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IDENTIFICATION AND CHARACTERIZATION OF CASSAVA MUTANT GENOTYPES WITH HIGH LEAF MINERAL CONTENT AT THE MV10 GENERATION

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SUMMARY

Cassava leaves' consumption as vegetables are common in several Asian countries. They contain various nutrients, such as, anthocyanins, carotene, minerals, and vitamins. New varieties with high mineral contents in leaves need development to increase the leaves' quality as vegetables. This study aimed to identify and characterize cassava genotypes of the MV10 generation resulting from gamma-ray-induced mutations based on the leaves' morphology, growth characteristics, and mineral contents, using two cultivars and 10 cassava genotypes. All genotypes planted in a randomized complete block design had three replications. The leaf's morphology and mineral contents underwent scrutiny on the third to fifth leaf below the apical shoot. The mineral content analysis used the Atomic Absorption Spectrophotometry method. Data analysis comprised the ANOVA test and descriptive analysis. This study revealed petiole length, leaf lobe length, and leaf lobe width were significantly different between genotypes at four months after planting (MAP). However, no meaningful difference showed for the plant height, stem diameter, and number of leaves among genotypes. In general, the leaf morphology consisted of purplish green apical leaves with present pubescence; the shape of the central leaflet is lanceolate; the petiole color is reddish to greenish; leaves are dark green with five to seven leaf lobes; leaf veins are reddish-green; smooth lobe margins; and horizontal petiole orientation. The genotypes affect mineral contents, as the genotype with the highest Mg contents was G6-2-15-5-3, and the one with the highest Zn contents was G2D1-422. Fe contents showed more variations between genotypes, and no genotype showed consistently high Fe contents. This research produced promising genotypes for Mg or Zn contents in leaves for future cassava varieties for vegetable production.

Keywords: magnesium, mutant putative potential, iron, leaves, zinc

Key findings: This study nominated the potential mutant genotype with a high Mg content, G6-2-15-5-3, and a high Zn content, G2D1-422. Genotype G3D2-413 may have a higher Fe content that still needs further confirmation.

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INTRODUCTION

Cassava is one of the staple foods and the main source of carbohydrates besides rice and maize in Asian and African countries. The 10 countries with high cassava production worldwide are Nigeria, Congo DR, Thailand, Ghana, Indonesia, Brazil, Vietnam, Angola, Cambodia, and Tanzania (Tridge, 2020). The tuber roots are the chief product of cassava as the prime source of carbohydrates for many people in several countries (Ceballos *et al*., 2012). Meanwhile, cassava is for tuber production and leaves as a vegetable for human consumption and cattle fodder (Hue *et al*., 2012; Lukuyu *et al*., 2014; Latif and Muller, 2015; Popoola *et al*., 2019). Cassava leaves contain a variety of nutrients, such as anthocyanins, carotene, magnesium, iron, zinc (Sandro, 2015), manganese, cobalt, natrium (sodium), nickel, calcium, phosphate, potassium, and other nutrients including protein, fat, carbohydrates, and vitamin C (Jamil and Bujang, 2016; Oresegun *et al*., 2016; Nguyen *et al.,* 2012, Ayele *et al.,* 2021). The mineral content in cassava leaves is comparable with other vegetable commodities, such as moringa leaves, which have an iron content of 20.49 mg 100 g^{-1} and Zn of 2.87 mg 100 g^{-1} (Manggara and Shofi, 2018), while cassava leaves have an iron content of 20 to 21 mg 100 g^{-1} and Zn of 12 to 15 mg 100 g^{-1} (Achidi *et al*., 2008). The high mineral contents in cassava leaves may encounter micronutrient and vitamin deficiency in human foodstuff.

A report stated the Pucuk Biru accessions in Indonesia contain a high antioxidant activity (approximately 88.09%) (Rachman *et al.,* 2016), as well as, variations in the mineral content of calcium, iron, potassium, and phosphate in the cassava cultivars SB1366, DVL12 and TL0101 (Nadjiam *et al.,* 2020). Therefore, the mineral contents of cassava leaves are one of the potential characteristics that can serve assessment in the cassava genotypes of developing cassava varieties with high mineral contents in leaves. The morphological characteristics of the plant genotypes are basic information in cassava plant breeding programs. It refers to the descriptors of Fukuda *et al*. (2010) from IITA (International Institute of Tropical Agriculture), which differ the cassava characters qualitatively and quantitatively.

The qualitative characteristics of the leaves include the color of and pubescence on apical leaves, the shape of the central leaflet, petiole and leaf color, lobe margins, and petiole orientation, while the quantitative characters of the leaves include the number, length, and width of the leaf lobes, petiole length, plant height, number of leaves, and stem diameter. The improvement of cassava plant characteristics can proceed through crosses, though the difference in receptive time between male and female flowers (about 14 days) (Richana and Waridah, 2013) and low seed viability cause obstacles to the success of conventional breeding in cassava plants. Mutation breeding through gamma irradiation can be an option for increasing diversity and improving character (Roslim *et al*., 2016; Sholihin *et al*., 2019; Nimlamai *et al*., 2020). Researchers use mutation breeding through gamma irradiation because the other mutation methods, such as, chemical mutagens (EMS, NMU, and NTG), showed no better results. It might be due to the low absorption capacity of plant vegetative tissues for mutagen chemical liquids, especially when using stem buds from the plant part (Maharani *et al*., 2015).

The series of studies related to inducing mutations in the cassava began with Maharani (2015) using five original cultivars that underwent evaluation for potential genotypes connected to tuber or storage root characters. Subsequent assessments of tuber character in the M1V3 to M1V5 generations by Agustina (2016), Yani (2016), Setiawan

(2017), and Sansurya (2018) followed. Evaluation of leaf and nutritional characters (especially mineral contents) took place for the M1V8 generation by Wulandari (2020). The authors' previous results (Pratama *et al*. 2021) showed that some cassava genotypes contained a significant level of Mg, Fe, and Zn in leaves based on the analysis of composite samples in M1V9 generation. The highest magnesium (Mg) content in leaves (about 3000 ppm) showed in G6-2-15-5-3 and G1D1-532 genotypes, with the highest Fe content of 182.48 and 101.32 ppm in G3D2-413 and G6-1-15-4-3 genotypes, respectively. Likewise, the highest zinc (Zn) content ranged at 450–500 ppm in G5D2-223, G6-2-15-3-3, G6-2-15-1-1, and G2D1-422 genotypes. The recent study continued to evaluate and confirm the cassava genotypes for their morphological, growth characteristics, and mineral contents in the leaves to find promising genotypes for new cassava varieties with high leaf mineral content.

MATERIALS AND METHODS

Experimental conditions

This experiment used two cultivars, namely, Ratim (G2) and Malang-4 (G4), and 10 cassava genotypes (putative mutant genotypes) from gamma rays-induced mutation in MV10 generation (Table 1). The plant materials are cuttings from M1V9 generation planted at the Cikabayan Experimental Garden, IPB University, Bogor, Indonesia (240 masl) from November 2020 to August 2021. The history of the mutant putative genotypes and description appears in Table 1.

Planting the cuttings (25 cm in length and 2–4 cm in diameter) of MV10 cassava generation proceeded in the field experiment, with genotypes as a single factor treatment in a randomized complete block design with three replications. Each replication consisted of 10 plants per genotype and a plant spacing of 70 cm × 70 cm. The applied fertilizers have the dosage of 200 (urea), 100 to 150 (SP-36), and 125 to 150 (KCl) kg per hectare. Fertilizer application comprised three steps based on Balitkabi's (2017) recommendation, which is at one week after planting (WAP) for all SP-36, at one-third amount of urea; then, two-thirds of urea one month after planting (MAP), with all KCl applied at three MAP.

Table 1. The wild type and mutant genotypes of cassava used in this study.

No.	Cultivars/Genotypes	Original cultivar/ Irradiation Treatment	Different characters to original cultivar*
1	Ratim (G2)	Local genotypes from Halmahera, North Maluku	-
2	Malang-4 (G4)	National Varieties: open-pollinated with female parent Adira-4	-
3	Mutant G1D1-532	Jame-jame/Irradiation dose: 15 gy	Lobe length, petiole color*3
4	Mutant G2D1-422	Malang-4/Irradiation dose: 15 gy	Lobe length, petiole color*3
5	Mutant G3D2-413	UJ5/Irradiation dose: 30 gy	Lobe length, petiole color*3
6	Mutant G4D1-222	Malang-4/Irradiation dose: 15 gy	First branch height, stem diameter, tuber weight*3
7	Mutant G4D3-113	Malang-4 Irradiation dose: 45 gy	First branch height*5
8	Mutant G5D2-223	Original cultivar*:* Adira-4 Irradiation dose: 30 gy	First branch height, stem diameter*5
9	Mutant G6-1-15-4-3	Original cultivar*:* Gajah Irradiation dose: 15 gy*5	Lobe width*2
10	Mutant G6-2-15-1-1	Original cultivar*:* Gajah Irradiation dose: 15 gy*5	Lobe width*2
11	Mutant G6-2-15-3-3	Original cultivar*:* Gajah Irradiation dose: 15 gy*5	Lobe width*2
12	Mutant G6-2-15-5-3	Original cultivar*:* Gajah Irradiation dose: 15 gy*5	Lobe width*2

The number after genotype and irradiation dose is part of the plant number of the previous generation; *1Maharani (2015); *2Wulandari (2020); *3 Yani (2016); *4Sansurya (2018); *5Agustina (2016); Subekti (2013), and Rambe (2017).

Data recorded

Morphological and growth characteristics of cassava leaves

The morphological characteristics of cassava assessment followed the cassava descriptor (Fukuda *et al*., 2010). The characters included the apical leaves color, pubescence on apical leaves, central leaflet shape, petiole, leaf, and leaf vein color, lobe margins, petiole orientation, leaf lobes number, leaf lobe length (in cm, measured from the base to the tip of the lobe vertically), leaf lobe width (in cm, measured in the widest part of the lobe horizontally), petiole length (in cm, measured from the base to the tip of the petiole), plant height (in cm, measured from the stem above the ground to the main shoot), leaf number (the total number of leaves on a plant), and stem diameter (in mm, measured at a stem that is 10 cm from the ground surface). This experiment consisted of three replicates, with each replicate having five samples. The leaves observed were the third to the fifth leaf below the apical shoot on each trial. The data collection occurred in three, four, and five MAP.

Mineral content analysis: magnesium, iron, and zinc

Analysis of mineral contents transpired on cassava leaves that have fully opened from each genotype in each replication at three and five MAP. The process occurred at the Testing Laboratory, Dept. Agronomy and Horticulture, IPB University, Bogor, Indonesia, using the Atomic Absorption Spectrophotometer (AAS) method and the steps referring to the research of Eviati *et al*., (2005), as follows: washing cassava leaves with ionized water removed ions from the leaf surface, then drying 100 g of wet samples. The mineral extraction used 0.25 g of dry sample. Creating a mixture of HNO_3, reagent 1.0 of 1000 ppm Mg, 2.5 ml of 1000 ppm Zn, and 10 ml of standard 1000 ppm Fe continued. Measuring Fe, Mg, and Zn went on by pipetting 1 ml of sample extract (add 9 ml of 0.25% La solution for Mg). Then measuring the target elements used the instrument PG-990 Atomic Absorption Spectrometer and UV-VIS (PG Instruments Ltd., Alma Park, Wibtoft, Lutterworth, LE17 5BH, United Kingdom) by the Testing Laboratory, Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia.

Statistical analysis

The morphological and growth features and mineral content data analyses employed SAS software version 9.1 ANOVA at a significant level of α = 5%, followed by the 'Duncan Multiple Range Test' for further validations. Analysis of the qualitative characteristics of the leaves used multivariate: principal component analysis (PCA) with RStudio version 2021.09.0 Build 351 software. In addition, the qualitative traits' descriptive evaluation used Microsoft Excel with the 'mode' function for measuring the trait that appears most often and the 'count' function (n) for calculating the number of individuals (plants) with a certain phenotype. Correlation analysis between morphological features and mineral content followed using Pearson Correlation in Minitab software version 21.1. A correlation value close to 1.0 indicates a significant correlation between traits. The * and ** signs indicate significantly correlated characteristics at the respective levels of α = 5% and α = 1%.

RESULTS AND DISCUSSION

Morphological and growth characteristics of cassava genotypes MV10 generation

The results of the analysis of variance on morphological and growth characteristics in 12 cassava genotypes in the experiment are in Table 2. Plant height, stem diameter, number of leaves, petiole length, leaf lobe length, and width were not significantly different between genotypes at three and five MAP. Meanwhile, only the petiole length, leaf lobe length, and width exhibited significant differences between genotypes at the four MAP.

Tables 3 and 4 show the mean of plant height, stem diameter, number of leaves, petiole length, leaf lobe length, and width of 12 cassava genotypes. The range of the plant

Table 2. Summary of ANOVA of the morphological quantitative characters and growth of MV10 generation cassava.

Characters	P-value		
	3 MAP	4 MAP	5 MAP
Plant height (cm)	0.65 ns	0.31 ns	0.87 ns
Stem diameter (mm)	0.94 ns	0.71 ns	0.71 ns
Leaves number (leaf blade)	0.53 ns	0.71 ns	0.66 ns
Petiole length (cm)	0.46 ns	0.02 *	0.15 ns
Leaf lobe length (cm)	0.61 ns	0.01 *	0.56 ns
Leaf lobe width (cm)	0.42 ns	0.05 *	0.81 ns

*: significant difference at the level of α = 5%; ns: no significant difference using ANOVA test at the level of α = 5%; MAP (months after planting).

Table 3. Mean of plant height, stem diameter, and number of leaves in 12 cassava genotypes MV10 generation.

Genotypes	Plant height (cm)			Stem diameter (mm)			Leaves number (leaf blade)		
	3 MAP	4 MAP	5 MAP	3 MAP	4 MAP	5 MAP	3 MAP	4 MAP	5 MAP
G5D2-223	78.7	88.1	98.3	11.8	11.8	17.7	28.3	66.0	101.7
G6-1-15-4-3	88.6	91.0	115.6	11.4	11.3	15.4	26.7	55.0	82.7
G6-2-15-1-1	112.4	102.9	124.0	12.6	12.6	20.0	24.3	51.7	69.7
G6-2-15-3-3	81.8	101.1	108.3	13.0	13.0	16.9	38.0	79.3	111.3
G6-2-15-5-3	88.8	97.4	119.3	33.0	11.6	14.6	31.7	62.0	101.3
G1D1-532	91.3	93.9	112.8	12.3	12.3	16.8	30.7	57.3	73.7
Malang-4 (G4)	80.0	95.9	106.0	13.9	13.9	18.2	35.3	81.0	114.3
G4D1-222	71.6	83.6	93.3	13.6	13.5	19.5	32.7	78.7	115.0
G4D3-113	83.1	90.2	114.0	15.1	15.1	18.9	39.7	78.7	79.0
Ratim (G2)	109.9	136.8	129.9	14.3	14.4	18.5	38.3	93.3	153.7
G2D1-422	94.7	100.5	104.3	47.1	12.6	17.4	26.3	77.0	104.3
G3D2-413	75.8	86.2	118.9	12.7	12.7	15.6	27.7	60.3	94.3

MAP (months after planting).

Table 4. The means of petiole length, length of leaf lobe, and width of the leaf lobe in 12 cassava genotypes MV10 generation.

Genotypes	Petiole length (cm)			Leaf lobe length (cm)			Leaf lobe width (cm)		
	3 MAP	4 MAP	5 MAP	3 MAP	4 MAP	5 MAP	3 MAP	4 MAP	5 MAP
G5D2-223	12.9	14.8bc	12.5	12.3	16.2a	15.2	2.7	3.9abcd	3.9
G6-1-15-4-3	13.2	16.6abc	15.6	12.0	17.0a	17.5	2.9	4.3ab	4.0
G6-2-15-1-1	13.3	19.9a	15.0	12.1	16.6a	16.3	2.9	4.0abcd	4.0
G6-2-15-3-3	11.0	14.7bc	13.7	12.7	15.6a	16.3	3.0	4.2abc	4.1
G6-2-15-5-3	15.9	13.9c	14.0	13.5	14.9a	15.8	3.1	3.5cd	4.0
G1D1-532	12.7	18.2ab	15.4	11.1	18.2a	16.5	2.7	4.2abc	4.0
Malang-4 (G4)	12.7	13.9c	11.9	12.0	14.7a	14.4	2.8	3.4d	3.6
G4D1-222	11.1	14.4bc	14.0	9.8	14.8a	13.9	2.5	3.6bcd	3.6
G4D3-113	17.9	19.5a	16.7	14.0	16.4a	16.2	3.5	4.4a	4.2
Ratim (G2)	14.9	17.7abc	14.0	14.0	18.3a	14.5	3.3	4.2abc	4.0
G2D1-422	13.9	15.0bc	10.0	13.8	15.4a	14.9	3.3	3.8abcd	3.8
G3D2-413	13.6	14.9bc	13.3	12.5	10.2b	15.6	2.9	3.6bcd	3.7

The numbers followed by the same letter in the same column were not significantly different based on the DMRT test at the level of α = 5%; MAP (months after planting).

height was 93.3–129.9 cm, the stem diameter was 14.6–20.0 mm, and the number of leaves was 69.7–153.7 at five MAP (Table 3). Averagely, the plant height increased by 10 to 12 cm, and the stem diameter increased by 0.05 mm per month, with a maximum increase of about 4 mm. The references on another cassava accession, Manggu, showed that the plant height increased by 10 cm at three MAP (Siswati, 2020). The stem diameter of the MV8 generation of putative mutant cassava increased by 5 mm per month at one to four MAP (Wulandari, 2020). The maximum vegetative growth in cassava plants was at three to six MAP, so plant growth characteristics, such as plant height, stem diameter, and the number and size of leaves, are probably maximum during that period (Balitkabi, 2017). The number of leaves increased by about 30 per month during three to five MAP.

The petiole length, leaf lobe length, and width displayed significant differences between genotypes at four MAP. The quantitative characteristics between genotypes, influenced by the interaction between genetics and the growing environment, mean that different genotypes will show varied performances depending on their specific responses in certain locations (Ismayani *et al*., 2016). Genotypes G4D3-113 and G6-2-15-1-1 had the highest petiole length (approximately 19.5–19.9 cm), and G4D3-113 had the most leaf lobe width compared with other genotypes. In addition, Ratim (G2) has the highest lobe length at about 18.3 cm, although it is not significantly different from other genotypes except with G3D2-413. The average increase in petiole length, leaf lobe length, and width at three to five MAP was approximately 1.0–3.0 cm, 1.5–4.0 cm, and 0.5–2.0 cm, respectively. Leaf area characteristics, such as, leaf lobe width, can increase the leaf area index so that the sunlight use efficiency for photosynthesis and available photosynthate for plant growth and development may intensify (Suwarto, 2013).

The description of the leaf morphology of 12 cassava genotypes displays in Tables 5, 6, and 7, and Figure 1. Based on the PCA analysis in Figure 1, the features of pubescence on apical leaves and lobe margins characterize the G3D2-413 genotype. The traits, petiole color, apical leaves, and leaf vein color are the marks of G5D2-223, G6-2-15-3-3, G6-2-15-5-3, and G2D1-422 genotypes. In addition, the petiole orientation character is a symbol of the Malang-4 (G4) and G4D1-222 genotypes, and leaf lobe number characters for genotypes G1D1-532, Ratim (G2), G6-2-15-1-

Table 5. Appearance of apical leaves color, pubescence on apical leaves, and the central leaflet shape of cassava genotypes MV10 generation.

Genotypes	Apical leaves color	Pubescence on apical leaves	Central leaflet shape
G5D2-223	Purplish green (14)	Present (17)	Lanceolate (22)
G6-1-15-4-3	Purplish green (16)	Present (13)	Lanceolate (22)
G6-2-15-1-1	Purplish green (16)	Present (16)	Lanceolate (21)
G6-2-15-3-3	Purplish green (19)	Present (15)	Lanceolate (21)
G6-2-15-5-3	Purplish green (15)	Present (21)	Lanceolate (21)
G1D1-532	Purplish green (21)	Absent (14)	Lanceolate (21)
Malang-4 (wt G4)	Dark green (11)	Present (18)	Lanceolate (23)
G4D1-222	Dark green (14)	Present (14)	Lanceolate (21)
G4D3-113	Purplish green (13)	Present (14)	Lanceolate (25)
Ratim (wt G2)	Purplish green (21)	Present (17)	Lanceolate (21)
G2D1-422	Purplish green (19)	Present (18)	Lanceolate (24)
G3D2-413	Purplish green (22)	Present (20)	Lanceolate (22)

Mode: the character that appears most often; (n): number of individuals (plants) with a certain phenotype; ([n/27] = three blocks*three replications*three plant age [3, 4 and 5 MAP]).

Table 6. Appearance of petiole color, leaf color, and leaf lobes number of cassava genotypes MV10 generation.

Genotypes	Petiole color	Leaf color	Leaf lobes number
G5D2-223	Red (9)	Dark green (21)	Five lobes (14)
G6-1-15-4-3	Red (15)	Dark green (16)	Seven lobes (16)
G6-2-15-1-1	Red (20)	Dark green (21)	Seven lobes (13)
G6-2-15-3-3	Red (23)	Dark green (15)	Five lobes (11)
G6-2-15-5-3	Red (15)	Dark green (21)	Five lobes (17)
G1D1-532	Red (13)	Dark green (20)	Seven lobes (16)
Malang-4 (wt G4)	Greenish (13)	Dark green (25)	Five lobes (17)
G4D1-222	Reddish-green (14)	Dark green (22)	Five lobes (16)
G4D3-113	Reddish-green (17)	Dark green (20)	Seven lobes (22)
Ratim (wt G2)	Greenish (12)	Dark green (17)	Seven lobes (14)
G2D1-422	Red (11)	Dark green (24)	Five lobes (19)
G3D2-413	Greenish-red (18)	Dark green (23)	Five lobes (16)

Mode: the character that appears most often; (n): number of individuals (plants) with a certain phenotype; ([n/27] = three blocks*three replications*three plant age [3, 4 and 5 MAP]).

Table 7. Appearance of leaf vein color, lobe margins, and petiole orientation of cassava genotypes MV10 generation.

Genotypes	Color of leaf vein	Lobe margins	Petiole orientation
G5D2-223	Reddish-green in less than half (18)	Smooth (24)	Horizontal (17)
G6-1-15-4-3	Reddish-green in more than half (13)	Smooth (23)	Horizontal (23)
G6-2-15-1-1	Reddish-green in less than half (18)	Smooth (26)	Horizontal (18)
G6-2-15-3-3	Reddish-green in less than half (15)	Smooth (23)	Horizontal (16)
G6-2-15-5-3	Reddish-green in less than half (12)	Smooth (18)	Horizontal (15)
G1D1-532	Reddish-green in less than half (21)	Smooth (18)	Horizontal (21)
Malang-4 (G4)	Reddish-green in less than half (19)	Smooth (25)	Inclined downwards (17)
G4D1-222	Green (18)	Smooth (26)	Inclined downwards (17)
G4D3-113	Reddish-green in less than half (20)	Smooth (14)	Inclined upwards (27)
Ratim (G2)	Reddish-green in less than half (16)	Smooth (18)	Horizontal (25)
G2D1-422	Reddish-green in less than half (17)	Smooth (18)	Horizontal (20)
G3D2-413	Reddish-green in less than half (16)	Winding (18)	Horizontal (23)

Mode: the character that appears most often; (n): number of individuals (plants) with a certain phenotype; ([n/27] = three blocks*three replications*three plant age [3, 4 and 5 MAP]).

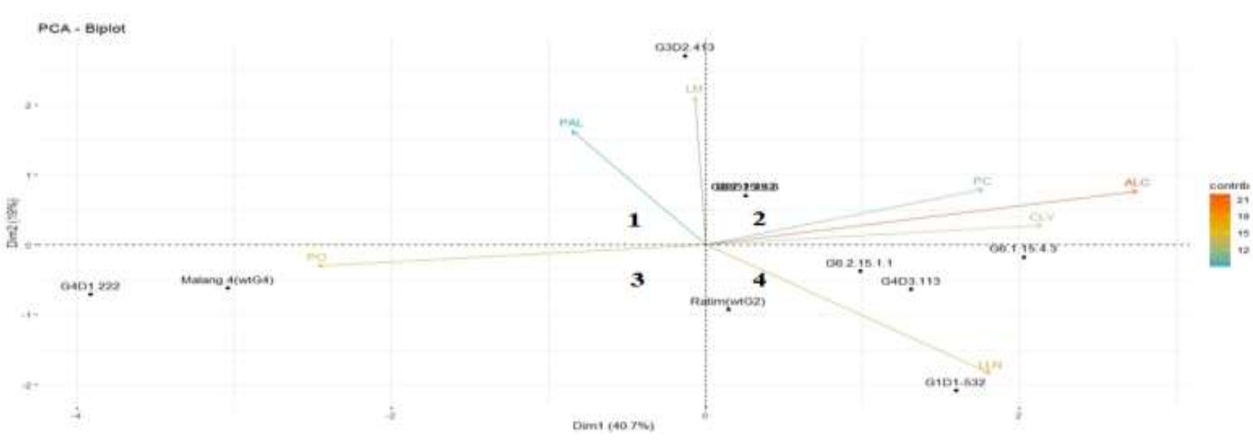

Figure 1. PCA analysis of leaf qualitative characters on several cassava genotypes of the MV10 generation. ALC (apical leaves color), PAL (pubescence on apical leaves), PC (petiole color), LLN (leaf lobes number), CLV (color of leaf vein), LM (lobe margins), PO (petiole orientation).

1, G4D3-113, and G6-1-15-4-3. The leaf performance emerges in Figures 2 and 3. In general, most genotypes had purplish green apical leaves with present pubescence, the shape of the central leaflet is lanceolate, the petiole color is reddish to greenish, the leaves are dark green have five to seven leaf lobes, reddish-green color of leaf veins, smooth lobe margins, and horizontal petiole orientation (Figure 4B). Meanwhile, several genotypes have different leaf morphological characteristics, for example, G4D1-222 has a dark green color of apical leaves, green leaf vein, and inclined-downwards of petiole orientation. Only the G1D1-532 genotype had absent pubescence on apical leaves, the G3D2-413 genotype with winding lobe margins, and the G4D3-113 genotype, an inclined-upwards petiole orientation (Figure 4C).

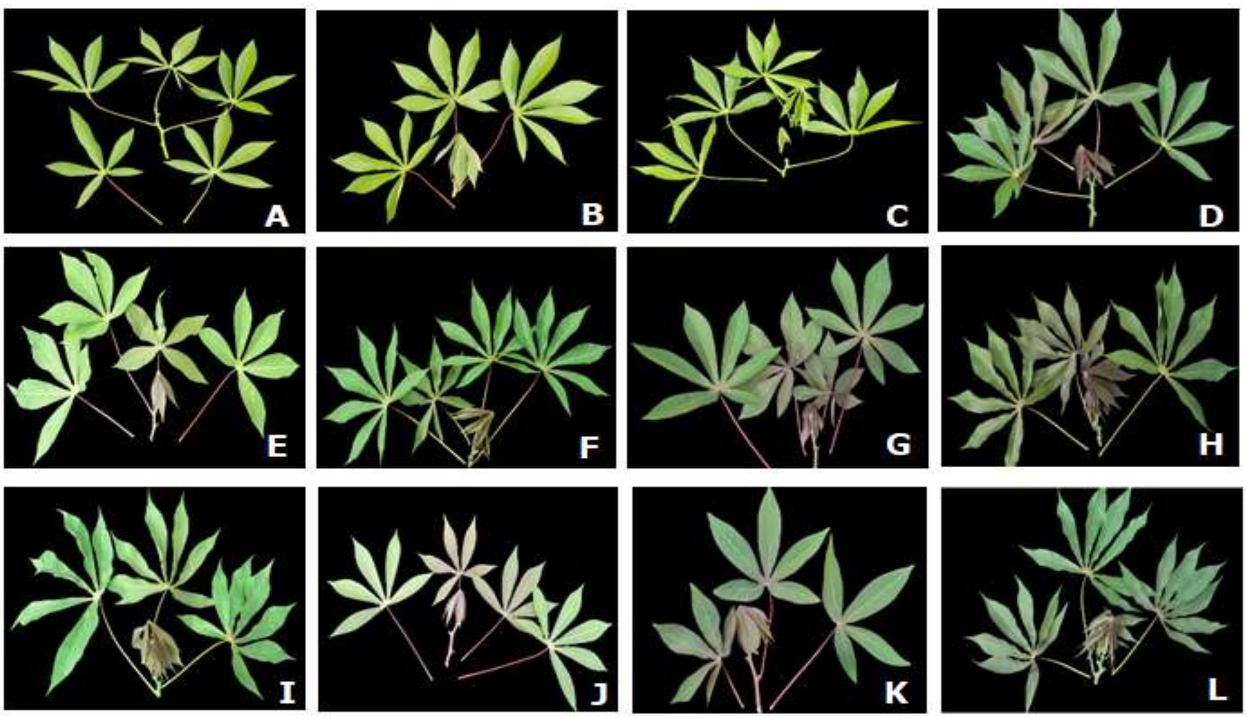

Figure 2. Leaf performance of several cassava genotypes in MV10 generation (A) G4D1-222; (B) G6D1-15-4-3; (C) G5D2-223; (D) G1D1-532; (E) G2D1-422; (F) G4D3-113; (G) G6-2-15-1-1; (H) Malang (wild type G4); (I) Ratim (wild type G2); (J) G6-2-15-3-3; (K) G6-2-15-5-3; (L) G3D2-413.

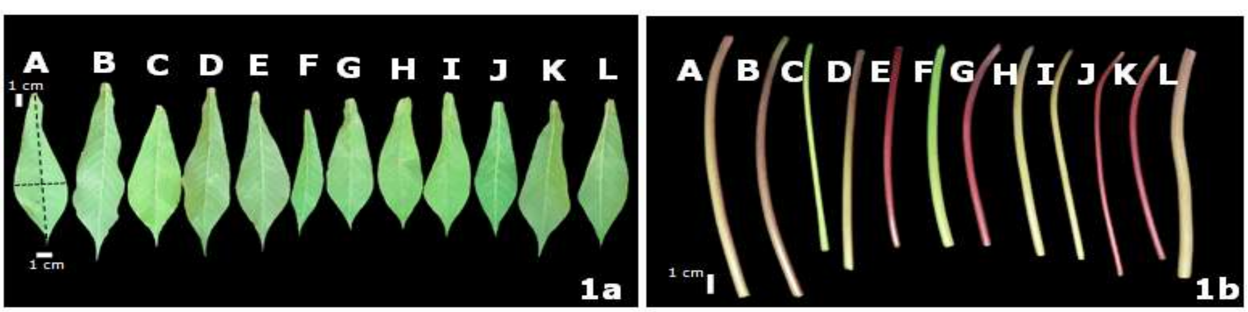

Figure 3. Performance of leaf lobe character (1a) and performance of petiole characters (1b) on several cassava genotypes. (A) G3D2-413; (B) G6-2-15-3-3; (C) Malang-4 (wild type G4); (D) G6-2-15-1-1; (E) G6-2-15-5-3; (F) Ratim (wild type G2); (G) G5D2-223; (H) G6-1-15-4-3; (I) G4D3-113; (J) G4D1-222; (K) G2D1-422; (L) G1D1-532.

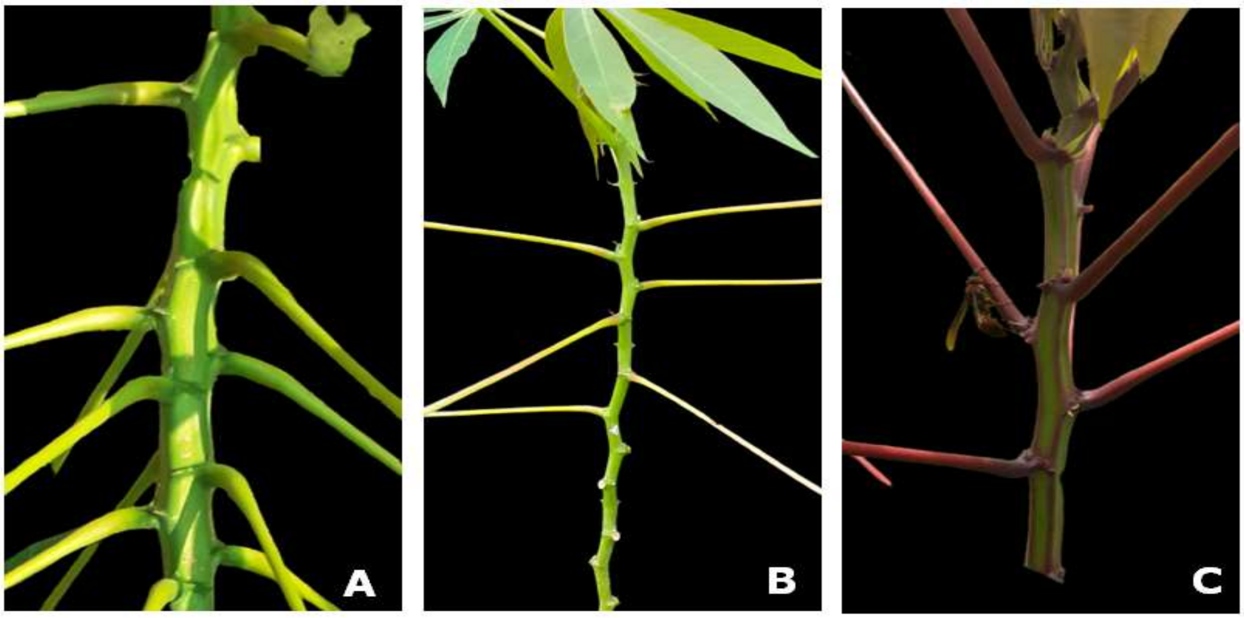

Figure 4. Petiole orientation of leaf cassava morphology (A) Inclined downwards, (B) horizontal, and (C) inclined upward.

Minerals content of cassava genotypes MV10 generation

The results of the analysis of variance of the mineral contents of cassava genotypes MV10 generation occur in Table 8. The Mg, Zn, and Fe contents received significant effects from genotypes at three and five MAP. Based on Table 9, the overall average content of Mg, Fe, and Zn in the 12 genotypes observed at three MAP was around 2786.11, 14.94, and 73.37 ppm, respectively, and Mg, Fe, and Zn content at five MAP were about 2777.79, 67.18, and 62.86 ppm, respectively. Genotypes with higher mineral content than the overall average value can categorize as high mineral content genotypes. Generally, Mg values at three and five MAP were higher in genotype G6-2-15-5-3, around 3100 ppm at three MAP and 3200 ppm at five MAP, compared with G6-1-15-4-3, which has a higher Mg content only at five MAP (3300 ppm) but with a lower Mg at three MAP (2166.7 ppm) (Table 9). The G6-2-15-5-3 has a consistently higher Mg content by the time of observation. Researchers also found a similar pattern on the G2D1-422 genotype, which had the highest Mg content at three MAP (3100 ppm) and five MAP (2966.7 ppm), hence nominating it and G2D1-422 as the genotypes with the highest Mg content. The G2D1-422 genotype also had the highest Zn contents at three and five MAP. In contrast, the genotype with the highest Fe contents at three MAP was G6-2-15-3-3 and G4D3-113 at five MAP.

The Zn and Fe content in several cassava varieties (TME1, 326, 505, 419, and 30,572 varieties) ranged around 36–68 and 321–458 ppm, respectively (Oresegun *et al*., 2016). On the other hand, in this study, the mineral contents of Zn and Fe ranged between 44–88 and 0.89–76 ppm. The study's results by Freitas *et al.* (2015) showed the Fe content in *Manihot esculenta* cv. 'IAC576-70,' using the abaxial and adaxial parts of the leaves, about 0.15% and 0.06% is around 0.3 mg 100 g-1 and 21 mg 100 g-1 leaves. The Fe contents may have a high variation between genotypes and observation time (3 MAP and 5 MAP). Alamu *et al*. (2022) reported research on 400 genotypes of cassava in Nigeria and found the iron in cassava leaves was in the range of 43–660 mg kg-1, zinc from 16 to 440 mg kg-1, and magnesium 1760–6500 mg kg-1, confirming that genetic factor influenced the mineral content of cassava leaves.

Table 8. Summary of ANOVA of the mineral contents of MV10 generation cassava genotypes.

Characters	P-value	
	3 MAP	5 MAP
Mg contents (ppm)	0.037*	0.050*
Zn contents (ppm)	0.012*	0.004**
Fe contents (ppm)	0.014*	0.012*

*: significant difference using ANOVA test at the level of α = 5%; **: significant difference using ANOVA test at the level of α = 1%; MAP (months after planting).

Table 9. The mean of leaf mineral content analysis using the AAS method on 12 genotypes of cassava generation MV10 at 3 and 5 MAP.

Genotypes	3 MAP			5 MAP		
	Mg	Fe	Zn	Mg	Fe	Zn
			--------------- ppm ---------------			
G5D2-223	2500.0 ab	3.1 bc	64.8 b	2733.3 ab	50.7 b	67.6 abcd
G6-1-15-4-3	2166.7 b	5.8 abc	68.8 b	3300.0 a	58.2 ab	66.9 abcd
G6-2-15-1-1	3000.0 ab	0.9 c	76.8 ab	3133.3 ab	74.6 ab	74.8 ab
G6-2-15-3-3	3000.0 ab	33.2 a	72.1 b	2033.5 b	67.3 ab	65.8 abcd
G6-2-15-5-3	3100.0 a	7.1 abc	73.7 ab	3200.0 ab	60.2 ab	68.3 abcd
G1D1-532	2800.0 ab	26.7 abc	69.8 b	2600.0 ab	72.2 ab	44.9 d
Malang-4 (G4)	2733.3 ab	30.7 ab	79.3 ab	2766.7 ab	62.9 ab	52.0 bcd
G4D1-222	2733.3 ab	11.9 abc	72.2 b	2666.7 ab	71.8 ab	70.1 abc
G4D3-113	2800.0 ab	12.2 abc	67.9 b	2533.3 ab	76.9 a	47.4 cd
Ratim (G2)	2800.0 ab	18.4 abc	74.9 ab	2700.0 ab	72.9 ab	56.5 abcd
G2D1-422	3100.0 a	8.0 abc	88.9 a	2966.7 ab	63.7 ab	79.2 a
G3D2-413	2700.0 ab	21.4 abc	71.2 b	2700.0 ab	74.7 ab	60.7 abcd
Average	2786.11	14.94	73.37	2777.79	67.18	62.86

MAP (months after planting); The numbers followed by the same letter in the same column were not significantly different based on the DMRT test at the level of α = 5%.

Various factors, including light intensity, can influence mineral contents, where the higher the light intensity, the higher the photosynthesis process and iron accumulation in leaf tissue (Hamzah and Yusuf, 2019). Other factors are soil pH, available minerals in the soil, soil temperature and humidity (nutrient leaching process), genes, genotypes' ability to absorb mineral ions, and others. Low temperatures of less than 10 °C can inhibit the growth and development of cassava (Bayitse *et al*., 2017). High temperatures can increase the transpiration rate that induces stomatal closing and chlorophyll damage, decreasing the photosynthesis rate (Schymanski *et al*., 2013). High temperature can also affect the chlorophyll-forming elements, such as, the mineral nutrients N, Mg, and Fe (Solikhah *et al*., 2019). In addition, high RH (>70%) can cause low nutrient absorption due to low transpiration rates, affecting the levels of Fe, Mg, and Zn minerals in cassava leaves. However, this experiment grew the genotypes in a uniform environment, so that the different responses between the genotypes are caused by the genetic factor or its interaction with the environments.

Further analysis of morphological traits and mineral contents progressed using Pearson correlation analysis shown in Tables 10 and 11. There is a significant positive correlation between the petiole length with the leaf lobe length, the petiole length with the leaf lobe width, and the leaf lobe length with the leaf lobe width at three and five MAP. Enlargement of leaf cells results in larger leaf sizes as influenced by the petiole as a support for the leaf. This study's results align with the research of Zanetti *et al*. (2017), which showed a significant positive correlation between leaf features with mid-lobe length and lobe width of cassava cultivars IAC 576-70.

In addition to stem diameter with Zn and Mg content, the number of leaves with Fe content had a significant positive correlation at three MAP. It showed that the mineral affects cell division, stem cell enlargement, and leaf formation at the start of growth. Minerals Mg, Fe, and Zn affect the growth characteristics of pear plants, such as, the number and area of leaves, stem diameter, and plant height, because iron actively functions in cell metabolism and enzyme activity in photosynthesis (El-Nasr *et al*., 2015). The Fe minerals act as metabolic activators and components of various enzymes involved in the forming chlorophyll and maintaining the stability of the structure and function of chloroplasts (Rout and Sahoo, 2015). The Zn minerals play a role in the biosynthesis of chlorophyll pigments, especially in young leaves (shoots) (Samreen *et al*., 2017). In addition, the Mg with Zn content has a significant positive correlation at three MAP, implying that Mg and Zn minerals both have synergistic interactions in plants (Sadeghi *et al*., 2021). The study results showed that the promising genotypes with high mineral contents in leaves are G6-2-15-5-3 (the highest Mg contents) and G2D1-422 (the highest Zn contents at three and five MAP). Contrastingly, genotype G6-2-15-3-3, which showed the highest Fe contents at three MAP, did not exhibit a similar pattern at five MAP, indicating the genotype has inconsistent high Fe contents in leaves.

Table 10. Correlation between morphology and growth characters with mineral contents in 12 cassava genotypes MV10 generation at 3 MAP.

	PH	SD	NL	PL	LLL	WLL	Mg	Fe
SD	0.161							
NL	−0.164	−0.214						
PL	0.267	0.286	0.247					
LLL	0.392	0.476	0.272	0.740**				
WLL	0.453	0.458	0.365	0.762**	0.911**			
Mg	0.304	0.572*	0.162	0.158	0.338	0.396		
Fe	−0.282	−0.271	0.572*	−0.325	−0.113	−0.102	0.135	
Zn	0.367	0.725**	−0.180	−0.038	0.260	0.312	0.577*	0.03

PH: (plant height); SD (stem diameter); NL (number of leaves); PL (petiole length); LLL (length of leaf lobe); WLL (width of leaf lobe); Mg content; Zn content, Fe content; * and **: significant difference using ANOVA test at the level of α = 5% and 1%.

Table 11. Correlation between morphology and growth characters with minerals content in 12 cassava genotypes MV10 generation at 5 MAP.

	PH	SD	NL	PL	LLL	WLL	Mg	Fe
SD	−0.173							
NL	−0.009	0.109						
PL	0.405	0.045	−0.436					
LLL	0.371	−0.441	−0.719**	0.596*				
WLL	0.494	−0.189	−0.345	0.645*	0.720**			
Mg	0.270	−0.221	−0.262	−0.015	0.181	−0.084		
Fe	0.400	0.421	−0.088	0.420	−0.038	0.131	−0.322	
Zn	−0.200	−0.006	0.040	−0.459	−0.100	−0.246	−0.353	−0.353

PH: (plant height); SD (stem diameter); NL (number of leaves); PL (petiole length); LLL (length of leaf lobe); WLL (width of leaf lobe); Mg content; Zn content, Fe content; * and **: significant difference using ANOVA test at the level of α = 5% and 1%.

CONCLUSIONS

There are differences between genotypes in petiole length, leaf lobe length, and width at four MAP. Inversely, plant height, stem diameter, and the number of leaves at three and five MAP are similar to the genotypes studied. In general, the leaf morphology of the observed genotypes had purplish green apical leaves with present pubescence, the shape of the central leaflet is lanceolate, the petiole color is reddish to greenish, the leaves are dark green, have five to seven leaf lobes on the fifth leaf after the apical shoot, leaf veins are reddish-green, smooth lobe margins, and horizontal petiole orientation. The genotypes with the highest Mg, Fe, and Zn content compared with the overall average mineral content were G6-2-15-5-3, G3D2-413, and G2D1-422, respectively. In addition, the Fe content in G3D2-413 requires further confirmation in other months, such as seven MAP.

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