Bhartiya Krishi Anusandhan Patrika, 36(3): 231-236 DOI: 10.18805/BKAP348

Evaluation of Genetic Diversity in *Ocimum* spp. using Agro-morphological Traits and RAPD Marker

Sachin Kumar[1], Ajay Kumar[2], Manoj Kumar Sharma[2], Manoj Kumar Sharma[1]

Department of Bioinformatics, Janta Vedic College, Baraut, Baghpat-250 611, Uttar Pradesh, India.

Received: August 2021 Accepted: September 2021

ABSTRACT

In the present study, morphological and genetic diversity is revealed among the four species of *Ocimum*. Among the morphological characters plant height, the number of leaves, leaf area, leaf width, leaf length, stem circumference and mitotic index studied and contributed a greater proportion of variations. RAPD markers revealed a high degree of polymorphism (88.89%) among the species of *Ocimum*. Dendrogram study revealed that the species *O. gratissimum* and *O. basilicum* are very closely related whereas *O. kilimandscharicum* and *O. sanctum* are distantly related in the phylogenetic history.

Key words: Genetic diversity, Morphological diversity, *Ocimum*, RAPD.

INTRODUCTION

India is one the main centre of diversity among the *Ocimum* species (Vieira and Simon, 2000). Genetic diversity has been created at inter and intraspecific levels in a crop germplasm by evolutionary forces (Stebbins 1957) and is an important parameter utilized for crop improvement either by selection or applying various breeding methodologies.

Different types of marker systems have been used for genetic analysis and characterization studies. These include (a) morphological markers: those based on visually assessable traits, (b) biochemical markers: those based on gene product and (c) molecular markers: those relying on a DNA assay. Usefulness and reliability of any genetic marker is dependent on its heritability and the level of polymorphism, it exhibits.

Williams *et al*., (1990) showed that the differences as polymorphisms in the pattern of bands amplified from genetically distinct individuals behaved as Mendelian genetic markers. Welsh and McClelland (1990) showed that the pattern of amplified bands so obtained could be used for genomic fingerprinting.

RAPD amplification is performed in conditions resembling those of PCR, using genomic DNA from the species of interest and a single short oligonucleotide (usually a 10-base pairs). The DNA amplification product is generated from a region that is flanked by a part of 10pb priming sites in the appropriate orientation. Genomic DNA from different individuals often produces different amplification patterns (RAPDs). A particular fragment generated for one individual but not for others represents DNA polymorphism and can be used as a genetic marker. They are visualized as bands; these bands constitute the RAPD loci.

A band is considered to be polymorphic if it is present in some individuals and absent in others and monomorphic if present in all the individuals or accessions. The most intense monomorphic band from each accession with each primer was used as reference to calibrate different lanes for the amount of DNA present when there are no monomorphic band the band with maximum frequency in each accession was used for calibration. In each lane, bands were scored present if their intensity was at least 10% of the monomorphic reference band within the same lane.

RAPD assay also has the advantage of requiring very small amount of genomic DNA without the need for blotting and radioactive detection (Cipriani *et al*., 1996; Atienzar *et al*., 2000) and are moderately reproducible.

The genus *Ocimum*, is an important genus of aromatic herbs, has been reported to grow widely throughout temperate regions of the world (Tchatchouang *et al*., 2017). *Ocimum* is reported to be the largest genera in Lamiaceae family, having more than 160 species worldwide, of which about 65 species are native to *Ocimum* and the rest should be considered as synonyms (Pushpangadan, 1995).

Though maximum number of species is found in the tropical rain forests of Africa, some species of Basil are native to India (Mishra *et al*, 2014). Nine species of *Ocimum* species are found in India so far, of which three species namely *O. americanum* L., *O. minimum L.* and *O. africanum* Lour are exotic (Balyan and Pushpangadan, 1988). The investigations on genetic diversity, inter-relationship and phylogeny of *Ocimum* species have also been reported (Sobti and Pushpangadan, 1977; Khosla, 1995; Viera *et al*., 2001) have analyzed the genetic diversity of *O. gratissimum* L. at DNA level using RAPD markers. The more polymorphic and

*Corresponding author's E-mail: sachinsuryan@gmail.com
[1]Department of Bioinformatics, Janta Vedic College, Baraut, Baghpat-250 611, Uttar Pradesh, India.
[2]Department of Botany, Janta Vedic College, Baraut, Baghpat-250 611, Uttar Pradesh, India.

heritable the trait the greater is its potential value for germplasm characterization (Portar and Smith, 1982).

Information on genetic diversity is valued for the management of germplasm and for evolving conservation strategies. Hence in the present study genetic diversity of four Indian species of *Ocimum i.e.*, *O. kilimandscharium*, *O. gratissimum*, *O. basilicum* and *O. sanctum* based on morphological and molecular markers.

MATERIALS AND METHODS

The seeds of three *Ocimum* species from CMAP Lucknow and one species from regional area of Jhansi were obtained. The growing plants were maintained in the Bundelkhand University Campus, Jhansi, India. The seeds were washed three times with distilled water and placed in sterile petri dish containing wet filter paper. The germinated seeds were transplanted in pots carefully filled with top soil. Thereby used for recording of different data.

Recording of the morphological data

Different qualitative and quantitative characters were measured *e.g.,* leaf characters, seed characters, growth habit among qualitative characters and plant height, number of leaves, leaf area, leaf width, stem circumference and mitotic index. The data was recorded at every 10 days interval of seeds germination.

Cytological observation

Mitotic index was calculated from the root tip cells. The root tips were harvested and fix in 3:1 ratio of alcohol and glacial acetic acid. After six hours fixation, the root tips were then preserved in 70% alcohol and were used for studying mitosis.

Mitotic index was calculated by using the formula-

$$\text{Mitotic index (MI)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells studied}} \times 100$$

Statistical analysis of morphological data

Results obtained were analysed statistically using various statistical parameters, *e.g.,* coefficient of range *i.e.,* (highest value of a parameter - it's lowest value) / (highest value of that parameter + that's lowest value), ANOVA, standard deviation, standard error *etc.*

Molecular analysis

Young leaves were picked for DNA extraction and analysis. Additional leaves were collected and placed in deep freezer for future use. Storage of plant tissues for DNA is important to avoid degradation of DNA. Preliminary studies were conducted on *Ocimum gratissimum* L. in order to establish the storage conditions for the collected samples before DNA extraction. Secondly, the aim was to determine the best protocol for the extraction of high-quality DNA, which would later be used for molecular analysis DNA can be extracted from the samples one month or longer time after field sampling (Matasyoh *et al*., 2008).

DNA isolation

The chemical and solution used in DNA isolation includes: CTAB buffer [1.4M NaCl, 100 mM Tris -HCl pH 8.0, 20 mM EDTA pH 8.0, 2% (w/v) CTAB and 1% β-mercaptoethanol (v/v)] Chloroform: isoamyl alcohol (24:1v/v), isopropanol, ethanol 70%, TE buffer (10 mM Tris-HCl, pH 8.0 and 0.1 mM EDTA), PVP (w/v) 0.1 g/g of the leaf tissue.

DNA was extracted from fresh leaves by using the cetyltrimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1990) with minor modifications.

Approximately 1g of fresh leaves was ground to powder in liquid nitrogen using a mortar and pestle along with 0.1 g of PVP. The pulverized leaves were quickly transferred to a 50 ml falcon tube with 20 mm of freshly prepared pre-warmed (65°C) CTAB buffer and shaken vigorously by inversion to form slurry. The tubes were incubated at 60°C in waterbath for 2 hours with intermittent shaking and swirling for every 30 min.

An equal volume of chloroform: isoamylalcohol (24:1) was added and mixed properly by inversion for 30 min. and centrifuged at 12000 rpm for 15 min. (Remi R-24) at room temperature to separate the phases. The supernatant was carefully decanted and transferred to a new tube and was precipitated with 0.6 volumes of cold isopropanol and gently mixed to produce fibrous DNA and incubated al 20°C for minimum 30 min.

The samples were centrifuged at 12000 rpm for 15 min at 4°C. The pellet was washed twice with 70% ethanol, air dried and resuspended in 3 ml of × TE buffer and 5 µl of RNase was added and incubated overnight at 37°C. Equal volume of the mixture of chloroform: isoamyl alcohol (24:1) was added, it was there after centrifuged at 8000 rpm for 10 min. at 4°C. The aqueous phase was taken and 0.6 volumes of isopropanol were added. It was then kept at -20°C for 10 min. The mixture was centrifuged at 8000 rpm for 5 min. at 4°C and supernatant decanted carefully.

The pellet was washed with 80% ethanol twice and dried at 37°C for 10 min. Finally, DNA pellet was dissolved in 50 µl of 1 × TE buffer.

Quality and quantity of DNA

DNA sample was treated with DNase free Ribonuclease A to remove RNA from isolated genomic DNA as the presence of large amounts of RNA in the DNA sample can reduce the yield of the PCR by chelating Mg^{2+} (Padmalatha and Prasad, 2006). The purity of DNA was determined by 260 /280 nm absorbance ratio (Sambrook *et al*., 2001).

DNA amplification

PCR was carried out with a single primer sequence "CCAGTGCTCT". The DNA concentration was adjusted to 12.5 ng/µl and the concentration of random primer sequence to 10 picomoles/µl. The method used for PCR amplification was similar to that reported by Williams *et al*., (1990). The reaction mixture (final volume of 50 µl) contained 5µl of 10× PCR buffer, 2.5 µl of 2 mM dNTPs mixture, 4 µl of 25 mM

$MgCl_2$, 2 µl decamer primer; 0.2 µl of Taq Polymerase (5 units/µl) and 2 µl genomic DNA. The final volume was made up with double distilled water.

Amplification was performed in a thermal cycler (Thermal Cycler Biocycler TC-S) programmed as follows: an initial cycle of 5 min. at 94°C, 1 min. at 40°C, 1 min. at 72°C; followed by 40 cycles each consisting of 1 min. at 94°C, 1 min. at 40°C, 1 min. at 72°C and final extension of 5 min. at 72°C, reaction was ended with an indefinite hold at 4°C.

Gel electrophoresis

Amplified PCR products were separated, on 1.4% (w/v) agarose gel in 1×Tris-Acetate EDTA (TAE) buffer containing ethidium bromide (0.5µg/ml). Amplification product were mixed with 6x gel loading dye, 0.25% bromophenol blue, 0. 25% Xylene Cyanol and 30% glycerol. The DNA was viewed under UV light and photographed using gel. 1 kb DNA ladder mix was used as a molecular size marker for calculations of fragment size.

Data scoring and dendrogram

Fragments that were clearly resolved on the gels were scored as 1 or 0 (*i.e.*, present or absent, respectively) across all the four species of *Ocimum*. Bands that could not be confidently scored were regarded as missing data. The results were analyzed based on the principle that a band is considered to be 'polymorphic' if it is present in some individua ls and absent in others and 'monomorphic' if present in all the individuals.

The data was assembled as binary matrix for RAPD markers and subjected to statistical analysis using NTSYSpc 2.02e (Rohlf 1998). Dice's similarity coefficient was employed to compute pairwise genetic similarities. Dice's similarity coefficient (Dice, 1945) defined as 2a/2a+u, where 'a' is the number of positive matches 'u' the number of non-matches was computed using the NTSYS-ps (Numerical Taxonomy System, Applied Biostatistics, Inc., New York, USA, software version 2.02e).

A dendrogram was constructed using the Unweighted Paired Group Method with Arithmetical Averages (UPGMA). The dendrogram was constructed employing an (UPGMA) using Sequential Agglomerative, Hierarchical and Nested Cluster (SAHN) (Sneath and Sokal 1973).

RESULTS AND DISCUSSION

Morphological and cytological studies

Mean performances and variability - Data recorded for various morphological traits were subjected to statistical analysis to ascertain the level of genetic variability present in the gene pool (Table 1, Fig 1). The analysis of variance was calculated separately for the characters like plant height, number of leaves, leaf area, leaf width, leaf length, leaf a rea, leaf width, leaf length and stem circumference, characters.

The highest value of mean for plant height (27.86) was found in *O. kilimandscharicum* and remaining all five attributes *i.e.,* number of leaves (86), leaf area (15.13 cm^2), leaf width (3.20 cm), leaf length (5.72 cm) and stem circumference (1.41cm) in *O. basilicum*.

Mean of mitotic index was highest in *O. basilicum* with the value of 14.72.

Table 1: Results of different morphological and cytological quantitative characters.

Character	Statistical parameters	*O. kilimandscharicum*	*O. gratissimum*	*O. basilicum*	*O. sanctum*
Plant height (cm)	Range	0.6 -62.1	0.5-22.8	1.6-51.3	0.4-30.1
	Mean ± SE	27.86±8.48	10.06±2.88	26.76±6.29	11.55±3.43
	Coefficient of range	0.9808	0.9570	0.9395	0.9737
No. of leaves	Range	4-136	2-54	4-174	2-66
	Mean ± SE	45.2±16.3	19.6±6.1	86.0±22.3	26.0±7.1
	Coefficient of range	0.9428	0.9285	0.9550	0.9411
Leaf area (cm^2)	Range	0.03-14.26	0.03-32.40	0.03-23.57	0.14-12.28
	Mean ± SE	6.92±1.84	13.16±4.14	15.13±3.19	6.44±1.55
	Coefficient of range	0.9958	0.9981	0.9732	0.9774
Leaf width (cm)	Range	0.2-3.5	0.2-5.1	0.6-4.4	0.3-3.1
	Mean ± SE	2.17±0.43	2.84±0.65	3.20±0.50	1.97±0.36
	Coefficient of range	0.8918	0.9245	0.7600	0.8235
Leaf length (cm)	Range	0.3-5.9	0.3-9.4	0.8-8.1	0.6-5.6
	Mean ± SE	3.53±0.72	4.77±1.17	5.72±0.97	3.60±0.64
	Coefficient of range	0.9032	0.9381	0.8202	0.8064
Stem circumference (cm)	Range	0.2-1.1	0.1-1.6	0.3-2.0	0.1-1.4
	Mean ± SE	0.90±0.12	0.84±0.20	1.41±0.21	0.80±0.17
	Coefficient of range	0.6923	0.8823	0.7391	0.8666
Mitotic index	Range	10.20-13.63	9.67-16.12	13.04-7.14	10.0-16.7
	Mean ± SE	11.21±0.53	12.53±0.86	14.72±0.65	12.02±0.98
	Coefficient of range	0.1439	0.2500	0.1202	0.2498
Seed germination	%	34	71	48	37

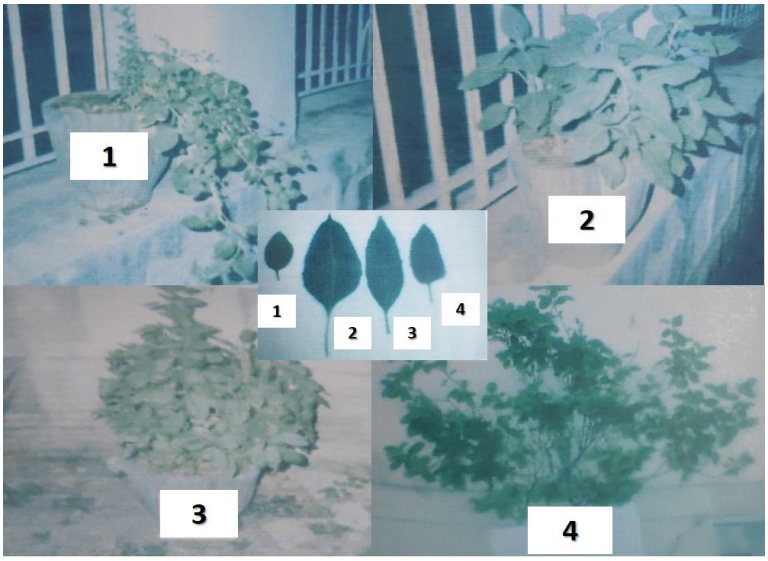

Fig 1: Picture showing growing plants of different *Ocimum* spp. in the pots and comparative shape and size of their leaves in the centre of picture. The labelled numbers represent different species *i.e.,* 1. Represents *O. kilimandscharicum,* 2. Represents *O. gratissimum* 3. Represents *O. basilicum* 4. Represents *O. sanctum.*

A wide range of variation was noticed for plant height (0.6-62.1 cm) in *O. kilimandscharicum* while for number of leaves (4-174) and stem circumference (0.3-2.0 cm) in *O. basilicum* and for remaining three characters, leaf area (0.03-32.40 cm), leaf width (0.2-5.1 cm) and leaf length (0.3-9.4 cm) in *O. gratissimum*.

A wide range for mitotic index was found in *O. gratissimum* from 9.67 to 16.12. The coefficient of range for all the characters studies showed highest coefficient of range for plant height (0.9808) in *O. kilimandscharicum*, number of leaves (0.9550) in *O. bosilicum* and highest coefficient of range with rest of four characters, leaf area (O.9981)' leaf width (0.9245), leaf length (0.9381) and stem circumference (0·8823) *O. gratissimum*.

Highest coefficient of range for mitotic index was found in *O. kilimandscharicum* (0.1439). Analysis of variance in relation to compare groups through ANOVA revealed that mean squares of genotypes were significant for plant height, number of leaves, leaf area, leaf width, leaf length and stem circumference. The statistical significance of mitotic index (mean square) due to total number of observations and mitotic index due to genotypes was tested against error mitotic index obtaining F values. The extent of standard error (SE) varied from 2.888 to 8.486 (Plant height), 6.07 to 22.28 (number of leaves), 1.5507 to 4.1442 (leaf area), 0.361 to 0.65l (leaf width), 0.635 to o.971 (leaf length) and 0.123 to 0.206 in stem circumference. This showed that there was enough genetic variability for various traits.

Mean squares were found to be significant for all the characters when tested against F value. "F statistic for genotypic mean squares in consideration of different traits, separately are as follows: 12.00, 12.51, 9.51, 13.59, 10.89 and 20.80 for plant height, number of leaves, leaf area, leaf width, leaf length and stem circumference respectively. P value for all the characters besides genotype p value in leaf area (0.02) are lesser than 0·0001, that indicate the highly significance of observation studied during compare groups *via* ANOVA.

O. basilicum with the highest mitotic index (14.72) correlate positively with all the highest value of morphological characters except plant height. The mitotic index of *O. kilimandscharicum* with lowest value (11.11) correlate negatively, with its plant height as it highest plant height. Mitotic index of remaining two plants *O. gratissimum* and *O. sanctum* treat moderately. During the comparative study between *O. gratissimum* and *O. sanctum*, we found that greater value of mitotic index of former (12.18) correlate in the same way with leaf area, leaf width, leaf length and stem circumference. *O. sanctum* with 11·50 mitotic index value perform higher degree of plant height and number of leaves rather than *O. gratissimum* with 12.18 mitotic index.

On the basis of qualitative characters, evaluated in four Ocimum species, different categories that have been revealed are mentioned in Table 2.

Molecular marker

The randomly selected primer generated a total of 9 RAPD bands most of which were polymorphic across genotypes. The primer was able to produce fragments that varied from 100 to 1500 bp in size. *Ocimum* species were analyzed using a randomly selected primer. Out of 9 bands generated, 8 were found to be polymorphic (88.89% polymorphism). The size of amplified products ranged from 100 to 1500 bp, while in number these are 9 in all four species (Table 3, Fig 2A). Three bands of *O. sanctum* are unique. RAPD analysis with the mentioned random decamer primer revealed Dice's coefficient of similarities based on RAPD data ranged from 0.20 to 0.80 (Table 4). The lowest genetic similarity was observed between *O. gratissimum* and *O. sanctum* even between *O. basilicum* and *O. sanctum* while the highest similarity belongs to *O. grotissimum* and *O. basilicum*.

Table 2: Different quantitative characters of different species.

Character	Categories	Species
Seed shape	Spherical	*O. gratissimum*
	Oval	*O. kilimandscharicum, O. sanctum*
	Globe Oval	*O. basilicum*
Testa colour	Brown	*O. gratissimum*
	Black	*O. kilimandscharicum, O. basilicum, O. sanctum*
Testa pattern	Mottled	-
	Non-mottled	*O. kilimandscharicum, O. gratissimum, O. basilicum, O. sanctum*
Growth Habit	Erect	*O. gratissimum, O. basilicum, O. sanctum*
	Decumbent	*O. kilimandscharicum*
Leaf size	Broad	*O. gratissimum*
	Medium	*O. basilicum*
	Narrow	*O. kilimandscharicum, O. sanctum*
Leaf shape	Ovate	*O. gratissimum, O. basilicum, O. sanctum*
	Cordate	*O. kilimandscharicum*
Leaf colour	Green	*O. basilicum, O. sanctum*
	Light Green	*O. kilimandscharicum*
	Dark Green	*O. gratissimum*
Stem pigmentation	Pigmented	*O. gratissimum, O. basilicum*
	Lightly pigmented	*O. kilimandscharicum*
	Non-pigmented	*O. sanctum*

Table 3: Total number of amplified bands and number of polymorphic bands generated by PC, using single randomly selected primer.

Primer sequence	Polymorphic bands	Monomorphic bands	% G+C content	Total bands	Fragment size range (bp)
5′CCAGTGCTCT3′	8	1	60	9	100-1500

Table 4: Dice's similarity coefficient matrix for *Ocimum* genotype based on RAPD data.

	O. kilimandscharicum	*O. gratissimum*	*O. basilicum*	*O. sanctum*
O. kilimandscharicum	1.0000			
O. gratissimum	0.8000	1.0000		
O. basilicum	0.8000	1.0000	1.0000	
O. sanctum	0.4000	0.2000	0.2000	1.0000

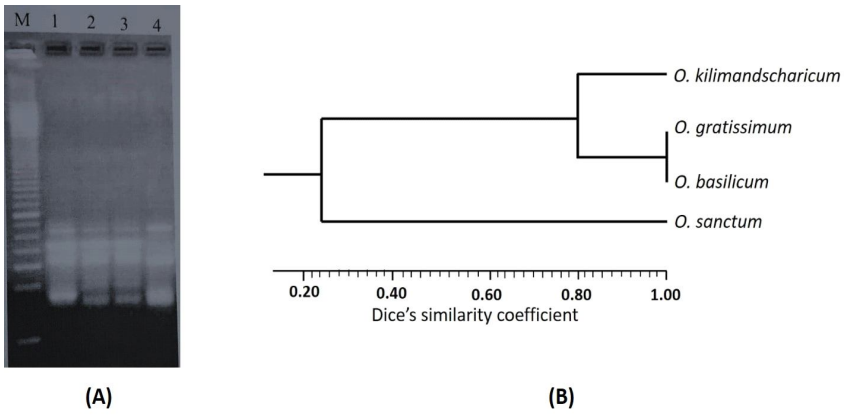

Fig 2: (A) RAPD profile of different *Ocimum* spp., the labelled numbers represent the lane of different species *i.e.,* 1. Represents *O. kilimandscharicum*, 2. Represents *O. gratissimum* 3. Represents *O. basilicum* 4. Represents *O. sanctum* while lane M is of 1 kb DNA ladder. (B) UPGMA-based dendrogram showing genetic relationship among *Ocimum* genotypes based on Dice's similarity, estimates for RAPD data.

Morphological variations present in *Ocimum* species with respect to qualitative and quantitative traits like, leaf colour, leaf size, plant height, *etc.* vary significantly that a high degree of taxonomical confusion prevails within the species with several synonyms at the species level, the molecular level analysis through RAPD helped to find the correlation between four experimental species of *Ocimum*. RAPD analysis found to be a robust and reliable method to detect inter-specific genetic diversity and study of phylogenetic relationship in the genus *Ocimum*.

Mitotic index value generally positively correlates with vegetative characters of all four experimental species. The species *O. basilicum* having highest vigorous vegetative growth (except plant height) also has highest mitotic index.

The investigations on genetic diversity Inter-relationship and *Ocimum* species have also been reported earlier (Sobti and Pushpangadan 1977; Khosla, 1995; Viera *et al*, 2001; Singh *et al*, 2004) have analyzed the genetic diversity using RAPD markers.

In present investigation RAPD markers revealed high degree of polymorphism (88.89%) among the four experimental *Ocimum* species. Dendrogram study revealed that the species *O. gratissimum* and *O. bassilicum* are very closely related whereas *O. kilimandscharicum* and *O. sanctum* are distantly related in the phylogenetic history (Fig 2B). The use of molecular markers helped in establishing the limits among the defined groups on a more objective basis.

CONCLUSION

In conclusion, both morphological and genetic variations exist among four species that were evaluated. Plant height, number of leaves, leaf area, leaf width, leaf length, stem circumference and mitotic index contributed a greater proportion of variations that exist among species. The dendrogram obtained from the molecular analysis was more discriminatory than the morphological and cytological parameters.

ACKNOWLEDGEMENT

Authors hereby acknowledge Late Dr. Rajdeep Kudesia, Department of Botany, Bundelkhand University Jhansi, India.

REFERENCES

Atienzar, F., Evenden, A., Jha, A., Savva, D. and Depledge, M. (2000). Optimized RAPD analysis generates high quality genomic DNA profiles at high annealing temperatures. Biotechniques. 28: 52-54.

Balyan, S.S. and Pushpangadan, P. (1988). A study of the taxonomical status and geographic distribution of the genus *Ocimum.* PAFAI. 10(2): 13-19.

Cipriani, G., Bella, R. and Testolin, R. (1996). Screening RAPD primer for molecular taxonomy and cultivar fingerprinting in genus Actinidia. Euphytica. 90: 169-174.

Mishra, D., Awasthi, A. and Mishra, P. (2014). Phylogenetic evolution studies on different varieties of genus *Ocimum* with special reference to Rewa district of Madhya Pradesh. Sci. Secure Journal Biotechnol. 3: 188-197.

Dice, L.R. (1945). Measure of the amount of ecological association between species. Ecology. 26: 297-302.

Doyle, J.J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. Focus. 12: 13-15.

Khosla, M.K., (1995). Study of inter-relationship, phylogeny and evolutionary tendencies in genus *Ocimum*. Ind. J. Genet. 55: 71-83.

Matasyoh, L.G., Wachira, F.N., Kinyua, M.G., Thairu Muigai, A.W. and Mukiama, T.K. (2008). Leaf storage conditions and genomic DNA isolation efficiency is *Ocimum gratissimum* L. from Kenya. African Journal of Biotechnology. 7: 557-564.

Padmalatha, K., Prasad, M.N.V. (2006). Optimisation of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. Afr. J. Biotechnol. 5(3): 230-234.

Pushpangadan, P., Bradu, B.L., Chadha, K.L., Gupta, R. (Eds.) (1995). Advances in Horticulture, Malhotra Publishing House, New Delhi.

Porter, W.M. and Smith, D.H. (1982). Detection of identification errors in germplasm collections. Crop Science. 22: 701-703.

Rohlf, F.J. (1998). NTSyS-p.c. Numerical Taxonomy and Multivariate Analysis System (Version 2.0). Exeter Software Publishers Ltd., Setauket.

Sambrook, J., Maccallum, P. and Russell, D. (2001). Molecular Cloning: A Lab-oratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press.

Singh, A.P., Dwivedi, S., Bharti, S., Srivastava, A., Singh, V. and Khanuja, S.P.S. (2004). Phylogenetic relationships as in Ocimum revealed by RAPD markers. Euphytica. 136: 11-20.

Sneath, P.H.A. and Sokal, R.R. (1973). Numerical Taxonomy. Freeman Press San Francisco, California, USA.

Sobti, S.N. and Pushpangadan, P. (1977). Studies in Genus *Ocimum*: Cytogenetics, Breeding and Produciton of New Strains of EconomicI. In: Cultivation and Utilization of Medicinal and Aromatic Plants. [C.K. Atal and B.M. Kapoor (ed)] (Regional Research Laboratory Jammu) pp. 273-285.

Stebbins, G.L. (Jr) (1957). Genetics, evolution and plant breeding. Proc Symp on Genet and Pl Breed in Southeast Asia, Jan. 1957, New Dehli. Ind. J. Genet. 17: 129-141.

Tchatchouang, S., Beng, V.P. and Kuete, V. (2017). Antiemetic African Medicinal Spices and Vegetables Medicinal Spices and Vegetables from Africa - Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases, Academic Press Pages 299-313. https://doi.org/10.1016/B978-0-12-809286-6.00011-X.

Vieira, R.F. and Simon, J.E. (2000). Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. Econ. Bot. 54: 207-216.

Vieira, R.F., Grayer, R., Paton, A. and Simon, J.E. (2001). Genetic diversity of *Ocimum gatissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. Biochem. Syst. Ecol. 29(3): 287-304.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18(24): 7213-7218. doi: 10.1093/nar/18.24.7213.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphism's amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18(22): 6531-6535. doi: 10.1093/nar/18.22.6531.