SAARC J. Agri., 11(2): 95-102 (2013)

MOLECULAR DIVERSITY ANALYSIS IN POTATO (*Solanum tuberosum* L.) THROUGH RAPD MARKERS

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) markers were used to study the molecular diversity of 12 popular potato varieties in Bangladesh. DNA was extracted from tender leaf sample for PCR amplification. The PCR amplified DNA profile was visualized on 2% agarose gel, staining with ethidium bromide. Eight RAPD primers were used to evaluate the genetic diversity of potato varieties. Some total of 36 DNA fragments were amplified and out of them 24 were polymorphic. Those primers generated 61.53% of polymorphic DNA band. The primer OPX 04 produced highest (9) number of DNA band and out of 9 amplicon 6 were polymorphic. Lowest number of amplification was observed in the primer OPA-17 and it was only 3. The highest Nei's genetic distance (0.9701) was noticed between the variety Granola and Provinto. The highest (0.8205) number of genetic identity/similarity was observed between the varieties Cardinal and Diamant. The unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's genetic distance revealed that the 12 varieties followed into two main clusters. The present finding showed that there was high level of genetic diversity among the varieties which can be used for parental selection in potato breeding program.

Key words: Molecular diversity, RAPD, potato.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a highly heterogeneous and vegetatively propagated crop. It is one of the important food crops of Bangladesh as well as in many other countries of the word. It produces more calories and protein per unit of land with minimum time than any other field crops (Upadhya, 1995). Because of its high yield potential and food value as compared to rice and wheat, it is cnsidered as a promising candidate crop for feeding the hungry people of the world (Pushkarnath,

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Received: 09.04.2013

1976). The yield level of potato in Bangladesh is lower than other potato growing countries of the world (BBS, 2010). The use of local seed and traditional varieties are the major constrains of low yield in potato. Development of high yielding varieties having good keeping quality is one of the challenges for potato breeders. Genetic variability has been considered as is prerequisite for crop improvement program. The quantification of genetic diversity made it possible to select diverse parents for successful hybridization program. In recent years, several molecular markers had been used to identify and assess the genetic diversity and phylogenies relationship in plant. The traditional methods based on morphological traits require more time, cost expensive and has large effect on environment. By the development of a wide range of molecular technique, marker assisted breeding is now used to enhance conventional breeding program for crop improvement. Among the different molecular markers RAPD technique (Williams et al. 1990) is reliable, faster and easier for exploiting molecular diversity analysis within and among species. RAPD markers have been widely used for identification of genetic relationship among cultivars (Tosti and Nejri, 2002). Hence, the present investigation was undertaken for molecular diversity analysis of some released potato varieties through RAPD markers and to identify the divergence genotypes for potato improvement program.

MATERIALS AND METHODS

The experiments were carried out at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. Twelve popular released potato varieties were collected from Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur and used as experimental materials. The potato varieties were Cardinal, Diamant, Granola, Asterix, Sagita, Courrage, Lady Rosetta, Felsina, Multa, Provinto, Petronige and TPS-1.

Seedling raising: Good quality, disease free, healthy tuber (breeder stock) were sown in plastic pots and kept in nets house. All management practices were done for raising quality seedlings from those materials. Fresh leaves were collected at 3-4 leaf stage of plant for isolation of DNA.

Eetraction and quantification of DNA: Total genomic DNA from each variety was isolated by CTAB method with slight modification according to Frey et al., (2004). The extracted DNA was purified by propanol and treated with 10µg/ml RNase A for 20-25 min at 37^{o}C to remove the RNA. The purified DNA was dissolved in TE buffer and quantification of DNA was done through electrophoresis on 1% agarose gel staining by ethidium bromide. The sample DNA was stored at -20^{o}C freezer for further use.

Primer selection and PCR amplification: Seventeen RAPD primers were selected on the basis of previous works to evaluate the molecular polymorphism among the potato varieties. PCR reaction was performed using BIONEER KIT

(korea). The PCR reaction having 20.0 µl mixture containing 3.0 µl sterile deionized water, l0X PCR buffer 4.0 µl, enzyme dilution buffer 4.0 µl, 20 mM $MgCl_2$, 3.0 µl dNTPs (10mM) 1.0 µl top DNA polymerase 0.5 µl, primer 2.5 µl and sample DNA (approx. 40-50 ng) 2.0 µl. The reaction mixture was subjected to the following thermal profile for amplification in a thermocycler : 5 min at 95^{o}C for initial denaturation, followed by 33 cycle of 1.10 min denaturation at 94^{o}C, 1.0 min at annealing and 1.30 min at 72^{o}C for extension. A final extension step was done at 72^{o}C for 7 min. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 2.0% agarose gel and amplified fragments were visualized by staining with ethidium bromide. The amplified bands were scored as present (1) and absent (0) for each primer. The score of bands were pooled to create a single data matrix. These were used to estimate polymorphic loci. Genetic distance and identity were calculated based on Nei's (1972)**.** Phylogenetic tree and dendrogram were established based on an unpaired group method of arithmetic means (UPGMA) using the software POPGENE (Version 1.31) (Yeh et al., 1999).

RESULTS AND DISCUSSION

Molecular diversity and polymorphism studies in 12 potato varieties of Bangladesh was done through RAPD primer. Seventeen RAPD Primers (10-mer) were initially screened on 12 popular potato varieties for their ability to amplify polymoric fragment of DNA. Out of them only eight primers viz. OPA-17, OPG-17, OPJ-13, OPP-12, OPX-01, OPX-04 and OPX-07 showed distinct polymeric DNA profiles. Some total of 39 bands were obtained from these primers with an average of 4.87 bands per primer. Among the amplified product 24 polymorphic DNA bands were observed. The polymorphic DNA fragments ranged from 2-6 in different RAPD oligomer. It was observed that the primer OPX-04 product had highest (9) number of polymorphic DNA band and it was lowest (2) in OPA-17 and OPX-07 primers. The percent of polymorphic DNA fragment was 61.53 under this present investigation (Table 2). The maximum DNA fragment was generated by the primer OPX-04 and it was minimum (3) in OPA-17. The DNA profile of 12 potato varieties using OPX-04, OPX-07 and OPX-17 primers are shown in figure 1 and 2, respectively. The number of polymorphic bands was considered appropriate to assess the genetic divergence of potato genotypes. It might be due to more amount of GC content (60-70%) of the primers used in this study. Fukuoka et al. (1992) observed an increased number of bands with increasing GC content of the primer. The explanation for the correlation between GC content and the number of bands may be the stability of base complementation of A with T. The amplified DNA profiling was scored according to the presence and absence of bands. Absence of bands might be failure of primers to anneal at a binding site in some genotypes due to nucleotide sequence differences or may be insertion or deletions of primer binding site. Rocha et al. (2010) reported on genetic diversity in potato cultivar by RAPD and SSR markers. They notice that, genomic DNA of 16 potato cultivars was amplified with 25 RAPD primers that

generated 92 polymorphic bands. The cultivar identification using RAPD markers is well documented in studies of molecular characterization (Bianchi et al, 2003). Fingerprinting based on RAPD marker type was used for identification and characterization of potato cultivars in North America (Sosinski and Donches, 1996). The genetic identity and genetic distance among the 12 potato varieties are presented in table 2. The Nei's genetic identity was the highest (0.82050) in the varietal pair Cardinal and Diamant and it was the lowest (0.333) in Provinto and Granola. The highest Nei's genetic distance (0.970) was noticed between Granola and Provinto. It was the lowest (0.137) in two different varietal pair viz, (a) Petronige and Provinto (b) Courrage and Provinto, respectively. However, high levels of genetic distance were also noticed in the varietal pairs: Lady Rossetta and Cordage (0.955), Provinto and Lady Rossetta (0.893), Courrage and Granola (0.8910). A dendrogram based on Nei's (1972) genetic distance using unmeasured pair group method of arithmetic mean (UPGMA) was established with 12 popular potato varieties (Figure 3). These varieties segregated into two main clusters. The variety Granola and Sagita were into one cluster and rest of the materials were in cluster-II. The cluster -II was sub-divided into two sub groups. Seven varieties were clustered in one sub-group and three varieties were clustered in second sub-group. Those sub-groups were further segregated in different sub-sub cluster group on the basis of their identity. The results indicated that, low and high level genetic distance exists between the varieties. The variety Granola, Provinto, Lady Rossetta and Courrage showed highest level of genetic diversity which can be used for further potato breeding program. Sawy et al. (2007) reported that, RAPD technique can be successfully applied to determine the genetic fidelity of potato plant. A limited study has been made on genetic divergence in potato either at tetraploid (Gaur et al. 1978 and Sidhu et al., 1981 or at diploid level (Grag 1988). Mondal (2007) reported that an understanding of the nature and magnitude of variability among the genetic stock is of prime importance to the breeders. Hence, it is important to analyze the genetic variability of parental materials. Molecular based analysis of present finding can provide information on actual genetic diversity among the potato cultivars.

CONCLUSION

Information on the genetic diversity allows to assist the parent selection and paving the way to genetic gains. The results of present study revealed the existence of high level of genetic diversity among the studied 12 popular widely grown potato varieties in Bangladesh. These varieties can further be used as parental material for fixation of heterosis in potato improvement program.

ACKNOWLEDGEMENT

The authors expressed deepest sense of appreciation and heartfelt thanks to Bangladesh Academy of Sciences (BAS) and United State Department of Agriculture (USDA) for financial support to conduct the research.

PCR Amplification with RAPD Primer OPX-07

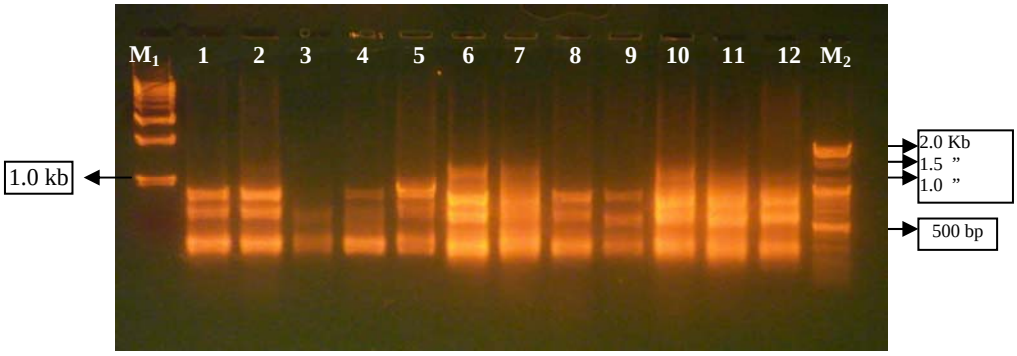

Figure 1: RAPD profile of 12 potato varieties using primer OPX-07. Lane: 1. Cardinal; 2. Diamant; 3. Granola; 4. Lady Rossetta; 5. Sagita; 6. Courrage; 7. Asterix; 8. Felsina; 9. Multa; 10. Provinto; 11. Petronige; 12. T.P.S. 1; M_1= Molecular marker 1kb (B.G. Nei, India) and M_2=100bp (Bioneer, Korea)

PCR Amplification with RAPD Primer OPX-04

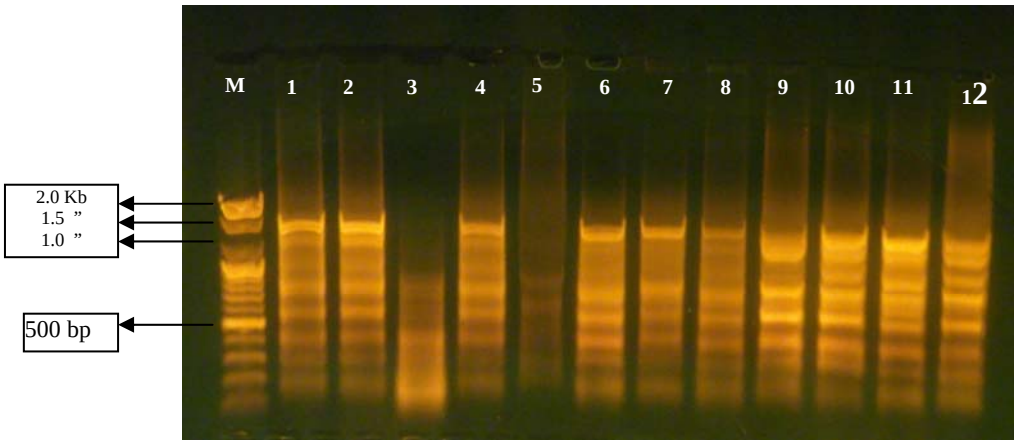

Figure 2: RAPD profile of 12 potato varieties using primer OPX-04. Lane: 1.Cardinal; 2. Diamant; 3. Granola; 4. Lady Rossetta; 5. Sagita; 6. Courrage; 7. Asterix; 8. Felsina;9. Multa; 10. Provinto; 11. Petronige; 12. T.P.S. 1; M= Molecular marker 100bp (Bioneer, Korea)

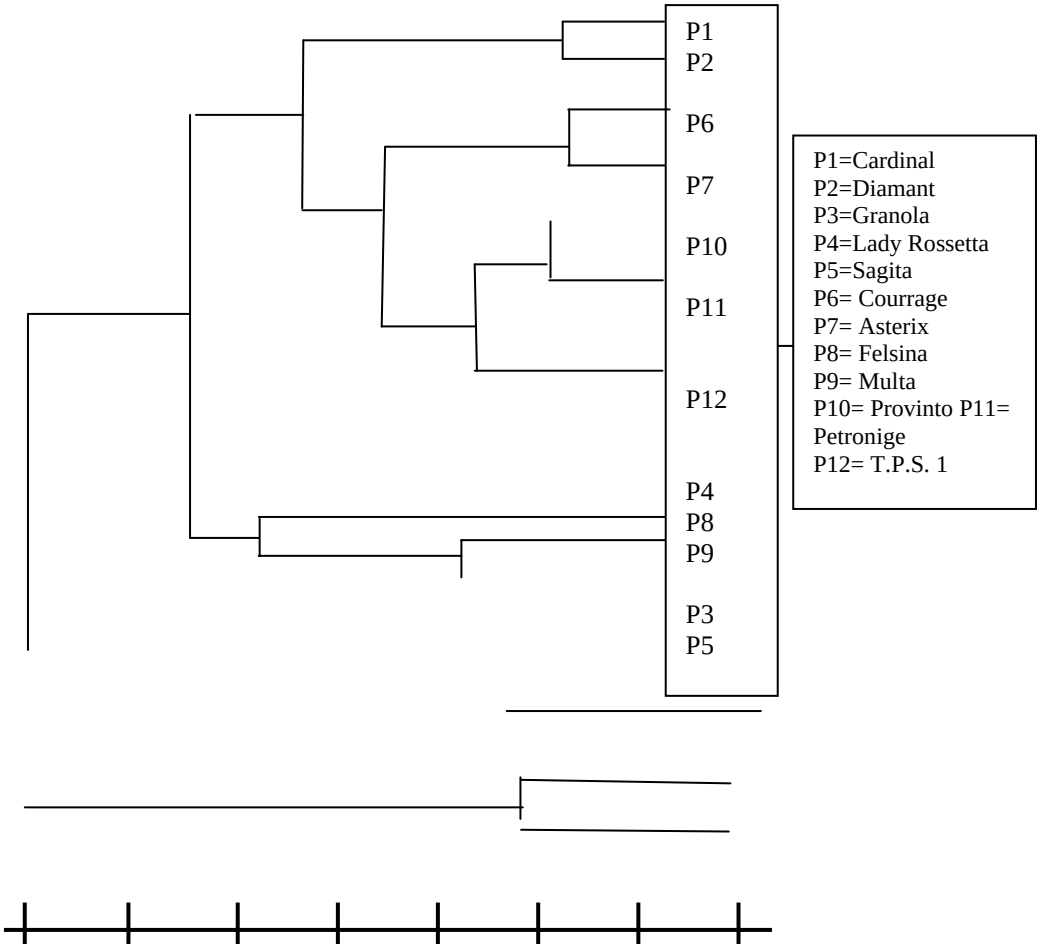

Figure 3: UPGMA dendrogram based on Nei's (1972) genetic distance, between 12 potato varieties according to RAPD analysis.

Table 1: Number and percentage of polymorphic loci obtained in 12 potato varieties

Name of RAPD primer	Sequence of the primer	GC content (%)	No. of bands scored	Size ranges (bp) observed	No. of polymorphic bands	Percentage of polymorphic loci
OPA17	GACCGCTTGT	60	3	278-735	2	66.66
OPG17	ACGACCGACA	60	6	249-1225	3	50.0
OPJ13	CCACACTACC	60	5	295-1491	4	80.0
OPP12	AAGGGCGAGT	60	5	400-1264	3	60.0
OPX01	CTGGGCACGA	70	7	144-934	4	57.14
OPX04	CCGCTACCGA	70	9	198-1579	6	66.66
OPX07	GAGCGAGGCT	70	4	301-1272	2	50.00
Total	-	-	39	-	24	61.53

Table 2: Genetic identity (above diagonal) and genetic distance (below diagonal) values among the twelve potato varieties

	Cardinal	Diamant	Granula	Asterix	Lady Rossetta	Courrage	Sagita	Felsina	Multa	Provinto	Petronige	TPS1
Cardinal	-	0.8205	0.5897	0.6154	0.5641	0.7692	0.6923	0.6410	0.5641	0.7436	0.6667	0.7692
Diamant	0.1978	-	0.6667	0.7436	0.5897	0.6410	0.5641	0.4615	0.5385	0.6667	0.7436	0.6923
Granula	0.5281	0.4055	-	0.6154	0.7692	0.4103	0.4359	0.5385	0.5641	0.3333	0.4615	0.4615
Asterix	0.4855	0.2963	0.4855	-	0.5897	0.5897	0.5641	0.6154	0.6923	0.6154	0.7436	0.6410
L.Rossetta	0.5725	0.5281	0.2624	0.5281	-	0.3846	0.4615	0.6667	0.6923	0.3590	0.4872	0.5385
Courrage	0.2624	0.4447	0.8910	0.5281	0.9555	-	0.8718	0.6154	0.5897	0.8718	0.7436	0.6923
Sagita	0.3677	0.5725	0.8303	0.5725	0.7732	0.1372	-	0.7436	0.6154	0.8462	0.7179	0.6667
Felsina	0.4447	0.7732	0.6190	0.4855	0.4055	0.4855	0.2963	-	0.7692	0.6410	0.5641	0.6667
Multa	0.5725	0.6190	0.5725	0.3677	0.3677	0.5281	0.4855	0.2624	-	0.6667	0.7436	0.6923
Provinto	0.2963	0.4055	0.9701	0.4855	0.8931	0.1372	0.1671	0.4447	0.4055	-	0.8718	0.8205
Petronige	0.4055	0.2963	0.7732	0.2963	0.7191	0.2963	0.3314	0.5725	0.2963	0.1372	-	0.7949
TPS1	0.2624	0.3677	0.7732	0.4447	0.6190	0.3677	0.4055	0.4055	0.3677	0.1978	0.2296	-

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