

## RAPD Characterization of Three Selected Cultivars OD-19, GRL-1 and Krishna of East Indian Lemongrass (*Cymbopogon flexuosus* Nees ex Steud) Wats

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**Abstract:** Three selected cultivars, two lemon scented OD-19 and Krishna and one rose scented GRL-1 of East Indian lemongrass (*Cymbopogon flexuosus* Nees ex Steud) Wats were characterized by RAPD markers. The genomic DNA isolated from fresh leaf tissues were amplified with 19 OPJ 10-mer arbitrary primers which produces an overall 60 scorable bands in the cultivars studied, 29 of which were polymorphic and 31 were monomorphic. Dendrogram constructed by cluster analysis of RAPD markers showed that cv. GRL-1 was very closely related with cv. OD-19 while Krishna slightly distant from cv. OD-19 than GRL-1. However, these cultivars differ greatly by their essential oil content and compositions. The cv. GRL-1 could be distinguished by the presence of high geraniol (92%) in essential oil from other two cultivars OD-19 and Krishna which are rich in citral 88 and 82 % respectively. Thus RAPD characterization of the lemongrass cultivars helped in their discrimination as morphological differences among them are almost blurred. Even if so, RAPD markers associated with essential oil quality could not be identified.

**Key words:** *Cymbopogon flexuosus* % Essential oil % GRL-1 % OD-19 % Krishna % PCR % RAPD

### INTRODUCTION

The genus *Cymbopogon* (Poaceae) is highly regarded due to their essential oil which is of high commercial value. It is reported to include about 140 species, 45 in India and more than 52 species in Africa [1]. Most of the *Cymbopogon* species on steam distillation of aerial parts produce essential oils rich in monoterpenes such as citral, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, geraniol, b-carophyllene, methyl heptenone, geranyl acetate and geranyl formate [2-4]. Palmarosa, (*Cymbopogon martinii*), lemongrass (*C. flexuosus*), citronella (*C. winterianus*) and jamrosa (*C. nardus* X *C. jwarancusa*) are the most important sources of commercial essential oils. *Cymbopogon flexuosus* (Nees ex Steud) Wats commonly known as East Indian lemongrass is tufted perennial grass with numerous stiff stems arising from short rhizomatous rootstock [5] which is characterized by citral [6-10]. Lemongrass oil is widely used in perfumery, cosmetics, soaps, detergents, confectionery and in synthesis of vitamin A. Several cultivars of *C. flexuosus* varying in essential yield and quality have been

developed by recurrent selection programs including those selected for the present study such as OD-19, GRL-1 and Krishna [10, 11]. Essential oils of the *Cymbopogon* species and cultivars have been abundantly investigated previously [3, 4, 10, 12-16]. Despite the significant variations in the essential composition, *Cymbopogon* species and cultivars are morphologically indistinguishable [2, 17]. The variation in the essential oils compositions has served as chemo-taxonomical marker to discern genetic diversity in the genus *Cymbopogon* but the taxonomic complications could not be completely solved using essential oil as the only taxonomic marker. Sangwan *et al.* [17] and Khanuja *et al.* [2] have introduced molecular marker such as RAPD to analyze genetic diversity in the genus *Cymbopogon*. Recently, Kumar *et al.* [18] have developed a set of simple sequence repeat markers from a genomic library of *Cymbopogon jwarancusa* for the identification of the species (including accessions) of *Cymbopogon*. In contrast, in our previous study we exploited polymorphism in the geranyl acetate esterase (GAE) isozymes for the identification of *Cymbopogon* sp. and cultivars [19].

The present study was undertaken for the assessment of the genetic diversity in three selected cultivars namely OD-19, GRL-1 and Krishna of East Indian lemongrass using RAPD technique. As a whole the study revealed that two entirely different cultivars, one lemon scented (cv. OD-19) and other rose scented (cv. GRL-1) are very closely related at the genetic level while cultivars Krishna and OD-19 having almost identical essential oil composition are genetically slightly distant from each other.

## MATERIALS AND METHODS

**Plant Material:** *Cymbopogon flexuosus* (Ness ex. Steud) Wats cultivars OD-19, GRL-1 and Krishna used in this study were grown in the Experimental Farm, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India.

**Gas Chromatographic (GC) Analysis of Essential Oils:** Essential oils isolated upon steam distillation of aerial parts (leaves) using mini Clevenger apparatus was subjected to gas chromatographic analysis using a Perkin Elmer 3920 B apparatus equipped with FID, stainless steel column (2 m x 3 mm) packed with 10% FFAP (free fatty acid phase) on chromosorb WAW (80-100 mesh) [3, 4].

**DNA Isolation and PCR Amplification:** DNA was isolated from fresh leaf tissues as per the procedure described previously [20]. The polymerase chain reaction was carried out in final volume of 25 µL containing 25 ng DNA, 0.6 U of *Taq* DNA polymerase (Perkin Elmer), 2.5 mM MgCl<sub>2</sub>, 100 µM each dNTPs and 5 pmol OPJ primers (Jennei, Bangalore). The DNA amplification was performed in the DNA Engine thermal cycler (MJ Research, USA) using the following conditions: complete denaturation (94°C for 5 min) and denaturation (94°C for 1 min) followed by 45 cycles of amplification

(94°C for 1 min, 35°C for 1 min and 72°C for 2 min) and the final elongation step (72°C for 5 min). All PCR products were separated on 1.8% (w/v) agarose gel containing ethidium bromide (0.5 µg ml<sup>-1</sup>). The gel was photographed with Image master VDS (Pharmacia).

**Data Analysis:** The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of similarity of Jaccard. The matrix of genetic distance was used for grouping the lemongrass cultivars based on the dendrogram constructed by UPGMA (unweighted pair group method with Arithmetic averages)

$$\text{Similarity Index} = \frac{2 \times \text{Number of common bands}}{\text{Total number of bands}}$$

## RESULTS

**Essential Oil Content and Composition:** The essential oil content and compositions of the three selected cultivars of East Indian lemongrass are presented in Table 1. As indicated in the Table 1, cultivars OD-19 and Krishna both had more or less similar essential oil content (%V/g fresh weight and %V/g dry weight) as well as essential oil composition. The GRL-1 had less essential content (%V/g fresh weight and %V/g dry weight) compared to cultivars OD-19 and Krishna. Moreover, GRL-1 had a unique essential oil composition dominated by the presence of very amount of geraniol.

**RAPD Analysis:** The RAPD patterns of genomic DNA of lemongrass cultivars GRL-1, OD-19 and Krishna were analyzed for polymorphism. 17 of 19 OPJ primers (Table 2) tested were able to amplify the genomic DNA and produced 60 scorable bands in the cultivars tested. 12 of the 17 primers produced 29 amplification products that were polymorphic. Thus, 55% of all primers tested

Table 1: Essential oil contents and compositions of the three selected cultivars, OD-19, GRL-1 and Krishna of East Indian Lemongrass (*Cymbopogon flexuosus* Nees ex Steud) Wats. Essential oils were isolated from fully expanded matured (50 d old) leaves upon steam distillation in Clevenger apparatus

<i>C. flexuosus</i> cultivars	Essential oil			Relative % in the oil			
	%V/FW	%V/DW	Geranyl acetate	Geraniol	Citral a	Citral b	Unknown
OD-19	0.88	2.10	0.43	1.73	51.04	39.01	7.79
GRL-1	0.45	1.30	1.75	92.30	0.83	0.55	4.57
Krishna	1.00	2.27	0.55	6.55	46.57	35.83	10.5

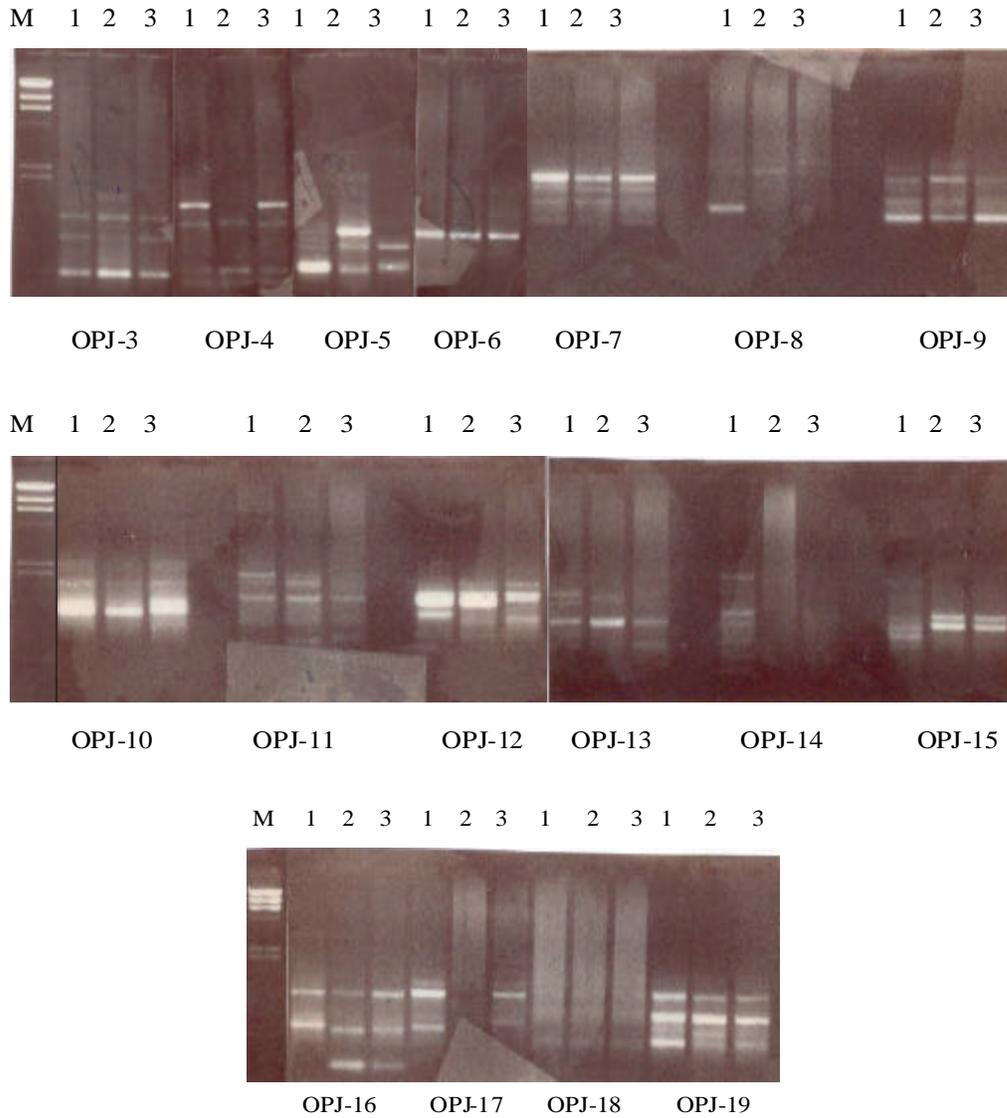


Fig. 1: RAPD profiles of three selected cultivars (1) OD-19 (2) GRL-1 and (3) Krishna of East Indian Lemongrass (*Cymbopogon flexuosus* Nees ex Steud) Wats obtained with OPJ primers (3-19)

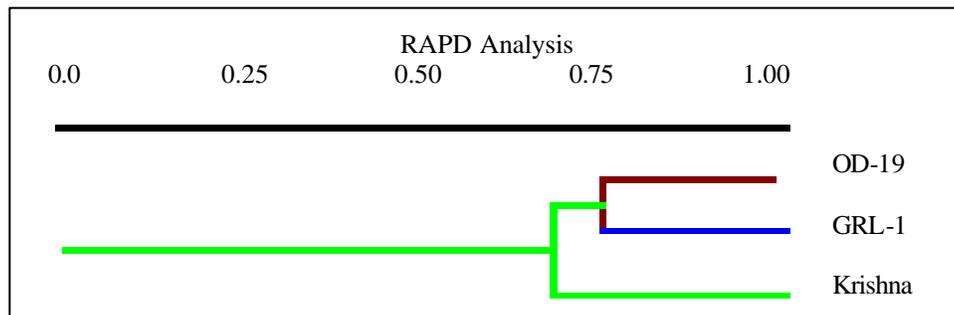


Fig. 2: Dendrogram showing genetic relationship among three selected cultivars, OD-19, GRL-1 and Krishna of East Indian Lemongrass (*Cymbopogon flexuosus* Nees ex Steud) Wats

Table 2: List of OPJ primers used for RAPD analysis and number of monomorphic and polymorphic bands obtained for each primer

Primer	Sequence (5'-3')	Numbers	
		Monomorphic bands	Polymorphic Bands
OPJ-1	CCCGGCATAA	1	3
OPJ-2	CCCGTTGGGA	-	-
OPJ-3	TCTCCGCTTG	3	1
OPJ-4	CCGAACACGG	3	3
OPJ-5	CTCCATGGGG	2	5
OPJ-6	TCGTTCCGCA	1	-
OPJ-7	CCTCTCGACA	4	-
OPJ-8	CATACCGTGG	2	1
OPJ-9	TGACGCTCAC	3	1
OPJ-10	AAGCCCGAGG	3	-
OPJ-11	ACTCTGCGA	2	2
OPJ-12	GTCCCGTGGT	-	6
OPJ-13	CCACACTTACC	2	2
OPJ-14	CACCCGGATG	-	-
OPJ-15	TGTAGCAGGG	1	2
OPJ-16	CTGCTTAGGG	2	2
OPJ-17	ACGCCAGTTC	-	-
OPJ-18	TGGTCGCAGA	-	-
OPJ-19	GGACACCACT	4	-

Note: Primer OPJ-14 failed to produce scorable bands in cv. OD-19 and Krishna while OPJ-17 failed in cv. OD-19

detected polymorphism for an average of 1.5 polymorphic bands per primer tested. Other 5 primers produced 31 mono-morphic bands (Table 2). The primer OPJ-14 that failed to produce scorable results in two cultivars OD-19 and Krishna was however, successful in the cultivar GRL-1. The primer OPJ-17 however, failed to produce scorable results in cv. OD-19. Figure 1 represents RAPD profiles obtained with 17 different primers of the three selected cultivars studied here. The average number of bands per genotype ranged from 4 to 9. Cultivar OD-19 could easily be distinguished from cultivars GRL-1 and Krishna on the basis of unique banding pattern obtained with primer OPJ-05. The dendrogram (Fig. 2) generated by cluster analysis of RAPD polymorphism indicated that GRL-1 is much closely related with cv. OD-19 while Krishna slightly distant from cv. OD-19. However, cv. Krishna is very closely related to GRL-1.

## DISCUSSION

RAPD is routinely used technique for deciphering genetic relationship among species cultivars and accessions. RAPD markers are highly efficient in

evaluation of genetic similarities and differences between cultivars and accessions even when a morphological description is blurred. Moreover, they can produce multi-locus profiles widely spanning the genome even in the absence of any prior genetic/ sequence information. Therefore we employed RAPD technique for the study of genetic diversity at the intra-species using three selected cultivars OD-19, GRL-1 and Krishna of the East Indian lemongrass (*C. flexuosus*). Two of the tree cultivars OD-19 and Krishna had almost similar essential oil composition, but both they differ broadly from cv. GRL-1 in essential oil composition. The RAPD profiles obtained with various OPJ-primers have shown that cv. GRL-1 closely resemblance to cv. OD-19 though GRL-1 did not match either of the cultivars OD-19 or Krishna with respect to the essential oil composition. Furthermore, cv. Krishna which was almost similar in essential composition to that of cv. OD-19, however, they were genetically slightly distant as shown in dendrogram (Fig. 2). The cv. GRL-1 was isolated from citral rich cv. OD-19 during the study of chemo-genetical improvement [10] hence genetic resemblance of cv. GRL-1 with OD-19 was expected. I has been presumed that cv. GRL-1 is deficient in the enzyme alcohol dehydrogenase which oxidizes geraniol into geranial (citral a) but not in cv. OD-19 and Krishna [4], therefore a broad variation were seen in the essential oil compositions of the selected cultivars. The numbers of amplification fragments produced per primer as well as their size ranged were analytically appropriate confirming to those recorded with certain other *Cymbopogon* grasses [2, 17]. The lower level of polymorphism in lemongrass cultivars tested here is expected as chemo-typically they display far less variability in essential oil composition. The results of RAPD analysis presented here are slightly different to the results of our previous study on geranyl acetate esterase isozymes polymorphism in *Cymbopogon* species and cultivars [19]. The GAE isozymes polymorphism clearly helps in precisely identification of lemongrass cultivars. However, the expressions of isozymes often vary in different tissues, developmental stages and environment [21].

In conclusion, RAPD profiles have been proven to be a very useful tool in assessment of genetic diversity at the intra-species level (in cultivars) in the three representative selected cultivars of East Indian lemongrass. The RAPD cluster analysis revealed marked similarities among these cultivars indicating that they have a single source of origin no matter whether they have likely or unlikely essential oil composition.

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