Molecular Characterization of Brassica Cultivars through RAPD Markers

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Abstract— This study intended to identify molecular characterization of Brassica through RAPD marker. The hereditary enhancement of Brassica cultivar is necessary for improved of yield and quality of various mustard varieties. Six Brassica cultivars have been used to assess inherent multiplicity and associations by PCR-based indiscriminate augmented Polymorphic DNA (RAPD) method. The available 6 varieties were BINAsarisha-4, BINAsarisha-5, Safal, Sampad, Rai-5, and Daulot. In this study, nine RAPD primers were used for assessment among them. 3 primers (OPA-02, OPB-01, and OPC-02) created 33 distinct polymorphic bands among 9. Different banding model generated by every primer with a standard of 11 score making bands. The primer OPA-02 formed an utmost quantity of the band (14) among 3 primers and the other 2 primers (OPB-01 and OPC-02) created 10 and 9 bands respectively. The cultivar Sampad (Brassica rapa L.) was very similar to Safal (Brassica campestris L.) with the lowest inherent space (0.0265). Sampad and Rai-5 (Brassica juncea, L.) showed the best hereditary remoteness (0.981). The findings will be helpful to take policy initiative for Brassica improvement program.

Keywords— DNA fingerprinting, biotechnology, RAPD marker, inherent dissimilarity, mustard.

I. INTRODUCTION

Brassica oilseed crops are very popular in the South Asian countries. The two vital Brassica oilseed crops are mustard (Brassica juncea L.) and rapeseed (B.rapa L,). Soybean Brassica species has a great popularity as oil seed producing crops overall all countries in the world [1],[2],[3],[4]. According to USDA (United States Department of Agriculture), , rapeseed oil production in the year 2016 was 150 (1000 MT) which was -17.13% i.e. 17.13% less than the previous year; in the year 2017 in Bangladesh, it was 162(1000 MT) 8.00% higher than the year 2016; in the year 2018, it was 166 (1000 MT), while it was 2.47% higher compared to the year 2017 (USDA-2018).

On the other hand, world rapeseed production around the year 2015/2016 was 68 million tons. Like all other crops to get better value and size of Brassica spp [5]. That's the difference of inheritance enough is very important [6].

There are various techniques for researching germ line changes, including morphological characteristics, general protein isolate, isoenzymes and many molecular markers [7],[8],[9],[10]. On the other hand, the DNA mark has been provided based on the prominence and reliability of the apparatus for the reasoning in the struggle, the variation, and the evolutionary interface [11],[12],[13].

Differentiation of molecular markers, including the RFLP, the simple SSR, the AFLP, and RAPD polar lengths are used to study the level of molecular markers [14],[15],[16],[17]. DNA molecules (RAPDs) are used broadly in genetic material testing owing to its rapidity and simplicity [18],[19],[20]. RAPD knowledge is a dependable, speedy and proficient tool for shaping the hereditary diversity of plant inherent property to get better yield [21],[22],[23]. RAPD requires barely a small DNA (5-20 ng) and a single primer (9-10 bp) with a geometric-speed randomized random order.

The multidimensional DNA (RAPD) is a new method and is extensively used to assess the hereditary interaction in different cultures of scientific significance [24],[25],[26]. RAPD analysis was extensively used to deed genetic difference in Brassicas [27],[28],[29],[28],[30],[31].

However, most studies of previous studies were conducted with B. napus and B. juncea and there was little information on the level of genetic variation in B. campestris/rapa using a DNA-based marking system. The findings of this study will be helpful for researchers, policymakers and practitioners to develop appropriate breeding strategies for future improved mustard varieties.

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II. MATERIALS AND METHODS

A. Plant Materials

Submit The field of Bangladesh Agricultural University (BAU) was selected as experimental field and molecular work was conducted at the Biological Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA). Six spp of Brassica variety such as Safal, Sampad, Binasarisha-4, Binasarisha-5, Daulot, and Rai-5 were recognized in the study using RAPD markers (Table 1). These seeds were collected from BINA and BAU.

Table 1. Characteristics of six cultivars and sources

Name of parents	Species	Flower color	Plant height	Days to maturity	Seed Source
Safal	B. campestris	Yellow	Medium	90-95	BINA
Sampad	B. campestris	Yellow	Medium	90-95	Dept. of GPB, BAU
Binasarisha- 4	B. napus	Yellow	Medium	100-105	BINA
Binasarisha -5	B. napus	Yellow	Medium	100-105	BINA
Daulot	B. juncea	yellow	Tall	100-105	BINA
Rai-5	B. juncea	yellow	Tall	100-105	BINA

B. Genotyping of Mustard Varieties

The DNA samples were quantitative, qualitatively assessed by means of a spectrophotometer and λ DNA (lambda) (marker concentration), respectively. Foliar samples were used to separate whole genomic DNA resulting in tiny CTAB technique custom-made procedure research. The polymerase chain reaction was created in 10 microliters volume containing 10X PCR Buffer 1 ul, 250 µM dNTP (mixture) 1 ul mask 10 µM primer 2.5 microliters 25 ng / DNA microliters 2 µl, Taq DNA polymerase 1 unit / µl or 0.2 µl, sterile deionizer water 3.3 µl. DNA amplification was done in a thermo cycler with the following profile: 940C for 3 min (initial denaturation), 94 ° C for 1 minute, annealing at 34 ° C for 1 min, elongation at 72oC for 2 min for 40 cycles with extension final at 72°C for 7 minutes. The expanded items were partitioned by 1.5% agarose gel electrophoresis in TBE buffer, were pictured by recoloring with ethidium bromide and UV transillumination under short wave light. Nine primers (OPA-01, OPA-02, OPB-01, OPB-02, OPC-01, OPC-02, 66AB10G6, 67AB10G7 and 69AB10G9) irregular arrangement were chosen in a sub-test of two arbitrarily chosen individual from six unique cultivars.

C. RAPD Data Analysis

Each band score was considered as single allele/locus and was scored as present (1) or missing (0) For hereditary decent variety investigation. The bivariate 1-0 information was utilized to assess hereditary separation (GD) following "Unweighted Pair Group of Arithmetic Mean (UPGMA)" techniques and to develop a dendrogram utilizing PC program "PopGene32" form 1.31 (http://www. Ualberta.ca./~Pyeh/fyeh).

III. RESULTS AND DISCUSSION

Six Brassica cultivars were assessed by RAPD markers utilizing nine primers. The example of intensified items created with the primers OPA-02, OPB-01, and OPC-02, individually, is appeared six cultivars (Figures 1-3). The chosen primers produced 33 particular groups with size going from 300-1000 bp. Every one of them (100%) was considered as polymorphic and no monomorphic band was discovered (Table 2).

Table 2. Total scorable bands and polymorphic bands

Primer codes	Sequences (5´ - 3´)	Total number of bands scored	Size range (bp)	No of polymorphic bands
OPA-02	TGCCGAGCTG	14	250->1000	14
OPB-01	GTTTCGCTCC	10	300->1000	10
OPC-02	GTGAGGCGTC	9	200->1000	9
Total		33		33

This extent of polymorphism is higher contrasted with some past RAPD investigation in Brassica, for example, 81.72% in mustard crops accessions9, 76% in Brassica napus germplasm4. This distinction can be credited to the primers utilized and the genotypes assessed. The three unique primers created different banding designs, on an average; 11 scorable bands delivered per primer and a similar 11 polymorphic RAPD markers for each pre primers. This abnormal state of polymorphism in Brassica spp. identified by the self-assertive primers was practically the same the past reports, for example, 11.5 scored per primer8 and 16 bands for each primer6. Among the three primers, the primer OPA-02 gave a most extreme number of bands. The most elevated polymorphic loci (24.24 %) were found in Daulot cultivar which gave eight polymorphic groups and the

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least 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).

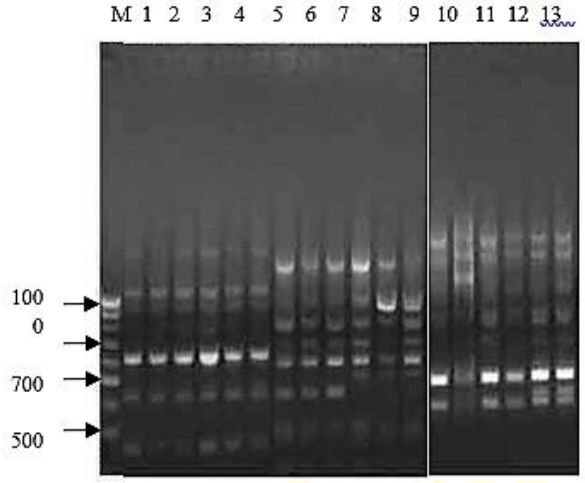

Figure 1. RAPD profiles of 6 cultivars of Brassica spp. using OPA-02 primer.

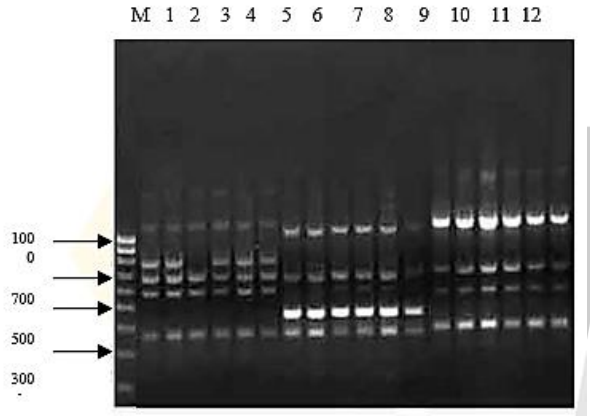

Figure 2. RAPD profiles of 6 cultivars of Brassica spp. using OPB-01 primer

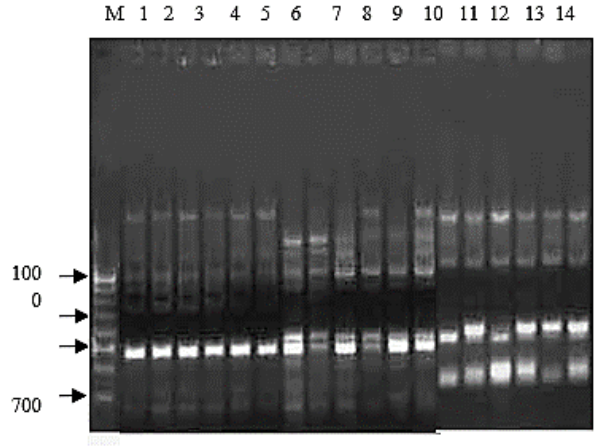

Figure 3. RAPD profiles of 6 cultivars of Brassica spp. using OPC-02 primer

Genetic Distance

Six Brassica spp was computed from combined data sets for the three primers (Table 3). The genetic distance value between the Sampad (B.rapa) and Rai-5 (B.juncea) cultivars was 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.

Table 3. Summary of Nei's15 genetic distance

Cultivar	Safal	Sampad	BINA-sharisha-4	BINA-sharisha-5	Daulot	Rai-5
Safal	****					
Sampad	0.0265	****				
BINA-sharisha-4	0.5521	0.5676	****			
BINA-sharisha-5	0.3673	0.3795	0.1640	****		
Daulot	0.9052	0.9590	0.9615	0.7446	****	
Rai-5	0.9690	0.9810	0.9546	0.7546	0.0704	****

The source of origin of Safal and Sampad varieties were derived from the same parent or closely related parents.

UPGMA Dendrogram

Six Brassica cultivars dependent on the information of three RAPD primers utilizing the UPGMA technique was utilized to develop 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 in cluster II. In cluster I, Safal and Sampad formed sub cluster I; Binasarisha-4 and Binasarisha-5, which represent B.napus species formed sub cluster II. In cluster I, the morphological characteristics such as seed color are probably indicated in Safal and Sampad (yellow), Binasarisha-4 and Binasarisha-5 (brown). Through cluster analysis, O [14] reported that 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. Sampad and Rai-5 were from a different origin (Brassica campestris and Brassica juncea, respectively) and have different seed colors too (yellow and reddish brown, respectively); showed highest genetic distance (0.981). Then again, Safal and Sampad demonstrated most reduced hereditary separation (0.0265). However, they are of a similar starting point (Brassica campestris) and contain same morphological characters, for example, plant tallness, days to development and yellow seed shading.

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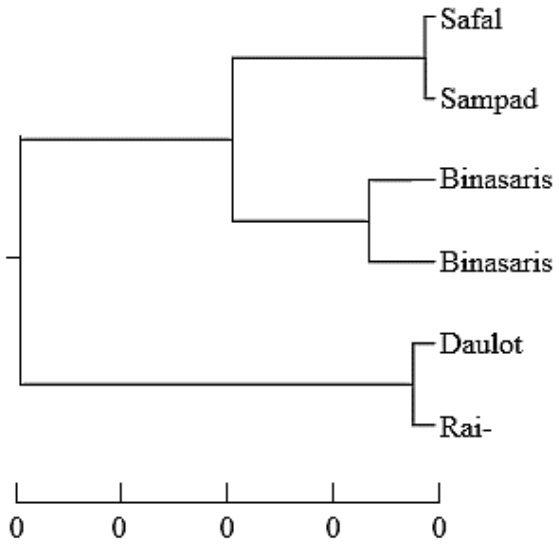

Figure 4. UPGMA dendrogram dependent of hereditary separationof Brassica spp.

The results show 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.

IV. CONCLUSION

DNA fingering and molecular depiction are the best approaches for the assurance of the decent variety. The RAPD marker used to identify the wide variety of genotypes and demonstrate the reasonableness of its application in Brassica species. This study revealed that PCR based tests like RAPD could be utilized viably to evaluate hereditary fluctuation in Brassica spp. It also explored that Rai-5 with Sampad variety showed the 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 the yield and quality characteristics of Brassica in the future breeding program. The findings of this study will be helpful for researchers, policymakers and practitioners to develop appropriate breeding strategies for future improved mustard varieties.

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