International Journal of Environment and Climate Change

Volume 13, Issue 8, Page 1529-1538, 2023; Article no.IJECC.99859
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Detection and Comparison of RAPD & SSR Primers in Genetic Diversity of *Ocimum sanctum*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i82102

Open Pee Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99859

Original Research Article

Received: 18/03/2023
Accepted: 20/05/2023
Published: 13/06/2023

ABSTRACT

Aims: To Analysis of genetic variability in Tulsi (*Ocimum sanctum*) genotypes by using RAPD & SSR Markers.
Place and Duration of Study: Department of Plant Biotechnology at K.K.Wagh College of Agricultural Biotechnology, Nashik.
Methodology: *Ocimum tenuiflorum* Linn., commonly known as Tulsi, is an aromatic plant with significant traditional and medicinal value. To assess the genetic diversity and relatedness of six Tulsi genotypes (Krishna, Ram, Lavangi, Pandharpuri, Daisil, and Kapoori), molecular techniques were employed. The genotypes were collected from Nagarjuna Medicinal and Aromatic Plant Park at Dr. P.D.K.V. Akola. DNA isolation was performed using alcohol fixation without liquid nitrogen, and the genotypes were analyzed using RAPD and SSR primers for molecular characterization.
Results: Genetic diversity analysis of six Tulsi genotypes (Krishna, Ram, Lavangi, Pandharpuri, Daisil, and Kapoori) was performed using RAPD and SSR markers. Five RAPD primers produced

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15 bands, with 11 bands showing polymorphism (73.3%) and 4 bands showing monomorphism (26.7%). The PIC value ranged from 0.28 to 0.49 (average: 0.40). Four SSR primers generated 9 bands, with 8 bands showing polymorphism and 1 band showing monomorphism. The PIC value ranged from 0.24 to 0.57 (average: 0.39). The Jaccard coefficient revealed moderate to high similarity in RAPD (0.40 to 0.73) and SSR (0.44 to 0.88) analyses. The UPGMA dendrogram separated the genotypes into two main clusters. Cluster 1 included Krishna, Lavangi, Ram, Pandharpuri, and Daisil Tulsi, while cluster 2 consisted of Kapoori Tulsi. The SSR dendrogram also formed two clusters, with Krishna, Lavangi, Ram, Daisil, and Kapoori genotypes in cluster 1, and Pandharpuri Tulsi showing dissimilarity and forming cluster 2.

Keywords: Tulsi; diversity; RAPD; SSR; RNase; DNA fingerprinting.

1. INTRODUCTION

"Ocimumtenuiflorum. (*Lamiaceae*) is an aromatic plant, and there are about 150 species. It is perhaps the most famous persistent spice native to India, widespread as a developed plant, kept away from weeds and covering the entire Indian subcontinent" (Kirtikar and Basu, 1984). "It is commonly found in Asia, Australia and West Africa. Distributed in some Arab countries, mainly in dry sandy soils" [1]. "*O. tenuiflorum* is also called O.tenuiflorum. Sanctuary. In English, it is called heavenly basil in India, ``Kala-Tulsi'' among Hindi-speaking individuals in India, and ``Efinrinwewe'' among Yoruba- speaking individuals in southwestern Nigeria" [2]. It is an erect tall underbush plant with bristly and square branches. The leaves are uncomplicated and vary in color from green to purple. Leaves can be clap, oval, loveless, or intensely shaped. The floating part has glandular hairs on the downstream and fixed organs that secrete labile oil. Inflorescences purplish-white, bisexual and zygotic flowers arranged in long racemes of tight whorls [3-5]. They produce a variety of small, dull brownish, globular hemispherical seeds with glossy seed coats. *O. tenuiflorum* is readily recognized by several species of the *Ocimum* family by its distinctly spreading flower stalk and inner glabrous calyx [6-8].

Ocimum, commonly known as basil, belongs to the *Labiatae* family. *Ocimum* has been dubbed the "Queen of Spices" due to its immense use in general drug frames, beauty care products, fragrances, drugstores, and the nutraceutical industry [9-11]. *Ocimum* L. *sanctum* has been used to treat bronchitis, bronchial asthma, malaria, dysentery, skin infections, arthritis, persistent fever, and insect bites. *Ocimum* exhibits antifertility, anticancer, antidiabetic, antifungal, antibacterial, hepatoprotective, cardioprotective, analgesic and adaptogenic properties.

Among various plant genetics, eugenol is known for its anticancer, antibacterial, sedative, anti-inflammatory, anticarcinogenic, neuroprotective, hypolipidemic, and antidiabetic properties [12-14]. Eugenol is derived from cinnamon bark, clove buds and leaves, turmeric, pepper, Tulsi leaves, ginger, oregano and thyme. Spices such as marjoram, basil, narrows, mace and nutmeg are also high in eugenol. Plant species such as cloves (5-90%), Tulsi (0-71%) and cinnamon (20%) contain the most notable substance eugenol.

Eugenol substances vary in different parts of the plant and also with the seasons. It is essentially obtained from raised parts such as leaves, bark, and flowers.Research confirms that eugenol production is higher in autumn than in late spring. Eugenol belongs to the class of phenylterpenoids ($C_{10}H_{12}O_2$). The phenolic accumulation of eugenol is responsible for its anti-cancer effects. The World Health Organization and Food and Agriculture Organization recommend a safe dose of 2.5 mg eugenol per kg body weight for humans.

Molecular techniques, especially random amplified polymorphic DNA (RAPD) markers, have been considered more valuable and accurate for ascertaining both inter- and intra-species genetic diversity in plants [15]. Studies of plant interspecific diversity have consistently produced not only phylogenetic but also plant phylogenetic data [16]. In recent years, fingerprinting frameworks have been gradually used in terms of RAPD investigations to identify genetic polymorphisms in several plant genera. The RAPD system has been used for cultivar testing of numerous therapeutic plant species due to its ease and speed of identification [17]. The PCR-based His RAPD marker has been commonly used to assess genetic diversity in the animal category by estimating genetic diversity in numerous species, including recreational plants.

The RAPD marker uses a single, short and inconsistent oligonucleotide precursor to improve enigmatic DNA assembly outcomes [18,19]. RAPD has found numerous applications in many scientific fields due to its simplicity of requiring no prior information about DNA inheritance. RAPD markers can recognize myriad genetic polymorphisms [20]. They can also be used to record diversity within plant populations [21,22], generate linkage maps, and trace the origin of hybrids [23-26,22]. Low cost, productivity to propagate large numbers of DNA markers in a short period of time, and the need for less sophisticated hardware have made the RAPD method important [27].

Simple Sequence Repeats (SSRs) are stretches of DNA containing pairwise rehash di-, tri-, or tetra nucleotide units that circulate throughout the eukaryotic genome [28]. SSRs are adaptive, polyallelic co-dominant markers, PCR-based, effectively reproducible, randomly and commonly cycled along the genome, and their interrogation can be automated. It is a decisive marker for many plant breeding projects because of its nature. Microsatellite polymorphism depends on differences in the amount of briefly warmed target at a given locus [29] segment on the gel. Basic sequence rehashing (SSR), also called microsatellites, is a short pairwise reprocessed subject that can vary the amount of rehashing at a particular location. SSR markers have many advantages over other atomic markers. B. Hereditary co-starch. They are polyallelic, commonly abundant, widely distributed throughout the genome, and have been effectively and spontaneously evaluated [29,30-32].

The use of molecular techniques in studying genetic diversity is supported by the finding that evolutionary forces such as natural selection and genetic drift produce distinct phylogenetic branches. This is recognizable because their underlying molecular sequences share a common ancestor. The aim of this study is to assess interspecific relationships and genetic variation between different *Ocimum* species using RAPD and SSR markers.

2. MATERIALS AND METHODS

2.1 Plant Material

Seedlings of six genotypes (Krishna, Ram, Lavangi, Pandharpuri, Daisil and Kapoori) of Tulsi were procured from Nagarjuna Medicinal and Aromatic Plant Park, Dr. Punjabrao Deshmukh Krishi Vidyapeeth (Dr. P.D.K.V.), Akola.

2.2 DNA Isolation and Purification

Total genomic DNA was carried from fresh leaf tissues by the method of Doyle and Doyle [24] with minor modifications as explained below.

The samples were prepared by putting approximately 200 mg of fresh leaf tissue into a mortar and pestle. The tissues were ground into fine powder. Thereafter, 600 µl of preheated plant extraction buffer was added incubation of tube was done at 65℃ for 60 min. After which the tubes were mixed by every 10 min of interval to homogenize the samples. The tubes were removed and allowed to cool, centrifuge at 10,000 rpm for 10 min at room temperature and the supernatant was then transferred into freshly labelled tubes. Then equal volume of phenol: chloroform:isoamylalcohol (25:24:1) was added. Again, centrifuge at 10,000 rpm for 10 min at room temperature. Repeated the phenol: chloroform:isoamylalcohol (25:24:1) treatment once. Aqueous layer was transferred into freshly labelled tubes and added equal volume of ice-cold isopropanol. Mix gently and incubated 4 ℃ for overnight to precipitate the DNA. It was then centrifuge at 10,000 rpm at 15 min at 4 ℃. The supernatant was decanted and added 500 µl of 70 % ethanol to wash the DNA palate, then centrifuge at 10,000 rpm for 15 min at 4 ℃. The supernatant was decanted and the palate was air dried until the ethanol smell disappears, 20 µl of molecular graded water was added to resuspend the DNA.1 µl of *proteinase K* was added and incubated at 37 ℃ for 60 min. In order to check the DNA quality and removal of protein, 0.8 % gel was prepared by boiling 0.8 gm of agarose in 100 ml of 1X TAE and allow to cool at 55 ℃. Then 0.2 µl of ethidium bromide was added and gently mix it. The solution was poured in the casting tray before it polymerizes.2 µl of DNA and 2 µl of loading dye were mixed together 4 µl of the mixture was loaded onto the 0.8 % agarose gel. The gel was run at 70 V for 45 min and the gel image was saved under the gel documentation system. When the protein and RNA was completely removed, it was then proceed to the Biospectrometer.

2.3 RAPD Amplification

A total of 20 primers were used in PCRamplification. RAPD in this study were

selected from the study of [33,34]. PCR amplification was carried out in 25 µlreaction volume containing 1 µl of template DNA (50ng), *Taq* PCR buffer with $Mgcl_2$(17.5 mm),1µl of *Taq polymerase* (1 U/µl), 4 µl dNTPs (2.5 mm) and 1 µl primer (10 pmol/µl).RAPD PCR was performed at an initial denaturation 94℃ for 2 mins, 36 cycles of 94 ℃ for 1 min, 35℃ for 1 min and 72℃ for 2min and final extension 72℃ for 7 mins.

For Polymerase chain reaction 5 RAPD primers. The list of primers given in the Table 1.

2.4 SSR Amplification

A total of 10 primers were used in PCR amplification.PCR amplification was carried out in 10µlreaction volume containing 1.5µl of template DNA (50ng), 1.6 µl*Taq* PCR buffer with $Mgcl_2$(17.5 mm),0.3µl of *Taq polymerase* (1 U/µl), 1.5µl dNTPs (2.5 mm) and 1 µl primer (10 pmol/µl). SSR PCR was performed at an initial denaturation 94℃ for 2.30 mins, 36 cycles of 94 ℃ for 30 sec, 52-59℃ for 1.30 min and 72℃ for 50 sec and final extension 72℃ for 7 mins.For Polymerase chain reaction 4SSR primers.The list of primers given in the Table 2.

2.5 Agarose Gel Electrophoresis

Amplified products of RAPD and SSR were electrophoresed in 1.2 % agarose in 1X TAE buffer and 3.5 % respectively. The gels were stained with ethidium bromide and documented using gel documentation system and data analysis was done.

2.6 Data Analysis and Scoring

Amplification profile of all cultivars compared with each other and bands of DNA fragments scored manually as (1) or (0) depending on the presence or absence of a particular band respectively. The data was analyzed using Numerical Taxonomy System of Multivariate Statistical Programme (NTSYS) software package (Roflh, 1998). The dendrogram was constructed using Unweight Pair Group Method of Arithmetic Averages (UPGMA).

To compare the efficiency of primer, polymorphic information content (PIC) was calculated by using the formula:

$PIC = 2f (1-f)$

Where f is frequency of present allele for RAPD marker.

$PIC = 1 - \sum Pi^2$

Where Pi is frequency of allele for SSR marker.

Table 1. List of RAPD primers

Sr. no.	Primer name	Sequences	Annealing Temperature
1.	OPA 01	5’-CAGGCCCTTC- 3’	35℃
2.	OPA 07	5’ -GAAACGGGTG- 3’	
3.	OPA 11	5’ –CAATCGCCGT- 3’	
4	OPD 16	5’ –AGGGCGTAAG-3’	
5	OP0 15	5’ –TGGCGTCCTT- 3’	

Table 2. List of SSR primers

Sr no.	Primer Name	Sequence	Annealing temperature
1	SSR-01	3’-F-GCGAAAACACAATGCAAAAA-5’ 5’-R-GCGTTGGTTGGACCTGAC-3’	54℃
2	SSR 04	3’-F-CTTTGTCTATCTCAAGACAC-5’ 5’-R-TTGCAGATGTTCTTCCTGATG-3’	55℃
3	SSR 06	3’-F-GCCTCGAGCATCATCATCAG-5’ 5’-R – CAACCTGCACTTGCCTGG-3’	53.2℃
4	SSR 08	3’-F-TTCCCTGTTAAGAGAGAAATC-5’ 5’-R-TGTATTTGGTGAAAGCAAC-3’	56.2℃

3. RESULTS

3.1 RAPD Analysis

In present study, six genotypes were studied with two marker system i.e. RAPD and SSR. For RAPD analysis among 20 arbitrary primers tested, 5 primers generated 62 bands of these 58 bands were polymorphic with an average polymorphism of 93.07 %. Out of 5 primers, 1 primer was showed 100 % polymorphism. The molecular size of the amplified RAPD products ranged from 100 bp (OPD16) to 700 bp (OPO15). The total no. of amplicons varied from 2 to 5 with an average of 3.5 per primer. The number of polymorphic amplicons ranged from 7 (OPA01) to 19 (OPA07) with an average of 13 bands per primer. OPO15 showed the highest PIC (0.49), while it was lowest for OPA11 (0.28) with an average of 0.40 as mentioned in Table 3.

3.2 SSR Analysis

Among 10 SSR primers used in this study 4 SSR primers detected a total of 42 bands in six genotypes out of which 30 bands were polymorphic (71%). Out of 4 SSR primer, 3 were 100% polymorphic. The number of total amplicons varied from 2 (SSR01, SSR06 and SSR 08) to 3 (SSR 04) with an average of 2.5 alleles per primer and six ranges from 150 bp (SSR 06) to 550 bp (SSR 04). The number of polymorphic amplicons ranged from 6 (SSR 06and SSR 08).

To 10 (SSR 04) with an average 8 polymorphic loci per primer marker. The PIC value ranged from 0.24 (SSR01) to 0.57 (SSR 04) with a mean of of 0.39 as mentioned in Table 4.

Table 3. Similarity index for the six Tulsi genotypesusing RAPD banding profiles

Sr. No.	Primers	Total no. of bands	Total no. of Polymorphic bands	Total no. of Monomorphic bands	Polymorphism (%)	PIC value
1.	OPA 01	8	7	1	50%	0.44
2.	OPA 07	20	19	1	80%	0.44
3.	OPA 11	10	10	0	100%	0.28
4.	OPD 16	14	13	1	67%	0.35
5.	OPO 15	10	9	1	67%	0.49
Total		62	58	4		

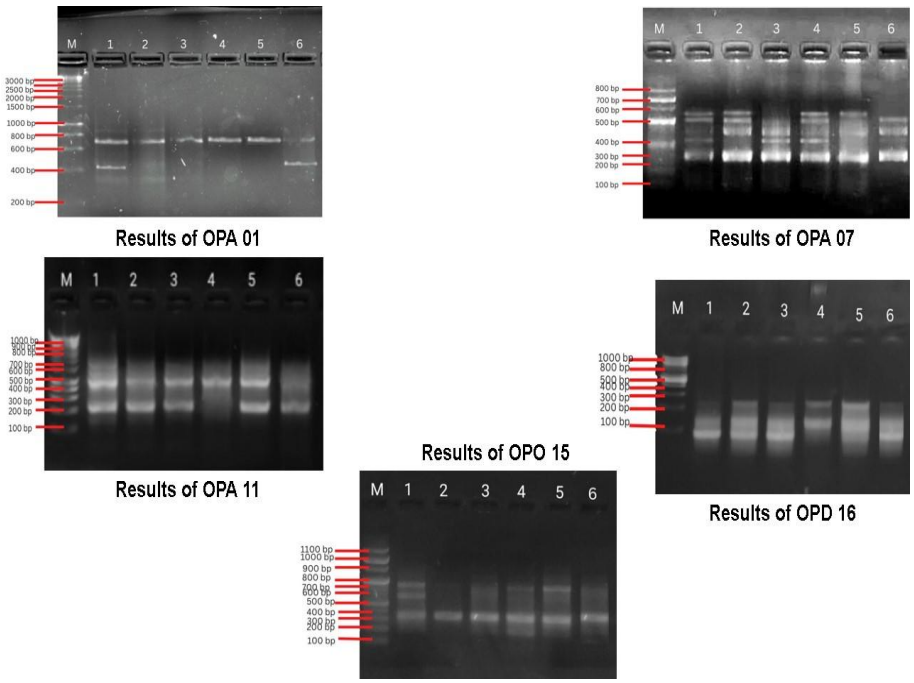

Plate 1. Gel photograph showing RAPD allelic profile of six genotypes with five primers

Table 4. Similarity index for the six Tulsi genotypesusing SSRbanding profiles

Sr. No.	Primers	Total no. of bands	Total no. of Polymorphic bands	Total no. of Monomorphic bands	Polymorphism (%)	PIC value
1.	SSR 01	10	8	2	80%	0.24
2.	SSR 04	14	10	4	71.4%	0.37
3.	SSR 06	9	6	3	66.6%	0.37
4.	SSR 08	9	6	3	66.6%	0.34
Total		42	30	12		

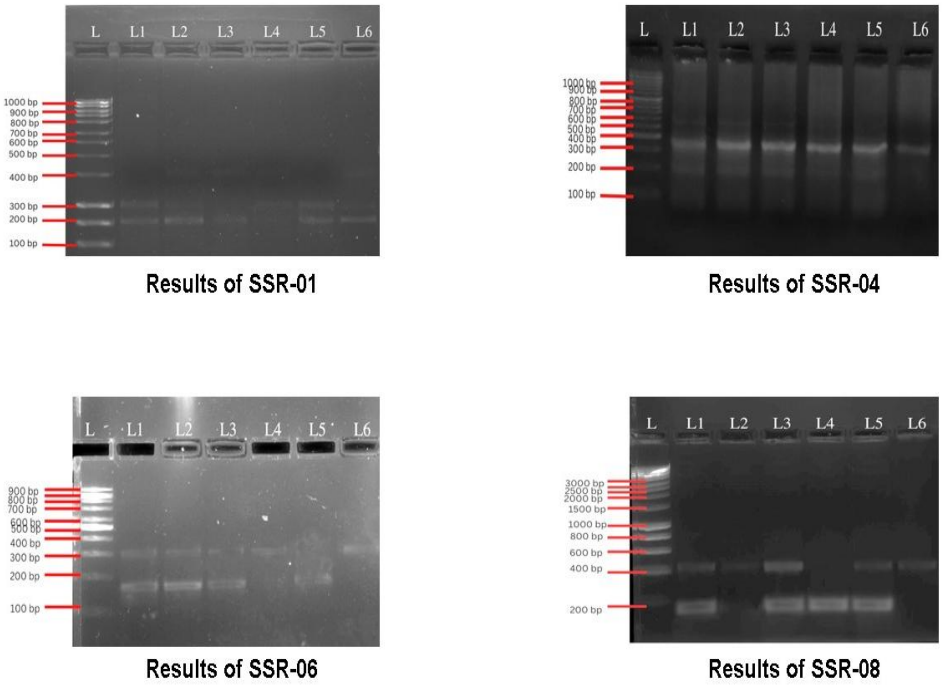

Plate 2. Gel photograph showing SSR allelic profile of six genotypes with four primers

3.3 RAPD-based Cluster Analysis

Jaccard's similarity coefficients based on RAPD markers amongtheallpair-wise combinations of genotypes ranged from 0.40 [between PandharpuriTulsi and Kapoori] to 0.73 (between Ramand Lavangi) with an average value of 0.39 as mentioned in Table 5. The dendrogram grouped into two main clusters on the basis of the reference line drawn at a similarity coefficient. The first cluster consists of similarity in Krishna, Lavangi, Ram, Pandharpuri and DaisilTulsi and second cluster consists of KapooriTulsi the First and Second cluster consists of slight variations between them. The KapooriTulsi was out grouped which shows high variations as shown in Fig. 1.

Table 5. Similarity index for the six Tulsi genotypes using RAPD banding profile

Variety	Krishna	Ram	Lavangi	Pandharpuri	Daisil	Kapoori
Krishna	1.00					
Ram	0.60	1.00				
Lavangi	0.60	0.73	1.00			
Pandharpuri	0.66	0.66	0.53	1.00		
Daisil	0.66	0.80	0.66	0.73	1.00	
Kapoori	0.60	0.73	0.73	0.40	0.53	1.00

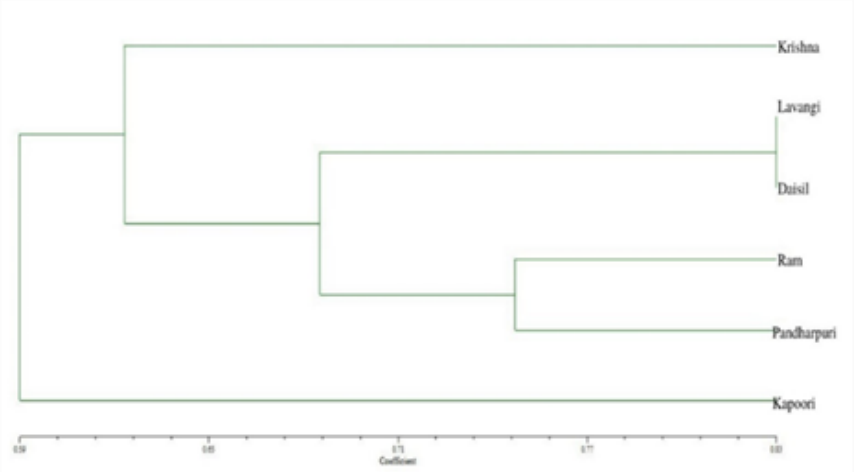

Fig. 1. Dendrogram for the Tulsi genotypes based on the data from five RAPD primer

3.4 SSR-based Cluster Analysis

Jaccard's similarity coefficients based on SSR markers among the allpair-wise combinations of genotypes ranged from 0.44 [Between Pandharpuri and Ram, Lavangiand Pandharpuri and Lavangi andKapoori] to 0.88 (between Ramand Krishna and Krishna andLavangi) with an average value of 0.65 as mentioned in Table 6. The dendrogram grouped into two main clusters on the basis of the reference line drawn at a similarity coefficient. The first cluster consists of Krishna, Lavangi, Ram, Daisil and Kapoori genotypes. The second cluster consists of PandharpuriTulsi shows less similarity with cluster first. The Pandharpuri shows dissimilarities with other genotypes and it is out grouped as shown in Fig. 2.

Table 6. Similarity index for the six Tulsi genotypes using SSR banding profile

Variety	Krishna	Ram	Lavangi	Pandharpuri	Daisil	Kapoori
Krishna	1.00					
Ram	0.89	1.00				
Lavangi	0.89	0.78	1.00			
Pandharpuri	0.55	0.44	0.44	1.00		
Daisil	0.78	0.67	0.67	0.55	1.00	
Kapoori	0.55	0.67	0.44	0.55	0.55	1.00

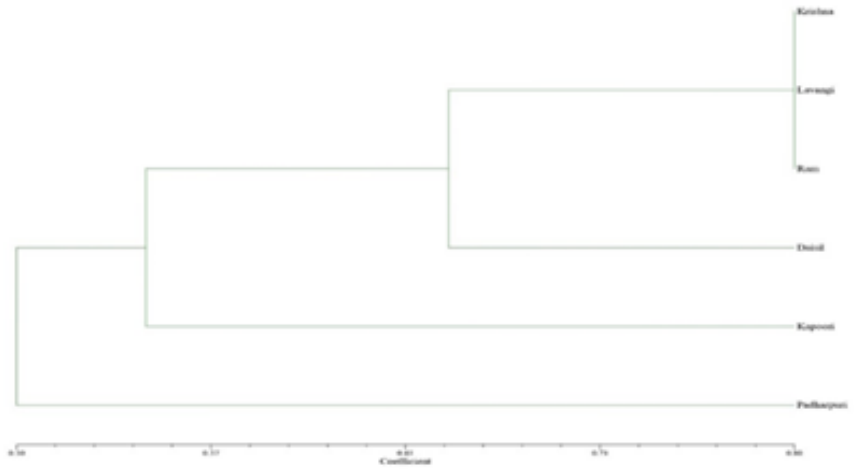

Fig. 2. Dendrogram for the Tulsi genotypes based on the data from fourSSR primer

4. DISCUSSION

"The information can be found about assessment of molecular diversity in *Ocimum*usinga PCR-based approach" [35,36]. "The intrinsic genetic diversity in present study on *Ocimum* accessions was apparent from the analysis of their RAPD and SSR profiles and from the dendrogram generated where all the accessions had unambiguously separated from each other. RAPD studies have been widely used for population genetic studies in both wild and cultivated plants" [28]. Generally, all these studies have reported that RAPD primers produce more reliable and reproducible bands than SSR primers. In the present study, however, it was observed that once the PCR conditions are well set, high reproducibility for both RAPD and SSR markers can be obtained. In general, all 9 markers used in this study produced clear consistent and reproducible amplification profiles.

In the present study, 6 *Ocimum* genotypes were studied with 2 different marker systems, i.e., RAPD and SSR for genetic diversity analysis. "RAPD showed the highest polymorphism level (93.07 %) than SSR (71.110 %). The results were consistent with genetic diversity analysis of *Ocimum* by" [36]. "High polymorphism of SSR and RAPD markers was also reported in many previous studies, for examples, RAPD of *Ocimum tenuiflorum"* [37]. Checked the efficiency of three PCR-based markers (RAPD) in Tulsi and reported higher polymorphism in RAPD markers. High levels of polymorphism found in the present work showed that both markers are suitable for genetic diversity studies and are equally effective to differentiate the closely related cultivars of *Ocimum*.

5. CONCLUSION

The identification of primers that can apparently generate species specific profiles is significant for further phylogenetic studies in *Ocimum*. RAPD and SSR techniques indicated that it is useful in estimation of polymorphism and phylogenetic relationships among *O. Sanctum* morphotypes. The polymorphism data generated can be used for further breeding and characterization of the species.

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented in the conference: 3rd International Conference IAAHAS-2023 "Innovative Approaches in Agriculture, Horticulture & Allied Sciences" on March 29-31, 2023 in SGT University, Gurugram, India. Web Link of the proceeding: https://wikifarmer.com/event/iaahas-2023-innovative-approaches-in-agriculture-horticulture-allied-sciences/

ACKNOWLEDGEMENTS

The authors are greatly thankful to Department of Plant Biotechnology, K. K. Wagh college of Agricultural Biotechnology, Nashik for providing laboratory facilities and financial support to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pistrick K. Phenology and genome variation in Allium L. - a tight correlation? Plant Biol. 2001;3:654-660
2. Mohan L, Amberkar M, Kumari M. *Ocimum sanctum* Linn. (Tulsi)- an overview. Int. J.Pharm. Sci. Rev. Res. 2011:7(1):51-53
3. Ahmad S, Khaliq I. Morpho-molecular variability and heritability in *Ocimum sanctum* genotypes from northern regions of pakistan. Pak. J. Biol. Sci. 2002;5:1084-1087
4. Biswas N, Biswas A. Evaluation of some leaf dusts as grain protectant against rice weevil Sitophilus oryzae (Linn). Environ. Ecol. 2005;23(3):485 – 488 553-571.
5. Borah R, Biswas S. Tulsi excellent source of phytochemicals. Int. J. Agric. Environ. Biotechnol. 2018;3(5):1732-1738.
6. 24.Matasyoh L, Matasyoh J, Wachira F, Kinyua M, Thairu M, Mukiama T. Chemical composition and antimicrobial activity of the essential oil of *Ocimum*gratissimum L. growing in Eastern Kenya. Afr J. Biotechnol. 2007:6(6): 760-765.
7. Mullis KB, Ferre F, Gibbs RA. The Polymerase Chain Reaction 1994.
8. Muluvi G, Sprent J, Soranzo N, Provan J, Odee D, Folkard G, McNicol J, Powell W. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in Moringa oleifera Lam. Mol Ecol. 1999:8:463-470
9. Cardoso S, Eloy N, Provan J, Cardoso M, Ferreira P. Genetic differentiation of Euterpe edulis Mart. populations estimated

by AFLP analysis. Mol. Ecol. 2000;9: 1753-1760.
10. Lal S, Mistry K, Shah S, Thaker R, Vaidya P. Genetic diversity assessment in nine cultivars of Catharanthus roseus from Central Gujarat (India) through RAPD, ISSR and SSR markers. J. Biol Res. 2011;1(8):667-675.
11. Lei Y., Gao H., Tsering T., Shi S., Zhong Y. Determination of genetic variation in Rhodiolacrenulata from the Hengduan Mountains Region, China using inter-simple sequence repeats. Genetics Mol Biol. 2006:(29)2:339-344.
12. Chen X, Yang J, Tang J. Species-diversified plant cover enhances orchard ecosystem resistance to climatic stress and soil erosion in subtropical hillside. J. Zhejiang Univ. Sci. A. 2004:5(10):1191-1198
13. Lemos J, Passos X, Fernandes O, Paµla J, Ferri P, Souza L, Lemos A, Silva M. Antifungal activity from *Ocimum*gratissimum L. towards Cryptococcus neoformans. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2005; 100(1):55-58.
14. Li Q., Xu Z., He T. Ex-situ genetic conservation of endangered Vaticaguangxiensis (Dipterocarpaceae) in China. Biol Conserv. 2002;106:151-156.
15. Goswami M, Ranade S. Analysis of variations in RAPD profiles among accessions of Prosopis. J. Genetics. 1999; 78:141-147.
16. Adams R, Demeke T. Systematic relationships in Juniperus based on random amplified polymorphic DNAs (RAPDs). 1993.Taxon 42
17. Lopez, Sanchez P, Batlle R, Nerin C. Solid and vapor phase anti-microbial activities of six essential oils susceptibility of selected food borne bacterial and fungal strains. J. Agric Food Chem. 2005:53(17):6939-6946
18. Pearson C, Sinden R. Trinucleotide repeat DNA structures: dynamic mutations from dynamic DNA. Curr. Opin. Struct. Biol. 1998;8:321–330.
19. TaleyzzamanM,Jain P, Verma R, Iqbal Z, Mirza M. Eugenol as a potential drug candidate: a Review. Bentham Sci. Pub. 2021;1804-1815.
20. Williams J., Kubelik A., Livak K., Rafalski J. and Tingey S. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990; 18:6531-6535.
21. Dawson J, Chalmers K, Waugh R, Powell W. Detection and analysis of genetic variation in Hordeum spontaneum populations from Isreal using RAPD markers. Mol. Ecol. 1993;2:151-159.
22. Hu J, Quiros J. Identification of broccoli and cauliflowercultivars with RAPD markers. Plant Cell Rep. 1991;10:505-511.
23. Crawford D, Brauner S, Cosner M, Stuessy T. Use of RAPD markers to document the origin of the intergeneric hybrid x Margyrocaeneskottsbergii (Rosoceae) on the Juan Fernandez Island. Amer. J. of Bot. 1993;80:89-92.
24. Doyle J, Doyle J. Isolation of plant DNA from fresh tissue. Phytochemical Bulletin. 1990;19:11-15
25. Gupta P, Mishra A, Lal R, Dhawan S. DNA fingerprinting and genetic relationships similarities among the accessions/species of *Ocimum* using SCoT and ISSR markers system. Springer. 2021;446-457.
26. Harisaranraj R, Prasitha R, Saravana S, Suresh K. Analysis of inter-species relationships of *Ocimum* species using RAPD markers. Ethnobot. Leafl. 2008;12: 609-13.
27. Bardakci F. Random amplified polymorphic DNA (RAPD) markers. Turk J. Biol. 2001;25:185-196.
28. Patel H, Foughat R, Kumar S, Mistry J. and Kumar M. Detection of genetic variation in *Ocimum* species using RAPD and ISSR markers. Springer. 2014; 10:1007.
29. Rafalski J, Morgante M, Vogel J, Powell W, Tingey S. Generating and using DNA markers in plants. In: Birren B. and Lai, E (eds). Non-mammalian Genome Analysis: a practical guide. AP, London New York. 1995;75-134.
30. Rohlf F. NTSYS-Pc. Numerical taxonomy and multivariate analysis system version 2.02e. Exeter Software. New York; 1997.
31. SairkarP, Vijay N, Silawat N, Garg R, Chouhan S, Batav N, Sharma R, Mehrotra NN. Inter-species association of *Ocimum* genus as revealed through random amplified polymorphic DNA fingerprinting. Sci Secure J. Biotech. 2012;1(1):1-8.
32. 35.Satyavathi C., Tiwari S., Bharadwaj C., Rao A., Bhat J., & Singh S.Genetic diversity analysis in a novel set of restorer lines of pearl millet [*Pennisetum glaucum* (L.) R. Br] using SSR markers. Vegetos. 2013:26(1):72-82.

33. Akande T, Omoigui L, Ikwebe J. Morphological variability and molecular characterization of thirty soybean genotypes using random amplified polymorphic DNA (RAPD) markers. Afr. J. Biotechnol. 2016;17(24).739747.
34. Bilal A. Molecular characterization of *Ocimum* species using random amplified polymorphic DNA method and gene identification using sanger sequencing method. J. Mol Biomark Diagn. 2020; 9: 423.
35. Jaccard P. Nouvelles Recherches sur la distribution florale. Bulletin Soc Vaud Sci Nat. 1908;44:223-270.
36. Chen S, Dai T, Chang Y, Wang S, Lin Y, Ku H. Genetic diversity among *Ocimum* specie as based on ISSR, RAPD and SRAP markers. Aust J Crop SCI. 2013; 1463-1471.
37. Opoola J, Oziegbe M. Characterization of *Ocimum tenuiflorum* (Linn.) Morpho-types Using RAPD Markers. Not. Sci. Biol. 2019; 11(4):417-420.

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