Molecular analysis of mutant of potato (*Solanum tuberosum* L.) cultivar Atlantic

E Mardiyah¹,², S Samipak³ and Suharsono²*

¹Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Jl Agatis Kampus IPB Dramaga, Bogor, 16680, Indonesia
²Research Center for Bioresources and Biotechnology, IPB University, Jl. Kamper Kampus IPB Darmaga, Bogor, 16680 Indonesia
³Department of Genetics, Kasetsart University, 50 Ngam Wong Wan Road, Lat Yao, Chatuchak, Bangkok, 10900 Thailand

*Corresponding author: sony-sh@apps.ipb.ac.id

Abstract. Gamma irradiation has been applied to potato (*Solanum tuberosum* L.) cultivar Atlantic and IPB CP1 cultivar is one of mutant clones had been obtained. RAPD was applied as molecular marker to analyze the difference between the potato cv IPB CP1 and cultivar Atlantic. A total of 24 primers composed of single random primers and combination of two random primers were used to amplify DNA fragments from the genomic DNA of potato cultivar IPB CP1 and Atlantic. These primers are considered as loci and the amplicon generated by this primer is considered as allele. The number of allele indicated by the number of amplified DNA fragment ranged from 3 to 16 alleles depending on the locus represented by the primer. The combination of two random primers produced the highest polymorphic fragments. The difference between potato cultivar IPB CP1 and Atlantic is determined by 4 loci among 24 loci of RAPD, as OPA-02, OPA-02 + OPA-03, OPA-02 + OPA-07, and OPA-01 + OPA-13. Locus OPA-02 + OPA-03 contains the highest genetic difference with 2 polymorphic fragments (22.22 %). This research had successfully detected molecular difference between potato cv Atlantic and IPB CP1 as its mutant using RAPD marker.

Keywords: IPB CP1, loci, potato cv Atlantic, RAPD, polymorphism

1. Introduction

Potato (*Solanum tuberosum* L.) is a staple food that play an important role in global food [1] Cultivated potatoes have spread from the Andes of South America where they originated to 160 countries around the world [2]. In 1811, the potato crops already spread over the high mountains of Indonesia [3]. Potato production in Indonesia in 2017 reached 1.16 million tons [4]. Potato production from 2011 to 2014 continued to increase. However, from 2015 to 2017 potato production continued to decrease [4]. Potato production in 2017 decreased by 3.98 percent compared to 2016 [4].

Crop improvement is one of the benefits offered by induced mutation [5]. The induction of mutation can be done by chemical or physical mutagens (the source agent that change the genetic material of an organism) [6]. For inducing variation and quality enhancement, the use of ionizing radiation, such as X-rays and gamma have been used in the improvement of major crops [7]. Mutation breeding is commonly used especially when there is nosource ofvariation in nature that could be introduced to varieties by ordinary breeding techniques or there is constraint in conventional breeding such as sexual incompatibility, inbreeding depression, and extensive breeding cycles [8].
Improving plants via mutation could produce the development of varieties [9]. Irradiation studies with different doses in potato have been found to make the mutants showed an increase in the average number of tubers, tuber size, and tuber weight in comparison with non-treated control [10], salt-tolerant potato plants were successfully created for in vitro tissue cultures by mutation induction using 15, 20, and 30 Gy gamma irradiation [11], and present studies were undertaken to induce variation for heat tolerance in two commercial cultivars of potato, ‘Kufri Jyoti’ and ‘Kufri Chandramukhi’ through gamma irradiation using 20 and 40 Gy [12].

The next step after mutation induction is to detect the changes in the selected mutant. The process of identifying mutant plants involving something used for distinguishing which is called a marker [13]. Molecular genetic markers have become a reliable tool in providing a relatively unbiased estimation of genetic diversity and phylogeny in plants [14]. Over the last decade, molecular genetic based on polymerase chain reaction (PCR) has become a main technique for several novel genetic assays based on selective amplification of DNA [15]. Random Amplification of Polymorphic DNA or RAPD is a type of PCR reaction in which the segments of DNA are randomly amplified [13]. The RAPD analysis is a molecular marker that generally used in genetic diversity studies [13]. A number of scientists have used RAPD markers to learn about polymorphism in different variety of plants [16]. RAPD is an effective way to differentiate between potato varieties and treatments [17].

Atlantic is potato variety, selected from the progeny of a cross between Wauseon and USDA B5141-6 (Lenape) [18]. It was released by the USDA breeding program in 1978. Atlantic has demonstrated the following characteristics: moderately large plants with thick and upright stems, bright, medium green, and smooth leaves, oval to round with light-to-heavy scaly netted skin, white flesh tubers, good processing quality, and acceptable fresh market quality [18].

IPB CP1 (Chip Potato 1) or Sipiwan potato cultivar is a new potato variety obtained by gamma irradiation. It was registered at Tanda Daftar Varietas Tanaman Hortikultura, Kementerian Pertanian No.019/Kpts/SR.120/D/2/7/1/2019. IPB CP1 potato cultivar has slightly round tuber shape, white bulbs skin color, white tubers, high starch content, and low sugar content [19].

Analyzing the differences by molecular markers is necessary to estimate genetic variation resulted from irradiation. The objective of this research was to analyze the difference between potato cv IPB CP1 and cv Atlantic using random amplified polymorphic DNA marker.

2. Materials and methods

2.1. Plant materials and primers
Potato cv Atlantic and its mutants, IPB CP1, were used as plant materials. MS medium [20] used for in vitro culture. Primer Tact-qF (ACATCTCTTATGCTTGA) and Tact-qR (GTGGAGCAT GGAAGGACCAG) were used to amplify the actin gene. Random primers from Operon Technologies, Inc. were used to compare between the potato cv Atlantic and its mutant.

2.2. Genomic DNA isolation
DNA isolation was done using the modified CTAB procedure. Three weeks old in vitro culture of potato plant wasground in the presence of liquid nitrogen in the mortar by using a pestle. The powder was transferred to 2% CTAB buffer solution, 5% polyvinylpyrrolidone (PVP), and 0.2% β-mercaptoethanol. The mixture was incubated at 65°C for 30 minutes. The mixture was extracted by chloroform-isoamyl alcohol (24:1) extraction, followed by centrifugation at 10 000 rpm for 10 minutes to denature proteins and facilitate phase separation. DNA was precipitated using an equal volume of isopropanol. The mixture was incubated in the freezer for 24 hours. The mixture was separated by centrifugation at low temperature (4°C) for 25 minutes. The pellet was washed by cold ethanol 70% and dried for 30 minutes and resuspended in 20 µl distilled water. Single-stranded RNA was digested with 1 µg/µl RNase for 30 minutes at 37°C.

DNA was verified in 1% agarose gel by loading the mixture of DNA and loading dye solution and quantified by measuring absorbance at 260 nm. After DNA quantification, DNA was diluted to make a template for PCR analysis.
2.3. Actin gene amplification

The integrity of DNA was confirmed by actin gene amplification using PCR. The PCR was carried out in 10 µl per tube containing 1 µl (100 ng) DNA, 5 µl master mix 2× AmpMaster™ Taq, 0.25 µl primer Tact-qF (2.5 pmol), 0.25 µl primer Tact-qR (2.5 pmol), and 3.5 µl H₂O. The PCR condition was: pre-PCR at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, primer annealing at 54°C for 1 minute 20 second, and extension at 72°C for 1 minute. Post-PCR was carried out at 72°C for 5 minutes. PCR products of actin gene amplification were run on 1.5% agarose gels to confirm the DNA integrity. 100 bp DNA ladder was used. The bands were visualized by ethidium bromide under UV transilluminator, photographed and analyzed by using Gel Doc UV Transilluminator. The size of the bands were estimated.

2.4. RAPD-PCR

RAPD was carried out by PCR using random primers. The PCR was carried out in 10 µl per tube, containing 1 µl DNA (100 ng), 5 µl master mix 2× AmpMaster™ Taq, 0.5 µl RAPD primer (10 pmol), and 3.5 µl H₂O. The PCR condition was: pre-PCR at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, primer annealing at 37°C for 1 minute, and extension at 72°C for 2 minutes. Post-PCR was done at 72°C for 5 minutes. PCR products of RAPD were run on 1.5% agarose gels to detect polymorphism between potato cv Atlantic and its mutant. 1 kb DNA ladder was used. The bands were visualized by ethidium bromide under UV transilluminator, photographed and analyzed by using Gel Doc UV Transilluminator. The polymorphism were estimated.

3. Results and discussion

3.1. Quality and quantity of DNA

The purpose of DNA extraction is to obtain DNA in a relatively pure which can be used for further investigations. The preparation of high quality DNA from polyphenolic containing plants such as field bean (Vicia faba), tomato (Lycopersicon esculentum), and potato (Solanum tuberosum) was challenging, because of the degraded DNA caused by secondary plant products such as tannins and phenolic terpenoids which may attract to DNA and/or RNA after cell lysis [21]. The use of higher polyvinylpyrrolidone (PVP) concentration (5%) as modification in the DNA extraction method following by cell lysis, precipitation and centrifugation selectively for deletion of PVP complexes, and DNA recovery has successfully isolated the DNA of potato (figure 1). High PVP percentage (6%) in the DNA extraction method is acceptable for the rapid isolation of many DNA samples [22].

For quantitating the amount of DNA, readings should be taken at wavelengths of 260 nm and 280 nm. The ratio between the readings at 260 nm and 280 nm provides an estimation of the purity of nucleic acid. The 260 nm/280 nm absorbance ratio of isolated genomic DNA of the potatoes is 1.4 and 1.5 for Atlantic and IPB CP1 cultivars respectively (table 1).

![Figure 1. Gel electrophoresis of genomic DNA. M: λ ladder, A: Atlantic cultivar, B: IPB CP1.](image)

| No | Cultivar | OD₂₆₀ (%) | OD₂₈₀ (%) | OD₂₆₀/₂₈₀ | DNA (ng/µl) |
|----|----------|-----------|-----------|------------|-------------|
| 1  | Atlantic | 24.50     | 16.9      | 1.40       | 8575        |
| 2  | IPB CP1 | 21.40     | 14.2      | 1.50       | 7490        |
3.2. Analysis of DNA integrity

Actin gene amplification is used to confirm DNA integrity. Actin is the most plentiful protein in most eukaryotic cells. It is highly conserved and takes part in more protein-protein interactions than any known protein [23]. By Tact-qF and Tact-qR primers, PCR resulted in the amplicon of 340 bp (figure 2). This result indicates that isolated genomic DNA contain intact actin gene, and has high integrity. Since the integrity of genomic DNA is high, it can be used as a template for PCR of other fragments of DNA.

![Figure 2. Gel electrophoresis of amplified actin gene. M: 100 bp ladder, A: Atlantic cultivar, B: IPB CP1.](image)

3.3. Analysis of RAPD

A total of 24 primers was used for RAPD. RAPD markers were used to estimate the structural alterations in DNA. We consider that every primer is a locus and the number of DNA fragments generated by each primer is allele. RAPD markers were efficient in differentiating somaclonal variants and mutants of potato induced by gamma radiation [10]. The DNA bands generated had been divided into two classification, monomorphic and polymorphic bands. Polymorphic bands gave important information in detecting changes in DNA. The change of DNA can be caused by the change of the DNA sequence due to the substitution of DNA and the change of length of DNA. The change of sequence DNA in the primer annealing sites cause the polymorphic DNA. Deletion or insertion changes the length of the DNA fragment without preventing its amplification [13].

3.3.1 Single primer. 1 primer produced polymorphism from the total of 14 RAPD primers (figure 3). Only OPA-02 primer produces polymorphism (Table 2). By the single random primer, Atlantic cultivar is almost similar to IPB CP1. From 14 loci of single primer we obtained 102 alleles, and only 1 allele is specific. To increase the polymorphism and the number of specific allele, we combined two random primers.

3.3.2 Combination of two random primers. The use of two random primers in this research increased polymorphism of DNA bands and several specific bands. PCR reaction by using only OPA-03 primer did not produce polymorphism, but when OPA-03 combined with OPA-02 primer, we obtained polymorphism of DNA bands. Our result showed that the combination of two random primers increases the polymorphism and the number of amplified bands (Table 3). The combination of two random primers (1:1) showed increase in the number of specific bands [24].

Among 10 combinations of two random primers, 3 combinations of primerproduced polymorphic bands, i.e. OPA-02 + OPA-03, OPA-02 + OPA-07, and OPA-01 + OPA-13 (figure 4). From 10 loci of random primer combination, we obtained 99 alleles, and only 4 alleles are specific (Table 3). The prospective of RAPD mapping would be expanded if additional "fingerprints" of PCR products could be produced by using combinations of random primers. The combination of two primers created new amplified DNA fragments that were not possible to observed when each primer was used individually[25].

Among 14 loci of single random primer and 10 loci of a combination of two random primers, only 4 loci produce polymorphic bands. The polymorphic bands ranged from one in loci OPA-02, OPA-02 + OPA-07, OPA-01 + OPA-13 to two in locus OPA-02 + OPA-03 (figure 5). These loci can be used to differentiate between Atlantic and IPB CP1 cultivars (table 4).
Figure 3. Gel electrophoresis of PCR products of Atlantic cultivar and IPB CP1 by using 14 RAPD primers. M: 1 kb marker, A: Atlantic cultivar, B: IPB CP1.

Table 2. Amplified DNA of Atlantic and IPB CP1 cultivars using single primers

| No | Locus | Total allele | Number of polymorphic band | Number of monomorphic band | Polymorphism percentage (%) | Molecular size range of allele (bp) |
|----|-------|--------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------------|
| 1  | OPA-01| 9            | 0                           | 9                           | 0                           | 500-2500                          |
| 2  | OPA-02| 7            | 1                           | 6                           | 14.28                       | 500-2000                          |
| 3  | OPA-03| 3            | 0                           | 3                           | 0                           | 1000-2000                         |
| 4  | OPA-05| 7            | 0                           | 7                           | 0                           | 250-1500                          |
| 5  | OPA-06| 8            | 0                           | 8                           | 0                           | 750-3000                          |
| 6  | OPA-07| 7            | 0                           | 7                           | 0                           | 750-3000                          |
| 7  | OPA-09| 6            | 0                           | 6                           | 0                           | 500-2500                          |
| 8  | OPA-10| 11           | 0                           | 11                          | 0                           | 250-2500                          |
| 9  | OPA-13| 5            | 0                           | 5                           | 0                           | 250-3000                          |
| 10 | OPA-18| 8            | 0                           | 8                           | 0                           | 250-2500                          |
| 11 | OPB-01| 7            | 0                           | 7                           | 0                           | 250-2000                          |
| 12 | OPB-11| 3            | 0                           | 3                           | 0                           | 750-1500                          |
| 13 | OPB-12| 11           | 0                           | 11                          | 0                           | 250-2500                          |
| 14 | OPB-18| 10           | 0                           | 10                          | 0                           | 250-2500                          |
|    | Total | 102          | 1                           | 101                         |                             |                                   |

Figure 4. Gel electrophoresis of PCR products of Atlantic cultivar and IPB CP1 by using combination of two random primers. M: 1 kb marker, A: Atlantic cultivar, B: IPB CP1.
Table 3. Amplified DNA of Atlantic and IPB CP1 cultivars using combination of two random primers.

| No | Locus                   | Total allele | Number of polymorphic band | Number of monomorphic band | Polymorphism percentage (%) | Molecular size range of allele (bp) |
|----|-------------------------|--------------|----------------------------|----------------------------|----------------------------|-----------------------------------|
| 1  | OPA-01 + OPA-02         | 9            | 0                          | 9                          | 0                           | 500-2000                          |
| 2  | OPA-01 + OPA-13         | 9            | 1                          | 9                          | 11.11                       | 250-1500                          |
| 3  | OPA-02 + OPA-03         | 9            | 2                          | 7                          | 22.22                       | 400-2250                          |
| 4  | OPA-02 + OPA-05         | 5            | 0                          | 5                          | 0                           | 750-2000                          |
| 5  | OPA-02 + OPA-07         | 13           | 1                          | 12                         | 7.69                        | 300-2250                          |
| 6  | OPA-02 + OPA-09         | 16           | 0                          | 16                         | 0                           | 250-2000                          |
| 7  | OPA-02 + OPA-10         | 9            | 0                          | 9                          | 0                           | 250-2000                          |
| 8  | OPA-02 + OPA-13         | 7            | 0                          | 7                          | 0                           | 250-1250                          |
| 9  | OPA-02 + OPA-18         | 13           | 0                          | 13                         | 0                           | 200-2250                          |
| 10 | OPB-07 + OPB-11         | 9            | 0                          | 9                          | 0                           | 500-1500                          |
|    | Total                   | 99           | 4                          | 95                         |                             |                                   |

Figure 5. Gel electrophoresis of PCR products which differentiate between Atlantic and IPB CP1 cultivars, 1: OPA-02, 2: OPA-02 + OPA-03, 3: OPA-02 + OPA-07, 4: OPA-01 + OPA-13. M: 1 kb marker A: Atlantic cultivar, and B: IPB CP1.

The use of primer combination in RAPD produce more fragments and had no homology to the bands amplified in single primer [26]. We observed that the primer combination tend to produce smaller fragment than their primer origin. For example, the biggest fragment amplified by OPA-07 primer is at range 3000-2500 bp. When OPA-07 combined with OPA-02, the biggest fragment amplified is at range 2500-2000 bp. The use of two primers could be a solution of the limited number
of primer as it is able to generate more fragment from the genomic region that not accessible when single primer reactions used.

Table 4. Amplified DNA using RAPD primers showed polymorphism.

| No | Locus       | Total allele | Allele          | Atlantic | IPB CP1 | Molecular size of allele (bp) |
|----|-------------|--------------|-----------------|----------|---------|-------------------------------|
| 1  | OPA-02      | 8            | OPA-02 (7)      | -        | +       | 1500                          |
| 2  | OPA-02 + OPA-03 | 9       | OPA-02 + OPA-03 (5) | +        | -       | 1300                          |
| 3  | OPA-02 + OPA-07 | 13      | OPA-02 + OPA-07 (13) | +        | -       | 2300                          |
| 4  | OPA-01 + OPA-13 | 9        | OPA-01 + OPA-13 (5) | -        | +       | 2250                          |

Note: (+): allele observed, (-): no allele observed

Molecular markers have become an effective tool to evaluate and characterize both intra-species and inter-species genetic diversity. Marker systems are discerned by the level of their informativeness, which in turn be determined on the extent of polymorphism. The concept of polymorphism is used to decide the genetic divergence in a population [17]. Gamma-ray irradiation resulted in DNA structural changes (breaks, transpositions, deletions) which produced band polymorphism [27]. The polymorphic bands had been successfully observed in potato cv Atlantic and IPB CP1 as its mutant provides the information about the genetic changes in a mutant caused by irradiation.

Conclusion

RAPD marker can be used to differentiate between potato cv Atlantic and cv IPB CP1 as its mutant. The use of a combination of two random primers in RAPD maximizes the chance to differentiate these two cultivars. Among 24 loci composed of single random primers and combinations of two random primers, only in four loci, the alleles of two cultivars is different. So, loci OPA-02, OPA-02 + OPA-03, OPA-02 + OPA-07, and OPA-01 + OPA-13 can be used to determine the different between potato cv Atlantic and its mutant, cv IPB CP1.

Acknowledgements

This research was financially supported by Penelitian Terapan (Penelitian Strategis Nasional) (Applied Research/Strategic National Research), Ministry of Research Technology and Higher Education, contract no 3/E1/KP.PTNBH/2019 on behalf of Prof Dr Ir Suharsono, DEA.

References

[1] Robertson T M, Alzaabi A Z, Robertson M D and Fielding B A 2018 Nutrients 10 1764
[2] Camire M E, Kubow S and Donnelly D J 2009 Crit Rev Food Sci 49 823
[3] Wattimena G A, Purwito A and Mattjik N A 2014 Research Progress in Potato Propagation and Breeding at Bogor Agricultural University (Bogor: Bogor Agricultural University) pp 28–43
[4] Badan Pusat Statistik 2017 Statistik Tanaman Sayuran Dan Buah-Buahan Semusim (Jakarta: Badan Pusat Statistik)
[5] Suprasanna P, Mirajkar S J and Bhagwat S G 2015 Springer 1 593
[6] Kodym A and Afza R 2003 Methods in Molecular Biology 236 189
[7] Ahloowalia B S and Maluszynski M 2001 Euphytica 118 167
[8] Ko D W, Nadakuduti S S, Douches D S and Buell C R 2018 Plos One 13 1
[9] Novak FJ and Brunner H 1992 IAEA Bulletin 4 25
[10] Afrasiab H and Iqbal J 2010 Pak J Bot 42 1629
[11] Yaycili O and Alikamanoğlu S 2012 J Biol 36 405
[12] Das A, Gosal S S, Sidhu J S and Dhaliwal H S 2000 Euphytica 114 205
[13] Williams J G K, Kubelik A R, Livak K J, Rafalski J A and Tingey S V 1990 Nucleic Acids Res 18 6531
[14] Clegg M T 1990 Molecular Diversity in Plant Populations, in Plant Population Genetics, Breeding, and Genetic Resources, ed AHD Brown et al (America: Sinauer Association Inc)
[15] Garibyan L and Avashia N 2013 J Invest Dermatol 133 1
[16] Ghislain M, Andrade D, Rodriguez F, Hijmans R J and Spooner D M 2006 Theor Appl Genet 11 1515
[17] El-Fiki A, Adam Z, Mohamed T, Sobieh S and Salah A 2018 Not Sci Biol 10 45
[18] Webb R E, Wilson D R, Shumaker J R, Graves B, Henninger M R, Watts J, Frank J A and Murphy H J 1978 Am Potato J 55 141
[19] Suharsono, Wattimena G A, Dahniar N, Indrawibawa D, Aji I, Nugraha A and Rapi’I H 2016 Registration Varieties of IPB CP1 Potato cv. (Bogor: Bogor Agricultural University)
[20] Murashige T and Skoog F 1962 Physiol Plant 15 473
[21] John M E 1992 Nucleic Acids Res 20 2381
[22] Pitch U and Schubert I 1993 Nucleic Acids Res 21 3328
[23] Dominguez R and Holmes K C 2011 Annu Rev Biophys 40 169
[24] Williams J G K, Hanafey M K, Rafalski J A and Tingey S V 1993 Method Enzymol 218 704
[25] Klein-Lankhorst R M, Vermunt A, Weide R, Liharska T and Zabel P 1991 Theor Appl Genet 8 108
[26] Hu J, van Eysden J and Quiros C F 1995 Genome Res 4 346
[27] Selvi B S, Ponnuswami V and Sumathi T 2007 J Appl Sci Res 3 1933