DNA fingerprinting and molecular characterization of Brassica cultivars using RAPD markers

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Abstract

The aim of this study is to explore the genetic variation and diversity among mustard and rape seed genotypes using RAPD marker. The production of Brassica germplasm with a wider genetic base is essential for utilizing in the genetic improvement of mustard. PCR-based Random Amplified Polymorphic DNA (RAPD) technique is used for assessing genetic diversity and relationships among six Brassica cultivars viz., Safal, Sampad, Binasarisha-4, Binasarisha-5, Daulot and Rai-5. A total of nine RAPD primers are used for this study. Out of nine, three primers (OPA-02, OPB-01 and OPC-02) generated 33 distinct polymorphic bands. Each primer generated various banding pattern with an average of 11 scorable bands per primer. Among the three primers, the primer OPA-02 produced a maximum number of the band (14) and the other two primers OPB-01 and OPC-02 generated 10 and 9 bands respectively.

The cultivar Sampad (Brassica rapa L.) close to the cultivar Safal (Brassica campestris L.) with the lowest genetic distance (0.0265) and the highest genetic distance (0.981) is found between Sampad and Rai-5 (Brassica juncea, L.). On the basis of the dendrogram, most diverse genotypes are identified that can be used in future for Brassica breeding program.

Keywords: RAPD marker, genetic diversity, mustard, Brassica spp.

Introduction

Mustard (Brassica juncea (L.) Czern. and Coss.) and rapeseed (B.rapa L.) are the two major Brassica oilseed crops in the Indian sub-continent. Brassica species are the most important sources of vegetable oil and the second most important oilseed crops in the international oilseed market after soybean5. These are important and the top-ranking oil seed crop of Bangladesh. In Bangladesh, rapeseed and mustard cover 0.532 million ha, 0.596 million MT (metric ton) with the yield of 1.12 MT (metric ton)/ha in 2013-14. Like any other crop species, to improve quality and quantity of Brassica spp., the presence of sufficient genetic diversity is very important. There are various techniques for studying the genetic variability of crop germplasm including morphological traits, total seed protein, isozymes and various types of molecular markers19. However, DNA-based markers provide powerful and reliable tools for discerning variations within crop germplasm and for studying evolutionary relationships20.

A variety of molecular markers including Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP) and RAPD have been used to study the extent of genetic variation among the diverse group of important crop species in the genus Brassica16. Among molecular markers, random amplified polymorphic DNAs (RAPDs) are increasingly being employed in genetic research owing to their speed and simplicity1.

RAPD technique is the most reliable, quick, efficient and cost-effective technology for the determination of genetic diversity in plant genetic resources for crop improvement17. RAPD requires only a small amount of DNA (5-20 ng) and single short (9-10 bp) primers of arbitrary sequence that would rapidly screen for polymorphism10. In addition, no prior knowledge of sequence is required. Random Amplified Polymorphic DNA (RAPD) is a relatively recent technique and has been widely used for the estimation of genetic relationships in various crops of agronomic importance2,3,11.

RAPD analysis has been extensively used to document the genetic variation in Brassicas12,14,16,18. However, most of the earlier studies were carried out with B. napus and B. junceae cultivars and not much information is available on the extent of genetic variation present in B. campestris/rapa using DNA based marker systems.

The present study was conducted to explore the genetic variation and diversity among mustard rape seeds genotypes (B. napus, B. juncea and B. campestris/rapa cultivars) using RAPD markers. The findings of the study will be helpful for researchers, academicians and practitioners to develop a proper breeding strategy for sustainable agricultural development.

Material and Methods

Plant Materials: All the planting was done in the experimental field of Bangladesh Agricultural University (BAU) and molecular works were done in the Biotechnology Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA) during growing season 2006-2007. Six high yielding Brassica spp. cultivars viz. Safal, Sampad, Binasarisha-4, Binasarisha-5, Daulot and Rai-5 were
Genotyping of Mustard Varieties: Leaf samples were used to isolate total genomic DNA following the protocol mini preparation modified CTAB method. DNA samples were evaluated both quantitatively and qualitatively using a spectrophotometer and λ (lambda) DNA (concentration marker) respectively. The polymerase chain reaction was carried out in a volume 10 μl containing 10X PCR Buffer 1 μl, 250 μM dNTP (mix) 1 μl, 10 μM primer 2.5 μl, 25 ng/μl DNA template 2 μl, Taq DNA polymerase 1 unit / μl or 0.2 μl, 250 μM dNTP (mix) 1 μl, 10 μM primer 2.5 μl, 25 ng/μl DNA template 2 μl, Taq DNA polymerase 1 unit / μl or 0.2 μl, sterile deionized water 3.3 μl. DNA amplification was performed in a thermal cycler with the following profile: 94°C for 3 min (initial denaturation), 94°C for 1 min, annealing at 34°C for 1 min, extension at 72°C for 2 min for 40 cycles with a final extension at 72°C for 7 minutes.

The amplified products were separated by electrophoresis in 1.5% agarose gel in TBE buffer, visualized by staining with ethidium bromide and transillumination under short-wave UV light. Nine primers (OPA-01, OPA-02, OPB-01, OPC-01, OPC-02, 66AB10G6, 67AB10G7 and 69AB10G9) of random sequence were screened on a sub sample of two randomly chosen individuals from six different cultivars to evaluate their suitability.

RAPD Data Analysis: For genetic diversity analysis, every scorable band was considered as single allele/locus and was scored as present (1) or absent (0). The bivariate 1-0 data were used to estimate genetic distance (GD) following “Unweighted Pair Group of Arithmetic Mean (UPGMA)” procedures described by Nei’s and to construct a dendrogram using computer program “PopGene32” version 1.31 (http://www.Ualberta.ca/~Pyeh/fyeh).

Results and Discussion
The genetic diversity and the relationships among six Brassica cultivars were evaluated by RAPD markers using nine primers. The pattern of amplified products generated with the primers OPA-02, OPB-01 and OPC-02, respectively, is shown in six cultivars (Figures 1-3). The selected primers generated 33 distinct bands with size ranging from 300-1000 bp. All of them (100%) were considered as polymorphic and no monomorphic band was found (Table 2).

This proportion of polymorphism is higher compared to some previous RAPD analysis in Brassica, e.g. 81.72% in mustard crops accessions, 76% in Brassica napus germplasm. This difference can be attributed to the primers used and the genotypes evaluated. The three different primers generated various banding patterns, on an average; 11 scorable bands produced per primer and the same 11 polymorphic RAPD markers per primer. This high level of polymorphism in Brassica spp. detected by the arbitrary primers was almost similar to the previous reports such as 11.5 scored per primer and 16 bands per primer.

Among the three primers, the primer OPA-02 provided maximum number of bands. The highest polymorphic loci (24.24 %) were found in Daulot cultivar which gave eight polymorphic bands and the lowest polymorphic loci were found in Safal (3.03%). The variety Daulot, a variety representing B. juncea species showed the highest level of gene diversity (0.1069).

Table 1
Characteristics of six cultivars used in the study and their source

<table>
<thead>
<tr>
<th>Name of parents</th>
<th>Species</th>
<th>Flower colour</th>
<th>Plant height</th>
<th>Days to maturity</th>
<th>Seed Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safal</td>
<td>B. campestris</td>
<td>Yellow</td>
<td>Medium</td>
<td>90-95</td>
<td>BINA</td>
</tr>
<tr>
<td>Sampad</td>
<td>B. campestris</td>
<td>Yellow</td>
<td>Medium</td>
<td>90-95</td>
<td>Dept. of GPB, BAU</td>
</tr>
<tr>
<td>Binasarisha-4</td>
<td>B. napus</td>
<td>Yellow</td>
<td>Medium</td>
<td>100-105</td>
<td>BINA</td>
</tr>
<tr>
<td>Binasarisha-5</td>
<td>B. napus</td>
<td>Yellow</td>
<td>Medium</td>
<td>100-105</td>
<td>BINA</td>
</tr>
<tr>
<td>Daulot</td>
<td>B. juncea</td>
<td>yellow</td>
<td>Tall</td>
<td>100-105</td>
<td>BINA</td>
</tr>
<tr>
<td>Rai-5</td>
<td>B. juncea</td>
<td>yellow</td>
<td>Tall</td>
<td>100-105</td>
<td>BINA</td>
</tr>
</tbody>
</table>

Table 2
Total scorable bands and polymorphic bands amplified by three RAPD primers in studied Brassica spp. Cultivars

<table>
<thead>
<tr>
<th>Primer codes</th>
<th>Sequences (5'-3')</th>
<th>Total number of bands scored</th>
<th>Size range (bp)</th>
<th>Number of polymorphic bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA-02</td>
<td>TGCCGAGCTG</td>
<td>14</td>
<td>250- &gt;1000</td>
<td>14</td>
</tr>
<tr>
<td>OPB-01</td>
<td>GTTTCGCCTCC</td>
<td>10</td>
<td>300- &gt;1000</td>
<td>10</td>
</tr>
<tr>
<td>OPC-02</td>
<td>GTGAGGCCTC</td>
<td>9</td>
<td>200- &gt;1000</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>33</td>
<td></td>
<td>33</td>
</tr>
</tbody>
</table>
Figure 1: RAPD profiles of six different cultivars of *Brassica* spp. using OPA-02 primer. M: Molecular weight marker (100 bp DNA ladder), Lane 1-3: Safal, Lane 4-6: Sampad, Lane 7-9: Binasarisha-4, Lane 10-12: Binasarisha-5, Lane 13-15: Daulot and Lane 16-18: Rai-5

Figure 2: RAPD profiles of six different cultivars of *Brassica* spp. using OPB-01 primer. M: Molecular weight marker (100 bp DNA ladder), Lane 1-3: Safal, Lane 4-6: Sampad, Lane 7-9: Binasarisha-4, Lane 10-12: Binasarisha-5, Lane 13-15: Daulot and Lane 16-18: Rai-5

Figure 3: RAPD profiles of six different cultivars of *Brassica* spp. using OPC-02 primer. M: Molecular weight marker (100 bp DNA ladder), Lane 1-3: Safal, Lane 4-6: Sampad, Lane 7-9: Binasarisha-4, Lane 10-12: Binasarisha-5, Lane 13-15: Daulot and Lane 16-18: Rai-5
**Genetic Distance:** The pair-wise comparison of Nei’s\(^{15}\) genetic distance between 6 *Brassica* spp was computed from combined data sets for the three primers. (Table 3). The genetic distance values between the Sampad (*B.rapa*) and Rai-5 (*B.juncea*) cultivars were found to be the highest (0.981) among the other pair-wise germplasm. The lowest genetic distance (0.0265) was revealed between Safal and Sampad, both belong to *B.rapa* and yellow mustard ecotype.

Thus, the source of origin of these two varieties, Safal and Sampad, was the same i.e. they derived from the same parent or closely related parents. The high genetic similarity supports the theory that they share a common origin\(^{5}\).

**UPGMA Dendrogram:** The 1-0 bivariate data dissimilarity coefficient matrix for six *Brassica* cultivars based on the data of three RAPD primers using the UPGMA method was used to construct a dendrogram (Figure 4). Based on the dendrogram analysis, the six *Brassica* cultivars can be categorized into 2 major groups i.e. Safal, Sampad, Binasarisha-4 and Binasarisha-5 grouped in cluster I, while Daulot and Rai-5 grouped in cluster II. In cluster I, Safal and Sampad formed subcluster I; Binasarisha-4 and Binasarisha-5 which represent *B.napus* species formed sub cluster II. In cluster I, the morphological characteristics such as seed colour are probably indicated in Safal and Sampad (yellow), Binasarisha-4 and Binasarisha-5 (brown). Through cluster analysis, yellow seeded *Brassica* cultivars clearly separated from brown seeded cultivars. In cluster II, Daulot and Rai-5 have almost the same characteristics including seed colour, days to flowering and days to maturity.

Safal and Rai-5 were from a different origin (*Brassica campestris* and *Brassica juncea* respectively) and have different seed colours too (yellow and reddish brown respectively); showed highest genetic distance (0.981). On the other hand, Safal and Sampad showed lowest genetic distance (0.0265) though they are of the same origin (*Brassica campestris*) and contain same morphological characters such as plant height, days to maturity and yellow seed color.

The analysis indicates that Rai-5 with Sampad variety showed the highest genetic variation and Safal and Sampad showed the lowest genetic variation. It is recommended that genetically distant lines observed among the 6 *Brassica* cultivars should be used in the future breeding program for improving yield and quality characteristics of *Brassica*.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Safal</th>
<th>Sampad</th>
<th>Bina-sharisha-4</th>
<th>Bina-sharisha-5</th>
<th>Daulot</th>
<th>Rai-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Safal</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Sampad</td>
<td>0.0265</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Bina-sharisha-4</td>
<td>0.5521</td>
<td>0.5676</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Bina-sharisha-5</td>
<td>0.3673</td>
<td>0.3795</td>
<td>0.1640</td>
<td>****</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Daulot</td>
<td>0.9052</td>
<td>0.9590</td>
<td>0.9615</td>
<td>0.7446</td>
<td>0.0704</td>
<td>****</td>
</tr>
<tr>
<td>6. Rai-5</td>
<td>0.9690</td>
<td>0.9810</td>
<td>0.9546</td>
<td>0.7546</td>
<td>0.0704</td>
<td>****</td>
</tr>
</tbody>
</table>

**Table 3**

Summary of Nei’s\(^{15}\) genetic distance (below diagonal) values among studied 6 *Brassica* varieties

Figure 4: UPGMA dendrogram based on Nei’s (1972) genetic distance, summarizing the data on the differentiation between *Brassica* spp. cultivars according to RAPD analysis.
Conclusion
The results of the RAPD marker explore the wide variation of genotypes and show the suitability of its application in Brassica species. DNA fingerprinting and molecular characterization are the best approaches for the determination of the diversity. This study reveals that PCR based assays like RAPD can be used effectively to estimate genetic variability in Brassica spp. and considering the easy handling of the technique, it is especially suitable for breeding programs where large number of lines/accessions have to be analyzed. It also explores that Rai-5 with Sampad variety shows highest genetic variation while Safal and Sampad showed the lowest genetic variation. The study suggests that genetically distant lines observed among the 6 Brassica cultivars should be used for improving yield and quality characteristics of Brassica in the future breeding program. The findings of the study will be helpful for researchers, academicians and practitioners to develop a proper breeding strategy for sustainable agricultural development.

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