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ANALYSIS OF GAMMA IRRADIATED FOURTH GENERATION MUTANT OF RODENT TUBER (TYPHONIUM FLAGELLIFORME LODD.) BASED ON MORPHOLOGY AND RAPD MARKERS

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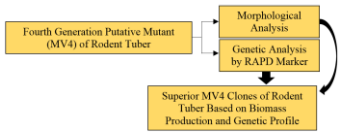
Graphical abstract



Abstract

Rodent tuber (*Typhonium flagelliforme* Lodd.) is an herbal medicinal plant with anticancer activity. The genetic diversity of rodent tuber is low due to vegetative propagation. Somatic cell population of rodent tuber from Bogor had been irradiated with gamma ray to increase genetic diversity. There were 37 clones of first generation putative mutant (MV1) which had been analyzed based on morphological and RAPD markers. Out of those 37 MV1 clones, there were 17 clones which had undergone genetic mutation and had a diversified genetic profile. MV1 had been regenerated to fourth generation putative mutant clones (MV4). This research was aimed to analyze the mutation stability of MV4 based on morphological and RAPD markers. Clone 6-1-2 had the highest increase of the number of shoots and leaves than control and the other MV4 clones, with 4.7 and 19.7 shoots and leaves, respectively. Clone 6-1-1-6 obtained the highest increase in plant height than control and the other MV4 clones, i.e. 25.2 cm. Clone 6-9-5 had the weightiest fresh and dry weight, i.e. 41.67 gram and 12.01 gram respectively. RAPD molecular marker analysis of MV4 by using 15 primers had produced 64 polymorphic DNA bands out of 146 total bands. OPD-10 primer produced the highest number of polymorphic bands, i.e. 15 polymorphic bands out of 17 total bands with sizes 200-2000 bp. RAPD profile of MV4 had showed 5 main clusters at similiarity coefficient cut-off 0.91. Morphological characterization and RAPD analysis had proved the stability of genetic mutation of MV4.

Keywords: *Typhonium flagelliforme* lodd, mutant, morphology, rapd molecular markers

1.0 INTRODUCTION

Rodent tuber (*Typhonium flagelliforme* Lodd.) is an herbal plant from Araceae family [1] which contains detoxification and anticancer compounds. Rodent tuber is a native Indonesian plants and has been used as traditional medicine for years. Bioactive compounds of rodent tuber are alkaloids, saponins, steroids, and glycosides [2]. Anticancer compounds can be found in all parts of a rodent tuber plant, including root, tuber, stem, and leaf [3].

Rodent tuber has cytotoxicity against cancer of lung, breast [4], liver [5], leukemia [6], colon, prostate, and cervix [7]. Rodent tuber's extract has also been proven to be able to prevent breast and cervical cancer [8]. Another biological activities of rodent tuber are antibacterial, antioxidant [9], toxic to *Artemia salina* [10], and able to induce apoptosis of cancer cells [5].

The development of Indonesian rodent tuber as anticancer drugs faces obstacle due to its low genetic diversity which is caused by conventional clonal propagation. Low genetic diversity is followed by low diversity of bioactive compounds of rodent tuber [11]. Diversity of rodent tuber's bioactive compounds can be increased by inducing mutation of in vitro somatic cell population or shoot culture. Mutation can be induced by irradiating with physical mutagens such as gamma ray.

Calli somatic cell population of rodent tuber [12] has been irradiated with gamma ray at dose of 6 Gy. The resultant in vitro putative shoots showed various growth response [13]. In vitro mutant plantlets of rodent tuber have undergone genetic mutation based on analysis with Randomly Amplified Polymorphic DNA (RAPD) molecular marker [14]. There were 37 clones of first generation putative mutant (MV1) of rodent tuber which had been analyzed based on morphological and RAPD markers. MV1 clones had a varied morphological characteristics [15]. Out of those 37 MV1 clones, there were 17 clones which had undergone genetic mutation and had a diversified genetic profile based on morphology and molecular markers [16; 17].

Genetic diversity can be detected with molecular markers such as RAPD, RFLP, AFLP, and SSR [18]. RAPD marker is able to detect genetic diversity of plants whose genome had not been sequenced yet [19], such as rodent tuber. Genetic characterization of plant's germplasm is a very important factor for maximizing the potency of genetic diversity [20].

Rodent tuber MV4 clones have been obtained by combination of gamma irradiation with somaclonal variation of in vitro culture. This research was aimed to analyze genetic mutation stability of the fourth generation putative mutant clones of rodent tuber (MV4) based on morphological and RAPD molecular markers.

2.0 EXPERIMENTAL

2.1 Plant Materials

This research analyzed the fourth generation putative mutant clones of rodent tuber (MV4) (collection of Sianipar *et al*., [21], in the patenting process). Rodent tuber mother plants were obtained from Bogor, West Java. MV4 clones have been obtained by combination of in vitro culture with 6 Gy-gamma irradiation on somatic cell population [13].

2.2 Morphological Characterization

Morphological characters observed were the number of shoots, number of leaves, plant heights, fresh and dry weights of rodent tuber control and mutant plants. Morphological characters were analyzed with NTSYS DIST coefficient and UPGMA.

2.3 Molecular Analysis with RAPD Marker

2.3.1 DNA Isolation

DNA isolation was done based on [22]. 2.5 gr of leaf sample was homogenized with PVP 0.1% and liquified nitrogen. Sample powder was added with 2 ml of CTAB buffer (CTAB 10% b/v, EDTA 0.5 M pH 8.0, Tris HCl 1M pH 8.0, NaCl 5M) and 10 µl of 1-merkaptoetanol 1% (b/v). Sample was homogenized with vortex, incubated at 60°C for 20 minutes, and cooled in room temperature. 750 µl of chloroform : isoamyl alcohol (24:1) solution was added to sample and then vortexed. Sample was centrifuged at 11.000 rpm for 10 minutes. Supernatant was added with 1 ml of choloroform : isoamylalcohol (24:1) and centrifuged at 11.000 rpm for 10 minutes. Supernatant was added with 750 µl of cold isopropanol, homogenized, and stored at -20°C for one night. Sample was centrifuged at 11.000 rpm for 10 minutes. DNA precipitate was dried in vacuum condition for one hour. Dried DNA sample was solubilized in 200 µl of buffer TE (Tris-HCl 1M pH 8.0; EDTA 0.5M pH 8.0). 200 µl of DNA solution was added with 20 µl of RNase (10 mg/ml) and incubated at 37 °C for one hour. DNA was incubated at 4 °C for one night. DNA solution was stored at -20 °C.

2.3.2 PCR-RAPD

DNA sample was amplified with 15 decamer primers by using Thermal Cycler Gene PCR (ABI 9700). The composition of 1x PCR reaction is 5 µl of DNA template (5 ng/ µl), 0.2 µl of dNTP 0.2 mM, 2.5 µl of PCR buffer + $MgCl_2$ (1x), 1 µl of primer 10 pmol, 0.2 µl of Taq polimerase 1U, and 16.1 µl of ddH_2O with total volume of 25 µl. PCR reaction thermal cycle was repeated for 45 times with stages as follows : 94 °C for one minute, 36 °C for one minute, 72 °C for 2 minutes, and 72 °C for 4 minutes. Extention time was conditioned at 72 °C for 4 minutes. PCR product was fractionated by electrophoresis method in 1,4% agarose gel (w/v) submerged in 40 ml of 1x TAE. Electrophoresis was runned at 75 volt for 1.5 hours. Agarose gel was submerged in ethidium bromide solution for 10 minutes and washed with distilled water. Electrophorized gel was visualized under UV light and documented with Kodak gel logic. Sizes of DNA bands were determined by comparison with 1 kb DNA ladder. Quantitative data were standarized based on [23]. DNA bands molecular data were converted to binary numbers (0 and 1) and formulated in matrix. Relationship between mutant clones was analyzed by SHAN clustering UPGMA.

Genetic distance and clones grouping were deterimined with NTSYS ver. 1.70

3.0 RESULTS AND DISCUSSION

3.1 Morphological Characterization

Morphological characters of 17 MV4 putative mutant clones were different from control based on the number of shoots, number of leaves, plant heights, fresh and dry weights (Figure 1 and Table 1). Clone 6-1-2 obtained the highest increase in the numbers of shoots and leaves than control and the other MV4 clones, with 4.7 shoots and 19.7 leaves respectively. Clone 6-1-1-6 obtained the highest increase in plant height than control and the other MV4 clones, i.e. 25.2 cm. Clone 6-9-5 had the weightiest fresh weight (41.67 gr) and the weigtiest dry weight (12.01 gr) than control and the other MV4 clones.

The higher biomass of MV4 clones than control is a crucial beneficial factor for commercial production of plants. The observed differences in morphological characters between control and MV4 plants were caused by gamma irradiation which produced free radicals in plant cells. Free radicals could induce chromosomal abberation, i.e. the changes of the number and structure of chromosome, somatic genetic cross-over, and changes of DNA sequence. Chromosomal abberation is able to induce changes of the structure and metabolism of plants which are followed by the changes of morphological characters [24]. Low dose of gamma irradiation has also been known to be able to induce morphological changes of potato plants [25].

Based on morphological characterization, many MV4 putative mutant clones were able to produce the higher number of shoots, number of leaves, and plant heights than control (Table 2). Gamma irradiation can increase the rate of mitosis [26], which is probably the reason of the increasing number of shoots, number of leaves, and plant heights of MV4 clones than control.

Figure 1 Rodent tuber plants at 8th week in green house
A= control; **B** = clone 6-3-2-5; **C** = clone 6-1-1-2; **D**= clone 6-9-1

Morphological characters of rodent tuber control and MV4 clones were analyzed with NTSYS program to make groupings of genetic similiarities. Dendogram showed 4 groups at similiarity coefficient cut-off 0.71 (Figure 2). Clones grouped as one cluster have a short genetic distance between each other. The diversity of morphological characters observed between MV4 putative mutant clones was due to random mutation induced by gamma irradiation which could change gene's structure and function in a different way among different plants [27; 28].

Genetic similiarity matrix based on morphological characters showed the genetic distance between the clones (Table 3). Similiarity coefficient of 1.00 (100%) indicated no genetic differences while similiarity coefficient less than 0.95 (95%) indicated genetic differences. MV4 putative mutant clones 6-3-3-6, 6-9-4, 6-1-1-2, 6-2-4-1, 6-6-3-7, 6-1-2, 6-1-1-6, and 6-9-5 had the lowest genetic similiarity (33%) with control. Based on morphological characters, MV4 putative mutant clones 6-2-8-2 and 6-3-3-10 had 100% similiarity with control. The other MV4 putative mutant clones had 56% (5 clones) and 78% (2 clones) genetic similiarities with control. The observed variation in genetic similiarities has proved the morphological diversity due to DNA mutation induced by gamma irradiation [29].

Table 1 Morphological characters of MV4 putative mutant clones and control plants

Num	Clone	Increase from 1st to 8th week			Total plant's weight (gr)	
		Shoot	Leaf	Height (cm)	Fresh weight	Dry weight
1	Control	1.0	9.0	13.2	12.91	4.72
2	6-3-3-6	0.7	2.7	8.1	6.83	2.68
3	6-9-3	1.7	8.3	16.1	34.97	9.65
4	6-9-4	3.0	13.0	20.0	31.17	8.68
5	6-2-5-3	1.3	4.0	8.8	28.94	10.52
6	6-3-2-5	1.0	4.7	11.8	9.20	3.22
7	6-1-1-2	0.0	4.3	21.2	12.40	4.01
8	6-9-1	0.0	3.7	11.1	12.01	3.62
9	6-2-4-1	0.3	0.7	3.9	6.24	2.02
10	6-6-3-7	0.0	0.0	4.3	6.46	1.97
11	6-6-3-6	0.0	3.3	13.1	8.14	2.34
12	6-2-7	2.7	7.3	19.5	17.54	4.62
13	6-2-6-3	2.3	8.3	18.0	24.34	8.06
14	6-1-2	4.7	19.7	16.2	20.72	7.82
15	6-1-1-6	0.3	4.7	25.2	8.74	3.70
16	6-2-8-2	2.0	6.7	14.5	12.87	3.54
17	6-9-5	3.0	15.7	21.5	41.67	12.01
18	6-3-3-10	1.7	8.7	14.7	9.59	3.13

Fresh and dry weights were a cumulative of roots, leaves, stems, tubers, and flowers weights

Table 2 Grouping of MV4 putative mutant clones based on morphological characters

Morphology	Interval	Clone
Increase of the number of shoots from 1st to 8th week	<1	6-3-3-6, 6-1-1-2, 6-9-1, 6-2-4-1, 6-6-3-7, 6-6-3-6, 6-1-1-6
	1-2	Control, 6-9-3, 6-2-5-3, 6-3-2-5, 6-2-8-2, 6-3-3-10
	>2	6-9-4, 6-2-7, 6-2-6-3, 6-1-2, 6-9-5
Increase of the number of leaves from 1st to 8th week	<5	6-3-3-6, 6-2-5-3, 6-3-2-5, 6-1-1-2, 6-9-1, 6-2-4-1, 6-6-3-7, 6-6-3-6, 6-1-1-6
	5-10	Control, 6-9-3, 6-2-7, 6-2-6-3, 6-2-8-2, 6-3-3-10
	>10	6-9-4, 6-1-2, 6-9-5
Increase of plant heights from 1st to 8th week (cm)	<10	6-3-3-6, 6-2-5-3, 6-2-4-1, 6-6-3-7
	10-15	Control, 6-3-3-10, 6-3-2-5, 6-9-1, 6-6-3-6, 6-2-8-2
	>15	6-9-4, 6-1-1-2, 6-2-7, 6-2-6-3, 6-1-2, 6-1-1-6, 6-9-5, 6-9-3

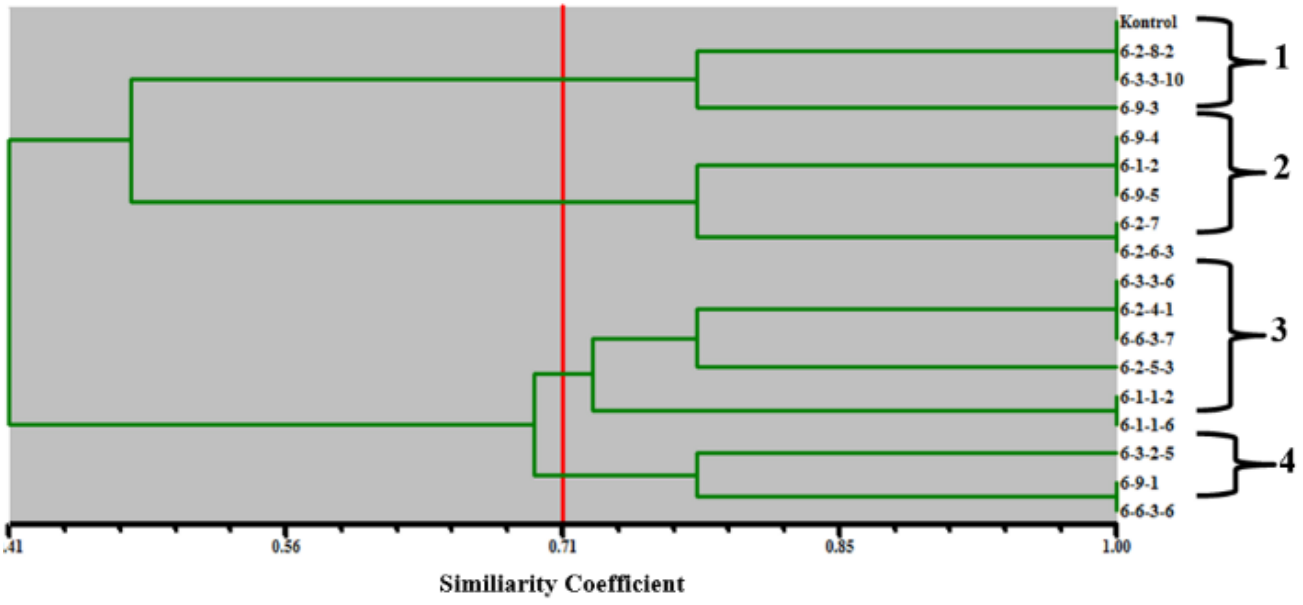


Figure 2 Dendogram of MV4 putative mutant clones based on morphological characters

Dendogram was made from binary scores of the number of shoots, number of leaves, and plant heights data by using NTSYS software at similiarity coefficient cut-off 0.71.

Gamma irradiation at dose of 6 Gy has been proven to be able to increase the number of shoots, number of leaves, and plant heights of rodent tuber MV4 putative mutant clones compared to control. Acoording to [30], the normal irradiation dose commonly applied to plant cells is at the range of 5-100 Gy. Morphological diversity induced by gamma irradiation has also been observed in mutant plants of yardlong bean (*Vigna unguiculata* (L.) Walp.) whose number of shoots, number of leaves, and plant heights were higher than control [31]. Potato var. Silana [32] and soybean [33] which had been induced by gamma irradiation also underwent the increase of plant heights compared to control. However, gamma irradiation at doses of 5-24 Gy on ginger vegetative plants (*Zingiber officinale*) [34] and at doses of 10 and 20 Gy on *Curcuma alismatifolia* [35] resulted in the lower number of leaves and shorter plants than control.

In line with MV1 [15], MV4 clones also have morphological variations due to gamma irradiation. However, environmental factors have an influence in morphological characters [36]. This limitation could be overcomed by combining morphological characterization with molecular marker in order to obtain the more accurate analysis of genetic diversity. RAPD molecular marker is able to detect DNA polymorphism without taking environmental factors into account [37].

Table 3 Genetic similiarity matrix of MV4 putative mutant clones based on morphological characters

	Control	6-3-3-6	6-9-3	6-9-4	6-2-5-3	6-3-2-5	6-1-1-2	6-9-1	6-2-4-1	6-6-3-7	6-6-3-6	6-2-7	6-2-6-3	6-1-2	6-1-1-6	6-2-8-2	6-9-5	6-3-3-10
Control	1.00																	
6-3-3-6	0.33	1.00																
6-9-3	0.78	0.33	1.00															
6-9-4	0.33	0.33	0.56	1.00														
6-2-5-3	0.56	0.78	0.56	0.33	1.00													
6-3-2-5	0.78	0.56	0.56	0.33	0.78	1.00												
6-1-1-2	0.33	0.78	0.56	0.56	0.56	0.56	1.00											
6-9-1	0.56	0.78	0.33	0.33	0.56	0.78	0.78	1.00										
6-2-4-1	0.33	1.00	0.33	0.33	0.78	0.56	0.78	0.78	1.00									
6-6-3-7	0.33	1.00	0.33	0.33	0.78	0.56	0.78	0.78	1.00	1.00								
6-6-3-6	0.56	0.78	0.33	0.33	0.56	0.78	0.78	1.00	0.78	0.78	1.00							
6-2-7	0.56	0.33	0.78	0.78	0.33	0.33	0.56	0.33	0.33	0.33	0.33	1.00						
6-2-6-3	0.56	0.33	0.78	0.78	0.33	0.33	0.56	0.33	0.33	0.33	0.33	1.00	1.00					
6-1-2	0.33	0.33	0.56	1.00	0.33	0.33	0.56	0.33	0.33	0.33	0.33	0.78	0.78	1.00				
6-1-1-6	0.33	0.78	0.56	0.56	0.56	0.56	1.00	0.78	0.78	0.78	0.78	0.56	0.56	0.56	1.00			
6-2-8-2	1.00	0.33	0.78	0.33	0.56	0.78	0.33	0.56	0.33	0.33	0.56	0.56	0.56	0.33	0.33	1.00		
6-9-5	0.33	0.33	0.56	1.00	0.33	0.33	0.56	0.33	0.33	0.33	0.33	0.78	0.78	1.00	0.56	0.33	1.00	
6-3-3-10	1.00	0.33	0.78	0.33	0.56	0.78	0.33	0.56	0.33	0.33	0.56	0.56	0.56	0.33	0.33	1.00	0.33	1.00

3.2 RAPD Molecular Marker Analysis

The concentrations of DNA extracts of rodent tuber putative mutant clones were ranged between 1301,0 - 2699,6 ng/µl. The purity of DNA extracts were ranged between OD_{260}/OD_{280} ratio 1,8 - 2,0, thus regarded as pure [38]. PCR-RAPD of DNA samples were done by using 15 primers (Table 4) which had been used for research about plants from *Typhonium* genus [39]. Primers used in this research were reproducibel according to RAPD profile analysis of rodent tuber MV1 clones [16]. The number of DNA bands produced by a primer was determined based on the number and sequence of complementary genome DNA [40]. There were 64 polymoprhic bands (200-3000 bp) out of the total 146 bands produced by 15 primers. OPD-10 primer produced the highest number of polymophic bands as well as total bands than the other primers. OPD-10 produced 15 polymorphic bands out of 17 total DNA bands. A single random primer has been known to be able to amplify DNA and show the polymorphism of mutant's DNA [41].

Amplification of MV4 DNA with OPB-18 primer produced polymorphic bands with sizes 390 bp, 750 bp, and 3000 bp (Figure 3), exactly the same as OPB-18 RAPD products of MV3. DNA polymorphism of MV4 clones was due to gamma irradiation on somatic cell population. According to [42], DNA mutation will be inherited to the next generations. This research has proved that DNA mutation of MV3 was inherited to MV4 plants.

RAPD molecular marker was able to show genetic diversity between control and MV4 clones. RAPD analysis of *Rhododendron* has shown the genetic differences between control and putative mutant clones irradiated with gamma ray at doses of 5 and 10 Gy)[43]. [44] has also proved that RAPD analysis was effective to detect DNA polymorphism of ginger plants (*Etlingera elatior*) irradiated with 10 Gy gamma ray. Gamma irradiation is able to induce the production of reactive free radicals which could

change the DNA sequence and break DNA double bonds. Gamma irradiation also caused chromosomal abberation such as deletion, invertion, translocation, and duplication [45].

Table 4 The number of DNA bands produced by RAPD amplification of MV4 DNA with 15 primers

Num	Primer	Sequence	Total bands	Polymorphic bands	Size (bp)
1	OPA-02	5'-TGCCGAGCTG-3'	9	3	270-1250
2	OPA-03	5'-AGTCAGCCAC-3'	10	5	350-3000
3	OPA-09	5'-GGGTAACGCC-3'	11	5	625-3000
4	OPA-14	5'-TCTGTGCTGG-3'	10	5	500-2500
5	OPB-18	5'-CCACAGCAGT-3'	15	6	300-3000
6	OPC-05	5'-GATGACCGCC-3'	14	7	375-2250
7	OPC-08	5'-TGGACCGGTG-3'	8	1	590-2250
8	OPC-11	5'-AAAGCTGCGG-3'	6	2	425-1140
9	OPC-14	5'-TGCGTGCTTG-3'	9	6	610-3000
10	OPD-08	5'-GTGTGCCCCA-3'	6	0	395-2500
11	OPD-10	5'-GGTCTACACC-3'	17	15	200-2000
12	OPD-18	5'-GAGAGCCAAC-3'	8	1	375-2250
13	OPD-20	5'-ACCCGGTCAC-3'	12	3	560-2330
14	OPE-03	5'-CCAGATGCAC-3'	5	4	340-1750
15	OPE-07	5'-AGATGCAGCC-3'	6	1	625-1500
Total			146	64	

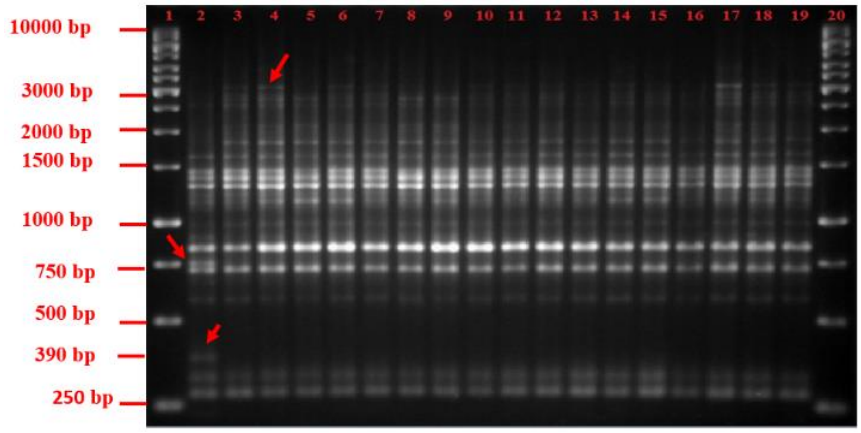


Figure 3 RAPD profiles of MV4 putative mutant clones amplified with OPB-18 primer
(1) Marker 1 Kb; (2) control; (3) 6-3-3-6; (4) 6-9-3; (5) 6-9-4; (6) 6-2-5-3; (7) 6-3-2-5; (8) 6-1-1-2; (9) 6-9-1; (10) 6-2-4-1; (11) 6-6-3-7; (12) 6-6-3-6; (13) 6-2-7; (14) 6-2-6-3; (15) 6-1-2; (16) 6-1-1-6; (17) 6-2-8-2; (18) 6-9-5; (19) 6-3-3-10; (20) Marker 1 kb.

Visualization of DNA amplified with OPB-18 primer showed that MV4 had new DNA bands as well as underwent the loss of DNA bands compared to control (Figure 3). The loss of DNA bands observed in the RAPD profile of MV4 clones was probably caused by destruction of DNA, rearragement of chromosomes, and deletion or insertion of DNA nucleotides. The existence of new DNA bands observed in RAPD profile of MV4 but not in control was probably caused by deletion or invertion of DNA nucleotides [44].

DNA mutation can change primer annealing sites which is followed by the change of DNA bands profile [46]. Polymorphism analysis of gamma-irradiated DNA has also been done to potato plants [47], *Hibiscus Sabdariffa* L. [48], and anthurium plants [49].

DNA bands produced by amplification with 15 RAPD primers were scored and analyzed for its genetic similiarity with NTSYS UPGMA and SHAN clustering to produce a dendogram. Dendogram depicts the genetic relationship between different plant varieties in the same species [20]. Dendogram of MV4 putative mutant clones based on RAPD analysis at similiarity coefficient cut off 0.91 showed 5 main clusters (Figure 4).

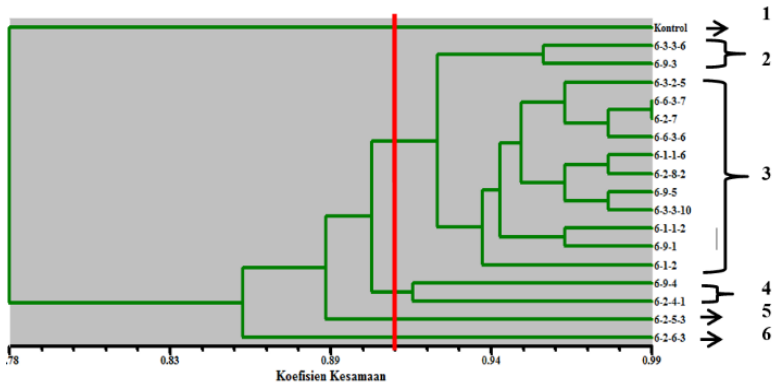


Figure 4 Dendogram of MV4 putative mutant clones based on RAPD profile
Dendogram was obtained from RAPD profiles scoring of 15 primers analyzed with NTSYS software at similiarity coefficient cut-off 0.91.

Genetic similiarity matrix showed the genetic distance between MV4 clones (Table 5). Matrix showed that there were no 100% genetic similiarity between 17 putative mutant clones and control. Mutant clone 6-2-6-3 had 72% genetic similiarity with control, which was the lowest among all clones. Clone 6-2-7 had 83% genetic similiarity with control, which was the highest among all clones. Based on Table 5, there were 2 clones with 75%, 3 clones with 77%, 2 clones with 78%, 3 clones with 79%, 2 clones with 80%, 1 clone with 81%, and 2 clones with 82% genetic similiarities with control. Genetic similiarity matrix (Table 5) showed the diversity of genetic changes between MV4 clones. According to [47], genetic variation and DNA repair mechanism of potato plants were varied from one cell to another. This genetic similiarity also indicated the random mutation caused by gamma irradiation [28].

Based on morphological markers, MV4 putative mutant clones 6-2-8-2 and 6-3-3-10 had 100% similiarity with control. While based on molecular marker, clones 6-2-8-2 and 6-3-3-10 had 79% and 82% genetic similiarity with control, respectively. The difference in genetic similiarity between MV4 putative mutant clones and control based on morphological and molecular markers wes due to the influence of environmental factors on morphological markers [36].

Table 5 Genetic similiarity matrix of MV4 putative mutant clones based on RAPD molecular markers

	Control	6-3-3-6	6-9-3	6-9-4	6-2-5-3	6-3-2-5	6-1-1-2	6-9-1	6-2-4-1	6-6-3-7	6-6-3-6	6-2-7	6-2-6-3	6-1-2	6-1-1-6	6-2-8-2	6-9-5	6-3-3-10
Control	1.00																	
6-3-3-6	0.77	1.00																
6-9-3	0.77	0.95	1.00															
6-9-4	0.75	0.87	0.90	1.00														
6-2-5-3	0.75	0.87	0.88	0.84	1.00													
6-3-2-5	0.79	0.90	0.91	0.88	0.91	1.00												
6-1-1-2	0.78	0.95	0.94	0.91	0.90	0.95	1.00											
6-9-1	0.81	0.90	0.92	0.91	0.90	0.95	0.96	1.00										
6-2-4-1	0.77	0.85	0.86	0.91	0.84	0.90	0.88	0.90	1.00									
6-6-3-7	0.82	0.91	0.92	0.89	0.90	0.97	0.94	0.97	0.94	1.00								
6-6-3-6	0.79	0.89	0.90	0.87	0.88	0.96	0.92	0.95	0.92	0.98	1.00							
6-2-7	0.83	0.90	0.92	0.89	0.89	0.95	0.92	0.95	0.94	0.99	0.97	1.00						
6-2-6-3	0.72	0.83	0.84	0.85	0.84	0.86	0.83	0.86	0.88	0.88	0.90	0.88	1.00					
6-1-2	0.78	0.90	0.91	0.90	0.90	0.93	0.93	0.93	0.89	0.94	0.92	0.95	0.86	1.00				
6-1-1-6	0.80	0.92	0.95	0.92	0.89	0.95	0.94	0.94	0.91	0.96	0.94	0.96	0.85	0.94	1.00			
6-2-8-2	0.79	0.92	0.96	0.92	0.89	0.92	0.92	0.94	0.88	0.93	0.91	0.93	0.85	0.92	0.97	1.00		
6-9-5	0.80	0.92	0.93	0.89	0.89	0.92	0.91	0.95	0.91	0.96	0.94	0.96	0.88	0.92	0.96	0.96	1.00	
6-3-3-10	0.82	0.91	0.92	0.89	0.88	0.94	0.92	0.95	0.92	0.97	0.95	0.97	0.86	0.94	0.97	0.95	0.97	1.00

According to [50], vegetative gamma-irradiated mutant plant is need to be analyzed for its genetic mutation stability until fourth generation (MV4). This is due to the chimera nature or instability of genetic mutation of mutant plant which was irradiated with gamma ray. There is a chance for mutant plants to be genetically reversed and become normal [51]. However, MV4 is a genetically-stable solid mutant generation [52; 53]. Rodent tuber MV4 clones have shown the stability of genetic mutation based on morphological and RAPD molecular markers.

4.0 CONCLUSION

MV4 putative mutant clones have undergone genetic changes from control based on morphological and RAPD profile. Based on RAPD molecular marker analysis, there were 5 genetic diversity clusters which proved that MV4 putative mutant clones were different from control. Putative mutant clone 6-2-6-3 had the highest genetic differences with control.

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