

RAPD and Protein Analyses Revealed Polymorphism in Mutated Potato Cultivars

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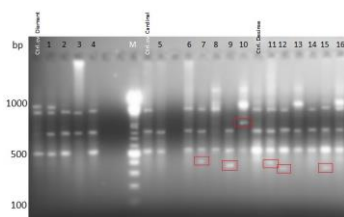
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Graphical abstract



Abstract

Genomic DNA of the mutant lines of the three potato cultivars, Cardinal, Diamant and Desiree, with respect to controls were isolated and analyzed for polymorphisms by using random amplified polymorphic DNA (RAPD) markers. Four 10 bp random fragment primers, S-13, S-18, S-19 and R-17 were studied and all of them gave the amplification of genomic DNA. All of the mutant lines gave different banding pattern against different primers with respect to control plants of the three varieties, and bands are present at 50 bp to 1500 bp. All these primers with specific banding pattern were unique in their polymorphic behavior. Different banding pattern of total protein contents were also observed by PAGE analysis of all the mutant lines as compared with the control plants. It is therefore suggested that RAPD and protein analyses would be important tools to detect the polymorphism in mutated lines of potato.

Keywords: Potato; RAPD; polymorphism; PAGE; mutation

Abstrak

DNA genomik barisan mutan daripada tiga kultivar kentang, *Cardinal*, *Diamant* dan *Desiree*, dengan aspek untuk mengawal telah diasingkan dan di analisis untuk polimorfisme, dengan menggunakan secara rawak, penanda DNA polimorfik (RAPD) yang telah di amplifikasikan. Empat 10 bp serpihan primer rawak, S-13, S-18, S-19 dan R-17 telah dikaji dan ia semua telah memberikan amplifikasi DNA genom sebagai hasil. Semua barisan mutan memberikan corak banding yang berbeza terhadap primer yang berlainan dengan aspek untuk mengawal tiga jenis tumbuhan berkenaan, dan ban-ban yang di gunakan adalah pada lingkungan 50 bp sehingga 1500 bp. Kesemua primer ini, dengan corak banding yang spesifik, adalah unik dalam tingkah laku polimorfik mereka. Corak banding berbeza daripada jumlah kandungan protein juga di kaji oleh PAGE analisa terhadap semua barisan mutan dibanding dengan tumbuh-tumbuhan kawalan. Dengan ini, ia di nasihatkan bahawa RAPD dan protein analisis merupakan mekanisma penting bagi mengesan polimorfisme di dalam barisan mutan kentang.

Kata kunci: Kentang; RAPD; polimorfisme; PAGE; mutasi

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1.0 INTRODUCTION

The cultivated potato (*Solanum tuberosum* L.) belongs to the family solanaceae, also called nightshade family. Potato enjoys a special place among vegetables in the world. The area and production in Pakistan has been tremendously increased since independence of the country. During 1947-48, the area under potato crop was only 2,800 ha and the total production was about 28,400 tons, whereas in 2006-2007 the area increased to 133,000 ha while production increased to 2,582,000 tons. (Agri. Stat. of Pak, 2006-2007).

In the last fifty years of conventional breeding, several potato cultivars were developed with good agronomic qualities, including high productivity and disease resistance. However, there are growing difficulties in developing new cultivars that have advantages over the existent in the market, using traditional methods (Vayada and Belknap, 1992). Genetic variability is essential for any breeding program aiming to select new plateaus of productivity and quality. Therefore, finding new methods for producing variability is of great importance, mainly if the genetic variability in the crop is low, as in potato (Kumar, 1994).

Plant cell and tissue culture has been seen as a valuable source of genetic variability for breeding programs. The regenerated plants may present *in vitro* genetic variation, induced during the cultivation phase. These changes include cariotypic alterations, changes in chromosome array and number and also point mutations (Larkin and Scowcroft, 1981). Associated to tissue culture mutagens can be used to increase the frequency of mutations (Hall *et al.*, 1992; Cuny *et al.*, 1993). Therefore, these techniques allow altering one or more desirable traits, improving already existing cultivars and developing new genotypes (Karp, 1989; Love *et al.*, 1996a). However, these mutagens can cause different effects at the cellular level, as changes and blocking of cellular division, they affect the *in vitro* regeneration of plants and even cause cellular death (Hell, 1983; Montalván and Ando, 1998).

Mutation breeding has been largely used in potato regarding improvements of tuber quality in amino acid composition, dry matter content, pulp color, starch composition, black spot susceptibility and sugar content (Love *et al.*, 1996b). To determine the occurrence of genomic variation, several techniques have been proposed and applied. Among them, RAPD (Random Amplified Polymorphic DNA), is a molecular marker technique that addresses polymorphism at the DNA level (Klerk, 1990; Welsh and McClelland, 1990; Willians *et al.*, 1990; Ferreira and Grattapaglia, 1996). This technology is based on the amplification of DNA segments at random, with ten base long primers, with arbitrary nucleotide sequences (Willians *et al.*, 1990). RAPD markers have been used in the construction of genetic maps, in the assisted selection of desirable traits, in determining genetic identity and diversity, in obtaining fingerprints for cultivar registration, in estimating genetic distances and in population genetics (Timmerman and McCallum, 1993).

Powell *et al.* (1995) reported that molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors and are easily accessible to breeders and biotechnologists. The RAPD is used for the characterization of plant species, and distribution of variability within their gene pools. This has resulted in RAPD becoming a favored method for analysis of mutants. Its results are very reliable and informative when the technique is carefully applied. It allows analysis of very small genetic differences that cannot be easily uncovered with alternative techniques. This technique has been used extensively in plant breeding studies and applications to potato, straw berries, wheat, oat, barley, soyabean, tomato and corn have all been reported by (Deragon *et al.*, 1992).

The present studies address to the induction of variation in imported Dutch varieties of potato (Cardinal, Diamant and Desiree) through the use of various treatments of Colchicine and Sodium azide as chemical mutagens and to analyze the genetic variability of mutant lines with control plant of potato by RAPD.

■ 2.0 EXPERIMENTAL

2.1 Plant Materials

Three potato (*Solanum tuberosum*) cultivars were used in this study as explant sources for tissue culture and genomic assessment by RAPD analysis namely Cardinal, Diamant and Desiree. Meristem was isolated from virus free potato plantlets, and the isolated apical meristem kept on MS media supplemented with 1% sucrose; 0.01% myoinositol, 0.01% casein hydrolysate and 0.2% phytagel.

2.2 Plant Mutation

Callus pieces of potato cvs. Cardinal, Diamant and Desiree of *in vitro* plantlets were mutated by Colchicine and Sodium azide chemical mutagens with different concentration (00 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM and 0.5 mM). Colchicine and Sodium azide were added after filter sterilization into 25 ml sterile distilled water. Callus pieces of 1cm³ size were put in each falcon tube and placed on rotary shaker for 1 hour, at 150 rpm, so that the callus pieces absorbed maximum amount of mutagen. After one hour the callus pieces were removed from the Colchicine and Sodium azide treated water and placed on regeneration media. For each mutagenic treatment 200 callus pieces of one cm³ were used.

2.3 DNA Extraction

Total genomic DNA was isolated from mutated cultivars and control potato plants by using the method described by Dellaporta *et al.* (1983).

2.4 Quantification of DNA

To check the quality and quantity of the extracted purified DNA, 0.8% agarose gel electrophoresis was done. DNA samples were compared with that of undigested λ DNA (0.5 μ g/1.5 μ l) and thus the exact quantity of each DNA samples was determined.

2.5 Amplification Conditions and Analyzing

To amplify genomic DNA of potato mutant plants with respect to control plants, the primers were designed for the amplification of 10 bp random fragment of genome. The sequences of the primers are shown in Table (1).

Table 1 Primer sequences used in this study

Primer	Sequence (5' to 3')
S-13	5'-GTGGTTCCTG-3'
S-18	5'-CTGGCGAACT-3'
S-19	5'-GAGTCAGCAG-3'
R-17	5'-CCGTACGTAG-3'

The amplification reactions were performed in volumes of 25 μ l containing 2.5 μ l (10X) PCR buffer (500 mM KCl, 15 mM MgCl₂, 100 mM Tris-HCl pH 8.8 and 10% triton 100X), 5 μ l (1 mM each) dNTPs, 1.0 μ l (two unit) Taq polymerase, 2.0 μ l (2.5mM) 10 mer primers, 5 μ l (10 ng/ μ l) genomic DNA and 9.5 μ l water (autoclaved double distilled). 2-3 drops of PCR mineral oil was added in each tube and kept in PCR thermocycler. Amplifications were carried out in a Thermal Cycler MP (TAKARA) and PTC100 Programmable Thermal Controller (MJ Research Inc., USA) as follows:

94 °C for 5 minutes followed by 35 cycles of 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for two minutes and ending with 5 minutes at 72 °C for the final extension.

The 5 μ l of 6X DNA loading dye (Bromo phenol blue mixed with 10% glycol, 0.1 M EDTA and 2% SDS) was added to the reaction mixture. 30 μ l of the reaction mixture was loaded

on 2% agarose gel with ethidium bromide in (IX) TBE buffer. The amplification products were viewed under UV transilluminator and photographed.

3.0 RESULTS AND DISCUSSION

3.1 Isolation of Genomic DNA & RAPD Analysis

Genomic DNA of the different potato varieties was isolated by using Dellaporta *et al.*, (1983) DNA extraction method. Quantification of extracted DNA was carried out by using 0.8% agarose gel along with uncut lambda DNA. The genomic size of the control and mutant lines DNA bands were compared with the band of lambda DNA (100 bp) in the gel. As DNA was very concentrated so it was diluted to 10 ng/μl. This template DNA was finally used for RAPD analysis. The template DNA of different lines was checked for four different primers S-13, S-18, S-19 and R-17. All four primers showed amplification of the template DNA.

All the mutant lines of the three potato varieties along with their respective control lines were selected and analyzed by the four primers in Table 1. All the four primers detected the polymorphic band of different frequencies. The mutants and control plants of each variety were subjected to RAPD analysis. The RAPD analysis was used to further confirm the genomic variations in mutant lines. In the entire mutant lines different DNA banding pattern was observed as compare to control indicating the presence of some additional bands with variable thickness indicating the genomic variability in the mutant lines. This observation was also supported by Forapani *et al.* (1999) and Taylor *et al.* (1995). They observed the same variation in banding pattern for DNA polymorphism in different crops.

There is a set of two bands of 300 bp in lane 12 and 15, a band of 350 bp in lane 9 and 11, a band of 400 bp in lane 7 and a band of 900 bp in lane 10 are present as additional bands in the genome of variants while a band of 800 bp in control cv. Diamant lane and lane 10 are the missing bands from the genomes of these plants where as present in all other mutant genomes amplified with same S-19 primer (Figure 1).

The genome of cv. Cardinal mutants in lane 4, 5, and 6 showed only a characteristic band of 1300 bp when amplified with S-13 primer while other mutants of the same variety showed amplification of more bands in the range of 400 bp to 1500 bp in control cv. Cardinal and lanes 1, 2 and 3 respectively (Figure 2).

The genome of cv. Desiree mutants in lane 2, 3 and 4 amplified a band of 50bp that is missing in other plants of the same variety. The genome of all mutants showed amplification in the range of 50 bp to 1000 bp with S-13 primer respectively (Figure 3).

A characteristic additional band of 50 bp in lane 9 of the cv. Diamant mutant is amplified with S-18 primer which is not present in all other mutants of the same variety. Similarly a band of 550bp is missing in lane number 1, 2, control cv. Diamant lane and lane 8 of cv. Cardinal and cv. Diamant mutants, which was present in all other mutants when amplified with the same primer. All the mutant plants of these both varieties showed amplification in the range of 50 bp to 1000 bp (Figure 4).

A band of 900 bp is missing in lane 5 which is present in the genome of other mutants of cv. Cardinal amplified with R-17 primer (Figure 5).

It is highly desirable to understand the molecular bases of mutational variation, so that it can be used with out fear of loss of genetic trait. Plant breeders need to be convinced that the stable mutant lines are softer to use in breeding new varieties.

These results are in agreement with previous studies that have also shown RAPD analysis to be useful in estimating reliable genetic relationships among potato cultivars (Isenegger, A. D. *et al.*, 2001).

3.2 PAGE Analysis

Table During the past 20 years protein revealed through electrophoresis have been the genetic markers most frequently employed in many crop species. Proteins are the most widely employed biochemical markers used for the identification of cultivars as well as for the characterization of induced mutations. (Damiano *et al.*, 1995). They could be assayed from a wide variety of organs and tissues, and analytical procedures were not exceptionally complicated (Weeden and Wendel., 1989).

In the present study, quantitative analysis of total soluble proteins was performed by PAGE in order to evaluate the variations in the mutant lines of the potato varieties cv. Cardinal, cv. Desiree and cv. Diamant.

Equal quantities of total soluble proteins were run on PAGE for the control and mutant lines of the three cultivars. In case of cv. Cardinal there are few characteristic protein molecules around 97 kDa in the mutant lines of cv. Cardinal in lane 1 and lane 5. These proteins are highly induced due to the effect of mutagenesis. This data shows that due to the in vitro induction of mutation in potato cultivars some genes become over expressed and thus producing their respective proteins in more quantity. Similarly there are some additional protein bands around 14 kDa observed in mutant line of cv. Cardinal in lane 1, 3 and 5 while they are absent in the control line of the same cultivar (Figure 6).

Two additional protein molecules around 75 kDa and 80 kDa were induced in the mutant lines of cv. Desiree in lane 1 and 5 while missing in the control line of cv. Desiree (Figure 7). Similarly in the mutant lines of cv. Diamant two additional protein molecules around 70 kDa and 75 kDa were induced due to the in vitro effect of mutagenesis while the same protein molecules are absent in the control line of the same cultivar (Figure 8).

Dye color	Cell form	% of COD removed
Mixed	Immobilized	63.49
	Free cell	29.25

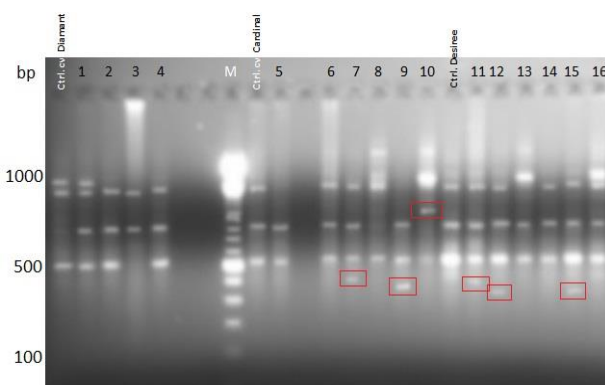


Figure 1 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Cardinal, Diamant and Desiree using S-19 Primer. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4 from lane 1-4, M1, M2, M3, M4, M5, M6 from lane 5-10 and M1, M2, M3, M4, M5, M6 from lanes 11-16, respectively. Lane M represents the ladder

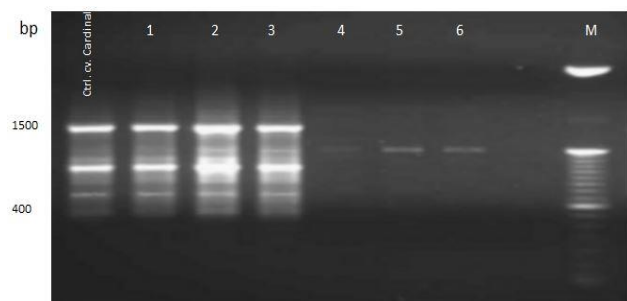


Figure 2 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Cardinal using S-13 Primer. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4, M5 and M6 from lane 1-6, respectively. Lane M represents the ladder

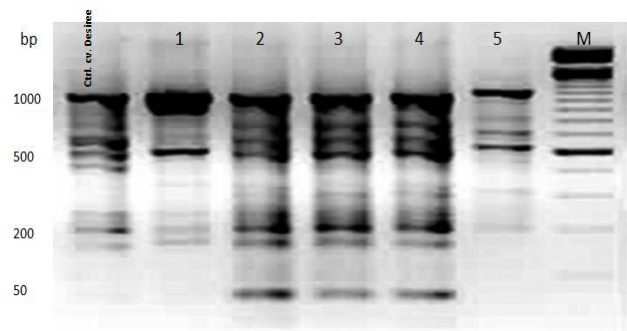


Figure 3 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Desiree using S-19 Primer. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4, M5 from lane 1-5, respectively. Lane M represents the ladder

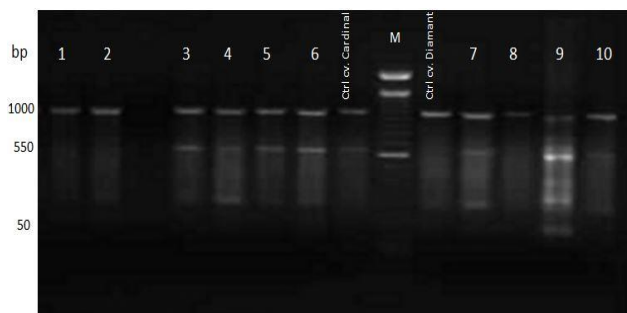


Figure 4 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Cardinal and Diamant using S-18 Primer. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4, M5, M6 from lane 1-6, M1, M2, M3, M4 from lane 10-13. Lane M represents the ladder

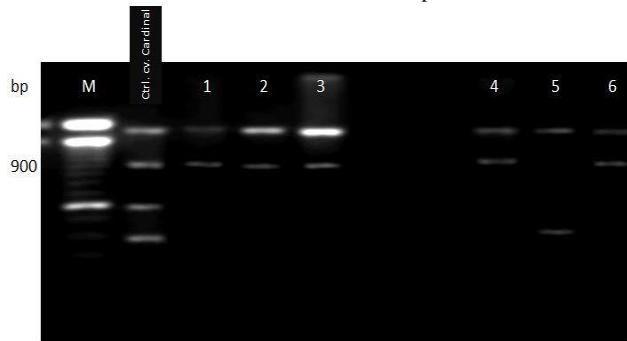


Figure 5 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Cardinal using R-17 Primer. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4, M5, M6 from lane 1-6. Lane M represents the ladder

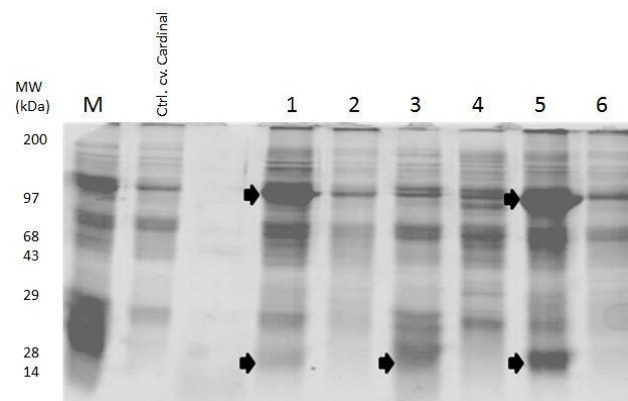


Figure 6 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Cardinal. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4, M5, M6 from lane 1-6. Lane M represents the ladder

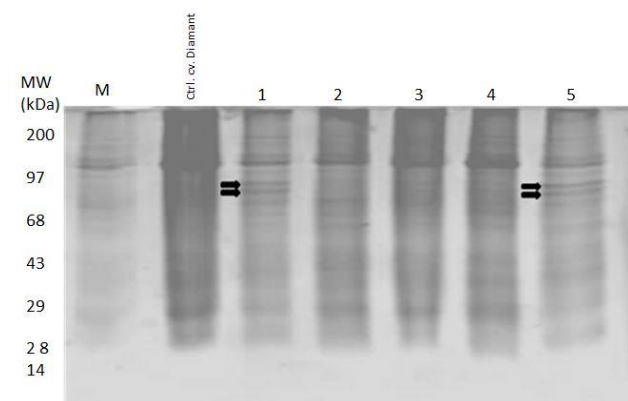


Figure 7 Agarose gel electrophoresis, RAPD Analysis of mutant cv. Desiree. Shown are the size differences of PCR products and RAPD patterns of M1, M2, M3, M4, M5 from lane 1-5, respectively. Lane M represents the ladder

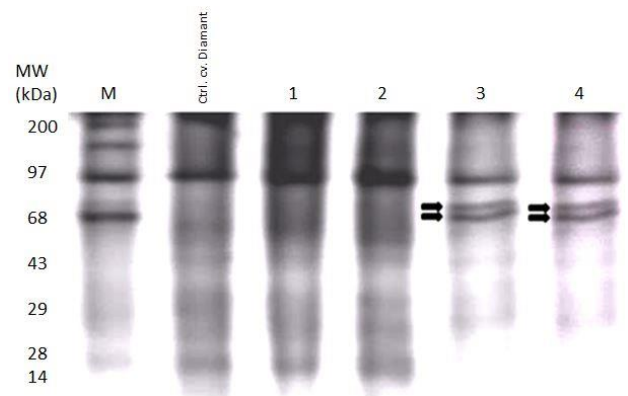


Figure 8 Agarose gel electrophoresis, RAPD Analysis of Diamant. The size differences of PCR products and RAPD patterns of M1, M2, M3, M4 from lane 1-4, respectively. Lane M represents the ladder

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