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Molecular Characterization of Potato Germplasm by Random Amplified Polymorphic DNA Markers

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Abstract: Random Amplified Polymorphic DNA (RAPD) analysis was used to study the genetic diversity of six cultivars of potato. Amplification with three decamer random primers generated 35 RAPD markers of which 33 (94.29%) were polymorphic. The proportion of polymorphic loci and the gene diversity estimates were 14.29% and 0.068, 28.57% and 0.138, 17.14% and 0.075, 51.43% and 0.217 and 54.29% and 0.245 for Cardinal, Diamant, Heera, Raja and TPS, respectively. The results indicated that relatively high level of genetic variation was observed in the TPS and Raja cultivars compared to other cultivars studied. No intra-cultivar genetic variation was observed in the Ailsa cultivar. The high level of cultivar differentiation (G_{ST} = 0.634) and low level of gene flow (N_m = 0.289) across all loci reflected that the level of genetic divergence among six cultivars was high. The UPGMA dendrogram based on the Nei's genetic distances segregated the cultivars into three clusters: Ailsa and Heera made one cluster, Cardinal and TPS made another cluster whereas Diamant and Raja grouped into another cluster. The RAPD markers were found to be useful in studying genetic diversity of potato cultivars.

Key words: RAPD, genetic diversity, polymorphism, potato

INTRODUCTION

Potato (*Solanum tuberosum* L.) belonging to the family Solanaceae is one of the world's most economically important crops originated in the Central Andean area of South America (Keeps, 1979). The crop is extensively grown in tropical and temperate countries. It has the forth place as the important staple crop after rice, maize and wheat. In Bangladesh 33.86 million tones of potatoes were produced from 6.06 lac hectares of land in 2002-2003 (BBS, 2003).

Despite of the commercial importance of the crop, genetic data on potato in Bangladesh are relatively scarce. Worldwide research activities at the aim of improvement of potato cultivars were initiated in 1960. The increasing number of potato cultivars and the importance of their choice make necessary to strengthen user's guarantees concerning purity and identity. The identification of different cultivars based on morphological markers implies culture inspection at different stages and is not very reliable because many traits of interest have low heritability and are genetically very complex. Molecular markers based on the DNA sequence are more reliable in this regard (Raghunathachari *et al*., 2000). The techniques may serve as valuable guide for effective collection and use of genetic resources, to conserve genetic resources for future breeding research and to improve and sustain genetic diversity of potato cultivars.

Randomly Amplified Polymorphic DNA (RAPD) markers have rapidly gained popularity to detect polymorphisms among different germplasms of potato (Kayim *et al*., 1998; Forapani *et al*., 1999; Polzerova, 2001). The technique is based on the amplification of discrete regions of the genome by Polymerase Chain Reaction (PCR) with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al*., 1990). The method is relatively simple and inexpensive compared to other methods (Ward and Grewe, 1995), as it does not require prior DNA sequences knowledge for the examination of genomic variations.

Knowledge of germplasm diversity among breeding materials and varieties is important for the genetic improvement of plants. It is therefore, useful for breeders to know the genetic background of the breeding materials and varieties. The aim of the study was to characterize six different cultivars of potato by RAPD markers.

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MATERIALS AND METHODS

The experiment was carried out in the Laboratory, Department of Biotechnology and Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh during January-May, 2004.

Sample collection: Six cultivars of potato viz., Ailsa, Cardinal, Diamant, Heera, Raja and TPS (True Potato Seed) were collected from Bangladesh Agricultural Research Institute, Gazipur. *In vitro* grown micro plants from sprouts were used as the source of plant material. A total of 30 individuals (five from each cultivar) were taken randomly to carry out the RAPD analysis.

DNA extraction: In brief, approximately 50 mg of fresh leaf tissue from each microplant were taken into a microcentrifuge tube, cut into small pieces, homogeneized and digested with RNAse A in extraction buffer [0.2 M Tris-HCl pH 8.5, 0.25 M NaCl, 25 mM EDTA, 0.5% (w/v)]. DNA was purified by successive extraction with phenol: chloroform: isoamyl alcohol (25:24:1; v:v:v) and chloroform: isoamyl alcohol (24:1; v:v), respectively. DNA was precipitated first using 0.6 volume of isopropanol, peletted by centrifugation, then resuspended in TE buffer (10 mM Tris. HCl, 1 mM EDTA, pH = 8.0). DNA was reprecipitated by adding two volumes of 70% ethanol and peletted by centrifugation. The pellets were then air-dried and resuspended in an appropriate volume of TE buffer. DNA quality was checked by electrophoresis in a minigel and quantified using a spectrophotometer (Spectronic® GENESIS™).

Primer selection: Initially, ten decamer primers (Kit A, Operon Technologies, Inc., USA) of random sequence were screened on a sub sample of two randomly chosen microplants from each cultivar, to test their suitability for amplifying potato RAPDs that could be accurately scored. Primers were evaluated on the basis of intensity or resolution of bands, repeatability of markers and consistency within individual and potential to differentiate cultivars (polymorphism). Finally, three primers (Table 1) were selected for the analysis of the whole sample set of the six cultivars.

PCR amplification and electrophoresis: PCR amplification was done essentially following a standard procedure (Williams *et al*., 1990). PCR reactions were performed on each DNA sample in a 10 μL reaction mix containing 1 μL of 10x Ampli Taq polymerase buffer, 2 μL of 10 μM primer, 1 μL of 250 μM dNTPs, 1 unit of Ampli Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) and 75 ng of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 34°C and extension at 72°C for 2 min. After the last cycle, a final step of 7 min at 72°C was added to allow complete extension of all amplified fragments. The amplified product from each sample was separated electrophoretically on 1% agarose gel (Nacalai tesque, Inc, KYOTO, Japan) containing ethidium bromide in 1XTAE buffer at 120 V for 1½ h. A molecular weight marker DNA (Lamda DNA-EcoT 14 I digest and/or 100 bp ladder) was run alongside the RAPD reactions. DNA bands were observed on UV-transilluminator and photographed by a Gel Cam Polaroid camera.

Genetic data analysis: Fragments were scored as 1 if present and 0 if absent by two independent persons. Bands not identified by the two readers were considered as non-scorable. The scores were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, gene diversity (Nei , 1973), gene flow (N_m), coefficient of gene differentiation or cultivar differentiation (G_{ST}), genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among cultivars using POPGENE (version 1.31) (Yeh *et al*., 1999)computer program. The similarity index values (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers according to the following formula:

$$\text{Similarity Index (SI)} = 2N_{AB} / (N_A + N_B)$$

Where, N_{AB} is the total number of RAPD bands shared by individuals A and B and N_A and N_B, are the numbers of fragments scored for each individual respectively (Lynch, 1990). Within population similarity (S_i) was calculated as the average of SI across all possible comparisons between individuals within a population. Between population similarity (S_{ij}) was calculated as the average similarity between randomly paired individuals from populations I and j (Lynch, 1991).

RESULTS AND DISCUSSION

All the three primers showed different banding patterns and the number of fragments amplified per primer varied (Table 1). Primer OPA02 yielded a maximum number of bands whereas the OPA15 produced the least number

Table 1: Parameters of the Operon random primers used for genotype analysis

Primer	Sequence (5′ to 3′)	(G + C) %	Total bands scored	No. of polymorphic bands
OPA02	TGCCGACGTG	70	13	12
OPA10	GTGATCGCAG	60	12	12
OPA15	TTCCGAACCC	60	10	9
Total	-	-	35	33

Table 2: Estimates of genetic variations: number and proportion of polymorphic loci and gene diversity in different potato cultivars

Cultivars	Polymorphic loci (No.)	Polymorphic loci (%)	Gene diversity
Ailsa	0	0	0
Cardinal	5	14.29	0.068
Diamant	10	28.57	0.138
Heera	6	17.14	0.075
Raja	18	51.43	0.217
TPS	19	54.29	0.245
All cultivars	33	94.29	0.338

Table 3: Intra-cultivar similarity indices (%) in the parentheses, inter-cultivar similarity indices (%) (above diagonal) and summary of Nei's (1972) genetic distance (below diagonal) values of the different cultivar pairs of potato

Cultivars	Ailsa	Cardinal	Diamant	Heera	Raja	TPS
Ailsa	(100.00)	75.32	68.10	84.32	70.96	67.90
Cardinal	0.333	(94.33)	65.30	72.00	68.90	74.46
Diamant	0.405	0.558	(89.03)	68.62	86.74	69.80
Heera	0.167	0.357	0.383	(94.27)	72.30	78.40
Raja	0.364	0.389	0.154	0.351	(79.03)	73.22
TPS	0.446	0.335	0.374	0.262	0.348	(78.46)

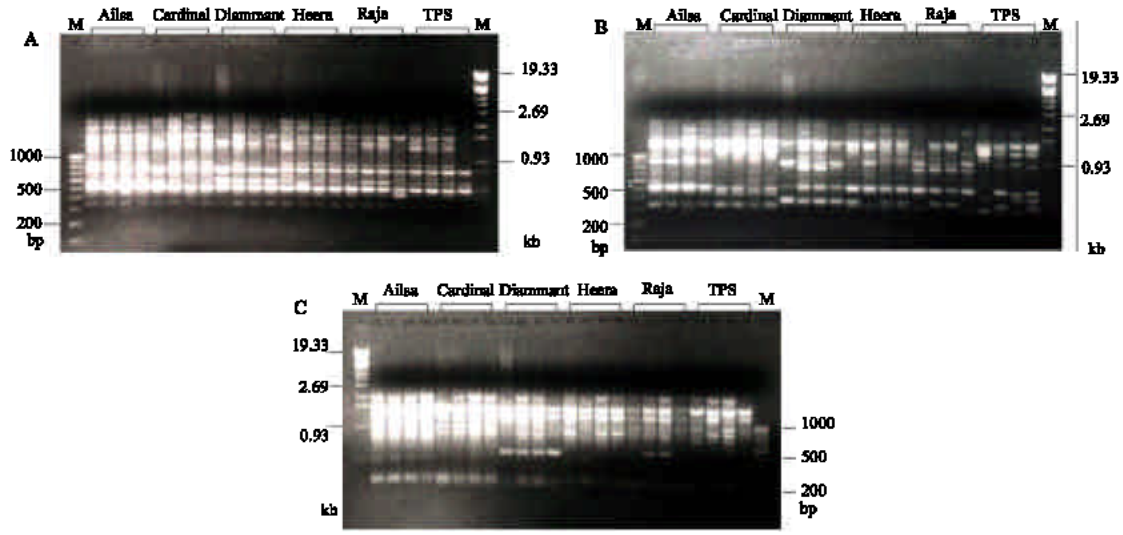

Fig. 1: RAPD profiles of six different potato cultivars using primers OPA02 (A), OPA10 (B) and OPA15 (C). Ms: Molecular weight markers (100 bp DNA ladder and lambda DNA EcoT 14 digest)

of bands. The banding patterns of six potato cultivars amplified by the three primers are shown in Fig. 1. The three primers yielded a total of 35 distinct bands of which 33 (94.29%) were considered as polymorphic (the frequency of the most common allele is 0.95 or lesser). The results of the preliminary RAPD analysis demonstrate that the RAPD method is capable of revealing nuclear DNA variation in potato cultivars. Like the present study, 57 potato cultivars with 28 polymorphic bands generated by 3 primers was discriminated (Forapani *et al*., 1999).

The intra-cultivar similarity indices (S_i) of different cultivar pairs ranged from 78.46 to 100% (Table 3). The S_i value for Ailsa was the highest (100%), which was followed by that of the Cardinal, Heera, Diamant, Raja and TPS cultivars, respectively. It was found that low level of similarity indices (ranging from 33 to 80%) among different pairs of 20 indian potato cultivars using 10 random primers compared to the present study and therefore, they found relatively high level of genetic variations among the cultivars (Chakrabarti *et al*., 2001). The inter-population

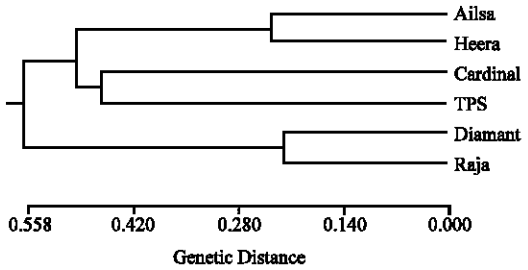

Fig. 2: UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between different cultivars of potato according to RAPD analysis

similarity indices (S_{ij}) were shown in the Table 3. The S_{ij} value for Diamant Vs Raja was higher (86.74%) than for all other between genotype comparisons. The lowest genetic similarity (65.30%) was observed between the Cardinal Vs Diamant genotypes.

The proportion of polymorphic loci and the gene diversity estimates were found to be high in the TPS, Raja and Diamant cultivars whereas the values for the Heera and the Cardinal were relatively low (Table 2). Ailsa cultivar showed no intra-cultivar genetic variability.

Cultivars showing higher intra-cultivar similarity and lower proportion of polymorphic loci are likely to have less heterozygosity i.e. possess lower level of genetic variation compared to those showing less intra-cultivar similarity and higher proportion of polymorphic loci. In other words, genotypes having higher similarity are more homogenous groups. Pair-wise comparison of DNA profile of the individuals within the Ailsa, Cardinal and Heera cultivars showed a high level of intra-cultivar similarity reflecting the lower genetic variability within each of those cultivars. On the other hand, relatively low level of intra-cultivar similarity indices for TPS and Raja indicated the higher genetic variation among individuals of the respective cultivars. The highest level of genetic variation studied in TPS cultivar could not be excluded as the cultivar was derived through hybridization.

Bandsharing based similarity indices were higher for intra-cultivar samples (average 89.19) than for all comparisons between cultivar samples (average 73.09). This implies that individuals within each cultivar are genetically more similar to each other, as is expected to be, than to individual from any other cultivar.

The D value for the Cardinal Vs Diamant cultivar pair was the highest (0.558) whilst the lowest D value (0.154) was observed between the Diamant and the Raja cultivars. Considering the genetic distance values, the results indicated that the cultivars were genetically different from each other (genetic distance value range from 0.15 to 0.56). Dendrogram based genetic distance (Nei, 1972) using UPGMA depicted the relationships among six cultivars of potato (Fig. 2). The lower level of overall gene flow (N_m = 0.289) and high level of cultivar differentiation (G_{ST} = 0.634) also supports the presence of sufficient polymorphisms in potato cultivars. However, information on pedigree of the six cultivars was not available as a result, it is difficult to make any comment on the causes of genetic variation and relatedness among the studied cultivars.

No information on genetic structure of the potato cultivars is available in Bangladesh. This is the first preliminary attempt to study genetic structure of potato cultivars in Bangladesh. Despite the fact that no cultivar specific marker was obtained in the study, the RAPD analysis discovered sufficient genetic variations among the potato cultivars. To obtain any cultivar specific markers, more primers should be used for larger samples in future.

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