MOLECULAR CHARACTERIZATION OF RICE (ORYZA SATIVA L.) GENOTYPES RESISTANT TO MOISTURE STRESS

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INTRODUCTION

Rice (Oryza sativa L.) is the world’s most important food crop and a primary source of food for more than half of the world’s population (Davla et al., 2013; Reddy et al., 2013). It has also become a model organism for genome analysis, having a diploid chromosome number of 24 and the smallest genome size of all major crop plants of 430 Mb. Although, rice is cultivating in large area but the final yield gain per unit area is very less due to biotic and abiotic stresses (Bala Krishna and Satyanarayana, 2013). Rice, generally grown under flooded conditions, is susceptible to drought stress owing to its shallow root distribution and limited capacity to extract water from deep soil layers (David, 1994). Yield of rainfed lowland rice, which occupies about 25% of the world’s rice areas, are drastically reduced by drought due to unpredictable, insufficient and uneven rainfall during the growing period. To reduce yield losses of rice crops in rain fed lowland areas and to increase overall rice production, new rice varieties with greater adaptation to drought are essential. Hence, the development of drought resistant cultivars with a higher yield potential is one of the main objectives of rain fed lowland rice breeding programme. A critical analysis of the genetic variability is a prerequisite for initiating any crop improvement programme and for adopting of appropriate selection techniques (Dhanwani et al., 2013). However, this selection process is labour intensive and slow as it requires cultivation of breeding populations under drought conditions. Marker-assisted selection (MAS) is cheaper and more convenient than phenotype-based selection and it presently is the only option to combine traits by gene pyramiding. DNA based markers can be derived from quantitative trait loci (QTL) and allow selection already in the seedling stage (Degenkolbe et al., 2013). Moreover, they allow the proper grouping of different populations and varietal groups and resolve doubts about the accession classification. This is of great advantage, because the high intra-specific variability and the environmental effects can hinder the differentiation of populations or varietal groups based only on the phenotypic evaluation (Souza et al., 2013).

In this context, an attempt has been made to study the molecular characterization of rice genotypes resistant to moisture stress. Also, genotyping can be extremely useful to breeders in their efforts to mange genetic resources by helping them to identify unique materials that need to be conserved. The purpose of this study was to identify the specific primers, which are likely to be efficient in revealing the diversity among the genotypes.

MATERIALS AND METHODS

The experimental material consisted of 26 rice genotypes comprising majority of upland and some low land varieties obtained from Main Rice Research Station, Anand Agricultural University, Nawagam (Table 1). Seedlings of all the genotypes were raised in pots. Fresh leaves were collected and further utilized for isolating genomic DNA to study genetic diversity and polymorphism by RAPD and SSR analysis. The DNA was extracted from the fresh leaves of four weeks old seedling by CTAB method as described by Ahmadikhah et al. (2007) with some modifications. To estimate quantity and quality (in terms of protein and RNA contamination) of isolated genomic DNA, spectrophotometry was performed and data were analyzed.
using Nanodrop N.D.1000 software (ver.3.3.0). The RAPD analysis was performed according to Williams et al., (1990) with minor modifications. The PCR reaction steps for RAPD are 94°C for 5 minutes (Initial denaturation), 45 cycles each of 94°C for 1 minute (Denaturation), 38°C or 40°C for 1 minute (Primers annealing), 72°C for 2 minutes (Extension of annealed primer) and 72°C for 10 minutes (Final Extension). For SSR analysis the PCR condition consisted of initial denaturation at 94°C for 7 minute, with 45 cycles of final denaturation at 94°C for 45 second, annealing at 55°C for 1 minute and extension at 7 min, followed by final extension at 72°C for 7 min. The amplified products for RAPD and SSR were analyzed electrophoretically using 1.8 % and 2.5 % agarose gel, respectively. The separated bands were visualized under UV transilluminator and photographed using syngene gene snap-G-box (Alpha Ease FC4.0.0 gel Documentation system). Each amplified product was scored across all the genotypes for its presence or absence. The scores 0 and 1 indicate the presence or absence of bands, respectively. The data were entered in to binary matrix and subsequently analyzed using NTSYSpc version 2.02. Coefficients of similarity were calculated as Jaccard’s similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithm under Sequential Agglomerative Hierarchical Nesting (SHAN) module of the NTSYS pc. Relationships among rice cultivars were graphically represented in the form of dendrograms. The cophenetic correlation analysis was carried out using COPH function of NTSYS pc. and dendrogram constructed based on the similarity coefficients. The PIC value for each locus was calculated on the basis of allele frequency (Anderson et al., 1993).

RESULTS AND DISCUSSION

The present investigation was carried out in the Biotechnology Laboratory of Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, to analyze the genetic diversity among 26 rice genotypes using RAPD and SSR markers.

In all, 60 different RAPD primers were used in the present study, out of which 15 produced polymorphic results (Table 2). The results obtained revealed different degree of polymorphism by different primers. Amplification of total genomic DNA from different varieties produced a total of 268 fragments, of which 267 (99.62%) were polymorphic in nature. The percentage of polymorphic bands shown by different primers ranged from 94.44 (OPC-8) to 100 (all except OPC-8). One of the reasons for this high level of polymorphism could be due to extensive intra-specific variation in rice. Although the majority of primers produced polymorphic bands, no single primer could clearly distinguish all the genotypes. RAPD marker OPC-15 produced maximum numbers of 247 bands, while OPK-18 amplified minimum numbers of 115 bands. The PIC values ranged from 0.875 (OPK-18) to 0.938 (OPK-20) with an average of 0.918 (Table 3). The similarity coefficient values ranged from 0.16 to 0.61 (Table 4). This indicated a fair range of variability in the similarity coefficient values, suggesting a fairly wide genetic base of 26 rice genotypes used in the experiment. The highest value of similarity coefficient (0.61) was found between the varieties GR12 and IR-64 (0.61) and between the varieties Lalat and Tapaswini (0.61). However, the lowest value of similarity coefficient (0.16) was observed between the varieties GR-5 and Tapaswini. In order to analyze the relatedness among the genotypes studied, the UPGMA-based dendrogram was constructed using paired matrix values for pooled RAPD data. Dendrogram generated for 13 RAPD primers against 26 rice genotypes formed two major clusters; ‘A’ and ‘B’ (Fig 1). Major cluster ‘A’ was further divided into two sub-clusters, ‘A1’ and ‘A2’. Sub-cluster ‘A1’ consisted of three varieties viz., Sathi 34-36, GR-5 and GR-9 (all upland cultivars), while sub-cluster ‘A2’ comprised of ten varieties viz., SK-20, Ashoka-200F, GR-8, AAUDR-1, DDR-22, Kallinga-III, Vandana, Annanda, GR-3 and GR-4. Cluster ‘B’ was also divided into three sub-clusters, ‘B1’, ‘B2’ and ‘B3’. Sub-cluster ‘B1’ comprised of six genotypes viz., GR-6, GR-11, GR-7, GR-12, IR-64 and Jaya. The subcluster ‘B2’ comprised of other six genotypes i.e. Swarna, TN-1, Lalat, Tapaswini, Pusa Basmati-1 and IET-18990, whereas sub-cluster ‘B3’ consisted of only one variety i.e. Samba Masuri. Most of cultivars included in cluster ‘A’ were upland varieties, while cluster ‘B’ comprised mostly transplanted varieties. The results revealed a moderate level of genetic variation among rice genotypes and led to the establishment of genetic relationships between them. The similarity matrix and dendrogram revealed that the upland paddy varieties viz., Sathi 34-36, GR-5, GR-9, SK-20, Ashoka-200F, GR-8 and AAUDR-1 are grouped into major cluster A, indicating that these genotypes may have similar genetic constitution and expression profiles for moisture stress. The varieties, GR-12 and IR-64 showed highest similarity in cluster B because of IR-64 is one of the two parents of GR-12. Tapaswini and Lalat showed minimum similarity with all the other genotypes, which can be used as parents in hybridization programme so as to generate maximum variability in segregating generations and

### Table 1: List of rice genotypes taken in the study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genotypes</th>
<th>Sr. No.</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sathi 34-36</td>
<td>14.</td>
<td>GR-6</td>
</tr>
<tr>
<td>2.</td>
<td>SK-20</td>
<td>15.</td>
<td>GR-7</td>
</tr>
<tr>
<td>3.</td>
<td>GR-5</td>
<td>16.</td>
<td>GR-11</td>
</tr>
<tr>
<td>4.</td>
<td>GR-8</td>
<td>17.</td>
<td>GR-12</td>
</tr>
<tr>
<td>5.</td>
<td>GR-9</td>
<td>18.</td>
<td>Jaya</td>
</tr>
<tr>
<td>7.</td>
<td>AAUDR-1</td>
<td>20.</td>
<td>IR-64</td>
</tr>
<tr>
<td>8.</td>
<td>DDR-22</td>
<td>21.</td>
<td>Swarna</td>
</tr>
<tr>
<td>10.</td>
<td>Vandana</td>
<td>23.</td>
<td>TN-1</td>
</tr>
<tr>
<td>11.</td>
<td>Annanda</td>
<td>24.</td>
<td>Tapaswini</td>
</tr>
<tr>
<td>12.</td>
<td>GR-3</td>
<td>25.</td>
<td>Pusa Basmati-1</td>
</tr>
<tr>
<td>13.</td>
<td>GR-4</td>
<td>26.</td>
<td>IET-18990</td>
</tr>
</tbody>
</table>

### Table 2: List of RAPD primers showed polymorphic results

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Primer</th>
<th>Sr. No.</th>
<th>Name of Primer</th>
<th>Sr. No.</th>
<th>Name of Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>OPA-2</td>
<td>6.</td>
<td>OPC-6</td>
<td>11.</td>
<td>OPK-8</td>
</tr>
<tr>
<td>2.</td>
<td>OPA-7</td>
<td>7.</td>
<td>OPC-8</td>
<td>12.</td>
<td>OPK-16</td>
</tr>
<tr>
<td>3.</td>
<td>OPA-8</td>
<td>8.</td>
<td>OPC-15</td>
<td>13.</td>
<td>OPK-17</td>
</tr>
<tr>
<td>4.</td>
<td>OPA-10</td>
<td>9.</td>
<td>OPC-11</td>
<td>14.</td>
<td>OPK-18</td>
</tr>
<tr>
<td>5.</td>
<td>OPC-2</td>
<td>10.</td>
<td>OPC-15</td>
<td>15.</td>
<td>OPK-20</td>
</tr>
</tbody>
</table>
increase scope of isolating desirable recombinants. On the whole, RAPD analysis revealed very useful information about genetic variability among cultivars, which can be used by plant breeders in their breeding programme. The present result is in accordance with the results of Islam et al. (2013) where genetic diversity of drought tolerant rice were carried out through RAPD analysis. Also, Ogunbayo et al. (2005) reported differentiation among rice genotypes was higher for RAPD markers than for morphological classification.

All the 26 rice genotypes were further analyzed by using 10 trait-specific SSR markers for genetic diversity analysis. Total 99 amplified products were obtained. The allele length for these 10 SSR markers varied from 80 to 238 bp. The highest allele length was recorded for RM 242 (238bp) for varieties Vandana and GR-3. The allele frequency produced by different markers was observed in the range of 0.038 to 0.500. The

Table 3: Analysis of RAPD patterns generated using 15 arbitrary primers for rice genotypes

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Primer</th>
<th>Maximum scorable bands</th>
<th>Polymorphic loci (P)</th>
<th>Total Loci (T)</th>
<th>Percentage polymorphism</th>
<th>PIC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>OPA-2</td>
<td>222</td>
<td>21</td>
<td>21</td>
<td>100</td>
<td>0.927</td>
</tr>
<tr>
<td>2.</td>
<td>OPA-7</td>
<td>135</td>
<td>19</td>
<td>19</td>
<td>100</td>
<td>0.928</td>
</tr>
<tr>
<td>3.</td>
<td>OPA-8</td>
<td>190</td>
<td>18</td>
<td>18</td>
<td>100</td>
<td>0.920</td>
</tr>
<tr>
<td>4.</td>
<td>OPA-10</td>
<td>178</td>
<td>22</td>
<td>22</td>
<td>100</td>
<td>0.933</td>
</tr>
<tr>
<td>5.</td>
<td>OPC-2</td>
<td>166</td>
<td>18</td>
<td>18</td>
<td>100</td>
<td>0.929</td>
</tr>
<tr>
<td>6.</td>
<td>OPC-6</td>
<td>162</td>
<td>19</td>
<td>19</td>
<td>100</td>
<td>0.930</td>
</tr>
<tr>
<td>7.</td>
<td>OPC-7</td>
<td>132</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>0.909</td>
</tr>
<tr>
<td>8.</td>
<td>OPC-8</td>
<td>190</td>
<td>17</td>
<td>17</td>
<td>94.44</td>
<td>0.914</td>
</tr>
<tr>
<td>9.</td>
<td>OPC-11</td>
<td>152</td>
<td>19</td>
<td>19</td>
<td>100</td>
<td>0.927</td>
</tr>
<tr>
<td>10.</td>
<td>OPC-15</td>
<td>247</td>
<td>20</td>
<td>20</td>
<td>100</td>
<td>0.934</td>
</tr>
<tr>
<td>11.</td>
<td>OPK-7</td>
<td>200</td>
<td>16</td>
<td>16</td>
<td>100</td>
<td>0.920</td>
</tr>
<tr>
<td>12.</td>
<td>OPK-16</td>
<td>164</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>0.909</td>
</tr>
<tr>
<td>13.</td>
<td>OPK-17</td>
<td>142</td>
<td>13</td>
<td>13</td>
<td>100</td>
<td>0.882</td>
</tr>
<tr>
<td>14.</td>
<td>OPK-18</td>
<td>115</td>
<td>13</td>
<td>13</td>
<td>100</td>
<td>0.875</td>
</tr>
<tr>
<td>15.</td>
<td>OPK-20</td>
<td>238</td>
<td>22</td>
<td>22</td>
<td>100</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>115-247</td>
<td>13-22</td>
<td>13-22</td>
<td>94.44-100</td>
<td>0.875-0.933</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>175.53</td>
<td>17.8</td>
<td>17.8</td>
<td>99.62</td>
<td>0.918</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>2634</td>
<td>267</td>
<td>267</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
**Table 4: Similarity matrix of 26 rice genotypes based on RAPD markers**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of Genotype(s)</th>
<th>Expected Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>2</td>
<td>Sathí 36-36</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>4</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>5</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>7</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>8</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>9</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
<tr>
<td>10</td>
<td>Sathí 34-36</td>
<td>0.88</td>
</tr>
</tbody>
</table>

**Table 5: Details of SSR markers used for molecular analysis**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of SSR marker</th>
<th>No. of Alleles</th>
<th>Range of allele length (bp)</th>
<th>Highest allele length observed in Genotype(s)</th>
<th>Allele frequency</th>
<th>Highest allele frequency observed in Genotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RM205</td>
<td>21</td>
<td>107-145</td>
<td>Sathí 34-36</td>
<td>0.047 - 0.476</td>
<td>DDR-22 and GR-9</td>
</tr>
<tr>
<td>2</td>
<td>RM213</td>
<td>24</td>
<td>109-134</td>
<td>SK-20</td>
<td>0.043 - 0.304</td>
<td>GR-12, Jaya, Samba Masuri, IR-64, Swarna, and Tapaswini</td>
</tr>
<tr>
<td>3</td>
<td>RM215</td>
<td>23</td>
<td>135-170</td>
<td>IET-18990</td>
<td>0.043 - 0.217</td>
<td>GR-5, AAUDR-1, DDR-22, Kalinga-III, and Vandana</td>
</tr>
<tr>
<td>4</td>
<td>RM223</td>
<td>24</td>
<td>141-168</td>
<td>Lalat</td>
<td>0.038 - 0.269</td>
<td>GR-7, GR-12, Jaya, and Samba Masuri, IR-64, Basmati and IET-18990</td>
</tr>
<tr>
<td>5</td>
<td>RM231</td>
<td>23</td>
<td>160 - 201</td>
<td>IR-64</td>
<td>0.043 - 0.173</td>
<td>GR-6, Jaya, TN-1, Tapaswini, GR-7, GR-11, GR-12, IET-18990, GR-8, GR-9, Ashoka-200F and AAUDR-1</td>
</tr>
<tr>
<td>6</td>
<td>RM234</td>
<td>26</td>
<td>109-134</td>
<td>SK-20</td>
<td>0.043 - 0.173</td>
<td>GR-6, GR-7, GR-12, Jaya, Samba Masuri, Lalat, TN-1, Pusa Basmati-1, IET-18990</td>
</tr>
<tr>
<td>7</td>
<td>RM242</td>
<td>19</td>
<td>184-238</td>
<td>Vandana, GR-3</td>
<td>0.045 - 0.181</td>
<td>Kalinga-III, Annada, Swarna, and Tapaswini.</td>
</tr>
<tr>
<td>8</td>
<td>RM318</td>
<td>25</td>
<td>116 - 144</td>
<td>Sathí 34-36</td>
<td>0.038 - 0.346</td>
<td>GR-6, GR-7, GR-12, Jaya, Samba Masuri, Lalat, TN-1, Pusa Basmati-1, IET-18990</td>
</tr>
<tr>
<td>9</td>
<td>RM420</td>
<td>23</td>
<td>165 - 181</td>
<td>Samba Masuri</td>
<td>0.041 - 0.500</td>
<td>Sathí 34-36, SK-20, GR-5, GR-9, AAUDR-1, GR-3, GR-11, Jaya, IR-64, Swarna, Lalat, IET-18990</td>
</tr>
<tr>
<td>10</td>
<td>RM3810</td>
<td>21</td>
<td>80 - 99</td>
<td>Pusa Basmati-1</td>
<td>0.045 - 0.181</td>
<td>Ashoka-200F, DDR-22, Kalinga-III, and Annada.</td>
</tr>
</tbody>
</table>
highest allele frequency (0.500) was recorded by the marker RM 420 for varieties Sathi 34-36, SK-20, GR-5, GR-9, AAUDR-1, GR-3, GR-11, Jaya, IR-64, Swarna, Lalat and IET-18990. The expected heterozygosity amongst 26 rice genotypes was observed in the range of 0.68 to 0.90, where in the marker RM 242 revealed the highest value of 0.90. It was observed that when 26 rice genotypes were analysed at molecular level using ten markers, Sathi 34-36 and Lalat amplified highest allele length, maximum number of times. Similarly, for allele frequency, varieties Jaya and GR-12 appeared maximum number of occasions (Table 5). Dendrogram generated for 10 SSR markers against 26 rice genotypes formed two major clusters ‘A’ and ‘B’ (Fig 2). Cluster ‘A’ comprised mostly upland cultivars and a few lowland cultivars, whereas cluster ‘B’ consisted of mostly transplanted cultivars. Cluster ‘A’ is further divided into two sub-clusters ‘A1’ and ‘A2’. Sub-cluster ‘A1’ comprised Sathi 34-36, SK-20, GR-8, GR-9, AAUDR-1 and Ashoka -200F, while sub-cluster ‘A2’ consisted of GR-5, Vandana, Annanda, GR-3, GR-4, DDR-22 and Kalinga-III. Sub cluster ‘A1’ included all upland rice varieties of Gujarat, except GR-5, whereas, sub-cluster ‘A2’ was a mixed group of upland and lowland varieties. Cluster ‘B’ has two sub-clusters ‘B1’
Figure 2: Dendrogram of genetic relationship among rice genotypes based on SSR markers.


