BIODIVERSITAS
Volume 20, Number 1, January 2019
Pages: 337-342

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200139

Short Communication: Early stability test of mutant candidates of Bangka local cassava, Indonesia

TRI LESTARI♥, ERIES DYAH MUSTIKARINI, RION APRIYADI, SAIPUL ANWAR
Faculty of Agriculture, Fisheries and Biology, Universitas Bangka Belitung. Desa Balunijuk, Kecamatan Merawang, Kbupaten Bangka 33172, Provinsi Bangka Belitung, Indonesia. Tel./Fax. +62-717-4260048, ♥email: trilestari25sm07@gmail.com

Manuscript received: 26 June 2018. Revision accepted: 30 December 2018.

Abstract. *Lestari T, Mustikarini ED, Apriyadi R, Anwar S. 2019. Early stability test of mutant candidates of Bangka local cassava, Indonesia. Biodiversitas 20: 337-342.* Cassava production in Indonesia has decreased due to decreased harvest area. Increasing production can be done by improving genetic diversity through gamma-ray irradiation to create high production mutant. The purpose of this study was to determine the optimum irradiation doses in relation to improve production of local cassava from Bangka Island, as well as morphological changes resulting from the irradiation. The research was conducted at the Experimental Garden, the University of Bangka Belitung, Indonesia in September 2017 until April 2018. This experiment was using Split Plot design, with the main plot consisted of 2 levels and subplot have 3 treatment levels, and 4 replications, in total there were 24 experimental units. The results showed that gamma-ray irradiation had very significant effect on plant height, number of leaves, young leaf color, stem color, and number of lobes. Gamma-ray irradiation on 3 Bulan accession showed an improved tuber production, whereas in Malang varieties gamma-ray irradiation decreased tuber production. Gamma-ray irradiation dose of 30 Gray was able to improve the number of tubers, tuber weight and starch content on 3 Bulan cassava accession.

Keywords: Cassava, characterization, irradiation

INTRODUCTION

Cassava plant (*Manihot esculenta* Crantz) is one of the plant sources of carbohydrate which are commonly consumed by people of Indonesia. The utilization of cassava as raw food material was accounted for 72%, for industrial raw material was around 13%, and for animal feed was less than 2%. Processed cassava products include cassava flour, tapioca, and *mocav* flour. Lestari and Apriyadi (2017) considered cassava has potency to be developed as the main ingredient for local food industry in Bangka Island.

Based on BPS data (2017), cassava production in Indonesia decreased to 7.02% in 2016 (20,255,867 ton) from 2015 (20,801,415 ton) with decreasing harvested area by 13.39% from 2015. This condition had made Indonesia imported cassava from other countries. Therefore, the production and productivity of cassava must be improved by using superior genotypes.

The efforts to develop superior genotypes can be done by increasing the diversity of plants genetics through mutation. Induction of mutations can be done by using chemical and physical mutagenic agents. The physical mutagen is a mutation caused by physical material, i.e., alpha, beta, and gamma-rays. While chemical mutagen is a mutation induced by chemical material, such as ethyl methanesulfonate, diethyl sulfate, ethylamine and colchicine (Sutapa and Kasmawan 2016). Irradiation has a possibility to increase only certain desired character, without changing the other characters. Aisyah et al. (2009) reported that mutation with irradiation in vegetative phase showed better results than those of chemical mutagens. This was caused by low absorption capacity of plant vegetative tissue to chemical liquids.

Induction of mutations by gamma-ray irradiation can induce mutations and alterations of chromosome arrangement (Sutapa and Kasmawan 2016). Gamma-ray irradiation is also known to increase the production of cassava plants (Maharani et al. 2015). Maharani et al. (2015) stated that 15 Gray irradiated doses treated on cassava accession Jame-jame from Halmahera can increase tuber weight, number of tubers, number of commercial tubers, and stem diameter higher than control and 30 Gray dosages.

Irradiation induction in plants has random effect in plants morphology. Random effect in plant morphology needs to characterize to obtain best genotype with several superior characters. Character selection from mutant induced by gamma-ray irradiation may have high genetic diversity in selected plant. The purpose of this study was to determine the optimum irradiation doses in relation to improve production of local cassava from Bangka Island, as well as morphological changes resulted from the irradiation.

MATERIALS AND METHODS

This research was conducted in the Experimental Garden of the Faculty of Agriculture, Fisheries and Biology, the University of Bangka Belitung, Indonesia from October 2017 until April 2018.

This experiment used Split Plot design, with the main plot was cassava accession that was consisted of 2 levels and 3 levels of subplot (irradiation dosages). The main plot consisted of R0: 0 Gray (Control), R1: 15 Gray, and R2: 30 Gray. The subplot composed of A1: 3 Bulan and A2: Malang. There were 6 combinations of treatment, each had 4 replications. Every replicate consisted of 4 samples, thus there were 24 experimental units and 96 plant samples in total. The size of each plot was 2 x 3 m^2 with 1m space between the plots.

The soil processed by hand tractor then formed into a 2 x 3 m^2 plot and was given chicken manure with a dosage of 5 tons/ha. Seedlings used were from stem cutting that has been chosen from the middle of the stem of plant aged 7 months old or older. The tip of the lower cuttings was cut sloping 45^o to expand the root area and as a sign of the planted part (Apriyadi 2011). The prepared cuttings then irradiated with gamma rays according to the selected dosages. Seed planting was done by placing ± ½ part of the cuttings in soil vertically. Planting maintenance included watering, weed control, and pest control. Harvesting was performed at 7-9 months after planting. The harvest time was characterized by the decreased of leaves' growth, in this case, the color of leaves was slightly yellow, and many leaves were fallen (Apriyadi 2011). Harvesting must be done carefully to reduce the damage.

Data were analyzed using F test with 95% significant level. If the data significantly different, then it will be followed by DMRT test at 95% significant level using SAS application (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

The result of variance analysis showed that the difference of irradiation dose had significant effect on plant height, number of leaves, number of tubers, but had no significant effect on stem diameter, weight of tubers and starch content. Differences in accession had a significant effect on all observation parameters, except for starch content. The interaction between the two levels had no significant effect on each parameter (Table 1).

Duncan Multiple Range Test (DMRT) showed that the stem diameter, weight of tubers, and starch content were not significantly different in all irradiation dosages. The number of leaves without irradiation levels (control) was significantly different with 15 Gray irradiation dose, but not significantly different with 30 Gray irradiation dose. The plant high and number of tubers of cassava plant without irradiation levels (control) was not significantly different with the irradiation dose of 15 Gray, but was significantly different with the irradiation dose of 30 Gray.

The number of tuber of cassava plant without irradiation dose (control) levels was not significantly different with the irradiation dose of 15 Gray, but was significantly different with the irradiation dose of 30 Gray (Table 2). The results of Maharani et al. (2015), that the number of tubers cassava plant was more at a dose of 15 Gray irradiation. These results are different allegedly because gamma-ray irradiation is random, so it can not be directed at a specific target.

The weight of cassava tubers without irradiation levels did not differ significantly with irradiation levels. The highest average weight of tuber was found in irradiation levels at 30 Gray, while the lowest weight of tuber was found in cassava without irradiation levels. Tuber weight in cassava plants is also influenced by the physiological cassava plant. The growth of leaf and roots as sources and sinks in cassava occur simultaneously, resulting in competition in obtaining photosynthesis. More dominant plant growth can inhibit the growth of underground plants (tubers). The results of Egesi et al. (2008) also show that overcrowded cassava (> 2 m) has a low harvest index.

The starch content of cassava without irradiation levels did not differ significantly with irradiation levels. The starch content of cassava is influenced by genetic, plant and physiological environment. Rahmiati et al. (2016) suggested that a genetic change in its potential to produce starch can occur in cassava genotypes that are induced by gamma rays. Li et al. (2016) stated that the accumulated starch content of fresh root is influenced by high stem transport flow stem flow rate (SFR) capacity, high starch synthesis (influenced by enzymatic activity) in the stem and low degradation at root, and high gene expression related transport sugar on the stem.

Table 1. Variant analysis of irradiation dose and accession to plant high, number of leaves, and stem diameter, number of tubers, weight of tuber and starch content of cassava plant

Parameters observed	Irradiation		Accession		Interaction		KK (%)
	F-Value	Pr>f	F-Value	Pr>f	F-Value	Pr>f	
Plant high	12.32**	0.0012	47.93**	0.0001	0.68^{tn}	0.5272	9.57
Number of leafs	6.32*	0.0133	114.13**	0.0001	1.15^{tn}	0.3505	15.87
Stem diameter	2.48^{tn}	0.1257	9.72**	0.0089	0.58^{tn}	0.5760	6.68
Number of tubers	11.83**	0.0015	16.43**	0.0016	0.77^{tn}	0.4862	19.23
Weight of tubers	0.32^{tn}	0.7327	15.84**	0.0018	0.25^{tn}	0.7846	25.05
Starch content	2.73^{tn}	0.1052	0.75^{tn}	0.4043	0.96^{tn}	0.4096	15.93

Note: KK: The coefficient of diversity, **: The effect is very real at the level of 1%, *: Significant effect on the level of 5%, tn: No real effect, Pr> F: Probability Value

Table 2. Average plant high, number of leaves, stem diameter, number of tubers, weight of tuber and starch content on levels of different gamma-ray irradiation doses

Irradiated dosage (Gray)	Plant high (cm)	Number of leaves (Sheet)	Stem diameter (cm)	Number of tubers (item)	Weight of tubers (kg)	Starch content (%)
0	164.47 a	111.66 a	2.50	14.75 a	10.35	42.20
15	154.16 a	88.60 b	2.41	14.37 a	9.87	40.81
30	129.88 b	87.80 b	2.32	9.37 b	10.91	35.26

Note: The numbers followed by the same letter in the same column show no significant difference at the 5% level

Table 3. Average plant high, number of leaves, stem diameter, number of tubers, weight of tubers and starch content at levels of different accession

Type of cassava	Plant high (cm)	Number of leaves (sheet)	Stem diameter (cm)	Number of tubers	Weight of tubers (kg)	Starch content (%)
3 Bulan	169.73 a	129.26 a	2.52 a	14.87 a	8.26 b	40.53
Malang	129.28 b	62.77 b	2.31 b	10.79 b	12.49 a	38.31

Note: The numbers followed by the same letter in the same column show no significant difference at the 5% level

Table 4. Crop morphology of cassava leaves at different irradiated doses and accessions

Irradiation dose (Gray)	Type of cassava	Form of lobes	No. of lobes	Color of young leaves	Color of old leaf	Color of petiole
0	3 Bulan	Lancet	3	Brown	Dark green	Red
	Malang	Lancet	5	Purple	Dark green	Light red
15	3 Bulan	Lancet	3	Violet	Dark green	Red
	Malang	Lancet	7	Violet	Dark green	Light red
30	3 Bulan	Lancet	3	Violet	Dark green	Red
	Malang	Lancet	7	Brown	Dark green	Light red

3 Bulan accession and Malang variety have significant differences on all observation parameters, except for starch content. Plant height, number of leaves, stem diameter, and number of tubers in accession 3 Bulan higher than those of Malang variety. Tuber weight and starch content in Malang variety are higher than accession of 3 Bulan (Table 3). 3 Bulan accession and Malang variety showed significant differences in all quantitative parameters, except for starch content. The average high of plant, number of leaves, stem diameter, and number of tubers was highest in accession of 3 Bulan, but with lower weight of tuber and starch content. Malang varieties have lower plant high, number of leaves, stem diameter and number of tubers, but with higher weight of tuber and starch content of 3 Bulan accession. Malang is national cassava varieties that have high production potential, while 3 Bulan is local accession of Bangka Belitung. Based on Lestari and Apriyadi (2017), local access to cassava from Bangka has different morphological characteristics that can be influenced by genetics and the environmental factors.

The interaction of the gamma-ray irradiation dose levels and the difference of accession were not significantly different in all levels. Average of plant height, number of leaves, stem diameter, and number of tubers of 3 Bulan accessions and Malang varieties decreased in irradiation dosages of 15 Gray and 30 Gray. The average weight of tubers and starch content of 3 Bulan accession are increasing in irradiation doses. The starch content of varieties of Malang decreased in the levels of irradiated doses (Figure 1).

The irradiation of gamma rays dosages on the 3 Bulan has differed effect on some quantitative characters. 3 Bulan accession treated in 30 Gray dose has lower plant height, leaves, tuber, and starch content, but higher tuber weight than those accessions treated in 15 Gray dosage. The highest weight of tuber from 3 Bulan accession was treated in 30 Gray doses (9.3 kg plant^{-1}), with starch content 34.7%. Rolland-Sabate et al. (2011) reported similar result in that an increase in quantity of tubers and starch content can be performed using gamma-ray irradiation. The results of Subekti et al. (2018) showed one of the genotypes of cassava mutants with irradiated doses of 15 Gray, having weight of tuber 5.3 kg plant^{-1} with starch content of 23.86%. Based on Hanafiah et al. (2010), that irradiation influences plant growth and development either through qualitative and quantitative that finally will influence plant's production.

Malang variety on irradiation treatment has higher value on plant height, number of leaves, number of tubers, weight of tubers but has lower starch content. Malang varieties without any treatment (control) have higher plant height, number of leaves and tubers, weight of tuber and starch content than those accessions treated in 15 Gray and 30 Gray dosages. Malang variety only experienced changes in the number of lobes and the color of young leaf (Figure 2). This is presumably because normal cells in Malang varieties are more dominant, whereas cells are mutated less and are not able to be expressed. Ceballos et al. (2008), the cassava genotype mutated using gamma rays undergoes a genetic change in its potential to produce starch, may be lower or higher than its parent.

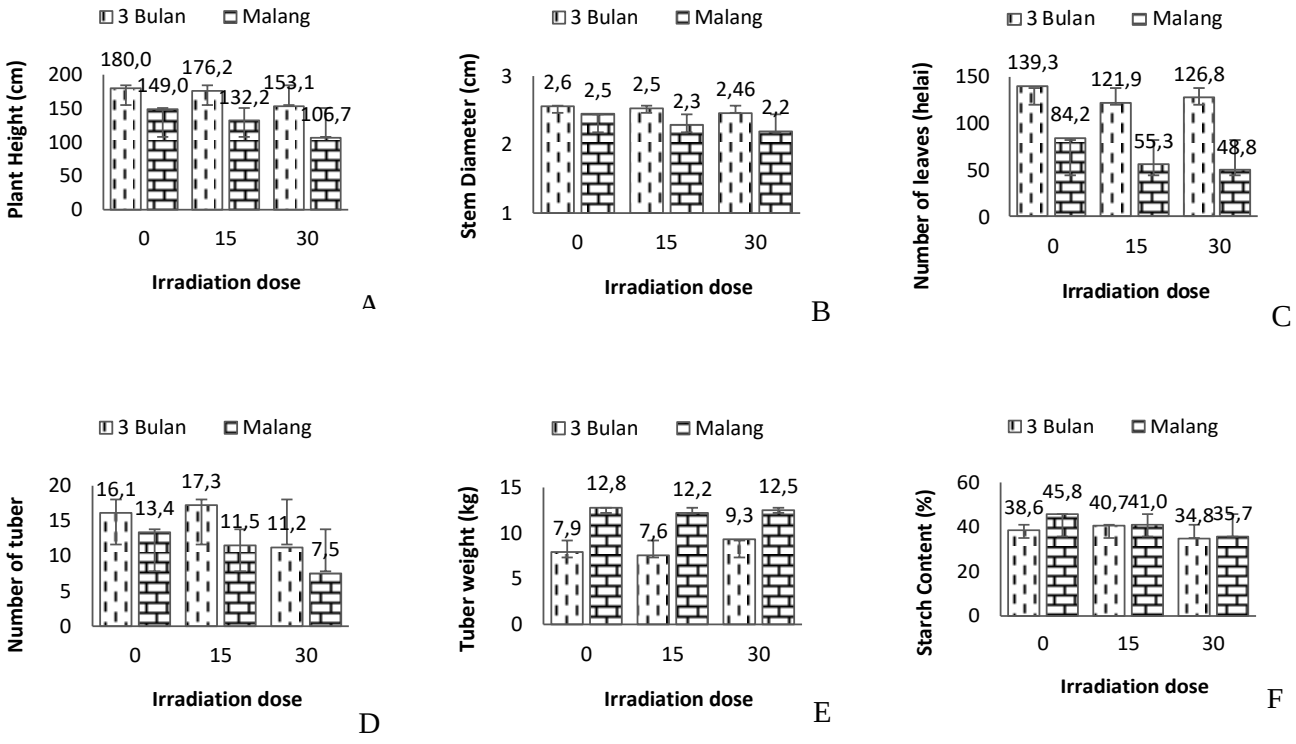

Figure 1. Interaction of different irradiation and accession doses on various observation parameters. A. Plant height, B. Number of leaf, C. Diameter of stem, D. Number of tubers, E. Weight of tubers, F. Starch content

The observation result showed that accession of 3 Bulan and Malang variety had lancet-form lobes and did not change at all irradiation dosages. The 3 Bulan accession has predominantly 3 lobes on all irradiated doses. Malang varieties have 5 lobes in control plants and at 15 Gray and 30 Gray irradiation doses are dominated by 7 strands. The color of young leaf on the 3 Bulan accession has a brown color in the control plants and on the levels of irradiated doses of 15 Gray and 30 Gray is dominated in purple. Malang varieties had the color of young leaf purple on plant without irradiation, levels of irradiation doses of 15 Gray dominated in purple, and on levels of 30 Gray irradiation doses predominantly brown (Table 4).

The color of old leaf on the accession of 3 Bulan and Malang varieties did not change color in all irradiation dose. 3 Bulan accession and Malang varieties dominated the same old leaf color that is dark green. The results showed that there was no change in the color of the 3 Bulan accession leaf stalk and the Malang varieties in all irradiation doses. The color of the leaf stalk on the accession of 3 Bulan is dominated red and the varieties of Malang are predominantly pink (Figure 2).

The color of the young stem on the 3 Bulan accession has a light green color on the control plant and the irradiation levels are 15 Gray, whereas in the levels the 30 Gray irradiation dose predominantly has green color. Malang varieties have the color of young stems dominated green in all levels of irradiation dose. The color of the old stems in the accession of 3 Bulan is dominated reddish brown. The color of old stems of Malang varieties dominated reddish brown on control plants and irradiation levels 15 Gray, while at 30 Gray irradiation doses predominantly gray. 3 Bulan accession and Malang variety have the same branching form of trichotomy. The outline of the branches did not change in either the 3 Bulan accession or the Malang variety on all irradiation doses (Table 5). Its showed irradiation doses can influence the morphological character changing in cassava. Based on Monikasari et al. (2018), gamma-ray irradiation can alter the morphological characteristics of plants. Maharani et al. (2015) stated that gamma radiation can improve genetic diversity through the alteration of gene structure, chromosome structure and number.

Tuber shape on accession of Three Month and Malang variety did not change at all irradiation dose levels. 3 Bulan accession and Malang variety have the same tuber form that is cone-cylindrical. The skin color and color of tuber meat of 3 Bulan accession and Malang variety did not change in all irradiation doses. 3 Bulan accession and Malang varieties have the same tuber skin color of beige. The color of tuber meat in the 3 Bulan accession and Malang variety has the same color that is white (Table 6). The results showed that cassava plants without irradiation (control) levels had different color of young leaf, stem color, and number of lobes from irradiated cassava plants (15 Gray and 30 Gray). This shows the levels of gamma-

ray irradiation doses allegedly causing changes in some morphological characters in cassava plants. Karyanti et al. (2015), indicating that the radiation changes the callus color of orange Garut due to the physiological phase. Plant morphology can also be influenced by the environment, such as the height of habitat. According to Tribadi et al. (2010), that the height of the cultivating site very much influences toward variations of root, stem and leaf morphology.

Malang 6 variety without irradiation treatment had higher plant height, more leaf, and number of tubers, tuber weight and higher starch content than irradiation treatment with a dose of 15 Gray and 30 Gray. Morphological characters of Malang 6 variety only had changes in the number of lobes and the color of young leaves. The number of lobes of Malang 6 variety was dominated by 7 strands at 15 Gray and 30 Gray irradiation doses, and the color of purple leaves became predominantly light purple on 15 Gray and chocolate color at 30 Gray irradiation doses. These results indicate that the effect of gamma-ray irradiation is only found on some morphological characters in Malang 6 variety. This is presumably because normal cells in Malang 6 varieties are more dominant, while mutated cells are less and unable to be expressed. Devy and Sastra (2016), stated that groups of normal cells were able to compete with mutated cells so that the occurrence of cell mutations had been selected. Ceballos et al. (2008), cassava genotypes that are mutated using gamma rays undergo genetic changes in their potential to produce starch, can be lower or higher than their ancestor.

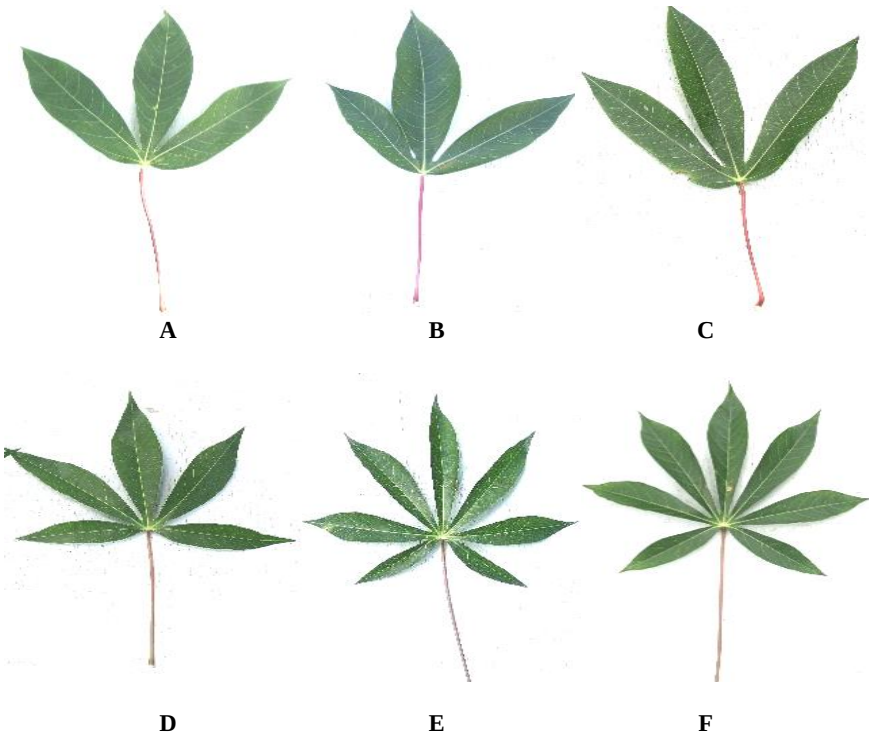

Figure 2. The shape of the lobes and the number of lobes on the old leaves of cassava plants with different irradiated doses. A. 3 Bulan Accession 0 Gray, B. Accession of 3 Bulan 15 Gray, C. Accession of 3 Bulan 30 Gray, D. Varieties Malang 0 Gray, E. Malang Variety 15 Gray, F. Malang Variety 30 Gray

Table 5. The morphology of cassava plant stems at different irradiated doses and accessions

Irradiation dosage (Gy)	Type of cassava	Form of branching	Color of young stem	Color of old stem
0	3 Bulan	Trichotomy	Light green	Reddish brown
	Malang	Trichotomy	Green	Reddish brown
15	3 Bulan	Trichotomy	Light green	Reddish brown
	Malang	Trichotomy	Green	Reddish brown
30	3 Bulan	Trichotomy	Green	Reddish brown
	Malang	Trichotomy	Green	Gray

Table 6. Cassava tuber morphology at different irradiated doses and accessions

Irradiation dosage (Gy)	Type of cassava	Shape of tuber	Color of tuber skin	Color of tuber
0	3 Bulan	Cylindrical cones	Cream	White
	Malang	Cylindrical cones	Cream	White
15	3 Bulan	Cylindrical cones	Cream	White
	Malang	Cylindrical cones	Cream	White
30	3 Bulan	Cylindrical cones	Cream	White
	Malang	Cylindrical cones	Cream	White

ACKNOWLEDGMENTS

The authors are grateful for financial support from Ministry of Research Technology and Higher Education, Republic of Indonesia for Applied Research Grant 2018.

REFERENCES

BPS. 2017. Produksi Ubi Kayu menurut Provinsi Tahun 2013-2016. Badan Pusat Statistik, Jakarta. [Indonesian].

Aisyah SI, Aswidinnoor H, Saefuddin A, Marwoto B, Sastrosumarjo S. 2009. Induced mutations in cutting of carnation (*Dianthus caryophyllus* Linn.) through Gamma Rays irradiation**.** Jurnal Agronomi Indonesia 37: 62-70.

Apriyadi R. 2011. Karakterisasi dan Pertumbuhan 10 Aksesi Ubi Kayu Lokal pada Lahan PMK dan Tailing Pasir Pasca Pernambangan Timah Bangka. [Hon. Thesis]. Universitas Bangka Belitung, Bangka. [Indonesian]

Ceballos H, Sanchez T, Denyer K, Tofindo AP, Rosero EA, Dufour D, Smith A, Morante N, Perez JC, Fahy B. 2008. Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). J Agric Food Chem 56 (16): 715-722.

Devy L, Sastra DR. 2016. Pengaruh iradiasi sinar gamma terhadap kultur in vitro tanaman jahe. Jurnal Sains dan Teknologi Indonesia. 8 (1): 1-14. [Indonesian]

Egesi CN, Ilona F, Ogbe M, Akoroda, Dixon A. 2008. Genetic variation and genotype x environment interaction for yield and other agronomic traits in cassava in Nigeria. Agron J 9 (9): 1137-1142.

Gomez KA, Gomez AA. 1984. Statistical Procedures in Agricultural Research, 2nd ed. Wiley, New York.

Hanafiah DS, Ningtyas T, Yahya S, Wirnas D. 2010. Induced mutations by gamma-ray irradiation to Argomulyo soybean (*Glycine max*) variety. Nusantara Biosci 2 (3): 121-125.

Karyanti, Purwito A, Husni A. 2015. Radiosensitivity and selection putative mutans mandarin cv. garut based on morphological markers. Jurnal Agronomi Indonesia 43 (2): 126-132.

Lestari T, Apriyadi R. 2017. Genetic potential of cassava biodiversity In Bangka Island, Indonesia. Cell Biol Dev 1 (2): 41-45.

Li YZ, Zhao JY, Wu SM, Fan XW, Luo XL, Chen BS. 2016. Characters related to higher starch accumulation in cassava storage roots. Sci Rep 6 (2): 1-17.

Maharani S, Khumaida N, Syukur M, Ardie SW. 2015. Radiosensitivity and variability of gamma irradiated cassava (*Manihot esculenta* Crantz). Jurnal Agronomi Indonesia 43 (2): 111 – 117.

Monikasari INS, Anwar S, Kristantanto BA. 2018. Variability of M1 sunflower ornamental plant affected by gamma-ray irradiation. Jurnal Agro Complex 2 (1): 1-11.

Rahmiati, TM, Purwanto YA, Budijanto S, Khumaida N. 2016. Physicochemical properties of cassava flour (*Manihot esculenta* Crantz) of 10 breeding genotypes. Agritech J 36 (4): 459-466.

Rolland-Sabate A, Sanchez T, Buleon A, Colonna P, Jaillais B, Ceballos H, Dufour D. 2011. Structural characterization of novel cassava starches with low and high-amylose contents in comparison with other commercial sources. Food Hydrocolloids 27 (1): 161- 174.

Subekti I, Khumaida N, Wahyuning AS, Syukur M. 2018. Yield and starch content evaluation of gamma irradiated cassava mutants at M1V4 generation. Jurnal Agronomi Indonesia 46 (1):64-70.

Sutapa GN, Kasmawan IGA. 2016. Efek induksi mutasi radiasi Gamma 60Co pada pertumbuhan fisiologis tanaman tomat (*Lycopersicon esculentum L.*). Jurnal Keselamatan Radiasi dan Lingkungan 1 (2): 5-11. [Indonesian]

Tribadi, Suranto, Sajidan. 2010. Variation of morphological and protein pattern of cassava (*Manihot esculenta*) varieties of Adira1 and Cabak makao in Ngawi, East Java. Nusantara Biosci 2 (1): 14-22.