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Analysis of Genetic Diversity in Potato (*Solanum tuberosum* L.) Genotypes through RAPD (Randomly Amplified Polymorphic DNA) Markers

Rajani* and Dhirendra Singh

Reseach Scholar, G. B. Pant University of Agriculture & Technology

*Corresponding Author E-mail: rawat.rajni08@gmail.com

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) markers were used to study the molecular diversity of 50 potato genotypes in Tarai region of Uttarakhand. DNA was extracted from tender leaves of potato genotypes for PCR amplification. The PCR amplified DNA profile was visualized on 1.5% agarose gel, staining with ethidium bromide. Twenty RAPD primers were used and out of twenty only eight were amplified and gave polymorphic results. Therefore, eight RAPD primers were used to characterize the genetic diversity among the 50 potato genotypes. A total 84 reproducible loci were obtained with 8 RAPD primers. Each primer varied greatly in their ability to resolve variability among the genotypes. The primers amplified minimum 6 (OPH-16) to maximum 13 (OPA-18) loci with an average of 10.5 loci per primer (Table 1). Out of 84 bands, 78 were polymorphic and 6 were monomorphic (Table 3). The polymorphism percentage ranged between 81.81 (OPX-02) to 100 per cent (OPH-16, OPH-19, OPA-12 and OPA-18). Average polymorphic band was recorded to be 10 per primer.

Key words: *Solanum tuberosum L., Genetic Diversity, Randomly Amplified Polymorphic DNA Cluster analysis, Dendrogram.*

INTRODUCTION

Potato is a highly heterogeneous and vegetatively propagated crop[7]. It is one of the important food crops of India as well as in many other countries of the word. The cultivated potato have narrow genetic base due to limited introduction of germplasm from their natural range in South America[14]. Most of the potato cultivars are autotetraploid (2n=4x=48), highly heterozygous and out breeding species, which suffer from inbreeding depression. The success of the breeding programme in potatoes largely depends on the identification of the amount and distribution of genetic diversity in the gene pool. The knowledge of the genetic diversity and relationship among the varieties are very useful in order to recognize gene pools, to identify the gaps in germplasm collection and to develop effective conservation and management strategies.

Molecular markers have become important tools in studies of genetic diversity[1], due to the high resolution and reliability in the identification of cultivars.

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RAPD (Random Amplified Polymorphic DNA) markers have the advantage of detecting polymorphism simply and quickly[6]. Thus genetic diversity in breeding for high yielding varieties has obvious importance as evidenced by earlier workers[10,2]. The knowledge of genetic diversity is important for successful selection of the parents for hybridization work. It is also established that degree of heterosis is related to the magnitude of genetic divergence between parental lines. Keeping all the above facts in view, the aim of this 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.

MATERIAL AND METHODS

PCR based molecular analysis of potato genotypes

For the studies of genetic divergence and establishment of distinctiveness the DNA from 50 genotypes of *Solanum tuberosum* L. was extracted from the 30 days old plants, at PCPGR Laboratory, G. B. Pant University of Agriculture and Technology, Pantnagar using RAPD markers. The genomic DNA was extracted by CTAB (Cetyl tri-methyl ammonium bromide) method described by Doyle and Doyle.

PCR based molecular analysis

Molecular marker technology provides information that can help to define the distinctiveness of germplasm and their ranking according to the number of close relatives and their phylogenetic position. An enhanced understanding of the temporal dynamics of intra-specific diversity is anticipated to improve the adequate priorities, methods and metrics. It is a complementary approach for genetic characterization. The present study is aimed for diversity analysis of 50 potato genotypes based on PCR related molecular markers i.e. RAPD and SSR.

RAPD PCR amplification

PCR Amplification[18], was performed with at bittail decamer primers (Table1).

Table 1: Reaction mixture for PCR amplification for RAPD

S.No.	Components Concentration	Final Concentration	Single tube (µL)
1.	DNA template (100ng/µL)	200-300ng	1µL
2.	dNTPs mix (235 mM each)	10Mm (2.5mMeach dNTP)	1.5µL
3.	*Taq* DNA polymerase (5U/ µL)	5U	0.1µL
4.	Primer (50ng/ µL)	150ng	1.5µL each
5.	Reaction buffer (10X)	10X	2µL
6.	Deionized water	6.6µL	6.6µL

Table 2: Detailed description of primer sequences of RAPD markers for potato

S.No.	RAPD Primercode	Primer sequence
1.	OPH-12	5'-ACGCGCATGT -3'
2.	OPH-13	5'-GACGCCACAC -3'
3.	OPH-14	5'-ACCAGGTTGG -3'
4.	OPH-16	5'-TCTCAGCTGG -3'
5.	OPH-17	5'-CACTCTCCTC -3'
6.	OPH-19	5'-CTGACCAGCC -3'
7.	OPA- 11	5'-CAATCGCCGT-3'
8.	OPA-12	5'-CAGCACCCAC-3'
9.	OPA-18	5' AGGTGACCGT-3'
10.	OPB-19	5'-ACCCCCGAAG-3'
11.	OPX-02	5'-TTCCGCCACC -3'
12.	OPX-04	5'-CCGCTACCGA -3'
13.	OPX-07	5'-GAGCGAGGCT- 3'
14.	OPX-08	5'-CAGGGGTGGA - 3'
15.	OPX-09	5'-GGTCTGGTTG - 3'
16.	OPG08	5'-TCACGTCCAC - 3'
17.	OPG10	5'-AGGGCCGTCT - 3'
18.	OPG13	5'-CTCTCCGCCA - 3'
19.	OPG17	5'-ACGACCGACA - 3'
20.	OPG19	5'-GTCAGGGCAA - 3'

Amplifications were performed in 25 µL volume containing the following components:

A set of 20 RAPD were employed for PCR amplification. A master mix (mince template DNA) was prepared to reduce pipetting error. The master mix was the distributed in each tube (11.5µL each) and finally 1.0 µL of template DNA was added each tube. The mixture was gently mixed and centrifuged for ten seconds. The PCR amplification was achieved in eppendorf thermocycler, programmed as follows:

Cycles	Denaturation		Annealing		Polymerization	
	Temp. (^{0}C)	Time (Min.)	Temp. (^{0}C)	Time (Min.)	Temp. (^{0}C)	Time (Min.)
First	94	3	-	-	-	-
Second	94	1	25-35 (RAPD)	45-50sec	72	1-2
Last	-	-	-	-	72	5-7

The amplification cycles used were initial denaturation at 94^{0}C for 3 min, followed by 40 cycles of denaturation at 94^{0}C for 1 min, annealing at 25-35^{0}C for RAPD for 45-50 sec and synthesis at 72^{0}C for 1 min and finally extension step of 7 min at 72^{0}C. The PCR products were electrophoreses on a 1.5% (RAPD) agarose gel.

RESULT

PCR optimization and primer screening

The polymerase chain reaction (PCR) amplification procedure was optimized by determining the most appropriate concentration of DNA template, Taq DNA polymerase dNTPs and Mg++ ion required to generate repeatable PCR amplification profiles. The random primers suitable for generation of polymorphic amplification profile among the genotypes of potato were identified by screening of 20 RAPD primers. However, only 8 RAPD primers generated polymorphic bands. PCR reaction was carried out in an Eppendorf Thermocycler. The amplified products of RAPD were made to run in 1.5 per cent agarose gel electrophoresis. Each amplified product was considered as a DNA marker. These were scored all 50 genotypes manually. Bands were recorded as present (1) or absent (0) across the lanes.

DNA polymorphism

Total number of RAPD loci generated by each primer and number of polymorphic loci for each primer are presented in Table 3.

Table 3: Analysis for polymorphism in RAPD markers

S. No.	Primer	Sample size	GC content	Annealing temperature (^{o}C)	Polymorphic bands	Monomorphic bands	Polymorphism (%)
1.	OPH-12	48.00	60.00	35	9	1	90.00
2.	OPH-16	48.00	60.00	35	6	0	100.00
3.	OPH-19	48.00	70.00	37	11	0	100.00
4.	OPA-12	48.00	70.00	37	12	0	100.00
5.	OPA-18	48.00	60.00	32	13	0	100.00
6.	OPX-02	48.00	70.00	35	9	2	81.81
7.	OPX- 04	48.00	70.00	35	8	1	88.88
8.	OPG-10	48.00	70.00	37	10	2	83.33

RAPD

Out of twenty RAPD primers used only eight were amplified and gave polymorphic results. Therefore, eight RAPD primers were used to characterize the genetic diversity among the 50 potato genotypes. A total 84 reproducible loci were obtained with 8 RAPD primers. Each primer varied greatly in their ability to resolve variability among the genotypes. Chakarbarti *et al.*[3], Pattanayak *et al.*[12], Singh *et al.*[16],etc. also used RAPD primer for assessing the genetic diversity of tetraploid potato germplasm.

The primers amplified minimum 6 (OPH-16) to maximum 13 (OPA-18) loci with an average of 10.5 loci per primer (Table 3). Out of 84 bands, 78 were polymorphic and 6 were monomorphic (Table 3). The polymorphism percentage ranged between 81.81 (OPX-02) to 100 per cent (OPH-16, OPH-19, OPA-12 and OPA-18). Average polymorphic band was recorded to be 10 per primer.

Genetic diversity analysis

Pair wise similarity matrix based on Jaccard's coefficient for 50 potato genotypes are presented in Appendix VIII for RAPD primers. The similarity value ranged from 54 to 100 per cent. TPSK-05-06-79 & C-11 (100%) and TPSK-05-06-86 & DPS-07 were found to be the most similar genotypes among the 50 studied genotypes/varieties.

Cluster analysis

The UPGMA (un-weighted pair group method with arithmetic mean) analysis was done and dendrogram was constructed using Jaccard's similarity matrix of RAPD markers involving data generated out of nine polymorphic primers on fifty genotypes of potato and was depicted in Fig.1.

Fifty genotypes were demarcated at 54 per cent similarity, and further separated into two major clusters I and II with approximately 64 and 63 per cent similarity, respectively. Cluster I comprised 11 genotypes while cluster II found 39 genotypes.

Cluster I further subdivided into two sub-clusters IA and IB each with approximately 74 and 67 per cent similarity, respectively. Sub-cluster IA contains three genotypes *viz* Kufri Arun Kufri Jawahar and EM-2 with 76 per cent similarity. Sub cluster IB further forked into two small clusters Bl and B2, cluster Bl genotypes had approximately 75.5 per cent similarity and contain five genotypes i.e. MS/95-1542, TPSK-05-06-98, Laddy Rosseta, MS/0-3740 and TPSK-05-06-83 while cluster B2 had approximately 73.5 per cent similarity and contain only three genotypes (MS/93-1344, Kufri Gaurav and Kufri Frysona).

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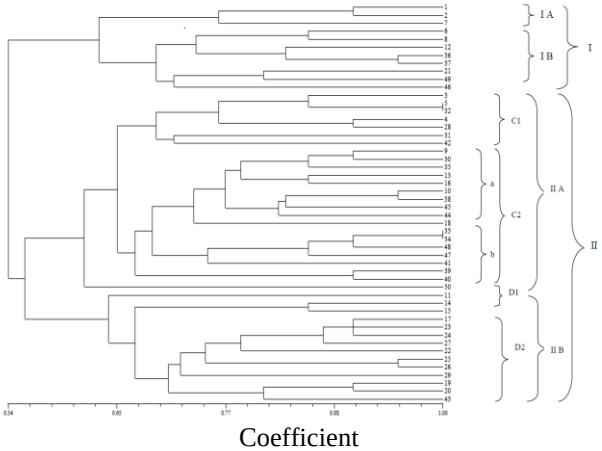

Fig. 1 Dendogram depicted the classification of the fifty genotypes of potato constructed using UPGMA method based on RAPD markers. The scale in the bottom is Jaccard's coefficient of similarity

Main cluster II was divided into two small clusters IIA and IIB with 63 and 64.5 per cent similarity. Small cluster IIA further forked into super small cluster C1 and C2 with 68 and 63 per cent similarity, respectively. Super small cluster C1 contains seven genotypes *viz* PMT-1, TPSK-05-06-79, C-11, TPSK-05-06-11 Kufri Jyoti, TPSK-05-06-85 and PH-3. However, super small clusters C2 further divided into two clusters i.e. cluster a and cluster b which contain 10 and 8 genotypes with 75 and 63 per cent similarity, respectively. Small cluster IIB is further forked into two super small cluster had approximately 64.5 per cent similarity. In super small clusters cluster D1 comprised only three genotypes *viz*. TPSK-05-05-95, EM-3 and Kufri Giriraj possess maximum similarity of approximately 86.5 per cent. Small cluster D2 perceived 11 genotypes i.e. Kufri Sadabahar, Kufri Badsah, TPSK-05-06-80, Kufri Khyati, TPSK-05-06-105 Kufri Pushkar, TPSK-05-06-44, TPSK-05-06-61 TPSK-05-06-117, Kufri Chipsona-2 and P-11 with 70 per cent similarity. Maximum similarity occurred between TPSK-05-06-79 and C-11 (100%) while TPSK-05-06-86 and DPS-07 also showed 100 per cent similarity with each other.

Out of eight random primers used, OPA-18 amplified in 13 loci with all polymorphic bands. Several workers reported similar type of observation on potato with RAPD primers. Rocha *et al.*[15],reported 1 to 8 bands with RAPD primers while Komy *et al* [9], observed 9 to 26 bands with RAPD primers. They also found approximately 71 per cent similarity between the genotypes Atlantica and Lady rosetta. Wild range of similarity coeffiecient i.e. 29 to 93 percent also reported by Das *et al.*[5]. Paz and Veilleux [13], also used pair wise similarity index or matrix based on Jaccard's coefficient to calculate the similarity among potato genotypes. RAPD markers were found to be sufficiently sensitive to detect genetic variation among germplasm as reported by Demeke *et al.*[6], and Sosinski and Douches[17]. Hosaka *et al.*[8], studied the genetic relationship of 73 Japanese potato cultivars; they concluded that banding pattern is reflection of the pedigree relationship. However, Demeke *et al.*[6], studied genetic diversity of 28 North American potato cultivars and recorded that cultivar with close kinship can often as genetically diverse as those with no immediate relationship. In this study it was also found that kinship relation could not be reflected in the similarity of banding pattern. This is probably due to highly heterozygous nature of the tetraploid genome. Similar results were also reported by Chakrabarti *et al.*[3], Chimote *et al.*[4]. The high levels of fragment polymorphism observed in the present study demonstrated the potential of this method in evaluating genetic diversity. However, the fact the potato genotypes/varieties differ in total number of fragments generated in all the genotypes indicates uneven distribution of RAPD markers in the genotypes tested; moreover, high number of fragments suggests that potato has a boarder genetic background which was also reported by Pattanayak *et al.*[11],and Chimote *et al.*[4].

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