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# Development of phenotypic markers and contrast genotype candidates of target minerals related to cassava

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**Abstract.** *Pratama SN, Sudarsono, Ardie W, Khumaida N, Sukma D. 2021. Development of phenotypic markers and contrast genotype candidates of target minerals related to cassava. Biodiversitas 22: 3049- 3056.* Cassava leaves have various macro and micro-nutrients with varying levels, such as magnesium, iron, and zinc. The differences in genotypes of cassava may result in different mineral content in cassava leaves. This study aims to determine the estimator phenotypic markers and contrast genotype candidate selection related to target minerals. This study used a one-factor treatment composed of 12 cassava genotypes and arranged in a randomized block design (RBD) which each treatment was repeated 3 times. The observation was conducted on the leaves' qualitative and quantitative characters such as plant growth, leaf morphology, and leaf mineral content characters. The observation was performed at 3 and 6 months after planting. The results showed that the quantitative and qualitative characters that could be used as estimator for mineral content in cassava leaves are the Soil Plant Analysis Development (SPAD)- Chlorophyll value and leaf color. Genotypes G6-1-15-4-3, G6-2-15-1-1, G6-2-15-3-3 (lowest) and Malang (G4D0), G4D1-222, G3D2-413 (highest) could be categorized as contrast genotypes based on SPAD-value and mineral content. The strong positive correlation between Mg and Fe content with SPAD suggested that SPAD is a possible marker for high Mg and Fe content in leaves. Adversely, a weak relationship between SPAD and Zn content in leaves showed that SPAD is a weak marker for Zn content in leaves. Further research related to molecular markers development can be carried out based on these results.

**Keywords:** cassava leaves, target minerals, phenotypic markers, contrast genotype.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a commodity originating from America , then distributed to Africa and other countries (Guira et al. 2016).It is the largest source of functional food and nutrition in many countries, as siger rice (mocaf) from cassava tuber (Analianasari et al. 2020), nutracetical by utilizing the antioxidant potential of polyphenols content from cassava leaves (Lay and Koubala, 2020). Cassava leaves could be mixed to enhance antioxidant feature, protein and fiber of rice noodles (Poonsri et al. 2019), In Indonesia, cassava is mainly cultivated in West Java, Central Java, Yogyakarta, East Java, Lampung, and East Nusa TenggaraSeveral genotypes commonly cultivated are UJ, Malang, Adira, CMM, Litbang, Cimanggu and others (Wulandari 2020).

All parts of cassava plants can be utilized for many purposes, especially leaves containing a potential source of nutrients with varying levels. Cassava leaves contain crude protein, beta carotene, lipid, carbohydrate, flavonoid compounds (clovin, myricetin-3-O-rutinoside, robinin, hyperoside, nicotiflorin, narcissin) (Tao et al. 2019), Ca, Mg, K, Na, Mn, Fe, Cu, Zn (Oresegun et al. 2016), and vitamin C (Da Silva Santos et al. 2019). The macro and micro-minerals are involved in various biochemical reactions, components of green leaf pigments, components of enzymes (Shar et al. 2012), structure and function of cell membranes, and sources of antioxidants as essential elements. These will influence the response of the physiological processes and metabolism of plant cells. Fe deficiency affected root length, biomass and chlorophyll synthesis in sorgum (Prity et al. 2021). In humans, zinc and iron minerals play an important role, including a body's immune (Skrajnowska and Bobrowska-Korczak (2019). Wessells et al. (2012) estimated 17.3% of the world's population is at risk of zinc deficiency. B Therefore, the study of cassava genotypes with a high mineral content is very important to be utilized as a functional and highly nutritious food.

Nutrition content in plant parts, including leaves, is influenced not only by the cultivation environment but also by the genotype; therefore, studying the genetic effect, the cultivation procedures must be consistent. An interaction between genotypes and location influenced vitamin C level and fiber in several spinach genotypes (Alamu et al. 2020). The differences in genotypes or cultivar SB1366, DVL12 and TL0101 indicated differences in calcium, iron, potassium, phosphorus level in cassava leaves, and different plant uses for mineral content analysis in leaves and root of cassava indicated differences mineral concentrations (Nadjiam et al. 2020). Each genotype may have a different mechanism and ability of mineral absorption, translocation, and accumulation in plant parts, especially in the leaves. Therefore, identifying the cassava genotype character as an estimator of mineral content in cassava leaves is very important.

The selection of a genotype in plant breeding programs can utilize the benefit of morphological or phenotypic, biochemical, and molecular markers. The leaves morphology can be used as a descriptor in identifying the diversity of cassava leaves. International Institute of Tropical Agriculture/ IITA has developed description guidance for cassava leaves (Fukuda et al. 2010). It covers morphological descriptors such as shoot color, leaf color, pubescence of apical leaves, flower and pollen, leaf orientation, leaf shape, number of leaf lobes, length and width of the middle leaf lobe and its ratio, leaf edge texture, petiole length, leaf bone color, petiole orientation, stem cortex and epidermal color, root shape and other characters (Carvalho et al. 2018; IITA 2019; Ha et al. 2016).

This study aims to determine the phenotypic estimator markers and the selection of cassava genotypes that contain high mineral content, especially magnesium, iron, and zinc minerals.

## MATERIALS AND METHODS

The research was conducted at Cikabayan Experimental Garden, Faculty of Agriculture, IPB University. The mineral analysis was conducted at the Testing Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Two commercial varieties and ten mutant genotypes generation M1V8 of cassava derived from mutation by using gamma-ray irradiation (Wulandari 2020), namely Malang, Ratim, G3D2-413 G4D1-222, G4D3-113, G2D1-422, G5D2-223, G2-15-5-3, D1G1-532, G2D0, G6-1-15-4-3, G6-2-15-1-1, and G6-2-15-3-3 were used in this study. To cultivate the cassava rootstock, the cow manure, Nitrogen urea fertilizer (Urea, 46 % N), Phosphate fertilizer (SP36, 36% $P_2O_5$) and Pottasium fertilizer (KCl, $K_2O$ 60%) were applied. The Soil Plant Analysis Development (SPAD) was performed to indicates the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions based on the light transmitted through the leaves.

The experiment tested a single factor treatment (plant genotypes) and designed at the randomized block design (RBD). The experiment was repeated five times (functioned as blocks) resulted in 60 experimental units. Each experimental block consisted of 10 plants per genotype, resulted 12 x 5 x 10 = 600 plants. Five plants were selected as plant samples for observation which produced 12 x 5 x 5 = 300 units of observation.

The plant materials were prepared by cutting the cassava stem into approx. 20-30 cm and kept in a humid place for a month before planting for bud initiation. Stem cuttings with five buds averagely were transplanted to the experiment plot. The plots were prepared by land clearing, plowing, harrowing, manuring (doses approx. 22 Kg $m^{-2}$), and mounds forming for planting. After planting, the plants were maintained with fertilization at one month after planting with N (Urea), K (KCl), and P (SP-36), with a dose of 3.5, 2.5 and 3.6 gram per plant respectively. The second fertilization was applied three months after planting with only urea and KCl, with a dose of 6.6 and 2.2 g per plant, respectively. The amount of fertilizer application was calculated based on the soil analysis and nutrient requirements for cassava, referred to Susila et al. (2010). Manual weeding and herbicide were applied for weed control once a month. Insecticide application is optional depending on pest and disease infestation in the field.

### Quantitative characterization of cassava leaves

The characterization of quantitative traits of cassava leaves was carried out using cassava descriptors guidance (Fukuda et al. 2010). The character observed were the number of leaf lobes, length of the middle lobe, the width of the middle lobe, petiole length, plant height, stem diameter, number of leaves, and SPAD-value (chlorophyll content) at 3, 5, and 6 MAP (months after planting). The leaf samples were observed from the full-opened leaves at the fifth node below the apical shoots. The number of leaves, stem diameter and plant height was observed at the sample plants in the experimental block. SPAD-values were analyzed using Chlorophyll Meter SPAD-502Plus Konica Minolta and observed in the morning at around 06.00-10.00 A.M, at the fifth leaf below the apical shoot with three measurement points, specifically tip, middle, and base of the leaf.

### Qualitative characterization of cassava leaves

The qualitative characterization of cassava leaves was carried out for the cassava descriptor (Fukuda et al. 2010) such as leaf color, petiole color, leaf lobe shape, shape and shoot color, leaf bone color, petiole orientation, and leaf edge surface at 3, 5, and 6 MAP (months after planting). The observation was conducted at the same leaves of quantitative character observation.

### Leaf mineral analysis

Leaf mineral content was analyzed using the Atomic Absorption Spectrophotometer (AAS) at 3, 5, and 6 MAP using sample collected from the fifth leaf below the apical shoot. The leaves were rinsed with ion-free water before extraction. Macro and micro-mineral content were calculated based on Eviati and Sulaeman (2009), using the formula below:

Mg content (%) = ppm curve x ml extract/1000 ml x 100/mg sample x DF x CF

Fe and Zn levels (ppm) = ppm curve x ml extract/1000 ml x 1000 g/g sample x CF

Where; ppm curve = sample rate obtained from the relationship curve between the standard series and the reading after correcting the blank; DF = dilution factor; CF = correction factor for moisture content = 100 / (100% moisture content) 100 = conversion to % (in% units); 1% = 10000 ppm.

**Data analysis**

The data were analyzed using Analysis of Variance (ANOVA) at the α level of 0.05 with the help of Microsoft Excel and SAS version 9.1 software. Correlation analysis was carried out to determine the relationship between observed variables and descriptive analysis on several qualitative characters. The DMRT (Duncan Multiple Range Test) is performed if there is a significant effect of the genotypes on cassava leaf character based on the F test at α =0.05.

## RESULTS AND DISCUSSION

**Quantitative characters**

The results showed no significant differences among genotypes in plant height and number of leaves at 3-6 MAP based on variance analysis (F-test). The plant height increase of all genotypes observed every month was about 10-20 cm (Figure 1). The number of leaves did not show a significant difference among all genotypes and plant ages (Figure 2). Thus, all genotypes have a similar growth rate of leaf numbers. The leaf number increased by approximately 20-50 leaves per month at 3-5 MAP (Figure 2). Adversely, genotypes affected the plant stem diameter significantly. The stem diameter among genotypes was significantly different at six months after planting (Figure 3). Genotype G6-2-15-5-3 showed the lowest stem diameter, about 13 cm, while Ratim showed the highest stem diameter, about 21 cm. The average increase of stem diameter every month was around 0.5 ± 3.0 cm.

The leaf characterization results showed no significant differences among genotypes in petiole length, length of leaf lobe, and width of leaf lobe in 3 to 6 months after planting. The increase of petiole length of all genotypes observed every month was about 0.05 - 2.0 cm (Figure 4). The length of the leaf lobe showed accretion approx. 0.6 – 6.0 cm for a month (Figure 5). Furthermore, the leaf lobe width accretion among 12 genotypes was about 0.05 – 1.4 cm (Figure 6).

A significant difference of the SPAD-values was observed among genotypes at 3 to 6 months after planting, estimating chlorophyll content of the base, middle and the tip of leaves with averages of 32 – 42, 32 – 41, and 33 – 48 units at 3, 5 and 6 MAP respectively (Table 1). Variance analysis and Duncan test showed that genotype G6-1-15-4-3, G6-2-15-1-1, and G6-2-15-3-3 showed the lowest SPAD-value in the period 3-6 MAP, while genotype Malang (G4D0), G4D1-222 and G3D2-413 showed the highest and increasing SPAD-value at 3-6 month.

**Qualitative characters of leaves**

Qualitative characters were analyzed using descriptive analysis to determine the dominance of scoring on the observed variables. Eight qualitative characters were observed, and the scoring of the characters was determined based on the Cassava Descriptor (Fukuda et al. 2010). The differences of characters were used to characterize certain genotypes. The twelve genotypes had relatively similar leaf characters (Table 2). Several genotypes have a purplish-green apical leaf, but Malang (G4D0) and G4D1-222 respectively had the light green and dark green color of apical shoots.

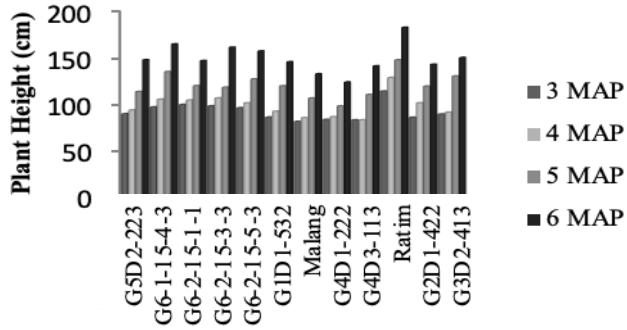



**Figure 1.** Plant height among 12 genotypes of cassava in the period of 3 to 6 months after planting (MAP)

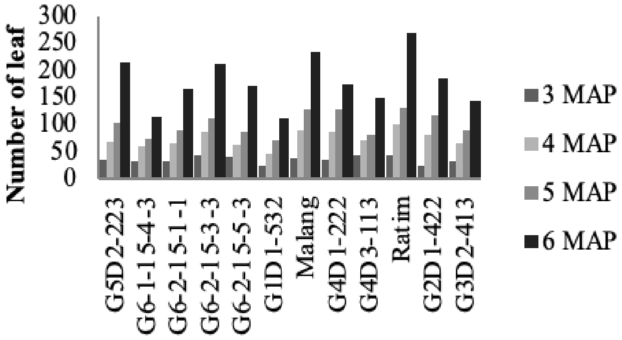



**Figure 2.** Number of leaves among 12 genotypes of cassava in the period of 3 to 6 months after planting

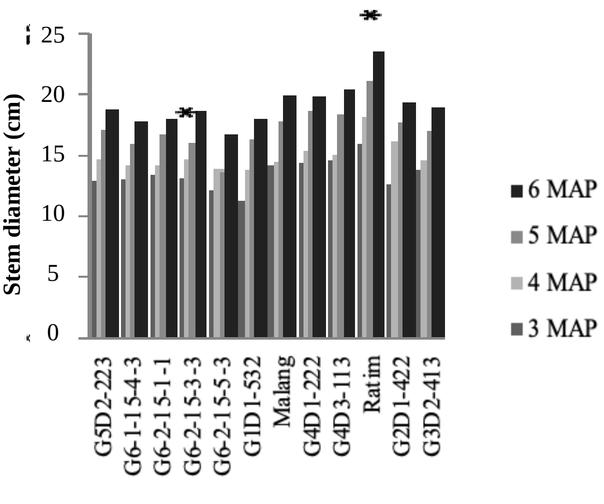



**Figure 3.** Stem diameters among 12 genotypes of cassava in the period of 3 to 6 months after planting

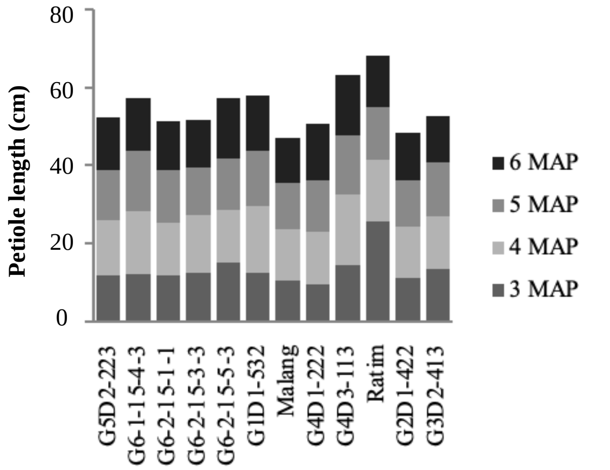



**Figure 4.** Petiole length among 12 genotypes of cassava in the period of 3 to 6 months after planting (MAP)

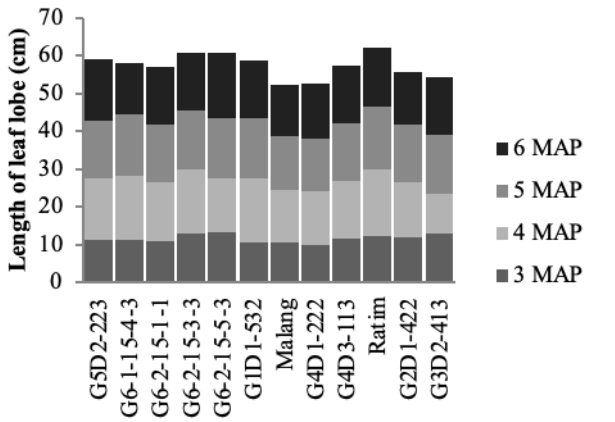



**Figure 5.** Length of leaf lobe among 12 genotypes of cassava in the period of 3 to 6 months after planting (MAP)

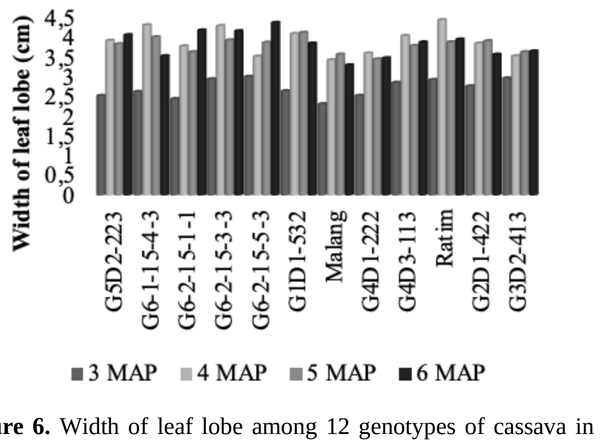



**Figure 6.** Width of leaf lobe among 12 genotypes of cassava in the period of 3 to 6 months after planting (MAP)

## Leaf mineral and the correlation with SPAD-values

The highest magnesium (Mg) content (about 3000 ppm) was found in G6-2-15-5-3 and G1D1-532 genotypes, adversely the lowest one was 2100 ppm in Malang (G4D0) and G4D3-113 (Table 3). The highest iron (Fe) content was 182.48 and 101.32 ppm in G3D2-413 and G6-1-15-4-3 genotypes, while the lowest was 51.11 ppm in the Malang (G4D0) genotype. The highest zinc (Zn) content was 450-500 ppm in G5D2-223, G6-2-15-3-3, G6-2-15-1-1 and G2D1-422 genotypes, while the lowest was 300 ppm in G3D2-413 and G4D3-113 genotypes. These results indicated that cassava's genotypes were planted in a uniform and controlled cultivation environment, obtaining different mineral content, especially magnesium, zinc, and iron.

There is a strong positive correlation between SPAD-value and Mg content in cassava leaves. It is shown by a high coefficient determination ($R^2$) level of 0.98, 0.97, and 0.97 at 3, 5 and 6 MAP (Figure 7). There is also a positive relationship between the SPAD value and the iron content of cassava leaves (Figure 8). Thus the SPAD-value at the three plant ages, especially at 6 MAP, can be used as the estimator for the highest iron content in cassava leaves. Contrasting to Mg and Fe, different zinc and SPAD relationships were observed (Figure 9). The coefficient determination ($R^2$) level of Zinc and SPAD correlation was 0.0026, 01.19 and 0.28 at 3, 5 and 6 MAP. The results show a weak relationship between SPAD value and zinc content in cassava leaves.

## Discussion

This research showed no significant differences among genotypes in plant height and the number of leaves at 3, 5, and 6 MAP based on variance analysis (Figures 1 and 2). At the same time, there is a significant difference in cassava's stem diameter (Figure 3). Rahmawati et al. (2017), showed that the large stem diameter due to the increase in cell size, so that will stimulate an increase in the growth of cassava leaves. The number of cassava leavesaffect the photosynthesis capacity and photosynthate production and transport to other developing parts such as shoots. No significant differences among genotypes in petiole length, length of leaf lobe, and width of leaf lobe in 3, 5 and 6 months after planting was also observed (Figure 4, 5, and 6). These results indicate similar photosynthesis capacity between the genotypes. Photosynthesis capacity between cassava genotypes is influenced by factors such as density of stomata, stomata aperture width, water content, chlorophyll content and photosynthesis rate (Amarullah et al. 2016). Higher photosynthesis capacity can promote various physiological processes in plants, such as nutrient absorption, translocation and chlorophyll formation in leaves.

The genotypes G6-1-15-4-3, G6-2-15-1-1, G6-2-15-3-3 and Malang (G4D0), G4D1-222, G3D2-413 can be categorized as contrast genotypes that have the lowest and highest chlorophyll content based on the SPAD-value. Sookchalearn and Abdullakasim (2017) showed a linear correlation between the SPAD-value and chlorophyll content and yielded cassava root with coefficient determination accuracy approx. 0.97, so that SPAD meter can used for estimated chlorophyll content in cassava plant. Wulandari (2020) found a correlation between the SPAD value and mineral content in leaves such as Fe, Mg, and others. Simao et al. (2013) showed that average levels of chlorophyll based on measurements using SPAD meter in 10, 12 and 14 months after planting indicated that there are differences of SPAD at the three plant ages in genotypes. Koshy et al. (2018) showed a correlation between SPAD units and total chlorophyll, chlorophyll a, chlorophyll b, and nitrogen content with a linear regression equation.

**Table 1.** SPAD-values for estimating leaf chlorophyll levels among 12 genotypes of cassava in the period 3 to 6 months after planting

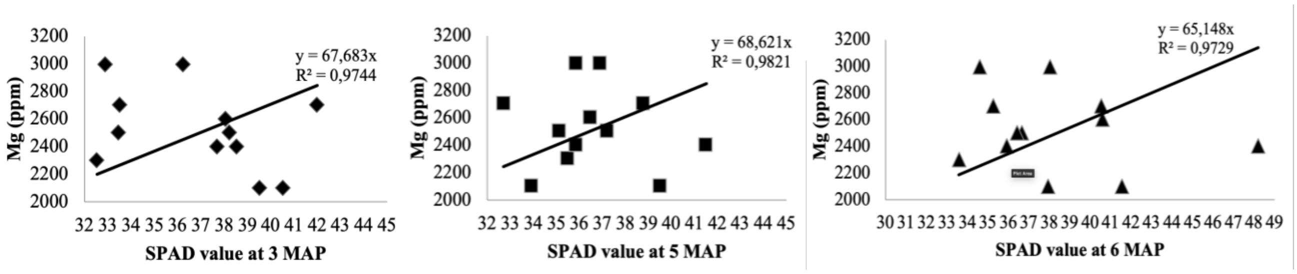
| Genotype | SPAD-value (units) | | |
|---|---|---|---|
| | 3 MAP | 5 MAP | 6 MAP |
| G5D2-223 | 38.07abcde | 36.5ab | 40.59ab |
| G6-1-15-4-3 | 32.53e | 35.48ab | 33.58b |
| G6-2-15-1-1 | 33.53cde | 32.7b | 35.28b |
| G6-2-15-3-3 | 33.44cde | 35.11ab | 36.67b |
| G6-2-15-5-3 | 32.89de | 36.91ab | 38.03ab |
| G1D1-532 | 36.25bcde | 35.86ab | 34.57b |
| Malang (G4D0) | 40.50ab | 39.53ab | 41.56ab |
| G4D1-222 | 42.00a | 38.8ab | 40.56ab |
| G4D3-113 | 39.53ab | 33.93b | 37.94ab |
| Ratim (G2D0) | 37.70abcde | 35.88ab | 35.91b |
| G2D1-422 | 38.20abcd | 37.2ab | 36.44b |
| G3D2-413 | 38.53abcd | 41.53a | 48.20a |

Note: The numbers followed by the same letter in the same column are not significantly different based on Duncan Multiple Range Test (DMRT) at α = 0.05; MAP (months after planting); SPAD-value (chlorophyll levels).

**Table 3.** Mineral content (magnesium, iron and zinc) of cassava leaves

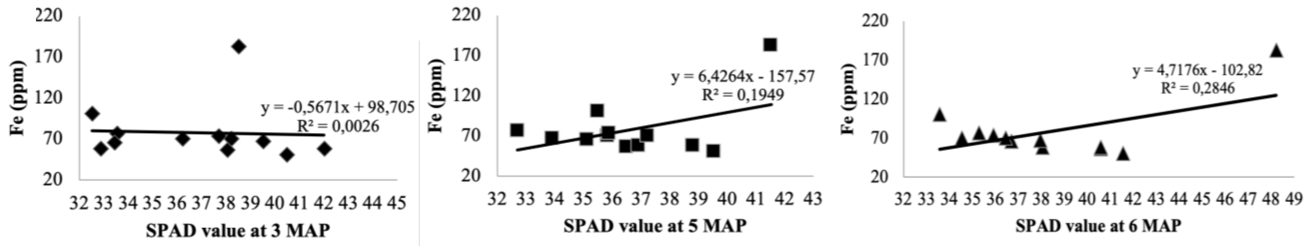
| Genotype | Mg (ppm) | Fe (ppm) | Zn (ppm) |
|---|---|---|---|
| G5D2-223 | 2600 | 57.46 | 452.6 |
| G6-1-15-4-3 | 2300 | 101.32 | 430.06 |
| G6-2-15-1-1 | 2700 | 76.89 | 476.96 |
| G6-2-15-3-3 | 2500 | 66.45 | 517.49 |
| G6-2-15-5-3 | 3000 | 57.73 | 426.5 |
| G1D1-532 | 3000 | 69.64 | 444.87 |
| Malang (G4D0) | 2100 | 51.11 | 377.19 |
| G4D1-222 | 2700 | 58.4 | 405.62 |
| G4D3-113 | 2100 | 67.25 | 311.75 |
| Ratim (G2D0) | 2400 | 73.59 | 426.71 |
| G2D1-422 | 2500 | 70.81 | 474.08 |
| G3D2-413 | 2400 | 182.48 | 351.13 |

Note: Analysis of target mineral using apical to fifth leaves of each genotype from the block in a composite; using Atomic Absorption Spectrophotometry method (AAS).



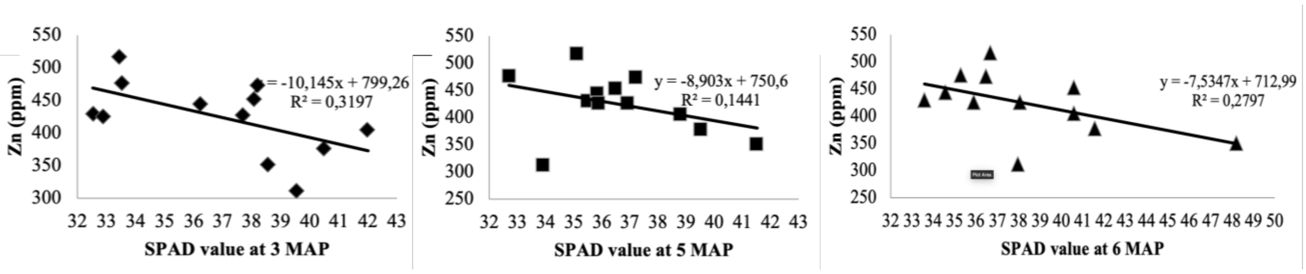

A B C

**Figure 7.** Correlation between the SPAD value (chlorophyll content) in months after planting (MAP) and Mg (ppm) content in cassava leaves (the fifth leaf below apical leaf). (A) correlation between SPAD value in 3 MAP with Mg content; (B) correlation between SPAD value in 5 MAP with Mg content; (C) correlation between SPAD value in 6 MAP with Mg content




A B C

**Figure 8.** Correlation between the SPAD value (chlorophyll content) in months after planting (MAP) and Fe (ppm) content in cassava leaves (the fifth leaf below apical leaf). (A) correlation between SPAD value in 3 MAP with Fe content; (B) correlation between SPAD value in 5 MAP with Fe content; (C) correlation between SPAD value in 6 MAP with Fe content




A B C

**Figure 9.** Correlation between the SPAD value (chlorophyll content) in months after planting (MAP) and Zn (ppm) content in cassava leaves (the fifth leaf below apical leaf). (A) correlation between SPAD value in 3 MAP with Zn content; (B) correlation between SPAD value in 5 MAP with Zn content; (C) correlation between SPAD value in 6 MAP with Zn content

**Table 2.** Qualitative traits of leaf characterization among 12 genotypes of cassava

| Genotypes | Colour of apical leaf | Pubescence on apical leaves | The shape of the central leaflet | Petiole color | Leaf color | Number of leaf lobes | Lobe margins | Colour of leaf vein | The orientation of the petiole |
|---|---|---|---|---|---|---|---|---|---|
| G5D2-223 | Purplish Green | Present | Lanceolate | Red | Dark Green | Seven | Smooth | Green | Horizontal |
| G6-1-15-4-3 | Purplish Green | Absent | Lanceolate | Red | Light Green | Seven | Smooth | Reddish Green (less) | Horizontal |
| G6-2-15-1-1 | Purplish Green | Present | Lanceolate | Red | Light Green | Seven | Smooth | Reddish Green (more) | Horizontal |
| G6-2-15-3-3 | Purplish Green | Present | Lanceolate | Red | Light Green | Seven | Smooth | Reddish Green (less) | Horizontal |
| G6-2-15-5-3 | Purplish Green | Present | Lanceolate | Red | Light Green | Seven | Smooth | Reddish Green (less) | Horizontal |
| G1D1-532 | Purplish Green | Present | Lanceolate | Reddish Green | Light Green | Seven | Winding | Green | Horizontal |
| Malang(G4D0) | Light Green | Present | Lanceolate | Greenish Red | Dark Green | Seven | Smooth | Green | Horizontal |
| G4D1-222 | Dark Green | Present | Lanceolate | Greenish Red | Light Green | Seven | Smooth | Green | Horizontal |
| G4D3-113 | Purplish Green | Present | Lanceolate | Greenish Red | Light Green | Seven | Winding | Reddish Green (less) | Inclined upwards |
| Ratim(G2D0) | Purplish Green | Absent | Lanceolate | Greenish Red | Dark Green | Seven | Winding | Reddish Green (less) | Horizontal |
| G2D1-422 | Purplish Green | Present | Lanceolate | Greenish Red | Dark Green | Seven | Smooth | Reddish Green (less) | Irregular |
| G3D2-413 | Purplish Green | Present | Lanceolate | Reddish Green | Dark Green | Seven | Winding | Reddish Green (less) | Horizontal |

Note: Data analysis using Descriptive Analysis (Median-Mode) method on Microsoft Excel

The highest magnesium, iron and zinc content in leaves, about 3000 ppm, 182.48 ppm and 500 ppm respectively, were found in G6-2-15-5-3 and G1D1-532; G3D2-413 and G6-1-15-4-3; G5D2-223, G6-2-15-3-3, G6-2-15-1-1 and G2D1-422, while the lowest of magnesium, iron and zinc levels were in Malang (G4D0) and G4D3-113; Malang (G4D0); G3D2-413 and G4D3-113 genotypes. The strong relationship between the SPAD-value with magnesium level at 3, 5, and 6 MAP and high iron level at 6 MAP equal with the research of Suharja and Sutarno (2009), Farhat et al. (2016), Trankner et al. (2018), which indicated that chlorophyll is composed of several elements, including magnesium. It also plays an important role in the formation of chlorophyll. Thus magnesium levels affected chlorophyll levels in leaf chloroplasts, and the photosynthesis process will remodel by these minerals. The high magnesium level on leaves increased the rate of photosynthesis in the leaf bones, due to high electron and energy transfer due to high magnesium levels in the thylakoid structure (Ye et al. 2019). Tuhenay (2018) states that leaf color and chlorophyll content can be used as an estimator of iron in leaves because there are associations of iron and leaf chlorophyll in chloroplasts. However, high zinc levels can be estimated only on 3 MAP, and low zinc levels in leaves can use leaves at 6 MAP. Kandoliya et al. (2018) showed an effect of providing zinc and iron micro fertilizers to the chlorophyll content of wheat leaves. With this additional fertilizer, the input for forming color pigments also increased, and the formation of chlorophyll a and b and the photosynthetic process will increase.Janke et al. (2018) showed that SPAD chlorophyll meter value significantly influenced of $ZnSO_4.7H_2O$ application.

In conclusion, the results of this study indicated that G6-1-15-4-3, G6-2-15-1-1, G6-2-15-3-3 and Malang (G4D0), G4D1-222, G3D2-413 genotypes could be categorized as contrast genotypes based on the highest and lowest chlorophyll content (SPAD value) and mineral content. The correlation between the target mineral and the SPAD value showed a strong positive relationship between the total SPAD value and magnesium and iron levels and a weak relationship for zinc. Estimating high magnesium content in leaves was carried out at 3, 5 and 6 MAP, but high iron content in leaves could be observed at 6 MAP.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