorphism	Genetic diversity	47
5.	*O. americanum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8
		Anand, Gujarat, India	RAPD , ISSR, SSR	100% polymorphism	Genetic diversity	47
		Taiwan	RAPD, SRAP, ISSR	97% polymorphism	Genetic diversity	52
		CIMAP, Lucknow, India	RAPD	98.20 % polymorphism	Interspecific diversity	40
		Southern India	RAPD	89% similarity	Interspecies relationship	42
6.	*O. gratissimum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8

		Anand, Gujarat, India	RAPD , ISSR, SSR	100 % polymorphic bands	Genetic diversity	47
		Taiwan	RAPD, ISSR,SRAP	97% polymorphic bands	Genetic diversity	51
		CIMAP, Lucknow, India	RAPD	98.20% polymorphic bands	Interspecific genetic diversity	40
		Southern India	RAPD	89% similarity	Interspecies relationship	42
		Bangalore, India	RAPD	13.84 to 3.07% polymorphism	Genetic diversity	23
		Chitrakoot and Bhopal, Madhya Pradesh, India	RAPD	92.31% polymorphism	Genetic diversity	58
7.	*O. killimandscharicum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8
		CIMAP, Lucknow, India	RAPD	98.20% polymorphism	Genetic diversity	40
		Southern India	RAPD	89% similarity	Interspecies relationship	43
		Bangalore, India	RAPD	13.84 to 3.07% polymorphism	Genetic diversity	23
		Chitrakoot and Bhopal, Madhya Pradesh, India	RAPD	92.31% polymorphism	Genetic diversity	58
8.	*O. campechianum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8
		Southern India	RAPD	89% similarity	Genetic diversity	43
9.	O. *africanum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Interspecific diversity	8
10.	*O. tenuiflorum*	University of Zagreb, Croatia	AFLP	44.7% polymorphism	Molecular and chemical characterization, genetic diversity	8
		Taiwan	SRAP, RAPD, ISSR	97% polymorphism	Genetic diversity	51
		CIMAP, Lucknow, India	RAPD	98.20% polymorphism	Genetic diversity	40
		Southern India	RAPD	89% similarity	Interspecies relationship	43
11.	*O. micranthum*	Southern India	RAPD	89% similarity	Genetic diversity	43
		Chitrakoot and Bhopal, Madhya Pradesh, India	RAPD	92.31% polymorphism	Interspecies association	58
12.	*O. canum*	Chitrakoot and Bhopal, Madhya Pradesh, India	RAPD	92.31% polymorphism	Inter species association	58

Table 3: Analyses of Intraspecific Genetic Diversity in *Ocimum basilicum* cultivars/varieties Through Molecular Markers

S. No.	Variety	Cultivar and its origin	Types of Marker	% polymorphism/% similarity	Reference
1.	*O. basilicum* var. *basilicum*	Genovese (Austria, Macedonia, Croatia, Italy and Slovakia)	AFLP	66.7% polymorphism	8
			RAPD	57.5% polymorphism	7
			AFLP	50.9% polymorphism similarity index: 0 to 0.18	27
			AFLP, RAPD	93% polymorphism	59

			RAPD, ISSR, SRAP	RAPD: 95% polymorphism, ISSR: 97%, SRAP: 93% polymorhism	51
		Sweet basil(Croatia, USA)	AFLP	66.7% polymorhism	8
			AFLP, RAPD	93% polymorphism	59
			RAPD	44.83% polymorphism	4
			RAPD, ISSR, SRAP	RAPD: 95% polymorphism, ISSR: 97%, SRAP: 93% polymorhism	52
		Green Gate	AFLP	85.18% polymorphism	25
		Canada	RAPD, ISSR, SRAP	RAPD: 95% polymorphism, ISSR markers showed 97% and SRAP markers showed 93% polymorhism	51
		Grosses gruenes	AFLP	85.18% polymorphism	25
		Oriental basil	AFLP	85.18% polymorphism	25
		Green globe	AFLP	66.7% polymorphism	8
		Canada	RAPD, ISSR, SRAP	RAPD markers showed 95% polymorphism, ISSR: 97%, SRAP: 93% polymorhism	51
		Napoletano Basil	AFLP	85.18% polymorphism	25
2.	*O. basilicum* var. *difforme*	Difforme	AFLP	85.18% polymorphism	25
		Benth (Italy)	RAPD	93% polymorphism	59
3	*O. basilicum* var. *thyrsiflorum*	Thai- Basilikum	AFLP	66.78% polymorphism	8
		Canada	RAPD, AFLP,SRAP	RAPD: 95% polymorphism, ISSR: 97% and SRAP: 93% polymorhism	51
		Benth	RAPD, AFLP	93% polymorphism	59
4.	*O. basilicum* var. *purpurascens*	Mexican Basil	AFLP	85.18% polymorphism	25
		Anise Basil	AFLP	85.18% polymorphism	25
			RAPD, ISSR, SRAP	RAPD: 95% polymorphism, ISSR: 97% and SRAP: 93% polymorphism	52
		Cinnamon Basil	AFLP	85.18% polymorphism	25
			RAPD	57.5% polymorphism	7
		Dark Opal (German, Russia	AFLP	66.7% polymorphism	8
		Slovakia	RAPD, AFLP	93% polymorphism	59
		Canada	ISSR,RAPD, SRAP	RAPD: 95% polymorphism, ISSR: 97% and SRAP: 93% polymorhism	51
			RAPD	57.5% polymorphism	7
		Rubin Basil	AFLP	85.18% polymorphism	25
5.	*O. basilicum* var. *minimum*	Bush Basil (Italy)	AFLP	85.18% polymorphism	25
		Canada	RAPD, ISSR, SRAP	RAPD: 95% polymorphism, ISSR: 97% and SRAP: 93% polymorhism	41
		Folgia di Lattuga (Italy)	RAPD	57.5% polymorphism	7
			RAPD	50.9% polymorphism; similarity index: 0 to 0.18	27
		Lemon Basil(Canada)	RAPD	57.5% polymorphism	7

RAPD, ISSR, SRAP		RAPD: polymorphism, ISSR: and SRAP: polymorphism	95% 97% 93%	51

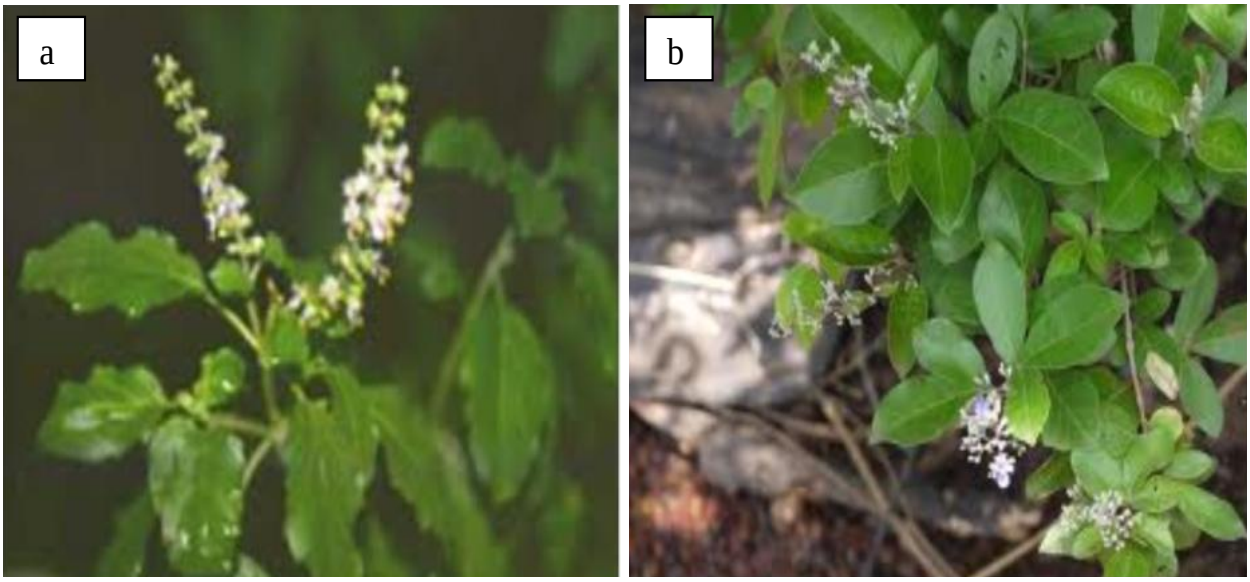

Figure 1: Similarity in morphological features of two different plants (a) *Ocimum sanctum* and (b) *Vitex negundo*

In the previous reports of diversity analyses eleven species of *Ocimum* have been divided into two groups- 'Basilicum' and 'Sanctum' on the basis of their morphology, cytology and oil charactersistics[22,41]. *O. basilicum, O. americanum* and *O. kilimandscharicum* were grouped as 'Basilicum', while *O. tenuiflorum* and *O. gratissimum* were grouped as 'Sanctum'. Paton[1] also placed *O. basilicum* with *O. americanum* and *O. kilimandscharicum* in one sub-section (*Ocimum* subsect. *Ocimum*). This shows, that the use of molecular markers helps in more objective classification. Chikkaswamy et al.[23] revealed the genetic relationships between six *Ocimum* species (two *O. basilicum*, two *O. sanctum*, one *O. kilimandscharicum*, one *O. gratissimum*) (Table 1). Of the 80 random primers screened 10 primers resulted in 73 RAPD bands of good amplification. The data structure included a total of 100 to 1000 base pairs marker levels. A dendrogram was constructed using Euclidean distances by Ward's method. Based on number of bands all the species were grouped into three clusters and the dendrogram maximum similarity between the *Ocimum* species. The highest distance was observed between *O. killimandscharicum* and *O. sanctum* and lowest distance was recorded between *O. basilicum* 1 and *O. basilicum 2* and intermediate distance was observed between *O. sanctum* 1 and *O. sanctum* 2. From the pattern of clustering, it was pertinent that RAPD technique was efficient in segregating species into different clusters.

In another study by Harisaranraj et al.[42] genetic relationship of seven species was estimated using 15 randomly amplified polymorphic DNA primers. It shows 89% close similarity of *O. basilicum* with *O. tenuiflorum* , *O. gratissimum* and *O. micranthum.* The results are surprising, but cannot be trusted upon as the data is based on only two primers. As, other thirteen primers utilised by these workers produced monomorphic bands across all the seven species. De Masi et al.[7] have collected 12 different accessions from nine different cultivars. RAPD analysis grouped them into two main clusters. One cluster comprised of cultivars which are suitable for food industry. Interestingly, the results of agro-morphological analysis matches with those of RAPD, but, the essential oil profiles differ from both of these results. Maybe due to difference in the key enzymes of the essential oil biosynthetic pathways. These reports prove that DNA-based markers may represent a routine tool to verify the identity and the quality of basil cultivars and basil-derived products.

AFLP Analyses of Ocimum species

This technique involves digestion of total cellular DNA with one or more restriction enzymes and ligation of adaptors to the sticky ends. This is followed by amplification of these fragments using two PCR primers that have sequences complimentary to the adaptors and restriction site[43]. The PCR products are finally resolved on PAGE matrix and visualized through autoradiography or chemiluminescence. There are various advantages of AFLP over other genetic markers; like high reproducibility, resolution, sensitivity at whole genome level, and no need of prior sequence information for amplification. AFLP also has the capability to amplify between 50 and 100 fragments at a time[32,44].

Carovic- Stanko et al.[8] have studied DNA fingerprinting and chemical analysis of essential oils in 22 *Ocimum* accessions belonging to five different species, viz., *O. basilicum, O. americanum/O. africanum, O. tenuiflorum,* and *O. gratissimum.* The chemical analysis identified 17 compounds as the main ones, and thus grouping the 22

accessions into 13 chemotypes. Whereas, AFLP data has further resolved the relationship between these accessions. 1,734 polymorphic bands were generated by Four AFLP primer combinations. The Dice's distance matrix and maximum parsimony (MP) analysis grouped these accessions into four clusters. The *O. campechianum* accession was not able to group together with other species, also proved by its unique essential oil composition with 1,8-cineole and b-caryophyllene as the main compounds. *O. gratissimum* and *O. tenuiflorum* are also separated out from other accessions. This is in congruence with the studies of Khosla[41], where both of them were placed in Sanctum group. The study also substantiated by the smaller number of chromosomes possess by *O. gratissimum* (2n = 40) and *O. tenuiflorum* (2n = 36) in comparison of *O. basilicum* (2n = 48) and *O. americanum* (2n = 72) (25).

Another study on nine accessions of *Ocimum basilicum* revealed 163 bands, 83 of which were polymorphic[27]. The accessions B and D show high genetic similarity with C and G. While, accessions A, H, I, E, and F are clearly separated from each other and from the other accessions, the accessions B, C, D, and G exhibit no genetic variability among individuals within the cultivar, suggesting them to be clones. The dendrograms exhibit low genetic variability thus showing closely related species. It may be because of autogamous reproduction and propagation in *O. basilicum.* The results suggest that it is not always necessary that the genomic similarity coincide with other morphological and physiological traits, such as oil composition, or agronomic traits etc. For example, although, cultivars B and D are quite different in their oil composition but they are genetically very similar.

ISSR and SSR Marker Analyses of Ocimum species

PCR-based techniques are much preferred among all the marker techniques because of their simplicity and requirement of small quantities of DNA template[18,32]. Non-anchored inter simple sequence repeats (ISSRs) are one of the PCR based markers dependendent on microsatellite loci. Like RAPD markers, they also do not require any prior genomic information[45]. The ISSR technique has one major advantage of its consistency across a wide range of PCR parameters[46].

In a study by Lal et al.[47] RAPD, ISSR and SSR markers for studying genetic relationship among six different species of *Ocimum.* Out of the total 209 bands amplified by three ISSR, all were polymorphic. Analysis of all three markers also showed 100% polymorphism among the *Ocimum* spp. RAPD and SSR analyses, revealed that *O. basillicum* and *O. polystachyon* are most similar (highest similarity index of 0.28000 and 0.61538, respectively). Through ISSR analysis, the highest similarity index of 0.32258 was observed between *O. americanum* and *O. basillicum*. The combined analysis of RAPD, ISSR and SSR revealed that *O. basillicum* and *O. americanum* were the most similar (highest similarity index is 0.2892) and *O. viride* and *O. americanum* are highly dissimlar (least similarity index is 0.01123). Information from all the three marker systems can be utilised to formulate a strategy for plant breeding and crop improvement programs by maintaining or enhancing the genetic diversity.

SRAP Marker Analyses of Ocimum species

Sequence-related amplified polymorphism (SRAP) is a simple and reliable method with moderate throughput ratios[48, 49]. It amplifies the coding sequences in the genome and results in a moderate number of co-dominant markers. Careful analysis of sequence data revealed that codominant markers are generated from fragment size changes due to insertions and deletions, wheras, dominant markers are generated from nucleotide changes. The SRAP marker system has been utilised for a variety of purposes like genetic diversity studies, map construction, gene tagging etc[50].

SRAP analysis of 37 basil accessions representing four *Ocimum* species (*Ocimum basilicum, O. americanum, O.gratissimum and O.tenuiflorum*, revealed highest mean value of polymorphic information content (PIC, 0.29) and resolving power (Rp, 30.19) which was much higher than those of RAPD (0.23, 5.13) and ISSR (0.19, 1.39)[51]. When ISSR and RAPD markers were used, all the accessions were clustered into four groups, but SRAP analysis grouped them into three clusters, indicating that the genetic diversity in different target regions of the tested basil genome was not the same. The SRAP dendrogram was correlated most with the combined data set indicating that SRAP markers could be better tool for genetic diversity analysis in basil than RAPD and ISSR markers[51].

DNA Barcoding of Ocimum species

DNA barcodes is the latest technique in DNA fingerprinting. It utilises the genome of mitochondria, chloroplast and nuclear regions and proved to be of great success in the validation of several plant families[52,53]. In the genus *Ocimum,* the three candidate barcodes of the chloroplast genome viz. matK, rbcL and psbA-trnH were analysed to access their ability to produce high sequence variability. The sequencing data revealed psbA-trnH region as the most suitable candidate barcode which presented an interspecific variation of 7.3%. Clear differentiation observed at the species level represent the maximum distance to be 0.264 among the dissimilar species. The phylogenetic analysis have identified the hybrids too. The DNA barcoding is proved to be ideal for interspecific differentiation of *Ocimum.*

CONCLUSIONS

The DNA based marker technologies are useful in assigning new unclassified accessions to pre-existing taxonomic groups and also rectifying any mistakes in the classification done by more primitive methods, like phenotyping and chemotyping. Hence, it is a more objective approach for phylogenetic studies. The newest technologies in the field of DNA genotyping, viz., DNA barcoding, Microarrays and Next Generation Sequencing (NGS) will add more substance to the existing approaches of phylogenetic studies. These approaches would be helpful not only in management of germplasm collections by avoiding redundancy but also in authentication of the genus *Ocimum.*

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