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Molecular Divergence Studies through RAPD Markers in Potato (*Solanum tuberosum* L.) Germplasm

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A B S T R A C T

Genetic diversity using Random Amplified Polymorphic DNA (RAPD) markers was assessed in forty eight Indian potato germplasm. Twenty RAPD markers amplified a total of ninety-one different loci that exhibited 82.30 per cent polymorphism among 48 potato germplasm. The PIC value ranged from 0.27 to 0.55. All the loci amplified by the primer which were found to be polymorphic varied in size from <50bp to >1500bp. The Jaccard's similarity coefficient was found to vary from 0.22 to 0.87. The maximum genetic similarity (0.87) was found between Kufri Badshah and Kufri Khyati and lowest (0.22) between Pant Sel.-01-15 and Pant Sel.-09-57. UPGMA based dendrogram ordered the populations of genotypes into nine clusters. The most diverse groups found were Cluster II and cluster IX followed by cluster I and VII with Cluster II. RAPD markers were found to be sufficiently sensitive to detect genetic variation among various germplasm, which will be useful for selecting genetically distinct germplasm in potato breeding programme.

Introduction

Potato (*Solanum tuberosum* L.) is an integral part of human diet. It belongs to the genus *Solanum*, which represents species with different ploidy states, varying from diploid (2n = 24) to hexaploid (2n = 72). Improvement in potato crop is essential as it is one of the most important cash crops of the country. Knowledge about germplasm diversity and genetic relationships among potato germplasm could be a valuable aid in crop improvement strategies and play a vital role for a successful breeding program. The amount of variability that exists in the germplasm collection of any crop is of the utmost importance towards breeding for better varieties.

Genetic diversity is also used to study the taxonomic relationship among genotypes and to choose varieties with good qualities and incorporate them into breeding programmes (Barandalla *et al.,* 2006).

Breeding trials are time-consuming and expensive due to self-incompatibility, multiple ploidy levels, and high heterozygosity observed in the Solanum genus (Slater *et al.,* 2013). Thus, researchers have started using molecular genetic markers to accelerate breeding programs (Moloney *et al.,* 2010; Finkers-Tomczak *et al.,* 2011; Slater *et al.,* 2013). Molecular markers that detect variation at the DNA level overcome most of the limitations of biochemical and morphological markers (Bered *et al.,* 2005). As confirmed by their use in a variety of plant species, molecular markers are most appropriate for assessment of genetic diversity and identification of varieties (Upadhyay *et al.,* 2004). Development of molecular markers associated with the traits of interest has the advantage that desirable genotype of the plants can be selected in the early stage of development (Gisbert *et al.,* 2007a). Some molecular marker techniques have been developed over the years, using RAPD (Randomly Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter-Simple Sequence Repeat), gene-based SSR (Simple Sequence Repeats) markers and SNPs. Among them, RAPD have shown promising results for germplasm evaluation and molecular aided breeding, is easy and quick, and requires no prior sequence information (Williams *et al.,* 1990; Tabkhkar *et al.,* 2012). They are widely distributed throughout the eukaryotic genomes in coding and non-coding regions. Their random genome distribution and use with low quality DNA have resulted into wide use in determination of genetic diversity, germplasm fingerprinting, genetic linkage mapping and phylogenetic studies (Yang *et al.,* 2015; Jian *et al.,* 2017; Duan *et al.,* 2018). RAPD markers have been used widely in potato for evaluating genetic diversity. The present study aimed at analyzing diversity of 48 potato genotypes based on RAPD markers, will certainly enrich the genomic-RAPD pool for potato. Besides, it will provide the scientists with more options for potato germplasm evaluation and designing of the breeding programs for producing superior cultivars of potato.

Materials and Methods

The experiment was conducted at PCPGR (Pantnagar Center for Plant Genetic Resource), Department of Genetics and Plant Breeding and "Molecular Lab", Department of Fish Biology, G.B.P.U.A.&T., Pantnagar, U.K. The materials used in this study consisted of 48 germplasm developed through selection in Pantnagar and few Kufri varieties released from CPRI, Shimla. The molecular divergence study was performed using 20 RAPD primers as illustrated in Table 1.

DNA isolation and PCR amplification

Tubers of 24 tetraploid Indian potato germplasm were grown in pots. The fresh and green leaves were collected and the genomic DNA was extracted by using the CTAB method of Doyle and Doyle (1990). RNaseA treatment was done for DNA purification. DNA concentration was measured by using UV spectrophotometer. The Polymerase Chain Reaction (PCR) was performed in eppendorf thermocycler. The amplification cycles used were initial denaturation at 94 ºC for 3 min, followed by 40 cycles of denaturation at 94 ºC for 1 min, annealing at 35-37 ºC for 45-50 sec and synthesis at 72 ºC for 1 min; lastly extension step of 5-7 min at 72ºC. Finally, the PCR products were run on 1.5 % agarose gel electrophoresis.

RAPD data analysis

Amplified RAPD profile of all the genotypes with each primer were documented using gel documentation system. DNA fragment

profiles were scored in a binary fashion with 0 indicating absence and 1 indicating presence of a band. Primers with null allele where an amplification product could not be detected were not considered in the analysis. The binary data were used to calculate genetic similarities based on Jaccard coefficients among the isolates by using NTSYS-pc version 2.11W (Rohlf, 2000). The SIMQUAL program was used to calculate the Jaccard's coefficients (Jaccard, 1908) and on the basis of these coefficients, dendogram was constructed using UPGMA (Unweighted pair group mean average) method.

Results and Discussion

Genetic diversity analysis and RAPD polymorphism

Out of twenty RAPD primers, eleven were found to be polymorphic, three were monomorphic and rest gave null results. Primers OPZ11, OPR 13, OPB12 yielded maximum of 10 to 12 bands (Figure 1 and 2). All the loci amplified by the primer which were found to be polymorphic varied in size from <50bp to >1500bp. A total of ninety-one loci were amplified that exhibited 82.30 per cent polymorphism. The PIC value ranged from 0.27 to 0.55 (Table 2). Based on the 20 RAPD marker data the Jaccard's similarity coefficients were estimated between pair of germplasm.

The similarity coefficient range was found to vary from 0.22 to 0.87 i.e. 22 to 87 percent. The highest value for genetic similarity (0.87) was found between Kufri Badshah and Kufri Khyati followed by Kufri Badshah and J-96-54 (0.83) and Pant Sel-09-46 and Pant Sel-09-43 (0.82). The lowest value for the similarity coefficient (0.22) was found between Pant Sel-01-15 and Pant Sel-09-57 and Pant Sel-09-57 and Kufri Frysona. Similar results in potato by using RAPD primers were reported by Collares *et al.,* (2004), Kujal *et al.,* (2005) and Komy *et al.,* (2012).

Cluster analysis

UPGMA ordered the populations of 48 genotypes into single cluster A subdivided into nine clusters (Figure 3). The biggest clusters with more number of genotypes found were Cluster II and III (Table 2). The main Cluster II consisted of sixteen genotypes namely Pant Sel-01-15, Pant Sel-09-33, Pant Sel-08-07-01(CT), Kufri Giriraj, Pant Sel-09-01, Pant Sel-09-07, Pant Sel-09-11, Pant Sel-09-50, Pant Sel-15/5, Pant Sel-09-19, J-97-242, J-96-54, J-96-288, Kufri Badshah, Kufri Khyati and J-95-225; which varied between very low to very high yielding types and showed wide genetic diversity. Cluster III consisted of seven genotypes namely Pant Sel-09-46, Pant Sel-09-43, Pant Sel-09-55, Kufri Surya, Kufri Ashoka, Pant Sel-09-18 and Kufri Arun; which varied between low to high yielding types. The smallest clusters were Cluster VIII having two genotype namely Pant Sel-09-03 and Kufri Bahar and cluster IX having single genotype namely Pant Sel-09-57. The most diverse groups found were Cluster II and cluster IX followed by cluster I and VII with Cluster II. Choosing parents/genotypes from these diverse clusters may produce heterosis in segregating generations which can be utilized for development of good and promising hybrids.

Wide range of similarity coefficient was also reported by Das *et al.,* (2010) and Rocha *et al.,* (2010). During the field analysis, germplasm namely Pant Sel- 08-02, Pant Sel-09-04, Pant Sel-09-43, Pant Sel-09-20, Pant Sel-09-11, Kufri Badshah, Kufri Sutlej, Kufri Chipsona-1 and Kufri Chipsona-2 showed high to moderate field resistance to late blight disease but no clear cut grouping was observed in resistant and susceptible genotypes by RAPD primers (Table 3).

Fig.1 Amplification pattern of primer OPZ 11

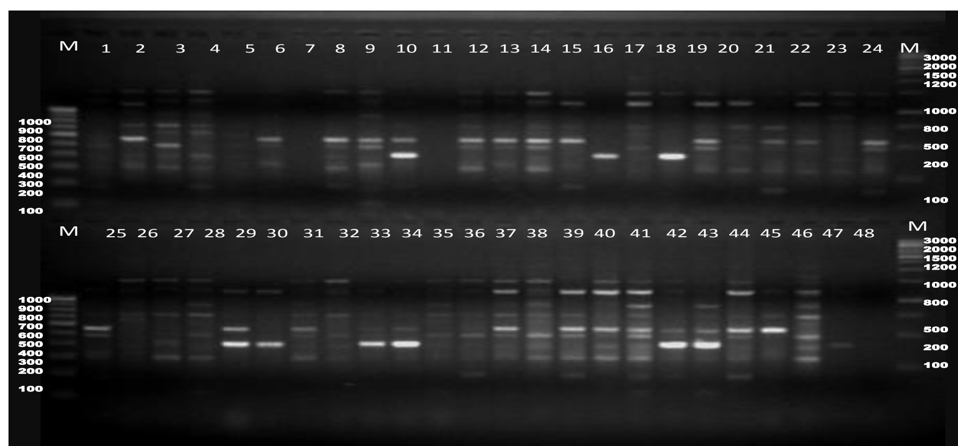


Fig.2 Amplification pattern of primer OPR 13

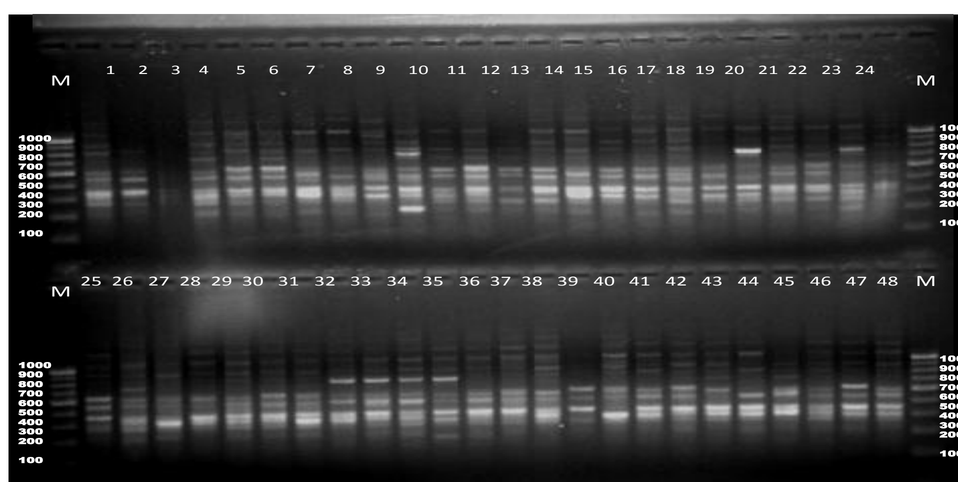


Table.1 Detailed description of primer sequences of RAPD marker

S. No.	RAPD Primer Code	Primer Sequence (5'to 3')	S. No.	RAPD Primer Code	Primer Sequence (5'to 3')
1.	OPR3	5'ACACAGAGGG3'	11.	OPB11	5'GTAGACCCGT3'
2.	OPR7	5'ACTGGCCTGA3'	12.	OPB12	5'CCTTGACGCA3'
3.	OPR9	5'TGAGCACGAG 3'	13.	OPB13	5'TTCCCCCGCT3'
4.	OPR13	5'GGACGACAAG3'	14.	OPB14	5'TCCGCTCTGG3'
5.	OPR12	5'ACAGGTGCGT3'	15.	OPB15	5'GGAGGGTGTT3'
6.	OPM4	5'GGCGGTTGTC3'	16.	OPB16	5'TTTGCCCGGA3'
7.	OPM5	5'GGGAACGTGT3'	17.	OPB17	5'AGGGAACGAG3'
8.	OPZ4	5'AGGCTGTGCT3'	18.	OPB18	5'CCACAGCAGT3'
9.	OPZ6	5'GTCTACGGCA3'	19.	OPB19	5'ACCCCCGAAG3'
10.	OPZ11	5'CTCAGTCGCA3'	20.	OPB20	5'GGACCCTTAC3'

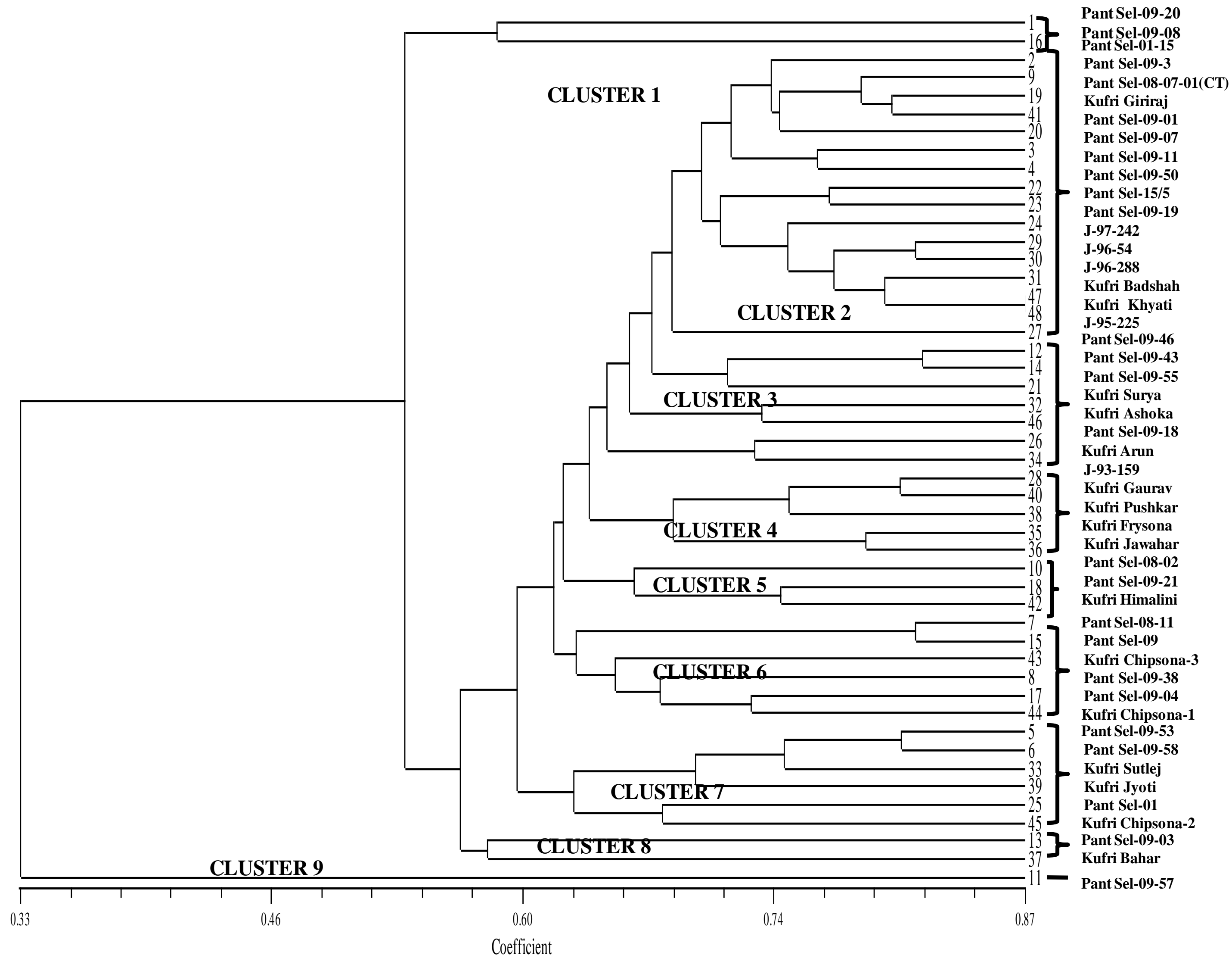
Table.2 Analysis for polymorphism in RAPD markers

S. no.	Marker	Allele Frequency	Sample size	Allele no.	Gene diversity	Amplified product size	GC content	Annealing Tm (^{0}C)	Polymorphic bands	Monomorphic bands	Polymorphism (%)	PIC value
1.	OPR3	0.78	48.00	5	0.32	50-600	60	35	5	0	100	0.27
2.	OPR7	0.90	48.00	10	0.17	100-800	60	35	9	1	90	0.55
3.	OPR12	0.69	48.00	8	0.38	150-1000	60	37	8	0	100	0.30
4.	OPZ4	0.90	48.00	8	0.16	200-800	60	37	8	0	100	0.14
5.	OPZ11	0.65	48.00	10	0.44	100-1000	60	37	10	0	100	0.54
6.	OPR13	0.82	48.00	12	0.37	200-1200	60	37	12	0	100	0.52
7.	OPZ6	0.74	48.00	6	0.35	200-1000	60	37	6	0	100	0.28
8.	OPB12	0.70	48.00	11	0.41	200-1500	60	37	11	0	100	0.43
9.	OPB17	0.65	48.00	9	0.43	100-900	60	37	9	0	100	0.34
10.	OPM4	0.90	48.00	5	0.15	200-700	70	32	4	1	80	0.13
11.	OPB15	0.75	48.00	5	0.33	200-800	60	37	5	0	100	0.26

Table.3 Grouping of 48 genotypes into 10 clusters using UPGMA method

Clusters	No. of Genotypes	Genotypes
Cluster I	2	Pant Sel-09-20 and Pant Sel-09-08
Cluster II	16	Pant Sel-01-15, Pant Sel-09-33, Pant Sel-08-07-01(CT), Kufri Giriraj, Pant Sel-09-01, Pant Sel-09-07, Pant Sel-09-11, Pant Sel-09-50, Pant Sel-15/5, Pant Sel-09-19, J-97-242, J-96-54, J-96-288, Kufri Badshah, Kufri Khyati and J-95-225
Cluster III	7	Pant Sel-09-46, Pant Sel-09-43, Pant Sel-09-55, Kufri Surya, Kufri Ashoka, Pant Sel-09-18 and Kufri Arun
Cluster IV	5	J-93-159, Kufri Gaurav, Kufri Pushkar, Kufri Frysona and Kufri Jawahar
Cluster V	3	Pant Sel-08-02, Pant Sel-09-21 and Kufri Himalini
Cluster VI	6	Pant Sel-08-11, Pant Sel-09, Kufri Chipsona-3, Pant Sel-09-38, Pant Sel-09-04 and Kufri Chipsona-1
Cluster VII	6	Pant Sel-09-53, Pant Sel-09-58, Kufri Sutlej, Kufri Jyoti, Pant Sel-01 and Kufri Chipsona-2
Cluster VIII	2	Pant Sel-09-03 and Kufri Bahar
Cluster IX	1	Pant Sel-09-57

Fig.3 Dendrogram illustrating the phylogenetic relationship among 48 potato genotypes based on UPGMA cluster analysis



However, Kufri Sutlej and Kufri Chipsona-2 shared a common cluster i.e. cluster VII. The dendrogram of the cluster analysis shows that germplasm of similar names the Kufri varieties and Pant Selection series were grouped into different clusters this may be because they share the place or method of cultivation only (Galretta *et al.,* 2007). Limited or low kinship relationship between morphological and molecular clustering was recorded in potato may be due to high heterozygosity, polyploidy and rigorous selection during breeding. Similar results were reported by Pattanayak *et al.,* (2010); Kumar *et al.,* (2017) and Hoque *et al.,* (2013). Kameswari and Girwani (2017) and Chaudhary *et al.,* (2018) concluded that, molecular marker technique can be successfully applied to determine the genetic fidelity of potato plant.

Conclusion

The RAPD markers used in the study were found to produce polymorphic bands and resulted into differentiation among various genotypes under study. Thus, by means of RAPD markers the genetic diversity assessed and the 48 potato genotypes analyzed in this study were identified. From this study, it may be concluded that significant diversity and variability was present among the genotypes and divergence analysis using RAPD markers was proved to be better than morphological data for discrimination among genotypes. It is clear that RAPD are useful for plant species that have an asexual means of propagation because genetic variation is fixed within a line. This characterization and documentation of diversity status of Indian varieties is likely to help in sustained potato improvement programme using diverse parents. The results so obtained through the present investigation at molecular level also elucidate the efficiency of the statistical methodologies used.

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