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COMPARATIVE GENETIC ANALYSIS AMONG GENERA *CYMBOPOGON* AND *VETIVERIA* BY RAPD, SOUTHERN HYBRIDIZATION AND SEQUENCING

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ABSTRACT

Two of the most important genera of family 'Poaceae' namely *Cymbopogon* and *Vetiveria* were investigated in this study with the objective to differentiate the species, determining the diversity and assessing the synteny among them. For this purpose, RAPD, southern hybridization and sequence analysis were used as tools. Combinely, after assessing all the parameter, a tentative pattern of evolution for different accessions of *Cymbopogon* and *Vetiveria zizanioides* was deduced in which all of the species were predicted to be evolved from a single ancestor through *C. confertiflorus* (CCo01) or *C. nardus* (L.) Rendle var. *confertiflorus* (CN01) type of genome. All species of *Cymbopogon* studied were having the basic chromosome number X=10. In the synteny study, *Vetiveria* types of grasses were analyzed to be derived from wild relatives of *Cymbopogon* type of genome. *Vetiveria* was further thought to be of recent origin in its development compared to *Cymbopogon*. The presence of similar sequence RAPD fragments in these two types of genomes were further confirmed through southern hybridization and sequencing.

KEYWORDS: *Cymbopogon; Vetiveria*; Genetic diversity; RAPD; Molecular markers; Southern hybridization; Sequencing; Synteny.

INTRODUCTION

The botanical family of grasses (Poaceae), consist of more than 10,000 species (Clayton and Renvoize, 1986). Their high level of adaptableness permits grass species to grow in the majority of the terrestrial habitats. In the past few thousand years, humans have taken benefit

of these natural resources by domesticating and breeding a small subset of the grass species. These hard works have resulted in many significant crop plants, such as wheat, rice, maize and sorghum. Many species, together with wheat, are grown in different environmental regions and ecological conditions, signifying the diversity in the gene pool of a single species. Wheat and rice each supply ~20% of the calories ingested by the world's population (FAOSTAT home page; http://apps.fao.org/). In whole, ~60% of the world's food production is acquired from grasses, which makes them efficiently undoubtedly the main crucial plant family. In terms of genome organization, grasses embody a extremely diverse family (Keller and Feuillet, 2000). Their chromosome number differs from 2n = 4 (Benett *et al*., 1995) to 2n = 266 (Hair *et al*., 1961). Their genome ranges fluctuate significantly (Arumuganathan and Earle, 1991). The grass subfamily Panicoideae contains ~3300 species in 206 genera (Grass Phylogeny Working Group, 2001) and is bigger than the majority of angiosperm families. At least seven tribes are usually documented in subfamily Panicoideae, of which certainly the biggest are Paniceae (101 genera; cf. Clayton and Renvoize, 1986) and Andropogoneae (85 genera). Other tribes comprise Arundinelleae (12 genera), Hubbardieae (1 genus), Neurachneae (3 genera), Isachneae (5 genera) and Steyermarkochloeae (1 genus) (Liliana *et al*., 2001).

Cymbopogon, a tufted stoloniferous and perennial leafy grass is a significant essential oil bearing plant (Annonymous, 1988 and Oyen, 1999). The genus *Cymbopogon* (tribe *Andropogoneae*, family *Gramineae*) contains almost 140 species spread in the tropics and subtropics of the old world (Jagadish Chandra, 1975a). Most of these species make distinctive aromatic essential oils in leaves except *Cymbopogon jwarancusa* subsp. *jwarancusa* (CJ01) which also can make these aroma chemicals in the roots (Mathela *et. al*., 1986, 1988). The oil has viable significance in perfumery, cosmetics and pharmaceutical relevance. The *Cymbopogon* essential oils are described by monoterpene components (Table-1) of which citral is one of the key ingradient of the oil present in several *Cymbopogon* species with ample industrial utilization such as raw stuff for perfumery, confectionery and vitamin A (Khanuja *et. al*., 2005). After Sprengel christianed this genus in 1815, several taxonomists have tried to categorize the species of *Cymbopogon*. Hackel (1887) and Hooker (1897) have classified this genus as a subgenus of *Andropogon*. However, Stapf (1906) elevated *Cymbopogon* to its unique status of a genus which has been established by all the afterward taxonomists. Taxonomically, the species of *Cymbopogon* have been alienated into three series viz. 'Shoenanthi', 'Rusae' and 'Citrati' (Stapf, 1906).

Vetiver, the wonder grass generally called Khus is found all over the tropical and subtropical plains. The species *Vetiveria zizanoides* (2n = 20) is a perennial grass and has thick clusters of gristly roots, which on distillation yield the Khus/Vetiver oil recognized for its therapeutic and aromatic properties (Annonymous,1885). Additionally, the plants are also cherished for the reason of soil conservation in saline, alkaline and marshy lands. The oil distilled from the roots is in substantial demand and is primarily used as a fixative in perfumery and for amalgamation in cosmetics and soap industries (Jain, 1991 and Khare, 2007).

The genus *Cymbopogon* and *Vetiveria* are both grasses belonging to the family Poaceae having similar type of morphology and reproductive structure (inflorescence) (Table-2). But, these two genera synthesize completely different types of monoterpenes. Further, *Cymbopogon* produces the monoterpenes mostly in the leaves but, *Vetiveria* biosynthesize these aroma chemicals (monoterpenes, sesquiterpenes and the others) in the roots (Annonymous, 1885, 1988). It is also reported that *Cymbopogon jwarancusa* subsp. *jwarancusa* (CJ01) also can synthesize these aroma chemicals in the roots (Mathela, 1986; Mathela *et al.*, 1988) thereby indicating that the spatial expression of these monoterpenes is not a constrain to share homology in the genome.

A most important modern discovery in plant genetics is the incidence of gene synteny that is the arrangement of genes is parallel in different plant genomes across taxa (Tanksley *et. al.*, 1988). Thus, the observable variations among the species and genera are more on account of alleles in spite of actual type of genes. Therefore, molecular taxonomy using DNA markers can be extremely practical in outlining the derivation of useful plants and ascertaining their phylogenetic association to the wild relatives. This information can offer the database to proceed as guiding feature for determining the potency of gene pools. Such database can then be gladly accessible for the exploitation in marker assisted breeding programmes by gene prospecting during cataloging of the valuable characters with molecular markers. Comparative genetics facilitate us to explore the genome organization in diverse species besides, comparative genetics offer the foundation for understanding genome evolution. Comparative genetics, akin to molecular mapping, has its roots in mammalian studies emerging from the effort with man-mouse hybrids in the late 1970s (Gale *et. al.*, 1996). The description and relevance of the information regarding the syntenous associations between increasingly vaguely allied species have, however, advanced more fastly in plants than in animals (Gale *et. al.*, 1996). Comparative linkage maps not only let further insight into

chromosome evolution but grant a source for construing genetic information among conflicting species (Ahns and Tanksley, 1993). For example, mice are currently a trendy model system for studying both single-gene and quantitatively inherited genetic disorders that influence humans, in part because of the accessibility of comparative maps for these two mammalian species (Darling and Abott, 1992). Further, molecular markers such as Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR-PCR) polymorphism studies developed earlier have offered novel insight to resolve such minutiae among closely related taxa (Zietkiewicz *et al.*, 1994 and Ballard, 2000). Further, the sequence data analysis is considered to be the most efficient mode to look on to these aspects of evolutionary entities. Thus, in the present present investigation RAPD and southern hybridization of the co-migrating fragments has been taken up with the aim to shed light on both the molecular divergence and the evolutionary interrelationships among the members of *Cymbopogon* species and *Vetiveria zizanioides* of family *Poaceae*. Perusal of the literature has revealed that these molecular markers were sensitive enough to resolve the systematic, evolutionary and phylogenetic intricacies in different groups of plants and animal systems (Nagaraju *et. al.*, 2001; Zietkiewics *et. al.*, 1994; Ballard, 2000). So in this investigation, eighteen different accessions of *Cymbopogon* species were analyzed along with three different accessions of *Vetiveria zizanioides* to know exactly the amount of genome sharing for determining the homology if any. An unspecified *Cymbopogon* species (CS01) and an unspecified outgroup species (UOS) were also taken in the study as positive and negative controls.

MATERIALS AND METHODS

Plant material

The genus *Cymbopogon* and *Vetiveria* both monocotyledonous plants constituted the material of study in this investigation. Eighteen accessions of *Cymbopogon* (belonging to eleven species, five varieties, one hybrid and one unidentified species) and three of *Vetiveria zizanioides* (from north and south India) along with an unidentified outgroup species were taken for this study. The representative accessions were collected either from the wild or received through the National Genebank in the genotype exchange programmes from other laboratories. These with individual identification are maintained in the National Genebank (field genebank) for medicinal and aromatic plants at Central Institute of Medicinal and Aromatic Plants (Sponsored by Department of Biotechnology, Government of India). The plants analysed in this study were also maintained in the glass house and are listed in Table-3.

All the accessions concerned in the study were generally propagated vegetatively through slips except *C. martinii*.

DNA isolation and Polymerase Chain Reaction (PCR)

DNA was isolated from leaf tissue essentially according to the protocol described earlier (Khanuja *et al.,* 1999). The concentration and quality of isolated DNA was checked by OD at 260/280 nm. Polymerase chain reactions (PCRs) for RAPD analysis were carried out in 25 µl volume. Each reaction tube contained 25 ng of DNA, 0.2 unit of Taq DNA polymerase, 100 µM of each dNTPs, 1.5 mM $MgCl_2$ and 5 pmol of decanucleotide primers. The amplifications were carried out using the DNA Engine thermal cycler (MJ Research, USA) using 94°C, 35°C and 72°C temperatures for 40 cycles (Khanuja *et al.*, 2000). The amplified products were separated on 1.2% agarose gel containing 0.5 μgml^{-1} of ethidium bromide and photographed with Image master VDS (Pharmacia). Custom-made decanucleotide primers were synthesised in the laboratory on Applied Biosystems 392 DNA–RNA Synthesizer and were designated as MAP01 to MAP20 (Table-4). Two other sets of commercially available primer kits of OPJ and OPT each consisting of twenty random decamer primers (Operon Technologies, USA) were also used. Amplification reactions were repeated thrice and the fragments appearing consistently were scored for the presence and absence of bands in RAPD profiles. In all the different amplifications, we selected the primers generating monomorphic fragments between accessions of both the genera for further analysis.

Probe development

A monomorphic fragment of 1.66 kb observed in all the accessions of both the genera with MAP-10 was amplified through PCR in replicates from *C. nardus* (L) Rendle var. *confertiflorous* (CN01) and eluted through low melting agarose gel according to the standard protocol of Sambrook *et. al.,* (1989). The eluted DNA fragment was further radio labeled with [α-32P]dCTP using random priming using ALL-IN-ONE^{TM} Random Prime DNA Labeling Mix (-dCTP) kit (Sigma-Aldrich, USA).

Blott preparation and probing

PCR products obtained with MAP10 primer were subjected to agarose gel electrophoresis and the profile was transferred to nylon membrane according to the standard protocol of Sambrook *et. al.*, (1989). Further, the blot was probed with a 1.66 kb radiolabeled probe through the standard procedure of Sambrook *et. al.,* 1989 under high stringency conditions.

Cloning and sequencing of monomorphic 1.66 kb fragment

The 1.66 kb fragment obtained during PCR of *C. nardus* (L.) Rendle var. *confertiflorous* (CN01) with MAP10 was subjected to restriction digestion with EcoR I enzyme and the product was cloned into phagemid vector, pBluescript II SK (+). The recombinant DNA was used to transform DHα strain of *E. coli* competent cells. Randomly selected white colonies were purified by using QIAGEN Plasmid Mini prep kits and the plasmid DNA was sequenced using ABI Prism® Big DyeTM Terminator cycle sequencing ready reaction kit (Perkin-Elmer Inc., USA). Computer-assisted analysis of the sequenced data was performed using both Sequence analysis and Sequence navigator *f* softwares version 3.3. The sequence data was further searched for homology with any of the gene(s) through Basic Local Alighnment Search Tool (BLAST) in National Centre for Biotechnology Information (NCBI) database.

Data analysis

RAPD profiles were analysed by scoring the presence and absence of bands from different accessions of both the genera. For quantification of similarity, pairwise comparisons of banding patterns were made by calculating indices of similarity using the matching coefficient method of Nei and Li (1979). The average similarity matrix was used to generate a tree for cluster analysis by Unweighted Pair Group Method with Arithmetic average (UPGMA) using NTSYS 2.1.

RESULTS AND DISCUSSION

Comparative RAPD profiling of genera Cymbopogon and Vetiveria for the estimation of genetic diversity

The comparative RAPD study among the genera *Cymbopogon* species and *Vetiveria zizanioides* with a set of sixty decamer primers generated a total of 162 fragments out of which 140 fragments were polymorphic (86.42% polymorphism), 10 were monomorphic (6.17% monomorphism) and 12 were unique (7.41% uniqueness) (Table-5).

A similarity range of 0-77.3% was observed in the analysis indicating 22.7-100% diversity (Table-6). Within the same genus of *Cymbopogon*, maximum similarity of 77.1%, was observed between *C. travancorensis* (CT01) and *C. flexuosus* var. *microstachys* (CF02). Within *Vetiveria*, maximum similarity (77.3%) was observed between VZ-20 from North India (New Delhi) and VZ-22 from South India (Kerala). And, maximum similarity (64.3%) was observed between both genus viz., accessions *C. nardus* (L.) Rendle var. *confertiflorus*

(CN01) and VZ-19 from North India (New Delhi). Within *Vetiveria*, minimum similarity of 70.1% between VZ-19 from North India (New Delhi) and VZ-22 from South India (Kerala). And, between both genus, a minimum similarity of 28.6% was observed between *C. flexuosus* var. *flexuous* (CF01) and VZ-19 from North India (New Delhi).

A similar pattern of clustering could be detected in the comparative analysis (Fig. 1) when compared with grouping patterns of RAPD analysis of only *Cymbopogon* species (Khanuja *et. al.,* 2005). All the accessions of *C. winterianus* (CW01 and CW02) and *C. nardus* (L.) Rendle (CN02 and CN03) except *C. nardus* (L.) Rendle var. *confertiflorus* (CN01) grouped together in a cluster. The grouping of *C. nardus* (L.) Rendle var. *confertiflorus* (CN01), *C. confertiflorus* (CCo01), unidentified species (CS01), *C. jwarancusa* subsp. *jwarancusa* (CJ01), *Jamrosa* (CH01), *C. travancorensis* (CT01), *C. flexuosus* var. *microstachys* (CF02), *C. caesius* var. narrow leaf (CCa01) and *C. caesius* var. broad leaf (CCa02) were exactly similar as in the clustering pattern for *Cymbopogon* species only. Interestingly, the accession *C. citratus* (CCi01) grouped along with all the *Vetiveria* accessions (VZ-19, VZ-20 and VZ-22) (Fig. 1). Whereas, *C. citratus* (CCi01) grouped with *C. flexuosus* var. *flexuosus* (CF01) in the RAPD cluster diagram got separated from *C. citratus* (CCi01) and clustered along with *C. martinii* group (CM01 and CM02), and formed a separate cluster. *C. pendulus* (CP01) outgrouping earlier from the other species with maximum diversity also out grouped from the rest in the comparative analysis.

Replacement of subcluster as in Khanuja *et. al.,* (2005) by *C. citratus* (CCi01) and all *Vetiveria zizanioides* accessions (VZ-19, VZ-20 and VZ-22) in the comparative analysis indicates the similarity in RAPD profile of *Vetiveria zizanioides* with *C. citratus* (CCi01). The genotype *flexuosus* (CF01), *martinii* (CM01 and CM02) and *pendulus* (CP01) were found to be much more diverse than *Vetiveria zizanioides* and *C. citratus* (CCi01). Being in the cluster of *C. nardus* (L.) Rendle var. *confertiflorus* (CN01), but showing similarity with *C. citratus* (CCi01), the *Vetiveria zizanioides* accessions can be correlated to have ancestory in some of the progenitors of *C. citratus* (CCi01) like genome. And hence, can be linked to the wild grasses of *Cymbopogon* not producing aromatic compounds in the leaves but synthesizing them in the roots (Mathela *et. al.,* 1986, 1988). In other words, the partitioning of terpene biosynthesis in shoots and roots might have occurred before the evolution of genus and the spatial specialization might be present in the ancient grasses. This finding can be supported by the presence of root terpenes expression in *C. jwarancusa* subsp. *jwarancusa*

(CJ01) (Mathela *et. al.*, 1986, 1988). Further, detection of low similarity *C. flexuosus* var. *flexuosus* (CF01) with *C. winterianus* Jowitt (CW01) and *C. confertiflorus* (CCo01) might indicate either separate line of evolution or evolution of *C. flexuosus* var. *flexuosus* (CF01) from *C. citratus* (CCi01) which might be a hybrid of *C. confertiflorus* (CCo01) type of genome with some wild ancestor. (Fig.1).

Generation of monomorphic fragments

A total of ten monomorphic fragments could be scored in the comparative profile analysis of *Cymbopogon* and *Vetiveria* (Table-5) out of which fragment of molecular weight 1.66 kb amplified by MAP-10 primer (Fig. 2) was randomly picked from *C. nardus* (L.) Rendle var. *confertiflorus* (CN01). Since, the fragment 1.66kb was found to be co-migrating in nature therefore it was further used to check the homology through Southern hybridization.

Southern hybridization for homology study

Southern hybridization was carried out to confirm the homology of the co-migrating fragments among different accessions of both genera *Cymbopogon* and *Vetiveria*. The co-migrating fragment of 1.66 kb size present in the PCR profile obtained upon amplication with MAP-10 in both the genera were targeted for hybridization with the prepared [α-32P]dCTP radio labeled probe (Fig. 3). It was observed that the probe hybridized only with all the co-migrating fragments in all the accessions of both the genera (Fig. 4) and not with any of the other fragments of the profile. Interestingly it was also observed that probe hybridized with the co-migrating fragment in unidentified *Cymbopogon* species (CS01) but not with unidentified outgroup species (UOS).

Origin, Evolution and Synteny

The family '*Poaceae*' is alienated into two major subfamilies '*Panicoideae*' and '*Poideae*' (Fig. 5) (Liliana *et. al.*, 2001). The subfamily '*Panicoideae*' again is separated into numerous 'Tribes' out of which the tribe '*Paniceae*' is the biggest (101 genera) followed by '*Andropogoneae*' (85 genera) (Liliana *et. al.*, 2001). The genus *Cymbopogon* and *Vetiveria* belong to the tribe '*Andropogonae*' (Hackel 1889). Several investigators have depicted the tribe '*Andropogonae*' as a monophyletic assemblage based on molecular sequence data (Mason-Gamer *et al*., 1998 and Spangler *et al*., 1999). This tribe is totally of C_4 type with the Maleic dehydrogenase enzyme boasting NADP as the co-factor. The genus *Cymbopogon* is depicted to be having about 140 species (Chase and Niles, 1962). Similarly, the genus *Vetiveria* is explained to contain 10 species (Gupta *et al*., 1995). The morphological

resemblances and variations between these two genera have been described in Table-2. Both of' should be there these genera have the fundamental chromosome number X=10 (Avdulov, 1931; Sikka *et al*., 1956; Celarier, 1959 and Fedorov, 1969).

The family '*Poaceae*' is a well studied phylogeny, however, the most rigorous phylogenetic work in '*Paniceae*' available nowadays is mostly on cladistic study of 67 exomorphological and anatomical features taken mainly from herbarium samples of 110 species (Zuloaga *et al*., 2000). A molecular study entailing sequences of the plastids *trn*L-F region of 32 species along with outgroups was performed by Gómez-Martinez and Culham 2000. Further, The molecular phylogeny of 57 species of '*Paniceae*' was explored by Duvall *et al*., 2001 using sequences from grass specific insert found in the plastid locus *rpo*C2. Moreover, DNA sequence data from the chloroplast gene *ndh*F were studied by Giussani *et. al*., (2001) to assess the phylogeny of subfamily '*Panicoideae*' with an emphasis on the tribe '*Paniceae*'. Thus, all the analysis on phylogeny of grasses aspire on diverse tribes in common and roughly categorizes them as monophyletic, paraphyletic and polyphyletic origin. But, these studies are quiet on the origin and evolution at the genus stage. The several great rearrangements that do distinguish grass genomes are normally inversions, translocations or duplications that engross all or nearly all of chromosome arms (Morrone *et al*., 1995).

The widespread conservation of gene contents and gene orders between maize and sorghum was not surprising, because only approximately 15-20 million years of autonomous descent distinguish these members of the tribe '*Andropogonae*'. However, successive comparative genetic maps of maize and rice (Ahn and Tanksley 1993; Ahn *et al*., 1993) and wheat and rice (Ahn *et al*., 1993; Kurata *et al*., 1994) using DNA probes too signified a long collinear section. Rice and maize had deviated some 60-80 million years before and thus symbolize a wide temporal array of grass genome partition (Clark *et al*., 1995). This surveillance of conserved gene content and order in the grasses lead to the model that, individual grass species could be observed best as demonstration of a sole grass genome and that, each of the strength of studies in different grasses could be used to promote all individual grass studies (Bennetzen and Freeling 1993).

It is also described that, despite various interesting exceptions, 'genome synteny' in the grasses provides a powerful set of tools for understanding and manipulating grass biology. In this present investigation, the individual genus *Cymbopogon* was studied for similarity and diversity, indicative of synteny among the individual members followed by comparison with

the allied genus *Vetiveria* of the same tribe. DNA sequence data from the gene *ndh*H and *ndh*F show monopoly of the subfamily '*Panicoideae*' and segregate the subfamily into three diverse clades (Giussani *et al*., 2001). One clade consisting of all '*Andropogonae*' having chromosome number X=10, we analyzed the genus *Cymbopogon* within this tribe which is separated into three main taxonomic series (Stapf. 1906), namely, 'Citrati', 'Rusae' and 'Schoenanthi'. A total of eighteen accessions mostly consisting of all 'Citrati' except *C. martinii* and *C. caesius* which belongs to 'Rusae' and *C. jwarancusa* subsp. *jwarancusa*, which belongs to 'Schoenanthi'. The first observation was that, the 'Citrati' series is being randomly dotted with individuals of other group in the cluster diagram (Fig. 1). All of the species taken were found to be highly similar to *C. confertiflorus* (CCo01) and *C. nardus* (L.) Rendle var. *confertiflorus* (CN01) in both RAPD and AFLP analysis (Lal and Awasthi, 2015). This indicates single source or origin of all the species of *Cymbopogon*. As reported earlier, the evolution of *C. winterianus* Jowitt (CW01) was found to be through *C. nardus* (L.) Rendle var. *Java II* (CN03) with its progenitor *C. nardus* (L.) Rendle var. *nardus* (CN02) ultimately evolving from *C. confertiflorus* (CCo01) and *C. nardus* (L.) Rendle var. *confertiflorus* (CN01) type genotypes (Shasany *et al.*, 1999). In the similar way, *C. citratus* (CCi01) with 2n=60 may be progenitor of *C. flexuosus* var. *flexuosus* (CF01) (2n=20) and *C. pendulus* (2n=40). In this case, a polyploid is predicted to be the ancestor of diploids and tetraploids. Polyploidy is common in the grass family. *C. citratus* (CCi01) might have evolved from *C. confertiflorus* (CCo01) with a different biochemical pathway differentiation in the due course of time than the evolution of *C. winterianus* Jowitt (CW01). Separate line of evolution was predicted for *C. jwarancusa* subsp. *jwarancusa* (CJ01) though, it was similar in its oil component with *C. caesius* var. narrow leaf (CCa01) (Khanuja *et. al.*, 2005). Similarly, *C. travancorensis* (CT01) as an endemic type evolved separately so as *C. caesius* var. narrow leaf (CCa01) and *C. caesius* var. broad leaf (CCa02). Some of the genotype like *C. jwarancusa* subsp. *jwarancusa* (CJ01), *C. travancorensis* (CT01), *C.caesius* var. narrow leaf (CCa01) and *C. caesius* var. broad leaf (CCa02) together might have given rise to *C. flexuosus* var. *microstachys* (CF02). *C. martinii* might have evolved again separately from the same ancestor or through *C. caesius* var. broad leaf (CCa02) (2n=20).

In *Cymbopogon* species, a large diversity of monoterpenes has synthesized in the leaf (Table-1). As expected 'Limonene' is the first major constituent being detected in almost all the *Cymbopogon* species. In *Vetiveria*, 'Limonene' is also being detected in trace amounts in the root in addition to different types of sesquiterpene conjugates and complexes (Table-1). The

major difference between these two genera in terms of expression of terpenoids is spatial in nature. The terpenoides are highly expressed in leaves in *Cymbopogon* whereas in *Vetiveria zizanioides*, this is in the roots. As grass genomes are portrayed to be monophyletic (Brown, 1810, 1814; Tateoka, 1962; Kellogg *et al*., 1987, 1998; Soreng *et al*., 1998 and GPWG, 2001) and the genus belonging to tribe '*Andropogonae*' are supposed to be belonging to the identical clad (Giussani *et al*., 2001), the demarcation for tissue specificity in terpenoid expression might have taken place after the genus differentiation. In comparative RAPD analysis (Table-6 and Fig. 1), the *Vetiveria* cluster interestingly demonstrated highest similarity with *C. citratus* (CCi01) (61.3% with VZ-20) and the whole subcluster was a part of the bigger cluster containing *C. nardus* (L.) Rendle var. *confertiflorus* (CN01), *C. confertiflorus* (CCo01), unidentified species (CS01), *C. jwarancusa* subsp. *jwarancusa* (CJ01), *Jamrosa* (CH01), *C. travancorensis* (CT01), *C. flexuosus* var. *microstachys* (CF02), *C. caesius* var. narrow leaf (CCa01) and *C. caesius* var. broad leaf (CCa02). We earlier predicted, progenitor of *C. citratus* (CCi01) is believed to be *C. nardus* (L.) Rendle var. *confertiflorus* (CN01) or *C. confertiflorus* (CCo01) or related wild species till now not being identified (Sashany *et. al.,* 2000). From this analysis, the evolution of *Vetiveria* may predicted to be after the evolution of *Cymbopogon* which contradicts our earlier statement that the *Vetiveria* expression pattern (root and shoot) was developed after the genus differentiation. It may be noted here, the aromatic sesquiterpenoids biosynthesis have also been reported in *C. martinii* var. *motia* (CM01) (Gaydouand and Raudriamiharisoa, 1987), and *C. proximus* (Elgamal and Wolff, 1987) and both monoterpenoids and sesquiterpenoids in the roots of *C. jwarancusa* subsp. *jwarancusa* (CJ01) (Mathela *et. al.,* 1986, 1988). Since, the intermediates exists and can be seen expressing the terpenoids both in roots and shoots, it may not be wrong to hypothesize that the *Vetiveria* type of grasses are the mixture of genotype of wild grasses along with *Cymbopogon* species and are of recent origin. It has been discussed that, much of the difference in genome size is attributable to variation in quantity of repetitive DNA (Flavell *et. al.,* 1974) such as bigger genomes of barely or wheat are composed of over 75% of repetitive DNA whereas small genomes of rice include less than 50% repetitive DNA (Flavell *et. al.,* 1974; Deshpande and Ranjekar, 1980). It has been also described that retrotransposons poped in between genes account for the majority of repetitive DNA in some huge genome grasses like maize (SanMiguel *et al*., 1996). We also found retrotransposons like element during sequencing of polymorphic fragments in *Cymbopogon* (data not shown). But in RAPD analysis, in addition to repetitive DNA, we may get unique sequences or middle repetitive sequences which might have provided the clustering pattern as indicated in

Fig. 1, containing *Vetiveria* as a more similar group to different species of *Cymbopogon* compared to *C. martinii* (CM01 and CM02), *C. pendulus* (CP01) and *C. flexuosus* var. *flexuosus* (CF01). The use of prevalent set of low copy number DNA markers, frequently coding sequences in the mapping of grass genomes has specified that the gene content of diverse grass species does not vary enormously (Hullbert *et. al*., 1990; Ahn *et al*., 1993; Kurata *et al*., 1994). For this reason, RAPD data could provide the better correlation between them. Hence, in the present study one of the co-migrating fragment (1.66 kb) was cloned from *C. nardus* (L) Rendle var. *confertiflorous* (CN01) from the amplified profile with MAP-10 having the restriction site of EcoR I, was sequenced and submitted to GenBank as gbIEU871430.1 was analyzed to be a segment from *ndh* locus of chloroplast DNA coding NADH specific dehydrogenases and showing homology with *ndh*D gene of *Zea mays, Hordeum vulgare, Hordeum murinum, Sorghum bicolor, Nicotiana sylvestris, Allium cepa, Aloe vera, Perityle lindheimeri, Palafoxia arida, Allium porrum, Polymnia canadensis, Oyedaea verbesinoides, Allium sativum* and *Phaneroglossa bolusii* species (Table-7). Being C_4 plant, *ndh*D genes play an significant part in former phylogeny (Giussani *et al*., 2001). Giussani *et al*. (2001) have used *ndh*F gene of chloroplast to analyze the phylogeny of grass subfamily '*Panicoideae*'. We took this fragment to know whether similar genes are present in all the *Cymbopogon* species at comparable molecular size position in the amplified profile gel with MAP-10 primer. Interestingly, only the co-migrating fragments for all *Cymbopogon species* along with *Vetiveria* at 1.66 kb position demonstrated hybridization and none of the other fragment in the profile lighted up (Fig. 4) thereby indicating the existence of considerable synteny among the two genera. Additionally, the hybridization with the co-migrating fragment in unidentified *Cymbopogon* species (CS01) positive control and not with unidentified outgroup species (UOS) as negative control further confirms the sequence specificity towards these two genera only.

Despite interesting exceptions, this investigation provides an insight into the genome synteny of the two genera as well as raises a new question whether *Vetiveria* is an ancient genus or of recent origin compared to *Cymbopogon*. The similarity with *C. citratus* (CCi01) some how indicate the existence of wild intermediates in the ancient time from which *Cymbopogon* and *Vetiveria* decended but *Vetiveria* genus kept its genome similarity with *C. citratus* (CCi01). The synteny analysis offers great set of paraphernalia for understanding and manipulating the biology with common gene content and analogous physiological development information and genes can be applied athwart species boundary (Bennetzen and Freeling, 1997).

Consequently, in future, the *ndhD* gene sequences from all the species can be cloned and analyzed to have a better understanding about the evolution of these C_4 type species in nature.

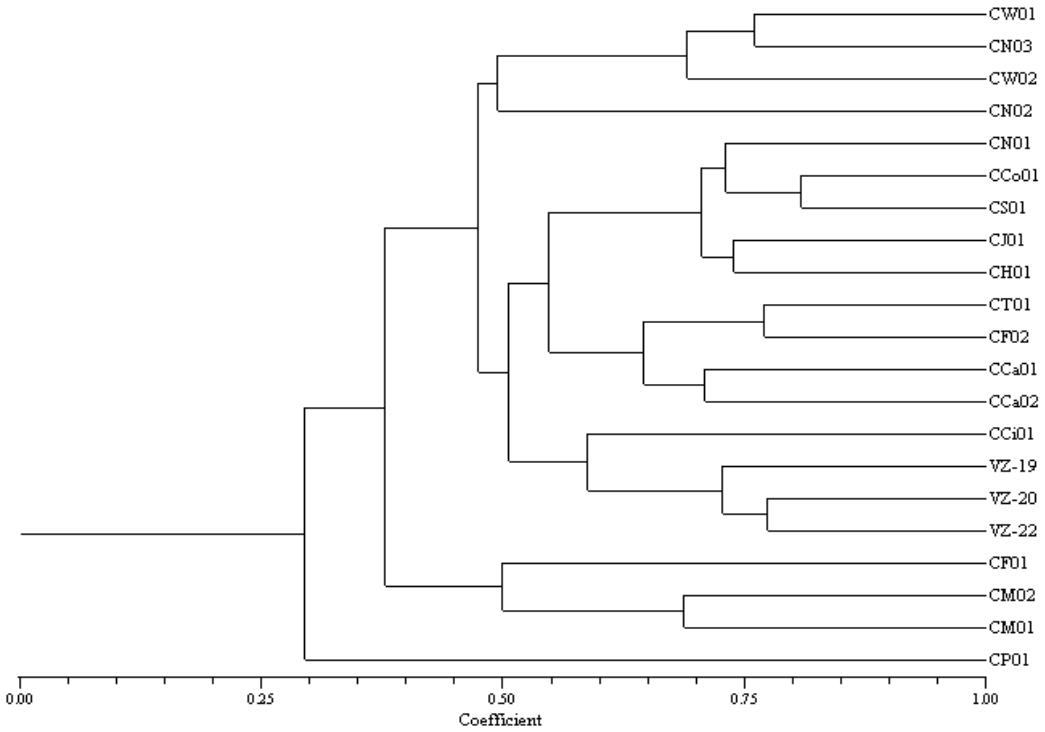

Fig. 1: Dendrogram showing comparative RAPD analysis of both genus *Cymbopogon* and *Vetiveria*.

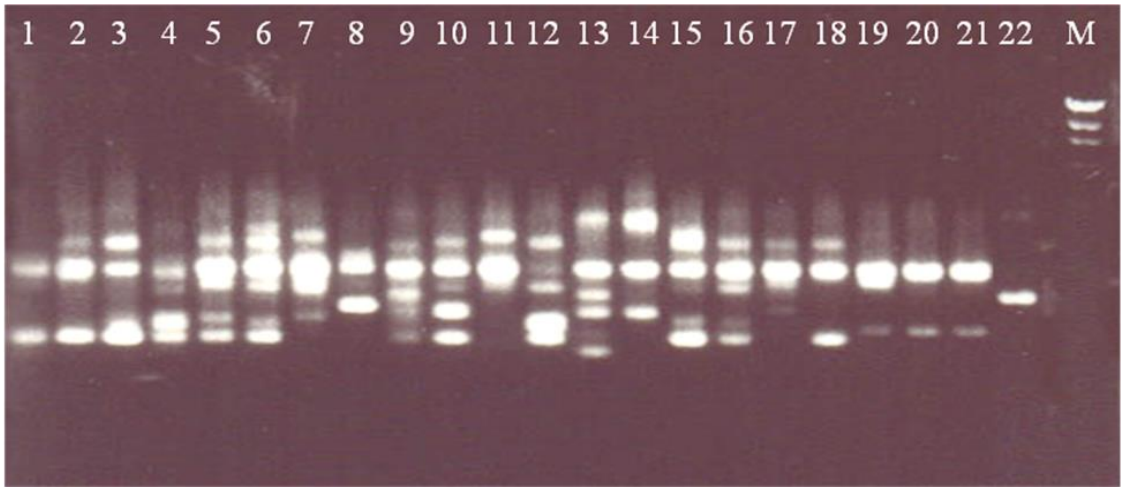

Fig. 2: RAPD profile of different accessions of *Cymbopogon* species and *Vetiveria zizanioides* with primer MAP-10 used in blot preparation for hybridization.

Lane 1- 18*:* *Cymbopogon* species

Lane 19- 21: *Vetiveria zizanioides*

Lane 22: Unidentified outgroup species (UOS).

M: λ Hind III single digest marker

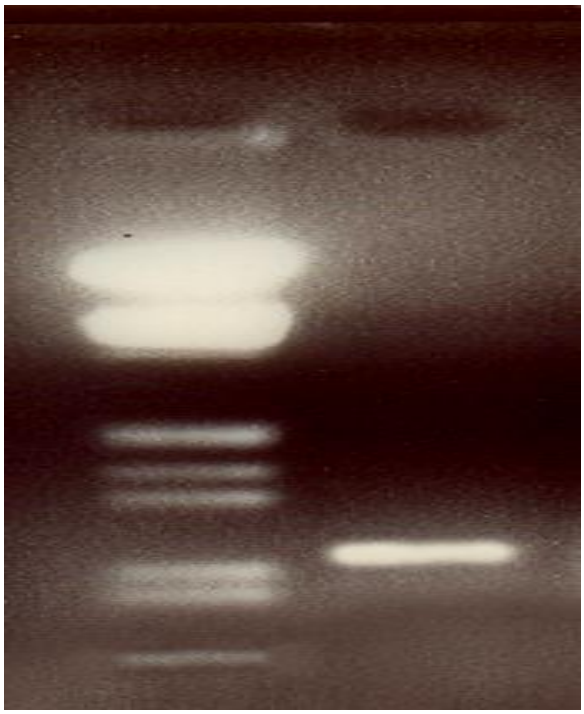

Fig. 3: Eluted DNA fragment of 1.66 kb used for probe preparation for southern hybridization.

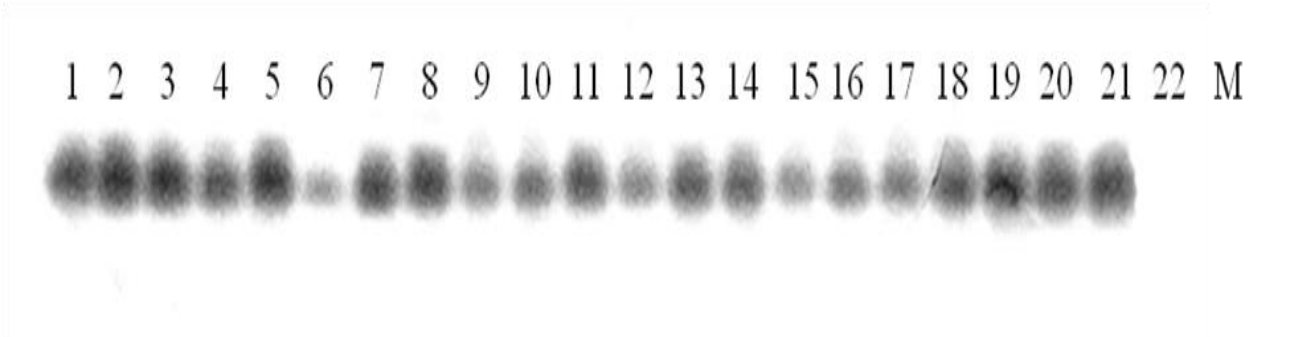

Fig. 4: Radioactive hybridization of monomorphic fragments in all the accessions of all *Cymbopogon* species and *Vetiveria zizanioides* and demonstrating synteny among them.

Lane 1-18: *Cymbopogon* species.

Lane 19-21: *Vetiveria zizanioides.*

Lane 22: Unidentified outgroup species (UOS).

M: λ Hind III single digest marker

.

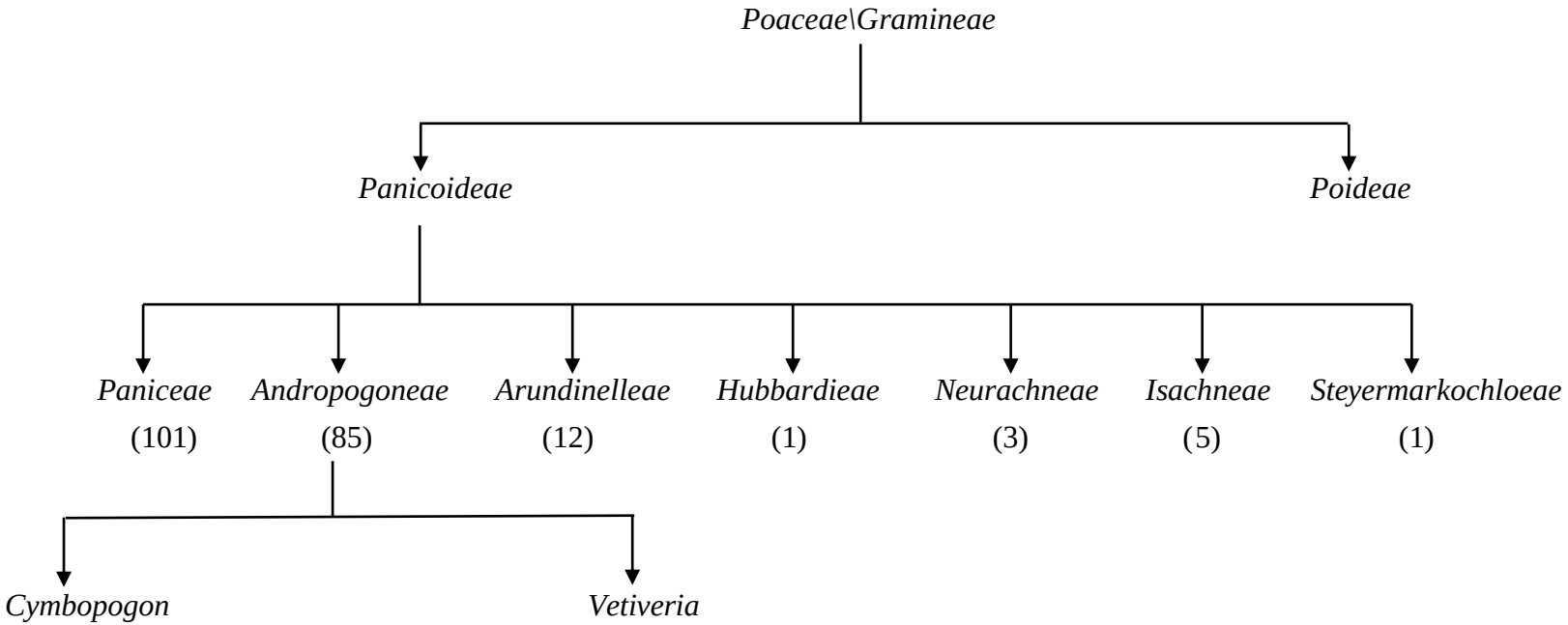

Fig. 5: Taxonomic classification of the family *Poaceae*.

Table-1: Yield and major constituents for the essential oils in the different accessions of *Cymbopogon* species and *Vetiveria zizanioides.*

Name of species	Oil yield (%)	Methyl heptenone (%)	Linalool (%)	Citral a (%)	Citral b (%)	Geraniol (%)	Elemol (%)	Geranyl acetate (%)	α-bisabalol (%)	1,8-cineole (%)	β-caryophyllene (%)	Citronellol (%)	Limonene (%)	Ocimene (%)	Perillyl alcohol (%)	Citronellal (%)	Khusimol (%)	Vetiselinenol (%)	β-eudesmol (%)	α-vetivone (%)	β-vetivone (%)	Vetiverol (%)	Epi-eudesmol (%)
CCa01	0.60 ± 0.20	-	-	9.79 ± 0.50	7.05 ± 1.30	3.76 ± 0.5	0.12 ± 0.01	2.00 ±0.28	0.39 ± 0.05	-	25.61 ± 2.10	2.54 ± 0.25	7.89 ± 0.60	-	-	-	-	-	-	-	-	-	-
CCa02	0.60 ± 0.20	-	-	9.78 ± 0.50	7.05 ± 1.30	3.75 ± 0.5	0.12 ± 0.01	1.99 ± 0.20	0.39 ± 0.03	-	25.62 ± 0.20	2.0 ± 0.10	7.00 ± 0.20	-	-	-	-	-	-	-	-	-	-
CCi01	0.80 ± 0.20	-	1.15 ± 0.30	52.40 ± 3.00	32.58 ±3.00	2.18 ± 0.6	0.57 ± 0.05	-	0.25 ± 0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCo01	0.60 ± 0.20	-	0.74 ± 0.10	7.12 ± 1.00	1.84 ± 0.30	67.78 ± 2.2	0.94 ± 0.05	7.12 ± 0.80	0.43 ± 0.03	2.68 ± 0.40	-	-	-	1.87 ± 0.02	-	-	-	-	-	-	-	-	-
CF01	0.50 ± 0.30	0.92 ± 0.22	1.17 ± 0.40	48.84 ± 2.20	32.15 ± 2.00	3.46 ± 0.5	1.13 ± 0.10	-	1.93 ± 0.30	-	1.12 ± 0.30	-	-	-	-	-	-	-	-	-	-	-	-
CF02	0.40 ± 0.20	-	1.01 ± 0.30	12.88 ± 1.50	7.34 ± 1.80	68.81 ± 1.2	0.78 ± 0.10	-	0.20 ± 0.04	2.24 ± 0.03	1.20 ± 0.04	-	-	-	-	-	-	-	-	-	-	-	-
CH01	0.40 ± 0.20	-	3.21 ± 0.50	1.26 ± 0.30	2.05 ± 0.40	68.7 ± 2.0	-	16.12 ± 1.20	0.09 ± 0.02	0.60 ± 0.05	-	0.51 ± 0.20	-	5.98 ± 0.35	-	0.48 ± 0.05	-	-	-	-	-	-	-
CJ01	0.40 ± 0.20	-	0.13 ± 0.03	14.96 ± 0.80	3.36 ± 0.30	2.93 ± 0.5	1.14 ± 0.30	1.64 ± 0.30	1.40 ± 0.30	-	23.25 ± 1.50	-	0.59 ± 0.06	-	14.53 ± 1.20	-	-	-	-	-	-	-	-
CM01	1.20 ± 0.30	-	0.74 ± 0.10	1.35 ± 0.30	0.38 ± 0.10	75.76 ± 2.0	0.60 ± 0.02	10.37 ± 1.00	-	-	-	-	0.49 ± 0.05	-	-	-	-	-	-	-	-	-	-
CM02	0.60 ± 0.20	-	0.12 ± 0.02	1.23 ± 0.20	2.37 ± 0.40	12.84 ± 1.2	-	4.68 ± 0.50	0.12 ± 0.01	2.59 ± 0.40	1.79 ± 0.02	-	9.61 ± 0.50	2.59 ± 0.30	17.19 ± 1.50	-	-	-	-	-	-	-	-
CN01	0.60 ± 0.20	0.26 ± 0.05	0.58 ± 0.30	8.85 ± 1.23	3.80 ± 0.50	46.01 ± 2.0	2.78 ± 0.30	1.55 ± 0.30	0.97 ± 0.03	1.22 ± 0.30	7.81 ± 0.40	-	0.62 ± 0.20	-	-	-	-	-	-	-	-	-	-
CN02	0.60 ± 0.20	0.01 ± 0.03	1.72 ± 0.30	2.38 ± 0.50	3.18 ± 0.80	30.95 ± 1.5	0.16 ± 0.01	2.43 ± 0.50	14.75 ± 1.20	2.13 ± 0.60	-	-	4.57 ± 0.50	1.07 ± 0.15	-	-	-	-	-	-	-	-	-
CN03	0.60 ± 0.20	0.13 ± 0.03	0.84 ± 0.15	9.80 ± 0.50	6.60 ± 1.20	36.5 ± 1.2	2.37 ± 0.30	4.77 ± 0.30	3.32 ± 0.50	0.09 ± 0.02	0.38 ± 0.060	6.06 ± 0.50	0.24 ± 0.05	-	-	13.95 ± 1.40	-	-	-	-	-	-	-
CP01	0.50 ± 0.20	1.19 ± 0.30	2.73 ± 0.50	48.18 ± 2.00	32.92 ± 2.30	4.74 ± 0.6	0.18 ± 0.02	-	0.13 ± 0.03	-	1.07 ± 0.05	-	-	-	-	-	-	-	-	-	-	-	-
CS01	0.50 ± 0.20	-	0.98 ± 0.10	5.06 ± 0.50	3.14 ± 0.80	69.37 ± 1.8	0.63 ± 0.10	5.29 ± 0.30	0.29 ± 0.02	2.95 ± 0.03	-	-	-	2.11 ± 0.24	-	-	-	-	-	-	-	-	-
CT01	0.30 ± 0.10	0.46 ± 0.15	-	3.35 ± 0.30	3.95 ± 0.3	10.54 ± 1.2	0.60 ± 0.02	-	0.07 ± 0.01	1.01 ± 0.04	-	7.60 ± 0.24	-	-	-	-	-	-	-	-	-	-	-
CW01	0.80 ± 0.20	0.97 ± 0.30	0.57 ± 0.05	-	-	23.3 ± 1.2	10.67 ± 1.20	3.27 ± 0.50	1.48 ± 0.40	-	-	9.92 ± 0.50	1.50 ± 0.40	-	-	35.94 ± 3.20	-	-	-	-	-	-	-
CW02	0.85 ± 0.20	0.99 ± 0.30	0.58 ± 0.05	-	-	23.32 ± 1.2	8.77 ± 1.00	3.28 ± 0.20	1.48 ± 0.30	-	-	14.06 ± 0.10	1.40 ± 0.20	-	-	42.97 ± 2.50	-	-	-	-	-	-	-
*Vetiveria**1 *zizanioides*	1.50-2.00	-	-	-	-	-	0.70-2.30	-	-	-	-	-	Trace	-	-	-	13.40-27.90	10.30-19.50	5.50-5.00	1.50-2.50	1.50-1.80	45-80*2	1.10-1.20

Other elements in *Vetiveria zizanioides3:** Khusilol, Khusene, Khushone, Khusenol, Khusilal, Khusimene, Khusimone, Khusimyl-acetate, Khusinol, Khusinoloxide, Khusiol, Khusitol,

Khusitone, L-d-cadinol, L-t-2-cadinene, L-r-cadinene, Laevojuneol, Laevojunessol, N-Eicosane, Norkhusinol-oxide, p-Cymene, Palmitic acid, Selina-4(14), 7(17)-diene, Sesquiterpene-ketones,
Tricyclovetivene, Veticadinol, Vetiselinene, Vetivene, Vetivenolene, Vetivenone, Vetivenyl-vetivenate, Zizanol, Zizanene, Zizanoic-acid, Zizanol.
*1Ref: Akhtar Hussain, (1994).
*2Ref: Gupta and Pareek, 1995.

Table-2: Morphological characters of genera *Cymbopogon* and *Vetiveria*.

S. No.	Topic(s)	*Cymbopogon*	*Vetiveria*
1.	**Nomenclature**	Derived from the Greek *kumbe* (boat) and *pogon* (beard), referring to many-awned inflorescences and boat shaped spathes.	From Tamil vetti (khus-khus or cus-cus) and ver (root), alluding to aromatic roots.
2.	**Habitat**	Usually perennial (rarely annual).	Perennial
3.	**Distribution**	Tropical and subtropical Africa and Asia, Australia. Mesophytic to xerophytic; species of open habitats; glycophytic. Savanna.	Tropical Africa, Asia, Australia. Helophytic; glycophytic. Floodplains and streambanks.
4.	**Taxonomy**	Panicoideae; Andropogonodae; Andropogoneae; Andropogoninae.	Panicoideae; Andropogonodae; Andropogoneae; Andropogoninae.
5.	**Cytology**	Chromosome base number, $x = 5$, or 10. $2n = 20$, 22, 40, and 60.	Chromosome base number, $x = 5$ and 10. $2n = 20$ and 40. Nucleoli persistent.
6.	**Vegetative morphology**	Caespitose, or rhizomatous and caespitose. ***Culms*** 15-300 cm high; herbaceous; usually unbranched above. Culm nodes glabrous. Culm internodes solid. The ***shoots*** aromatic. ***Leaves*** not basally aggregated; non-auriculate. ***Leaf blades*** linear (from broadly so to filiform); broad, or narrow; cordate, or not cordate, not sagittate; setaceous, or not setaceous; flat, or folded; without cross venation; persistent; rolled in bud; an unfringed membrane to a fringed membrane. ***Contra-ligule*** absent.	Often with aromatic ***roots***; forming large clumps from stout rhizomes. ***Culms*** 50–300 cm high; herbaceous; unbranched above. Culm nodes glabrous. Culm internodes solid. ***Leaves*** mostly basal; non-auriculate. Sheath margins free. The lower sheaths compressed. ***Leaf blades*** linear; broad, or narrow; without cross venation; persistent; a fringed membrane to a fringe of hairs.
7.	**Reproductive organization**	Plants bisexual, with bisexual spikelets; with hermaphrodite florets. The ***spikelets*** of sexually distinct forms on the same plant; hermaphrodite and male-only (usually), or hermaphrodite and sterile; overtly heteromorphic (the pedicelled spikelets not depressed abaxially, awnless); in both homogamous and heterogamous combinations (the lowermost pair of the lowest raceme, or of each raceme, homogamous and imperfect). Plants outbreeding. ***Inflorescence*** paniculate (decompound, leafy); non-digitate. Rachides hollowed, or flattened, or winged, or neither flattened nor hollowed, not winged. Inflorescence spatheate; a complex of 'partial inflorescences' and intervening foliar organs. ***Spikelet-bearing axes*** 'racemes' (short, spikelike, each pair with a spatheole); paired (the raceme bases short to more or less connate, flattened, often widely spreading or deflexed); with very slender rachides; disarticulating; disarticulating at the joints. *'Articles'* linear; appendaged, or not appendaged; densely long-hairy to somewhat hairy. ***Spikelets*** paired (or with a terminal triplet); not secund; sessile and pedicellate; consistently in 'long-and-short' combinations; in pedicellate/sessile combinations. ***Pedicels*** of the 'pedicellate' spikelets free of the rachis, or discernible, but fused with the rachis and free of the rachis (sometimes the pedicel of the homogamous pair being swollen and more or less fused with the internode). The 'shorter' spikelets hermaphrodite. The 'longer' spikelets male-only (usually), or sterile. ***Female-sterile*** spikelets pedicellate spikelets never depressed or canaliculate on the back; only the L_1	Plants bisexual, with bisexual spikelets; with hermaphrodite florets. The ***spikelets*** of sexually distinct forms on the same plant; hermaphrodite and male-only, or hermaphrodite and sterile; homomorphic; all in heterogamous combinations. ***Inflorescence*** of spicate main branches, or paniculate (a panicle with slender, whorled, simple or rarely compound racemes); open; espatheate; not comprising 'partial inflorescences' and foliar organs. ***Spikelet-bearing axes*** 'racemes'; the spikelet-bearing axes with 2-3 spikelet-bearing 'articles' to with 6-10 spikelet-bearing 'articles', or with more than 10 spikelet-bearing 'articles' (typically with many spikelet pairs); spikelet-bearing axes with very slender rachides; disarticulating; disarticulating at the joints. *'Articles'* linear; not appendaged; disarticulating transversely. ***Spikelets*** paired; secund (rarely), or not secund; sessile and pedicellate; consistently in 'long-and-short' combinations; in pedicellate/sessile combinations. Pedicels of the 'pedicellate' spikelets free of the rachis. The 'shorter' spikelets hermaphrodite. The 'longer' spikelets male-only, or sterile. ***Female-sterile*** **spikelets** pedicelled, male spikelets similar to the sessile ones, or slightly smaller. ***Female-fertile spikelets*** 4.5-10 mm long; compressed laterally; falling with the glumes (and with the joint and pedicel). Rachilla terminated by a female-fertile floret. Hairy ***callus*** present,

		present, hyaline, 2-nerved, its floret usually male but occasionally sterile or suppressed. The male spikelets 1 floreted. The lemmas awnless. ***Female-fertile spikelets***. Spikelets 3-7 mm long; compressed laterally, or not noticeably compressed, or compressed dorsiventrally; falling with the glumes. Rachilla terminated by a female-fertile floret. Hairy callus present. ***Callus*** short; blunt. Glumes two; more or less equal; long relative to the adjacent lemmas; awnless; very dissimilar (the lower bicarinate, the upper naviculate). ***Lower glume*** two-keeled (the keels sometimes winged apically); flattened on the back to sulcate on the back; not pitted; relatively smooth; 1-5 nerved. ***Upper glume*** 1-5 nerved. ***Spikelets*** with incomplete florets. The incomplete florets proximal to the female-fertile florets. ***Spikelets with proximal incomplete florets***. The proximal incomplete florets 1; epaleate; sterile. The proximal lemmas awnless; 2 nerved; less firm than the female-fertile lemmas to similar in texture to the female-fertile lemmas; not becoming indurated (hyaline). ***Female-fertile florets*** 1. Lemmas hyaline to firm-stipitate beneath the awn; less firm than the glumes; not becoming indurated; apically incised; 2 lobed; awnless, or awned. Awns when present, 1; from a sinus; geniculate; hairless (glabrous); much shorter than the body of the lemma to much longer than the body of the lemma. ***Lemmas*** hairless; non-carinate; 1-3 nerved. ***Palea*** absent. ***Lodicules*** present; 2; free; fleshy; glabrous. ***Stamens*** 3. Anthers not penicillate. ***Ovary*** glabrous. Styles free to their bases. Stigmas 2; red pigmented.	or absent. Callus pointed to blunt. ***Glumes*** two; more or less equal; long relative to the adjacent lemmas; awned (G_2, sometimes), or awnless; very dissimilar (the lower rounded on the back, the upper naviculate). ***Lower glume*** convex on the back; not pitted; spinulose; 5 nerved. ***Upper glume*** 3 nerved. ***Spikelets*** with incomplete florets. The incomplete florets proximal to the female-fertile florets. ***Spikelets with proximal incomplete florets***. The proximal incomplete florets 1; epaleate; sterile. The proximal lemmas awnless; 2 nerved; similar in texture to the female-fertile lemmas (hyaline); not becoming indurated. ***Female-fertile florets*** 1. ***Lemmas*** less firm than the glumes (hyaline); not becoming indurated; incised; not deeply cleft (bidentate); awnless, or mucronate, or awned. Awns when present, 1; from a sinus; geniculate; hairless (glabrous); much shorter than the body of the lemma to much longer than the body of the lemma. Lemmas hairless; non-carinate; 1-3 nerved. ***Palea*** present, or absent; when present, very reduced; apically notched; awnless, without apical setae; not indurated (hyaline); nerveless. ***Lodicules*** present; 2; free; fleshy; glabrous. ***Stamens*** 3. Anthers not penicillate. ***Ovary*** glabrous. Styles free to their bases. Stigmas 2.
8.	**Friut, embryo and seedling**	***Fruit*** free from both lemma and palea; small; compressed dorsiventrally (subterete to planoconvex). ***Hilum*** short. ***Embryo*** large; waisted. ***Endosperm*** hard; without lipid; containing compound starch grains. Embryo without an epiblast; with a scutellar tail; with an elongated mesocotyl internode. ***Embryonic leaf*** margins overlapping. ***Seedling*** with a long mesocotyl. First seedling leaf with a well-developed lamina. The lamina broad; curved; 21-30 veined.	***Fruit*** small; not noticeably compressed. ***Hilum*** short. ***Embryo*** large. ***Endosperm*** containing only simple starch grains. Embryo without an epiblast; with a scutellar tail; with an elongated mesocotyl internode. ***Embryonic leaf*** margins overlapping. First seedling leaf with a well-developed lamina. The lamina broad; curved; 21-30 veined.
9.	**Abaxial leaf blade epidermis**	***Costal/intercostal zonation*** conspicuous. ***Papillae*** present (rarely), or absent. Intercostal papillae when present, consisting of one oblique swelling per cell. ***Long-cells*** similar in shape costally and intercostally (narrow); of similar wall thickness costally and intercostally. Mid-intercostal long-cells rectangular (long); having markedly sinuous walls, or having straight or only gently undulating walls (rarely). ***Microhairs*** present; panicoid-type, or chloridoid-type (rarely); (26-)30-48(-60) microns long; 6-7.5 microns wide at the septum. Microhair total length/width at septum 4.8-7. Microhair apical cells (3-)13-22(-24) microns long. Microhair apical cell/total length ratio (0.17-)0.33-0.48-0.48. ***Stomata*** common; 21-22.5(-24) microns long. Subsidiaries variously low or high dome-shaped, or triangular, or dome-shaped and triangular. Guard-cells overlapping to flush with the interstomatals. ***Intercostal short-cells*** common, or absent or very rare; in cork/silica-cell pairs (and solitary); not silicified (usually), or silicified. Intercostal si lica bodies when present, tall-and-narrow, or cross-shaped, or vertically elongated-nodular. ***Costal short-cells*** conspicuously in long rows, or neither distinctly grouped into long rows nor predominantly paired. Costal silica bodies 'panicoid-type'; cross shaped, or butterfly shaped, or dumb-bell shaped, or nodular (occasionally).	***Costal/intercostal zonation*** conspicuous. ***Papillae*** absent. ***Long-cells*** similar in shape costally and intercostally; of similar wall thickness costally and intercostally. Mid-intercostal long-cells rectangular; having markedly sinuous walls (the sinuosities very tight in *V. elongata*). ***Microhairs*** present; panicoid-type (but often balanoform - the thin walled apical cells quite broad and blunt); (39-)48-51(-54) microns long; 9-12.6 microns wide at the septum. Microhair total length/width at septum 3.8–5.5. Microhair apical cells (25–)27–30(–33) microns long. ***Microhair*** apical cell/total length ratio 0.55-0.63. ***Stomata*** common; 27-33 microns long. Subsidiaries low dome-shaped, or triangular. Guard-cells overlapped by the interstomatals (the interstomatal end walls very thickened in *V. elongata*). ***Intercostal short-cells*** common; in cork/silica-cell pairs; silicified. Intercostal silica bodies tall-and-narrow, or cross-shaped. Costal zones with short-cells. ***Costal short-cells*** predominantly paired. Costal silica bodies tall-and-narrow (exclusively, in *V. elongata*), or 'panicoid-type'; cross shaped (in *V. zizanioides*); not sharp-pointed.
10.	**Transverse section of leaf blade, physiology**	C_4; biochemical type NADP-ME (*C. citratus*); XyMS-. PCR sheath outlines uneven. PCR cell chloroplasts centrifugal/peripheral. ***Mesophyll*** with radiate chlorenchyma. ***Leaf blade*** adaxially flat. ***Midrib*** conspicuous; with one bundle only, or having a conventional arc of bundles; with colourless mesophyll adaxially (the colourless tissue often extending across the adaxial part of the blade). Bulliforms not present in discrete, regular adaxial groups (in irregular groups); occasionally, irregularly associated with colourless mesophyll cells to form deeply-penetrating fans. Many of the smallest vascular bundles unaccompanied by sclerenchyma. Combined sclerenchyma girders present; forming 'figures'. Sclerenchyma all associated with vascular bundles.	C_4; XyMS–. PCR sheath outlines even. PCR cell chloroplasts with reduced grana; centrifugal/peripheral. ***Mesophyll*** with radiate chlorenchyma. ***Leaf blade*** adaxially flat. ***Midrib*** conspicuous; having a conventional arc of bundles; with colourless mesophyll adaxially (the adaxial mesophyll of the rest of the blade also extensively colourless in *V. elongata*, and with large intercellular lacunae in *V. zizanioides*). Bulliforms not present in discrete, regular adaxial groups (except in association with the midrib). Many of the smallest vascular bundles unaccompanied by sclerenchyma. Combined sclerenchyma girders present. Sclerenchyma all associated with vascular bundles.

11.	**Disease**	***Rusts and smuts***. Rusts — *Puccinia*. Taxonomically wide-ranging species: *Puccinia nakanishikii*, *Puccinia eritraeensis*, '*Uromyces*' *schoenanthi*, *Puccinia versicolor*, and '*Uromyces*' *clignyi*. Smuts from Ustilaginaceae. Ustilaginaceae — *C. refractus*.	***Smuts*** from Tilletiaceae and from Ustilaginaceae. Tilletiaceae — *Tilletia*. Ustilaginaceae — *Ustilago*.
12.	**Economic importance**	Commercial essential oils: *C. nardus* and *C. winterianus* (citronella oil), *C. flexuosus* (East Indian Lemon-grass), *C. citratus* (West Indian Lemon-grass), *C.martinii*. *C. citratus* used as a culinary herb.	Commercial essential oils: *V. zizanioides* (from the roots). *V. zizanioides* is valuable for hedging, and as a guard against soil erosion (it also 'repels pests such as rats and snakes' - O. Sattaur 1989, *New Scientist* **1664**, 16–17).
13.	**Refrences**	Morphological/taxonomic: Soenarko 1977. Leaf anatomical: Metcalfe 1960; http://biodiversity.uno.edu/delta/grass.	Leaf anatomical: Metcalfe 1960; http://biodiversity.uno.edu/delta/grass.

Table-3: Germplasm collection of genera *Cymbopogon* and *Vetiveria*.

S. No.	Accession(s)	Name of species	Taxonomic series (Stapf, 1906)
1	CIMAP/CCa01	*Cymbopogon caesius* (Nees) var. narrow leaf.	Rusae
2	CIMAP/CCa02	*Cymbopogon caesius* (Nees) var. broad leaf.	Rusae
3	CIMAP/CCi01	*Cymbopogon citratus* (D.C.) Stapf.	Citrati
4	CIMAP/CCo01	*Cymbopogon confertiflorous* (Steud.) Stapf.	Citrati
5	CIMAP/CF01	*Cymbopogon flexuosus* (Steud.) Wats. var. *flexuosus.*	Citrati
6	CIMAP/CF02	*Cymbopogon flexuosus* (Steudel) Wats. var. *microstachys* (Hook f.) Bor.	Citrati
7	CIMAP/CH01	*Jamrosa*	-
8	CIMAP/CJ01	*Cymbopogon jawarancusa* (Jones) Schult.subsp. *jawarancusa*	Schoenanthi
9	CIMAP/CM01	*Cymbopogon martinii* (Roxb.) Wats. var. *motia* (B.K. Gupta).	Rusae
10	CIMAP/CM02	*Cymbopogon martinii* (Roxb.) Wats. var. *sofia* (B.K.Gupta) .	Rusae
11	CIMAP/CN01	*Cymbopogon nardus* (L.) Rendle var. *confertiflorous* (Steud.) Stapf..	Citrati
12	CIMAP/CN02	*Cymbopogon nardus* (L.) Rendle var. nardus.	Citrati
13	CIMAP/CN03	*Cymbopogon nardus* (L.) Rendle var. *Java II.*	Citrati
14	CIMAP/CP01	*Cymbopogon pendulus* (Nees ex Steud.) Wats.	Citrati
15	CIMAP/CS01	Unidentified *Cymbopogon* species	-
16	CIMAP/CT01	*Cymbopogon travancorensis* Bor.	Citrati
17	CIMAP/CW01	*Cymbopogon winterianus* Jowitt.	Citrati
18	CIMAP/CW02	*Cymbopogon winterianus* var. *manjari.*	Citrati
19.	CIMAP/VZ-19	Pusa Hyb-28	-
20.	CIMAP/VZ-20	Pusa Hyb-8	-
21.	CIMAP/VZ-22	MBR-4B	-
22.	CIMAP/UOS	Unidentified outgroup species	-

Table-4: Primers synthesized in the laboratory.

Primers	Sequences
MAP01	5’ AAATCGGAGC 3’
MAP02	5’ TGCGCGATCG 3’
MAP03	5’ GTCCTACTCG 3’
MAP04	5’ GTCCTTAGCG 3’
MAP05	5’ AACGTACGCG 3’
MAP06	5’ GCACGCCGGA 3’
MAP07	5’ CACCCTGCGC 3’
MAP08	5’ CTATCGCCGC 3’
MAP09	5’ CGGGATCCGC 3’
MAP10	5’ GCGAATTCCG 3’
MAP11	5’ CCCTGCAGGC 3’
MAP12	5’ CCAAGCTTGC 3’
MAP13	5’ GTGCAATGAG 3’
MAP14	5’ AGGATACGAG 3’
MAP15	5’ AAGATAGCGG 3’
MAP16	5’ GGATCTGAAC 3’
MAP17	5’ TTGTCTCAGG 3’
MAP18	5’ CATCCCGAAC 3’
MAP19	5’ GGACTCCACG 3’
MAP20	5’ AGCCTGACGC 3’

Table-5: Fragments generated through comparative RAPD analysis in different both genera *Cymbopogon* and *Vetiveria*.

Primers	No. of Polymorphic fragments	No. of Monomorphic fragments	No. of Unique fragments	Total No. of fragments
MAP	43	3	2	48
OPJ	43	4	5	52
OPT	54	3	5	62
Total	140	10	12	162

Table-6: Average similarity index generated during comparative RAPD analysis in both the genera *Cymbopogon* and *Vetiveria*.

	CCa01	CCa02	CCi01	CCo01	CF01	CF02	CH01	CJ01	CM01	CM02	CN01	CN02	CN03	CP01	CS01	CT01	CW01	CW02	VZ-19	VZ-20	VZ-22
CCa01	1.000																				
CCa02	0.708	1.000																			
CCi01	0.037	0.468	1.000																		
CCo01	0.352	0.660	0.534	1.000																	
CF01	0.056	0.000	0.571	0.071	1.000																
CF02	0.762	0.621	0.427	0.540	0.033	1.000															
CH01	0.598	0.657	0.491	0.722	0.600	0.582	1.000														
CJ01	0.355	0.628	0.524	0.633	0.054	0.528	0.738	1.000													
CM01	0.457	0.624	0.414	0.548	0.500	0.452	0.460	0.489	1.000												
CM02	0.374	0.580	0.347	0.542	0.500	0.505	0.406	0.500	0.688	1.000											
CN01	0.341	0.559	0.506	0.680	0.054	0.511	0.685	0.731	0.523	0.439	1.000										
CN02	0.236	0.528	0.442	0.580	0.023	0.505	0.589	0.426	0.473	0.472	0.510	1.000									
CN03	0.193	0.462	0.382	0.469	0.041	0.539	0.587	0.473	0.445	0.444	0.506	0.461	1.000								
CP01	0.200	0.440	0.260	0.225	0.133	0.363	0.490	0.203	0.327	0.403	0.225	0.187	0.155	1.000							
CS01	0.524	0.626	0.552	0.809	0.571	0.590	0.742	0.724	0.505	0.464	0.781	0.540	0.555	0.450	1.000						
CT01	0.541	0.659	0.496	0.553	0.060	0.771	0.680	0.549	0.450	0.442	0.454	0.465	0.347	0.290	0.668	1.000					
CW01	0.338	0.483	0.431	0.517	0.000	0.452	0.487	0.489	0.316	0.381	0.476	0.521	0.761	0.147	0.483	0.418	1.000				
CW02	0.336	0.537	0.508	0.518	0.000	0.457	0.497	0.530	0.374	0.428	0.543	0.502	0.663	0.380	0.512	0.431	0.717	1.000			
VZ-19	0.421	0.579	0.585	0.579	0.286	0.458	0.446	0.580	0.491	0.377	0.643	0.473	0.504	0.311	0.527	0.470	0.593	0.487	1.000		
VZ-20	0.445	0.625	0.613	0.547	0.500	0.489	0.517	0.555	0.564	0.477	0.602	0.481	0.523	0.335	0.565	0.484	0.530	0.488	0.753	1.000	
VZ-22	0.443	0.608	0.562	0.540	0.600	0.455	0.517	0.489	0.534	0.416	0.593	0.536	0.447	0.353	0.497	0.509	0.440	0.385	0.701	0.773	1.000

Table-7: Details of blast result of co-migrating fragment (1.66 kb) cloned from *Cymbopogon nardus* (L.) Rendle var. *confertiflorous* (CN01) amplified profile with MAP-10.

S. No.	Gene Bank Accession Nos.	Organisms (Score (bits), Expect value, Match Size (Gene size)	Domain
1	gi|50080438|gb|AE009947.2	*Saccharum* hybrid cultivar (605, e-170, 355)(32545-32901)	chloroplast, complete genome
2	gi|49659489|dbj|AP006714.1	*Saccharum officinarum* (605, e-170, 355) (112102-112458)	chloroplast, complete genome
3	gi|11990232|emb|X86563.2|ZMA86563	*Zea mays* (589, e-165, 353) (111348- 111704)	complete chloroplast genome
4	gi|12421|emb|X13159.1|CHZMNDHD	Maize (589, e-165, 353) (1812- 1456), Gene : ndhE : 382-687 cds; psaC : 1155-1400 cds; ndhD : 1475-3022 cds.	chloroplast genes ndhD, ndhE and psaC
5	gi|13928184|dbj|AB042240.3	*Triticum aestivum* (533, e-148, 346) (107654- 108010)	chloroplast DNA, complete genome
6	gi|4150866|emb|AJ011848.1|HVU011848	*Hordeum vulgare* (517, e-144, 344) (6702- 6346), Genes : rps 15 : 1-164 cds; ndhH : 311-1492 cds; ndhA : 1494-3615 cds; ndhI : 3717-4259 cds; ndhG : 4510-5040 cds; ndhE : 5253-5558 cds; psaC : 6039-6284 cds; ndhD : 6385-7912 cds.	chloroplast rps15 (partial), ndhH, ndhI, ndhG, ndhE, psaC, ndhD and ndhA genes
7	gi|2145423|emb|Y12258.1|CHHVNDHD	*H.vulgare* (509, e-141, 333) (353- 57), Gene : ndhD : 61-1563 cds.	chloroplast ndhD gene
8	gi|42795537|gb|AY522330.1	*Oryza sativa* (japonica cultivar-group) cultivar Nipponbare (505, e-140, 328) (107630-107926)	chloroplast, complete genome
9 10	gi|42795473|gb|AY522329.1; gi|11957|emb|X15901.1|CHOSXX	*Oryza sativa* (indica cultivar-group) (505, e-140, 328) (107579- 107875); (505, e-140, 328) (107614- 107910)	chloroplast, complete genome
11	gi|49614971|dbj|AP006728.1	*Oryza nivara* (505, e-140, 328) (107573- 107869)	chloroplast DNA, complete genome
12 13	gi|52076896|dbj|AP004989.3; gi|52076613|dbj|AP003728.3	*Oryza sativa* (japonica cultivar-group) genomic DNA (505, e-140, 328) (148509-148805); (505, e-140, 328) (101763- 102059)	genomic DNA, chromosome 6
14 15 16	gi|31431454|gb|AE017082.1; gi|21104881|gb|AC122148.1; gi|18693402|gb|AC099402.2	*Oryza sativa* (japonica cultivar-group) (498, e-138, 327) (125858- 125562); (498, e-138, 327) (105697- 105401); (498, e-138, 327) (39761- 39465)	chromosome 10
17	gi|25956171|emb|BX000560.1|CNS08CE6	*Oryza sativa* (japonica cultivar-group) (498, e-138, 327) (29329- 29033)	chromosome 12
18	gi|50510199|dbj|AP005438.5	*Oryza sativa* (japonica cultivar-group) genomic DNA, (498, e-138, 327) (92387- 92091)	chromosome 7
19	gi|34897597|ref|NM_185256.1	*Oryza sativa* (japonica cultivar-group) (410, e-111, 274) (287- 50)	mRNA

20	gi|8919614|emb|AJ278355.1|HMU278355	*Hordeum murinum* (377, e-101, 238) (344- 106), Genes : psaC : 1-45 cds; ndhD : 165-351cds.	chloroplast partial psaC gene for PSI 9 KDa protein and partial ndhD gene for NADH dehydrogenase subunit 4
21	gi|21927774|gb|AY083667.1	*Sorghum bicolor* cultivar (343, 2e-91, 193) (277- 98), Gene : ndhD : 150-281 cds.	NADH dehydrogenase subunit 4 ndhD) gene, partial cds; chloroplast gene for chloroplast product
22 23	gi|38175650|dbj|AP005383.3; gi|38175587|dbj|AP004694.3	*Oryza sativa* (japonica cultivar-group) (210, 2e-51, 147) (134364- 134245); (210, 2e-51, 147) (40501- 40382)	genomic DNA, chromosome 8
24	gi|20068310|emb|AJ316582.1|ABE316582	*Atropa belladonna* (174, 1e-40, 245) (119424- 119661)	complete chloroplast genome
25	gi|31580916|dbj|AB098223.1	*Nicotiana sylvestris* (167, 3e-38, 244) (549- 312), Gene : psaC : 1-138 cds; ndhD : 254-707 cds.	psaC, ndhD genes for PSI 9 kDa protein, NADH dehydrogenase subunit 4, partial cds
26	gi|2924257|emb|Z00044.1|CHNTXX	*Nicotiana tabacum* (167, 3e-38, 244) (118733- 118970)	chloroplast genome DNA
27	gi|50080242|gb|AC098832.3	*Oryza sativa* (japonica cultivar-group) (161, 2e-36, 143) (96219- 96100)	chromosome 5
28	gi|31580959|dbj|AB098244.1	*Nicotiana tomentosiformis* (159, 7e-36, 243) (565-328)' Gene : psaC : 1-154; ndhD : 246-714 cds.	psaC, ndhD genes for PSI 9 kDa protein, putative NADH dehydrogenase ND 4 subunit, partial cds
29	gi|167037|gb|L06607.1|BLYDLPSAC	*Hordeum vulgare* (137, 3e-29, 85) (456-368)	photosystem I subunit C (PsaC) gene, complete cds; NADH dehydrogenase (ndhD) gene, complete cds
30	gi|8919759|emb|AJ278350.1|ACE278350	*Allium cepa* (131, 2e-27, 164) (350-172), Genes : psaC : 1-45 cds; ndhD : 166-351cds.	chloroplast partial psaC gene for PSI 9 KDa protein and partial ndhD gene for NADH dehydrogenase subunit 4
31	gi|8919843|emb|AJ278353.1|AVE278353	*Aloe vera* (129, 6e-27, 191) (350- 172)	chloroplast partial psaC gene for PSI 9 KDa protein and partial ndhD gene for NADH dehydrogenase subunit 4
32	gi|27885316|gb|AF384510.2	*Perityle lindheimeri* (115, 9e-23, 150) (185- 10), Gene : ndhD : 1-1408 cds.	NADH dehydrogenase subunit 4 (ndhD) gene, partial cds; chloroplast gene for chloroplast product
33	gi|27885312|gb|AF384508.2	*Palafoxia arida* (115, 9e-23, 150) (185- 10), Gene : ndhD : 1-1408 cds.	NADH dehydrogenase subunit 4 (ndhD) gene, partial cds; chloroplast gene for chloroplast product
34	gi|8919840|emb|AJ278352.1|APO278352	*Allium porrum* (115, 9e-23, 162) (350- 159), Genes : psaC : 1-45 cds; ndhD : 166-351cds.	chloroplast partial psaC gene for PSI 9 KDa protein and partial ndhD gene for NADH dehydrogenase subunit 4
35	gi|27885330|gb|AF384517.2	*Polymnia canadensis* (111, 1e-21, 234) (293- 10)	NADH dehydrogenase subunit 4 (ndhD) gene, partial cds; chloroplast gene for chloroplast product
36	gi|32399358|emb|AJ428413.1|CFE428413	*Calycanthus fertilis* var. *ferax* (111, 1e-21, 239) (117693- 117983)	complete chloroplast genome

37	gi|27885310|gb|AF384507.2	*Oyedaea verbesinoides* (107, 2e-20, 149) (185- 10)	NADH dehydrogenase subunit 4 (ndhD) gene, partial cds; chloroplast gene for chloroplast product
38	gi|8919837|emb|AJ278351.1|ASA278351	*Allium sativum* (107, 2e-20, 161) (350- 159)	chloroplast partial psaC gene for PSI 9 KDa protein and partial ndhD gene for NADH dehydrogenase subunit 4
39	gi|27885324|gb|AF384514.2	*Phaneroglossa bolusii* (103, 4e-19, 233) (293- 10)	NADH dehydrogenase subunit 4 (ndhD) gene, partial cds; chloroplast gene for chloroplast product

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