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GENOTYPIC VARIABILITY IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ) MUTANTS (M_1V_4) USING GAMMA IRRADIATION

SHOLIHIN, K. NOERWIJATI and M.J. MEJAYA

Indonesian Legumes and Tuber Crops Research Institute, Malang Jl. Raya Kendalpayak, Indonesia
*Corresponding author's email: sholhalim@gmail.com
Email addresses of coauthors: tika_iletri@yahoo.com; mmejaya@yahoo.com

SUMMARY

The aim of this study was to evaluate the cassava mutants (M1V4) and a total of 27 mutantsand six checks were screened during 2017 at Muneng Experiment Station, Probolinggo, Indonesia. The testing of cassava genotypes for various growth and yield traits was carried out through randomized complete block designs (RCBD), with three replications. Every mutant and check were planted in a plot size of 4x 4 m with planting distance of 1.0x 0.8 m. Plants were fertilized by 750 kg of fertilizer i.e., *phonska*(N 15%, P 15%, K 15%). Data were recorded on plant height in three and seven months, fresh tuber yield in eight months, starch content, starch yield, intensity of mite attacks, fresh tuber yield, starch content, starch yield and taste of steamed tuber. Results revealed that the fresh tuber yield in eight months ranged 32.73 to 66.63t/ha with average of 45.97 t/ha, starch content ranged 17.3 to 22.1% with average of 19.94%, starch yield ranged 5.88 to 12.7 t/ha with average of 9.14 t/ha. Mutation increased the fresh tuber yield, starch yield, plant height, and the resistance to mites; however, it reduced the HCN content. The mutants revealed greater variability and there is a chance to get new cultivar from these mutants. Results suggested to carryout the chemical analysis for these mutants to know the potency of these mutants and also molecular analysis to know the genetic variance based on molecular markers.

Keywords: Cassava,gamma irradiation, mutant, variability

Key findings: Mutation is an alternative method to get new cultivar in cassava. This study proved that mutation can increase the fresh tuber yield, starch yield and plant height. Mutant CId50-233 was the highest in the fresh tuber yield and strach yield and siginficantly higher than the original cultivar (CecekIjo). The plant height of mutant CId50-233 was also higher than the original cultivar.

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INTRODUCTION

The productivity of cassava in Indonesia is still low.National productivity of cassava was 22.9 t/ha in 2015 with range 10.5-39.1 t/ha. A good cultivar is important thing in increasing productivity.There are twelve released cultivars of cassava in Indonesia. Many farmers in Lampung plant cultivar UJ3, UJ5 and Litbang UK2, and many farmers in East Java planted cultivar UJ5, Adira 4, Malang 6 and Malang 4(Wijanarko*et al.*, 2014). New cultivar should be superior to the released cultivar and the productivity should be higher than the national productivity.

Mite *(Tetranychus bimaculatus)*is the main insect for cassava. This insect can decrease the productivity of cassava. Population of mite during dry season is higher than that in rainy season, and the population of mite in the area with dry climate is higher than that in area with wet climate.The mite attack can reduce the yield range 15 to 73%; depend on the cultivars, 15% for resistance cultivar and 73% for susceptible cultivar (Byrne *et al.*, 1982).Indiati (1999) reported that the mite attack could reduce the yield till 95%. Usually, the farmer plant the resistance cultivar for reducing lost of yield because of mite attacks. Root rot is the disease for cassava. This disease also can decrease the cassava productivity. A few pathogens cause root rot, one of them is *Fusarium* spp. This pathogen is as a soil microbe. This pathogen can attack the cassava from the beginning till post-harvest. Mite attack should be considered in development of new cultivar of the cassava.

The released cultivar is important in increasing productivity of cassava. Number of released cultivar is relatively limited, so development of new cultivar should be encouraged. There is a disadvantage in development of new cultivar via crossing between parents. There is a tendency that flowering parent is branching parent, so there is a tendency progenny resulted is branching progeny.This condition is not expected by farmer. There is an alternative method in development of new cultivar that is mutation. There are few mutagenic agents; one of them is gamma irradiation. To determine the optimum dosage of gamma irradiation is impotant to get the maximum genetic variability. Maharani *et al.* (2015) reported thatlethal dose (LD) 50 for UJ5 30.8 Gy, Adira 4 33.36 Gy, Malang 4 19.52 Gy, varietas local Ratim 30.49 Gy, danvarietaslokalJame-jame 30.24 Gy. There is a challenge to develop new cassava cultivars through mutation.

Mutation breeding at ILETRI (Indonesian Legume and Tuber Crops Research Institute) was started in 2013 with radiation using gamma irradiations with dose 30 – 75 GY. The original cultivars were UJ3, UJ5 and local cultivar (Cecek Ijo). Source of radiation was Cobalt 60 with rate of radiation 40 gray/hour. Evaluation of M1V1- M1V3 mutant was done during 2014-2016. The objective of this study was to evaluate the M1V4 mutants performance derived from three source cultivars UJ3, UJ5 and local Cecek Ijo.

MATERIALS AND METHODS

A total of 27 mutantand six checks were tested during 2017 at MunengExperiment Station, Probolinggo, Indonesia. These mutans were selected mutant from the evaluation of M1V3 mutants. Radiation was done in 2013 at BATAN, Jakarta usinggammairradiations with dose 30 – 75 GY. The original cultivars were UJ3, UJ5 and local cultivar (Cecek ijo). Source of radiation was Cobalt 60 with rate of radiation 40 GY/hour. The testing of M1V4 mutants was done using a randomized complite block designs (RCBD), with three replications. Every mutant and check was planted on a plot with size 4 x 4 m with planting distance 1 x 0.8 m. Plants were fertilized by 750 kg of *phonska*(N 15%, P 15%, K 15%). Application of fertilizer is in 2, 6, and 10 weeks after planting. Data presented were tuber yield in eight months, starch content, starch yield, number of tuber/plant, taste of steamed tuber, response to mite attack. Starch yield was a multiplication of fresh tuber yield and starch content. The starch content was measured based on the specific gravity (SG). Data were analysed using MSTATC program to obtain the combined analysis of variance.Dendogram was developed using Minitab program.

Specific gravity(SG) = (fresh tuber weight in the air)/ (fresh tuber weight in the air-fresh tuber weight in the water)

Starch content = SG x 112.1-106.4.

The damage intensity due to the mite attacks was evaluated using the scoring system shown in Table 1. The damage intensity of the mite attacks was calculated as follows:

$$I = \sum \frac{nxv}{NxV} x100\%$$

Where, I=the damage intensity of the miteattack;

N= number of leaves/plant;

V = the highest score (5);

n = number of leaves in each score category;

v = category score (from 0 to 5) (Table 1)

The level of resistance to red mites was determined based on the standard deviation method that was developed by Doreste *et al.*(1979):

Highly resistant(HR) = $I < \left(\bar{I} - 2\delta\right)$,

Resistant (R) = $\left(\bar{I} - 2\delta\right) < I < \left(\bar{I} - \delta\right)$,

Moderately resistant (MR) = $\left(\bar{I} - \delta\right) < I < \left(\bar{I} + \delta\right)$

Susceptible (S) = $I > \left(\bar{I} + \delta\right)$

where: $\left(\bar{I}\right)$=mean of the damage intensity of the mite attack;

$\left(\delta\right)$= standard deviation

Table 1. Score of leaf damage due to red spider mites.

Score	Damage intensity
0	No symptoms
1	Initiation of yellowish spots on some of the lower and/or middle leaves
2	Fairly abundant yellowish spots on lower and/or middle leaves
3	Considerable damage: many spots; small necrotic zones and curling of leaves, especially the basal and middle leaves; yellowing and loss of some leaves
4	Severe damage: heavy defoliation in the lower and middle part of the plant; a large number of mites as well as webs can be observed
5	Total defoliation of the plant; shoot reduced in size with large number of webs; death of plant

Source:Bellotti and Schoonhoven, 1978.

RESULTS

The fresh tuber yield in eight months of tested mutants/cultivars ranged from 32.73 to 66.63 t/ha with average 45.97 t/ha. Mutant CId50-223 had the highest yield, whereas UJ5d50 gave the lowest yield (Table 2). The taste of steamed tuber of Mutant CId50-223 was bitter, while that of Mutant UJ5d50-75 was not bitter. Mutant CId50-223 is as result of mutation from local cultivar (CecekIjo) with gamma radiation 50 Gy. Tuber yield of CecekIjo was 47.73 t/ha, significantly lower than that of mutant CId50-223. Mutant CId50-143, UJ3d30-140, CId50-223, CId50-263, CId50-144, CId50-205, UJ5d50-75, and released cultivar UJ3, and UK1 Agritan had the fresh tuber yield similar to mutant CId50-223, their yield ranged between 53.7 to 58.17 t/ha.

Starch contents of tested mutants/cultivars varied from 17.3% to 22.1% with average value of 19.94%. Starch content of mutant UJ5d50-55 was the highest 22.1%, and Adira 4 had low starch content, 17.3%. Mutants UJ5d50-17, UJ5d50-49, UJ5d50-54, UJ5d50-26, UJ5d50-50, UJ5d50-35, UJ5d50-11, UJ5d50-67, UJ5d50-64, UJ5d50-72, UJ5d50-78, UJ5d50-75, UJ5d50-55, UJ5d50-14, UJ5d50-37, UJ5d50-13, UJ5d50-119, UJ5d50-120, and cultivar UJ5 had starch content similar to mutant UJ5d50-55, their starch content ranged 20.23 to 22%. Starch yield of mutants/cultivars tested ranged 5.88 to 12.7 t/ha with average 9.142 t/ha. Starch yield of mutant CId50-233 was the highest 12.7 t/ha and Adira 4 had low starch yield 5.88 t/ha.

Mite is main insect for cassava and attack of this insect can decrease the productivity of cassava. Resistance to mite is controled by genes. A few number of report on inheritance study of resistance to mite in cassava because cassava is monocious and protogynous, so cassava has high degree of heterozygosity. So it is more difficult to study inheritance study of some characters in cassava compare to other crops like rice, soybean and maize. Beside that, growth periode of cassava is longer than the other crops like rice, soybean and maize. Mutation can change the genes that control the resistance to mite attack. Planting the resistant or moderately resistant cultivar can reduce the mite attack. Response of tested mutants/cultivars varied (Table 2).Mutants UJ5d50-118 and UJ5d50-119 were resistant to mite, while CecekIjo, mutants CId50-143, UJ5d50-17, UJ3d30-140, and UJ3d30-99 were susceptible to mite. A total of fourteen mutants/cultivars were moderately resistant to mite.

Plant height in seven months of tested mutants/cultivars ranged from 153 to 220 cm with the average value of 182 cm. UJ5d50-11 had the highest plant height in seven months, while mutant CId50-143 had the lowest plant height in seven months. Mutant CId50-143, UJ3d30-140, UJ3d30-99, UJ5d50-35, CId50-152, CId50-202, CId50-263, CId50-144, CId50-205, CId50-208, UJ5d50-64, UJ5d50-75, UJ5d50-120, and cultivar UJ3 had similar plant height in seven months with CId50-143, their plant height 156 - 177 cm. Plant height in three months of mutants/cultivars tested ranged60-98 cm with average 79 cm. Cultivar UK1 Agritan had the highest plant height in three months, while mutant CId50-143 had the lowest plant height in three months. Mutant CId50-152, CId50-202, CId50-223, CId50-263, CId50-144, CId50-205, CId50-208 and local cultivar (CecekIjo) had similar plant height in three months

Table 2. Plant height in three and seven months, fresh tuber yield in eight months, starch content, starch yield, mite intensity and taste of steamed tuber, 2017.

No.	Mutants / Cultivars	Mark	Plant height in 3 months (cm)	Plant height in 7 months (cm)	Fresh tuber yield in 8 months (t/ha)	Starch content %**	Starch yield (t/ha)	Mite intensity, 8 months*	Taste of steamed tuber
1	CId50-143	Mutant	60 k	153 k	53.70 a-e	18.20 jk	9.783 b-f	6.60/S	bitter
2	UJ5d50-17	Mutant	84 b-e	201 a-d	44.40 c-k	20.83 a-e	9.207 b-f	5.22/S	bitter
3	UJ3d30-140	Mutant	83 b-e	177 c-k	54.97 abc	19.57 c-j	10.76 a-d	6.15/S	bitter
4	UJ3d30-99	Mutant	85 bcd	171 e-k	43.83 c-k	19.30 d-j	8.483 b-g	5.61/S	bitter
5	UJ5d50-49	Mutant	85 bcd	186 b-h	42.57 c-k	21.40 abc	9.140 b-f	3.06/MR	Not bitter
6	UJ5d50-54	Mutant	82 b-e	186 b-h	39.97 f-k	21.10 a-d	8.483 b-g	5.39/S	bitter
7	UJ5d50-26	Mutant	85 bcd	196 a-e	43.60 c-k	20.23 a-h	8.850 b-f	3.34/MR	Not bitter
8	UJ5d50-50	Mutant	74 c-j	189 b-h	43.03 c-k	20.23 a-h	8.730 b-f	1.99/MR	Not bitter
9	UJ5d50-35	Mutant	84 b-e	183 b-j	49.97 b-i	22.00 ab	10.98 abc	3.09/MR	Not bitter
10	CId50-152	Mutant	65 jk	177 c-k	48.17 b-j	19.17 e-k	9.220 b-f	4.35/MR	Not bitter
11	CId50-202	Mutant	67 h-k	166 f-k	52.47 b-f	19.17 e-k	10.09 a-e	3.98/MR	Not bitter
12	CId50-223	Mutant	70 f-k	183 b-i	66.63 a	19.33 d-j	12.70 a	4.39/MR	bitter
13	CId50-263	Mutant	66 ijk	168 e-k	50.80 b-h	18.00 jk	9.173 b-f	2.59/MR	bitter
14	CId50-144	Mutant	67 h-k	165 g-k	49.10 b-i	18.57 h-k	9.113 b-f	1.88/MR	Not bitter
15	CId50-205	Mutant	64 jk	166 f-k	*54.37 a-d*	18.30 i-k	10.00 a-e	*1.94*/MR	Not bitter
16	CId50-208	Mutant	72 e-j	156 ijk	52.43 b-g	17.90 jk	9.423 b-f	2.11/MR	Not bitter
17	UJ5d50-11	Mutant	84 b-e	220 a	38.87 g-k	21.20 a-d	8.280 c-g	2.70/MR	Not bitter
18	UJ5d50-67	Mutant	83 b-e	185 b-h	45.63 b-k	20.80 a-e	9.537 b-f	2.27/MR	Not bitter
19	UJ5d50-64	Mutant	73 d-j	161 h-k	35.07 jk	21.13 a-d	7.487 e-g	2.47/MR	bitter
20	UJ5d50-72	Mutant	77 b-j	186 b-h	38.70 h-k	20.67 a-g	8.073 d-g	3.34/MR	Not bitter
21	UJ5d50-78	Mutant	82 b-e	187 b-h	45.23 b-k	21.50 ab	9.750 b-f	2.39/MR	bitter
22	UJ5d50-75	Mutant	74 d-j	174 d-k	32.73 k	21.30 a-c	7.010 fg	2.06/MR	Not bitter
23	UJ5d50-55	Mutant	88 ab	203 abc	48.27 b-j	22.10 a	10.66 a-d	3.16/MR	Not bitter
24	UJ5d50-14	Mutant	85 bcd	187 b-h	40.50 e-k	20.70 a-f	8.410 b-g	2.21/MR	bitter
25	UJ5d50-37	Mutant	82 b-f	185 b-h	39.00 f-k	21.40 a-c	8.380 b-g	2.51/MR	bitter
26	UJ5d50-13	Mutant	86 bc	188 b-h	41.23 d-k	21.17 a-d	8.817 b-f	2.87/MR	bitter
27	UJ5d50-118	Mutant	80 b-g	206 ab	36.63 i-k	20.17 b-i	7.340 e-g	1.43/R	Not bitter
28	*UJ5d50-119*	Mutant	78 b-g	185 b-h	*42.90 c-k*	20.53 a-g	8.880 b-f	1.47/R	Not bitter
29	UJ5d50-120	Mutant	75 c-j	179 b-k	44.83 b-k	21.33 abc	9.480 b-f	2.29/MR	Not bitter
30	Malang 1	Variety	88 ab	182 b-j	51.50 b-h	18.77 g-k	9.720 b-f	2.40/MR	Not bitter
31	Adira 4	Variety	87 ab	194 a-f	33.57 k	17.30 k	5.880 g	1.85/MR	bitter
32	UJ3	Variety	84 b-e	168 e-k	58.17 ab	18.83 f-k	10.94 abc	2.31/MR	bitter
33	UJ5	Variety	83 b-e	190 b-g	41.77 c-k	21.40 abc	8.953 b-f	2.77/MR	bitter
34	Cecekijo	Variety	69 g-k	155 jk	47.73 b-j	17.73 jk	8.420 b-g	4.54/MR	bitter
35	Litbang UK-2	Variety	82 b-e	190 b-g	44.27 c-k	17.67 jk	7.873 efg	3.37/MR	Not bitter
36	UK1 Agritan	Variety	98 a	191 b-g	58.17 ab	18.97 e-k	11.09 ab	2.51/MR	Not bitter
Means		78.6	182	45.966	19.94	9.142			
LSD5 %		11.85	28.1	13.58	1.92	2.779			
C.V (%)		9	10	18	6	18			

Note:*: R=Resistant; MR=moderately resistant; S=susceptible, **: Based on the specific gravity

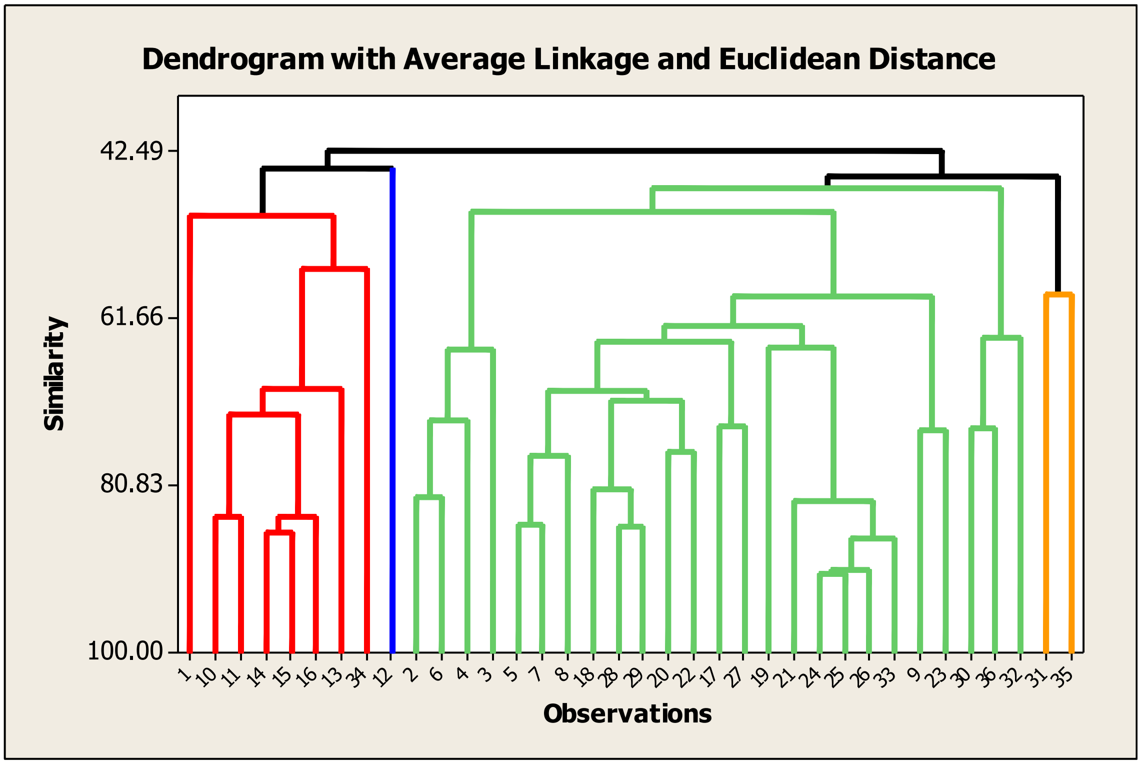

Figure 1. Grouping of mutants/varieties based on plant height three months and 7 months, intensity of mite attack in 8 months, fresh tuber yield, starch content, starch yield, taste of steamed tuber.

with CId50-143, their plant height in three months 64 -70 cm. The taste of steamed tuber can be used as indicator for HCN content; the bitter mutant/cultivar has HCN content ≥ 40 ppm.The non-bitter mutant/cultivar has HCN content <40 ppm. There were twelve bitter mutants and seventeen non-bitter mutants.

Dendogram analysis of 36 mutants/cultivars based on a few parameters (Plant height in three and seven months,fresh tuber yield in 8 months, starch content,starch yield,intensity of mite attackand taste of steamed tuber) is presented on Figure 1. If cutting is done with similarity 45.47%, then four clusters will be got (Table3). There are eight mutants/cultivars in the first cluster. The characteristics of these mutants/cultivars were medium tuber yield and starch yield, medium starch content and low plant height. There is one mutant in second cluster. The characteristics of this mutantwas high tuber yield and starch yield, medium starch content, medium plant height, moderately resistant to mite and bitter taste of steamed tuber. There are twenty five mutants/cultivars in third cluster. The characteristics of these mutants/cultivars were moderately tuber yield and starch yield, medium to high starch content and high plant height. There are two cultivars in fourth cluster. The characteristics of these mutants/cultivars were low tuber yield and starch yield, low starch content, high plant height, and moderately resistant to mite.

Table 3. Grouping of mutants/varieties based on plant height three months and 7 months, intensity of mite attack in 8 months, fresh tuber yield, starch content, starch yield, taste of steamed tuber.

Group	Mutants / varieties	Characteristics of group
1	1; 10; 11; 14; 15; 16; 13; 34	medium tuber yield and starch yield, medium starch content, low plant height,
2	12	high tuber and starch yield, medium starch content, medium plant height, moderately resistant to mite, and bitter taste of steamed tuber.
3	2; 6; 4; 3; 5; 7; 8; 18; 28; 29; 20; 22; 17; 27; 19; 21; 24; 25; 26; 33; 9; 23; 30; 36; 32	medium tuber yield and starch yield, medium to high starch content, high plant height
4	3; 35	low tuber and starch yield; low starch content, high plant height, moderately resistant to mite,

DISCUSSION

Mutant CId50-223 had the highest tuber yield. It was signifiantly higher than the original cultivar (Cecek Ijo). So mutation could increase the yield. Maharani *et al.* (2015) reported that mutation also could increase the yield. Many factors affected the yield, one of them was plant height. Plant heigh at seven months after plantingof CId50-233 was 183 cm higher signifiantly than that of the original cultivar (Cecek ijo) 155 cm (Table 1). If clone has high plant height, the number of leaf will be high, clone with high leaf number, the photosyntate produced will be high. So, if the clone has high photosyntate, the yield will be high.Beside that, Mutan CId50-233 had starch content 19.33%, signifiantly higher than released cultivar UJ3 (18,83%). The price of the tuber with high starch content is higher than tuber with low starch content. This is useful for farmers. For starch industry, it is useful for increasing the efisiency. Cultivar UJ3 was major relesed cultivar planted by farmer in Lampung (Anonymous, 2014). And Mutant CId50-233 had more superior compared to UJ3.There is opportunity to release this promising mutant.

Plant height in seven months of mutant CId50-223 was 183 cm, it was significantly higher than the original cultivar (CecekIjo). So mutation could increase the plant height.However, Sobrizal (2008) reported that mutations could decrese the plant height in rice. The starch yield of mutant CId50-233 was the highest, significantly higher than the original cultivar (CecekIjo). So mutation can increase the starch yield. Aryanti*et al.* (2010) reported that mutation could increase starch content in sweet potato.

Mutant UJ5d50-118 and UJ5d50-119 were resistant mutants to mite, while the original cultivar (UJ5) was moderately resistance.So mutation could increase the resistance to mite. Chalwe*et al.* (2015) reported that additive and non-additive gene affects play a role in the expression of cassava green mite density and cassava green mite leaf damage. Chipeta MM *et al.* (2013) also reported that both additive and non-additive gene action play a role in influencing the resistance to cassava green mite. Chipeta MM *et al.* (2013)also reported that the non additivegene action was more important than the additive one. Mutan UJ5d50-118 and UJ5d50-119 had starch content 20.17% and 21.33%, respectively, higher than UJ3 (18.83%). Beside that, Mutant UJ5d50-118 and UJ5d50-119 were not bitter mutants, while the original cultivar (UJ5) was bitter cultivar. So mutation can reduce HCN content. The similar result was reported by Maharani *et al.* (2015) that there were mutants that were not bitter that were as a result of mutation of bitter cultivar. These mutants can be used as parents in crossing block for development of new variety resistance to mite, high starch content and not bitter cultivar.

Information of chemical characteristic of cultivar is needed as a consideration in planting any cultivar in wide scale such as total sugar content. The users want cultivar with high total sugar content when the tuber is used for raw material for bioethanol industry. In Brazil, mutant that can accumulate sugar 100 times than commercial cultivar was indentified. Rahmiati*et al.* (2016) reported that water content of flour, ash content, fat content, protein content, carbohydrate content, amylosa content, gelatinization temperature, the peak viscosity, breakdownviscosity, setback viscosity, and whiteness of flour of mutant could be lower or higer than the original cultivar. Ceballos*et al.* (2007) reported that mutant had lower amylose than normal level in its starch. Mutant cassava starch had larger values for peak viscosity, gel breakdown, and setback compared with normal cassava starch. Solubility was considerably reduced, while the swelling index and volume fraction of the dispersed phase were higher in the mutant. No change in starch granule size or shape was observed. Rahmiati*et al.* (2016) reported that mutation could increase or decrease water content, ash content, fat content, protein content, carbohydrate content, whitenessvalue, starch content, amylose content,peak viscosity, andend of viscosity of cassava flour. Arefrad*et al.* (2012) reported that mutation could increase the oil content of soybean.

Some researchers reported that the genotypes interacted to the environment for the fresh tuber yield, starch yield, and starch content (Sholihin, 2009, 2011, 2013, 2015, 2017; Noerwijati, 2017). Beside that, to release the new cultivar, the promising genotype should be tested in some locations/years at least six location/years (Irianto, 2018). Multi-location trial should be conducted in central area of cassava. So the multi-location trial for the some promising mutants in this research should be done for the future, So those promising mutants can be release as new cultivar.

CONCLUSION

Themutants varied and there is a chance to get new cultivar from these mutants. The multi-location trial for the some promising mutants in this research should be done for the future. Mutant UJ5d50-118 and UJ5d50-119 can be used as parents in crossing block for development of new cultivar resistance to mite, high starch content and not bitter cultivar..

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