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GENETICS AND GENOMICS

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# Molecular analysis of genetic diversity in elite Indian cultivars of essential oil trade types of aromatic grasses (*Cymbopogon* species)

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**Abstract** Eleven elite and popular Indian cultivars of *Cymbopogon* aromatic grasses of essential oil trade types – citronella, palmarosa and lemongrass – were characterized by means of RAPDs to discern the extent of diversity at the DNA level between and within the oil biotypes. Primary allelic variability and the genetic bases of the cultivated germplasm were computed through parameters of gene diversity, expected heterozygosity, allele number per locus, SENA and Shannon's information indices. The allelic diversity was found to be in the order: lemongrass > palmarosa > citronella. Lemongrasses displayed higher (1.89) allelic variability per locus than palmarosa (1.63) and citronella (1.40). Also, RAPDs of diagnostic and curatorial importance were discerned as 'stand along' molecular descriptors. Principal component analysis (PCA) resolved the cultivars into four clusters: one each of citronella and palmarosa, and two of lemongrasses (one of *C. flexuosus* and another of *C. pendulus* and its hybrid with *C. khasianus)*. Proximity of the two species-groups of lemongrasses was also revealed as they shared the same dimension in the three-dimensional PCA. The molecular distinctions are discussed in relation to oil-chemotypic variations.

**Keywords** Aromatic grasses · *Cymbopogon* · Essential oil crops · Genetic diversity · RAPD

**Abbreviations** *FPI*: Fragment prevalence index · *PCA*: Principal component analysis · *PIC*: Polymorphic information content · *SENA*: Sum of effective number of alleles

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## Introduction

Plant-derived essential oils form the basis of many large chemical, pharmaceutical and perfumery industries and make up a significant proportion of the agro-chemical trade worldwide. Chemically, the oils are diverse mixtures of terpenes and/or phenylpropenes (Sangwan et al. 2000). Essential oils of the *Cymbopogon* species (commonly known as aromatic grasses) have become profitable export products for many developing agrarian nations. Their cultivation in terms of area cultivated has rapidly expanded during the past few decades. While some elite cultivars for the aromatic grasses have become available, genetic resource support to the development of cultivars has remained limited compared to the situation in traditional crops where enormous genetic resource knowledge and even saturated linkage maps have become available.

*Cymbopogons* are highly stress-tolerant plants that are adapted to diverse edapho-climatic conditions, occurring widely throughout the tropics and subtropics (Sangwan NS et al. 1994; Sangwan RS et al. 1993). Although more than 50 species of the genus occur in nature (Soenarko 1977), only a few of them – *C. flexuosus, C. pendulus, C. winterianus* and *C. martinii* var. *motia* – are commercially cultivated as modern cash crops for essential oil production. Elite cultivars such as Pragati, Krishna and Cauvery of *C. flexuosus*, Manjusha, Mandakini and Bio-13 of *C. winterianus,* Trishna, Tripta and PRC-1 of *C. martinii*, Praman of *C. pendulus* and interspecific hybrid (*C. pendulus* × *C. khasianus*) CKP-25 (Table 1) have become popular for commercial cultivation on the Indian subcontinent (Anonymous 1986; Kulkarni et al. 1997; Mathur et al. 1988; Sharma et al. 1987a, b, 1988, 1989, 1997). Although these cultivars differ in oil content and quality at the intra- and inter-species levels, morphological differences among them are often blurred, particularly at the intra-species level.

DNA based markers reveal differences and relatedness between individuals and discern genomic diversity. The techniques have also been employed for cultivar

**Table 1** Major elite Indian cultivars of *Cymbopogon* aromatic grasses and their phenotypic, essential oil chemotypic features, genetic background and agronomic attributes

| Cultivar | *Cymbopogon* species | Chemo-taxonomic series | Chromosome number (2n) and ploidy | Morphological distinctions | Major oil constituents | Developed through: | Developed at: | Reference |
|---|---|---|---|---|---|---|---|---|
| Mandakini | *C. winterianus* (Citronella Java) | Citrati | 20, diploid | Smaller, slightly wider leaves | Cintronellal, Citronellol | Repeated clonal selection | CIMAP, Lucknow | Sharma et al. (1988) |
| Manjusha | " | " | 20, diploid | Long, drooping leaves | " | " | " | Sharma et al. (1988) |
| Bio-13 | " | " | 20, diploid | " | " | Somaclonal selection | " | Mathur et al. (1988) |
| Trishna | *C. martinii* (Palmarosa) | Rusae | 20, diploid | Long inflorescence compact | Geraniol | Synthetic breeding | " | Sharma et al. (1987b) |
| Tripta | " | " | 20, diploid | Shorter inflorescence, early dwarf | " | Half-sib selection | " | Sharma et al. (1997) |
| PRC-1 | " | " | 20, diploid | Long inflorescence | " | Composite breeding | " | Anonymous (1986) |
| Pragati | *C. flexuosus* (East Indian lemongrass) | Citrati | 20, diploid | Deep purple pigments on leaf-sheath, dark-green leaves | Citral | Cloline breeding | " | Sharma et al. (1987a) |
| Cauvery | " | " | 20, diploid | Narrow, long leaves for South India | " | Cloline breeding | " | CIMAP (1989) |
| Krishna | " | " | 20, diploid | Narrow and slender leaves with little flower | " | Recurrent selection | CIMAP FS (Bangalore) | Kulkarni et al. (1997) |
| Praman | *C. pendulus* (North Indian lemongrass) | " | 40, tetraploid | Narrow leaves, fast regeneration, prolific tillering | " | Repeated clonal selection | CIMAP, Lucknow | Sharma et al. (1989) |
| CKP-25 | *C. pendulus* × *C. khasianus* | " | 60, hexaploid | Very narrow leaves, prolific tillering | " | Interspecific hybridization | RRL, Jammu | Rao and Sobti (1991) |

identification, phylogenetic analysis, genetic mapping, estimation of out-crossing rates and population differentiation in a number of food, forage and fibre crops (Chalmers et al. 1992; Fritsch and Rieseberg 1992; Kresovich et al. 1994). Amongst such molecular tools, the randomly primed polymerase chain reaction (PCR) provides a simple and fast approach to detecting DNA polymorphism, with allelic [randomly amplified polymorphic DNA (RAPD) marker] variations being detected as a plus or minus allele (Welsh and McClelland 1990). In particular, the approach provides multilocus profiling of DNA sequence differences of genotypes when genetic knowledge is lacking. We report here the RAPD characterization of major elite and popular *Cymbopogon* cultivars as a means to discern : (1) genetic diversity within and across the species/ essential oil trade types, (2) RAPDs helpful in cultivar description, curatorial considerations, assignment of chemotypic clustering and (3) genetic issues of management and improvement of the oil grasses.

## Materials and methods

### Plant materials

In our investigation we used 11 elite and most abundantly cultivated Indian cultivars of *Cymbopogon* species, namely Pragati, Cauvery and Krishna of East Indian lemongrass (*C. flexuosus*); Praman of North Indian lemongrass (*C. pendulus*); PRC-1, Trishna and Tripta of palmarosa (*C. martinii* var. motia); Mandakini, Manjusha and Bio-13 of Java citronella (*C. winterianus*) and CKP-25, an interspecific hybrid (*C. pendulus* × *C. khasianus*). These were raised from slips of their mother plants in earthen pots in a glasshouse at the Central Institute of Medicinal and Aromatic Plants, Lucknow (26.5°N, 85.5°E, 120 msl) India. In the world of commerce, cvs. Praman and CKP-25 are also designated as lemongrass as both yield citral-rich oil. The phenotypic, chemotypic, pedigree, agronomic traits and other background information of the cultivars is presented in Table 1.

### Isolation of DNA

Total plant DNA was isolated from young leaves essentially by using the CTAB procedure with some minor modifications (Sangwan NS et al. 1998; Sangwan RS et al. 1999). In the case of composite (cross breeding) varieties of *C. martinii* potential errors in the algorithm analysis derived from heterozygosity were minimized by sampling pooled leaf material from several individuals in each entry. At least three independent DNA preparations were made, with the quantity and quality of the DNA samples being estimated by a comparison of band intensities on agarose gel as well as by fluorometry (DyNA Quant 200, Pharmacia) using Hoechst 33258 as the fluorochrome.

### PCR assay

Thirty 10-mer oligonucleotides of arbitrary sequences with varying GC content (50–70%) were used as primers for the PCR reactions. The optimal assay composition and amplification conditions were arrived at through a factorial experiment described in detail by Sangwan et al. (1999). Consistency and reproducibility of the RAPDs were discerned with three templates (replicates) of a variety using one primer. The reproducibility of profiles under the optimized conditions was high (consistency factor >0.98). The reaction mixture (25 µl) contained 400 µ*M* of each dNTP, 1.0 m*M* $MgCl_2$, 10 pmol primer, 0.25 U *Taq* polymerase, 2.5 µl *Taq* buffer (Bangalore Genei, India) and 50 ng of genomic DNA. PCR amplifications were carried out essentially as described earlier (Sangwan et al. 1999): a pre-PCR cycle (94°C, 5 min; 35°C, 1.5 min; 10°C, 15 min), 40 PCR cycles (each cycle consisting of 94°C, 1.5 min; 35°C, 1.5 min; 72°C, 1.0 min) and, finally, a 5-min incubation at 72°C in a PCR machine (Perkin-Elmer, PE-2400). Following the PCR, the amplification products were separated electrophoretically on 1.4% agarose gels in 1× TAE buffer, visualized over a UV transilluminator (NightHawk, *pdi*, Huntington Station, N.Y.) after staining with ethidium bromide. As a molecular-size marker of the PCR products, a *Hin*dIII digest of lambda DNA was co-electrophoresed. Images of DNA profiles on the gels were captured into the PC files using Video Camera Module (NightHawk, *pdi*, Huntington Station, N.Y.) online with the computer using the Diversity Database Software Package (*pdi*, Huntington Station, N.Y.). The images were retrieved as hard copy and also processed for the analyses.

### Data analysis

Amplification profiles were recorded, and the molecular size of the PCR products was estimated using a calibration curve based on the marker lane. The profiles were matched using the NightHawk System equipped with Diversity Database software and analysed to compute polymorphic information content (PIC), heterozygosity, fragment prevalence index (FPI), Shannon's index, etc. using the appropriate mathematical derivations of population studies (Excoffer 1992; King and Schaal 1989). A three-dimensional (3-D) scatter diagram (principal component analysis, PCA) and Neighbour Joining tree were generated using the Diversity Database software. PCA is a method used for reducing the number of variables in a complex set of data. In Diversity Database, it is used to reduce the number of dimensions of the sample vectors, which have a high number of dimensions, to three. The samples can be represented as a 3-D point (*x*, *y*, *z*) and plotted on a 3-D set of axes either manually (Green 1979 and references therein) or software-assisted (*pdi* 1996; Rohlf et al. 1974).

## Results

The RAPD patterns of genomic DNA of essential oil grass cultivars were analysed with respect to: (1) size

**Table 2** Primer-wise score of PCR amplification products scored in the elite Indian cultivars of major trade types of *Cymbopogons*

| Primer number | Primer nucleotide sequence (5′→3′) | Number of PCR amplification fragments generated in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Citronella | | Palmarosa | | Lemongrass | | All genotypes | |
| | | Monomorphic | Polymorphic | Monomorphic | Polymorphic | Monomorphic | Polymorphic | Monomorphic | Polymorphic |
| AG-01 | CGCAGTACTC | 4 | 6 | 4 | 9 | 1 | 11 | 0 | 19 |
| AG-02 | CGCTGTTACC | 3 | 8 | 3 | 12 | 2 | 9 | 0 | 15 |
| AG-03 | CTAGGTCTGC | 8 | 4 | 6 | 8 | 3 | 9 | 3 | 14 |
| AG-04 | TGCCGAGCTG | 0 | 8 | 4 | 5 | 0 | 8 | 0 | 15 |
| AG-05 | GCAGGATAG | 6 | 2 | 6 | 5 | 0 | 12 | 0 | 15 |
| Total score | | 21 | 28 | 23 | 39 | 6 | 49 | 3 | 78 |

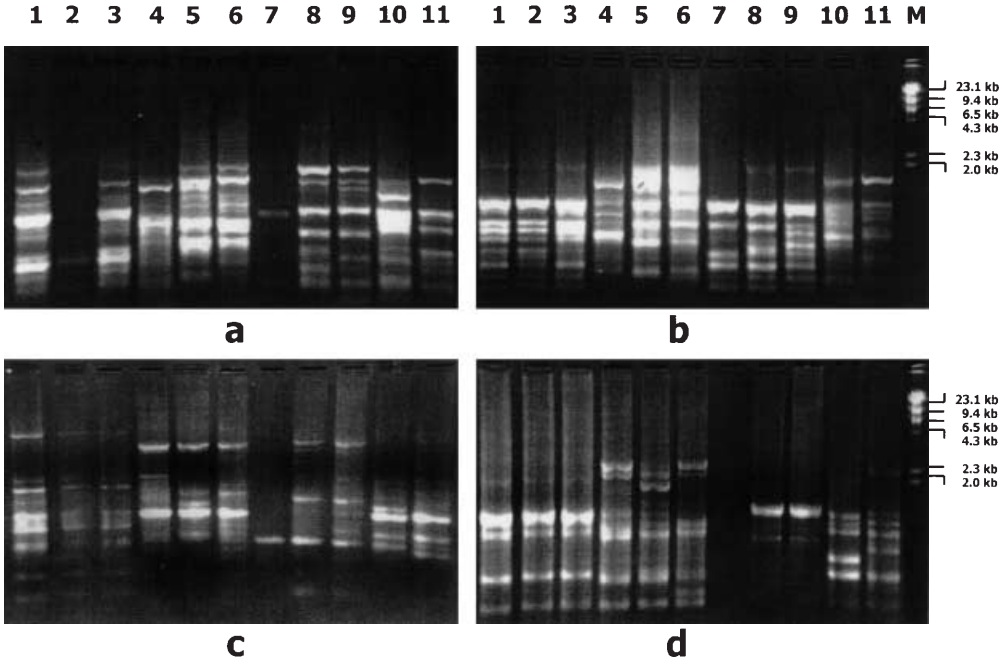


**Fig. 1** Selected RAPD profiles of *Cymbopogon* cultivars. *Lanes 1–3* Citronella cultivars (Mandakini, Manjusha and Bio-13), *lanes 4–6* palmarosa cultivars (Trishna, Tripta and PRC-1), *lanes 7–11* lemongrass cultivars (Pragati, Cauvery, Krishna, Praman and CKP-25). The amplification profiles *a*, *b*, *c* and *d* of the cultivars were generated by primers AG-02 (5′-CGCTGTTACC-3′), AG-03 (5′-CTAGGTCTGC-3′), AG-01 (5′-CGCAGTACTC-3′) and AG-05 (5′-GCAGGATAG-3′), respectively. *Lane M* is the molecular-size marker (*Hin*dIII digest of lambda DNA)

and distribution of fragments, (2) informativeness of the markers and partitioning of polymorphism, (3) computation of genetic diversity within and across the species and (4) their implications in diagnostics and genetic upgrading of cultivars.

## RAPD marker size and patterns

Eighty-one RAPD loci were scored in the *Cymbopogon* cultivars (Table 2). The most responsive primers (in terms of number of amplification products and/or responding genotypes) are also listed in Table 2. Fifteen to 19 types of amplification fragments (monomorphic + polymorphic) were produced by each primer in different cultivars (Table 2). Figure 1 shows the amplification profiles obtained with four of the primers (AG-01, AG-02, AG-03 and AG-05). Most (79) of the PCR products were in the size range 0.05–2.7 kb. The average number of bands per genotype was 11, 16 and 20 in lemongrass, citronella and palmarosa, respectively. FPI values varied only slightly (from 0.216 to 0.292, i.e. within 20% of the overall average of 0.241) in the cultivars except for the low (0.099) value for Pragati lemongrass. Comparative FPI reflected an almost normal pattern of distribution of the markers.

## Polymorphic information and genetic diversity

Wide genetic variation between cultivars of the species was evident from the high number of polymorphic markers and unique bands, even though the survey was limited by the small number of cultivars available. Only 3 out of the 81 band types were monomorphic, thereby giving an estimate of profound (>96%) polymorphism. On an all-genotype basis, all of the PCR products primed by AG-01, AG-02, AG-04 and AG-05 were polymorphic (Table 2). However, polymorphism differed substantially within the discrete groups of cultivars and was approximately 57% (28/49) in citronella, 63% (39/63) in palmarosa and 89% (49/55) in lemongrasses (Table 2). Polymorphism in lemongrasses stemmed mainly from *C. flexuosus* scions (Pragati, Cauvery and Krishna), which produced 35 markers with 74% polymorphism. Our analysis of RAPDs (Table 3) revealed that lemongrass had more allelic variability (A) per locus (1.89) than citronella (1.40) and palmarosa (1.63). Frequency-weighing of both alleles (presence, absence) at each locus (A′ or frequency-corrected A) displayed a similar trend but with a lower magnitude of variation (Table 3). Similarly, gene diversity values, computed as an average of each individual RAPD marker, were recorded as 0.41, 0.47 and 0.68 for citronella, palmarosa and lemongrass, respectively. The sum of effective number of alleles (SENA) was also minimal for citronella (22.4) and maximal for lemongrass (34.5). The pattern of diversity as determined by PIC (maximum possible being 1.0) was citronella < palmarosa < lemongrass with a very high (0.753) average value across the full set of cultivars (Table 3). Within lemongrasses, *C. flexuosus* cultivars had higher PIC (0.555) than *C. pendulus* forms (0.349). Further, against the maximum feasible heterozygosity (0.50), the average expected heterozygosity ($H_{av}$) values were much lower in citronella and palmarosa than in lemongrass. The same order of genetic heterogeneity was discerned through Shannon's information index (Table 3).

**Table 3** Genetic variability across the major Indian elite *Cymbopogon* genotypes as well as within the discrete biotypic groups of cultivars with respect to essential oil trade type as discerned through randomly primed PCR

| Parameter | Diversity value for cultivars of the *Cymbopogon* biotypes | | | |
|---|---|---|---|---|
| | Citronella | Palmarosa | Lemongrass | Allgenotypes |
| Percentage of polymorphic loci (P) | 57.1 | 62.9 | 89.1 | 96.3 |
| Frequency [unweighted average no. of alleles per RAPD locus (A)] | 1.40 | 1.63 | 1.89 | 1.96 |
| Frequency [weighted average no. of alleles per RAPD locus (A′)] | 1.46 | 1.50 | 1.62 | 1.59 |
| Sum of effective no. of alleles (SENA) | 22.4 | 31.2 | 34.5 | 47.3 |
| Polymorphic information content (PIC) | 0.425 | 0.465 | 0.686 | 0.753 |
| Total expected heterozygosity ($H_{av}$) | 0.254 | 0.279 | 0.355 | 0.310 |
| Shannon's information index | 0.182 | 0.204 | 0.220 | 0.279 |

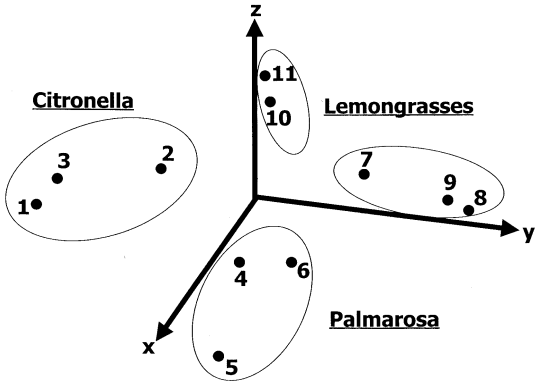


**Fig. 2** RAPD marker-based three-dimensional Principal Coordinate Analysis (PCA) of cultivars of *Cymbopogon* covering three oil trade types (citronella, palmarosa and lemongrass) of the aromatic grasses. The *numbers* (*1–11*) represent the cultivars: *1* Mandakini, *2* Manjusha, *3* Bio-13, *4* Trishna, *5* Tripta, *6* PRC-1, *7* Pragati, *8* Cauvery, *9* Krishna, *10* Praman, *11* CKP-25

### PCA and phylogenetic relationships

PCA provides a field representation of the variability in a 3-D set of axes. It is very useful analysis for visually inspecting the similarity of samples since dissimilar samples will appear to be further apart than highly similar samples. A three-dimensional PCA of the RAPD data separated the 11 cultivars into four clusters: one each of citronella, palmarosa and two of lemongrasses – one of the *C. flexuosus* types and another of *C. pendulus* and its hybrid (Fig. 2). Nevertheless, both clusters of lemongrasses resided in the same (*z-y*) field/dimension. A Dice matrix-based Neighbour Joining tree of phylogenetic relationships (figure not shown) also exhibited a similar pattern of clustering of the species and biotypes.

### Cultivar identification through diagnostic RAPDs

The randomly primed PCR approach not only facilitated molecular distinction of the genotypes (cultivars) of *Cymbopogon* species but also provided some biotype-specific markers. The biotype-specific markers (by presence) included $AG01_{(5,000\ bp)}$ for the citronella cultivars and $AG02_{(290\ bp)}$ for the palmarosa cultivars. However, no such positive marker could be scored for lemongrasses. Variety-wise, 0, 3, 3, 4 and 8 unique bands (by presence) could be scored for lemongrass cultivars Pragati, Cauvery, Krishna, Praman and CKP-25, respectively. Cultivars Trishna, Tripta and PRC-1 of palmarosa could be specified by the presence of 7, 7 and 9 unique bands, respectively (Table 4). Citronella cvs. Mandakini, Manjusha and Bio-13 displayed 9, 3 and 2 unique bands (+), respectively. The cultivar-specific diagnostic bands (by presence as well as absence) are presented in Table 4 along with their molecular size and generating primer.

## Discussion

DNA-based molecular markers can demonstrate similarities and differences between cultivars and accessions even when a morphological description is severely limited. Among these, RAPDs, despite having certain disadvantages (dominant nature and stringent optimization of assay), can produce multilocus profiles widely spanning the genome even in the absence of any prior genetic/sequence information. Also, they may be helpful in defining parental combinations (for distant gene introgression) to obtain better agronomic and oil trait cultivars. Therefore, we employed RAPDs in the investigation presented here to estimate genetic relatedness and diversity among the cultivars of different essential oil trade types of *Cymbopogon* grasses. To our knowledge, this is the first report of a DNA-based polymorphism assay with respect to a genetic analysis of elite cultivars of *Cymbopogons*. The number of amplification fragments produced per primer as well as their size range were analytically appropriate, conforming to those recorded with certain other plants examined analogously (Ho et al. 1997). The normal distribution of PCR products that was observed among the genotypes allowed us to measure the observations.

**Table 4** *Cymbopogon* cultivar-wise unique (by presence or absence) RAPD markers. The size of the markers are specified (in base pairs) as a subscript to the generating primer

| *Cymbopogon* | Unique RAPD markers by: | |
|---|---|---|
| Cultivars | Presence | Absence |
| Mandakini | AG-01$_{(190, 410, 700)}$<br>AG-02$_{(320, 680)}$<br>AG-04$_{(320, 500, 810)}$<br>AG-05$_{(420)}$ | AG-01$_{(880)}$ |
| Manjusha | AG-03(250)<br>AG-04$_{(100)}$<br>AG-05$_{(700)}$ | AG-02(140, 360, 540, 770, 1,600)<br>AG-03$_{(1,800)}$<br>AG-04$_{(110, 160, 220, 280)}$ |
| Bio-13 | AG-02(870)<br>AG-03(70) | AG-01(130)<br>AG-03(280) |
| Trishna | AG-01$_{(880)}$<br>AG-02$_{(100, 540)}$<br>AG-03$_{(360, 600)}$<br>AG-04$_{(150)}$<br>AG-05$_{(420)}$ | AG-02$_{(200, 680, 780, 1,600)}$<br>AG-03$_{(1,500, 1,800)}$<br>AG-04$_{(810, 1,000)}$<br>AG-05$_{(1,000,1,400)}$ |
| Tripta | AG-01$_{(320)}$<br>AG-02$_{(140, 170)}$<br>AG-03$_{(770)}$<br>AG-04$_{(140, 900)}$<br>AG-05$_{(1,600)}$ | AG-01$_{(100)}$<br>AG-02$_{(320)}$<br>AG-03$_{(900)}$ |
| PRC-1 | AG-01$_{(150, 170, 440, 1,400)}$<br>AG-02$_{(150, 600)}$<br>AG-03$_{(170, 440)}$<br>AG-05$_{(310)}$ | AG-01$_{(140, 1,600)}$<br>AG-02$_{(870)}$<br>AG-05$_{(980)}$ |
| Pragati | | AG-02$_{(770)}$<br>AG-03$_{(600)}$<br>AG-05$_{(980)}$ |
| Cauvery | AG-01$_{(500)}$<br>AG-04$_{(250, 280)}$ | AG-03$_{(540)}$ |
| Krishna | AG-01$_{(170, 700, 4,700)}$ | |
| Praman | AG-01$_{(320, 440, 500)}$<br>AG-05(500) | AG-03$_{(170)}$ |
| CKP-25 | AG-01$_{(100)}$<br>AG-03$_{(110, 160, 440, 500)}$<br>AG-05$_{(170, 310, 1,700)}$ | |

## Intra-and inter-specific molecular differentiation in *Cymbopogon*s

The observed high proportion of polymorphic loci suggests that there is a profound genetic heterogeneity in the species. The lower level of polymorphism in citronella is understandable, as rare and scanty flowering might have limited outcrossing-mediated heterozygosity enhancement. Chemotypically also, citronella displays far less variability in oil composition than the other *Cymbopogon* species (Sangwan and Sangwan 2000). However, the lower genotypic diversity observed in the palmarosa cultivars may be ascribed to the open-pollinated improved bulk or composite nature of the varieties, all of which have a common origin (Sharma et al. 1987b, 1997). Consequently, oil chemotypic diversity within the cultivated palmarosa is very low. However, palmarosa forms with an enormous diversity in their oil composition have been encountered in the wild (Sangwan et al. 2000). Lemongrasses possess the most diversified oil compositions in both wild types as well as in cultivars under domestication (Sangwan and Sangwan 2000).

The number of alleles per locus obtained through frequency-unweighted allele assignments (A) as well as frequency-weighted (presence and absence alleles, A′) of RAPD markers has revealed a very high level of heterogeneity in the *Cymbopogons* (A=1.96 and A′=1.59; a value of 2.0 means complete heterozygosity for RAPDs. Unlike restriction fragment length polymorphisms (RFLPs), values of A for RAPDs based on only polymorphic bands and without frequency weighing are barely informative (being calculated as 2.0) (Liu and Furnier 1993). Actually, this value should be less than 2.0 as: (1) RAPDs are dominant in nature; hence, almost all copies of low-frequency recessive alleles present in heterozygotes go undetected in the assay and (2) deletion and insertion events occurring within the primer-bracketed region produce a band type that is scored as an independent locus rather than an allele. Thus, although A or A′ may reflect allelic richness, their numerical values also

depend on the scoring of rare alleles which tend to escape in a sample of limited size. Hence, alternative estimates of genetic diversity, such as PIC and SENA, were made. The values, comparable to those observed in certain large and rich germplasms of other cultivated and wild plants (Powell et al. 1996), appeared to be meaningful with respect to genetic differentiation in the *Cymbopogons*. The pattern of allelic variations (lemongrass > palmarosa > citronella) is reliable since the numbers of loci polymorphic in the genus *Cymbopogon* (all species/cultivars put together), but monomorphic within a particular species or cultivar group, was low, thereby indicating a low error, if any, due to hidden heterozygotes.

### Diagnostic RAPD markers

The 'DUS' criteria for cultivar identification entail: *d*istinctness (distinguishable inter-varietal variation), *u*niformity (minimum intra-varietal variation) and *s*tability (environmental steadiness coupled with experimental reproducibility). With respect to the first criterion, the level of variation detected for RAPDs in the *Cymbopogon* cultivars is amongst the highest revealed with any known marker system. Although we have not examined the level of intra-clonal variability in detail, as the plants are vegetatively propagated and are somatically stable, the experiments reported fulfil the third criterion.

The cultivars can be distinguished visibly at the interspecies level. The identity of plants as biotypes of lemongrass, citronella and palmarosa is also quite clear. The distinctions remain intact even when extremes of oil chemotypic variants are encountered for the species. Accordingly, we feel that invoking molecular techniques for biotype assignment in *Cymbopogons* may be unwarranted. However, at intra-species (inter-cultivars) levels morphological distinctions are very minute and may require a suitable score of unique RAPD markers (positive) as 'stand along' (if not 'stand alone') fingerprints to aid in cultivar recognition. Our study has provided useful diagnostic markers to this end.

### Genetic resource management and improvement

Molecular diagnoses strongly suggest that the cultivars of citronella and palmarosa differ very little among themselves. Also, our observations suggest that the genetic base utilized in their breeding programmes has been restricted and that the introgression of genes from unexploited sources deserves attention. Therefore, their wild counterparts of the Indian subcontinent (centre of genetic diversity) should be considered for utilization in the plant improvement programme, especially for minimizing the imbalance of recessive alleles in the heterozygous state. RAPDs, in combination with agronomic, morphological and oil chemotypic characteristics, can provide a catalogue of *Cymbopogon* cultivars for the identification of duplicate accessions, thereby defining core collections and strengthening exploitation of their genetic resources for horticultural and curatorial needs.

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