Identification of potentially high yielding irradiated cassava 'Gajah' genotype with different geographic coordinates

I Subekti, N Khumaida* and SW Ardie
Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University (IPB), Jl. Meranti Kampus IPB Darmaga, Bogor

E-mail: nkhumaida@yahoo.com

Abstract. Cassava is one of the main and important carbohydrate producing crops in Indonesia. Thus cassava production and its tuber quality need to be improved.'Gajah' genotype is a local genotypes cassava from East Kalimantan, has high potential yield (> 60 ton Ha^{-1}). However, the harvest time of this genotype is quite long (>= 12 months). The objective of this research was to identify the high yielding cassava mutants from the gamma rays irradiated 'Gajah' genotype at M1V3 population and potential yield at different location. Several putative cassava mutants (12 mutants) were planted in Cikabayan Experimental Field, IPB from March 2015 to March 2016 and the yields compared with the same genotype grown at different location by seeing its coordinates to observe the potential yield. Our result showed that the fresh tuber weight per plant of some putative mutants could reach more than 8 kg (yield potential of 64 ton Ha^{-1}). The harvested tubers also had sweet flavor, although the tubers of some putative mutants were bitter. Based on previous research study, the different geographic coordinate has resulted variability on fresh tuber yield. It seems that it needs to observe the stability of 'Gajah'- irradiated mutants in several location in Java Island.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the main carbohydrate sources after rice and maize. In Indonesia, mainly cassava is used as food (58%), as raw material for industry (28%), exported in form of cassava chips (8%), as feed (2%), and the rest (4%) is agricultural waste that has not been utilized [1]. As the main utilization of cassava is as food, it is important to match cassava quality with the demand. Important character of cassava as food are high productivity (cassava productivity of several national varieties is around 20-40 ton ha^{-1}), high starch content (starch content of several released varieties is 25-31%), and low cyanide acid (HCN) content [2]. HCN mostly found in cassava leaves and tuber, but the level of HCN content is depend on genotype and environmental factors (Salvador *et al*. 2014), with the maximum HCN content to be safe as food is 10 mg/kg [1]. Ferrero and Villegas [3] classified tuber based on HCN content, which were HCN $\leq$ 50 ppm as *innocuous*, 50-100 ppm as *moderately toxic* and $\geq$ 100 ppm as *dangerously toxic*, so it is better if consumed cassava has HCN content around 10 mg/kg. In irradiation research on cassava by Khumaida *et al*. [4], cassava mutants with bitter tuber taste and had 10-20 kg tuber mass per plant was resulted. Therefore, new genotype that adaptive and has sweet tuber taste should be developed so cassava tuber is suitable as raw material for food industry.

Cassava germplasm in Indonesia that has been started to be developed is 'Gajah' genotype. This genotype has been listed as variety with serial number of 183/PVHP/2013/18 November 2013, has high yield potential in its origin area, East Kalimantan (Samarinda) and even has higher yield potential than other local varieties. Beside of high yielding property, this genotype has quite sweet tuber taste, making 'Gajah' suitable for further research and development. However, 'Gajah' genotype gave different yield potential in different area, which suspected because the absent of adaptive property. One of means to improve adaptive property of a genotype is by improving diversity of potential genetic material and continued with selection. Schwartz [5] explained, improving genetic diversity and variability on performance of species through changing the DNA chain can be done by induction mutation approach using gamma ray irradiation. Furthermore, the yield potential information of the genotype and also other varieties in several areas is needed to understand the interaction between gen and environment in order to determine the best environment for the genotype. Fransisco *et al.* [6] tested *Brassica rapa* in several different areas to understand environment or genotype effect on secondary metabolite and production. The objective of this research is to analyze yield potential of Gajah genotype in different areas and identify several cassava mutants resulted from gamma ray irradiation on M1V3 generation.

2. Materials and methods

The planting material used in this study was 12 genotypes of mutant resulted from gamma ray irradiation of 'Gajah' genotype on M1V3 generation.

The research was conducted by planting 12 mutant genotypes and one background genotype (Gajah) using Completely Randomized Block Design with three replications in experimental field Cikabayan IPB. Maintenance was performed on the plant including fertilizing, pruning and weeds control. Observations were made on morphological characters refer to Fukuda et al. [7], as well as physicochemical characters analysis performed on harvested tubers of certain number genotypes which were genotypes with high yield potential. Analysis of variance was done on quantitative data and Kruskal-Wallis analysis based on qualitative data. To determine the yield potential of 'Gajah' cassava in different distribution areas, data collection of 'Gajah' genotype cassava was carried out, in several regions of Indonesia, followed by Pearson correlation analysis between the plantation locations coordinates and yield potential. Data analysis was perform using Statistical Tool for Agricultural Research (STAR) software.

3. Result and discussion

Distribution of 'Gajah' genotype cassava in Indonesia has not been spread evenly, because it was less than ten years since its release as new variety. However based on search result, this genotype has been spread in several area in Indonesia, namely in Kalimantan, Sumatera, and Java with yield potential per plant vary. When viewed in the map, the spread of 'Gajah' genotype cassava was in different coordinate points (Table 1) and had different yield potential. Yield potential in origin area (East Kalimantan – Samarinda) was the same with West Kalimantan – Pontianak, which was 35 kg/plant, followed by Central Java – Wonogiri, and North Sumatera.

Based on Pearson correlation analysis between coordinate points with yield potential, coordinate point was not correlated with yield potential (Figure 1), which meant that the change in coordinate point will not affect resulted tuber yield potential. However, based on analysis on Table 1, there was difference on harvested yield potential between planting area. This difference on yield based on environment showed that there was interaction between 'Gajah' genotype genetic and environment (GxE). Harvested yield is a quantitative character and this character is highly affected by environment. If the environment or location effect is significant, the genotype can be developed as location specific variety and if insignificant, the genotype can be developed as widely adaptive variety. Syukur *et al.* [8] stated that genetic potential and interaction between genotype x environment (GxE) are needed in developing plant variety in order to determine gen stability on tested genotype. Based on this study, 'Gajah' genotype was suspected as specific location variety, thus wide adaptation of 'Gajah' genotype needs to be improved. Khumaida et al. [9] conduct a research on cassava irradiation, and the study resulted that yield of cassava background and mutant in two different areas has not significant. It means, by irradiation a genotype can be change the level of adaptation. So that, the irradiation on 'Gajah' genotype expected to improve their adaptation and other important characters.

Table 1. Different coordinate points and yield potential of Gajah genotype cassava

Location	Latitude	Longitude	Harvest potential (kg/plant)*
Samarinda	$0^0$28' LS	$117^0$11' BT	35
Pontianak	$0^0$05' LS	$109^0$22' BT	35
Palembang	$2^0$59' LS	$104^0$47' BT	15
North Sumatera	$3^0$38' LU	$98^0$38' BT	20
Wonogiri	$7^0$5' LS	$110^0$55' BT	25
Surabaya	$7^0$15' LS	$112^0$45' BT	15
Bogor	$6^0$37' LS	$106^0$48' BT	8**

* Data was compilled from various sources; yield each plant based on information and news

**Data obtained from experiment using Completely Randomized Block Design

Latitude			
Longitude	0.6202		
Yield potential	-0.179	0.2855	
	Latitude	Longitude	Yield potential

Figure 1. Pearson correlation analysis result of coordinate point- yield potential

Research on 'Gajah' genotype from gamma ray irradiation has been conducted until M1V3 generation on both qualitative and quantitative character. This article contains results from observation on qualitative and quantitative character of harvested tuber. Quantitative character observed was tuber mass, number of tuber per plant and number of commercial tuber (tuber length > 20 cm). Results showed that there was no significant difference on quantitative character of mutant genotypes (Table 2).

Table 2. Average value of three quantitative characters on 13 cassava genotypes

Genotype	Tuber mass (kg)	Number of tuber	Number of commercial tuber
	P value		
	1.09ns	0.79ns	1.40ns
U1-0-2	2.7	7.22	2.69
U1-15-2	4.11	7.11	3.66
U1-15-4	3.23	8.08	4.5
U1-15-5	3.88	5.06	3.17
U2-15-1	3.67	7.33	4.33
U2-15-3	2.81	7.42	3.08
U2-15-4	3.89	7.38	4.64
U2-15-5	3.1	7.72	4.08
U3-15-1	2.94	5.83	3.72
U3-15-2	3.73	6.96	3.48
U3-15-3	2.43	6.22	3.33
U3-15-4	3.17	7.25	3.42
U3-15-5	3.31	6.86	3.31

*ns= no significant

On tuber mass per plant character, the highest tuber mass was 4.11 kg and the lowest was 2.43 kg, but on individual observation there were mutants with tuber mass of 8 kg per plant. That tuber mass was obtained on plant resulted from 15 Gy irradiation (mutant plant), which were U1-15-2, U2-15-1 and U3-15-4 genotypes. On number of tuber per plant, the highest number of tuber was 8 and the lowest was 5. But same as tuber mass, individual observation result showed that there were mutants with number of tuber > 10, even reach 14 tubers, namely on mutants U1-15-2, U1-15-4, U2-15-1, U2-15-3, U2-15-5, U3-15-2, and U3-15-4. Analysis result for number of commercial tuber showed that observed mutants had 2 – 4 commercial tubers per plant. On individual observation, there was mutant with 10 commercial tubers, which was U2-15-1 genotype (Figure 2). Khumaida *et al*. [4] reported that highest number of tuber from mutant plant was 35 tubers, which was higher than its background genotype of 18.78 tubers.

A. B.

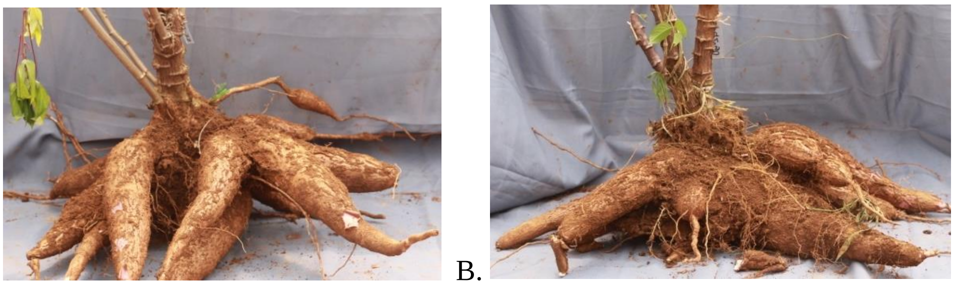

Figure 2. Cassava genotype with superior quantitative characters. A. U2-15-1 genotype with 14 tubers, 10 commercial tubers and tuber mass of 5.8 kg; B. U3-15-2 genotype with 14 tubers, 8 commercial tubers and tuber mass of 7.6 kg.

Observation on qualitative character was conducted on characters: tuber level from stem, tuber constriction, tuber shape, and outer tuber color, tuber color, and cortex color, easiness to peel, tuber skin texture, tuber taste, and cortex thickness. Result of Kruskall-Wallis analysis on quantitative characters showed that there was no significant difference between mutant genotypes (Table 3). On tuber level from stem character, the most common was *pedunculate*, which meant tubers were not attached directly to stem, but had stalk. On tuber constriction, all tubers had no constriction or smooth. On tuber shape character, analysis result showed that overall tuber shape was *conical-cylindrical*, but individual observation still showed diversity among mutants. There were found mutants with tuber shape of *conical, cylindrical*, and *conical-cylindrical* (Figure 3).

A. B. C.

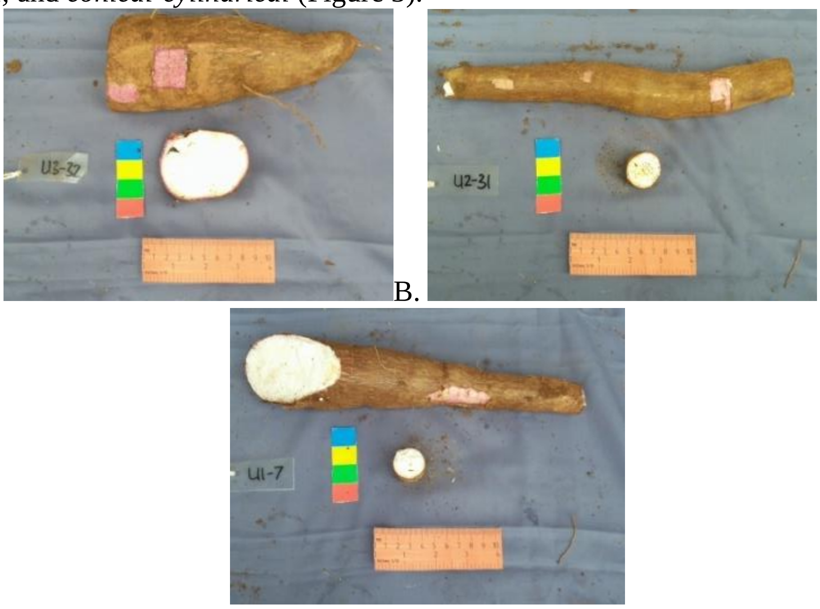

Figure 3. Observed tuber shape of cassava mutant, A. *Conycal*; B. *Cylindrical*; C. *Conical cylindrical*

Next characters observed on all mutants were outer tuber color (dark brown), tuber color (white), cortex color (purple), easiness to peel (easy), and cortex tuber (intermediate). For tuber taste character, the most common was sweet and intermediate, but there were some numbers with bitter tuber taste. The change in tuber taste could wither be sweet to bitter like in this research or bitter to sweet, as shown in study from Khumaida *et al.* [4]. Roslim *et al.* [10] reported that partial gene mutation of *Meisal* (gen that responsible of coding *isoamylaseI* enzyme that involved in starch metabolism in cassava) happened in intron cannot affect tuber taste. Based on this study, it was suspected that occurred mutation was not partial and happened on exon, thus can affect tuber taste.

Tabel 3. Kruskall-Wallis analysis results of qualitative characters from 13 cassava mutant genotypes

Genotipe	Extent of root peduncle	Root constrictions	Root shape	External color of storage root	Color of root pulp (parenchyma)	Color of root cortex	Cortex: ease of peeling	Texture of root epidermis	Root taste	Cortex thickness
	P Value									
	0.81ns	0.63ns	0.51ns	0.20ns	0.44ns	0.16ns	0.44ns	0.25ns	0.13ns	NaN
U1-0-2	Sessile to pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Intermediate	Intermediate
U1-15-2	Pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Rough	Sweet	Intermediate
U1-15-4	Pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Rough	Intermediate	Intermediate
U1-15-5	Sessile to pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Intermediate	Intermediate
U2-15-1	Sessile to pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Sweet	Intermediate
U2-15-3	pedunculate to mixed	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Intermediate	Intermediate
U2-15-4	Sessile to pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Intermediate	Intermediate
U2-15-5	Pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Intermediate	Intermediate
U3-15-1	Pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Rough	Intermediate	Intermediate
U3-15-2	Sessile to pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate to rough	Sweet	Intermediate
U3-15-3	Pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Pink	Easy	Intermediate to rough	Intermediate	Intermediate
U3-15-4	Pedunculate	Few to none	Conical-cylindrical	Dark brown	White	Purple	Easy	Intermediate	Intermediate	Intermediate
U3-15-5	pedunculate to mixed	Few to none	Conical-cylindrical	Dark brown	White	Pink	Easy	Intermediate to rough	Intermediate	Intermediate
H	10.15	-	-	-	-	4.71	-	7.91	6.43	-

*ns= no significant

Observation of the physicochemical character of tubers was conducted to see the difference in chemical content of tubers in the mutant plant tubers and its background genotypes. Observations were made on some characters, such as starch content, amylose, amylopectin, moisture content, whiteness and HCN (Table 4). Mutant tuber starch content ranging from 77.17-83.01%, higher compared to the background genotype, which was 75.615%. Amylose content also found higher in the mutant plants ranging from 26.2-26.9%, while the background genotype was 23.5%. On amylopectin content, background genotype had higher levels, namely 53%, whereas in the mutant plants 51 and 55%. According Rawel and Kroll [11], the levels of amylopectin and amylose ranges from 83% to 17% of total starch tubers, while Keeling and Myers [12] states amylopectin ranges from 70-80% and amylose ranges from 20-30% of total starch content. The results of this study indicate amylose content was 30% and amylopectin was 70% of the total starch content. The highest water content observed was 8.06% and the lowest was 6.8%. Highest whiteness character of the observed in the mutant plants was 100%, but the background genotype plant has low degree of whiteness, which was 97.6%. Whiteness indicate the level of β-carotene, the yellow color of the tuber indicate higher β-carotene content. However, the content of β-carotene is negatively correlated with the production of tubers, although not very significant, as reported by Akinwale *et al*. [13], the greater the weight of the tuber, the lower levels of β-carotene. In Indonesia, the degree of tuber whiteness affects the level of consumer preferences, because consumers prefer white tubers, making mutants with white tuber potential to be developed in

Indonesia. The last character was the HCN content, which the lowest observed was 8.5% in mutant plants. This value was lower than the background genotype HCN content, which was 12.9%. Wilson and Dufour [14] states HCN content negatively correlated with starch content of the tuber, the lower the HCN content, the higher starch content. Thus, the low HCN levels causing sweet tuber taste.

Table 4. Physicochemical analysis results of severeal selected genotypes tubers

Genotype	Starch (%)	Amylose (%)	Amylopectin (%)	Water content (%)	HCN (ppm)*	Whiteness (degree)
U2-2	75.61	23.57	53.91	7.58	12.98	97.6
U3-19	77.17	26.56	51.72	8.06	14.02	96.2
U2-32	83.01	26.98	55.32	6.8	10.57	99.8
U3-49	78.11	26.27	51.85	7.28	8.57	100.05

*ppm= part per million, physicochemical analysis was done base on dry base

4. Conclusion

Planting location affect different yield potential, as in cassava 'Gajah' genotype showed different yields potential between regions, it suspected that 'Gajah' genotype was specific location variety. Observations on harvested yield of M1V3 population resulted from gamma ray irradiation, showed that some mutant genotypes had higher tuber weight with lower HCN content, and had sweet tuber taste. Thus, some mutant from 'Gajah' genotypes with high yield potential, low HCN and sweet tubers taste need to be further developed to support the food industry.

Acknowledgments

The authors wish to acknowledge The Ministry of Research, Technology and Higher Education, Republic of Indonesia, for the funding of this research at 2016.

References

[1] [Dirbudkabi] Direktorat Budidaya Kacang-kacangan dan Umbi-umbian 2007 *Budidaya Ubi Kayu* (Jakarta: Direktorat Jenderal Tanaman Pangan)
[2] Sudarmonowati E, Hartati N S and Amzal A 2012 *Perbaikan Sifat Ubi Kayu dan Pengembangannya untuk Ketahanan Pangan dan Nutrisi*. http://www.wnpg.org/frm_index.hp?pg=informasi/info_makalah.php&act=edit&id=73
[3] Ferrero M T and Villegas L 1992 *Proc. CBN (Colombia)* (Cali: CIAT) p 433-7
[4] Khumaida N, Ardie S W and Syukur M 2015 *Procedia Food Sci* **3** 27–34
[5] Schwartz S 2014 *'Fast-forward Genetics' Induces Mutations to Produce Higher-Yielding Crops* http://www.pbs.org/wgbh/nova/next/nature/fast-forward-genetics-induces-mutations-produce-higher-yielding-crops/
[6] Fransisco M, Cartea M E, Butron A M, Sotelo T and Velasco P 2012 *J. Agric. Food Chem*. **60** 5507-14
[7] Fukuda W M G, Guevara C L, Kawuki R and Ferguson M E 2010 *Selected morphological and agronomic descriptors for the characterization of cassava* (Nigeria: International Institute of Tropical Agriculture)
[8] Syukur M, Sujiprihati S and Yunianti R 2012 *Teknik pemuliaan tanaman* (Jakarta: Penebar Swadaya)
[9] Khumaida N, Evii, Ardie S W and Sopandie D 2016 *Procedia Env Sci.* **33** 70-7
[10] Roslim D I, Nisa F and Herman 2016 *J. Of Biology & Biology Edu., Biosaintifika* **8** 103-10
[11] Rawel H M and Kroll J 2003 *DLR* **99** 102-10
[12] Keeling P L and Myers A M 2010 *Annu. Rev. Food Sci. Technol.* **1** 271–303
[13] Akinwale M G, Aladesanwa R D, Akinyele B O, Dixon A G O and Odiyl A C 2010 *Intl. J. of Genetics and Mol. Biol* **2** 198-201
[14] Wilson W M and Dufour D L 2002 *Economic Botany* **56** 49–57

[15] FAO/WHO 1991 *XII Suppl. 4ed*. FAO 1-42
[16] Salvador E M, Steenkamp V and McCrindle C M E 2014 *J. Of Agric. Biotech. And Sustainable Dev*. **6** 29-38