Original Research Article

Molecular diversity analysis of indigenous potato cultivars using RAPD markers

ABSTRACT

The study was undertaken to identify genotypic diversity at molecular level of potato for varietal improvement program at the Advanced Plant Breeding Laboratory, Department of Genetics and Plant Breeding, Bongobondhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Eight cultivars of potato viz. Lalpakri, Sindurkouta, Indurkani, Ausha, Patnai, Sadaguti, Shilbilati, Challisha were collected from Bangladesh Agricultural Research Institute (BARI), for studying genotyping divergence. Genomic DNA was extracted from young leaves of the cultivars and PCR reaction was performed. The PCR amplified DNA profile was visualized on agarose gel, staining with ethidium bromide. Three RAPD primers named OPA 01, OPA 03 and OPD 18 were used to evaluate the genetic diversity of potato varieties. All the primers were polymorphic and the primers produced the highest number of alleles. The genetic diversity value in Lalpakri with all cultivars except Sadaguti was found to have the highest genetic distance (1.0). The amount of genetic diversity within potato germplasm is quite distinct as revealed by the genetic similarity coefficients. The results indicate that, high level of genetic distance exists among the cultivars. The Primers OPA 01, OPA 03 and OPD 18 showed the highest level of genetic diversity and PIC value while the Lalpakri and Sadaguti cultivars had the highest genetic distance among other cultivars which could be used for further potato breeding program.

Keywords: Cultivar, diversity, indigenous, potato and RAPD marker

1. INTRODUCTION

Hereditary decent variety of farming biodiversity gives species the capacity to adjust with changing condition and develop, by expanding their resilience to high temperature, dry spell and water-logging, just as their protection from specific sicknesses, vermin and parasites. This is especially critical in regards to environmental change. The development of agro-assorted variety, and our survival, for the most part relies upon this yields decent variety. The significance of tuber crops biodiversity includes socio-social, financial and ecological components. All trained tuber crops are come about because of human administration of biodiversity, which is always reacting to new difficulties to keep up and increment profitability under continually differing conditions. Under different tuber yields, for example, potato, sweet potato, aroids, cassava and yams are assuming vital job in nourishment security of Bangladesh [1].

Potato is really a worldwide yield and is the fourth real sustenance harvest of the world by rice, wheat and maize. In Bangladesh, potato is the third vital yield beside rice and wheat

however it is second just to rice as far as creation. Sub-atomic portrayal is an imperative biotechnological apparatus in plant rearing projects. This is on the grounds that atomic techniques uncover contrasts in genotypes, that is, in a definitive dimension of variety encapsulated by the DNA groupings of an individual and uninfluenced by condition.

RAPD markers have quickly picked up prominence to recognize polymorphism among various germplasms of potato [2]. By the advancement of a wide scope of atomic procedure, marker helped rearing is currently used to upgrade regular reproducing program for harvest improvement. Among the distinctive atomic markers RAPD method [3] is solid, quicker and simpler for misusing atomic decent variety investigation inside and among species and is an extremely helpful device in the investigation of biodiversity, hybridization, quality mapping and hereditary guide development [4]. RAPD system is being utilized effectively to recognize, portray and gauge hereditary dissimilarity of potato cultivars [5, 6, 7, 8, 9 and 10]. ID of hereditary assorted variety utilizing DNA markers in potato can give experiences into the hereditary structure and decent variety among assortments from various geological starting points. At the point when the size and nature of hereditary assorted variety is assessed ahead of time, a reasonable choice procedure is arranged by heritability of hereditary characteristics [11]. A blend of identification information and hereditary decent variety data from sub-atomic markers would in this manner upgrade the development of germplasm stocks and could be valuable apparatus in the estimation of hereditary separation of the potato genotypes. Thus, the present examination was embraced to analyze the molecular diversity of eight traditional potato cultivars through RAPD markers and to identify diverse genotypes for potato varietal improvement program.

2. MATERIALS AND METHOD

2.1 Sample collection

The examination was led at the Advanced Plant Breeding Laboratory, Department of Genetics and Plant Breeding, BSMRAU, Gazipur. Eight cultivars of potato viz. Lalpakri, SindurKouta, Indurkani, Ausha, Patnai, Sadaguti, Shilbilati and Challisha were gathered from Bangladesh Agricultural Research Institute (BARI), and were utilized as exploratory materials. New leaves gathered from plants were utilized as the wellspring of plant material.

2.2 RAPD markers

Three RAPD markers PA 01, OPA 03 and OPD 18 with clear amplifications were selected for genetic diversity analysis of eight potato cultivars [12].

2.3 DNA extraction

Genomic DNA was extracted according to modified CTAB (Cetyl trimethyl ammonium bromide) method portraited by Murray and Thampson [12]. Around 20g of potato leaf tissue was ground to an extremely fine powder by mortar and pestle. Around 20 mg new youthful delicate leaf was taken into Eppendorf tube and 700 µl extraction support and 100 µl 20% SDS arrangement was included. Hundred µl 5M NaCl and 100µl CTAB(10X) were included and hatched at 65^{o}C for 5 min. To expel any strong particles centrifugation were done at 12000 rpm for 5 min. At that point 900 µl chloroform: isomaylalcohol (24:1) were included and centrifuged at 12000 rpm for 5 min. After that 500 µl of isopropanol was included and

centrifugation were done at 13000 rpm for 30 min. The supernatant were flushed with 70% ethanol. At that point it was centrifuged again at 12000rpm for 5min. After that ethanol was disposed off and DNA pellets were dried. Finally, the pellets were suspended in 150µl 1XTBE cushion.

2.4 Polymerase Chain Reaction (PCR) amplification

DNA was extracted from leaves of potato cultivars. PCR was performed using three ten-mer RAPD markers (Cina Gene, Tehran, Iran). 25 µl mixture was prepared for the PCR reaction containing 2.5µl template DNA, 2.5 µl of 10X buffer, 2.5 µl of 2.5 mM dNTPs, 2 µl of 25 mM $MgCl_2$, 2 µl of each of the primers, and 0.25 µl of Taq polymerase. DNA Molecular Weight Marker (100 bp ladder; Roche) was used to estimate PCR fragment size. The PCR reaction was performed at 94°C for 5 min; then for 42 cycles of 94°C for 1 min; 36°C for 1 min; 72°C for 2 min followed by 72°C for 10 min. The resulting products were electrophoretically analyzed through 1% agarose gel stained with ethidium bromide (5 µg /ml) in TAE buffer. After staining in 0.5 µg/ml ethidium bromide solution, gels were photographed on a UV transilluminator.

Threeten-merrandom primers were initially screened for the presence of bands. Three primers (OPA 01, OPA 03 and OPD 18) were selected as they produced polymorphic and reproducible banding profiles.

2.5 RAPD data scoring and analysis

Since RAPD markers are overwhelming, it's a supposition that each band spoke to the phenotype at a solitary allelic locus [3]. Two sub-atomic weight markers, 1kb and 100 bp DNA stepping stool were utilized to assess the extent of the intensified items by looking at the separation gone by every piece with known estimated sections of sub-atomic weight markers. All the particular groups or pieces (RAPD markers) were there by given ID numbers as indicated by their on gel and scored outwardly based on their essence (1) or nonappearance (0), independently for every person and every groundwork. The scores acquired utilizing all preliminaries in the RAPD investigation were then pooled to make a solitary information framework. This was utilized to gauge Polymorphic loci, Nei's [13] quality decent variety. Hereditary separation and to build an UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populaces utilizing a PC program. Nei's hereditary separation was processed from frequencies of polymorphic markers to gauge hereditary connection between the concentrated 8 (eight) potato genotypes utilizing the Unweighted Pair Group Method of Arithmetic Means (UPGMA) [14].

3. RESULTS AND DISCUSSION

Molecular diversity and polymorphism studies in eight potato cultivars of Bangladesh were carried out through RAPD primers. All the three primers were polymorphic which made molecular distinctness among the studied potato genotypes.

A similarity matrix based on the proportion of shared RAPD fragments was used to establish the level of relatedness between potato varieties. Pair-wise estimates of similarity ranged from 0.00 to 0.500. The highest genetic similarity revealed in RAPD analysis was determined

between Sindurkouta and Indurkani (0.50), Sindurkouta and Patnai (0.50), Lalpakri and Sadaguti (0.50), Sindurkouta and Shilbilati (0.50), and Indurkani and Patnai (0.50). The amount of genetic similarity within potato ~~germplasm~~ cultivar is quite distinct as revealed by the genetic similarity coefficients.

Table 01. Similarity matrix of potato ~~genotypes~~ cultivars analyzed using Nei's original measures of genetic identity

Cultivars	Lalpakri	Sindurkouta	Indurkani	Ausha	Patnai	Sadaguti	Shilbilati	Challisha
Lalpakri	1	0.000	0.000	0.000	0.000	0.500	0.000	0.000
Sindurkouta		1	0.500	0.000	0.500	0.000	0.500	0.000
Indurkani			1	0.000	0.500	0.000	0.200	0.333
Ausha				1	0.333	0.000	0.000	0.000
Patnai					1	0.000	0.000	0.333
Sadaguti						1	0.000	0.000
Shilbilati							1	0.000
Challisha								1

The cultivars identification using RAPD markers were well documented in studies of molecular characterization [15]. Fingerprinting based on RAPD marker type was used for identification and characterization of potato cultivars in North America [16]. The genetic distance among the eight potato varieties are presented in Table 02. The highest Nei's genetic distance (1.000) was observed in Lalpakri with all cultivars except Sadaguti, Sindurkouta with Ausha, Sadaguti and Challisha, Indurkani with Ausha and Sadaguti, Ausha with Sadaguti, Shilbilati and Challisha~~.~~; Patnai with Sadaguti and Shilbilati~~.~~; Sadaguti with Shilbilati and Challisha~~.~~ and Shilbilati with Challisha.

Table 02. Distance matrix of potato ~~genotypes~~ cultivars analyzed using Nei's original measures of genetic identity

Cultivars	Lalpakri	Sindurkouta	Indurkani	Ausha	Patnai	Sadaguti	Shilbilati	Challisha
Lalpakri	0	1.000	1.000	1.000	1.000	0.500	1.000	1.000
Sindurkouta		0	0.500	1.000	0.800	1.000	0.500	1.000
Indurkani			0	1.000	0.500	1.000	0.800	0.667
Ausha				0	0.667	1.000	1.000	1.000
Patnai					0	1.000	1.000	0.667
Sadaguti						0	1.000	1.000
Shilbilati							0	1.000
Challisha								0

Table 03. Distribution of eight potato ~~genotypes~~ cultivars into three clusters

Cluster	Number of ~~genotypes~~ cultivars	~~Genotypes~~ cultivars
1	4	Sadaguti, Lalpakri and Shilbilati, Challisha

2	3	Indurkani, Sindurkouta and Patnai
3	1	Ausha

The results indicated that, low and high level of genetic distance exists between the ~~varieties~~ cultivars. The Primer OPA 01 showed the high level of genetic diversity among Sindurkouta, Indurkani, Patnai and Shilbilati. The Primer OPA 03 showed the high level of genetic diversity among Lalpakri, Sindurkouta, Indurkani and Shilbilati. The Primer OPD 18 showed the high level of genetic diversity among Lalpakri, Sindurkouta, Indurkani, Ausha, Patnai and Shilbilati (Figure 2). In case of Challisha cultivar primers used in this study OPA 01, OPA 03 and OPD 18 didn't show any band in gel electrophoresis.

3.1 Genetic distance-based analysis

A dendrogram based on Nei's [13] genetic distance using unmeasured pair group method of arithmetic mean (UPGMA) was established with 08 popular potato ~~varieties~~ cultivars (Figure 01). These ~~varieties~~ cultivars segregated into three clusters. The cluster-I was sub-divided into two sub groups. One sub group is divided into two sub-group. Sadaguti and Lalpakri are in one sub sub-group and Shilbilati is in another sub sub-group. Challisha is in another sub-group. Three ~~varieties~~ cultivars were clustered in Cluster II (Indurkani, Sindurkouta, Patnai) and one ~~variety~~ cultivar was clustered in cluster III (Ausha).

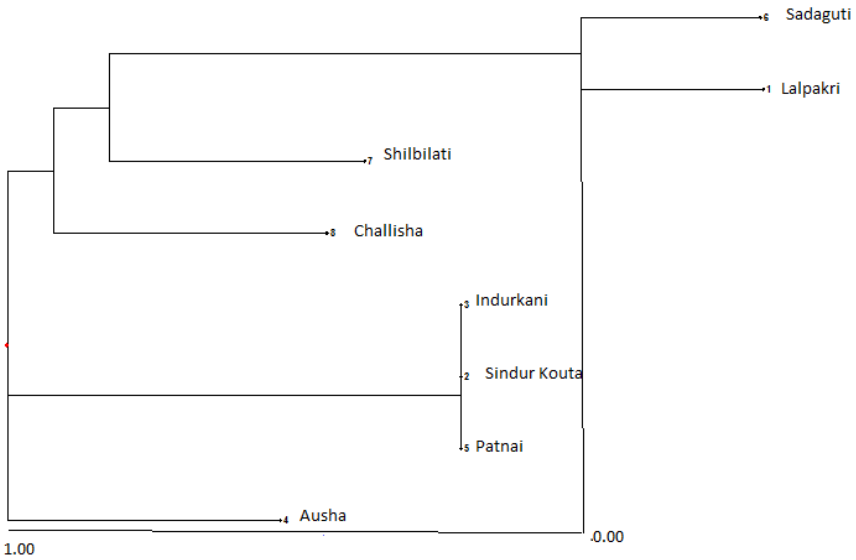

Figure 1. UPGMA cluster dendrogram showing the genetic relationship among eight potato ~~genotypes~~ cultivars based on the alleles detected by three RAPD markers

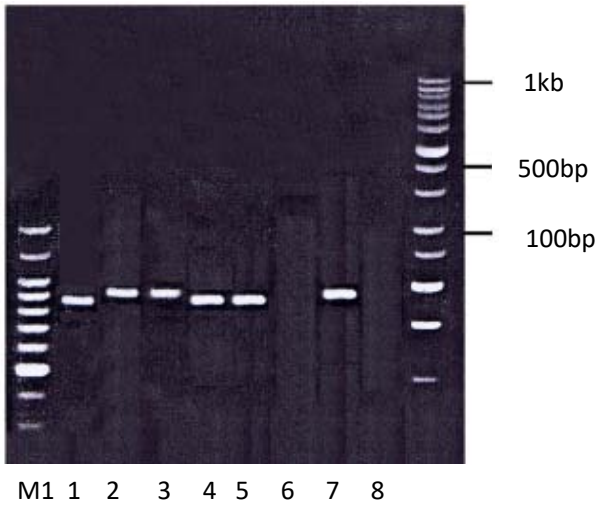

Figure 2. RAPD profile of eight potato ~~varieties~~ cultivars using primer OPD 18 Lane: 1. Lalpakri; 2. Sindurkouta 3. Indurkani; 4. Ausha; 5. Patnai; 6. Sadaguti; 7. Shilbilati; 8. Challisha; M1= Molecular marker 100bp and 1kb (Promega, USA)

4. CONCLUSIONS

From this study, the following conclusion may be drawn:

- High level of genetic diversity was observed among the traditional potato cultivars under study except Challisha.
- The cultivars Lalpakri and Sadaguti showed the highest genetic diversity.

The following recommendations may be given based on the results:

- Lalpakri and Sadaguti cultivears can be utilized for further potato varietal improvement program of Bangladesh.
- As Challisha didn't respond to the primers used in the current study it is highly recommended to use other primers for its molecular study.

REFERENCES

1. Kawochar MA, Mohanta HC, Hossain MA, Goswami BK, Uddin MJ. Diversity of Tuber Crops in Bangadesh. 2014; 53.
2. Polzerova H. The use of molecular genetic tecniques to potato variety identification. *Rostlinna Výroba.* 2001; 47(11): 78-87.
3. Williams JGK, Kubelik KJ, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphism amplified by arbitrary primers are useful genetic markers. Nucleic Acids Research. 1990;18:6531-6535.
4. Sharma K, Mishra AK, Misra RS. A simple and efficient method for extraction of genomic DNA from tropical tuber crops. African Journal of Biotechnology. 2008;7(8):1018-1022.

5. Miller JT, Spooner D. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (*Solanum sp*. Sect. Petota): molecular data. Plant Systematic and Evolution. 1999;214:103-130.
6. Isenegger DA, Taylor PJ, Ford R, Franz P, Mcgregor GR. DNA fingerprinting and genetic relationships of potato cultivars (*Solanum tuberosum* L.) commercially grown in Australia. Australian Journal of Agricultural Research. 2001;52:911-918.
7. Moisan TM, Hingrat YL, Kerlan MC. Potato cultivars identification using molecular markers. Acta Horticulturae. 2001;546:471-477.
8. Sun GG, Wang-pruski M, Mayich M, De jong H. RAPD and pedigree-based genetic diversity estimates in cultivated diploid potato hybrids. Theoretical and Applied Genetics. 2003;107:110-115.
9. Spooner DM, McLean KG, Fau–Ramsay K R, Ramsay G. A single domestication for potato based on multi-locus amplified fragment length. Proceedings of the National Academy of Sciences of the United States of America. 2005;102:1494-1499.
10. Rocha AE, Luciano VP, Humberto HC and Claudia TG, Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. Crop Breeding and Applied Biotechnology. 2010;10:1984-7033.
11. Ghislain MD, Andrade F, Rodríguez RJ, Hijmans, Spooner, DM. Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja group using RAPDs and nuclear SSRs. Theoretical *and* Applied Genetics. 2006;113:1515-1527.
12. Das AB, Mohanty IC, Mahapatra D, Mohanty S, Ray A. Genetic variation of Indian potato (*Solanum tuberosum L.)* genotypes using chromosomal and RAPD markers. Crop Breeding and Applied Biotechnology. 2010;10:238-246.
13. Murray MG, Thampson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 1980;8:4321-4325.
14. Nei M. Genetic distance between populations. The American Naturalist. 1972;106: 283-292.
15. Sneath PHA, Sokal RR. Numerical taxonomy: the principles and practice of numerical classification. San Francisco: Freeman. 1973;573.
16. Bianchi VJ, Fachinello JC, Schuch MW. RAPDs na caraterizacao geneticomolecular eno estudo da variabilidade genetica de cultivares de ameixeira. *Revista Brasileira de Fruticultura*. 2003;25:272-274.
17. Sosinski B. Douches DS. Using polymerase Chain reaction based DNA amplification to fingerprint North America potato Cultivars. Horticultural Science. 1996;31:130-133.