Isozyme, ISSR and RAPD profiling of genotypes in marvel grass (*Dichanthium annulatum*)

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Abstract: Genetic analysis of 30 accessions of marvel grass (*Dichanthium annulatum* Forsk.), a tropical range grass collected from grasslands and open fields of drier regions, was carried out with the objectives of identifying unique materials that could be used in developing the core germplasm for such regions as well as to explore gene (s) for drought tolerance. Five inter-simple sequence repeat (ISSR) primers [(CA)₄, (AGAC)₄, (GACA)₄]; 27 random amplified polymorphic DNA (RAPD) and four enzyme systems were employed in the present study. In total, ISSR yielded 61 (52 polymorphic), RAPD 269 (253 polymorphic) and enzyme 55 isozymes (44 polymorphic) bands. The average polymorphic information content (PIC) and marker index (MI) across all polymorphic bands of 3 markers systems ranged from 0.419 to 0.480 and 4.34 to 5.25 respectively. Dendrogram analysis revealed three main clusters with all three markers. Four enzymes namely esterase (EST), polyphenol oxidase (PPO), peroxidase (PRX) and superoxide dismutase (SOD) revealed 55 alleles from a total of 16 enzyme-coding loci. Of these, 14 loci and 44 alleles were polymorphic. The mean number of alleles per locus was 3.43. Mean heterozygosity observed among the polymorphic loci ranged from 0.406 (SOD) to 0.836 (EST) and accessions wise from 0.679 (IG3108) to 0.743 (IGKMD-10). Though there was intermixing of few accessions of one agro-climatic region to another, largely groupings of accessions were with their regions of collections. Bootstrap analysis at 1000 iterations also showed large numbers of nodes (11 to 17) having strong clustering (>50 bootstrap values) in all three marker systems. The accessions of the arid and drier regions forming one cluster are assigned as distinct core collection of Dichanthium and can be targeted for isolation of gene (s) for drought tolerance. Variations in isozyme allele numbers and high PIC (0.48) and MI (4.98) as observed with ISSR markers indicated their usefulness for germplasm characterization.

Key words: Cluster analysis, Dichanthium annulatum, Genetic resources, Genetic similarity, Marvel grass, Polymorphism

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Introduction

* Dichanthium annulatum* (Forsk.) commonly known as Marvel grass is an important perennial grass species of tropical and subtropical regions. It is an important component of two major grass covers of India i.e., *Dichanthium-Cenchrus-Lasiurus* and *Sehima-Dichanthium* (Dabadghao and Shankamaranayan, 1973). To date around 20 species of the genus have been reported, eight of these found in India in various agro-ecological zones (Arora et al., 1975). Two species viz., *D. annulatum* and *D. caricosum* are widely used for forage production. The basic chromosome number in *Dichanthium* is 10, however *D. annulatum* complex shows different ploidy levels with chromosome number 2n = 2X = 20, 4X = 40 and 6X = 60 with distinct morphological characters (Mehra, 1961). It is largely apomictic in nature but line showing facultative apomixes is also observed. Despite of this, a high level of polymorphism has been reported (Chandra et al., 2004, 2006) along with considerable agro-morphological variations among accessions collected from similar eco-geographical situations (Agarwal et al., 1999). In India a collection of more than 250 accessions (collected from different parts of the country) is maintained at Indian Grassland and Fodder Research Institute (IGFRI), Jhansi. Despite such germplasm holding, only one variety (Marvel-8) has been released in India.

The characterization of germplasm is required to maintain identity and purity for proper conservation and management. Further, this helps to identify unique lines of crop species, especially those growing unabatedly under harsh environmental conditions. Morphological, phenological and agronomical characteristics are often used for estimating genetic variations. These traits however, are often polygenic and/or influenced by environmental conditions. Despite high variability at morphological level variations in isozyme alleles in *Dichanthium* germplasm of the drier regions has not been studied. The allozyme technique (Hunter and Markert, 1957) provides an opportunity to establish genetic relationships among crop species and cultivars, and used to estimate genetic diversity in many crops (Freitas et al., 2000; Bhandari et al., 2006; Jain et al., 2006).

DNA markers, such as random amplified polymorphic DNA (RAPD), have been used in genetic and breeding studies in many plant species (Williams et al., 1993). Of the various molecular diagnostic techniques available, RAPD and ISSR are easy to perform and cost-efficient, does not require radioactive compounds and analysis can reveal a high degree of polymorphism. Compared to restriction fragment length polymorphism (RFLP) marker, RAPD and ISSR can generate markers more rapidly but with some loss of information, because
these markers are usually dominant markers. RAPD and RFLP (Smith et al., 1993) have been employed for developing markers for identification and fingerprinting of Napier grass (Pennisetum purpureum), however limited information is available on RAPD polymorphism in Dichanthium. Our earlier studies in Dichanthium indicated a high level of variations with RAPD markers (Chandra et al., 2004, 2006). DNA markers, especially those based on microsatellites are useful in assessing large numbers of accessions, quickly and reliably (Sarala et al., 2002). Inter-simple sequence repeat-PCR (ISSR-PCR) is a simple, cost efficient, robust, multilocus marker method, which is extensively useful in determining genetic variability (Sarala et al., 2002), however choice of primers used in ISSR amplification is critical for obtaining high levels of polymorphism. The objectives of our study are to assess, estimate and compare genetic variability obtained with isozyme, ISSR and RAPD in thirty accessions of Dichanthium largely collected from drier regions of the country.

Materials and Methods

Plant materials: Thirty Dichanthium accessions used in the present investigation represent collections from the central north (Bundelkhand) and the southern plateau and hills (Dharwad and Bangalore) of India. These accessions were collected from their natural habitats, i.e., grasslands and open fields, and maintained in experimental fields of the Indian Grassland and Fodder Research Institute, Jhansi (25°27′N, 78°35′E). All the accessions used in present study were tetraploid (Saxena and Chandra, 2006). The fresh and young leaves from three plants of each accession were used for enzyme extraction and total genomic DNA isolation.

Enzyme extraction and isozyme analysis: Fresh and young leaves from each accession were homogenized in three (v/w) fold volume of cold extraction buffer (50 mM Tris-HCl, pH 7.5, 10% sucrose, 1.0 mM EDTA and 1 mM 2-mercaptoethanol, added freshly). The extract was centrifuged (10,000 rpm) for 10 min at 4°C and resultant supernatant was used as enzyme source. Isozyme analysis of peroxidase (PRX, E.C. 1.11.1.17), esterase (EST, E.C. 3.1.1.2), polyphenol oxidase (PPO, E.C. 1.14.18.1) and superoxide dismutase (SOD, E.C. 1.15.1.1) was performed using native polyacrylamide gel (10%) electrophoresis (PAGE) at 4°C. Gels were stained as described by Veech (1969) for peroxidase and as described by Wendel and Weedel (1989) for esterase, superoxide dismutase and polyphenol oxidase.

Plant DNA extraction: For DNA extraction fresh and young leaves from three plants were used for isolation of genomic DNA following CTAB method (Iqbal et al., 1997) with suitable modifications (Chandra et al., 2004). DNA was quantified using UV spectrophotometer and also checked on 0.7% agarose gels. The stock DNA was finally diluted to 5 ng µl⁻¹ for use in polymerase chain reaction (PCR).

RAPD analysis: RAPD-PCR reactions was performed in 20 µl of reaction mixture containing 67 mM Tris-HCl pH 8.0, 16.6 mM (NH₄)₂SO₄, 0.45% v/v BSA, 3.5 mM MgCl₂, 150 µM of each dNTP, 7.5 pmoles (15 ng) primer, 0.5 unit Taq polymerase and 25 ng genomic template DNA. The reaction was performed in a thermal cycler (PTC 200, MJ Research, USA) with a cycling program of 94°C for 1 min, 37°C for 1 min, 72°C for 2 min for 40 cycles, followed by 41st cycle at 94°C for 1 min, 37°C for 1 min and finally at 72°C for 10 min primer extension step. PCR products were separated by electrophoresis on 1.6% agarose gel, and visualized using ethidium bromide.

ISSR analysis: In the present study five ISSR primers with the basic repeats of (CA)n, (AGAC)n, (GACA)n were used to identify markers with high levels of polymorphism. The reaction mixtures contained 2.5 µl of 10x buffer, 2.0 µl of 25 mM MgCl₂, 1.5 µl of 2.5 mM dNTP, 2.0 µl primer (0.5 µM), 0.1% gelatin, 1 unit Taq polymerase and 30 ng genomic DNA in a final volume of 25 µl. The PCR reactions were performed on thermal cycler (PTC 200, MJ, Research USA) with cycling program consisting 94°C for 4 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing for 1 min at 50°C, extension at 72°C for 90 s, and final extension at 72°C for 7 min. The amplification product was separated by electrophoresis on 1.6% agarose gel and detected by ethidium bromide staining. The molecular weight marker (100 bp ladder) was used for band sizing.

The zones of activity were defined for each of four enzyme systems. These activity zones were designated as locus (1 to 5) having different numbers of bands corresponding to alleles. Taking this into consideration the parameters estimated for genetic variations were percentage of polymorphic loci (Pp), mean number of alleles per polymorphic locus (Ap) (Hamrick and Godt, 1997), mean expected heterozygosity based on unbiased estimate of Nei (1978) and allele frequency. Allele frequencies were also used to estimate expected heterozygosity, however the genetic interpretation in terms of individual as heterozygous/ homozygous for a particular locus was not attempted due to apomictic nature of the crop. Genetic diversity (Hs = Hardy-Weinberg expected heterozygosity) (Weir, 1989) was calculated for each locus (including monomorphic and polymorphic loci) by:

$$H_s = 1 - \sum x_i^2$$

where $x_i$ is the mean frequency of the ith allele pooled across the accessions.

The PIC value was calculated employing the formula of Roldan-Ruiz et al. (2000): PIC = 2f(1-f), where f is the frequency of the amplified allele (band present) and (1-f) is the frequency of the null allele (band absent) of marker i. MI was determined as the product of PIC and the number of polymorphic bands per assay unit (Powell et al., 1996). The binary data generated on the basis of presence (1) and absence (0) of the bands in both ISSR and RAPD was analyzed for genetic similarity among the accessions based on Dice’s similarity coefficient, which was also converted to distance measures (d) using the formula d = 1-s. Amplification failure of a sample or missing data was coded as 9. Dendrogram was constructed by Sequential Agglomerative Hierarchical and Nested
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Results and Discussion

Isozyme patterns and cluster analysis: The four enzyme systems generated a total of 16 isozyme loci with 55 alleles. 80% of the alleles were polymorphic (Table 1). Number of loci varied from 3 to 5 whereas alleles at a particular locus from 2 to 7. Locus four of esterase (EST4) was polymorphic in 96.6% of the accessions followed by PPO3 in 90%. PRX1 locus was least polymorphic (16.6%). Although both the SOD2 and PRX2 loci possessed two alleles and both of these were either present or absent in accessions and thus the polymorphism was in the form of a null allele. At the SOD2 locus both alleles were present in 5 and absent in 25 accessions. This was also true with the PRX2 locus but the accessions were different. The polymorphic loci within accessions ranged from 37.50 to 75%. Accessions IG97-121, IG97-244, IG97-245 and IG97-144 depicted the least polymorphism (37.5%) whereas accession IG97-234 showed the maximum polymorphism (75%). The number of monomorphic loci also varied from maximum 10 in 6 accessions and minimum 4 in only one genotype, IG97-234. Minimum allele frequency (0.037) was observed at the EST4 locus for allele 4 whereas the maximum (0.75) was observed at SOD3 for allele 1. Esterase was the most polymorphic enzyme, generating 22 alleles of which 20 were polymorphic. Locus 4 of esterase yielded 7 alleles which were polymorphic in 29 of the accessions examined.

The dendrogram of 30 accessions based on 55 bands of four isozyme systems (EST, PPO, PRX and SOD) showed 3 main clusters (Fig. 1A). The genetic distance ranged from 0.00 to 0.253 between three clusters. Cluster-1 was further subdivided into two sub-clusters comprising 11 accessions in total. Two accessions, viz., IG97-241 and IG95-25 originally collected from the close vicinity of south India, exhibited 67.0% similarity with Cluster-2 and these two clusters together showed 79.0 and 83.0% similarity with the rest of the members of this cluster. Cluster-3 showed 72.0% similarity with Cluster-2 and these two clusters together exhibited 67.0% similarity with Cluster-1.

Table - 1: Number of alleles at different locus and percentage (%) of accessions polymorphic to the locus

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number of alleles detected</th>
<th>Number of polymorphic locus</th>
<th>Mean number of alleles / polymorphic locus</th>
<th>% of accessions polymorphic to the locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>EST1</td>
<td>7</td>
<td>4</td>
<td>1.90</td>
<td>80.00</td>
</tr>
<tr>
<td>EST2</td>
<td>3</td>
<td>4</td>
<td>2.00</td>
<td>46.66</td>
</tr>
<tr>
<td>EST3</td>
<td>5</td>
<td>4</td>
<td>3.06</td>
<td>60.00</td>
</tr>
<tr>
<td>EST4</td>
<td>7</td>
<td>4</td>
<td>3.51</td>
<td>96.66</td>
</tr>
<tr>
<td>PRX1</td>
<td>2</td>
<td>3</td>
<td>1.00</td>
<td>16.66</td>
</tr>
<tr>
<td>PRX2</td>
<td>2</td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>PRX3</td>
<td>4</td>
<td>3</td>
<td>2.60</td>
<td>83.33</td>
</tr>
<tr>
<td>PPO1</td>
<td>3</td>
<td>5</td>
<td>2.00</td>
<td>43.33</td>
</tr>
<tr>
<td>PPO2</td>
<td>3</td>
<td>5</td>
<td>1.43</td>
<td>23.33</td>
</tr>
<tr>
<td>PPO3</td>
<td>4</td>
<td>5</td>
<td>1.85</td>
<td>50.00</td>
</tr>
<tr>
<td>PPO4</td>
<td>3</td>
<td>5</td>
<td>1.00</td>
<td>46.66</td>
</tr>
<tr>
<td>PPO5</td>
<td>2</td>
<td>5</td>
<td>1.00</td>
<td>50.00</td>
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<td>SOD2</td>
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<td>2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>SOD3</td>
<td>3</td>
<td>2</td>
<td>1.00</td>
<td>80.00</td>
</tr>
</tbody>
</table>
Fig. 1: Dendrogram of 30 Dichanthium accessions derived from Dice similarity values based on isozyme (A), RAPD (B) and ISSR (C) markers data. In total, 15 nodes in isozyme, 17 in RAPD and 11 in ISSR were observed having strong clustering (>50 bootstrap values).
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An average PIC value of 0.46 across all scored RAPD bands, as well as an average MI of 4.34 across all primers obtained with Dichanthium accessions was different than that of AFLP-based genetic diversity studies in various crops (Powell et al., 1996; Muminovic et al., 2004). Though both AFLP and RAPD are dominant markers, the easiness associated with RAPD analysis as well as high PIC and MI obtained with Dichanthium justifies its use for fingerprinting and identification of cultivars for different agroclimatic zones. ISSR yielded high MI (5.50) with each primer which was more than RAPD (4.3) indicating more usefulness of such marker (Table 4).

All three marker systems (Isozyme, RAPD and ISSR) generated three main clusters. Cluster-2 obtained with two DNA-based markers was observed largest cluster among three clusters. Isozyme based clusters also indicated two groups each comprising 4 and 2 accessions depicting 100% similarity. Such situation was not observed with RAPD and ISSR presumably because of high number of markers generated from both coding and non-coding regions of the genome. Though the level of polymorphism was high with DNA-based markers, the clustering patterns of accessions were comparable between isozyme and DNA-based markers. Even bootstrap values also indicated strong clustering at comparable number of nodes (11 to 17) with three sets of markers. However, the difference lies with the placement of accessions of south India particularly in Cluster-2 and 3. The dendrogram based on DNA-based markers data-set indicated a closer relationship between accessions of south India. At inter-specific level, the reliability of DNA-based data is comparable to that of isozyme data indicated such markers are more valuable in assessing the genetic variations in populations (Liu and Furnier, 1993).

Due to less discriminatory power of isozyme marker, 18 of 30 Dichanthium accessions yielded more than 90% similarity and 12 accessions from 67 to 90%. All 18 accessions in question were from central north India. Out of twelve genotypes that have shown similarity from 67 to 90%, six accessions were from south India exhibited a high level of diversity among them. Two closest accessions of this region were IG3108 and IGBANG-D-1 having 88% similarities. Clustering patterns based on isozyme banding patterns clearly indicated that accessions of south India clustered together though having higher level of diversity among them. Four accessions belonging to central north India possessing 100% similarity intermixed with the members of south India. All six accessions belonging to south India were highly divergent and two nearest accessions in this category was IG3108 and IGBANG-D-1. A high level of diversity in isozyme banding patterns have been reported in guinea grass (Jain et al., 2006), where authors emphasized evolution of new types through sexual recombination or other means namely hybridizing of indigenous materials with sexual/apomictic exotic lines. Guinea grass (Panicum maximum Jacq.) consists largely of apomictic population, although many sexual plants are
### Table 2: Sequence and amplified products of twenty seven arbitrary primers (Operon) used to generate RAPD markers in Dichanthium

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>No. of bands</th>
<th>No. of polymorphic bands</th>
<th>PIC</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPE01</td>
<td>5’CCCAAGGTCC3’</td>
<td>10</td>
<td>10</td>
<td>0.487</td>
<td>4.87</td>
</tr>
<tr>
<td>OPE08</td>
<td>5’TACCAACGGT3’</td>
<td>7</td>
<td>7</td>
<td>0.464</td>
<td>3.25</td>
</tr>
<tr>
<td>OPF01</td>
<td>5’ACGGATCCTG3’</td>
<td>12</td>
<td>10</td>
<td>0.500</td>
<td>5.00</td>
</tr>
<tr>
<td>OPF04</td>
<td>5’GGTTGATCAGG3’</td>
<td>7</td>
<td>6</td>
<td>0.206</td>
<td>1.24</td>
</tr>
<tr>
<td>OPF06</td>
<td>5’GGGAAATTCCG3’</td>
<td>10</td>
<td>10</td>
<td>0.482</td>
<td>4.82</td>
</tr>
<tr>
<td>OPG02</td>
<td>5’GCCACTGAGG3’</td>
<td>6</td>
<td>6</td>
<td>0.499</td>
<td>2.99</td>
</tr>
<tr>
<td>OPG12</td>
<td>5’CAGGCTACAGA3’</td>
<td>5</td>
<td>5</td>
<td>0.496</td>
<td>2.48</td>
</tr>
<tr>
<td>OPH04</td>
<td>5’GAAATGTCGCC3’</td>
<td>8</td>
<td>7</td>
<td>0.296</td>
<td>2.07</td>
</tr>
<tr>
<td>OPH05</td>
<td>5’AGTTGCTCCTC3’</td>
<td>7</td>
<td>7</td>
<td>0.448</td>
<td>3.14</td>
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<tr>
<td>OPH09</td>
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<td>10</td>
<td>10</td>
<td>0.481</td>
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<tr>
<td>OPH13</td>
<td>5’GAGGCCACAC3’</td>
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<td>9</td>
<td>0.462</td>
<td>4.16</td>
</tr>
<tr>
<td>OP07</td>
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<td>21</td>
<td>19</td>
<td>0.377</td>
<td>7.18</td>
</tr>
<tr>
<td>OP08</td>
<td>5’TTTGGCGGGTG3’</td>
<td>8</td>
<td>7</td>
<td>0.485</td>
<td>3.40</td>
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<tr>
<td>OP14</td>
<td>5’TGACGGCGGT3’</td>
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<td>10</td>
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<td>4.97</td>
</tr>
<tr>
<td>OP18</td>
<td>5’TGGCAGCTCTC3’</td>
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<td>5</td>
<td>0.468</td>
<td>2.81</td>
</tr>
<tr>
<td>OPA01</td>
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<td>13</td>
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<td>OPA07</td>
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<td>11</td>
<td>0.443</td>
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<tr>
<td>OPN04</td>
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<td>9</td>
<td>7</td>
<td>0.487</td>
<td>3.41</td>
</tr>
<tr>
<td>OPN06</td>
<td>5’GACCGACCA3’</td>
<td>8</td>
<td>7</td>
<td>0.460</td>
<td>3.24</td>
</tr>
<tr>
<td>OPN09</td>
<td>5’GTGTCGCCGA3’</td>
<td>8</td>
<td>8</td>
<td>0.438</td>
<td>3.57</td>
</tr>
<tr>
<td>OPQ06</td>
<td>5’CCGTCGGTAG3’</td>
<td>12</td>
<td>11</td>
<td>0.472</td>
<td>5.19</td>
</tr>
<tr>
<td>OPQ07</td>
<td>5’ACTGGCCTAG3’</td>
<td>13</td>
<td>12</td>
<td>0.483</td>
<td>5.19</td>
</tr>
<tr>
<td>OPQ08</td>
<td>5’CCCCTGCTCT3’</td>
<td>15</td>
<td>15</td>
<td>0.488</td>
<td>7.33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>269</td>
<td>253</td>
<td></td>
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</tr>
<tr>
<td>Average</td>
<td></td>
<td>9.962</td>
<td>9.370</td>
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</table>

**PIC** = Polymorphic information content, **MI** = Marker index

### Table 3: ISSR primers used in 30 Dichanthium accessions

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Total bands</th>
<th>Polymorphic bands</th>
<th>% Polymorphic bands</th>
<th>Monomorphic bands</th>
<th>PIC</th>
<th>MI</th>
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<tbody>
<tr>
<td>ISSR80</td>
<td>GC[CA]4</td>
<td>15</td>
<td>14</td>
<td>93.33</td>
<td>1</td>
<td>0.499</td>
<td>6.99</td>
</tr>
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<td>ISSR81</td>
<td>GT[CA]4</td>
<td>06</td>
<td>08</td>
<td>100</td>
<td>-</td>
<td>0.500</td>
<td>4.00</td>
</tr>
<tr>
<td>ISSR82</td>
<td>[AGAC]GC</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
<td>0</td>
<td>0.405</td>
<td>4.05</td>
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<tr>
<td>ISSR83</td>
<td>AC[GACA]</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
<td>0</td>
<td>0.499</td>
<td>4.90</td>
</tr>
<tr>
<td>ISSR84</td>
<td>[GACA]GT</td>
<td>14</td>
<td>10</td>
<td>71.42</td>
<td>0</td>
<td>0.500</td>
<td>5.00</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>61</td>
<td>52</td>
<td>85.24</td>
<td>1.8</td>
<td>0.480</td>
<td>4.98</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>12.2</td>
<td>10.4</td>
<td>85.24</td>
<td>1.8</td>
<td>0.480</td>
<td>4.98</td>
</tr>
</tbody>
</table>

**PIC** = Polymorphic information content, **MI** = Marker index

### Table 4: Comparative analysis of banding patterns generated by ISSR, RAPD and isozyme markers system for 30 Dichanthium accessions

<table>
<thead>
<tr>
<th>Components</th>
<th>ISSR</th>
<th>RAPD</th>
<th>Isozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of assay units</td>
<td>5</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Total number of bands</td>
<td>61</td>
<td>269</td>
<td>55</td>
</tr>
<tr>
<td>Mean number of bands / per assay unit</td>
<td>12.2</td>
<td>9.96</td>
<td>13.75</td>
</tr>
<tr>
<td>Total number of polymorphic bands</td>
<td>52</td>
<td>253</td>
<td>44</td>
</tr>
<tr>
<td>Number of polymorphic bands per assay unit</td>
<td>10.4</td>
<td>9.37</td>
<td>11.0</td>
</tr>
<tr>
<td>Mean (%) polymorphism per assay</td>
<td>85.24</td>
<td>94.07</td>
<td>74.51</td>
</tr>
<tr>
<td>Number of monomorphic bands per assay</td>
<td>1.8</td>
<td>0.59</td>
<td>2.75</td>
</tr>
<tr>
<td>Mean PIC per assay</td>
<td>0.480</td>
<td>0.463</td>
<td>0.419</td>
</tr>
<tr>
<td>Mean Marker Index (MI)</td>
<td>4.98</td>
<td>4.34</td>
<td>5.25</td>
</tr>
</tbody>
</table>

**ISR** = Inter simple sequence repeat, **RAPD** = Random amplified polymorphic DNA, **PIC** = Polymorphic information content
also available in nature (Assienan et al., 1993). In case of Dichanthium though being largely apomictic in nature the increased variability in sub-humid dry regions of south India might be due to the presence of major grasslands as well as favorable climate for natural crossing. Assienan and Nioirt (1995) have reported that apomixis does not lead to the reduction in the diversity of grasses. The Dichanthium annulatum complex, having different ploidy levels and growing wild in natural habitats, exhibits large phenotypic variations. Even diverse forms have been reported from material collected at the same locations and the clustering patterns based on agro-morphological attributes in marvel grass indicated independent groupings with their geographical distribution (Aga rwal et al., 1999). Genetic variation in Dichanthium annulatum genotypes by RAPD and ISSR corroborated that diversity was independent of geographical distribution as genotypes from different areas clustered in the same group and vice versa (Chandra et al., 2004). However, in present study when accessions of only two contrasting regions were analyzed, most of the accessions of South India centered to one part of the phenogram (Fig. 1).

Contrary to isozyme, RAPD revealed eight accessions possessing more than 90% similarity and rest from 47 to 90%. In Cluster-2, single accession of south India got mixed otherwise this cluster as well as Cluster-1 comprised accessions only from central north India. The minimum level of similarity in case of RAPD was 47% whereas in case of isozyme 67%. This difference clearly provided edge of RAPD over isozyme in diversity study as RAPD has the potential to detect polymorphism at more loci and detect both coding and non-coding sequences in the genome. However, the estimates of genetic variation as obtained with RAPD may be higher than those actually existing because of the dominant nature and one-band/one-locus assumption of these markers. Higher estimates of genetic diversity with RAPD markers over isozyme have been reported in other crops also (Liu and Furnier, 1993; Kongkiatngam et al., 1995).

When results of all marker systems were taken together, accessions namely IGKMF-D-1, IGTGD-4 and IGBANG-D-2 of south India and IG97-189, IG97-132, IG97-121, IG97-244, IG97-245 and IG97-144 of central north India were identified as distinct as they have depicted high level of variations and unique clustering patterns to the rest of the accessions. The three south Indian accessions (IGKMF-D-1, IGTGD-4 and IGBANG-D-2) identified as distinct; either got intermixed with the rest of the north Indian genotypes or formed separate cluster. With RAPD, IGBANG-D-2 accession of south India showed 47% similarity to the rest of the genotypes, whereas with isozyme it has shown 70 and 81% similarity with two other clusters. Both marker systems revealed that genotype IGKMF-D-1 of south India was closest to the central north Indian accessions. Study resulted the grouping of accessions based on their genetic distances and both protein and DNA-based markers categorized Dichanthium accessions based on their agro-climatic regions. The closely related genotypes as well as diverse and unique accessions can be used for evaluation under specific ecological and environmental conditions.

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