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Research Article

Morphological traits and Zn content of several cassava genotypes in nutrient solution culture

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ABSTRACT

Leaf morphological characters and nutrient content in leaf tissue can vary depending on plant genotype. The nutrient solution culture support plant to produce leaves with good nutritional content. Therefore, this research aimed to evaluate and validate cassava putative mutants' morphological traits and Zn content using a nutrient solution culture system. This research was carried out from January to May 2022 at the greenhouse of Cikabayan Experimental Garden, IPB University, Bogor. This study used two cassava cultivars and ten mutant genotypes from the cassava research team of IPB University, resulting from mutation breeding by gamma irradiation. The plant characters observed in this study included the number of leaves, length and width of the leaflet, leaf color, plant height, fresh and dry weight leaf, and analysis of Zn content. The data were analyzed using ANOVA at a significant level of α = 5%, followed by Tukey's test. The results showed that the G2D1-422 genotype had a higher Zn content than the wild type (Ratim/G2) and the other genotypes. The high Zn genotypes (G2D1-422) and low Zn genotypes (Ratim (G2) had not significantly different in fresh and dry leaf weight. Meanwhile, the plant genotype did not affect other growth characteristics such as plant height, the number of leaves, and the length and width of the leaflets, and overall, the characters were similar between the observed genotypes.

Keywords: Cassava leaf; growth; leaf biomass; mutation breeding; zinc

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INTRODUCTION

Cassava plants have various morphological characteristics between genotypes. Morphological diversity of cassava leaves based on the descriptor from Fukuda et al. (2010) showed that leaf color ranged from green to purplish, leaf shape was oval to linear, petiole color was greenish yellow to purple, and the number of leaflets went from 3 to 11 leaflets. In addition, Ha et al. (2016) research demonstrated the morphological diversity of several cassava cultivars in Vietnam seen in the characteristics of apical leaf color, leaf color, leaf retention, leaf shape, number of lobes and leaf size. Genotypes (plant genetics) may affect the essential mineral content in leaves. Several studies showed the differences of Zn content between genotypes, for example, genotypes MZUB04012 vs MZUB04030 around 87 vs 158 mg kg^{-1} (Burns et al., 2012), genotypes G2D1-422 vs Ratim (wild type G2) around 88 vs 74 ppm (Pratama, 2022), genotype 96/1414 vs IRAD4115 around 13 vs 29 µg g^{-1} (Koubala et al., 2015).

The Cassava research team of IPB University has developed cassava genotypes through mutation breeding, but the research is still limited to tuber characters (Subekti,

2013; Khumaida et al., 2015; Maharani et al., 2015; Agustina, 2016; Rambe, 2017; Sansurya, 2018; Yani et al., 2018), while leaf characters still need to be studied. This research related to the biofortification approach of the nutritional character of leaves by evaluating and selecting putative mutant genotypes, especially regarding leaf morphological characters (including leaf biomass) and essential micromineral content (especially Zn content). In Indonesia, cassava leaves can be used as a vegetable (Firdausni & Anova, 2015) and animal feed (Artanti and Andriani, 2020). The relatively high content of Zn in cassava leaves has the potential to make cassava leaves an affordable high-nutrition vegetable. Zn (zinc) is an essential micromineral that acts as a cofactor for more than 1,000 transcription factors in the process of gene expression and 300 enzymes (polymerase, protease, etc.), as well as maintaining and influencing the function of T-helper cells which are related to the human immune system (Suzuki et al., 2021).

Cassava plants for tubers and leaves production are usually cultivated on crumbly textured soil media with a pH of around 4.5 to 8.0, such as alluvial, latosol and podzolic soil types, with elevation of 10 to 1,500 m asl, sunshine of about 10 hours per day, annual rainfall of about 1,500 to 2,500 mm, air temperature of 25 °C to 28 °C and relative humidity of 60 to 65% (Ministry of Agriculture, 2019). Besides being cultivated in soil media, cassava plants for leaf production can be grown using nutrient solution media with a hydroponic system. In hydroponic system, pH and TDS values ranged from 5.5 to 6.0 and 980 to 1260 ppm, respectively, or equivalent to electrical conductivity (EC) of 1.4 to 1.8 mS/cm are optimum media for cassava (Susilawati, 2019). The hydroponic system using nutrient solution culture has been used in several cassava studies, including the Castañeda-Méndez et al. (2017) to increase the survival rate and propagation of early cuttings of cassava resulting from in vitro culture and Khongchiu et al. (2014) for *MeZIP* gene expression studies.

In addition, cultivating cassava using a nutrient solution media at a greenhouse can be used to minimize environmental effects in studying plant genetic effects (plant genotypes), especially on the mineral content and leaf morphological characters; therefore, this study uses this method. This study aimed to evaluate and validate cassava putative mutants' morphological traits and Zn content using a nutrient solution culture system.

MATERIALS AND METHODS

The experiment was conducted from January to May 2022 in the greenhouse at Cikabayan Experimental Station, Department of Agronomy and Horticulture, IPB University. The plant material used stem cuttings (7 to 8 months after planting) resulting from the M_1V_{11} generation gamma irradiation mutation. The genotypes consisted of two wild types (Ratim (wild type G2) and Malang-4 (wild type G4)) and ten putative mutant genotypes of cassava consisting of G1D1-532 (G1; originated from Jame-jame; local genotypes from Halmahera, North Maluku), G2D1-422 (G2; originated from Ratim; local genotypes from Halmahera, North Maluku), G3D2-413 (G3; originated from UJ5; an introduction variety from Thailand), G4D1-222, G4D3-113 (G4; originated from Malang-4; Indonesian national variety), G5D2-223 (G5; originated from Adira-4; Indonesian national variety), G6-1-15-4-3, G6-2-15-1-1, G6-2-15-3-3, and G6-2-15-5-3 (G6; originated from Gajah; local genotypes from East Kalimantan. The stem cuttings had a length of ± 25 cm and cuttings were grouped by diameters of 2.0-2.5 cm (upper stem), 3.0-3.5 cm (middle stem), and 4.0-4.5 cm (bottom stem) (Figure 1a).

This experiment used a growing bucket (volume growing bucket about 4 L), corrugated board (as supporting stem cuttings), TDS meter, pH meter, measuring cup, RHS color chart, mini studio, and camera. The growing bucket used has a height of 17 cm, a top diameter of 26 cm, and a corrugated board measuring 26 cm x 26 cm (shown in Figure 1). AB-mix fertilizer consisted of 150 g nutrient A dan 150 g nutrient B (composition of the media shown in Table 1) with a concentration of each nutrient A and B was 5 mL in 1 L of water at plant age 1 to 4 weeks after planting (WAP) and 10 ml in 1 L of water at 5 to 8 WAP.

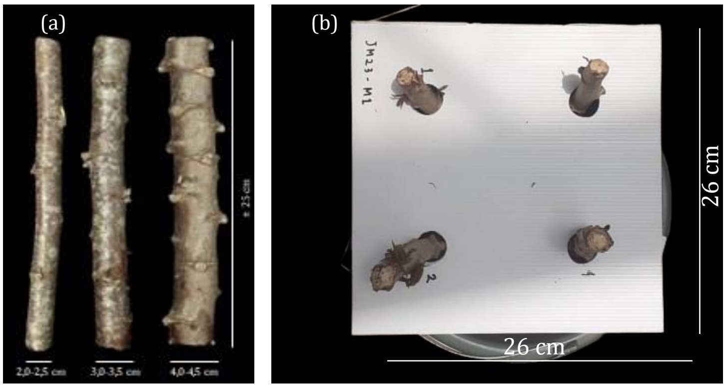


Figure 1. (a) The size of stem cuttings and (b) growing bucket with the nutrient solution inside and corrugated board to hold the cassava cuttings which is used in this study.

Table 1. Composition of AB-mix fertilizer.

Nutrients	Compositions in percentage (%)	Compositions in ppm
N total	20.70	207,000
Ca	14.50	145,000
K	24.80	208,000
Mg	5.10	51,000
S	8.90	89,000
P	5.10	51,000
Fe	0.10	1,000
Mn	0.05	500
Cu	0.05	500
B	0.03	300
Zn	0.02	200
Mo	0.001	10

The experiment used a randomized complete block design (RCBD) single factor based on genotypes. In this experiment, the repetition or grouping of cuttings was based on the size of the cuttings, where the group was done to reduce the experimental error due to the different sizes of the cuttings (Figure 1). Each replicate consisted of three growing buckets, and each bucket consisted of four cuttings, so a total of 1,296 plant cuttings were used. The additive linear model that fits the experiment is:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \rho_k + \varepsilon_{ijk}$$

Y_{ijk} : Response value of genotype-i, size cutting-j, and replication-k
μ : General mean
α_i : Effect of genotype-t (i=1,2,3,...,12)
β_j : Effect of size cutting-j (j=1,2,3)
$(\alpha\beta)_{ij}$: Effect of interaction between genotype-i and size cutting-j
ρ_k : Effect of replication-k (k=1,2,3)
ε_{ijk} : Experimental error of genotype-i, size cutting-j, and replication-k

Experimental preparation was carried out by punching holes in the corrugated board (4 holes per board) for each growing bucket. The solution was prepared by dissolving each nutrient A (150 g) and nutrient B (150 g) with water to make stock solutions, each 500 mL. The working solution was a mixing of 1 L of water with the stock solution according to the dose at each planting age (5 mL A and 5 mL B at 1 to 4 WAP; 10 mL A and 10 mL at 5 to 8 WAP) for each growing bucket. The pH ranged from 5.5 to 6.5, and TDS was 980 to 1260 ppm. Replacement of nutrient solution at plant age 1 to 4 WAP was done once a week, while at 5 to 8 WAP was done twice a week. Planting was carried out by inserting

cassava cuttings into the hole in the supporting board, which each growing bucket consisted of 4 plants. Environmental conditions such as light intensity, temperature, humidity, and photoperiod were under the natural conditions in the greenhouse.

The traits observed in this experiment consisted of the number of leaves, the length, and width of leaflets on the first, third, and fifth leaves after shoots, leaf color on the first, third, fifth, seventh, and ninth leaves (if any) referring to the RHS color chart); plant height, leaf biomass of fresh and dry weight (all leaves that have fully opened after budding and leaving three leaves near the base of the stem are weighed from each plant for each genotype. The fresh and dry weights of leaves were observed. The dry weight was observed by measuring the collected leaves per growing bucket (4 plant/bucket) for each genotype, then dried in an oven at 65 °C for two days. Zn content (in ppm) was measured at 8 WAP using the AAS (Atomic Absorption Spectrophotometry) method on 0.25 g dry samples of each genotype with three replications. Zn content analysis was carried out at the Department of Agronomy and Horticulture, IPB University. Zn was evaluated from fully opened leaves between young shoot and before three last leaves from stem's base.

The quantitative data were analyzed using analysis of variance (ANOVA). Post hoc test was performed using Tukey's for the source of variation with a significant effect at the level of $\alpha = 5\%$. Data analysis used Minitab software version 21.1. and descriptive analysis was used for the qualitative data.

RESULTS AND DISCUSSION

Overall, cassava genotypes grew well and produced healthy and normal leaves. Each cutting stem developed about 2 to 3 buds, which emerged around 1 WAP. Rooting started from 1 WAP. The general condition of cassava plants in nutrient solution culture is shown in Figure 2. The use of simple hydroponic techniques, such as in this study, can be used as a method for producing cassava leaves with good nutritional quality on a household scale. In addition, several studies have used modified hydroponic techniques for various purposes, such as the study by Nansahwang et al. (2022) in studying plant nutrient accumulation on several cassava genotypes using the sand hydroponic hybrid technique. Castañeda-Méndez et al. (2017) used a liquid hydroponic system, and Tokunaga et al. (2019) used aeroponic culture using young stems for the propagation of cassava cuttings (planting material) in a relatively short time, with high survival rate and disease free.

Analysis of variance (ANOVA) showed that the genotype effect was significant for Zn content and fresh and dry leaf weight (Table 2) average Zn content in all observed genotypes was about 128 ppm. The genotype with the highest Zn content was the putative mutant G2D1-422 (about 300 ppm), while the wild type of the putative mutant, Ratim (G2), had a low Zn content (about three times lower than the mutant genotype). These results indicate that the validated putative mutant G2D1-422 can accumulate Zn compared to other genotypes. This result supports our previous research on Pratama et al. (2021), in which the putative mutant G2D1-422 has Zn levels in the field cultivation of about 88 ppm (88 mg kg^{-1}), while Ratim (wild-type G2) is about 74 ppm (74 mg kg^{-1}). In addition, based on several studies, the Zn content of several cassava genotypes in field cultivation, such as MZUB04012 vs. MZUB04030 is around 87 vs. 158 mg kg^{-1} (Burns et al., 2012), genotype 96/1414 vs. IRAD4115 around 13 vs. 29 µg g^{-1} (equal to 13 vs. 29 mg kg^{-1}) (Koubala et al., 2015).

Differences in plant genotypes indicated the different abilities of plants to accumulate minerals in specific plant tissues (Nouri et al., 2009). In addition, other factors that affect the accumulation of minerals in plant tissues are the pH of the media and nutritional status. The nutrient status of Zn in the AB-mix fertilizer was around 0.02%, and the pH value of the media ranged from 5.5 to 6.5. The pH of the media dramatically affects Zn solubility in the nutrient solution, and roots need a pH of around 5.5 to 7.0 to absorb soluble Zn (Nouri et al., 2009; Gupta et al., 2016). Based on the value of Zn content from the results of this study, the genotypes had good Zn uptake ability because leaf growth was average and Zn content in the leaves was relatively high.

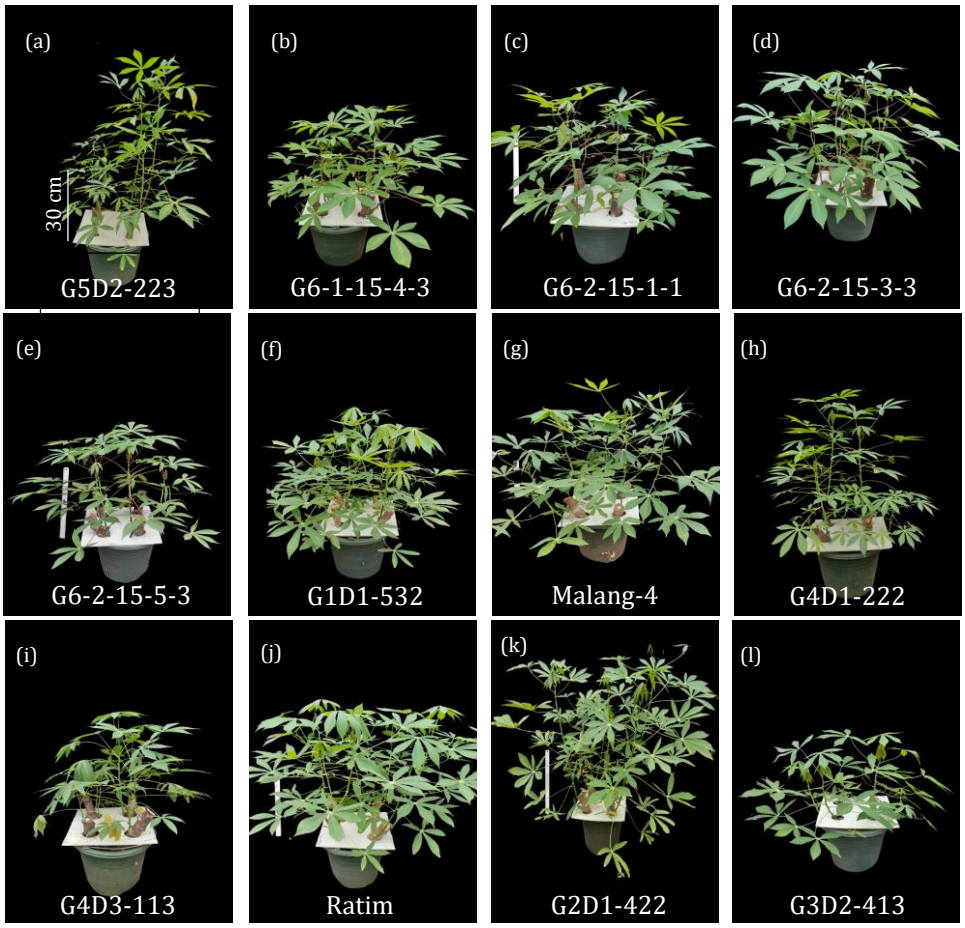


Figure 2. General condition of cassava genotypes in nutrient solution culture at 8 WAP. (a) G5D2-223; (b) G6-1-15-4-3; (c) G6-2-15-1-1; (d) G6-2-15-3-3; (e) G6-2-15-5-3; (f) G1D1-532; (g) Malang-4 (wild type G4); (h) G4D1-222; (i) G4D3-113; (j) Ratim (wild type G2); (k) G2D1-422; (l) G3D2-413.

Based on the results of the analysis of variance and further tests in Table 2, the wild-type or original genotype of Ratim (G2) had not significantly different fresh and dry leaf weights from the mutant genotype G2D1-422. The high Zn genotypes (G2D1-422) had about 6.5 g/plant fresh weight and 4.2 g/growing bucket for dry weight. The low Zn genotypes (Ratim (G2)) had around 8.2 g/plant fresh weight and 7.9 g/growing bucket for dry weight. In addition, the putative mutant G1D1-532 had the highest fresh and dry leaf weights compared to the other genotypes, around 8.5 and 10.5 g, respectively.

Cassava leaves were generally green on the first until the seventh leaves after young shoots. In the RHS color chart shown in Table 3, the dominant color of the first leaf was medium yellow-green, the third was moderate to grayish olive green, and the fifth and seventh were medium olive green. The wild-type Ratim (G2) genotype and the putative mutant genotype G2D1-422 had different RHS color chart color codes in the first and third leaves, while the fifth and seventh leaves were the same. The first and third leaves of the Ratim (G2) genotype were medium olive green and grayish olive green, while the first and third leaves of the G2D1-422 mutant genotype were moderate yellow-green and moderate olive green. The study by Eze et al. (2016) also found the diversity of leaf color between cassava genotypes where Nigeria varieties have purple leaves (varieties TME419, 30572, 01-1368, and 01-1371) while other varieties are greenish (varieties 01-1412 and 01-693). The cause of color differences in leaf are related to chlorophyll content and concentrations of chlorophyll constituent minerals such as Fe, Mn, Mg (Erdal et al., 2016) and Zn (Samreen et al., 2017), ratio of carotenoid content (Yuan et al., 2021) and anthocyanin

components (acylated and glucosylated cyanidins) to chlorophyll content in leaves from the yellow or purple to green stages (Song et al., 2020; Tang et al., 2020).

Table 2. Zn content, fresh and dry weight of several cassava genotypes at 8 WAP in nutrient solution culture.

Genotypes	Zn content (ppm)[z]	Fresh weight per plant (g)[z]	Dry weight per pot (g)[z]
G1D1-532	148.08b	8.53a	10.55a
Ratim (G2)	104.35b	8.19ab	7.95abc
G2D1-422	302.32a	6.53abc	4.19abc
G3D2-413	126.41b	7.68abc	3.95abc
Malang-4 (G4)	67.13b	7.88abc	10.21ab
G4D1-222	140.91b	5.32abc	2.36c
G4D3-113	163.89ab	4.56abc	2.19c
G5D2-223	94.61b	4.21abc	3.61abc
G6-1-15-4-3	79.98b	3.45abc	2.21c
G6-2-15-1-1	164.24ab	2.75c	2.53bc
G6-2-15-3-3	105.56b	6.61abc	3.35abc
G6-2-15-5-3	79.41b	4.32abc	2.16c
Mean	128.78	5.83	4.61

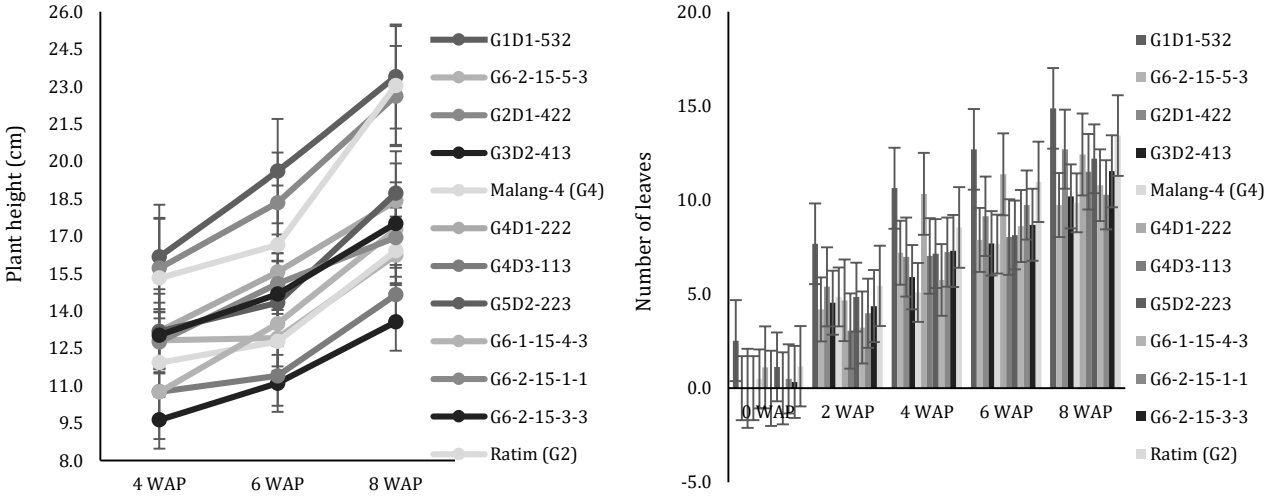
Note: [z] Numbers followed by the same letter in the same column are not significantly different based on Tukey test α = 5%.

Table 3. The color of the first, third, fifth and seventh leaves on the cassava genotypes refers to RHS color chart.

Genotypes	1st leaf	3rd leaf	5th leaf	7th leaf
G1D1-532				
	147B	137B	137A	NN137B
Ratim (G2)				
	146A	NN137A	137A	NN137A
G2D1-422				
	146B	137A	137A	NN137C
G3D2-413				
	146B	NN137B	137B	137B
Malang-4 (G4)				

	NN137B	NN137B	137A	137B
G4D1-222				
	137B	137B	NN137D	146B
G4D3-113				
	137B	NN137B	NN137A	NN137B
G5D2-223				
	146B	137B	137A	NN137C
G6-1-15-4-3				
	146A	NN137A	NN137B	137A
G6-2-15-1-1				
	NN137C	137C	137B	137A
G6-2-15-3-3				
	NN137C	NN137B	137C	137A
G6-2-15-5-3				
	146B	137B	137C	137B

Note: 137A – B and 146A (moderate olive green); 137C – 146B, 147B (moderate yellow green); NN137A-C (greyish olive green).



(a) (b)

Figure 3. Growth character of cassava plant in nutrient solution culture. (a) Plant height of the cassava genotypes at 4 to 8 WAP; (b) Number of leaves of the cassava genotypes at 0 to 8 WAP; Bar±SE.

Based on the analysis of variance (ANOVA), the genotype effect was not significant on plant height and number of leaves; therefore, the average plant height and number of leaves between the genotypes were similar at 4 to 8 WAP (data not shown). The increment in plant height at 4 to 6 WAP was around 1 to 2 cm, whereas after 6 WAP, the average plant height increased by about 3 to 6 cm in 2 WAP (Figure 3a). The weekly increase in plant height in the local Manggu genotype at the early vegetative age is 10 cm (Siswati et al., 2019). These results are similar to the average increase in plant height for the genotypes observed in this study. In addition, the increase in the number of leaves at the beginning of growth (0 to 2 WAP) was around 3-5 leaves, while after 6 WAP, the average leaf increase was around 2 (shown in Figure 3b). The high Zn genotype (G2D1-422) in 8 WAP has a number of leaves and plant height respectively about 12 leaves and 22 cm.

Silva et al. (2020) pointed out in genotypes of coffee (*Coffea canephora*) that plant height is predominantly influenced by environmental factor than plant genotype. The growing media composition is one factor that influences growth characteristics, including plant height and the number of leaves. Research by Carvajal et al. (2023) showed that N, P, K, Ca, Mg, and S greatly affected plant height and the number of leaves because the omission of minerals treatment reduced plant height and the number of leaves by around 29% and 66.7%, respectively. Based on Howeler (2017), the range of media composition that can be used for cassava growth in nutrient solution cultures consists of 80 ppm N in $Ca(NO_3)_2.4H_2O$, 4 ppm P in $NaH_2PO_4.2H_2O$, 40 ppm K in K_2SO, 57, 25 ppm Ca, 20 ppm Mg in $MgSO_4.7H_2O$, and 42.97 ppm S, and 0.1 ppm Mn in $MnSO_4.4H_2O$, 0.05 ppm Mo in $Na_2MoO_4.2H_2O$, 0.2 ppm B in H_3BO_3, 0, 1 ppm Zn in $ZnSO_4.7H_2O$, 0.05 ppm Cu in $CuSO_4.5H_2O$, and 2 ppm Fe in $FeCl_3.6H_2O$. In this study, the growing media composition was optimum for cassava growth; therefore, resulting in uniform cassava growth.

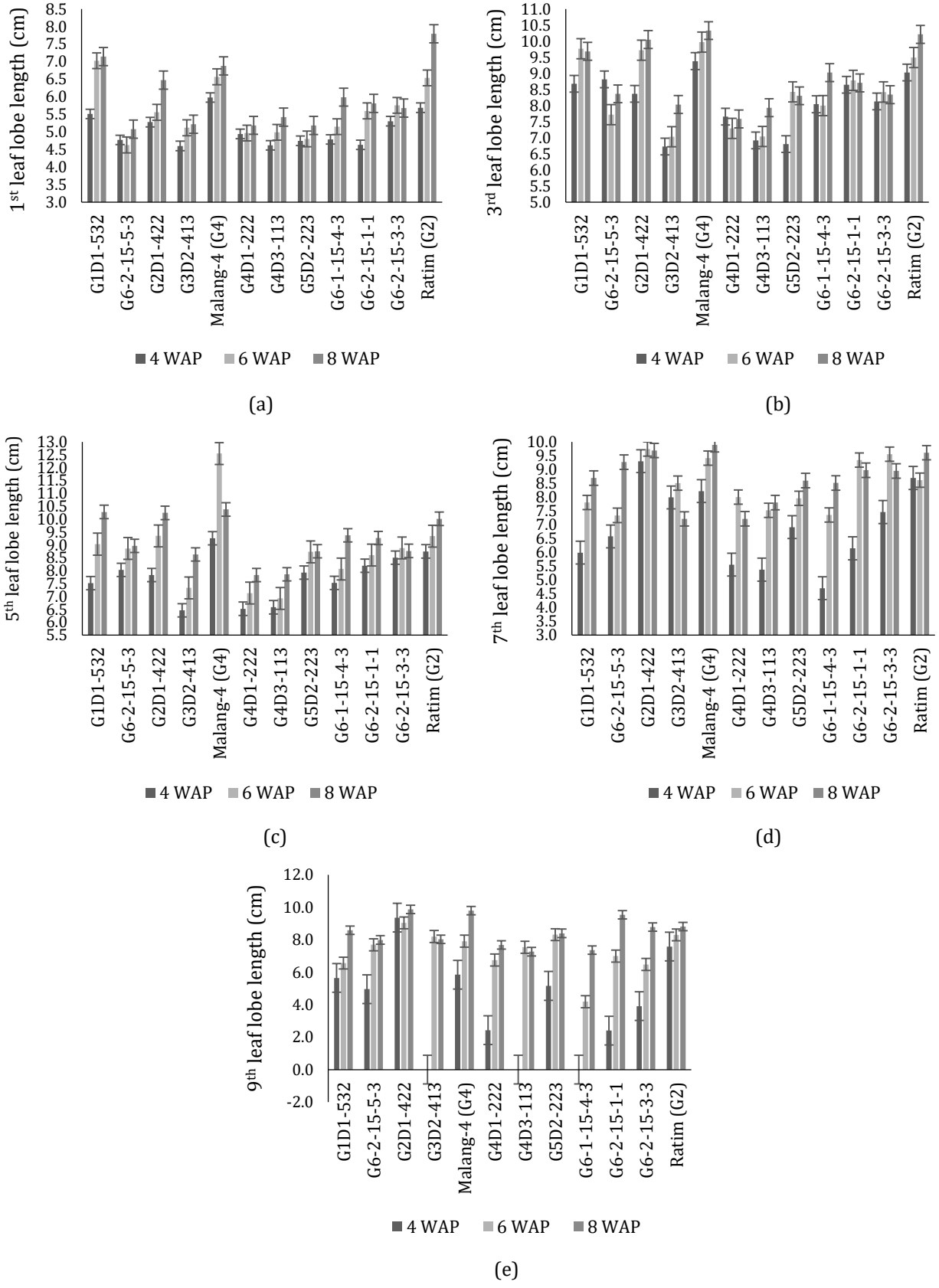


Figure 4. The lobe length of cassava leaves at 4 to 8 WAP grown in nutrient solution culture. (a) 1st leaf lobe length; (b) 3rd leaf lobe length; (c) 5th leaf lobe length; (d) 7th leaf lobe length; (e) 9th leaf lobe length; Bar±SE.

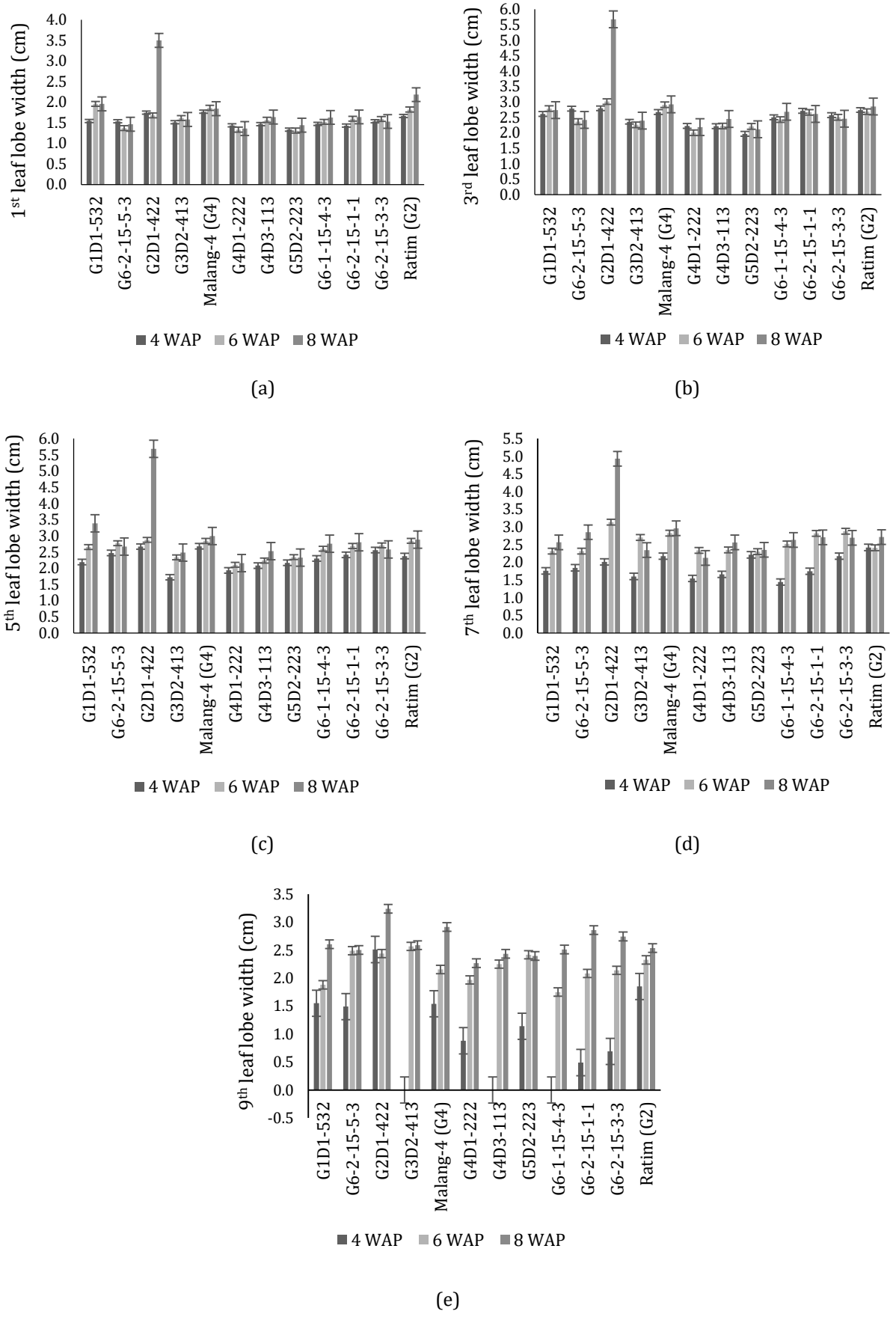


Figure 5. The lobe width of cassava leaves at 4 to 8 WAP in nutrient solution culture. (a) 1st leaf lobe width; (b) 3rd leaf lobe width; (c) 5th leaf lobe width; (d) 7th leaf lobe width; (e) 9th leaf lobe width; Bar±SE.

The analysis of variance showed that the genotype effect was not significant on the lobe length and lobe width; therefore, these characters did not differ between the observed genotypes. The average increase in the length of the first, third, fifth, seventh, and ninth leaf lobes from 4 to 8 WAP, respectively, was about 0.3 to 1.2 cm (Figure 4a); 0.3 to 1.2 cm (Figure 4b); 0.3 to 3.2 cm (Figure 4c); 0.3 to 2.0 cm (Figure 4d); and 0.8 to 1.8 cm (Figure 4e). The average increase in the width of the first, third, fifth, seventh, and ninth leaf lobes from 4 to 8 WAP, respectively, was about 0.1 to 1.8 cm (Figure 5a); 0.1 to 2.6 cm (Figure 5b); 0.1 to 2.8 cm (Figure 5c); 1.0 to 1.8 cm (Figure 5d); and 1.0 to 0.8 cm (Figure 5e). The composition of the media influences growth characteristics such as leaf length and width. Research Carvajal et al. (2023) on *Arracacia xanthorrhiza* which was treated with the removal of N, Ca, and S, and P resulting in the formation of relatively small leaves where the leaf area index was low so that the length and width of the leaves narrowed. Therefore, using media with optimal composition can produce normal leaf formation.

CONCLUSIONS

The nutrient solution culture system can be used in cassava cultivation for many purposes, such as producing leaves with good nutritional content. The G2D1-422 genotype accumulated Zn better than the wild type (Ratim G2) and other genotypes in nutrient solution culture. Based on the results of the further test analysis, the high Zn genotypes (G2D1-422) and low Zn genotypes (Ratim (G2) had not significantly different in fresh and dry leaf weight. Meanwhile, the plant genotype did not affect other growth characteristics such as plant height, the number of leaves, and the length and width of the leaflets, so overall, the characters were the same between both genotypes. Therefore, the G2D1-422 (high accumulation Zn genotype) and Ratim (G2) (low accumulation Zn genotype) genotypes can be used for further molecular studies (such as transcriptomic studies) to compare the genes involved in Zn accumulation in the two contrasting genotypes.

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