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Genetic diversity analysis in *Cymbopogon* species using DNA markers

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Abstract

Genetic diversity of 25 accessions of *Cymbopogon* aromatic grasses including eight species, two hybrids and one mutant strain were analyzed using DNA markers generated by employing 20 primer pairs derived from cDNAs containing simple sequence repeat (SSR) of rice genome. A total of 151 bands were produced ranging from 3 to 12 per primer pair. The polymorphic information content values varied from 0.143 to 0.916 with an average 0.715. Jaccard's similarity coefficient ranged from 64 to 87% among the paired accessions. The level of diversity among different taxa/accessions observed during the present study was, however, low relative to the diversity level obtained due to RAPD markers in earlier studies. The pattern of genetic diversity neither matched with the known taxonomic classification, nor did it always match with the distribution of chemical constituents of the essential oils available in these accessions. Thus, present investigation though revealed poor correlation between the molecular and chemical diversity, indicating that chemical diversity in medicinal and aromatic species is not only result of genetic variability, but it also depends on a number of other factors. Thus this study may prove useful in several ways in *Cymbopogon* conservation and breeding programs and in the development of perfect markers though association mapping for genes involved in controlling agronomically important traits.

Keywords: *Cymbopogon* aromatic grasses; monoterpenoids; molecular markers; DNA polymorphism; genetic diversity and phylogenetic relationship; expressed sequence tag-simple sequence repeats (EST-SSRs).

Introduction

Cymbopogon is one of the most important essential oil yielding genera of the Poaceae. The genus comprises ~140 species that are widely distributed in semi-temperate to tropical regions of Asia, Africa and America. Approximately 45 species have been reported to occur in India. The *Cymbopogon* species that produce volatile oils are called aromatic grasses (Rao, 1997). Different types of essential oils, such as palmarosa oil, lemongrass oil, citronella oil and ginger grass or rusa oil, are very popular in perfumery (Rao, 1997; Sangwan et al. 2001a). *Cymbopogon* species display wide variation in morphological attributes and essential oil composition at inter- and intraspecific levels (Rao, 1997). Knowledge of

germplasm diversity is important for plant conservation and improvement, therefore there is interest in determining the genetic diversity in *Cymbopogon* germplasm.

Although, morphological traits can be used to assess genetic diversity they are strongly influenced by environment conditions and show little variation at the intra-specific level (Stapf, 1906; Sharma et al. 2000; Sangwan et al. 2001a). Therefore, molecular markers are often used to obtain more reliable estimates of genetic diversity. Amongst them, RAPDs have been widely employed for the identification of cultivars (Sangwan et al. 2001a), ancestors (Shasany et al. 2000), and discerning the genomic diversity (Sangwan et al. 2001a; Khanuja et al. 2005) in *Cymbopogons*. However, RAPDs have some limitations as molecular markers, the most important being poor reproducibility of the profiles across the laboratories (Marmiroli et al. 1998; Sangwan et al. 2001a).

Simple sequence repeats (SSR) markers have features that make them particularly suitable for assessing the genetic diversity among cultivated lines or germplasm including that they are co-dominant, multiallelic, locus specific and highly reproducible (Rafalski and Tingey, 1993; Jones et al. 1995; Smith et al. 1997). Comparative genetic analyses have shown that different plant species share orthologous genes for similar function and have highly conserved gene content and gene order (Devos and Gale, 2000). Since expressed sequence tag-simple sequence repeats (EST-SSRs) are derived from the transcribed portion of the genome, they are expected to be more conserved compared to anonymous/random sequences (Scott, 2001; Eujayl et al. 2002; Thiel et al. 2003). Therefore, EST-SSRs possess considerable potential not only for genome mapping within species but also for comparative studies between species (Cordeiro et al. 2001; Thiel et al. 2003; Bandopadhyay et al. 2004; Varshney et al. 2004). Moreover, EST-SSR markers are known to provide better estimates of functional diversity existent in a gene pool (Bandopadhyay et al. 2004). Hence, it would be of interest to explore the feasibility of using EST-SSRs from related model grass species like rice for the genomic analyses of less studied grass species such as *Cymbopogon* aromatic grasses. Accordingly, in this study, eight species (selected on the basis of availability of seed, their adaptation to local production areas and their commercial importance), two cultivated hybrids and one mutant of *Cymbopogon* volatile oil grasses, were investigated for genetic diversity analyses using EST-SSR markers from rice EST database.

Materials and methods

Plant material and Chemicals

Twenty-five accessions belonging to 8 species of *Cymbopogon* (Table 1) were collected from different geographical zones of India, transplanted through tussocks (slips) in experimental beds at Field Research Station of Regional Research Laboratory, Jammu, (India). Different chemotypes/varieties were either phenotypically selected from vegetatively raised progenies or bred by crossing between cultivars or closely related species. Ten plants of each accession were sampled randomly for analyzing their essential oils and DNA polymorphism profiling. Twenty primer pairs from rice microsatellite loci (available on http://www. dna-res. kazusa. or.jp /3/ 4/ 02/HTMLA/) as described by Miyao et al. (1993) were used for this study. These primers, presented in Table 2, belonged to cDNA/EST derived SSR loci distributed over 8 different chromosomes of rice.

DNA isolation

Genomic DNA was isolated by grinding the pooled leaves of the 10 plants of each accession according to the method reported by Prabhu et al. (1998). The concentration and purity of the genomic DNA was determined with a Perkin Elmer spectrophotometer.

Extraction and analysis of essential oils

The essential oils extracted from pooled aerial parts (collected in the month of January) of the 10 randomly selected plants per accession were analyzed following the methods described by Sharma et al. (2000). Essential oil isolation and analysis was performed using three independent replicates of each accession. The mean oil yield and composition of the 3 replications computed. The oil composition was presented as relative proportion (%) of the constituents.

PCR amplification and fragment analysis

Polymerase chain reaction (PCR) to amplify the SSR loci was performed in a 20 µl assay mixture

Table 1. Details of *Cymbopogon* species and their accessions subjected to diversity analyses

Species	Accessions	Method of Development	Collection site in India	Major chemical constituent (s)*
A. SERIES *Scheonanthi*				
1) *C. jwarancusa* (Jones) Schult 2n=20	Rajasthan	Site collection	Rajasthan, India	Piperitone (68-70)
	Kashmir	Site collection	Kashmir, India	Piperitone (72-75)
B. SERIES *Rusae*				
2) *C. martinii* (Roxb.) Wats var. *motia* 2n=20, 40	Wild	Site collection	Madhya Pradesh, India	Geraniol (80-85)
C. SERIES *Citrati*				
3) *C. flexuosus* (Steud.) Wats 2n=20,40,60	RRL (J)-CF-PTK-W	Site collection	Punjab, India	Citral (80-82)
	RRL (J)-CF-PTK-1	Phenotypic selection	RRL,Jammu, India	Citral (84-86)
	RRL (J)-CF-HP-W	Site collection	Himachal Pradesh India	Geraniol (30-32) + Neointer-medeol (18-20) + ∝–bis-abolol (6-7)
	RRL (J)-CF-HP-1 (CT)	Phenotypic selection	RRL, Jammu, India	Geraniol (30-32) + Neointer-medeol (23-25) + ∝–bis-abolol (9-10)
	RRL (J)-CF-HP-1(CM)	Phenotypic selection	RRL, Jammu, India	Geraniol (30-32) + Neointer-medeol (23-25) + ∝–bis-abolol (9-10)
	RRL (J)-CF-HP –2	Phenotypic selection	RRL, Jammu, India	Geraniol (30-32) + Neointer-medeol (23-25) + ∝–bis-abolol (9-10)
	RRL(J)-CF-100	Site collection	Southern forest of Kerala, India	∝–bis-abolol (30-35)
	RRL (J)-HSR	Phenotypic selection	Haryana, India	(+)-1-bisabolone (35-40)
	OD-19	Phenotypic selection	LRS, Oddakali, Kerala, India	Citral (80-85)
	Pragati	Phenotypic selection	CIMAP, UP, India	Citral (80-85)
	JorlabL2	Phenotypic selection	RRL, Asam, India	Citral (80-85)
4) *C.citratus* (DC.) Stapf. 2n=20, 40	RRL (J)-CCA-W	Site collection	Madhya Pradesh, India	Citral (72-75)
	RRL (J)-CCA_{12}	Phenotypic selection	RRL, Jammu, India	Citral (80-85)
5) *C. commutatus* (Steud) Stapf 2n=20, 40	RRL (J)-CC-W	Site collection	Jammu, India	Geraniol (35-40) + geraniol acetate (25-30)
	RRL (J)-CC-I	Phenotypic selection	RRL, Jammu, India	Geraniol (75-80) + geraniol acetate (30-35)
6) *C. pendulus* (Nees ex Steud.) Wats 2n=60	RRL (J)-16	Site collection	Sub-Himalayan regions of India	Citral (75-80)
7) *C. nardus* var. *conferti flor- us* (Steud.) Stapf 2n=20, 40	RRL (J)-CN-5	Site collection	Himachal Pradesh, India	Geraniol (75-80) + geraniol acetate (30-35)
	RRL (J)-CN-17	Phenotypic selection	RRL, Jammu, India	Geraniol (80-85) + geraniol acetate (30-35)
8) *C. winterianus* Jowitt 2n=20	Java citronella	Site collection	Assam, India	Geraniol (26-30), Citronellol (24-28) + Citronellal (10-15)
9) Hybrids (*C. khasianus* × *C. pendulus*)	CKP-25	Hybridization	RRL, Jammu, India	Citral (80-85)
	$F_2$38	Hybridization	RRL, Jammu, India	Citral (75-80)
10) Mutant Jamrosa (*C. jwar- a ncusa* × *C. nardus* var. *confertiflorus)*	RRL (J)-931	Mutation	RRL, Jammu, India	Geraniol (75-78)

*Percentage of chemical constituents is given in parenthesis

consisting of 30ng genomic DNA, 1× PCR buffer (with 15 mM $MgCl_2$), 200 µM of each of dATP, dTTP, dGTP and dCTP (Banglore Genei, India), 0.2 µM of each forward and reverse primer (Sigma, USA) and 1U *Taq* polymerase (Banglore Genei, India). The thermal cycling conditions in an Eppendorf Mastercycler were: 3 min at 94^0C followed by 39 cycles amplification (each cycle comprising 94^0C for 30s, annealing for 60s, 72^0C for 90s) and a final extension for 10 min at 72^0C. The annealing temperature employed for PCR with each primer pair is given in Table 2. The amplified products were resolved by electrophoresis in a 2.5% agarose gel run at 150V for 45 minutes.

Data analysis

The amplicons obtained with different microsatellite primers were scored as present (1) or absent (0) in for all of the accessions in the study. The number of alleles per locus was determined and the polymorphic information content (PIC) values were calculated using the following formula, as suggested by Cordeiro et al. (2001)

$PIC = 1-\Sigma P_{ij}^2$

Where P_i is the relative frequency of j^{th} allele for i^{th} marker, and summed over n number alleles. The calculations were based on the number of alleles /primer pair. Genetic similarities were computed for genetic diversity assessment and cluster analysis. The data in binary format were used to compute pair wise similarity coefficient, utilizing the SIMQUAL (similarity for quantitative data) method in NTSYS-pc software. A similarity coefficient matrix was developed for phylogenetic analysis with UPGMA using NTSYS-PC software (Rohlf, 1992)

Results and discussion

Selection of primers and DNA polymorphism analysis

Rice is considered the best genetic resource to serve for genomic analyses of neglected or underutilized /less studied grass species. Hence, in this investigation, the primer pairs that flanked the various SSR motifs in the transcribed regions (cDNAs) of the rice genome were selected for profiling the genetic similarity-dissimilarity in the *Cymbopogon* aromatic grasses. The SSR motifs of these regions have been shown to be polymorphic and often mapped on the 5'-untranslated region near the initiation codon in the rice cDNA sequences, which encode proteins important for the maintenance of plant growth (see Miyao et al. 1993). In addition, these transcribed regions are conserved across the genera having large differences in genome size and chromosome number (Devos and Gale, 2000; Gutierrez et al. 2005). Each primer pair resulted in successful amplification suggesting the usefulness of recruitment of cDNA/EST-SSR markers for analyzing and understanding genetic diversity in the grass species. A representative of amplification profile is presented in Fig 1.

Although each of the selected primer pairs indicated transferability of rice SSR markers across the genera, it could not be sequence-confirmed whether or not the amplicons contained SRR motif. It has been suggested that even though rice SSR markers generate the amplicons in other grass genera but all amplicons may not contain SSR motifs (Wang et al. 2005). All amplicons generated by a primer pair have been used as DNA markers, despite higher transferability of EST-SRR across the genera/species (Cordeiro et al. 2001; Thiel et al. 2003; Varshney et al. 2004).

In the 25 accessions of different *Cymbopogon* species, the 20 primer pairs produced a total of 151 amplicons, with number of amplicons per primer pair ranging from 3 to 12 and an average of 7.6 (Table2). Out of these, 122 (~81%) loci were found polymorphic. The PIC values displayed by different primer pairs individually ranged from 0.143 to 0.916. As nineteen of the 20 primer pairs, gave a PIC value of more than 0.5, they could be classified as very informative (Table 2). The observed high proportion of polymorphic loci suggested that there was profound genetic heterogeneity at inter-species and intra-species level, as also previously noted (Sangwan et al. 2001a).

The size of these amplicons also differed from the original amplicons (from primer-designed species). Wang et al. (2005) reported earlier that transferable genomic-SSR or EST-SSR markers generate amplicons of different size and number due to the cross-genus or cross species differences in gene order and content. Thus polyploid nature of *Cymbopogon* species, which can differ in sequence content from rice has resulted similar results in our case.

Genetic relationships

Of the 45 *Cymbopogon* species found in India, only few like lemongrass, palmarosa and citronella types are cultivated for commercial production of essential oil for the industry. Conventional breeding efforts have been made for improvement of the cultivars belonging to several species of *Cymbopogon* but these efforts did not involve assessment and consideration of genetic diversity for the selection of parents. Knowledge of molecular marker aided genetic diversity profiles, parallel to morphological and biochemical relatedness and differences among the *Cymbopogon* species (Sharma et al. 2000), could offer added advantages of strategic combination of traits and exploitation of the germplasm diversity. In order to establish genetic relationships between the

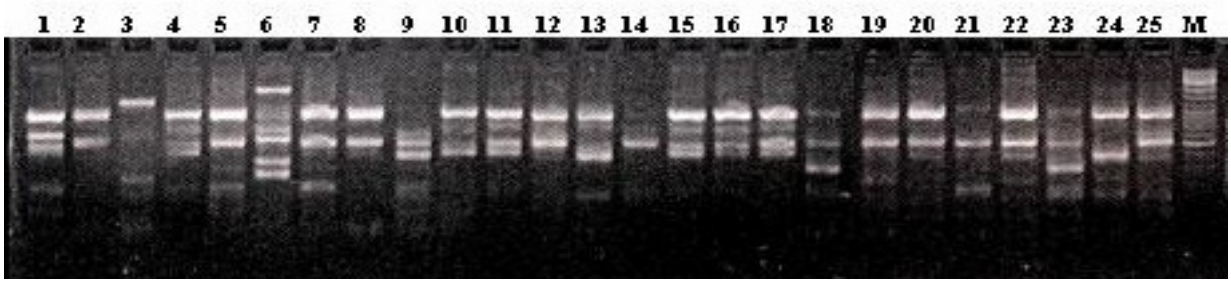

Fig 1. A representative of amplification profile of *Cymbopogon* accessions generated by primer pair CM2930. Lane 1-25: Java citronella RRL (J)-CF-PTK-1, *C. jwarancusa* (Kashmir), RRL (J)-CF-HP-1 (CT) , RRL (J)-CCA-W, RRL (J)-16, *C. martinii*, RRL (J)-931 $F_2$38, RRL(J)-CF-100, RRL (J)-CF-HP –2, RRL (J)-CC-1, RRL (J)-CN-17 , *C. jwarancusa* (Rajasthan), RRL (J)-CF-HP-W, RRL (J)-CF-HP-1(CM), RRL (J)-HSR, RRL (J)-$CCA_{12,}$ OD-19, Pragati, JorlabL2, RRL (J)-CF-PTK-W, CKP-25, RRL (J)-CN-5, RRL (J)-CC-W. Lane M: 1 kb DNA marker.

Cymbopogon accessions, genetic similarity was calculated using Jaccard's similarity coefficient, which ranged from 64 to 87%. The values were higher than those previously reported on the basis of phytochemicals (3.12 to 75 %) and RAPDs (38 to 70%) in these species (Sharma et al. 2000; Khanuja et al. 2005). This is probably because markers derived from functional regions generate higher genetic similarity owing to higher stringency of conservation of functionality (Bandopadhyay et al. 2004). The GS values based clustering of 25 accessions of *Cymbopogons* resulted in two major clusters, cluster-I comprising of 24 accessions and cluster-II having a solitary accession of *C. nardus* var. *confertiflorus* (Fig 2). Cluster-I was divided into two sub-clusters (i.e. sub-cluster-Ia comprising 21 accessions and sub-cluster-Ib represented by only 3 accessions pertaining to different species). The 21 accessions of sub-cluster-Ia were further divided into two groups, one with 20 accessions of *C. flexuosus* and other with a solitary accession of *C. martinii* belonging to series *Rusae*. The *C. flexuosus* group contained several clusters having accessions of either same or other species. This group also contained as solitary sub-cluster of accession belonging to '*Scheonanthi*' series (Fig 2).

Genetic relationships in relation to chemical groupings

In *Cymbopogons,* holistic essential oil chemical compositions share significant qualitative commonality across the species (Rao, 1997; Sangwan et al. 2001b). On the basis of chemical similarities for the major chemical constituents (geraniol, geraniol acetate, citral, piperitone and citronellol), present accessions were grouped into three different phytochemical/monoterpene groups and matched with genetic clusters developed on the basis of DNA marker profiles, assuming that these markers have resolved a part of functional diversity available in the *Cymbopogon* genome (Bandopadhyay et al. 2004). The genus *Cymbopogon* is divided into three Series (Citrati, Rusae and Schoenanthi) on the basis of constituents of essential oils derived from them (Table 1; Stapf, 1906). However, the circumscription of individual species is largely based on morphology (Stapf, 1906). It can be noticed from the dendrogram that the accessions belonging to *Citrati* series formed a large group that was interrupted by the accessions of other series forming solitary groups (Fig 2). For example, the accessions of *C. jwarancusa* (rich in piperitone) analyzed in this study belonging to series *Scheonanthi* collected from different agroclimatic conditions of India (Dhar et al. 1981; Shahi and Sen, 1989) did not cluster together but instead showed closer relationships with chemically different species of series *Citratti* (*C. nardus* var. *confertiflorus* or *C. flexuosus*). Similar results also observed in an earlier study based on RAPD analysis (Khanuja et al. 2005).

A cross compatibility between these species belonging to different series (*C. jwarancusa* vs. *C. nardus* var. *confertiflorus* and *C. jwarancusa* vs. *C. flexuosus*) might be responsible for closer genetic relationship observed.

For studying the interspecific relationships according to chemical composition of essential oils, we have examined six species of *Citrati* series and found that

Table 2. DNA marker information, distribution and polymorphic information content (PIC) of 25 *Cymbopogon* accessions

Primer pair code	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing Tm (^{0}C)	Marker size ranged (bp)	Total markers	Polymorphic markers	PIC
CM0102	TGCACGGGGAAGAGGAGAGA	AACCGAAGCGCAAGAACCCA	60	318-1095	7	6	0.715
CM0304	ACCCCAGCGCGATCCGAGGT	AGCGGCGTCTCCAGCCCGAA	60	565-855	8	7	0.776
CM0506	CGCCGAAGTGGTAGAGGCAA	TGCAGGAGGCAGGAGGAGAA	60	564-984	7	6	0.716
CM0910	CGCCGCCGTACTGCTCCATC	GCGGAGGAGACCTGCGGGT	60	413-1065	7	6	0.785
CM1112	GAATCCGATCCATCCATTGG	CACGAACACGCGACGCACGA	60	499-1873	4	4	0.712
CM1314	ACTTTGCGTGCGAGGCGAGG	CGAGGTCGAGTAGAAGGCGT	60	973-1870	10	7	0.846
CM1516	ACTTTGCGTGCGAGGCGAGG	CGAGGTCGAGTAGAAGGCGT	60	222-1317	8	4	0.753
CM1718	CTCGCCGTCGAATCCGCCAT	CACTCTCCTCTCCTGGCCCG	60	299-2333	8	8	0.822
CM1920-	CCCAATTTTACGCTAAACCC	CACTCTCCTCTCCTGGCCCG	55	607-889	8	6	0.790
CM2122	CTCCCCCTCCTCCTCCTCCC	CGACCGGCCGGAATGGATGC	60	436-630	9	8	0.808
CM2324	GTTTCTCACGTCTCTCGCTG	TCCTCCTCCTACGGCTTCTC	55	189-1147	11	10	0.823
CM2728	ATCTCTTCGCAGATCCACCT	GCTGAGACGCGCGGGTCGGA	60	228-803	12	11	0.916
CM2930	TTTATCCGCGTCCCTAGCTT	GCCGCCGGGGTCACAGGTCA	60	149-1736	11	9	0.791
CM3132	GAGAGGATTCCGATACCCTT	TCGGCCTCTCGCCCCCCGA	55	533-1211	5	4	0.529
CM3334	CGTGCTCGTGGATCCCCATC	CACCGTCGAATCGAATCCAA	60	973-1212	6	5	0.726
CM3536	GACACCACCAGGTTGGCTCC	TGGCTCCTCTCGGGTGACGGA	60	105-1011	9	9	0.814
CM3738	ATATATCCAGCCAGCCGCAT	GGGCCGTGCCGTGCCTCACC	60	170-785	8	5	0.683
CM3940	CTCCTCGATCTTCCTCTACT	TCGACCCCATCACAAATCCA	55	204-467	5	2	0.376
CM4142	CCTCTGTTTCGCCTCCTAGT	CCGGCCTGACCGACTAACTG	55	240-951	5	5	0.770
CM4344	GGGATGATCATCTCCGATGC	CCCTTCTCCCACTCTTCTCC	55	393-663	3	0	0.143
	Total				151	122	14.294

the two citral-rich accessions of *C. citratus* grouped with several accessions of *C. flexuosus*. These two species are also close on the basis of other features like morphology, chemical constituents, geographical distribution and RAPD analysis (Sharma et al. 2000; Sangwan et al. 2001a; Khanuja et al. 2005). It is also known that *C. winterianus* is a major source of citronellol and differs from *C. nardus* var. *confertiflorus*, which is rich in geraniol, rather than in citronella. This species clustered with the mutant strain of Jamrosa (*C. jwarancusa* × *C. nardus* var. *confertiflorus*), which is rich in geraniol and contains citronellol in traces amounts (Kak et al. 2000). Thus, both on the basis of essential oil profile and molecular marker analysis, *C. winterianus* grouped uniquely, although this species is known to have ancestral relationship with *C. nardus* var. *confertiflorous* (Bor, 1960; Shasany et al. 2000). On the basis of dendrogram, *C. nardus* var *confertiflorous* seems to be closely related to *C. commutatus*, both taxa being rich in geraniol and geraniol acetate, and both being clustered together in the present study (Fig 2). It also showed close relationship with the accessions of *C. flexuosus* having geraniol as main chemical constituent in the volatile oil. These genetic relationships corroborate the observations of similarity made on the basis of morphological parameters (Sharma et al. 2000).

Two cultivated hybrids (CKP25 and $F_2$38) of common parentage derived from a cross between *C. khasianus* and *C. pendulus* (Rao et al. 1992; Anonymous, 2003) have citral rich essential oil.

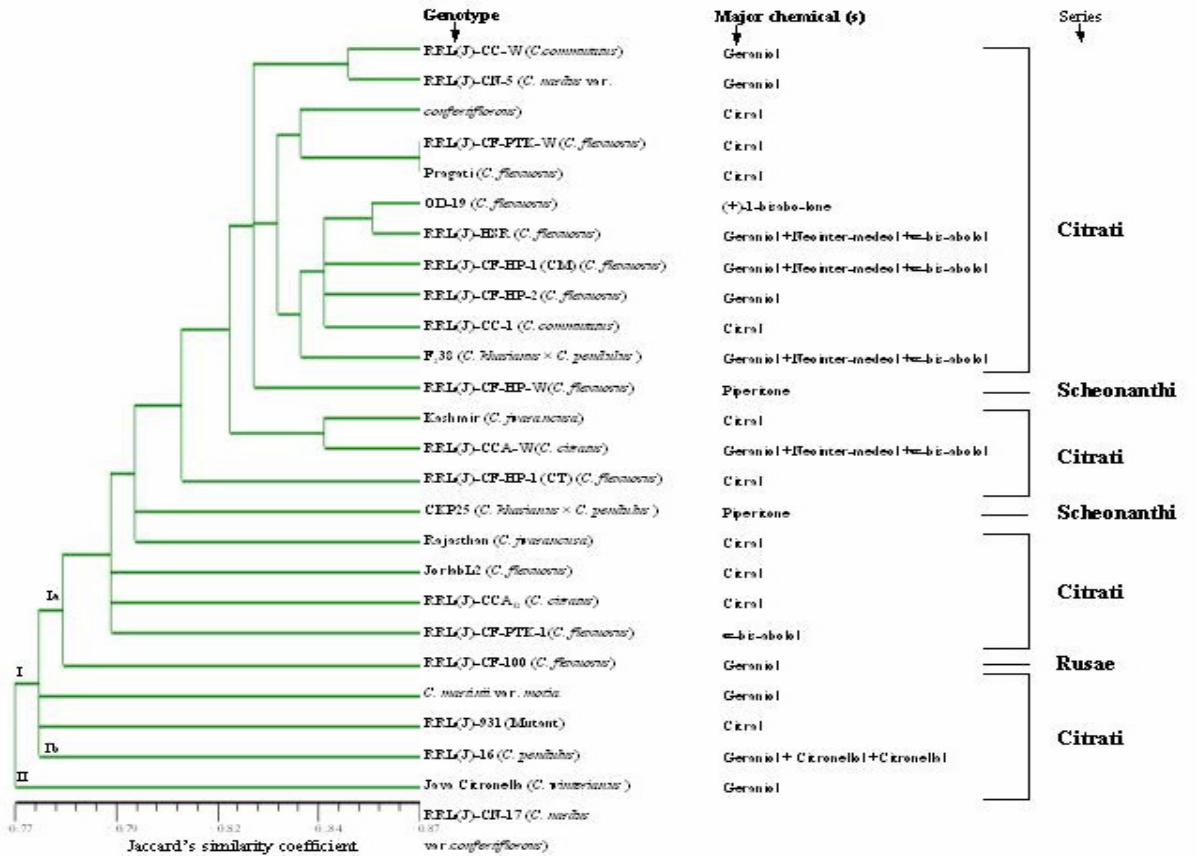

Fig 2. The dendrogram (UPGMA method, NTSYS generated) representing genetic relationships amongst 25 accessions of *Cymbopogon* species based on 151DNA markers.

Of them CKP25 has been reported as the most consistent-performing hybrid cultivar compared to $F_2$38 (Bhan et al. 2005). In present study, these two varieties also discerned genomic differences on basis of molecular markers derived from rice genes involving in maintenance of growth. Because such genes have shown to be conserved across the genera and hence it might be possible that these differences have been resolved from similar genes. Thus a better consistency of CKP25 over the $F_2$38 clearly indicates that this variety carries certain different useful genes for breeding new consistent-performing varieties as reported earlier on the basis of phenotypic observations (Bhan et al. 2005).

Lemongrasses possess the most diversified oil composition in both wild types as well as in cultivars under domestication (Sangwan and Sangwan, 2000; Sangwan et al. 2001a; Sangwan et al. 2001b). Thus, the improved cultivars having a particular kind of economically important chemical constituent, other than citral, in their essential oils have been developed in cultivated species - *C. flexuosus*, *C. commutatus*, *C. nardus* var. *confertiflorus*, *C. citratus,* and *C. jwarancusa*. The results pertaining to these species (except *C. pendulus*) for genetic relationships at the intraspecific level revealed that only, a part of the diversity pattern conformed to the history of the accessions and the chemical constitution of these accessions. For instance, the accessions Pragati and OD-19 belonging to *C. flexuosus* clustered together, as one would expect from the fact that Pragati was selected from the progeny of OD-19 and that both these accessions are rich in citral (Sharma et al. 1997). However, contrary to expectation, in majority of other cases involving the above five species, accessions of the same species clustered with

accessions of other species with different chemical constitution. It can be seen in case of accessions belonging to *C. flexuosus*, which were found scattered in several sub-clusters such as one citral-rich genotype (JorlabL2) grouped with piperitone-rich *C. jwarancusa* in a sub cluster, and the remaining 8 accessions with diverse chemical constitution grouped either with three other species (citral-rich *C. citratus*, and geraniol-rich *C. commutatus*) or with two citral-rich accessions developed by hybridization of the *C. khasianus* and *C. pendulus* (Anonymous, 2003).

Thus no matching between molecular and chemical groupings was observed in present study, although an earlier study based on RAPD analysis showed groupings of *Cymbopogon* cultivars in accordance with three oil trade types (citronella, palmarosa and lemongrass) of *Cymbopogon* grasses. The use of locus specific EST-SSR markers in this study probably discerned genomic differences only from those genes that are not directly responsible for biosynthesis of phytochemicals present in these accessions. However, an accession (RRLCN-17) of *C. nardus* var. *confertiflorus* pertaining to lemongrass group has been placed distantly from the rest of the accessions including those that belonged to palmarosa grass (*C. martinii var. motia*) and citronella grass (*C. winterianus*). These findings suggest that species of lemongrass group have higher molecular diversity (genetically highly heterogeneous in nature) compared to other grasses like palmarosa, citronella that makes this group chemotypically most diverse than the other two groups (Sangwan et al. 2001a; Sangwan et al. 2003). However in some of other cases, the results did not match even with the known history, despite similar chemical constitution. For instance, RRL (J)-CF-HP1 (CT), did not group with its ancestor accession, RRL (J)-CF-HP-W, although both were rich in geraniol, neointer-medeol and α-bisabolol, rather it grouped elsewhere. Similarly, two citral-rich accessions of *C. flexuosus* [RRL (J)-PTK-W and RRL (J)-PTK-1] did not cluster together. Thus no clustering between the cultivated and wild (from which cultivated accessions were developed through phenotypic selection) accessions has been observed in these cases. It is understandable because a population of wild accession was raised from a number of slips collected from different places of a particular region of India, and previous reports indicate that natural population of a *Cymbopogon* species particularly *C. flexuosus* has a lot of outcrossing-mediated heterozygosity (Sangwan et al. 2001).

Moreover, accessions belonging to *C. flexuosus* had the very different phytochemicals [RRL(J)-HSR and RRL(J)-CF-HP-1 (CM)] despite to higher similarity (0.86) compared to those[RRL(J)-HSR and RRL(J)-CF-100] that had very similar phytochemicals but with less genetic similarity (0.77). These inconsistency between the molecular and chemotypic diversity observed among the accessions of lemongrass species of present study suggests that genotype and environment interactions also led the diversification of chemical constituents, other than genotypic differences (Sangwan et al. 1993; Sangwan et al. 1994; Shasany et al. 2000; Trapp and Croteau, 2001; Sangwan et al. 2001b). As can be seen that this study is based on limited number of molecular marker, which thought to be resolved a part of functional diversity derived or not derived from genes governing phytochemicals and hence further analysis with more number of functional markers particularly from genes involving in biosynthetic pathways of phytochemicals can give better picture of genetic relationships among the accessions of *Cymbopogon* in relation to phytochemicals.

In conclusion, the study emphasizes the utility of DNA markers, particularly EST-SSRs profiled using the functional genome information of taxonomically close counterpart (driver), like rice for grasses, in elucidating genetic relationships among the tester species and would helped to know insights of the genomic structure of a neglected *Cymbopogon* species. The genetic diversity scenario presented for the *Cymbopogon* species and variants therein is not only valuable for conservation of their germplasm but would also be useful for breeding new or novel varieties/chemotypes of *Cymbopogons*. The sets of such chemotypes are immensely important genetic materials for developing recombinant inbred lines (RIL), like those available for maize, and understanding inheritance patterns of biosynthetic pathways or specific metabolic steps therein.

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