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# Molecular Analysis of Hexaploid Wheat (*Triticum aestivum* L.) Cultivars by RAPD Markers

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## Abstract

RAPD assay was conducted for molecular genetic analysis of six wheat cultivars, such as, Kanchan, Sourav, Gourab, Shatabdi, Pavon and BAW-1006 to observe genetic variability and relatedness among these cultivars. Three out of 12 decamer random primers showed distinctly polymorphic bands when used to amplify genomic DNA. The primers yielded a total of 23 RAPD markers of which 14 were considered as polymorphic. The proportion of polymorphic loci and gene diversity (h) values were 34.78% and 0.153 for BAW-1006, 30.43% and 0.124 for Kanchan, 26.09% and 0.127 for Shatabdi, 26.09% and 0.127 for Pavon, 26.09% and 0.111 for Gourab, 21.74% and 0.098 for Sourav, respectively. The coefficient of gene differentiation ($G_{st}$) and gene flow ($N_m$) values across all the loci were 0.50 and 0.50, respectively indicating genetic divergence among populations. The UPGMA dendrogram based on Nei's genetic distance, grouped six cultivars into two main clusters: Kanchan, Sourav, Gourab and Shatabdi in cluster I; Pavon and BAW-1006 in cluster II. The cluster I was further separated: Kanchan alone in sub-cluster I and Sourav, Gourab, Shatabdi in sub-cluster II; furthermore, Sourab and Gourab grouped together in sub-sub-cluster I of sub-cluster II with the lowest genetic distance of 0.035. Thus, RAPD offer a potentially simple, rapid and reliable method to evaluate genetic variation and relatedness among six wheat cultivars.

## Introduction

Wheat is one of the most important cereal crops grown widely and intensively all over the world. It ranks first in area as well as production among the crops globally grown and is in second position next to rice in Bangladesh. Wheat contains about 35 genera including *Triticum, Aegilops, Thinopyrum, Dasypyrum,*

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*Lophopyrum* and *Secale.* Of the three ploidy levels the most important wheat is the hexaploid bread wheat (*Triticum aestivum* L. Syn. *T. vulgare* Host) and is of winter and spring types. In Bangladesh, only the spring type wheat is commercially grown. Conventional cereal breeding is time consuming and dependent on environmental conditions. Molecular markers are rapidly being adopted in crop improvement programs as an effective and appropriate tool for basic and applied studies addressing biological components in agricultural production systems (Jones et al. 1997). RAPD is a PCR-based technique commonly used as molecular marker in genetic diversity studies. RAPD has been used widely because it requires no DNA probe and no sequence information for the design of specific primers. In wheat, RAPD methods have allowed fast and effective approaches for detecting polymorphism at the DNA level (Sivolap-Yu et al. 1997, Zvingila et al. 1998). RAPD analysis can be used to identify genetic variation (Bered et al. 2001, Taghian et al. 2003), genetic relatedness (Monte et al. 1999, Shan et al. 2001), genetic diversity analysis (Zheng et al. 2001, Maric et al. 2004) and phylogenetic relationship (Tsuji et al. 2000, Goriunova et al. 2004). Genetic variation refers to the differences in the hereditary constitutions of the individuals of a species. Genetic diversity is a level of biodiversity that refers to the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary. It was well-known that genetic diversity in crop species is a fundamental tool in hybrid wheat breeding programs. It is therefore, useful, for breeders to know the genetic background of the breeding materials and varieties. The aim of the study was to determine the genetic variation and establish the genetic relationship at molecular level of six wheat cultivars by RAPD markers. The goal of the study was inspired from above mentioned research and emphasized on genetic variation and relatedness among the cultivars. To our knowledge, this was the first preliminary attempt to study the molecular analysis of wheat cultivars in Bangladesh.

**Materials and Methods**

Six high yielding hexaploid wheat cultivars, namely Kanchan, Sourav, Gourab, Shatabdi, Pavon and BAW-1006 were collected from the Wheat Research Center (WRC) of Bangladesh Agricultural Research Institute (BARI). Genomic DNA was extracted from fresh leaf samples of 15-day-old-seedlings and a total of 18 individuals (three from each cultivar) were taken randomly to carry out the RAPD analysis.

Twelve primers of random sequence (Genei Pvt. Ltd., Banglore, India) were screened to evaluate their suitability for amplifying DNA sequences, which could be accurately scored. Primers were selected on the basis of band resolution

intensity, presence of smearing, consistency within individuals and potential for population discrimination. Finally, three primers (Table 1) were selected for the analysis of the whole sample set of the six cultivars.

PCR reactions were performed on each DNA sample in a 10 µl reaction mix containing 1 µl of 10x ampli taq polymerase buffer, 2.5 µl of 10 µM primer, 1 µl of 250 µM dNTPs, 1 unit of ampli taq DNA polymerase and 3 µl of genomic DNA and a suitable amount of sterile deionized water (Williams et al. 1990). DNA was amplified in a thermal cycler (Master Cycler Gradient, Eppendorf, Germany) and the reaction mix was performed at 94ºC for 3 min followed by 40 cycles of 1 min denaturation at 94ºC, 1 min annealing at 37ºC and elongation at 72ºC for 2 mins. After the last cycle, a final step of 7 min at 72ºC was added to allow complete extension of all amplified fragments. The amplified product from each sample was separated electrophoretically on 1.4% agarose gel containing ethidium bromide in 1xTBE buffer. A molecular weight DNA marker (Lambda DNA with *Hind* III digest and/or 100 bp DNA ladder) was loaded in the first lane of the gel. DNA bands were observed on UV-transilluminator in the Image Documentation System and the image was viewed on the monitor and saved in a diskette, as well as printed on thermal paper.

All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The scores obtained using all primers in the RAPD analysis were then pooled to create a single data matrix. This was, then, used to estimate polymorphic loci (Nei 1973), gene diversity, population differentiation ($G_{st}$), gene flow ($N_m$), genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) (Yeh et al. 1999).

The similarity index (SI) values were defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel and calculated as below

$$\text{Similarity index (SI)} = 2\ N_{xy}/(N_x + N_y)$$

where, $N_{xy}$ is the number of RAPD bands shared by individuals x and y respectively, and $N_x$ and $N_y$ are the number of bands in individual x and y, respectively (Chapco et al. 1992, Wilde et al. 1992 and Lynch 1990). The SI value range from 0 to 1; when SI=1.0, the two DNA profiles are identical and when SI is 0.0, there are no common bands between the two profiles. Intra population similarity ($S_i$) was calculated as the average of SI across all possible comparisons between individuals within a population. Inter population similarity ($S_{ij}$) was calculated as the average similarity between each paired individuals of population i and j (Lynch 1991). The highest and the lowest $S_i$ values reflect a low

genetic variability and a high genetic variability, respectively among the individuals. On the other hand, the highest and the lowest $S_{ij}$ values reflect a low genetic distance and a high genetic distance, respectively between the cultivar pairs. High genetic variability within populations and significant genetic differentiation between populations indicate rich genetic resources of a species.

## Results and Discussion

Three out of 12 primers, 71AB10G11, 72AB10G12 and 73AB10T13 produced comparatively maximum number of high intensity bands with minimal smearing. The banding patterns of six wheat cultivars using primer 71AB10G11, 72AB10G12 and 73AB10T13 are shown in Figs. 1 - 3.

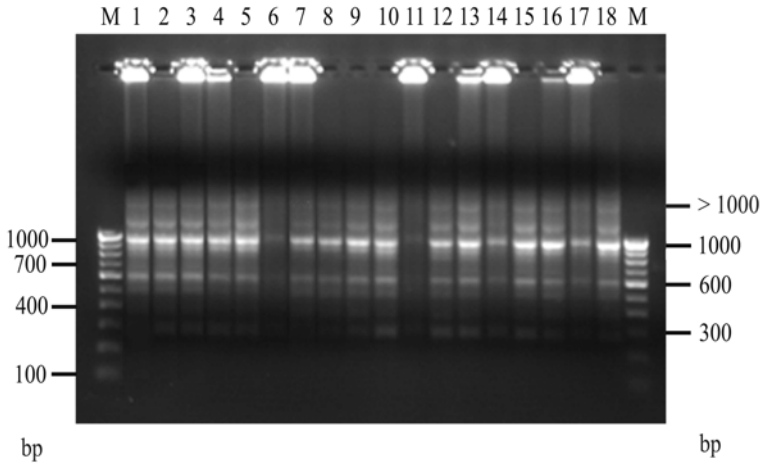


Fig. 1. RAPD profiles of wheat cultivars using primer 71AB10G11. Lanes 1 - 3: Kanchan, lanes 4 - 6: Sourav, lanes 7 - 9: Gourab, lanes 10 - 12: Shatabdi, lanes 13 - 15: Pavon, lanes 16 - 18: BAW-1006. M = molecular weight marker (100 bp DNA ladder).

The three primers yielded a total of 23 distinct bands of which 14 (60.87%) were considered as polymorphic (either occurring in or absent in < 95% of all individuals). The primer 73AB10T13 produced maximum number of bands (10), whereas, 71AB10G11 generated the least number (3) of polymorphic bands. The three primers generated 7.67 bands per primer and 4.67 polymorphic RAPD markers per primer (Table 1).

This was relatively a high level of polymorphism detected by the arbitrary primers compared to the other previous RAPD studies on wheat e.g., three polymorphic bands per primer in Pakistani wheat cultivars (Anwar et al. 1998), 1.7 polymorphic bands per primer in China wheat cultivars (Zheng et al. 2001).

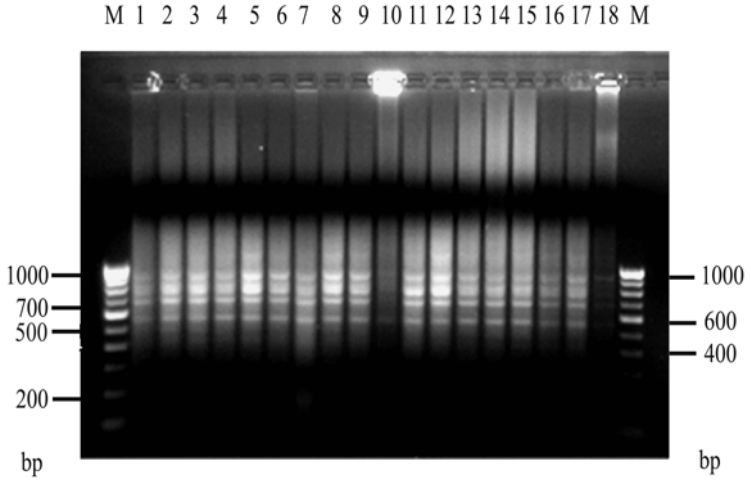


Fig. 2. RAPD profiles of wheat cultivars using primer 72AB10G12. Lanes 1 - 3: Kanchan, lanes 4 - 6: Sourav, lanes 7 - 9: Gourab, lanes 10 - 12: Shatabdi, lanes 13 - 15: Pavon, lanes 16 - 18: BAW-1006. M: Molecular weight marker (100 bp DNA ladder).

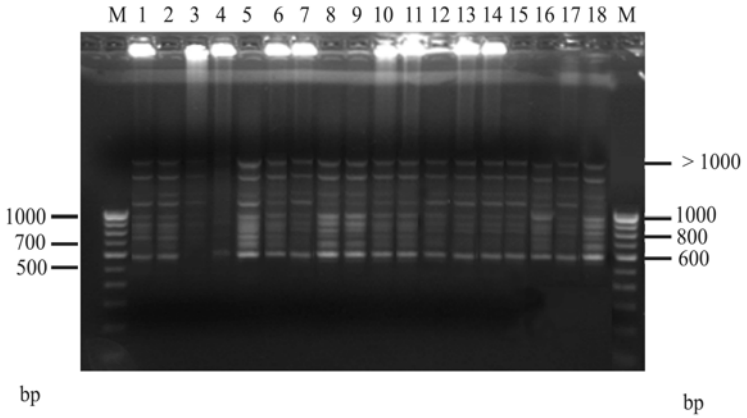


Fig. 3. RAPD profiles of wheat cultivars using primer 73AB10T13. Lanes 1 - 3: Kanchan, lanes 4 - 6: Sourav, lanes 7 - 9: Gourab, lanes 10 - 12: Shatabdi, lanes 13 - 15: Pavon, lanes 16 - 18: BAW-1006. M: Molecular weight marker (100 bp DNA ladder).

**Table 1. Parameters of the random primers used for genetic analysis.**

| Primer codes | Sequences ( 5'-3') | Total number of bands scored | Size range (bp) | Number of polymorphic bands |
|---|---|---|---|---|
| 71AB10G11 | AGCGCCATTG | 5 | 550-950 | 3 |
| 72AB10G12 | AGGGCGTAAG | 8 | 290->1000 | 6 |
| 73AB10T13 | CTGGGGACTT | 10 | 580->1000 | 5 |
| Total | | 23 | | 14 |
| Average | | 7.67 | | 4.67 |

The highest intra-cultivar similarity indices ($S_i$) value was found in Sourav (91.78%) followed by that of Gourab, Pavon, Shatabdi, Kanchan, respectively (Table 2). The $S_i$ value for BAW-1006 was the lowest (84.22%). The higher $S_i$ values reflect lower genetic variability within the individuals of Sourav cultivar. The inter-cultivar similarity indices ($S_{ij}$) between Sourav and Gourab was found to be the highest (92.96%), indicate the genetic distance between the cultivar pair was low. The lowest band sharing value (77.81%) was observed between Sourav and BAW-1006 cultivar combinations indicate a greater genetic distance was existed between these populations (Table 2).

**Table 2. Summary of band sharing based similarity ($S_i$) between individuals of the same population (Intra-) and pair wise inter-variety similarity indices ($S_{ij}$) among six wheat cultivars.**

| Intra and inter cultivar combinations | | Percentage of similarity |
|---|---|---|
| Cultivars | BAW-1006 | 84.22 |
| | Kanchan | 84.67 |
| | Shatabdi | 87.56 |
| | Pavon | 90.11 |
| | Gourab | 90.67 |
| | Sourav | 91.78 |
| | **Average** | **88.17** |
| Cultivar | Sourav and BAW-1006 | 77.81 |
| combinations | Kanchan and BAW-1006 | 79.89 |
| | Kanchan and Pavon | 81.00 |
| | Shatabdi and BAW-1006 | 82.33 |
| | Kanchan and Shatabdi | 82.56 |
| | Gourab and Pavon | 84.96 |
| | Gourab and BAW-1006 | 85.33 |
| | Kanchan and Sourav | 85.52 |
| | Kanchan and Gourab | 86.22 |
| | Shatabdi and Pavon | 86.67 |
| | Sourav and Shatabdi | 87.41 |
| | Sourav and Pavon | 87.63 |
| | Pavon and BAW-1006 | 90.59 |
| | Gourab and Shatabdi | 92.00 |
| | Sourav and Gourab | 92.96 |
| | **Average** | **85.53** |

The values for intra-cultivar similarity indices ($S_i$ ) were higher (average 88.17%) than inter-cultivar similarity indices ($S_{ij}$) (average 85.53%) indicating less genetic variation between two individuals of same cultivars than between two

individuals of different cultivars. Genetic variability is important in maintaining the developmental stability and biological potential of plant species. Here, BAW-1006 line and Sourav variety contained the highest genetic variation and the lowest genetic variation, respectively which could be used as germplasm for future breeding programs.

The number and proportion of polymorphic loci was found to be the highest in BAW-1006 cultivar that were 8 and 34.78%, respectively (Table 3). From the viewpoint of the lowest intra-cultivar similarity value (84.22%), the highest gene diversity value (h = 0.153) and the highest proportion of polymorphic loci (34.78%), BAW-1006 was likely to be the most diversified cultivar among six cultivars. On the other hand, Sourav was found to be the least diversified cultivar compared to the others as it comprised the highest value of $S_i$, the lowest value of gene diversity and proportion of polymorphic loci as well.

**Table 3. Number and proportion of polymorphic loci, gene diversity obtained in different wheat cultivars.**

| Cultivars | Number of polymorphic loci | Proportion of polymorphic loci ( % ) | Gene diversity (h) |
|---|---|---|---|
| 1. BAW-1006 | 8 | 34.78 | 0.153 |
| 2. Kanchan | 7 | 30.43 | 0.124 |
| 3. Shatabdi | 6 | 26.09 | 0.127 |
| 4. Pavon | 6 | 26.09 | 0.127 |
| 5. Gourab | 6 | 26.09 | 0.111 |
| 6. Sourav | 5 | 21.74 | 0.098 |

The Nei's (1972) genetic distance (D) of different cultivar pairs are shown in Table 4. A comparatively higher distance (0.297) was observed between Sourav vs. BAW-1006 cultivar pair than other cultivars combination. The lowest genetic distance (0.035) was found in Sourav vs. Gourab cultivar pair. Considering the genetic distance values, the results indicated that the cultivars were genetically different from each other (genetic distance value range from 0.035 to 0.297) (Table 4).

**Table 4. Summary of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) values between 6 wheat cultivars.**

| Cultivars | Kanchan | Sourav | Gourab | Shatabdi | Pavon | BAW-1006 |
|---|---|---|---|---|---|---|
| Kanchan | *** | 0.837 | 0.867 | 0.845 | 0.757 | 0.743 |
| Sourav | 0.178 | *** | 0.966 | 0.899 | 0.779 | 0.743 |
| Gourab | 0.143 | 0.035 | *** | 0.948 | 0.783 | 0.761 |
| Shatabdi | 0.168 | 0.106 | 0.054 | *** | 0.825 | 0.792 |
| Pavon | 0.278 | 0.250 | 0.244 | 0.192 | *** | 0.928 |
| BAW-1006 | 0.296 | 0.297 | 0.274 | 0.233 | 0.075 | *** |

Dendrogram based on Nei's (1972) genetic distance using unweighted pair group method of arithmetic means (UPGMA) indicated segregation of six cultivars of wheat into two main clusters (Fig. 4), in which, four cultivars Kanchan, Sourav, Gourab and Shatabdi constitute cluster-I.

These cultivars are probably identical based on morphological and genetical characters (BARI 2004 and BARI 2005). The other two cultivars Pavon and BAW-1006 formed cluster-II because these two cultivars contain rust resistance gene and a high 1000 grain weight.

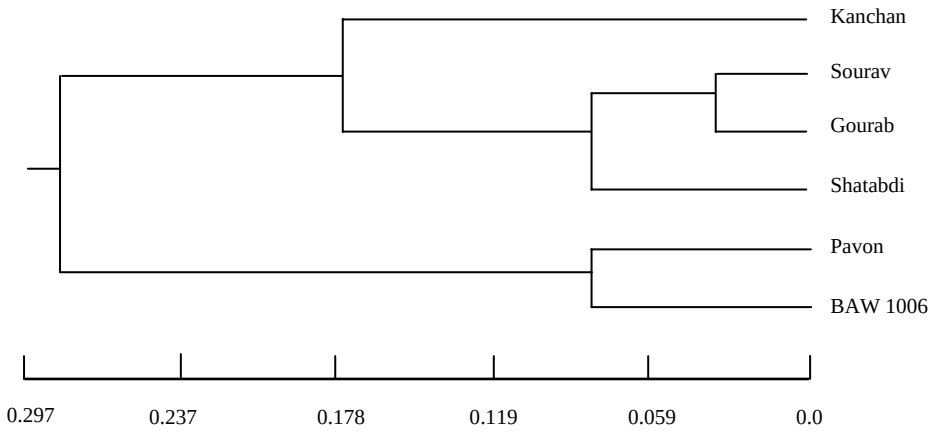


Fig. 4. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between six wheat cultivars according to RAPD analysis.

Cluster-I was also observed to divide into two sub-clusters. Kanchan alone in sub-cluster-I: Sourav, Gourab and Shatabdi grouped in sub-cluster II based on heat tolerant and BpLB disease resistance. Sourav and Gourab again formed together in sub-sub-cluster I of sub-cluster II with the lowest genetic distance of 0.035. Therefore, the special genetic or morphological characters might be similar between those; however, the available information was not obtained. The highest genetic distance (0.297) was found between Sourav and BAW-1006 cultivars. Here, Sourav is heat tolerant and has BpLB disease resistant variety; on the other hand, BAW-1006 is rust resistant and contains a high 1000 grain weight advanced line. No cultivars specific marker was obtained in the study. Thus, necessity was felt to use more markers to obtain any cultivars specific markers for larger samples.

In conclusion, RAPD markers have been proved to be powerful tools for molecular genetic analysis of wheat cultivars for plant breeding program to assess available genetic diversity. This study indicated that BAW-1006 line and Sourav variety contained the highest genetic variation and the lowest genetic variation, respectively which could be used as germplasm for future cross

breeding programs with the goal to improve wheat variety. Moreover, these markers allow for the production of new wheat varieties that are aimed at crop productivity improvement enabling them to withstand biotic and abiotic stresses.

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