SSR and RAPD-Based Genetic Diversity in Cotton Germplasms

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Summary This study aimed to evaluate the genetic diversity and to characterize 11 Gossypium hirsutum L. (cotton) germplasms by RAPD and SSR markers. The genomic DNA from the 11 cotton germplasms was amplified with 10 oligonucleotide primers and five microsatellite primer pairs for RAPD and SSR assay, respectively. The 10 RAPD primers generated 335 distinct bands with 100% polymorphisms indicated highly diversified nature. In addition to polymorphism, 29 unique RAPD sequences were identified in 11 cotton germplasms. The five SSR primer pairs generated 69 distinct bands of which 39 were considered as polymorphic (56.52% polymorphisms). Moreover, four unique SSR sequences were identified in three germplasms, namely CB-1, CB-5 and CB-9. The combined RAPD and SSR dendrogram made CB-11 germplasm distinct from the rest and placed alone in a separate cluster that correlated with its phenotypic and agronomic features. Thus, by means of RAPD and SSR markers the genetic diversity was assessed and the 11 cotton germplasms could be characterized authentically.

Key words RAPD, SSR, Cotton, Genetic diversity.

Cotton is a kind of bast fibre obtained from the epidermis of the seeds. Commercial cotton fibres are mainly obtained from different Gossypium species. Out of about 50 Gossypium species, only four are cultivated such as Gossypium herbaceum L., G. arboreum L., G. barbadense L. and G. hirsutum L. (Brubaker et al. 1999). The species Gossypium hirsutum L. was named due to its hairiness (hirsute) around seeds. Among the cultivated species, G. hirsutum has the highest yield potential and is the largest globally cultivated species with about 90% contribution to the world cotton market.

Cotton is one of the important cash crops in Bangladesh. It is the main raw material of the textile industry. Being an important cash crop, breeders of different national agencies are attracted to this crop. As a consequence, the Bangladesh Cotton Development Board (CDB) has been conducting research since 1991 and was able to release so far 11 germplasms (Upland Cotton) of G. hirsutum, namely CB-1, CB-2, CB-3, CB-4, CB-5, CB-6, CB-7, CB-8, CB-9, CB-10 and CB-11, through selection based on morphological and physiological features (yield, fiber quality, resistance against certain pests and diseases, etc.; Iqbal et al. 1997) (Table 1).

Successful breeding programs depend on the complete knowledge and understanding of the genetic diversity within and among genetic resources of the available germplasms. This will enable plant breeders to choose parental sources that generate diverse populations for selection (Esmail et al. 2008).

Molecular markers have become important tools in studying genetic diversity (Bered et al. 2005). The utility of molecular markers are generally determined by the technology that is used to reveal DNA-based polymorphisms. Recent studies have shown that simple sequence repeats (SSRs) and random amplified polymorphic DNA (RAPDs) are able to detect a certain degree of polymorphism in different plant species (He and Prakash 1997, He et al. 2003, Hopkins et al. 1999, Subramanian et al. 2000, Raina et al. 2001).

RAPD and SSR molecular markers have been used for revealing genetic diversity among some germplasms and genotypes in cotton (Esmail et al. 2008, Maleia et al. 2010, Saravanan et al. 2006, Hussain et al. 2007, Menzel and Brown 1978, Kumar et al. 2003, Vafaie-Tabar et al. 2003, Mehetre et al. 2004). Moreover, genetic analysis using DNA markers have been used to reveal genetic bases of both qualitative and quantitative traits in different crop plants including cotton (Saha et al. 1998). These DNA markers have an advantage over morphological traits as they are polymorphic with no pleiotropic or epistatic effects and are not affected by environmental conditions. Hence, they are used to exploit the available cotton gene pools enhance germplasm resources (Cantrell et al. 1999).

Although some PCR-based marker analyses of cotton have been undertaken, these were scattered and not used for characterization. As a result, our germplasms have not yet been characterized. Genomic characterization is necessary for successful breeding and patenting each germplasms to prevent illegal trade.

Therefore, this study aimed to evaluate the genetic
diversity and to characterize 11 Gossypium hirsutum L. (cotton) germplasms based on RAPD and SSR markers.

Materials and methods

Eleven germplasms of Gossypium hirsutum L., viz. CB-1, CB-2, CB-3, CB-4, CB-5, CB-6, CB-7, CB-8, CB-9, CB-10 and CB-11, released by CDB, Bangladesh were investigated in this study. Seeds were initially collected from the gene bank of BARI and sown in the Botanic garden, Department of Botany, University of Dhaka.

DNA isolation

Leaves were harvested and total genomic DNA was extracted by using a modified CTAB method (Doyle and Doyle 1987). DNA concentration was quantified through spectrophotometer (Analylykiena, Specord 50, Germany). The A 260/280 readings for DNA samples were 1.6–1.8.

PCR amplification and primer survey

The PCR reaction mixture for 25 μL contained template DNA (25 ng) 2 μL, de-ionized distilled water 18.8 μL, Taq buffer A 10× (Tris with 15 mM MgCl2) 2.5 μL, primer (10 μM) 1.0 μL, dNTPs (2.5 mM) 0.5 μL and Taq DNA polymerase (5 U μL−1) 0.2 μL. PCR amplification was done in an oil-free thermal cycler (Biomera UNOII, Germany) for 46 cycles after initial denature at 94°C for 5 min, denature at 94°C for 1 min, annealing at 34–36°C for 30 s, extension at 72°C for 3 min and final extension at 72°C for 5 min. In the present study, 10 oligonucleotide primers and five microsatellite primer pairs were used for RAPD and SSR assay, respectively (Tables 2, 3).

Gel electrophoresis

The amplified products were separated electrophoretically on 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide (10 mg mL−1) 8 μL and 100 mM 1×TAE buffer. Agarose gel electrophoresis was conducted in 1×TAE buffer at 50V and 100mA for 1.5h. DNA ladder (1 kb) was electrophoresed alongside the RAPD and SSR reactions as marker. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system.

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**Table 1.** Major morphological features and agronomic performance of 11 Gossypium hirsutum L. varieties (According to Cotton Development Board, Bangladesh, 2014).

<table>
<thead>
<tr>
<th>Variety</th>
<th>CB-1</th>
<th>CB-2</th>
<th>CB-3</th>
<th>CB-4</th>
<th>CB-5</th>
<th>CB-6</th>
<th>CB-7</th>
<th>CB-8</th>
<th>CB-9</th>
<th>CB-10</th>
<th>CB-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB Registration No.</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
<td>05(06)</td>
</tr>
<tr>
<td>Days to first boll split</td>
<td>127</td>
<td>130</td>
<td>117</td>
<td>136</td>
<td>122</td>
<td>117</td>
<td>117</td>
<td>124</td>
<td>119</td>
<td>114</td>
<td>112</td>
</tr>
<tr>
<td>Number of boll per plant</td>
<td>56</td>
<td>33</td>
<td>43</td>
<td>42</td>
<td>47</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>44</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>Single boll weight (g)</td>
<td>4.8</td>
<td>5.4</td>
<td>6.4</td>
<td>6</td>
<td>5.1</td>
<td>5.6</td>
<td>5.6</td>
<td>4</td>
<td>5.8</td>
<td>5.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Plant height at harvest (cm)</td>
<td>151</td>
<td>121</td>
<td>130</td>
<td>134</td>
<td>132</td>
<td>129</td>
<td>121</td>
<td>105</td>
<td>106</td>
<td>126</td>
<td>106</td>
</tr>
<tr>
<td>Seed cotton yield (ton/ha)</td>
<td>1.75–2.00</td>
<td>1.50–1.80</td>
<td>1.75–2.00</td>
<td>1.32–1.50</td>
<td>1.75–2.00</td>
<td>1.50–2.00</td>
<td>1.50–2.50</td>
<td>2.00–2.50</td>
<td>1.75–2.00</td>
<td>2.10–3.50</td>
<td>—</td>
</tr>
<tr>
<td>Fibre length (2.5%)</td>
<td>31</td>
<td>25–2000</td>
<td>37</td>
<td>100–6000</td>
<td>31</td>
<td>200–2500</td>
<td>31</td>
<td>Jassid insect resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jassid insect resistancy</td>
<td>—</td>
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<td>Jassid insect resistant</td>
<td></td>
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</tr>
</tbody>
</table>

**Table 2.** Compilation of RAPD analysis in 11 germplasms of Gossypium hirsutum L.

<table>
<thead>
<tr>
<th>Primer codes</th>
<th>Sequences (5′–3′)</th>
<th>Total bands</th>
<th>Size ranges (bp)</th>
<th>Number of polymorphic bands</th>
<th>Number and size (bp) of germplasms specific unique bands</th>
<th>Polymorphisms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA-1</td>
<td>5′-CAG GCC CTT C-3′</td>
<td>31</td>
<td>250–2000</td>
<td>31</td>
<td>CB-4 (2000), CB-9 (1400, 400, 300)</td>
<td>100</td>
</tr>
<tr>
<td>OPA-3</td>
<td>5′-AGT CAG CCA C-3′</td>
<td>37</td>
<td>600–7000</td>
<td>37</td>
<td>CB-3 (1600), CB-11 (1500, 750, 600)</td>
<td>100</td>
</tr>
<tr>
<td>OPA-4</td>
<td>5′-AAT CGG GCT G-3′</td>
<td>65</td>
<td>300–4000</td>
<td>65</td>
<td>CB-11 (4000, 3000, 1700)</td>
<td>100</td>
</tr>
<tr>
<td>OPA-6</td>
<td>5′-GGT CCC TGA C-3′</td>
<td>11</td>
<td>500–2000</td>
<td>11</td>
<td>CB-9 (2000), CB-11 (1300)</td>
<td>100</td>
</tr>
<tr>
<td>OPA-7</td>
<td>5′-GAA ACG GGT G-3′</td>
<td>09</td>
<td>500–1700</td>
<td>09</td>
<td>CB-6 (500)</td>
<td>100</td>
</tr>
<tr>
<td>OPA-10</td>
<td>5′-GTG ATC GCA G-3′</td>
<td>44</td>
<td>500–2400</td>
<td>44</td>
<td>CB-10 (1400)</td>
<td>100</td>
</tr>
<tr>
<td>Primer-1</td>
<td>5′-GAA ACG GGT G-3′</td>
<td>31</td>
<td>850–2750</td>
<td>31</td>
<td>CB-1 (1600), CB-11 (2750, 1000)</td>
<td>100</td>
</tr>
<tr>
<td>Primer-2</td>
<td>5′-GTT CGG ATC C-3′</td>
<td>21</td>
<td>200–2500</td>
<td>21</td>
<td>CB-9 (450), CB-10 (2500)</td>
<td>100</td>
</tr>
<tr>
<td>Primer-18</td>
<td>5′-GTT CGG ATC C-3′</td>
<td>49</td>
<td>400–5000</td>
<td>49</td>
<td>CB-9 (600, 500), CB-10 (1750), CB-11 (1600)</td>
<td>100</td>
</tr>
<tr>
<td>Primer-23</td>
<td>5′-GTC AGG GCA A-3′</td>
<td>37</td>
<td>600–2000</td>
<td>37</td>
<td>CB-1 (2000, 1000, 800, 650), CB-9 (600)</td>
<td>100</td>
</tr>
</tbody>
</table>

Grand total: 335 200–7000 335 29 100
Scoring and data analysis

The PCR products were analyzed after gel electrophoresis. The photographs were critically discussed on the basis of presence (1) or absence (0), size of bands and overall polymorphism of bands. These were carried out for further investigation. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei (1972) gene diversity, genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the specimen using computer program POPGENE (Version 1.31).

Results and discussion

The 11 cotton germplasms selected for the present study represented a broad spectrum of variation for several phenotypic traits. DNA from the 11 cotton germplasms was studied with 10 oligonucleotide primers and five microsatellite primer pairs for RAPD and SSR asay, respectively.

Polymorphism as detected by RAPD analysis

In this study, a total of 25 RAPD primers were screened on template DNA of 11 cotton germplasms. Only 10 out of 25 were selected because they revealed multiband fingerprinting, which were easily scorable and reproducible. The primer sequence, band size and banding pattern of 11 cotton germplasms are shown in Table 2 and Figs. 1–10. Both light and bright bands were produced in the RAPD reactions (Figs. 1–10). Light bands produced from low homology between the primer and the pairing site on the DNA strand whereas bright bands showed strong affinity (Thormann et al. 1994). The 10 primers generated 335 distinct polymorphic bands ranging from 200 to 7000 bp and thus showed 100% polymorphisms (Table 2).

A diverse level of polymorphism in different crops have been reported earlier such as Chickpea 98.14% (Rasool 2013), Brassica 98.03% (Ghosh et al. 2009), Eggplant 57.89% (Biswas et al. 2009) and Chilli 90% (Paran et al. 1998). A wide range of RAPD polymorphism in cotton germplasms was also reported. Esmail et al. (2008) scored a high degree of polymorphism in 21 cotton genotypes from Egypt using RAPD markers. The molecular weight of bands analysed by them ranged from 100 and 1500 bp of which 84.95% were polymorphic. Maleia et al. (2010) reported 90.96% polymorphism among 21 cotton cultivars. Saravanan et al. (2006) scored a high degree of polymorphism in 10 cotton genotypes using RAPD markers and observed 69.37% polymorphism. Hussain et al. (2007) reported 63.20% polymorphism among 11 cotton genotypes. In this study, 100% polymorphism was recorded for the first time in cotton (Table 2). Therefore, the 11 germplasms used in this study were highly diversified from each other. On the other hand, low level of DNA polymorphism in cotton germplasms was not observed by any previous workers. Therefore, broad range of polymorphism revealed wide diversity in cotton germplasm. The diversification would be useful for improved breeding programme in cotton.

Unique RAPD markers

In addition to polymorphism, 29 unique RAPD sequences were identified in 11 cotton germplasms using 10 different primer combinations (Table 2, Figs. 1–10). The term unique sequence means that the sequence found in a germplasm with a certain primer was absent in other germplasms. In the earlier literature, there was no information about unique bands (Esmail et al. 2008, Maleia et al. 2010, Saravanan et al. 2006). The earlier authors considered all bands as polymorphic bands. In this study, mentioning unique bands was a new parameter for RAPD analysis. The unique bands were stable and specific for the respective germplasms and thus could be used as a tool for characterization.
Polymorphism as detected by SSR analysis

The five primer pairs generated 69 distinct bands of which 39 were considered as polymorphic and thus showed 56.52% polymorphism which indicated a moderate level of polymorphisms (Table 3, Figs. 11–15). The sizes of bands ranged from 50 to 2000 bp. Out of five, the primer pair BA00175683/BA00175684 produced the highest number (20) of polymorphic bands followed by 16 polymorphic bands with the primer pair BA00175685/BA00175686 (Figs. 13, 15, Table 3). In contrast, the primer pair BA00175685/BA00175686 did not generate any polymorphic bands (Fig. 14, Table 3).

Only one common band of 50 bp was observed in two primer pairs, viz. BA00175681/BA00175682 and BA00175687/BA00175688 (Figs. 12, 15, Table 3). The common band indicated the sharing of similar DNA fragments among 11 cotton germplasms.

Unique SSR markers

In addition to polymorphism, four unique SSR sequences were identified in 11 cotton germplasms using five different primer pair combinations. Primer pair BA00175681/BA00175682 produced one unique band of 738 bp in CB-9 (Table 3, Fig. 12, arrow). With primer
pair BA00175683/BA00175684, the germplasm CB-9 showed one unique band of 1968 bp (Table 3, Fig. 13, arrow). Primer pair BA00175687/BA00175688 produced one unique band of 123 bp in CB-1 and 492 bp in CB-5 (Table 3, Fig. 15, arrow). The unique bands were stable and specific for the respective germplasms and thus could be used as markers. Except polymorphic bands, no reports of unique SSR bands were available for cotton (Esmail et al. 2008, Maleia et al. 2010, Saravanan et al. 2006). Therefore, this is the first report about unique SSR bands for cotton germplasms.

**Genetic relationships among 11 cotton germplasms**

The values of pair-wise Nei’s (1972) genetic distances analyzed by using computer software “POPGENE32” among 11 germplasms of cotton were computed from combined data obtained from 10 RAPD primers and five SSR primer pairs. The values ranged from 0.0991 to 0.8348. The highest genetic distance (0.8348) was found between CB-11 with CB-5 and CB-9. On the other hand, the lowest (0.0991) genetic distance was observed between CB-7 and CB-8 (Fig. 16). The difference between the highest and the lowest values of genetic distance revealed the wide range of variability persisting among the 11 cotton germplasms. High genetic distance values between germplasm pairs were found due to differences in genetic constituent (Thormann et al. 1994). The germplasms of lowest genetic distance can be used as parental source for breeding line to improve cotton germplasms.

**CB-11 is distinct from the rest**

The germplasm CB-11 is different from the other 10 germplasms in various morphological and agronomical aspects. Flowers and fruits come faster in CB-11 compared to other germplasms. This plant is about eight feet high whereas the other 10 germplasms are about four feet. The shapes of the leaves are totally different from the other cotton germplasms. CB-11 showed the highest yielding capacity (2.10–3.50 ton cotton/hectare) from the rest (1.32–2.50 ton cotton/hectare). This germplasm is different in respect of RAPD fingerprinting. Several unique sequences were found in CB-11 with various primers (Table 2, Figs. 1–10). The combined RAPD and SSR dendrogram placed CB-11 alone in a separate cluster (C₁₁) (Fig. 16). The above data made CB-11 distinct from the other 10 germplasms of *Gossypium hirsutum*.

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**References**


Kumar, P., Singh, K., Vikal, Y., Randhawa, L. S. and Chahal, G. S.


