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Molecular Diversity Analysis of Somaclonal Variants of Potato (*Solanum tuberosum* L.) by Random Amplified Polymorphic DNA Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Author MS assisted in designing the experiment, conducted the experimental work, collected, analyzed and interpreted the data and drafted the manuscript. Author MSI assisted in conducting the experiment. Author DKB took part in preparing and critical checking of this manuscript. Author MEH designed the experiment and supervised throughout the experimental work. All the authors finally approved for publication of the article.

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ABSTRACT

Aims: An experiment was conducted to analyze the DNA fingerprinting and genetic diversity of nine potato (*Solanum tuberosum* L.) somaclonal variants and three check varieties.
Place and Duration of Study: The experiment was carried out at the Biotechnology laboratory of the Department of Biotechnology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during November, 2013 to December, 2014.
Methodology: The somaclonal variants investigated were SIP-3, SIP-5, SVP-6, SVP-18, SVP-19,

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SVP-25, SVP-55, SVP-56 and SVP-68, while the check varieties were Cardinal, Diamant and Asterix. Six RAPD primers were used to perform PCR reaction after genomic DNA was extracted from young leaves.

Results: The selected 6 primers produced total 54 distinct and differential amplified DNA bands of size range 88 bp to 3265 bp, where 47 bands (~87%) were polymorphic and 7 bands (~13%) were monomorphic. The pair-wise inter-genotype similarity indices were ranged from 61.59% to 93.55% with an average of 74.31%. The Nei's genetic distance among 12 potato genotypes was estimated from 0.0972 to 0.6217 whereas, genetic identity was between 0.5370 and 0.9074. Here, the distantly linked accessions among the somaclonal variations with check varieties were SVP-6 (to Cardinal and Diamant) and SVP-25 (to Asterix). In addition, the UPGMA dendrogram segregated the 12 potato genotypes into two broad clusters containing 8 and 4 genotypes, respectively. Furthermore, the dendrogram also displayed the highest genetic distance between SVP-6 vs SVP-68 genotype pair.

Conclusion: Significant relationship and diversity were found among the studied 12 genotypes. This genetic diversity among the potato genotypes would be utilized for further potato improvement.

Keywords: DNA fingerprinting; genetic distance; molecular characterization; molecular marker; PCR; RAPD; somaclonal variation.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important members of family Solanaceae and genus *Solanum* [1]. More than 4000 edible potato varieties are cultivated throughout the world whereas, 27 local and 90 BARI released potato varieties are grown in Bangladesh [2]. The yield rate of potato in Bangladesh is too low when compared with that of other leading countries even in case of high yielding or modern varieties [3].

Mainly, genetic impurity, susceptible to different diseases and pest, use of traditional varieties and environmental differences are the vital causes of lower yield of potato in Bangladesh [4]. These problems can be solved by developing high yielding varieties having others good qualities like resistant to insect and diseases and/or improving the local varieties for higher yield [5].

Potato has a narrow genetic base which hampers its improvement in respect of disease resistance and other agronomic traits through conventional breeding [6,7]. Commercial cultivation of potato from true potato seeds (botanical seeds) is not possible as it is highly heterozygous and autotetraploid in nature and shows poor germination rate and higher variation in segregate generations, which is the main limitation for potato improvement in the conventional method [8,9]. Moreover, conventional breeding is time consuming, expensive, laborious and can be affected by inbreeding depression. Somatic embryogenesis, somaclonal variation and potato tissue culture make these challenges easy for the breeders [10,11]. So, instead of conventional crop improvement procedure, somaclonal variations can be used widely for potato. There are several examples of using somaclonal variation to create desirable characteristics and successful transmission of them to the progeny crops [12].

Sometimes, the term "tissue or culture-induced variation" is used instead of somaclonal variation. When genetic variation is observed within the *In vitro* culture regenerated plants, then it is termedas somaclonal variation [13]. Somaclonal variation method is a good alternative to conventional crop improvement procedure since the desirable variant characteristics obtained through it is successfully transmitted to the progeny [10,13].

To select the desirable progeny, somaclonal variations detection is very important as both useful and unfavorable traits can be produced through this process [14]. Somaclonal variations as well as other genetic variation can be detected by morphological, biochemical, cytological or molecular marker methods but nowadays, molecular markers are widely used as they are stable, relatively more informative, simple, quick, less laborious and not affected by environment [15,16]. Numerous molecular markers viz. random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat (ISSR) markers have been used to characterize potato and as well as to analyze genetic diversity and somaclonal variation [10]. While, using RAPD

markers has several advantages since RAPD markers are usually dominant in nature, they are technically easy and fast to perform with a little amount of DNA, and radioactive labelling as well as previous genomic information is not needed. Furthermore, this technique is reliable and comparatively inexpensive too [17,18].

Several scientists have been used RAPD technique successfully to analyze somaclonal variations in different plant species, for example banana, chili, pepper, ginger, maize, sugarcane, tomato and potato [16].

In Bangladesh, molecular data on potato is very limited and there is no reported work on molecular characterization and diversity analysis of potato somaclonal variants till date. Hence, it is essential to develop and accumulate molecular data on potato somaclonal variation.

Therefore, the present study has been undertaken to estimate the genetic diversity and relation among some somaclonal potato genotypes and their standard check varieties.

2. MATERIALS AND METHODS

2.1 Name and Sources of Plant Materials

Twelve potato genotypes were used in this research. Among these Cardinal, Diamant and Asterix are popular varieties in Bangladesh, collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. Other genotypes i.e., SIP-3, SIP-5 and SVP series (SVP-6, SVP-18, SVP-19, SVP-25, SVP-55, SVP-56 and SVP-68) are somaclonal variant potatoes, which were generated in the "Laboratory of Biotechnology, Sher-e-Bangla Agricultural University (SAU)". In addition, the source materials for the somaclonal variants were Cardinal, Diamant and Asterix.

2.2 Extraction of Genomic DNA

The source of genomic DNA was fresh and young potato leaves which were collected at 3-4 leaf stage of each genotype. Genomic DNA was extracted from the leaf samples following minor modified protocol of Phenol-Chloroform-Isoamyl alcohol method of DNA extraction described by Sultana et al. [19] in their study.

2.3 DNA Confirmation and Quantification

To confirm the quality of extracted DNA, electrophoresis was conducted after loading DNA from each sample on 1% agarose gel and placing the gel in the gel chamber (Continental Lab product. Inc.) containing 1X TBE buffer. Better quality band showing DNA samples were taken for quantification and working solution preparation. Finally, a spectrophotometer was used to determine the quantity of DNA.

2.4 Primer Selection

Nine decamer RAPD primers viz. OPA-18, OPA-20, OPB-04, OPB-06, OPB-08, OPC-01, OPD-02, OPF-08 and OPW-01 (Operon Technologies, Inc., Alameda, California, USA) were screened for PCR reaction on 12 genotypes of potato.

2.5 PCR Reaction and Thermal Profile

PCR reactions were performed using 2X Taq Mastermix (GeneON, Germany). The composition of PCR reaction was as follows: 2X Taq Master Mix = 12.5 µL; RAPD primer = 2.5 µL; sterile deionized water = 7.5 µL; Genomic DNA (25 ng/ µl) 2.5 µL and total reaction volume = 25 µL.

An oil-free thermal cycler (Esco Technologies Swift™ Mini Thermal Cyclers) was used to perform DNA amplification. PCR reaction was conducted following pre-denaturation at 95℃ for 5 minutes, then denaturation at 95℃ for 45 seconds, annealing at 30℃ for 30 seconds and elongation or extension at 72℃ for 1 minute and run for 33 cycles. Finally, complete extension of all amplified fragment was done at 72℃ for 5 minutes.

2.6 Electrophoresis of the Amplified PCR Products

PCR products of each sample were visualized and confirmed by running on 1.5% agarose gel (containing 1 µL of 10 mg/L ethidium bromide) in 1X TBE buffer in gel electrophoresis at 85V for 50 minutes. Two molecular weight markers 100 bp (BIONEER, Cat. No. D-1030, South Korea) and 1kb (BIONEER, Cat. No. D-1040, South Korea) DNA ladder was also loaded on the left and right side of the gel, respectively.

2.7 Amplified DNA Samples Documentation

After completing the electrophoresis, the gel was gently removed from the gel chamber. Then, to check the DNA amplification (observed as a

band) the gel was put on a high-performance ultraviolet light box (UV transilluminator) and a 'Gel Cam Polaroid' camera was used to take photograph.

2.8 RAPD Bands Scoring and Data Analysis

All the RAPD bands were scored manually for their visualization. Each primer on each genotype was scored as one decimal number for the presence of the band and zero (0) for the absence of the band. A computer program namely DNAfrag (version 3.03) [20] was used to calculate the sizes of the band length.

Matrix data was created by pooling all the score counted from each RAPD primer, and used to estimate Genetic distance (GD), polymorphic loci, Nie's [21] gene diversity, frequencies of polymorphism were also used to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among genotypes investigated, with the help of a computer software namely POPGENE (version 1.31) [22]. The homogeneity in different locus between genotype pairs was tested using the same program.

The genetic similarity values using the formula described by Lynch [23]: Similarity index $(SI)_{=} \frac{2Nxy}{Nx+Ny}$

Where, Nxy is the number of RAPD bands shared by individuals x and y respectively, and Nx and Ny are the number of bands in individuals x and y, respectively.

3. RESULTS AND DISCUSSION

3.1 RAPD Banding Pattern with Their Size and Polymorphism

Among 9 RAPD primers screened/pretested, 6 primers were selected for further amplification (Fig. 1) on the basis of their ability to amplify polymorphic patterns.

The selected six primers were OPA-18, OPB-08, OPC-01, OPD-02, OPF-08 and OPW-01 (Table 1). Each of the primer produced separate RAPD patterns (bands) in 12 potato genotypes (Figs. 2-5). These 6 primers generated total 54 effective bands i.e. average 9 bands per primer and 4.5 bands per genotypes. The DNA fragments size were from 88 to 3265 bp. The maximum number (13) of bands were produced by the primer OPF-08 followed by OPB-08 (11) and OPD-02 (9). Whereas, the primer OPC-01 and OPW-01 produced same number (8) of bands and OPA-18 produced the least number (5) of bands. Out of total 54 bands, 47 bands showed polymorphic amplification and rest of the DNA fragments were monomorphic. The result gave an average of 7.83 polymorphic and 1.16 monomorphic bands per primer and 3.9 polymorphic bands per genotype. Meanwhile, the primer OPC-01 amplified the highest percentage of (100%) polymorphic bands, followed by OPF-08 (92.31%), OPB-08 (90.91%), OPD-02 (88.89%), OPW-01 (75.0%) and least polymorphic bands was produced by OPA-18 (60%).

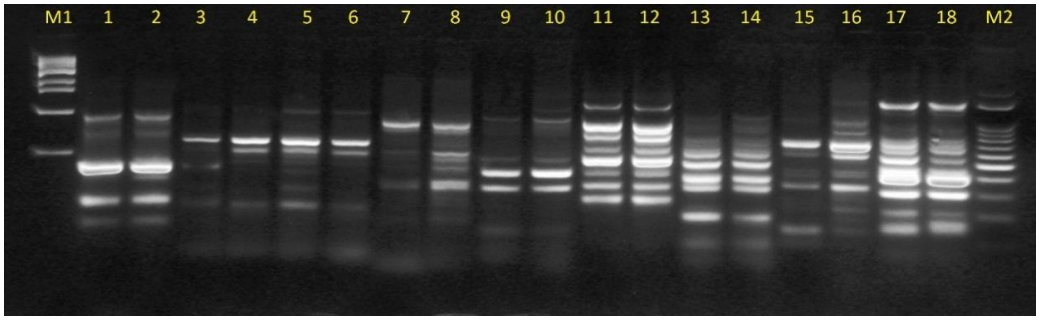

Fig. 1. Primer test: Amplified PCR products of 9 decamer RAPD primers using DNA of randomly selected two genotypes

(SL# 1-2: OPA-18; SL# 3-4: OPA-20; SL# 5-6: OPB-04; SL# 7-8: OPB-06; SL# 9-10: OPB-08; SL# 11-12: OPC-01, SL# 13-14: OPD-02; SL# 15-16: OPF-08 and SL# 17-18: OPW-01. M1= 1kb ladder and M2= 100bp ladder; BIONEER, South Korea)

Table 1. Features of RAPD primers and their amplification result in 12 potato genotypes

Primer codes	Sequences (5′- 3′)	GC-content (%)	Total amplified band numbers	Size ranges of the scored bands (bp)	No. of polymerphic bands	Polymorphic loci (%)
OPA-18	AGGTGACCGT	60	05	155-1040	03	60.00
OPB-08	GTCCACACGG	70	11	193-1868	10	90.91
OPC-01	TTCGAGCCAG	60	08	201-1172	08	100.00
OPD-02	GGACCCAACC	70	09	194-3265	08	88.89
OPF-08	GGGATATCGG	60	13	120-2007	12	92.31
OPW-01	CTCAGTGTCC	60	08	88-827	06	75.00
Total	-	380	54	-	47	-
Average	-	63.33	9.0	-	7.83	87.04

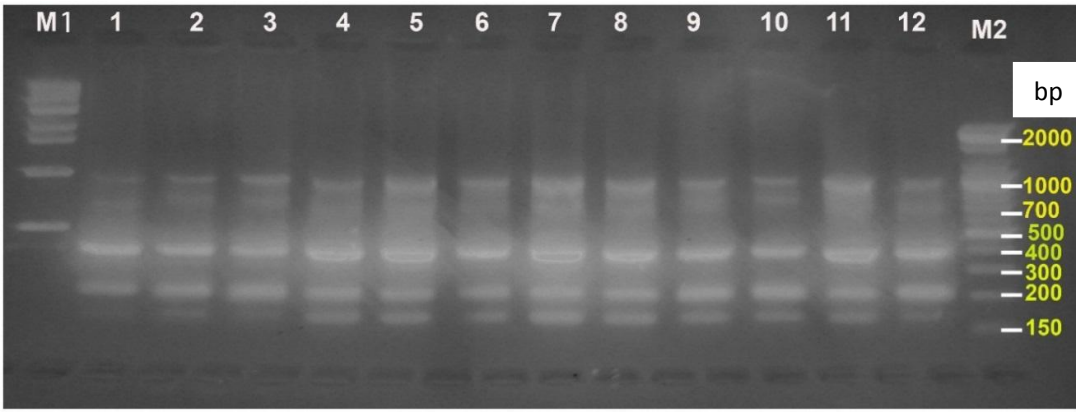

Fig. 2. Amplified DNA pattern of 12 potato genotypes generated by primer OPA-18
(SL# 1: SIP-3; SL# 2: SIP-5; SL# 3: SVP-6; SL# 4: SVP-18; SL# 5: SVP-19; SL# 6: SVP-25; SL# 7: SVP-55; SL# 8: SVP-56; SL# 9: SVP-68; SL# 10: Cardinal; SL# 11: Diamant and SL# 12: Asterix. M1 and M2: Molecular weight marker (1 kb and 100 bp, respectively; BIONEER, South Korea)

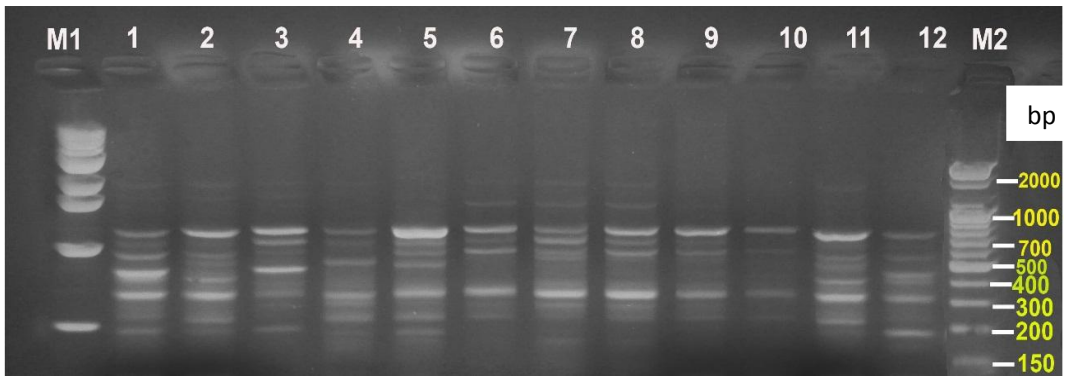

Fig. 3. Amplified DNA pattern of 12 potato genotypes generated by primer OPB-08
(SL# 1: SIP-3; SL# 2: SIP-5; SL# 3: SVP-6; SL# 4: SVP-18; SL# 5: SVP-19; SL# 6: SVP-25; SL# 7: SVP-55; SL# 8: SVP-56; SL# 9: SVP-68; SL# 10: Cardinal; SL# 11: Diamant and SL# 12: Asterix. M1 and M2: Molecular weight marker (1 kb and 100 bp, respectively; BIONEER, South Korea)

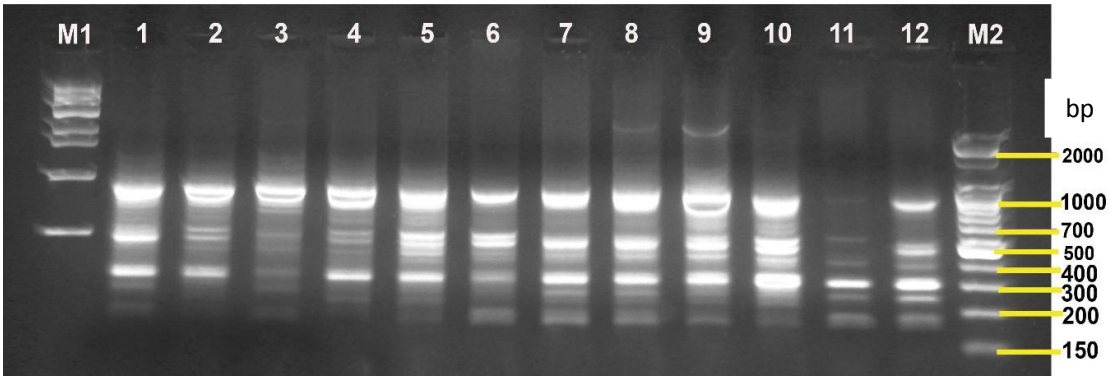

Fig. 4. Amplified DNA pattern of 12 potato genotypes generated by primer OPD-02
(SL# 1: Cardinal; SL# 2: Diamant; SL# 3: Asterix; SL# 4: SIP-3; SL# 5: SIP-5. SL# 6: SVP-6; SL# 7: SVP-18; SL# 8: SVP-19; SL# 9: SVP-25; SL# 10: SVP-55; SL# 11: SVP-56 and SL# 12: SVP-68. M1 and M2: Molecular weight marker (1 kb and 100 bp, respectively; BIONEER, South Korea)

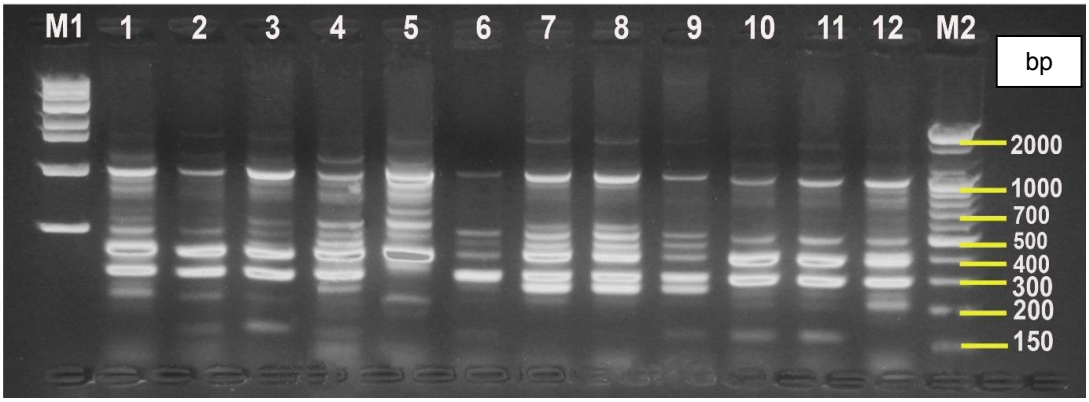

Fig. 5. Amplified DNA pattern of 12 potato genotypes generated by primer OPF-08
(SL# 1: Cardinal; SL# 2: Diamant; SL# 3: Asterix; SL# 4: SIP-3; SL# 5: SIP-5. SL# 6: SVP-6; SL# 7: SVP-18; SL# 8: SVP-19; SL# 9: SVP-25; SL# 10: SVP-55; SL# 11: SVP-56 and SL# 12: SVP-68. M1 and M2: Molecular weight marker (1 kb and 100 bp, respectively; BIONEER, South Korea)

3.2 Gene Frequency and Frequency of Polymorphic Loci

The primers used here showed different levels of gene frequency and different frequency of polymorphic loci (Table 2). Gene frequency valued from 0.083 to 1.00 and the frequency of polymorphic loci was ranged from 0 to 0.917.

The highest gene frequency (1.00) i.e. the lowest frequency of polymorphic loci (0) was shown by the primer OPA-18 (at 400 and 240 bp), OPB-08 (at 794 bp), OPD-02 (at 340 bp), OPF-08 (at 1047 bp) and OPW-01 (at 521 and 673 bp). On the other hand, the lowest gene frequency (0.083) i.e. the highest frequency of polymorphic loci (0.917) was shown by the primer OPF-08 (at 658 bp).

3.3 Inter-genotype Similarity Indices (Sij)

In our study, the inter-genotype means of the pair-wise similarity indices (Sij) was from 61.59% to 93.55% and the average was 74.31% (Table 3).

The highest similarity index (93.55%) was between SVP-55 vs. SVP-56 genotype pair. Thus, genetic distance between this pair of genotypes was lower than rest of the genotype pairs. On the other hand, Asterix vs SIP-3 pair showed the lowest inter- genotype similarity indices (61.59%). Hence, genetic distance between that genotype pair was higher than rest of the pairs.

Table 2. Frequencies of polymorphic loci by RAPD primers in 12 potato genotypes

RAPD marker	Locus no.	Locus size (bp)	Gene frequency	RAPD marker	Locus no.	Locus size (bp)	Gene frequency
OPA-18	1	1040	0.9167	**OPD-02**	4	615	0.9167
	2	719	0.7500		5	532	0.9167
	3	**400**	**1.0000**		6	443	0.9167
	4	**240**	**1.0000**		7	**340**	**1.0000**
	5	155	0.7500		8	287	0.8333
OPB-08	1	1868	0.6667		9	194	0.9167
	2	1156	0.5000	**OPF-O8**	1	2007	0.9167
	3	**794**	**1.0000**		2	1302	0.6667
	4	662	0.8333		3	**1047**	**1.0000**
	5	580	0.9167		4	876	0.5833
	6	476	0.9167		5	813	0.5833
	7	391	0.3333		6	**658**	**0.0833**
	8	335	0.3333		7	553	0.5833
	9	289	0.3333		8	433	0.9167
	10	238	0.8333		9	306	0.9167
	11	193	0.5833		10	265	0.9167
OPC-01	1	1172	0.3333		11	224	0.5000
	2	678	0.7500		12	153	0.5833
	3	550	0.5833		13	120	0.4167
	4	494	0.8333	**OPW-01**	1	1527	0.8333
	5	422	0.3333		2	1261	0.8333
	6	297	0.5000		3	1069	0.8333
	7	238	0.5000		4	832	0.9167
	8	201	0.2500		5	**673**	**1.0000**
OPD-02	1	3265	0.1667		6	**521**	**1.0000**
	2	1036	0.9167		7	384	0.3333
	3	794	0.2500		8	238	0.8333

Table 3. The highest and the lowest average RAPD band sharing percentage inter-genotype similarity indices among the 12 potato genotypes across six primers

Genotype name	The lowest average inter-genotype similarity showing pair (%)	The highest average inter-genotype similarity showing pair (%)	Overall, the lowest average inter-genotype similarity showing pair (%)	Overall, the highest average inter-genotype similarity showing pair (%)
Cardinal	Cardinal vs SIP-3 (67.92)	Cardinal vs SIP-5 (81.76)	Asterix	SVP-55
Diamant	Diamant vs Asterix (64.14)	Diamant vs SVP-55 (73.39)	vs	vs
Asterix	Asterix vs SIP-3 (61.59)	Asterix vs SVP-55 (80.79)	SIP-3	SVP-56
SIP-3	SIP-3 vs SVP-19 (66.23)	SIP-3 vs SVP-68 (71.48)		
SIP-5	SIP-5 vs SVP-68 (70.57)	SIP-5 vs SVP-55 (84.19)	(61.59)	(93.55)
SVP-6	SVP-6 vs SVP-68 (65.30)	SVP-6 vs SVP-19 (83.43)		
SVP-18	SVP-18 vs SVP-68 (76.21)	SVP-18 vs SVP-19 (86.73)		
SVP-19	SVP-19 vs SVP-68 (73.75)	SVP-19 vs SVP-55 (85.50)		
SVP-25	SVP-25 vs SVP-68 (82.45)	SVP-25 vs SVP-56 (83.78)		
SVP-55	SVP-55 vs SVP-68 (73.24)	SVP-55 vs SVP-56 (93.55)		

Table 4. Nei's (1972) genetic distance and genetic identity values among 12 genotypes at below diagonal and above diagonal respectively

Genotypes	Cardinal	Diamant	Asterix	SIP-3	SIP-5	SVP-6	SVP-18	SVP-19	SVP-25	SVP-55	SVP-56	SVP-68
Cardinal	***	0.722	0.778	0.741	0.704	0.57	0.759	0.722	0.648	0.704	0.722	0.815
Diamant	0.325	***	0.685	0.722	0.611	0.593	0.630	0.630	0.667	0.722	0.704	0.759
Asterix	0.251	0.378	***	0.667	0.667	0.611	0.648	0.648	0.574	0.741	0.685	0.741
SIP-3	0.300	0.325	0.406	***	0.703	0.574	0.648	0.612	0.648	0.630	0.611	0.815
SIP-5	0.351	0.493	0.406	0.352	***	0.648	0.574	0.685	0.648	0.741	0.685	0.630
SVP-6	0.555	0.523	0.493	0.555	0.434	***	0.704	0.778	0.667	0.722	0.667	0.537
SVP-18	0.275	0.463	0.434	0.434	0.555	0.351	***	0.815	0.741	0.648	0.667	0.722
SVP-19	0.325	0.463	0.434	0.493	0.378	0.251	0.205	***	0.778	0.722	0.667	0.648
SVP-25	0.434	0.406	0.555	0.434	0.434	0.406	0.300	0.251	***	0.722	0.741	0.648
SVP-55	0.351	0.325	0.300	0.463	0.300	0.325	0.434	0.325	0.325	***	0.907	0.704
SVP-56	0.325	0.351	0.378	0.493	0.378	0.406	0.406	0.406	0.300	0.097	***	0.722
SVP-68	0.205	0.275	0.300	0.205	0.463	0.622	0.325	0.434	0.434	0.351	0.325	***

3.4 Nei's (1972) Genetic Distance and Genetic Identity

Pair-wise comparisons of Nei's (1972) genetic distance among 12 potato genotypes was from 0.0972 to 0.6217 (Table 4). The highest Nei's genetic distance (0.6217) was found in SVP-6 vs SVP-68 genotype pair and the lowest genetic distance (0.0972) was found in SVP-55 vs SVP-56 genotype pair. Whereas, genetic identity value was from 0.5370 to 0.9074. The highest Nei's genetic identity (0.9074) was seen in SVP-55 vs SVP-56 genotype pair and the lowest genetic identity (0.5370) was found in SVP-68 vs SVP-6 genotype pair.

Here, SVP-68 was very close to the three varieties among the somaclonal variations. In particular, for Cardinal to other somaclonal variants Nei's (1972) genetic identity ranged from 0.5741 (at SVP-6) to 0.8148 (at SVP-68), while for Diamant it ranged from 0.5962 (at SVP-6) to 0.7593 (at SVP-68) and in the case of Asterix it ranged from 0.5741 (at SVP-25) to 0.7407 (at SVP-55 and SVP-68.

On the other hand, SVP-6 (to Cardinal and Diamant) and SVP-25 (to Asterix) is distantly linked accessions among the somaclonal variations. Furthermore Nei's (1972) genetic distance between Cardinal and somaclonal variations ranged from 0.2048 (at SVP-68) to 0.5550 (at SVP-6). Whereas for Diamant, it ranged from 0.2754 (at SVP-68) to 0.5232 (at SVP-6) and in case of Asterix it ranged from 0.3001 (at SVP-55 and SVP-68) to 0.5550 (at SVP-25).

3.5 Cluster Analysis Based on UPGMA Dendrogram

The UPGMA dendrogram showed the segregation of 12 potato genotypes into two broad clusters: A and B (Fig. 6). The broad cluster A had 8 genotypes i.e. Cardinal, SVP-68, SIP-3, Diamant, Asterix, SIP-5, SVP-55 and SVP-56, while the broad cluster B contained 4 genotypes and these were SVP-6, SVP-18, SVP-19 and SVP-25.

Moreover, the broad cluster A was split into two sub-cluster: AI and AII. Sub-cluster AI contained 5 genotypes i.e. Cardinal, SVP-68, SIP-3, Diamant and Asterix; while Sub-cluster AII contained 3 genotypes i.e. SIP-5, SVP-55 and SVP-56. Again, the broad cluster B was split into two sub-cluster: BI and BII. Sub-cluster BI contained only one genotype (SVP-6), and Sub-cluster BII contained 3 genotypes (SVP-18, SVP-19 and SVP-25).

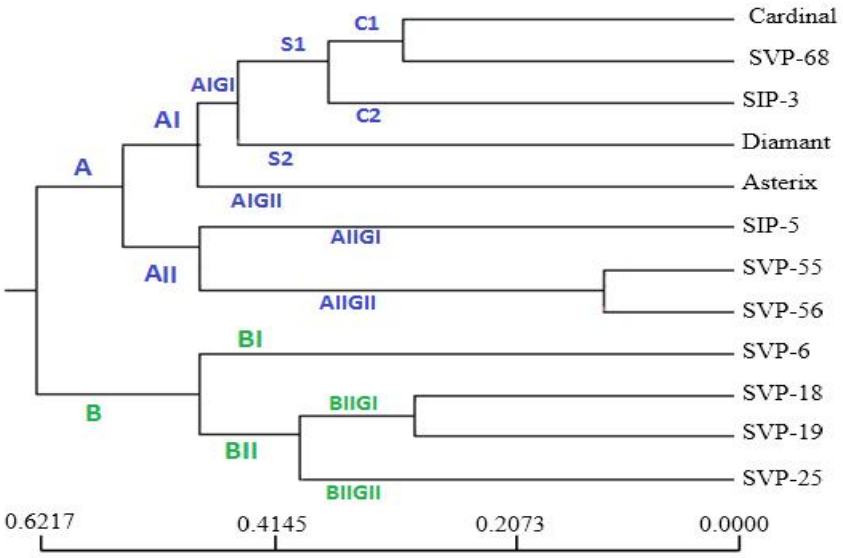

Fig. 6. UPGMA dendrogram displaying the phylogenetic relationship among 12 potato genotypes based on Nei's (1972) genetic distance

4. DISCUSSION

To characterize, identify and analyze genetic diversity of somaclonal variants in potato genotypes RAPD markers are very useful as they can be performed easily with small amount of DNA and able to show polymorphism at high level. Moreover, RAPD primers are universal for all crops and are not species specific. Therefore, without prior knowledge about the sequence, DNA probes and hybridization, the RAPD primer can be designed [24,25].

Although only 6 primers are not sufficient to provide information of full genome of the selected genotypes but they can detect the genetic variation efficiently. As like as the present study, AL-Salihy et al. [26] screened 6 RAPD primers on 4 *In vitro* propagated potato and selected 5 primers to study genetic diversity. Moreover, Iuliana and Cerasela [27] screened 6 RAPD primers on 6 potato genotypes (3 potato cultivars and their 3 somaclonal variants) and selected 3 RAPD primers for final amplification and successfully determined the genetic diversity.

Our selected 6 primers generated average 9 DNA bands per primer and 4.5 bands per genotypes and most of the bands were between 150 to 2000 bp. Afrasiab and Iqbal [6] estimated 123 clear and easily storable bands using 24 RAPD primers in 9 potato genotypes (3 somaclonal variant of cultivar Desiree and 6 gamma mutant lines) size ranged from 200 to 3000 bp and average 5.12 bands per primers and 13.67 bands per potato cultivar. Their scored bands size range supported our investigation though, their studied bands per primer is lower and bands per genotypes is higher than ours. Again, using 7 RAPD primers in 12 mother potato plants and their 12 somaconal regenerants Tiwari et al. [24] detected 54 scorable bands i.e., 7.71 bands per primer and 2.25 bands per genotypes. They scored fewer bands per genotypes and per primers than the current study. This variation may be due to the difference in genotypes and primers used. Ahmad et al. [28] studied 4 RAPD primers in mutant lines of Cardinal, Diamant and Desiree and found bands were present between 50 bp to 1500 bp. Which is less than our overall band size range but close to majority band size range. They studied a smaller number of primers which may be cause of scoring less size range bands. Moreover, Verma and Singh [1] reported 91 bands while screening 48 Indian potato by 20 RAPD markers i.e. 4.55 bands per primer and 1.9 bands per potato cultivar. DNA fragments size and number are determined by the primer sequence. Different factors, such as- the primer sequence, thermocycler type, template quality and quantity and polymerase concentration influenced the reproducibility of the RAPD technique [13].

In the current study about ~87% polymorphic and ~13% monomorphic bands were detected which indicates that genetic variation level among our studied genotypes is high. OPF-08 primer amplified the highest number (12) and OPA-18 primer amplified the lowest number (03) of polymorphic bands although, in case of percentage polymorphic band production, OPC-01 primer is at the highest position. Afrasiab and Iqbal [13] screened 22 RAPD primers on 9 potato genotypes (5 gamma irradiant potato and 4 somaclonal variant potato of variety Diamant). They estimated 140 (74.86%) polymorphic bands and 47 (25.14%) monomorphic bands out of total 187 bands amplified. The result gave an average 6.36 polymorphic and 2.14 monomorphic bands per primer. The present study was almost similar to their study in respect of average bands per primers but their counted polymorphic bands percentage and average polymorphic bands per primer was lower than ours. Indeed, Khatab and El-Banna [17] used five RAPD primers in 14 somaclonal variants of potato and revealed 38 (62.29%) polymorphic and 23 (37.71%) monomorphic bands. The result showed an average 7.6 polymorphic and 4.6 monomorphic bands per primer. Polymorphism percentage close to our study (~82%) was detected by both Onamu et. al. [18] and Verma and Singh [1] in 35 potato accessions by 19 RAPD primers and 48 potato germplasm by 20 RAPD primers, respectively. Lower level of average polymorphism (~53) was found by Salem and Hassanein [11] 3 potato genotypes by 11 RAPD primers.

Khatab and El-Banna [17] found the gene frequency range from 0.056 to 1.00 and the frequency of polymorphic loci was from 0 to 0.944. The result was very close to the outcome of the current study.

Isenegger et al. [29] studied 64 potato cultivars in Australia and found 67% to 90% similarity among them. The results of the above study were very close to the present study. Again, Das et al. [30] detected a wide range of similarity values (ranged from 29% to 93%) in 30 Indian potato cultivars with 13 RAPD primers. On the other

hand, Gauchan et al. [31] reported 55.2% to 69% similarity value among 4 Nepali local potato cultivars studied with 10 RAPD primers, which was lower than the present study. This may happen due to cultivar variations and variation in primers used.

In the current study, we observed low to moderate level of genetic variation and genetic identity. Chakrabarti et al. [32] detected 0.33 to 0.80 similarity value among 20 potato cultivars with 10 RAPD primers. On the other hand, Yasmin et al. [33] reported Nei's (1972) genetic identity from 0.6530 to 0.8674 and genetic distance from 0.154 to 0.558 among 6 potato cultivars. They also reported that Nei's (1972) genetic identity and genetic distance between Cardinal and Diamant was 0.6530 and 0.558 respectively. Their result was very close to the present study. Use of some common varieties in both studies might be the reason of that. Again, Hoque et al. [34] reported very higher genetic variation (0.55 to 1.0) among 8 potato genotypes. They studied both indigenous and high yielding varieties, which might be the reason of their higher genetic variation.

In our study we found very diverse relationship among the studied genotypes. From the dendrogram, five somaclonal variants and the three source materials were grouped in cluster A, whereas, the remaining four somaclonal variants were grouped in cluster B. The result indicates that the somaclonal variants of cluster B might possess higher genetic variation from other somaclonal variant genotypes. The source materials are also closely related to one another than their progeny four somaclonal variants of cluster B. Likewise, Brenna [35] showed the genetic relationship among 12 potato varieties with a dendrogram. The dendrogram segregated his studied varieties into two main clusters and then different sub-clusters. Again, Hoque et al. [3] studied a UPGMA dendrogram based on Nei's genetic distances among 12 potato varieties. These varieties were divided into two broad clusters. Then the clusters into sub-clusters. They also reported that Cardinal, Diamant and Asterix belonged to the same sub-cluster like the present study.

5. CONCLUSION

Significant diversity and relationships were present among the 12 genotypes studied. It indicates that the RAPD marker techniques could be used for detecting the genetic variation as well as DNA fingerprinting in potato somaclone. The higher level of variation showing genotypes (SVP-6 and SVP-25) and the lowest Inter-genotype similarity showing genotype (SIP-3) might be used in future potato improvement program. Furthermore, the findings of the present study could be the guideline for future fingerprinting and genetic diversity study of potato. However, a larger number of genotypes and more RAPD primers, along with other molecular markers such as AFLP, SNP, SSR etc. would be needed to develop a precise relationship and diversity but the present type of study is widely acceptable in all concerns.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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