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Essential oil studies and RAPD markers in sUV-B treated *Cymbopogon flexuosus* (Nees ex Steud) wats

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Abstract

The wild plants of *Cymbopogon flexuosus* (Nees ex Steud) Wats were subjected to sUV-B treatment to analyze essential oil variation and DNA polymorphism. Significant difference in essential oil yield and citral content was observed. Plants treated with sUV-B for different intervals of time (15min, 30min, 1h, 1.5h, 2.0h, 2.5h, 3.0h, 3.5h and 4.0h) along with control were subjected to RAPD analysis with 10 random decamer primers. Five primers (OPA-01, OPA-09, OPY-18, OPY-09 and OPG-10) produced polymorphism generating 57 amplicons, of which 27 were polymorphic and 30 were monomorphic in nature. Primer OPA-01 showed highest polymorphism (60%) and OPA-09 showed lowest polymorphism (12.5%). High genetic similarity was observed in treatment with 1.5h and 2.5h and least genetic similarity in treatment with 3.5h and control plants. Dendrogram constructed on genetic similarity coefficient showed two clusters viz., cluster I and II. The study indicates the significant variation at intraspecies level with respect to essential oil yield, citral content and DNA polymorphism in sUV-B treated plants which can be further exploited for developing high yielding chemotypes in *C. flexuosus*.

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Abbreviations: sUV-B - Supplemental ultraviolet-B radiation, RAPD - Random amplified polymorphic DNA.

Introduction

Cymbopogon flexuosus (Nees ex Steud) Wats is widely grown medicinal and aromatic plant belonging to the family Poaceae. The plant yield one of the top ten essential oils in the world. The essential oil contains a mixture of complex volatile constituents used in flavour, fragrance, food and pharmaceutical industries (Parikh and Desai, 2011). The major constituents of the oil are citral (isomer of geranial and neral), limonene, linalool, geranyl acetate, nerol and caryophyllene oxide. Citral, the active compound in essential oil determines quality of the oil. Many cultivars of *C. flexuosus* have been developed by phenotypic recurrent selection programs which show variation in quality and quantity of essential oil (Kulkarni and Ramesh 1992; Kulkarni, 1994).

Earlier studies indicated the development of new and significant alterations in plant characteristics through induced mutations which increased the production of yield. Direct use of these mutants or utilizing these in hybridization technique (Ahloowalia *et al*., 2004) overcome yield plateaus and generates desirable traits. There is significant contribution in mutation breeding for plant improvement resulting in production of many varieties. In India, through this method nearly 300 cultivars are developed in different plant species (Kharkwal*et al*., 2004). Conventional breeding in *Cymbopogon* through mutations are used as alternative method for crop improvement. Variability in quality and quantity of inherited traits obtained through induced mutations are studied in*C. flexuosus* and other crops. (Kulkarni *et al*., 1991; Maluszynski *et al.,* 1995; Muduli and Mishra, 2007).

RAPD method is used as investigation tool and useful biomarker assay to study the genetic polymorphism in plants. RAPD characterization of *C. flexuosus* cultivars having similar morphology expressed low polymorphism and displayed low level of variability in the composition of essential oil (Ganjewala D, 2008). In elite germplasms of *C. winterianus,* RAPD analysis was efficient in detecting polymorphism, loci scoring and polymorphic information content (PIC) values. However, the resolving power, shannon's index, mean coefficient of gene differentiation and gene flow estimates were better studied in ISSR markers (Bhattacharya *et al*., 2010). RAPD analysis helped to resolve the taxonomic complexities in *Cymbopogon* taxa (Khanuja *et al*., 2005). In *Cymbopogon* accessions, the SSR markers showed low level of polymorphism and poor correlation between molecular and chemical diversity and indicated that chemical diversity is not only a result of genetic variability but also depend on number of other factors (Kumar *et al*., 2009). New SSR markers were developed in *Cymbopogon* species showed higher levels of allelism and polymorphism, which can be utilized to improve quality of essential oil, to control agronomically important traits and marker assisted breeding (Kumar *et al*., 2007). Thus, several studies made in species of *Cymbopogons* using RAPD markers were aimed at detecting genetic diversity at inter and intra species level. To the best of our knowledge there is no information available on sUV-B induction studies to increase the essential oil yield in *C. flexuosus*. Hence, the present study was undertaken.

Material and methods

Collection and Maintenance

The wild collections of *C. flexuosus* used in the present study were made from Himavadgopala hills in Karnataka. The germplasm was maintained in the departmental garden of Microbiology and Biotechnology, Bangalore University, Bangalore for further investigations. The plants were divided into three sets for different stress treatments.

sUV-B Stress induction

sUV-B radiation for different intervals of time (15 min, 30 min, 1hr, 1.5h, 2.0h, 2.5h, 3.0h, 3.5h and 4.0h))were administered in growth chamber. The growth chamber was fitted with UV-B fluorescent tubes (TL40W/12 RS UV-B Medical, Philips) with the output of 312 nm. Cellulose acetate of 0.13mm and polyester films were used to filter the transmission of wavelength below 290nm. Control plants were exposed to direct sunlight.

Extraction of essential oil

Aerial part collected from treated plants along with the control was used for essential oil studies. The herbage was air dried at room temperature for three days and subjected to hydrodistillation for 3 hours using Clevenger's apparatus (Clevenger JF, 1928).

The essential oils were collected and stored under anhydrous sodium sulphate and kept at 4°C for further analysis.

Analysis of essential oil

Essential oil yield

The yield of essential oil from sUV-B treated and control plants were calculated on the basis of dry weight of the material (v/w) and chemical analysis were performed using Gas Chromatography-Mass Spectroscopy (GC-MS).

GC-MS analysis

The GC/MS analysis was performed on a Thermo GC-trace ultra ver: 5.0, Thermo MS DSQ II using DB 5-MS Capillary Standard Non-Polar Column (30mts x 0.25mm x 0.25µm). The temperature program was 70°c (6 min) rising to 260°C at a rate of 6°C/min. Injector and detector temperature was 260°C. Helium was used as carrier gas at a flow rate 1.0ml/min.

Identification of the compounds was carried out by comparison of the mass spectral fragmentation patterns with those stored in MS database (National Institute of Standards and Technology).

RAPD Assay

The leaf samples from sUV-B treated plants and control were used for DNA extraction using standard protocol (Chaudry B *et al.*, 1999). For RAPD analysis genomic DNA were diluted by taking 4µl of genomic DNA (template DNA 25 (ng)) mixed with 1.5µl of Taq assay buffer, 0.3µlof $MgCl_2$, 5µl of Primer (1pmoles/µl) and Taq polymerase.0.3µl of the above reaction mixture was made up to 15 µl with water and amplification was performed in a programmable Thermal Controller (Eppendorf personal master cycler) for an initial denaturation of 4 min at 94°C, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 38°C for 1min, and extension for 2 min at 72°C. A final extension for 7 min at 72°C was included after the last cycle. Amplified products along with DNA molecular weight markers were separated in a horizontal gel electrophoresis using 1.2 % agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. Based on their scoring matrix, data were subjected to analysis using MVSP-32 version RAPD analysis software. The genetic similarity matrix was calculated using Jaccards coefficient, distance matrix and dendrogram was based on Eucledian linkage using UPGMA (Unweighted Pair Group Method with Arithmetic average).

Results

Analysis of essential oil

Quantitative and qualitative analysis of essential oils from sUV-B treated plants showed variation in percentage and its constituents compared to control. Essential oil yield and citral content of sUV-B treated plants with control are given in Table 1. High essential oil yield (1.65%) and citral content (83.40%) were obtained from treatment with1.5h compared to control (1.27% of essential oil and 64.98% of citral). sUV-B treatment for 3.0hshowed reduced essential oil yield (0.95%)and treatment for 4.0 h showed reduced citral content(22.32%).The variations observed in percentage of perfumery important compounds in essential oils of sUV-B treated plants along with control are listed in Table 2.

RAPD analysis

DNA amplification was carried out with 10 random decamer primers, out of which five produced polymorphism (OPA-01, OPA-09, OPY-18, OPY-09 and OPG-10) (Fig. 1). The primer sequences and total number of amplicons generated are listed in Table 4. An overall of 57 amplicons were produced for the tested plants out of which 27 were polymorphic and 30 were monomorphic in nature.OPA-01 showed high polymorphism (60%) and OPA-09 showed low polymorphism (12.5%). High genetic similarity was observed between sUV-B treatment for 1.5 h and 2.5 h and there was least similarity between treatment for

3.5 hand control. Based on the dendrogram constructed on genetic similarity coefficient, stress induced plants were divided into two main clusters i.e. cluster I and II. Cluster I consisted of treatment with 3.5hand cluster II with other treatments (15min, 30min, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h and 4.0 h).Cluster II was further divided into two sub clusters i.e. IIA and IIB. IIA consists of treatment with 4.0 h and IIB consists of remaining treatments (15min, 30min, 1.0 h, 1.5 h, 2.0 h, 2.5 h and 3.0 h).

Table 1. Essential oil yield and citral content.

Sl.No.	Treatment	Essential oil yield (%)	Citral content (%)
1.	Control	1.27	64.98
2.	sUV-B-15min	1.37	68.74
3.	sUV-B-30min	1.55	81.80
4.	sUV-B-1h	1.68	67.83
5.	sUV-B-1.5h	1.65	83.40
6.	sUV-B-2.0 h	1.31	68.05
7.	sUV-B-2.5 h	1.14	63.12
8.	sUV-B-3.0 h	0.76	68.34
9.	sUV-B-3.5 h	0.77	33.70
10.	sUV-B-4.0 h	0.87	22.32

Table 2. Yield of perfumery compounds (%).

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X
Geranial	35.13	40.12	48.11	39.45	51.42	39.12	34.91	38.49	15.96	12.03
Neral	29.85	28.62	33.69	28.38	31.98	28.93	28.21	29.85	17.74	10.29
Nerol	2.85	3.17	1.52	2.55	1.57	2.26	1.40	2.41	-	-
β-caryo phyllene	1.63	1.29	1.44	0.20	0.17	0.12	0.02	0.09	0.21	0.20
Geranyl acetate	0.22	0.16	0.06	0.15	0.09	0.08	0.05	0.04	0.09	0.25
Linalool	0.16	0.10	0.14	0.18	0.19	-	-	-	0.15	-

I-control, II-sUV-B 15min, III -sUV-B 30min, IV sUV-B 1h, V-sUV-B 1.5h, VI-2.0h,VII -2.5h, VIII-Zn-3.0h, IX-3.5h, X-4.0h.

Table 3. List of primers and amplified bands.

Primer	Sequence	Total amplicons	Polymorphic amplicons	Monomorphic amplicons	% of polymorphism
OPA-01	**5'-CAG GCC CTT C-3'**	10	6	4	60%
OPA-09	**5'-GGG TAA CGC C-3'**	8	1	7	12..5%
OPY-18	**5'-GTG GAG TCA G-3'**	19	10	9	52.6%
OPY-09	**5'-AGC AGC GCA C-3'**	10	5	5	50%
OPG-10	**5'-AGG GCC GTC T-3'**	10	5	5	50%

Discussion

In the present investigation sUV-B treatment of *C. flexuosus* showed variation in essential oil yield, citral content and genetic polymorphism. Treatment for 1.5 h gave high yield of essential oil and citral content. The findings are in accordance with the previous reports in *C.citratus* where sUV-B treatment for 3h had increased the percentage of essential oil and z-citral (Rimakumari*et al*., 2009). Increase in essential oil yield was also observed in *Ocimum basilicum* L

(Jhonson *et al.*, 1999, Nitz and Schnitzler, 2004), *Mentha piperita* (Maffei M and Scannerini S, 2000) and *M. spicata* (Karousou *et al.*, 1998) where plants were exposed to short daily treatments with sUV-B. The study also indicated the variation in its essential oil constituents.

Table 4. Cluster Analysis.

UPGMA										
Jaccard's Coefficient										
Similarity matrix										
	plant1	plant 2	plant3	plant4	plant5	plant6	plant7	plant8	plant9	plant10
plant1	1									
plant 2	0.627	1								
plant3	0.766	0.585	1							
plant4	0.714	0.604	0.932	1						
plant5	0.686	0.582	0.891	0.956	1					
plant6	0.735	0.593	0.955	0.977	0.935	1				
plant7	0.787	0.574	0.932	0.911	0.872	0.933	1			
plant8	0.771	0.564	0.955	0.933	0.894	0.956	0.977	1		
plant9	0.755	0.554	0.933	0.913	0.875	0.935	0.956	0.978	1	
plant10	0.771	0.564	0.955	0.933	0.894	0.956	0.977	1	0.978	1
	plant1	plant 2	plant3	plant4	plant5	plant6	plant7	plant8	plant9	plant10

Table 5. Jaccard'sCoefficientSimilarity Matrix.

UPGMA										
Standardized Euclidean										
Distance matrix										
	plant1	plant 2	plant3	plant4	plant5	plant6	plant7	plant8	plant9	plant10
plant1	0									
plant 2	13.101	0								
plant3	8.464	13.269	0							
plant4	9.163	13.127	3.51	0						
plant5	10.196	13.868	5.685	4.472	0					
plant6	8.926	13.289	2.835	2.07	4.928	0				
plant7	8.248	13.404	3.489	3.99	5.993	3.411	0			
plant8	8.504	13.563	2.808	3.411	5.625	2.711	2.07	0		
plant9	9.073	13.927	4.229	4.651	6.453	4.165	3.78	3.162	0	
plant10	8.504	13.563	2.808	3.411	5.625	2.711	2.07	0	3.162	0
	plant1	plant 2	plant3	plant4	plant5	plant6	plant7	plant8	plant9	plant10

The genetic polymorphism in *Cymbopogons* has been well studied using RAPD markers at inter and intra species level (Khanuja *et al.*, 2005, Bhattacharya *et al.*, 2010 and Adikhari *et al.*, 2013). Different cultivars of *C.flexuosus* characterized by RAPD markers showed difference in their DNA polymorphism (Ganjewala D, 2008). In the present study sUV-B treatment of *C. flexuosus* for different time intervals showed 60% polymorphism at genetic level. Correlation between essential oil yield, citral content and DNA polymorphism could not be established as also observed in earlier studies (Khanuja *et al.*, 2005 and Kumar *et al.*, 2009). The study signifies the importance of sUV-B treatment on *C. flexuosus,* which can be further exploited for developing high yielding chemotypes.

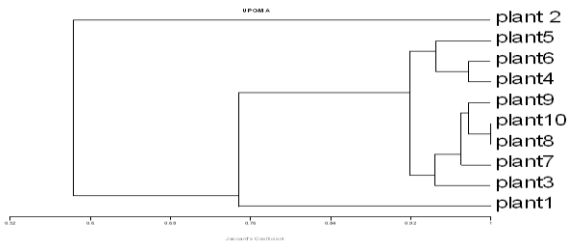

Fig. 1. Where plant 1-control, plant 2-sUV-B 15min, plant 3-sUV-B 30min, plant 4- sUV-B 1h, plant 5-

sUV-B 1.5h, plant 6 - 2.0h, plant 7-2.5h, plant 8-Zn-3.0h, plant 9-3.5h, plant 10-4.0h.

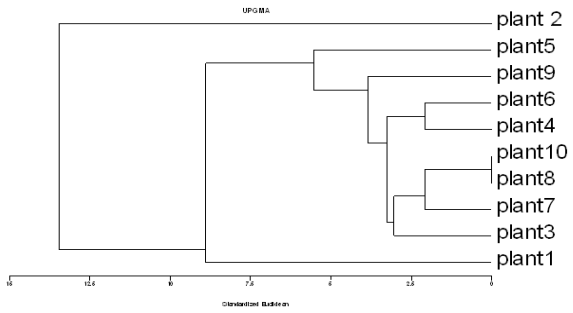

Fig. 2. Jaccard'sCoefficientDistance matrix.

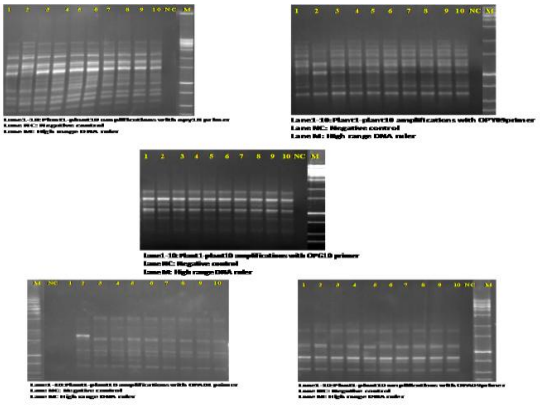

Fig. 3. RAPD profiles of five different primers.

References

Adikhari S, Bandopadhyay TK, Ghosh PD. 2013. Assessment of genetic diversity of certain Indian elite clones of *Cymbopogon* species through RAPD analysis. Indian Journal of Biotechnology **12,** 109-114.

Ahloowalia BS, Maluszynski M, Nichterlein K. 2004. Global impact of mutation-derived varieties. Euphytica **135(2),** 187-204. http://dx.doi.org/10.1023/B:EUPH.0000014914.85465.4f

Bhattacharya S, Bandopadhyay TK, Ghosh PD. 2010. Efficiency of RAPD and ISSR markers in assessment of molecular diversity in elite germplasms of *Cymbopogon winterianus* across West Bengal, India. Emirates Journal of Food and Agriculture **22(1),** 13-24.

Chaudry B, Yasmin A, Husnain T, Riazuddin S. 1999. Mini-scale Genomic DNA Extraction from Cotton. Plant Molecular Biology Reporter **17,** 280-281.

Clevenger JF. 1928. Apparatus for the determination of volatile oil. Journal of the American Pharmaceutical Association **17(4),** 345-349. http://dx.doi.org/10.1002/jps.3080170407

Ganjewala D. 2008. RAPD characterization of three selected cultivars OD-19, GRL-1 and Krishna of East Indian Lemongrass (*Cymbopogon flexuosus* Nees ex Steud) Wats. American-Eurasian Journal of Botany **1(2),** 53-57.

Girija M, Gnanamurthy S, Dhanavel D. 2013. Genetic diversity analysis of Cowpea mutant (*Vigna unguiculata* (L.) Walp) as revealed by RAPD marker. International Journal of Advanced Research **4,** 139-147.

Johnson CB, Kirby J, Naxakis G, Pearson S. 1999. Substantial UV-B mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). Phytochemistry **51,** 507-510. http://dx.doi.org/10.1016/S0031-9422(98)00767-5

Karousou R, Grammatikopoulos G, Lanaras T, Manetas Y, Kokkini S. 1998. Effects of enhanced UV-B radiation on *Mentha spicata* essential oils. Phytochemistry **49,** 2273-2277. http://dx.doi.org/10.1016/S0031-9422(98)00385-9

Khanuja SPS, Shasany AK, Pawar A, Lal RK, Darokar MP, Naqvi AA, Rajkumar S, Sundaresan V, Lal N, Kumar S. 2005. Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* Spreng.(Poaceae).Biochemical systematics and ecology **33,** 171-186. http://dx.doi.org/10.1016/j.bse.2004.06.011

Kumar J, Verma V, Shahi AK, Qazi GN, Balyan HS. 2007. Development of simple sequence repeat

markers in *Cymbopogon* species. Planta Medica. **73(3),** 262-6. http://dx.doi.org/10.1055/s-2007-967121.

Kumar J, Verma V, Goyal A, Shahi AK, Sparoo R, Sangwan RS, Qazi GN. 2009. Genetic diversity analysis in *Cymbopogon* species using DNA markers. Plant Omics Journal **2(1),** 20-29.

Kharkwal MC, Pandey RN, Pawar SE. 2004. Mutation breeding for crop improvement. In: Jain HK, Kharkwal MC, ed. Plant breeding: Mendelian to molecular approaches. Narosa Publishing House Private Limited, 601-645.

Kulkarni RN, Mallavarapu GR, Ramesh S. 1992. The oil content and composition of new variants of *Cymbopogon flexuosus*. Journal of Essential Oil Research **4(5),** 511-514. http://dx.doi.org/10.1080/10412905.1992.9698118

Kulkarni RN, Ramesh S. 1992. Development of lemongrass clones with high oil content through population improvement. Journal of Essential Oil Research **4(2),** 181-186. http://dx.doi.org/10.1080/10412905.1992.9698040

Kulkarni RN. 1994. Phenotypic recurrent selection for oil content in East Indian lemongrass. Euphytica **78(1-2),** 103-107. http://dx.doi.org/10.1007/BF00021404

Maffei M. Scannerini S. 2000. UV-B effect on photomorphogenesis and essential oil composition in peppermint (*Mentha piperita* L.). Journal of Essential oil Research **12,** 523-529. http://dx.doi.org/10.1080/10412905.2000.9712150

Maluszynski M, Ahloowalia BS, Sigurbjornsson B. 1995. Application of in vivo and in vitro mutation techniques for crop improvement. Euphytica **853,** 303-315. http://dx.doi.org/10.1007/978-94-011-0357-2_36

Muduli KC, Mishra RC. 2007. Efficacy of mutagenic treatments in producing useful mutants in finger millet (Eleusine coracana Gaertn.). The Indian Journal of Genetics and Plant Breeding **67(3),** 232-237.

Nitz GM. Schnitzler WH. 2004. Effect of PAR and UV-B radiation on the quality and quantity of the essential oil in sweet basil (*Ocimum basilicum* L.). Acta Horticulturae (International Society for Horticultural Science) **659,** 375-381.

Parikh JK, Desai MA. 2011. Hydrodistillation of Essential Oil from *Cymbopogon flexuosus*. International Journal of Food Engineering **7(1),** 1-9. http://dx.doi.org/10.2202/1556-3758.2067

Kumari R, Agarwal SB, Sarkar A. 2009. Evaluation of changes in oil cells and composition of essential oil in lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) due to supplemental ultraviolet-B irradiation. Current science **97(8),** 1137-1142.