



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2018; 6(2): 3073-3076

© 2018 IJCS

Received: 16-01-2018

Accepted: 17-02-2018

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Molecular markers play key role to evaluate and distinguish different cultivars of potato (*Solanum tuberosum* L.) on the basis of genetic diversity

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Abstract

Twenty Random Amplified Polymorphic DNA (RAPD) markers were used for assessing the genetic diversity and molecular characterization of 41 potatoes genotypes/cultivars. Out of 20 RAPD primers only 9 were amplified and produced polymorphic bands. Total 81 scorable fragment were recorded whereas, 72 polymorphic and only were 9 monomorphic. Similarities among the genotypes/varieties were ranged from 69 to 94 percent. Primers OPA-02, OPA-11 and OPA-18 produced 100 percent polymorphic bands.

Keywords: *Solanum tuberosum* L., RAPD, genetic diversity, primers

Introduction

Potato (*Solanum tuberosum* L.), is an important edible tuber crop belonging to family Solanaceae. It is believed that the cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) originated from another tetraploid subspecies, *S. tuberosum* ssp. *andigena*, which probably evolved from its wild diploid ancestors in the basin of Lake 'Titicaca' in Peru – Bolivian borders (Cadmen, 1942) [2]. The cultivated potato has narrow genetic base due to limited introduction of germplasm from their natural range in South America (Rauf *et al.*, 2010) [11]. Most of the potato cultivars are autotetraploid (2n=4x=48), highly heterozygous and out breeding species which suffer from inbreeding depression. An increase in its heterozygosity results in producing more hybrid vigour (Tarn and Tai, 1977) [15]. Heterosis in potato perpetuates because of its vegetative propagated nature.

Traditionally, morphological characterization is the first step in description and classification of genetic resources (Smith and Smith, 1989) [13] and the traits such as leaf type, tuber shape, skin colour and flower colour *etc.* have been used to identify potato genotypes (Chimote *et al.*, 2007) [4]. However, these traits can be influenced by many factors like environment, leading to lack of reliability and reproducibility in this method. Therefore, characterization of genotypes using molecular markers has become important tools in studies of genetic diversity (Rocha *et al.*, 2010) [12], due to the high resolution and reliability in the identification of cultivars. It is greatly used in breeding programmes for improving the traits because such techniques directly reveal genetic variability through DNA analysis (Staub and Serquen, 1996) [14] and therefore their detection is not influenced by environmental effects. It also helps in cultivar protection, mainly in seed tuber of potato, as well as ensures the trademark and intellectual property rights (Coombs *et al.*, 2004) [5].

Mostly the RFLP, RAPD, AFLP, ISSR and SSR markers are suitable for assessing the genetic diversity of genotypes (McGregor *et al.*, 2000) [10] as their common origin is point mutation or chromosome rearrangements, which accumulates during the evolution of the species without any negative influence in their survival and reproduction. RAPD markers have the advantage of detecting polymorphism in a simple way simply and quickly, but it lacks reproducibility (Chakrabarti *et al.*, 2001) [3], while SSR (simple sequence repeat) markers or microsatellites provide high reproducibility and genetic information (Braun and Wenzel, 2005) [1]. The choice of the most suitable marker to be used depends on the labor required for their detection, possibility of revealing single or multiple loci, dominant or co-dominant nature and costs. The use of molecular markers in potato breeding offers new opportunities for the selection of genotypes and today many markers linked to useful traits have been found.

Materials and Methods

The genomic DNA was extracted according to CTAB (Cetyl Trimethyl Ammonium bromide) method as described by Doyle and Doyle (1990) [7] with some modifications from fresh young leaves. About 5 gm fresh leaves were weighed and cut into small pieces. These were frozen in liquid nitrogen and grounded to a fine powder in a pre-chilled mortar. The powder was transferred to a 50 ml centrifuge tube containing 15 ml of DNA extraction buffer and incubated at 65 °C for 30 min. The tubes were cooled at room temperature. Equal amount of chloroform: iso amylalcohol (24: 1 volume) was added and mixed thoroughly by gentle inversion and centrifuged at 8,500 rpm for 20 min. at 27 °C. Then the upper layer was transferred to a fresh centrifuge tube and 0.6 v/v of chilled isopropanol and 0.1 volume of 3 M sodium acetate were added to it and kept overnight at -20 °C. The tubes were mixed thoroughly by quick gentle inversion. The tubes were spun at 8,500 rpm for 20 min at 4 °C and pellet so obtained was washed with 70 percent ethanol and dried by inverting the tube on paper towel for 10 min. Finally, the pellet was dissolved in TE buffer and kept at -20 °C.

Five µl RNase (10 mg/ml) was added to 100 µl of dissolved DNA and incubated at 37 °C for 1 hour. Equal volume of tris saturated phenol: chloroform: iso amylalcohol (25: 24: 1) was added and mixed gently by inverting the tubes. Centrifuge the tubes at 8,000 rpm for 5 minutes and the aqueous layer (i.e. upper layer) was collected. To this layer equal amount of chloroform + isoamyl alcohol (24: 1) was added. The tubes were then spun at 8,500 rpm for 5 min and top layer of DNA was removed. To this, 3 M sodium acetate (1/10 vol, pH = 5.2) and chilled absolute ethanol was added. After that the contents were mixed and kept at -20 °C for 30 min.

Centrifuge the content to obtain the pellet. Finally the pellet was washed with 70 percent ethanol, dried and dissolved in 100 µl TE buffer. The qualities of DNA were assessed using 1% agarose gels (El_Komy *et al.*, 2012) [8].

PCR Amplification (Williams *et al.*, 1990) [16] was performed with arbitrary decamer primers obtained from IDT. Amplifications were performed in 25 µl volume which perceived 1 µl 10µM primer, 0.3 µl *Taq* DNA polymerease (5 U µl⁻¹), 2.5 µl 10 X PCR buffer, 1µl 10 mM MgCl₂, 1 µl 2 mM dNTPs (of each), 1 µl of template DNA (approximately 50 ng) and 18.2 µl sterilized deionized water. The RAPD reaction was done in Eppendorf Thermocycleras with following programmes: one cycle of 94 °C for 5 min as initial denaturation, 35 cycle of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min and final extension cycle at 71 °C for 10 min. The PCR product was analyzed by electrophoretic separation in 1.2% agarose gels which was stained with 0.5 µg ml⁻¹ ethidium bromide.

Results and Discussion

Out of 20 RAPD primers only nine were amplified and gave polymorphic results. These 9 RAPD primers were further used for assessing the genetic diversity of 41 potato genotypes and they produced a total 81 reproducible loci. Out of 81 bands, 72 were polymorphic and only 9 were monomorphic (Table 1). Each primer varied greatly in their ability to identify variability among the genotypes/varieties. The primers were amplified minimum 5 (AC-15 and AC-20) to maximum 13 (OPA-18) loci with an average of 9 loci per primers. The polymorphism percentage was ranged between 60 (AC-20) to 100 percent (OPA-02, OPA-11 and OPA-18). Average polymorphic band were recorded to be 8 per primer.

Table 1: Analysis of RAPD markers

S.NO	PRIMER	SEQUENCE (5'-3')	% GC	Number of bands			% Polymorphic bands
				Total bands	Mono bands	Poly. Bands	
1	OPA-01	CAGGCCCTTC	70	11	2	9	81.81
2	OPA-02	TGCCGAGCTG	70	11	0	11	100.00
3	OPA-11	CAATCGCCGT	60	7	0	7	100.00
4	OPA-18	AGGTGCCGTT	60	13	0	13	100.00
5	AC-15	TGCCGTGAGA	60	5	1	4	80.00
6	AC-20	ACGGAAGTGG	60	5	2	3	60.00
7	TIBMBB-17	ACACCGTGCC	70	9	2	7	77.77
8	TIBMBB-02	TGCTCGGCTC	70	11	1	10	90.09
9	TIBMBB-16	CTGGTGCTCA	60	9	1	8	88.88

Genetic Diversity Analysis

The clustering was done with the help of similarity matrix based on Jaccard's coefficient, which ranged from 58 to 94 percent. Pant Sel-01 and Pant Sel-09-20 (94%) were found most similar genotypes among the 41 studied genotypes/varieties, followed by TPSK-05-06-007 and Pant Sel-08-07-1(CT) with 93 percent similarity (Fig.1 and Fig. 2). Forty-one genotypes were separated into two major clusters I and II at the demarcation of 69% similarity, which was further divided into two sub clusters each with approximately 74 and 72 percent similarity, respectively. Cluster I comprised 18 genotypes while cluster II found 23 genotypes. Cluster I further subdivided into two sub-clusters IA and IB each with approximately 75 and 77.5 percent similarity, respectively.

Main cluster II further divided in to two sub cluster IIA and IIB. IIB comprised two genotypes (Lady Rosetta and Pant Sel-09-33), whereas IIA cluster further divided into two small sub clusters IIA1 and IIA2 with approximately 82.5 and 76.5 percent similarity.

Out of 9 random primers used, OPA-18 were amplified 13 loci with all polymorphic bands. OPA-1 primer were amplified 81.81% polymorphic bands (Fig. 2). Several workers reported similar type of observation on potato with RAPD primers. Rocha *et.al* (2010) [12] reported 1 to 8 bands with RAPD primers while El_Komy (2012) [8] observed 9 to 26 bands and found approximately 71 percent similarity between the genotypes Atlantica and Lady Rosetta.

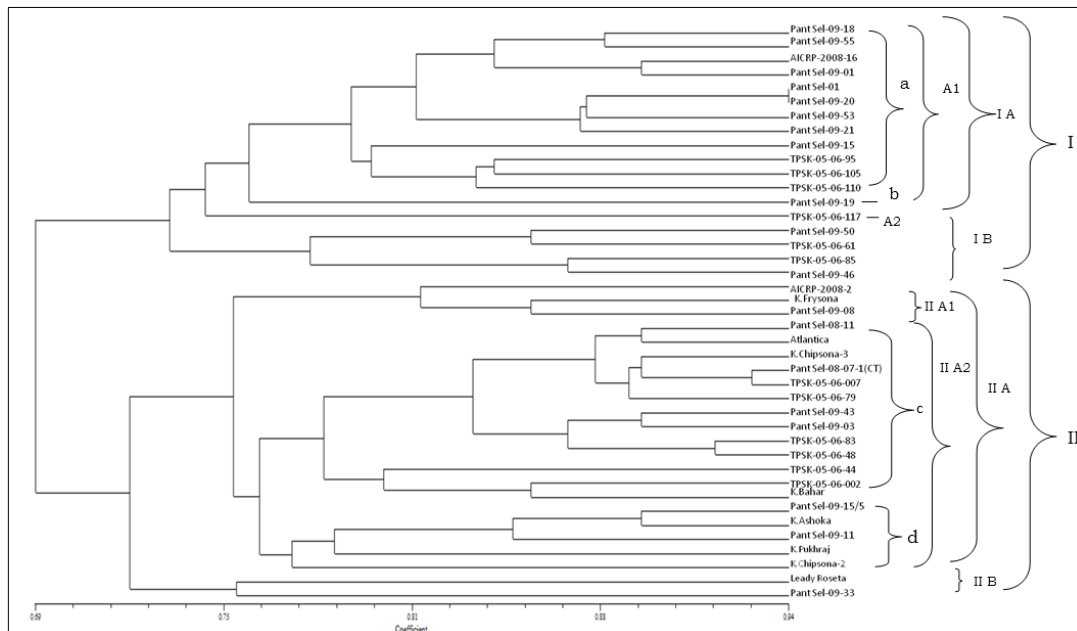


Fig 1: Dendrogram depicting the classification of the 41 Genotypes/ varieties of Potato (*Solanum tuberosum L.*) constructed using UPGMA method and based on RAPD. The scale at the bottom is Jaccard's coefficient of genetic similarity

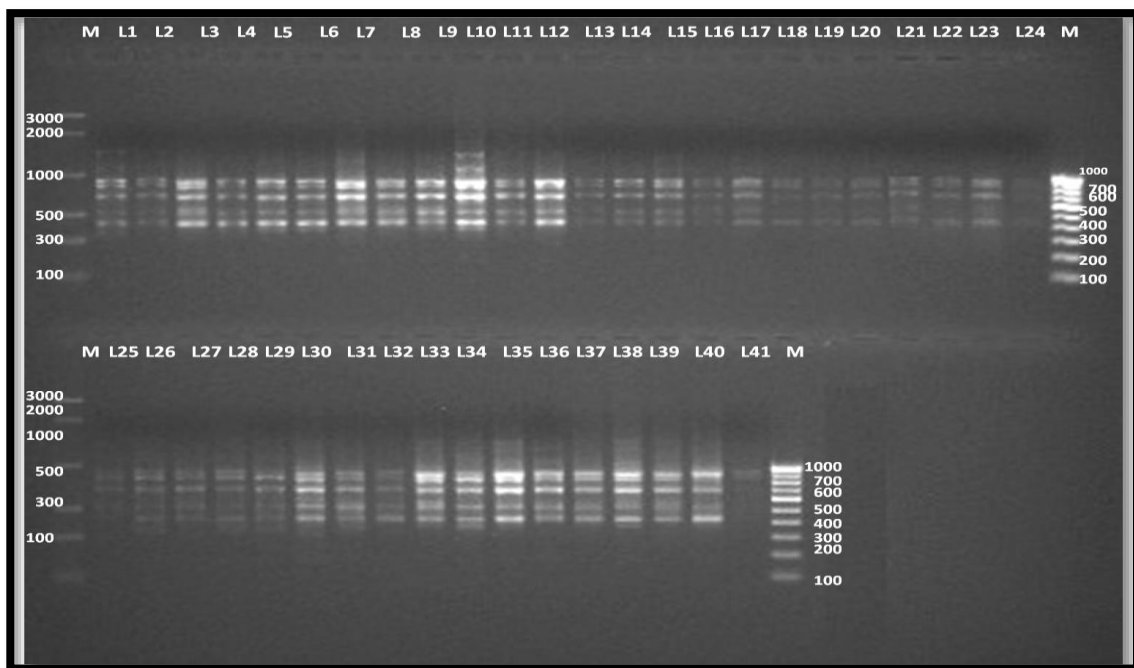


Fig 2: PCR amplification of 41 Potato genotypes/varieties by RAPD primer OPA-01

Hosaka *et al.* (1994) ^[9] studied the genetic relationship of 73 Japanese potato cultivar and reported that RAPD banding pattern of closely related were cluster together and they concluded that banding pattern are reflection of the pedigree relationship however Demeke *et al.* (1996) ^[6] studied genetic diversity of 28 North American potato cultivars and observed exception to be observation and recorded that cultivar with close kinship can often as genetically diverse as those with no immediate relationship. In this study also found that kinship relation could not reflected in the similarity of banding pattern. This is probably due to highly heterozygous nature of the tetraploid potato genome.

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