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Cassava (*Manihot esculenta* Crantz.) Improvement through Gamma Irradiation

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Abstract

Cassava (*Manihot esculenta* Crantz., Euphorbiaceae) is an important dietary carbohydrate source for approximately 800 million people in the tropics. Cassava breeding through conventional approaches are hampered with some limitations which resulted in a low number of superior varieties. The objective of this research was to generate several mutant lines with higher yield and starch content. According to field studies it was found that several cassava mutant lines have higher yield (root fresh weight >10-20 kg plant^{-1}). Two mutant lines has a high starch content (>39%). However, diversity on some variables are still found in M1V2 generation of cassava.

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Introduction

The import value of wheat based products is still on the first rank of food and agricultural products list in Indonesia, and it continues to increase year by year. In 1990 wheat flour consumption in Indonesia was 9.17kg/capita/year and almost doubled in 1999 (14.29 kg/capita/year). It is clear that wheat consumption per capita is constantly rising. This resulted in the increase of wheat import volume. In 2012, Indonesia has imported 6 million tons of wheat. Therefore, a breakthrough and efficient strategy are needed to reduce wheat import. One effort that

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can be done is to optimize and raise alternative sources of carbohydrates from tuberous crops such as cassava (*Manihot esculenta* Crantz., Euphorbiaceae). In Indonesia, from 2006-2011 the average of cassava planting area 1,199,313 ha, with production of 21,858,879 ton, and average productivity of 18 ton/ ha.

Cassava is an important dietary carbohydrate source for approximately 800 million people in the tropics [1]. Africa is the major producer of cassava worldwide, followed by Asia and Latin America, with total production around 200 million ton [2]. In the global trade of cassava, Thailand and Indonesia are the major cassava exporters; exporting mainly cassava chips, cassava pellets, cassava starch, and flour. More recently, it has gained importance as a possible fuel commodity not only in Indonesia but also in Philippines, China, Thailand, and other countries which have more advanced national bio-fuel programs.

The storage root of cassava is rich in starch (31%) [3]. Susilawati *et al*. [4] further reported that the starch content of Kasetsart variety reached 35.9% at 10 month after planting in Gunung Agung Village, Sekampung Udik sub district, Lampung Province. However, the starchy root of cassava is very poor in protein and contains linamarin/ cynogenic potential [5, 6]. Starchy root of cassava can be directly consumed if the cyanide (HCN) content ≤50 mg (kg fresh weight)$^{-1}$. Cassava for tapioca industry does not have any specific protein and HCN level requirement, since most of the HCN will be removed during processing.

With the development of cassava processing industry today, improvement of both yield and nutritional quality and reduction in cyanide content are among of agronomic characters need to be targeted in cassava breeding program. Cassava breeding through conventional approach faces some limitations, such as ploidy level, high heterozigosity, inbreeding depression, and low genetic variability caused by clonal propagation commonly applied for this plant. High genetic variability is one of important determinants in successful breeding of clonally propagated crops such as cassava. Mutation induction using gamma irradiation is one strategy to increase genetic variability. Joseph *et al*. [7] reported that gamma irradiation (Co^{60}) at the rate of 50 Gy could successfully induced mutation in cassava variety PRC-60a *in vitro*. That study reported that more than 50% mutant lines showed variability in morphology compared to the wild type plants. The objectives of this research were to generate several cassava mutant lines with higher yield and starch content. Some cassava mutant lines with higher yield and starch content, and also lower HCN content will be used for further studies, especially for multi location trials to develope the best lines as new varieties.

Materials & Methods

Stem cuttings of several mutants of M1V2 generation were planted in Cikabayan Experimental Field (240 m asl) in a planting space of 1 m x 1 m. Fertilizers were applied with the rate of 200 kg urea ha^{-1}, 150 kg SP-36 ha^{-1}, and 150 kg KCl ha^{-1}. Full rate of SP-36 was applied at the day of planting, while only 1/3 rate of urea was applied at the day of planting. At 1 month after planting (MAP), 2/3 rate of urea was applied, and full rate of KCl was applied at 2 MAP. Tuber morphological characters were observed at harvest time and scored according to IITA (*International Institute of Tropical Agriculture*) [8].

The broad sense heritability estimation (h^2bs) is obtained by using the following formula [9]:

$$h^2bs = \frac{\sigma^2 c}{\sigma^2 c + \sigma^2 e}$$

h^2bs = broad sense heritability

$\sigma^2 c$ = clones variance

$\sigma^2 e$ = environment variance

Heritability grouping according to the heritability value if 0% - 20% categorized as low, 20% - 50% as medium and more than 50% as high [10]. Measurement of starch content refers to the method of Luff Schoorl, while amylose content refers to Juliano [11].

Results & Discussion

In the non-irradiated cassava population of M1V1 generation (data not shown), the root fresh weight varies between genotypes. UJ-5 genotype had the highest root fresh weight (10.84 kg $plant^{-1}$), followed by Ratim (10.28 kg $plant^{-1}$), and Malang-4 (8.70 kg $plant^{-1}$). Several irradiated plants showed root fresh weight > 20 kg $plant^{-1}$ (Table 1), thus they are potentially developed as new cassava variants with higher yield. Water content of several mutants ranged between 57.94-60.13%, furthermore the starch content on several mutants ranged between 33.33-40.17%, and cyanide content ranged between 135.02-307.95 mg kg^{-1}.

Table 1. The weight of tubers per plant and tuber chemical character of some potential mutant M1V1 generation

Potential mutants	Root fresh weight/ plant (kg)	Water content (%)	Starch content (%)	Cyanide content (mg/kg)
V1D1-4(1)	15.5	57.94	40.17	181.51
V2D0-1(1)	20.5	60.13	39.09	172.55
V3D2-2(1)	9.0	61.02	36.92	307.95
V3D4-1(1)	26.0	59.92	33.33	135.02
V4D4-1(1)	5.5	61.09	36.50	171.19

Note : V1 = Jame-jame, V2= Ratim, V3=UJ-5, and V4=Malang-4.; D0 = 0 gy, D1= 15 gy, D2= 30 gy, D4= 60 gy.; numbers in the bracket indicate number of plant

Quantitative Character Pre-Harvest and Harvest Time of M1V2 Generation

Table 2 shows the results of mean values on harvest variables from the Ratim (V2) mutant genotypes. Mutant genotype with the highest plant height is V2D1-2(1)365.89 cm, this genotypes not significantly different from Ratim genotype, 290.87 cm. Mutant genotypes V2D1-1(3) and V2D1-4 (3) have mean value 237.11 cm and 237.78 cm for height to the first branching variable, and not significantly different from Ratim genotype (143.72 cm). Highest number of tubers from mutant genotypes was 14.0 tubers on V2D1-4(2) genotypes, which was not significantly different from Ratim genotypes (Ratim) (12.22 tubers). The lowest number of tubers was 2 tubers on V2D2-2(2). The highest number of economic tubers was 11.0 tubers from V2D1-4(1), which is significantly higher than the Ratim genotype (6.17 tubers). Based on these data, appears that the highest number of tubers is not followed by the number of economic tubers anyway, because the tubers that calculated as the number of tubers are the tubers with a length more than 5 cm. Genotypes with the thick cortex wasV2D1-5(3) with 0.152 cm which was not different from Ratim genotype (0.145 cm), while the thinnest cortex was on V2D1-4(2) genotypes.

Table 2. Average plant height, height to the first branching, number of tubers, number of economic tubers, and the cortex thickness from various Ratim mutant genotypes M1V2 generation

Genotype	Variable[1]				
	Plant height (cm)	HFB (cm)	Number of tuber	NET	CT (mm)
Ratim (V2D0)	**290.87 abcd**	**143.72 abc**	**12.22 ab**	**6.17 cde**	**1.45 ab**
V2D1-1(2)	309.22 abcd	121.11 abcd	8.00 def	5.00 cdef	0.93 f
V2D1-1(3)	334.22 abcd	**237.11 a**	10.67 bcd	4.67 def	1.17 cde
V2D1-2(1)	**365.89 a**	194.50 abc	7.67 ef	6.00 cde	1.15 de
V2D1-2(2)	246.67 dc	135.00 abcd	12.00 ab	4.00 efg	0.80 fg
V2D1-2(3)	328.11 abcd	215.00 ab	9.00 cdef	3.33 fg	1.33 bcd
V2D1-3(2)	265.78 bcd	100.28 abcd	12.00 ab	7.00 bcd	1.10 e
V2D1-3(3)	279.17 abcd	153.56 abc	9.00 cdef	3.00 fg	0.85 fg
V2D1-4(1)	257.50 bcd	127.89 abcd	13.00 ab	**11.00 a**	1.16 de
V2D1-4(2)	272.33 abcd	99.44 abcd	**14.00 a**	9.00 ab	**0.73 g**
V2D1-4(3)	336.67 abc	**237.78 a**	10.33 bcde	4.00 efg	1.36 abc
V2D1-5(1)	354.33 ab	190.67 abc	8.67 cdef	4.33 efg	1.26 cde
V2D1-5(2)	261.33 bcd	**60.44 cd**	7.33 ef	5.00 cdef	1.32 bcd
V2D1-5(3)	334.78 abcd	180.89 abc	6.00 f	5.00 cdef	**1.52 a**
V2D1-6(1)	271.33 abcd	105.89 abcd	7.50 ef	4.50 ef	1.21 cde
V2D2-1(3)	**237.50 d**	95.33 bcd	11.00 bc	7.33 bc	1.43 ab
V2D2-2(2)	240.11 dc	139.72 abc	**2.00 g**	**2.00 g**	1.18 cde

[1] Value from the same coloumn followed with the same letter is not significantly different in 5 % test level (Duncan Multiple Range Test). HFB: height to the first branching, NER: number of economic tubers, CT: cortex thickness; V2 =Ratim ....; D0 = 0 gy, D1= 15 gy, D2= 30 gy; numbers in the bracket indicate number of plant

Height to the first branching from UJ-5 mutant with the highest value was founded on V3D1-3(3) with 295.67 cm and significantly different from UJ-5 genotype (83.75 cm). The lowest value is 0.00 cm that means the mutant plant does not have any branches founded on V3D1-1(3), V3D1-2(1), V3D2-3(2), V3D1-4(1), and V3D4-1(1).

The heaviest tuber weight was 10.62 kg obtained in V3D2-4 (1) genotype, which was significantly different from control genotypes (7.38 kg). Highest number of tubers per plant were 35 tubers obtained in mutant V3D1-3 (3), significantly different from the UJ-5 genotype (18.78 tubers). Highest number of economic tubers was 15.67 and found in V3D2-4 (1) genotypes with the total number of tubers is 20.33. The highest value of cortex thickness was 0.163 cm in V3D1-4(2) genotypes, while the thinnest was on V3D1-3(2) genotype (0.065 cm), the data presented in the Table 3.

Table 3. Average height to the first branching, number of branching, tuber weight, number of tuber, number of economic tuber, and cortex thickness from various UJ-5 mutant genotypes M1V2generation

Genotype	Variable[1]				
	HFB (cm)	TW (kg)	NT	NET	CT (mm)
UJ-5 (V3D0)	**83.75 bc**	**7.38 bcd**	**18.78 def**	**10.00 bcd**	**0.93 fg**
V3D1-(1)	33.33 c	5.96 bcdef	14.00 hij	6.33 efg	0.83 gh
V3D1-1(3)	**0.00 c**	5.03 def	24.00 b	9.00 bcde	1.09 defg
V3D1-2(1)	**0.00 c**	5.69 cdef	12.00 ij	4.33 ghi	1.37 abcd
V3D1-2(2)	194.89 abc	6.15 bcdef	16.00 fgh	3.00 hi	1.22 cdef
V3D1-3(2)	261.33 ab	6.49 bcde	18.00 efg	10.00 bcd	**0.65 h**
V3D1-3(3)	**295.67 a**	8.55 ab	**35.00 a**	12.00 b	1.03 efg
V3D1-4(2)	196.67 abc	7.37 bcd	17.00 efgh	6.00 efgh	**1.63 a**
V3D1-5(2)	147.50 abc	6.69 bcd	23.00 bc	8.00 cdef	1.10 defg
V3D1-5(3)	94.44 abc	5.74 bcdef	18.67 def	5.33 fgh	1.42 abc
V3D1-6(1)	**50.00 c**	5.32 cdef	11.00 j	7.67 def	1.33 bcde
V3D2-(1)	188.78 abc	3.48 fg	15.00 ghi	12.00 b	1.57 ab
V3D2-1(1)	133.33abc	7.03 bcd	14.33 hij	9.33 bcde	1.09 defg
V3D2-1(2)	154.22 abc	7.55 bcd	22.00 bcd	8.00 cdef	1.42 abc
V3D2-1(3)	144.50 abc	6.60 bcd	22.00 bcd	12.00 b	1.27 cde
V3D2-3(2)	**0.00 c**	3.81 efg	11.67 ij	**2.00 i**	1.27 cde
V3D2-4(1)	**0.00 c**	**10.62 a**	20.33 cde	**15.67 a**	1.58 ab
V3D2-4(2)	120.00 abc	**2.06 g**	**7.00 k**	**2.00 i**	0.95 fg
V3D2-4(3)	120.00 abc	6.84 bcd	11.00 j	8.00 cdef	1.12 defg
V3D2-6(1)	**50.00 c**	7.93 bc	19.00 def	11.33 bc	1.47 abc
V3D4-1(1)	**0.00 c**	6.88 bcd	16.00 fgh	9.00 bcde	1.30 bcde

[1] Value from the same coloumn followed with the same alphabet is not significantly different in 5 % test level (Duncan Multiple Range Test). HFB: height to the first branching, TW: Tubers Weight, Number of tubers, NER: number of economic tubers, CT: cortex thickness. D0 = 0 gy, D1= 15 gy, D2=30 gy, D4= 60 gy.; numbers in the bracket indicate number of plant

Mutants from Malang-4 genotype M1V2 generation has height to the first branching that does not different from the origin genotypes (131.44 cm), the highest value founded on V4D1-2(2), V4D2-2 (3) and V4D3-4 (3) with the range of values 243.11 cm- 302.44 cm. Other mutant plants have a height to the first branching score 0.00 cm, which means that the mutant plants do not have any branches (Table 4).

Table 4. Average height to the first branching, number of branching, tuber weight, number of tuber, number of economic tuber, and cortex thickness from various Malang-4 mutant genotypes M1V2 generation

Genotype	Variable[1]				
	HFB(cm)	TW	NT	NET	CT (mm)
Malang-4 (V4D0)	**131.44 ab**	**7.51 abc**	**12.00 bcde**	**8.17 abc**	**1.33 bc**
V4D1-(1)	**0.00 b**	4.81 de	**8.00 e**	**5.00 c**	**2.43 a**
V4D1-1(1)	**0.00 b**	**3.78 e**	8.33 de	**4.67 c**	1.35 bc
V4D1-1(3)	**0.00 b**	4.93 cde	10.33 cde	6.00 bc	1.37 bc
V4D1-2(2)	**243.11 a**	6.67 bcd	15.00 abc	8.00 abc	**1.02 e**
V4D1-4(3)	**0.00 b**	5.11 cde	12.00 bcde	7.67 abc	1.41 bc
V4D1-5(1)	**0.00 b**	5.70 bcde	9.00 de	**4.00 c**	1.43 b
V4D2-1(1)	**0.00 b**	6.82 abcd	15.00 abc	**11.00 a**	1.26 cd
V4D2-1(2)	**0.00 b**	7.36 abcd	11.67 bcde	8.67 abc	1.43 b
V4D2-2(2)	207.39 ab	7.19 abcd	13.00 bcd	10.00 ab	1.17 d
V4D2-2(3)	**280.44 a**	6.62 bcd	15.67 ab	**11.33 a**	1.48 b
V4D3-4(3)	**302.44 a**	7.94 ab	**18.00 a**	10.00 ab	1.40 bc
V4D4-(1)	**0.00 b**	**9.33 a**	**8.00 e**	**5.00 c**	1.33 bc

[1] Value from the same coloumn followed with the same alphabet is not significantly different in 5 % test level (Duncan Multiple Range Test). HFB: height to the first branching, TW: Tubers Weight, Number of tubers, NER: number of economic tubers, CT: cortex thickness. D0 = 0 gy, D1= 15 gy, D2= 30 gy, D3= 45 gy, D4= 60 gy.; numbers in the bracket indicate number of plant

The weight of tubers per plant from Malang-4 genotype was 7.51 kg, which is not different from the mutant with the highest tuber weight (9.33 kg) on V4D4-(1). Mutants with the lowest tuber weight (3.78 kg) was V4D1-1(1). Highest number of tubers found in V4D3-4(3) with 18.00 tubers, significantly different from the origin genotypes (12.00 tubers). The number of economic tubers in V4D2-1(1) and V4D2-2(3) are 11.00 and 11.33 tubers. These results did not different withthe origin genotype (8.17). The high value of cortex thickness founded in V4D1-(1) (0.243 cm) and V4D1-2(2) is the lowest value of cortex thickness (0.102 cm), while the origin genotypes cortex thickness was 0.133 cm.

Shape, Type, and Tuber Taste

Ratim genotype has a sessile tuber types, the tuber types became mixed in some M1V1dan M1V2 generation mutant (Table 5). UJ-5 Genotype has sessile tuber types. In M1V1 generation tuber type onV3D1-5(3) and V3D2-1 (3) genotypes does not change, while the other mutants changed from sessile become mixed. Tubers from M1V2 generation show the diversity on type of tuber from cuttings source, which became sessile, mixed, and penduncculate. Malang-4 genotype type of tuber are sessile, but in M1V1 generation especially V4D2-1 (1) genotypes has a mixed tuber type and V4D3-4 (3) has penduncculate type. In M1V2 generation the tuber type still show diversity and the average type of tubers are sessile and mixed.

Tuber shape that being measured was the overall shape of tubers from the base to the tip of tuber. Ratim tuber shape has a cylindrical shape, whereas M1V1 generation mutant has three shape (conical shape, cylindrical, and irregular). M1V2 generation mutants show a variety of shape from the cuttings source, which is conical-cylindrical, cylindrical, and irregular. UJ-5 tubers shape was conical, whereas M1V1 generation mutant showdiversity become conical, conical-cylindrical, cylindrical, and irregular. In M1V2 generation mutant, tuber shape dominated with cylindrical and V3D1-2(1), V3D2-1(1) and V3D2-4 (1) has a conical tubers shape on plants from tip cuttings. Malang -4 Genotype have conical-cylindrical tuber shape, while the M1V1 generation occurs tuber shape diversity which are conical, conical-cylindrical, and cylindrical. Mutant tuber forms in M1V2 generation was dominated by cylindrical shape.

Table 5 Diversity on tuber type, tuber shape, and tuber taste of M1V1 and M1V2 generation

Variable	Origin genotype		M1V1 mutant	M1V2 mutant
Tuber Type	Ratim	*sessile*	*mixed*	*Mixed*
	UJ-5	*sessile*	*sessile*	*Mixed* and *penduncculate*
	Malang-4	*sessile*	*Mixed and penduncculate*	*Sessile and mixed*
Tuber Shape	Ratim	cylindrical	Conical, cylindrical, irregular	Conical- cylindrical, cylindrical, irregular
	UJ-5	conical	Conical, conical- cylindrical, cylindrical, irregular	Dominant cylindrical
	Malang-4	Conocal-cylindrical	Conical, conical- cylindrical, cylindrical	Dominant cylindrical
Tuber Taste	Ratim	Sweet	Sweet to intermediate	Sweet to intermediate
	UJ-5	Bitter	Intermediate to bitter	Intermediate to bitter
	Malang-4	Bitter	Sweet to bitter	Sweet to bitter

Tuber taste is one of the qualitative characteristics that determine the utilization of cassava tubers by consumers, which is due to HCN content. Ratim genotypes has a sweet tuber taste, and genotypes V2D1-2(2), V2D1-5 (3), V2D2-1 (3), and V2D2-2 (2)in M1V1 generation has an intermediate taste. The M1V2 generation has a tuber taste from sweet to bland. UJ-5 tuber taste was bitter and M1V1 generation mutant genotypes have an intermediate taste. In M1V2 generation of V3D2-1(1), V3D2-4 (1), V3D1-2 (1) V3D1-6 (1), V3D2- (1), and V3D2-

6 (1) has an intermediate taste. Tuber taste from Malang-4 genotype was bitter, and the M1V1 generation mutants taste ranging from sweet to bitter. Tuber taste in M1V2 generation generally intermediate to bitter, genotypes of V4D3-4(3) has an intermediate taste (Table 5). Irradiation doses were effective in suppressing HCN, determined by the cassava varieties used. Genetic changes caused by physical mutation in cassava plants can alter the character of agronomic and chemical content of cassava randomly, so radiation treatment can give various results [12]. The gamma-ray irradiation may lead to diversity, which is caused by the interaction of genetic and environmental factors [13].

Broad Sense Heritability Estimation

Broad sense heritability of the Ratim (V2), UJ-5 (V3), and Malang-4 (V4) mutant genotype are presented in Table 6. Heritability estimation value of Ratim mutant genotypes on plant height, height to the first branching and the number of branching were 99.02%, 62.31%, and 72 70%. Heritability estimation value on the cortex thickness was 91.28%, while the value for number of tubers and the number of economic tubers were 95.06%, and 87.87%. Tuber weight variable has a heritability estimation value 88.27% (Table 6). Based on these results, it can be stated that all of these variables can be used as selection criteria in the mutants from Ratim genotypes for the next generation.

However, not all of the observed variables can be used as selection criteria in the next mutant generation from the UJ-5 genotype. Based on the broad sense heritability estimation value, only plant height variables, number of tuber, and number of economic tuber that can be used as selection criteria in the mutants from UJ-5 on next generation. In Malang-4 genotype appears that height to the first branching and tuber weight per plant have a 0.00% broad sense heritability estimation values, which indicates no more diversity showed on these variables.

Table 6.Broad sense heritability (H^2bs) estimation value on the Ratim, UJ-5, and Malang-4 mutant genotype

Variable	Ratim	UJ-5	Malang-4
	----------------------------------	(%)	------------------------------
Plant height	99.02	97.12	41.64
Height to first branching	62.31	45.92	0.00
Number of branching	72.70	39.16	84.53
Cortex thickness	91.29	0.00	90.20
Number of tubers	95.06	99.35	93.20
Number of economic tubers	87.87	90.46	77.72
Root weight per plant	88.28	48.18	0.00

Another factor that assume for resulting low diversity on several variables for Malang-4 and UJ-5were because UJ-5 is the introduced varieties from Thailand and Malang-4 is national varieties, so the genetic constitution of those two varieties are difficult to be changed. Heritability estimation values were high on Ratim genotype, could be caused by Ratim is a Halmahera local genotype so the genetic constitution easily changing. High heritability estimation values on observation variable showed that the phenotypic variables are influenced by genetic factors and selection activity in cassava mutant can be based on these variables [14].

Conclusion

Gamma irradiation induced some morphological changes and variability in yield. Several irradiated plants (M1V1 generation) showed root fresh weight > 10-20 kg plant^{-1}, thus they are potentially developed as new cassava variants with higher yield. Diversity on some variables are still found in M1V2 generation cassava both on pre-harvest and harvest quantitative variables. Heritability values from Ratim mutant genotypes were obtained as high, while the UJ-5 and Malang -4 mutant genotypes has a low to high heritability value. Number of tubers and number of economic tuber variable from three mutant origin has a high heritability estimation values, so it can be used as a third-generation (M1V3) mutant selection variables.

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