

Variation of Wild *Cymbopogon martinii* (Roxb.) in Different Ecotypes using Molecular Markers

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Abstract: The study of the genetic diversity of wild plant populations is essential for their management and conservation. In-order to provide a better comprehension of the results obtained and a comparison between markers which will help to choose tools for future studies in natural populations of *Cymbopogons*. This study used both Isozymes and Amplified fragment length polymorphism (AFLP) to assess genetic diversity and genetic structure of six populations, pointing to similarities and divergence of each marker and evaluating the relative importance of the results for studies of population genetics and conservation. Suitable identification and characterization of medicinal plants/aromatic plants are necessary for conservation of plant resources, investigations of genes associated with desirable traits, and understanding of evolutionary relationships. Therefore, various molecular marker techniques such as AFLP and Isoenzymes have been improved to provide detail information about genomes. This brief review represents usage of these markers for molecular diversity analysis of medicinally important plants (SevgiMarakli 2018). Several DNA – based marker systems are available for genetic finger printing of plants but information on their relative usefulness for characterization of *Cymbopogon* germplasm is lacking. The efficiency of AFLP markers for the assessment of genetic relationships. This species of different ecotypes, were investigated. Dendrogram is produced based on band pattern scores using UPGMA metho (Nirupama Lal and S.K. Awasti 2014). Genetic diversity of 6 accessions estimated using amplified Fragment length polymorphism (AFLP) marker technology and specific activity of Isoenzymes using spectrophotometric methods. Six ecotypes *Cymbopogon martinii* in the study five AFLP primer combinations generated the bands and showing average polymorphic. The UPGMA method was employed to construct a dendrogram based on the Jaccard's similarity co-efficient. Based on these co-efficient it was concluded that these accessions show genetic variation. These results have significant implications in the crop breeding programmes, the results demonstrates the great value of AFLP and Isoenzyme markers in the management of genebanks. Several isoenzymes are present in plants and their relative composition changes during to exposure to stress. Several methods are available for the quantitative determination of the enzymes. Based on the spectrophotometric detection and quantification, the enzyme activity is calculated by determining the concentration compounds in solution (M.S. Negi, V. Sabharwal et al, 2003)

1. Introduction

Genetic diversity is the fundamental source of biodiversity. In 1989, the world wildlife fund defined biodiversity as the richness of life on earth-millions of plants, animals and microorganisms, including the genes which they carry and complex ecosystems that create the environment. Currently the issue of maintaining genetic diversity as a component of the conservation of biodiversity has been accepted at an international level. One of the main concerns of scientific research activities is conserving genetic diversity of local breeds especially those of economic interest. Genetic diversity among individuals reflects the presence of different alleles in the gene pool and hence different genotypes within the populations (MahmutCaliskan 2012).

The genus *Cymbopogon* (Poaceae) is known to include about 140 species. Among these, more than 52 have been reported to occur in Africa, 45 in India, six each in Australia and South America, four in Europe, two in North America and the remaining are distributed in South Asia (Jagadish Chandra, 1975b). Most of these species produce characteristic aromatic essential oils that have commercial importance in perfumery, cosmetics and pharmaceutical applications. The important components of essential oil present in several species of *Cymbopogons* with wide industrial uses such as raw material for perfumery, confectionery and vitamin A (Hackel 1887 and Hooker 1897). *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) and aromatic grasses have become available as genetic resource. *Cymbopogon martinii* (Roxb) wats also called Palmarosa is a tropical grass growing wild throughout India and abundantly in

South India (Tharasaraswathi et al. 2014). It is an industrially important multi harvest crop belonging to Rusae series of the family poaceae. For instance, *C. martinii* var. Sofia and *C. martinii* var. motia, are morphologically almost indistinguishable, but show distinct chemotypic characteristics in-terms of oil constituents (Guenther, 1950). The plant is indigenous to India. It is an industrially important multi harvest crop belonging to Rusae series of the family Poaceae. The Palmarosa oil of commerce is one of top ten essential oils in the world. The oil finds extensive application in perfumery, cosmetic, flavor and aroma therapy (Bhasker et al., 2015). It is best known by the common name Palmarosa as it smells sweet and rose-like. Other common names include Indian geranium, ginger grass, rosha and rosha grass.

Herbal medicines, aromatic plants *Cymbopogon* Species play a significant role as an alternative to synthetic pharmaceuticals. For this reason conservation of endemic, threatened and endangered medicinal species, improving high-quality cultivars with desirable traits and even knowledge of the germplasm diversity have gained importance in the past decades (Baruah, J.et al., 2017). Hence different molecular markers have been commonly used for these purpose to give detailed information about genomes which is not possible with phenotypic methods. Ideal DNA markers should be highly polymorphic in nature, co-dominant inheritance, frequent occurrence in the genome, fast and easy testing high reproducibility and even easy exchange of data among laboratories (Joshi S.P. et al., 1999). AFLP (AFLP) technique depends on the PCR amplification of restriction fragments obtained as a result of digestion of genomic DNA. Among molecular markers, AFLP (Amplified fragment length polymorphism) and Isoenzymes produces much more reproducible information,

hence this technique is an important method to detect genetic variability of plants. (Aversano, R.F., *et al.*, and Mehta, R.V., Sharma *et al.*, 2011). The ability of AFLP markers for efficient and rapid detection of genetic variations at the species as well as intra-specific level, qualifies it as an efficient tool to estimate genetic similarity (variation) in plant species and for effective management of genetic resources. High reproducibility, rapid generation and high frequency of identifiable polymorphisms make AFLP analysis a suitable technique for identifying polymorphisms (N.S. Sangwan *et al.*, 2001)

The usefulness of Isoenzymes for species identification assessing genetic distances between intra and inter (Taxa) and in analyses of systematic relationships has been demonstrated in a large number of crops (Cooks 1984). Genetic variability in cultivars of *Vitis vinifera* was determined using Isozyme electrophoresis by Sandra *et al.*, (2005). Pasquet and Vandereborgh (2000) have reported the use of Isoenzymes to assess the genetic distance between 21 *Vigna* accessions. Isoenzymes have shown their potential for rapid cultivar identification in onion and lily (Cooke, 1986) have described an efficient procedure for identification of tulip cultivars based on polymorphism in Isozymes of esterase. Though the species differ at the intra and interspecies level, morphological differences often blurred at the intra species level (Deepak Ganjewal *et al.*, 2007).

It is an important and valuable model grass to investigate the genetic diversity due to its commercial importance, wide distributed limits and resistance to diverse stress. Such investigations are essential to understand the biological processes within and among populations during historic events. Generally polyploidy populations with a broad geographically distributed range are better suited to survive and colonize new niches in diverse environments, because they occupy greater heterozygosity and variation, lower inbreeding depression. The specific environmental conditions, such as elevation, latitude, recitation, sunlight may lead to different physiological challenges for species, which in-turn results in morphological and molecular adaptation to local environment. So the genomes of populations in different environments may genetically differ at a few key sites and contain valuable alleles. Thus assessing the level of genetic diversity among different geographical regions benefits for forage crop breeding, germplasm collection and protection of genotypes from genetic erosion. Marking on individual DNA is more effective to distinguish geographically distinct populations which could differ in the level of genetic diversity or in the distribution of diversity within and among regions (Rita costa *et al.*, 2016). Generally neutral evolution and natural selection partly have the joint effects on population divergence and speciation (Rohlf F.J 1995). Where gene flow is broken off, divergence and differentiation among populations would intensify if random drift driven and divergent selection might lead to local adaptation (Mantel N 1967).

Totally six wild *Cymbopogon* population from six geographical regions were used to research their genetic diversity and structure patterns and develop basis for the

collection, protection and utilization of some excellent germplasm resources. From these studies we can estimate the genetic diversity and structure pattern of populations; we can study the geneflow/genetic drift among regions and evaluate the co-relation of genetic diversity and environmental factors. In our investigation we used six different wild ecotypes of *Cymbopogon martinii* collected from different ecotypes of Karnataka (India)

2. Materials and Methods

2.1 DNA preparation

For DNA extraction, 1 g of fresh leaf tissue was ground in liquid nitrogen and added to 10 mL of extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA, 0.5 M NaCl, 0.2% β -mercaptoethanol, 1% PVP and 3% CTAB). The samples were incubated at 65°C for 60 min. To this mixture add equal volume of Chloroform: isoamylalcohol (24:1) to each tube, mix well for 25-30 times by inverting. Spin the mixture at 10000 rpm for 10 min. Transfer the supernatant to fresh tube and repeat the process until a clear supernatant is obtained. To this pooled mixture add half the volume of 3M sodium acetate and mix well, to this add one volume of ice cold isopropanol to precipitate. The solution was precipitated for 2 hr at -20°C and centrifuged at maximum speed of 12,000 rpm for 15 min. After one washing with 70% ethanol the pellet was re-suspended in 10 ml of TE buffer (pH - 8.0) and treated with 4 μ g of RNase for 1 hr at 37°C.

Amplified fragment length polymorphism (AFLP) analysis was performed as per the technical instructions of the manufacturer (Invitrogen, Life Technologies). Genomic DNA (100 ng) was restricted with *EcoRI* (10 U/ μ l) and *MseI* (4 U/ μ l) in a restriction buffer (50 mM Tris-HCl, pH 7.5, 50 mM Mg Chloride, 250 mM K-acetate) in total volume of 50 μ l. *MseI* and *EcoRI* adapters were subsequently ligated to digested DNA fragments. The adapter ligated DNA was preamplified using the following PCR parameters: 20 cycles of 94°C for 30 s, 56°C for 60 s, 72°C for 60 s and 10°C for 30 s. The pre-amplified DNA was diluted 50 folds and used as a template for selective amplification reaction using *EcoRI* and *MseI* primers with three selective nucleotides at 3' end. Selective amplification was carried out initially at 94°C for 3min, 94°C for 30s, annealing at 55°C for 30s and 72°C for 60s; this step is repeated up-to 10 cycle was followed by a touch-down cycling protocol where the amplification is carried out initially at 94°C for 30s, 45°C for 30s and 72°C for 60s; this step is repeated up-to 28 cycles. After that amplification carried out for 72°C for 10 min and 12°C for 15hrs. The reaction was stopped by addition of gel loading dye (98% formamide dye) and the samples were resolved on 6% denaturing poly-acrylamide gel stained by silver staining.

2.2 Isoenzyme analysis

For isoenzyme analysis six plants of *Cymbopogon martinii* were analysed. Isoenzymes were extracted by macerating young leaves in pre-chilled pestle-mortar and ice cold 10mM tris-HCl buffer (pH 8.0) and centrifuging at 10000 rpm @ 4 degree for 15min. and the extract was concentrated by

ammonium sulfate precipitation(80% saturation) and Dialysis is performed.

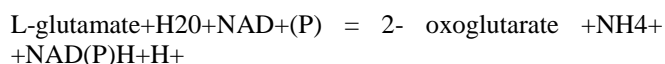
Crude sample was subjected to ammonium sulphate precipitation by slowly adding 5.614g (80%) of ammonium sulphate, carried out under cold conditions with constant stirring. This Solution was allowed to stand for 15 min. @ 4 degree C. This was centrifuged @ 10, 000 rpm for 10min or @ 3000 rpm for 40min and record the volume of supernatant.

The pellet was re-dissolved in 7ml of 0.1mm Tris buffer (in which sample was extracted) or phosphate buffer (PH.7.0) overnight. The dialysis is done almost 18 to 20 hours. After dialysis, keep the solution and store it @ -20 degree C, for further use.

2.3 Isoenzyme analysis

Glutamate dehydrogenase (GDH)

GDH which catalyzes the reductive amination of α -KG was originally considered or implicated to be the most important enzyme in NH_3 assimilation in root nodules, although other enzymes have been considered to be of some importance. The enzyme has higher K_m for ammonia (10-80mM). it is found in both bacteroid and cytosol fractions of root nodules. The reaction catalysed by the enzyme is as follows:

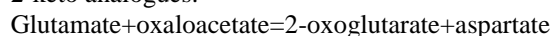


Enzyme assay:

Prepare the reaction mixture (3ml) consisting of 1.6ml of 0.1M Tris-HCL buffer (pH 7.5), 0.1ml of 0.33M 2-oxoglutarate (pH 6.0), 0.1ml of 3M NH_4Cl , 0.2ml of 10^{-3} M NADH and 1.0ml of enzyme extract. Add 0.1 ml of water in the blank instead of 2-oxoglutarate and incubate at 37°C for 15-30 min. Record the change in absorbance at 340nm.

Glutamate Oxaloacetate Transaminase (GDH):

Glutamate oxaloacetate transaminase catalyzes the reversible inter-conversions between glutamate and aspartate and their 2-keto analogues.



The enzymatic transfer of amino groups plays an important role in many metabolic processes where the inter-conversion of nitrogen-containing molecules is involved. Nitrogen, following its initial assimilation into glutamine and glutamate can be distributed to any other compounds including aspartate by the action of amino-transferases.

Enzyme assay:

Incubate 0.05ml of NADH, 0.5ml of enzyme preparation and 2.3ml of phosphate buffer containing 0.25M aspartate at 25°C. Start the reaction by adding 0.1ml of the 2-oxoglutarate solution, mix and record the decrease in absorbance at 340nm at different time intervals over a period of about 5 min. Run the blank similarly except the 2-oxoglutarate in place of which add 0.1ml of water. Plot a graph (OD vs time) and calculate the decrease in absorbance from linear part of the curve.

Esterase:

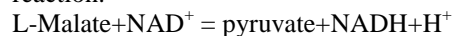
Esterases are widespread in nature, but considerably less work has been done on plant esterases. Esterases are a family of enzyme that catalyse the hydrolysis of variety of esters, peptides, amides and halides. Esterases have been purified from a wide variety of sources, animal tissue in particular, while those present in higher plants have received relatively little attention. Their physiological functions in intermediary metabolism in many cells are unclear. To 4.6ml of 0.05M phosphate buffer/tris buffer, pH 8.0 in a tube, add 2.0ml of 10^{-3} M indophenyl acetate. Incubate at 30°C and initiate the reaction by adding 0.2ml of enzyme. Run a blank similarly except that the enzyme solution is replaced by additional 0.2ml buffer. Measure the absorbance at 625nm against the blank at different time intervals 30, 60, 90, 120, 150, 180 sec.

Enzyme assay:

To 4.6ml of 0.05M phosphate buffer/tris buffer, pH 8.0 in a tube, add 2.0ml of 10^{-3} M indophenyl acetate. Incubate at 30°C and initiate the reaction by adding 0.2ml of enzyme. Run a blank similarly except that the enzyme solution is replaced by additional 0.2ml buffer. Measure the absorbance at 625nm against the blank at different time intervals 30, 60, 90, 120, 150, 180 sec. Plot the graph (OD vs time) and calculate the activity from the initial linear part of curve.

Malate dehydrogenase (MDH)

Malate dehydrogenase (MDH) is one of the key enzymes and ubiquitous to all animal cells. It catalyzes the following reaction:



It exists in multiple molecular forms which are presumed to be tetramer molecules and are referred to as isoenzymes. It occupies an important position in cell metabolism as it forms the centre of balanced equilibrium between the catabolism and anabolism of carbohydrates.

Enzyme assay:

Pipette out 1ml of buffered sodium pyruvate, 1ml of NADH and 0.1ml of enzyme extract (suitably diluted). Incubate at 37°C for 20 min. Terminate the reaction by adding 1ml of 2, 4-dinitrophenyl hydrazine. After 20min, add 10ml of 0.4N NaOH and mix. Read the absorbance at 520nm against reagent blank after 5min but within 30min after adding NaOH.

3. Results and Discussion

Amplified fragment length polymorphism:

The total fragments were generated on average, with the used primer combination. Only minimum percentage of AFLP marker were polymorphic with these could be distinguished in the species, while in a previous study RAPD study no polymorphic markers were obtained. For the different genotypes/ecotypes average fragments were scored per primer combination. No single primer pair could distinguish all different genotypes on the basis of the presence or absence of variety specific bands. Minimum primer pairs were found to be most informative and had discriminatory power.

DATA analysis:

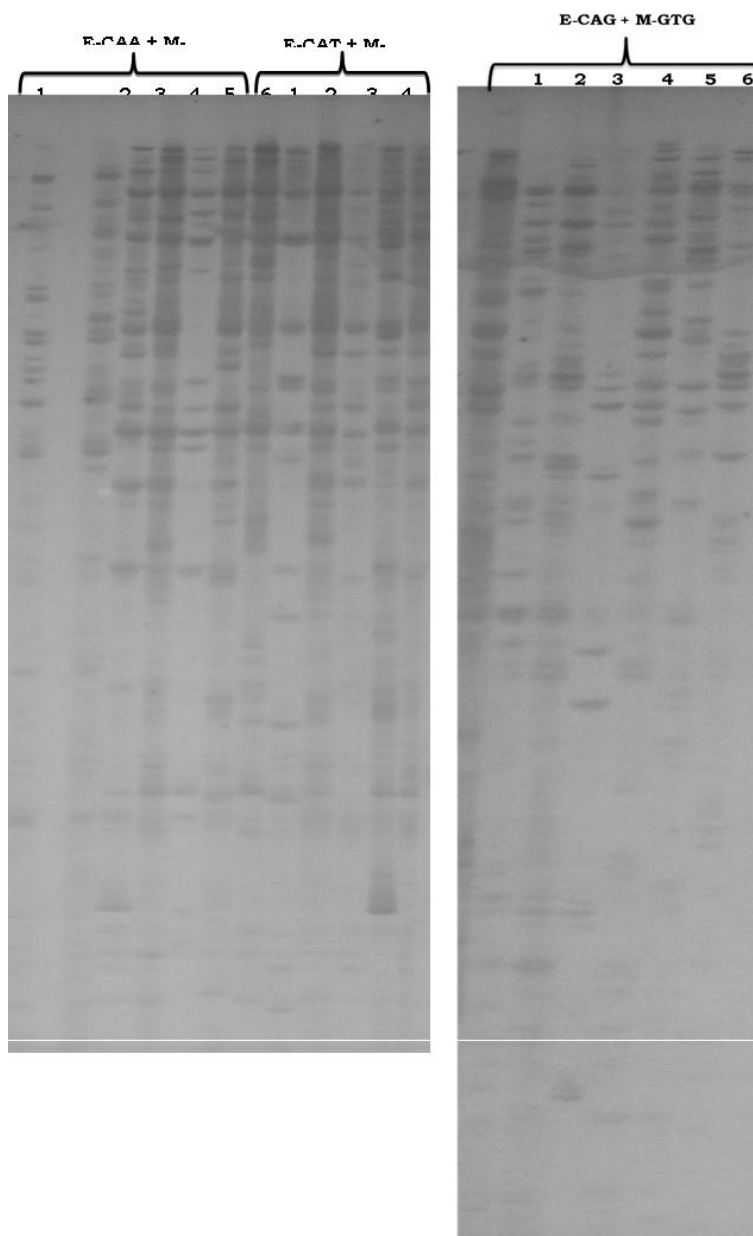
Each primer combination was tested for its ability to generate an AFLP banding pattern. The total number of bands was determined and monomorphic bands were discarded from the analysis. The ability of the most informative primers to discriminate between accessions was assessed by calculating their resolving power R_p (Prevost and Wilkinson-1999) which has been reported to correlate between accessions according to formula of Gilber et al. (1999).

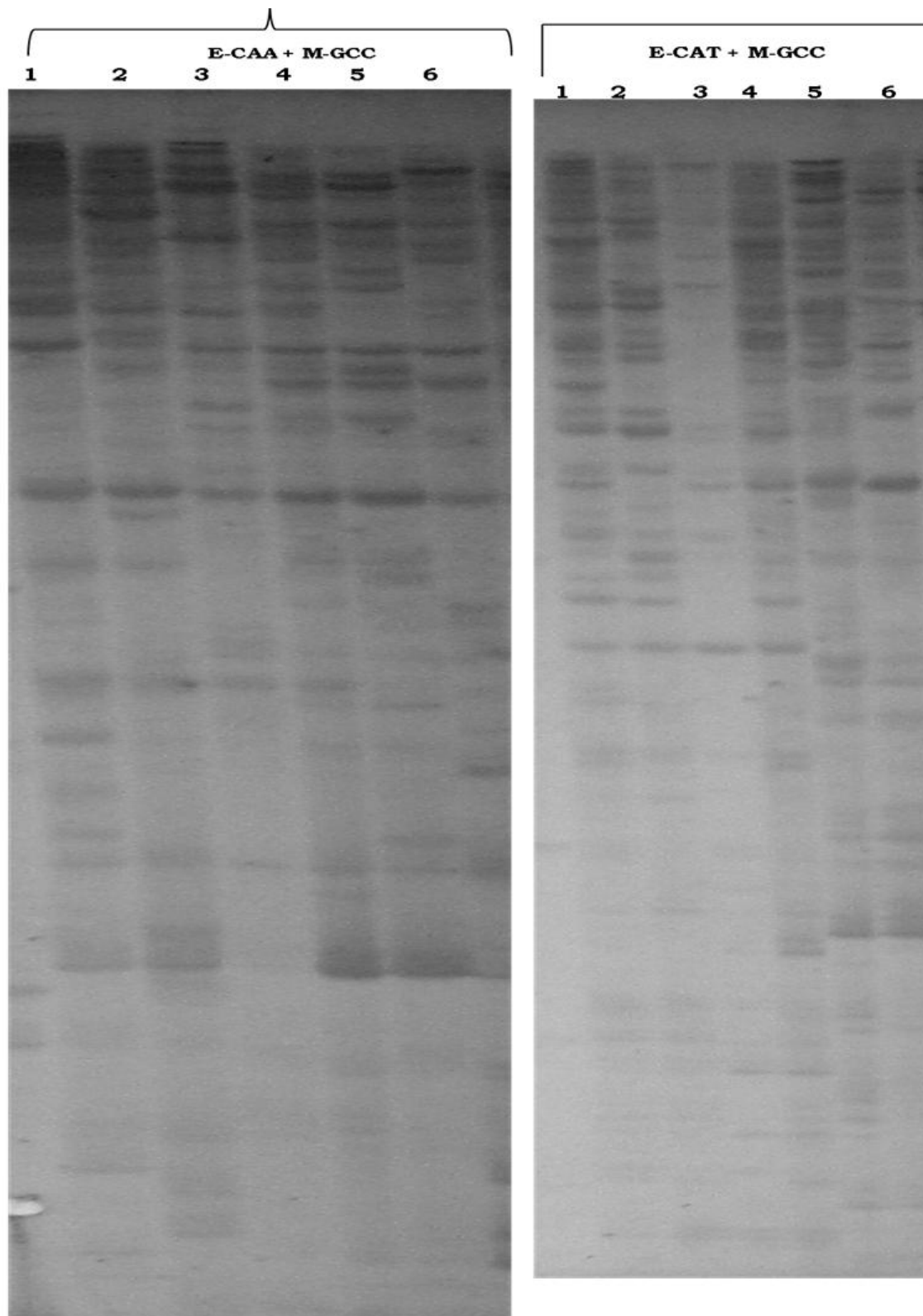
The amplified fragments in each of the 6 samples with 5 primer combinations were scored manually for their presence (denoted as '1') or absence (denoted as '0') (Sneath and Sokal 1973) for each primer combination. The similarity matrices were subjected to UPGMA (Unweighted Pair Group Method of Arithmetic averages) method of clustering in order to generate the dendrograms. All the above-mentioned statistical analyses were performed using NTSYS-pc software (version 2.02) (Rohlf 1992).

4. Statistical Analysis

Genetic similarity among 6 genotypes based on Nei's coefficient

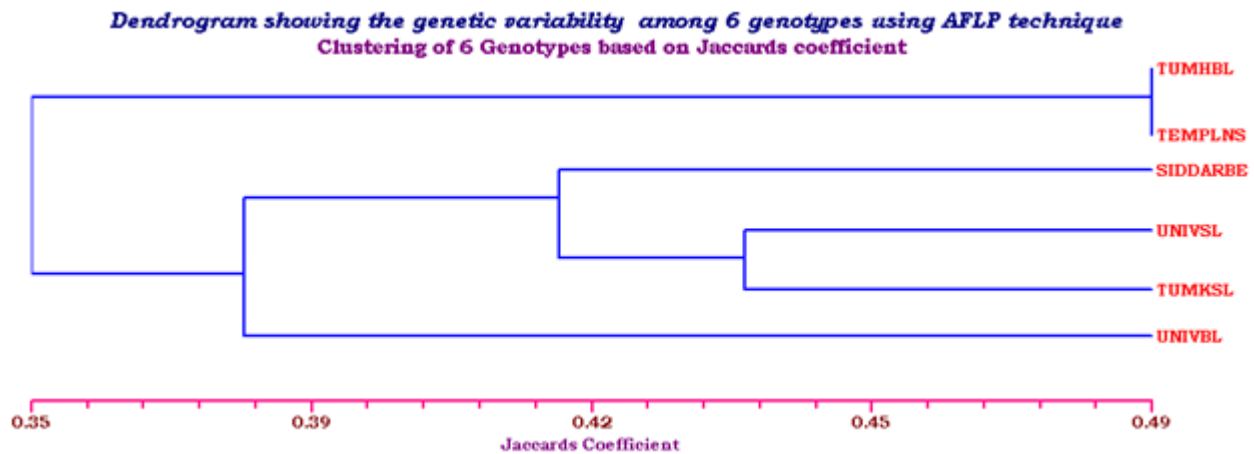
	TUMHBL	TEMPNS	SIDDARBETTA	UNIVSL	UNIVBL	TUMKSL
TUMHBL	0.00					
TEMPNS	0.42	0.00				
SIDDARBETTA	0.62	0.83	0.00			
UNIVSL	0.53	0.54	0.52	0.00		
UNIVBL	0.61	0.66	0.67	0.51	0.00	
TUMKSL	0.67	0.79	0.53	0.49	0.62	0.00





The genetic similarity among 6 genotypes was determined using Nei's coefficient. The study reveals there is maximum similarity between the Tumkur small leaves and Temple Narasimha swamy (0.79) followed by Tumkur small leaves and Tumkur highway big leaves, University big leaves and Siddarabetta (0.67) the least similarity was found between Temple Narasimha swamy and Tumkur high way big leaves. The clustering was based on the Nei's genetic distance which clustered the genotypes into 2 main clusters cluster 1

consists of four genotypes, which further clustered into 3 clusters. Cluster A consisted of genotypes Tumkur small leaves and University small leaves, genotype Siddarabetta leaves formed a solitary cluster with cluster A forming cluster B and genotype University big leaves formed a solitary cluster with Cluster B and The main cluster 2 consisted of 2 genotypes (Tumkur highway big leaves and temple Narasimha swamy) which formed with the main cluster 1.



Starting from a set of local ecotypes, the primers tested in this study showed a average polymorphic bands among a total bands generated, these primers were characterized by high Rp values. Using only four primer sets 66.6% of polymorphic bands were generated within the six accessions studied. This result that *Cymbopogon* germplasm is characterized by having a genetic diversity at the DNA level. Starting from a set of local ecotypes, the primers tested in this study showed a average polymorphic bands among a total bands generated, these primers were characterized by high Rp values. Using only four primer sets 66.6% of polymorphic bands were generated within the six accessions studied. This result that *Cymbopogon* germplasm is characterized by having a genetic diversity at the DNA level.

The AFLP technique detects polymorphisms arising from changes (pressure or size) in the restriction sites or adjacent to these. Different restriction enzymes can be used and different combinations of pre-selective nucleotides will increase the probability of finding useful polymorphisms. The more selective bases, the less polymorphism will be detected. Bands are usually scored as either present or absent. Heterozygous versus homozygous bands may be detected, based on the thickness, although this can be tricky. AFLP allow a quick scan of the whole genome for polymorphism. Because of the large number of bands generated, each marker gives a highly informative fingerprint. These are also highly reproducible. This study shows the ability of AFLP to detect geographic patterns in genetic variation, which information is necessary for developing a strategy for optimal conservation. Adaptors are ligated to ends of restriction fragments followed by amplification with adaptor homologous primers. AFLP has capacity to detect thousands of independent loci and can be used for DNA's of any origin or complexity. The number of amplified fragments is controlled by the cleavage frequency of the rare cutting enzyme and the number of selective bases. Most AFLP fragments correspond to unique position on the genome and hence can be exploited as landmarks in genetic and physical mapping. The AFLP approach has been widely adopted by plant geneticists because it requires no previous sequence characterization of the target genome. In higher plants AFLP may be the most effective way to generate high density maps. The AFLP markers can also be used to detect corresponding cDNA fragments. AFLP technique is reliable since stringent reaction conditions are

used for primer annealing. The present paper illustrates the development of AFLP markers to generate DNA fingerprinting of 6 (six) *Cymbopogon* accessions.

In addition, the topology of the dendrogram and the distribution of ecotypes. The analysis showed that a typically continuous genetic diversity characterizes *Cymbopogon* germplasm. In fact the ecotypes were clustered independently of their geographic origin, suggesting narrow genetic basis among the ecotypes studied in spite of their phenotypic distinctiveness. Thus our data confirm the unique origin of domestication of *Cymbopogon* (Wrigley.1995). The present study also illustrates that AFLP is a powerful method to discriminate *Cymbopogon* genotypes and to assess genetic diversity. Obviously, this would be enhanced by using more primer sets and/or a large number of ecotypes.

Isoenzymes:

Specific activity of soenzymes

Glutamate dehydrogenase(GDH):

Nitrogen fixation in root nodules is to a large extent controlled by ammonia assimilation in that nitrogenous synthesis is expressed by excess ammonium ions, the end product of N-fixation. Currently, one of the enzymes glutamate dehydrogenase (GDH) is implicated in efficacy of NH_3 assimilation. The principle behind is like other dehydrogenase, GDH activity is measured by following the oxidation of the reduced coenzyme, NADH at 340nm.

Glutamate oxaloacetate transaminase(GOT):

Glutamate oxaloacetate transaminase catalyses the reversible interconversions between glutamate and aspartate and their 2-keto analogues. The enzymatic transfer of amino groups plays an important role in many metabolic processes where the inter-conversion of nitrogen-containing molecules is involved. Nitrogen, following its initial assimilation into glutamine and glutamate can be distributed to any other compounds including aspartate by the action of aminotransferases. The enzyme is assayed in the direction of aspartate utilization measuring the rate of oxaloacetate formation with malate dehydrogenase and NADH. The method given below is based on that Bergmeyer and Bernt (1974).

Esterase:

Esterases are widespread in nature, but considerably less work has been done on plant esterases. Esterases are a family of enzyme that catalyse the hydrolysis of variety of esters, peptides, amides and halides. Esterases have been purified from a wide variety of sources, animal tissue in particular, while those present in higher plants have received

relatively little attention. Their physiological functions in intermediary metabolism in many cells are unclear. The enzyme activity is measured using indophenyl acetate as the substrate colorimetrically at 625nm and the change in absorbance due to hydrolysis of the substrate is taken as basis for calculation of activity.

Isoenzymes	TUMHBL	TEMPNS	SIDDERABETTA	UNIVSL	UNIVBL	TUMKSL
Esterase	0.0189U/mg	0.0185U/mg	0.0398U/mg	0.0410U/mg	0.0329U/mg	0.0189U/mg
Glutamate oxaloacetate transaminase (GOT)	0.0165U/mg	0.0132U/mg	0.0182U/mg	0.0310U/mg	0.0282U/mg	0.0421U/mg
Glutamate dehydrogenase (GDH)	0.0310 μ U/mg	0.0518U/mg	0.0412U/mg	0.0343U/mg	0.0418U/mg	0.0512U/mg
Malate dehydrogenase (MDH)	0.0808U/mg	0.0529U/mg	0.0369U/mg	0.0682U/mg	0.0495U/mg	0.0162U/mg

The table below includes data, the specific activity of enzymes pertaining to the standard curve solutions created and obtained along with their absorbance readings.

Malate dehydrogenase (MDH)

Malate dehydrogenase is one of the key enzymes and ubiquitous to all animal cells. It exists in multiple molecular forms which are presumed to be tetramer molecules and are referred to as isozymes of isoenzymes. It occupies an important position in cell metabolism as it forms the centre of balanced equilibrium between the catabolism and anabolism of carbohydrates. The enzyme activity is measured as the amount of pyruvate reduced in the presence of NADH and determined colorimetrically by treating with 2, 4-dinitrophenyl hydrazine with alkali at 520nm.

The specific activity is a method for measuring enzymatic activity and the enzyme purity in a mixture. In order to determine the specific activity of an enzyme, the units of enzyme activity per mg of protein present, the amount of the enzymes activity and protein content in an unknown mixture is needed. In the present article an efficient procedure for the identification of variation of *C.martinii* wild species based on enzyme activity of isoenzymes, described that enzymes hydrolyze chemical linkage of different metabolites and are presented ubiquitous in all developmental stages of plants many isoforms (Raso et.al., 1999) and changes in their expression and activity were observed under abiotic (de carval ho et al., 2003) and biotic stress (muralidharan et al., 1996). All *Cymbopogon martinii* cultivars studied showed almost identical or average enzyme activity of different isoenzymes indicating that they were belong to same species and showing the variation of different ecotypic conditions. From the measurements of specific activity of isoenzymes for the six different ecotypes can be observed. *Cymbopogon* has increasingly formed a very sound base for export economics of many developing nations. Their cultivation area has expanded rapidly during last few decades. The genetic support to the cultivar development program has still remained limited considering some of the traditional crops. Molecular markers can demonstrate genetic similarities and differences than that of the morphological characteristics.