Analysis of mutant of potato (*Solanum tuberosum* L.) cultivar Kennebec

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Abstract. Potatoes are horticultural commodity that have high economic value. Kennebec is one potato (*Solanum tuberosum* L.) cultivar suitable for potato chips, therefore it is able be used as genetic resource to develop superior potato cultivars. By gamma ray irradiation at a dose of 15 Gy to cv. Kennebec, one mutant clone, i.e. clone 53.1, had been obtained. This research had an objective to analyse the clone 53.1 as a mutant of potato cv Kennebec. Molecular analysis by RAPD marker using 9 primers namely OPA 1, OPA 2, OPA 5, OPA 7, OPA 8, OPA 9, OPA 10, OPB 8 and OPB 18 showed that the mutations occurred at all nine loci and 36.67% alleles had mutated. The highest level of mutation occurred at the OPA 2 locus. Mutations also significantly affected morphological characters in the form of plant height at 45 and 90 days after planting (DAP), tuber diameter, tuber number, tuber weight, shoot weight, root weight, color of tuber eye and color of basal stem of sprouting tuber. The plant height and the tuber productivity of clone 53.1 as a mutant are higher than that of potato cv Kennebec as an original cultivar.

Keywords: Kennebec, mutant, potato, RAPD marker, tuber productivity.

1. Introduction

Potatoes are very important commodity due to the high economic value. They are widely used as a staple food in some countries and a raw materials for the food industry. In fulfilling national industrial potato needs, Indonesia still imports potatoes from other countries. One effort to meet these needs is to increase potato tuber production. Tuber seeds are a critical factor for the tuber production. The high quality of tuber seeds can be obtained by creation of new cultivar of potato. Mutation is widely used to improve genetically the potato plants [1]. Recently, new technique for mutation applied in potato is CRISPR/Cas9 [2].

Mutations can be induced physically by gamma ray irradiation. Mutation induction by gamma irradiation has been done to increase the variations in plant height, number of nodes, number of tubers, tuber size and weight of tuber in potato cv Desiree and cv Diamant [3], and the salinity tolerance in potato cv Marfona [4]. Potato clone 53.1 had been obtained by induction of gamma irradiation at 15 Gy to potato cv Kennebec. Evaluation of this mutant is very important. Morphological and molecular analysis is needed to determine the difference between mutant and their original cultivar.
Random amplified polymorphic DNA (RAPD) is polymorphism of DNA generated by amplification of random DNA segments with single primers of arbitrary nucleotide sequence [6]. RAPD could be applied to differentiate between two different organisms. Analysis of mutant plants resulted by gamma ray induction using RAPD markers had been widely carried out including mutant of potato cv Desiree and cv Diamant [3], and cv Marfona [4].

This experiment had an objective to analyse the mutant of potato cv Kennebec. Molecular analysis carried out by using RAPD marker and morphological analysis covered vegetative growth and tuber productivity.

2. Materials and methods

2.1. Materials
G0 tuber seeds were cultivated in polybag containing cocopeat media and placed in the greenhouse. A pair of primers composed of Kact-qF (GAAAATCCTGACCGAAAGAGG) and Kact-qR (CAAGTATGGCTGGAATAAGA) was used to amplify a part of actin gene. Analysis of RAPD was carried out by using some single primers covering OPA 01 (CAGGCCCTTC), OPA 02 (TGCCGAGCTG), OPA 05 (AGGGGTCTTG), OPA 07 (GAAACGGGTG), OPA 08 (GTGACGTAGG), OPA 09 (GGTAAACGCC), OPA 10 (GTGATCGCAG), OPB 08 (GTCCACACGG), and OPB 18 (TGGGGGACTC).

2.2. Methods

2.2.1. Experimental design. This experiment was conducted using a completely randomized design (CRD) with 3 replications and 1 treatment factor, namely genotype. We used two genotypes called cultivar Kennebec and clone 53.1. Each replication was consisted of 5 polybags for each genotype. In each polybag, 2 tubers were planted, therefore the total plant number was 30 plants for each genotype. These plants were placed in the greenhouse at 1200 m asl (above sea level).

2.2.2. Isolation of genomic DNA. Genomic DNA was isolated from leaves using cetyl-trimethyl ammonium bromide method as described [6].

2.2.3. Evaluation of genomic DNA. The quality of genomic DNA was evaluated based on DNA purity and DNA integrity. The purity of DNA was evaluated by using spectrophotometer in comparing the absorbance value at a wavelength of 260 nm with absorbance at a wavelength of 280 nm (OD$_{260}$/OD$_{280}$). Integrity of genomic DNA was determined by using PCR of part of actin gene. The PCR mixture of actin gene was composed of 1 µl (100 ng) genomic DNA, 5 µl 2x KAPA master mix (0.2 units/µl Taq DNA polymerase pH 8.5, 200 µM dATP, 200 µM dGTP, 200 µM dCTP, 200 µM dTTP, 1.5 mM MgCl2), 2.5 pmol primer Kact-qF, 2.5 pmol primer Kact-qR in the total volume of 10 µl. The PCR was carried out 35 cycles with a condition of pre-PCR at 94°C for 5 minutes, denaturation at 94°C for 1 minute, denaturation at 55°C for 45 seconds, extension at 72°C 1 minute, and post-PCR at 72°C for 5 minutes.

2.2.4. RAPD analysis. The composition of PCR for RAPD analysis was the same as PCR for actin gene except the primer where RAPD used only one primer at 5 pmol. The condition of PCR was pre-PCR at 95°C for 5 minutes, denaturation 95°C for 1 minutes, annealing at 37°C for 1 minutes, extension at 72°C for 2 minutes and post-PCR at 72°C for 7 minutes. The PCR was carried out for 40 cycles.

2.2.5. Electrophoresis. DNA was migrated in 1% agarose gel (b/v) in TAE 1X buffer solution (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) at 100 volts for 30 minutes. The DNA in the agarose gel was stained by 0.05% EtBr visualized using Gel Doc.
2.2.6. Morphological analysis. Observations of morphological plants were carried out at 45 days after planting (DAP) and at 90 DAP. Observation at 45 DAP was carried out on plant height, number of leaf, and the size of leaflet. At 90 DAP, the morphological observation covered plant height, fresh and dry weight of shoots and roots, the number and the weight of tuber produced per plant, the number of leaf and size of leaflet, the diameter of tuber, and the color of basal sprouting shoots. Morphological data were then analyzed using T Test at a 95% confidence with SPSS 16.0 software.

3. Results and discussion

3.1. Integrity of genomic DNA

Genomic DNA of potato plants was successfully isolated from young leaves of G1 plants. Electrophoresis of genomic DNA showed that genomic DNA had a high size (figure 1a) of smear band indicating that the genomic DNA had a high integrity. The integrity of genomic DNA can be detected based on the DNA integrity of housekeeping genes. Housekeeping genes is a group of genes that are present in many copies, in every cell and constitutively expressed in the cells [7], even though its expression level is depended on the condition of experiment [8]. These genes are commonly used as internal controls, such as actin (ACT), b-tubulin, cyclophilin, elongation factor 1-a (ef1a), 18S rRNA, adenine phosphoribosyl transferase (aprt), cytoplasmic ribosomal L2 protein, ubiquitin (UBQ), and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) [9, 10]. In this experiment, actin gene was used as internal control. Actin is a globular protein composing cell cytoskeleton. This protein has a very important role in many cellular processes which simultaneously regulate cell growth and morphology [11]. PCR by using specific primer for actin gene, Kact-qF and Kact-qR, showed that the region of actin gene in 250 bp length can be amplified by using this genomic DNA as template (figure 1b). The presence of actin gene in the genomic DNA indicates that the genomic DNA has a high level of integrity.

The ratio value of OD$_{260}$/OD$_{280}$ is relatively low, 1.53 for Kannebec DNA, and 1.52 for clone 53.1 DNA. This result indicated that the purity of DNA is relatively low, it may be contaminated by protein. The presence of protein contaminant can be due to the incompletely extraction by organic solvent such as phenol. The high purity of DNA is determined by 1.8 rasio of OD$_{260}$/OD$_{280}$ [12]. Even though genomic DNA is not pure, it can be used to amplify the DNA of actin gene by PCR, therefore it is able to be used also for RAPD analysis.

3.2. RAPD analysis

The random single primers anneal to the nucleotide sequences of the genome, therefore each single random primer in the RAPD is considered as a locus, while the DNA fragments generated by PCR using this primer is scored as alleles. The level of the mutation is based on the allele differences found in Kannebec and clone 53.1, therefore the polymorphic alleles reflect the level of mutation.

By using nine single random primers, RAPD of population composed of cultivar Kannebec and clone 53.1 produced 90 bands consisting of 57 monomorphic bands and 33 polymorphic bands (figure
2, table 1). Monomorphic bands describe non-mutant alleles, whereas polymorphic bands describe mutant alleles. RAPD can reflect deletions and insertions (indel) in the genome described by polymorphic alleles [5].

![Figure 2. RAPD of cultivar Kennebec (K) and clone 53.1 (M) using nine random primers namely OPA and OPB.](image_url)

**Table 1. Polymorphic alleles in nine loci of RAPD**

| Primer/Locus | Number of band/alleles | Polymorphic alleles (%) |
|--------------|------------------------|-------------------------|
|              | total | monomorphic | polymorphic |        |
| OPA 01       | 12    | 10          | 2           | 16.67  |
| OPA 02       | 10    | 3           | 7           | 70.00  |
| OPA 05       | 7     | 6           | 1           | 14.29  |
| OPA 07       | 11    | 7           | 4           | 36.36  |
| OPA 08       | 9     | 4           | 5           | 55.56  |
| OPA 09       | 7     | 5           | 2           | 28.57  |
| OPA 10       | 12    | 7           | 5           | 41.67  |
| OPB 08       | 11    | 6           | 5           | 45.45  |
| OPB 18       | 11    | 9           | 2           | 18.18  |

Total: 90 | 57 | 33 | 36.67

Mutations occurred at all nine loci. Mutation at every locus varied from 14.29% to 70%. There are 36.67% polymorphic alleles in nine loci of the potato population consisted of cultivar Kennebec and its mutant, clone 53.1 indicating that there are 36.67% mutations occurred in nine loci. This mutation can be caused by insertion, deletion and substitution in the primer annealing sites. The insertion and deletion of some nucleotides in the region flanked by the primer annealing sites can also cause the changes of alleles. The highest level of mutation was occurred in locus of OPA 02. These mutations are random, can be found in the coding and non-coding region of DNA.

Mutation in the primer annealing sites in the genome by indel and substitution cause loss of the alleles. In the other side, mutation in the non-annealing sites in the genome can create new alleles. The mutation from cultivar Kennebec to be clone 53.1 showed that some alleles were changed, not only the
loss of alleles but also the creation of new alleles (table 2, figure 3). These alleles can be used to identify the difference between cultivar Kennebec and clone 53.1 as its mutant. The mutation of cultivar Kennebec to become clone 53.1 caused the creation of 20 new alleles and loss of 13 alleles. It means that the creation of new primer annealing sites is dominant in this mutation. At some loci, in the same time, mutation caused loss and creation of alleles, but in locus OPA 09, mutation caused only the loss of alleles. In the other side, at locus OPA 02 and OPB 18, mutation of cultivar Kennebec resulted only creation of new alleles.

| Locus | Alleles     | Cultivar Kennebec | Clon 53.1 | Size of amplicon DNA (bp) |
|-------|-------------|-------------------|-----------|---------------------------|
| OPA 1 | OPA 1.10    | +                 | -         | 300                       |
|       | OPA 1.11    | -                 | +         | 250                       |
| OPA 2 | OPA 2.1     | -                 | +         | 1500                      |
|       | OPA 2.2     | -                 | +         | 1250                      |
|       | OPA 2.3     | -                 | +         | 1050                      |
|       | OPA 2.4     | -                 | +         | 1000                      |
|       | OPA 2.5     | -                 | +         | 750                       |
|       | OPA 2.9     | -                 | +         | 250                       |
|       | OPA 2.10    | -                 | +         | 100                       |
| OPA 5 | OPA 5.1     | +                 | -         | 1500                      |
| OPA 7 | OPA 7.3     | -                 | +         | 1250                      |
|       | OPA 7.4     | -                 | +         | 1200                      |
|       | OPA 7.8     | +                 | -         | 500                       |
|       | OPA 7.10    | -                 | +         | 250                       |
| OPA 8 | OPA 8.1     | +                 | -         | 2000                      |
|       | OPA 8.2     | +                 | -         | 1500                      |
|       | OPA 8.4     | +                 | -         | 1100                      |
|       | OPA 8.7     | -                 | +         | 550                       |
|       | OPA 8.9     | -                 | +         | 300                       |
| OPA 9 | OPA 9.1     | +                 | -         | 1250                      |
|       | OPA 9.2     | +                 | -         | 1400                      |
| OPA 10 | OPA 10.6    | +                 | -         | 900                       |
|       | OPA 10.7    | -                 | +         | 800                       |
|       | OPA 10.8    | -                 | +         | 600                       |
|       | OPA 10.9    | -                 | +         | 550                       |
|       | OPA 10.10   | +                 | -         | 500                       |
| OPB 8 | OPB 8.1     | +                 | -         | 2250                      |
|       | OPB 8.2     | +                 | -         | 1750                      |
|       | OPB 8.3     | +                 | -         | 1500                      |
|       | OPB 8.4     | -                 | +         | 1400                      |
|       | OPB 8.10    | -                 | +         | 750                       |
| OPB 18 | OPB 18.4    | -                 | +         | 1600                      |
|       | OPB 18.8    | -                 | +         | 900                       |

Number of +: 13, 20

Note: + : present; - : absent
Figure 3. The difference between cultivar Kennebec and clone 53.1 in some loci of RAPD by using OPA 1 (A), OPA 2 (B), OPA 5 (C), OPA 7 (D), OPA 8 (E), OPA 9 (F), OPA 10 (G), OPB 8 primers (H), OPB 18 (I). M: 1 Kb Ladder marker, 1: Kennebec cultivar, 2: clone 53.1.

3.3. Morphological analysis

The plant height of clone 53.1 mutant was higher than the original cultivar, Kennebec, whereas other morphological characters such as number of leaves and the size of leaflet were not significantly different at 45 DAP (table 3). Eventhough it was no significant different, the number of leaves and leaflet size of clone 53.1 tends to be higher than that of Kennebec cultivars. It means that mutations cause an increase the morphological characters especially the height of plant. Vegetative growth of potato is usually recorded at 45 DAP as in potato cv Kufri Pukhraj, cultivated in India, which the vegetative growth was observed at 45 and 75 DAP [13].
Table 3. Vegetative characters of plants at 45 DAP

| Genotype   | Plant height (cm) | Number of leaves | Size of leaflet (cm) |
|------------|-------------------|------------------|----------------------|
|            |                   |                  | Length   | Width   |
| Kennebec   | 25.87<sup>a</sup> | 13.27 ± 2.2      | 5.89 ± 0.7 | 4.53 ± 0.6 |
| Clone 53.1 | 32.87<sup>b</sup> | 13.6 ± 1.8       | 6.09 ± 0.6 | 4.54 ± 0.3 |

Note: Number followed by the different letter in the same column is significantly different based on T test at confidence interval of 95 %

All vegetative characters including plant height and shoot and root weight, of mutant clone 53.1 were higher than that of the original cultivar Kennebec at 90 DAP (table 4). It means that mutation in cultivar Kennebec induced the increase of vegetative growth. These mutation may degenerate the inhibitor proteins that inhibit the vegetative plant growth. Without the inhibitors, the genes responsible for vegetative growth can be expressed constitutively.

Table 4. Vegetative characters of plants at 90 DAP

| Genotype   | Plant height (cm) | Fresh weight (g) | Dry weight (g) |
|------------|-------------------|------------------|---------------|
|            |                   | shoot           | root          |
|            |                   | shoot           | root          |
| Kennebec   | 28.75<sup>a</sup> | 3.54<sup>a</sup> | 0.56<sup>a</sup> | 1.45<sup>a</sup> | 0.19<sup>a</sup> |
| Clone 53.1 | 39.30<sup>b</sup> | 7.41<sup>b</sup> | 0.95<sup>b</sup> | 3.34<sup>b</sup> | 0.40<sup>b</sup> |

Note: Number followed by the different letter in the same column is significantly different based on T test at confidence interval of 95 %

The production characters including the productivity, number and the size of tuber of mutant was different than the original cultivar. Clone 53.1 had a tuber productivity and tuber size higher than cultivar Kennebec, although the number of tuber of clone 53.1 was lower than that of cultivar Kennebec (table 5).

Table 5. Production characters of plant 90 DAP

| Genotype   | Productivity (g/plant) | Number of tuber | Size of tuber |
|------------|------------------------|-----------------|---------------|
|            |                        |                 | Diameter (cm) | Weight (g)   |
| Kennebec   | 33.53<sup>a</sup>      | 3.16<sup>a</sup> | 1.85<sup>a</sup> | 10.61<sup>a</sup> |
| Clone 53.1 | 61.95<sup>b</sup>      | 2.71<sup>b</sup> | 2.16<sup>b</sup> | 22.86<sup>b</sup> |

Note: Number followed by the different letter in the same column is significantly different based on T test at confidence interval of 95 %

The increasing vegetative plant growth (table 4) due to the mutation in cultivar Kennebec supports the increasing tuber productivity of plant (table 5). The plant with high vegetative growth has a high rate of photosynthesis and then the photosynthate is stored in the tuber form. The chlorophyll contained in the shoot capture the solar energy to be converted to the sugar as a photosynthate. The root of plant is responsible to absorb the nutrient and water to be used for photosynthetic process. Since the clone 53.1 mutant has a higher vegetative growth than the original cultivar Kennebec, therefore the productivity of tuber including the weight and the size of tuber of clone 53.1 is higher than that of cultivar Kennebec. It is very interesting to investigate the different rate of photosynthesis between these two genotypes. Since clone 53.1 has a better character especially the tuber productivity, this clone can be developed to be new superior cultivar for chipping potato. It needs to realize the multi-location trial to release this clone.

Mutations also occurred in the character of the color of the buds and the basal shoot of sprouting tubers. The color of buds in Kennebec cultivars is the same as the skin color of the tuber, which is
white-yellowish, while the buds of clone 53.1 have red color. The color of basal shoots of sprouting tuber of cultivar Kennebec is white-purplish whereas that of clone 53.1 is red (figure 4). The mutation may increase in anthocyanin pigment in clone 53.1 mutant. Mutations by gamma ray irradiation at a dose of 200 Gy in cultivar Zarevo also increased the anthocyanin content characterized by changes in unpigmented protoplasts to form pigmented protoplasts [14]. In addition, mutations in cultivar Cara, Red Cara, and Pink Kerss at a dose of 20 Gy changed the color of tubers and buds of sprouting tuber [15].

Figure 4. The color of budding eye and basal shoot of sprouting tuber. A: cultivar Kennebec having white-purplish basal shoot, B: white-yellowish budding eye of cultivar Kennebec, C: red color of basal shoot of clone 53.1, D: red color of budding eye of clone 53.1. Pt: basal shoot, mt: budding eye. Bar= 1 cm

4. Conclusion
Analysis of mutant of potato cv Kennebec showed that mutations occurred in nine loci namely OPA 1, OPA 2, OPA 5, OPA 7, OPA 8, OPA 9, OPA 10, OPB 8 and OPB 18. A total of 36.67% alleles were mutated. Mutations increased vegetative growth, tuber productivity and tuber size. Clone 53.1 as a mutant of cultivar Kennebec has a better character, therefore it is potential to be developed as a new superior cultivar of chipping potato.

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