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Research article

Characterization of macro and micro-minerals in cassava leaves from genotypes planted in three different agroecological locations in Nigeria

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ABSTRACT

Diversity in the mineral composition of cassava leaves bred in sub-Saharan Africa has not been fully investigated. This study characterized macro and micro-minerals in 400 genotypes of Cassava leaves planted in three different agroecological environments in Nigeria. Laboratory analysis of the leaves was done using an Inductively Coupled Optical Emission Spectrometer. Across all three locations sampled in this study, the iron content ranged from 43 to 660 mg/kg, zinc from 16 to 440 mg/kg, Manganese 16–61mg/kg, Copper 0.7–14 mg/kg, Aluminum 5.3–630 mg/kg. Among the macro elements, Calcium ranged from 3600 to 17600 mg/kg, Magnesium 1760–6500 mg/kg, Sodium 0.4–720 mg/kg, Potassium 3100–27000 mg/kg. When the location effect was tested, there was a significant difference among the genotypes for all elements. Cluster analysis resulted in five clusters containing 187, 147, 60, 2, and 4 genotypes, respectively. Cluster 2 contained eight varieties (01/0046, 94/0020, 93/0098, 88/112-7(3X), I00/0017, 91/00417, I00/0017, 88/112-7(3X)) that possessed the highest mineral compositions in Fe, Al, Ti, Na, K, S, Mn, and B, respectively. Genotypes 93/0681(4X), 92/0430, and 95/0460 in cluster 3 had the highest concentrations of Mg, Na, and Zn, respectively. The correlation results showed a notable positive relationship among iron with zinc, copper, aluminum, and titanium (r = 0.33, 0.39, 0.48, and 0.56, respectively), zinc with nickel, titanium, and sulphur (r = 0.52, 0.3,2 and 0.51, respectively) while calcium negatively correlated with potassium (r = – 0.31), phosphorus (r = –0.41). This study provides evidence that genotypic diversity exists for mineral composition in cassava leaves and, therefore, can be exploited for genetic improvement by breeders seeking solutions to reduce persistent mineral deficiencies in sub-Saharan Africa.

1. Introduction

Cassava (*Manihot esculenta* Crantz), which originated from South America, is one of the primary sources of dietary energy in the sub-Saharan African diet, ranking highest with rice, wheat, and maize (Ceballos et al., 2004; FAO, 2022). Africa, especially Nigeria, is the world's highest cultivator of the crop. As of 2020, world cassava production was about 303 million tons, with Africa contributing about 60% and Nigeria contributing a third of Africa's production (FAO, 2022). Cassava is tolerant of diverse weather conditions and soil types (Ceballos et al., 2004) and is grown year-round. After maturity, it can be left for a year and harvested when needed, thereby playing a proven role in improving food security (Montagnac et al., 2009).

The roots and leaves are the two main products that are the nutritively beneficial parts of a mature cassava plant (Ceballos et al., 2004). The roots (an important source of carbohydrate) and leaves (a great source of proteins and micronutrients) constitute the bulk of the food utilization of the cassava plant (Montagnac et al., 2009), with the roots playing a more dominant role. In sub-Saharan African countries, the leaves are consumed as a vegetable, either as a soup eaten with starchy dishes or as cooked green vegetables (Achidi et al., 2005; Latif and Müller, 2015). Being rich in protein, it contributes to protein intake in developing countries (Ayele et al., 2021) and has an amino acid profile similar to some animal-sourced protein foods (Babu and Chatterjee, 1999; Popoola et al., 2019). However, its shortcoming has been its composition of anti-nutritional compounds, including phytates, saponins, oxalates, and especially cyanogenic compounds, which is why they have not been extensively incorporated into the food system (Latif. and Müller, 2015; Ayele et al., 2021). They are also a known rich source of minerals and vitamins, with some reports suggesting that they have

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substantial potential to reduce deficiencies if consumption is taken to scale (Montagnac et al., 2009; Aregheore, 2012). Minerals are elements critically needed by the body as regulators of biochemical processes. They are broadly divided into trace and macro elements based on their quantitative contribution to dietary and nutrient intake. Microelements include Iron (Fe), Zinc (Zn), Manganese (Mn), Boron (B), Copper (Cu), Nickel (Ni), Aluminum (Al), and Titanium (Ti). Macro elements include Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Phosphorus (P), and Sulphur (S). Various sources in the literature provide compositional information on some genotypes and products of cassava leaves prepared into food or animal feed (Chávez et al., 2000; Nassar and Marques, 2006; Achidi et al., 2008; Nguyen et al., 2012; Popoola et al., 2019) but evaluation of a large number of genotypes is not common. A recent mineral compositional evaluation of large germplasms of cassava roots grown in sub-Saharan Africa has been reported (Alamu et al., 2020a, 2020b, 2021, 2022). Also, an extensive collection of leaves was evaluated for genotypic effects on amino acids, carotenoids, and cyanogenic properties in Latin America (Ospina et al., 2021). However, this report did not provide compositional data on macro and trace elements contained in genotypes of cassava leaves. Another notable knowledge gap is the performance of genotypes across different environments since plants (in this case, cassava) are known to give significant differential genotypic responses under varying environmental conditions (Ceballos et al., 2004; Maroya et al., 2012). Therefore, this study characterized 14 micro- and macro-elements of cassava leaves from 400 genotypes planted in three different agroecological locations in Nigeria to provide information for plant breeding purposes and existing food compositional databases within sub-Saharan Africa and worldwide.

2. Materials and methods

2.1. Background information on fresh cassava leaves

Four hundred (400) genotypes of cassava leaves (Supplementary file 1) were collected in a genetic gain roots and leaves trial in readiness for on-farm trials before official release. They were collected from three locations—Ibadan (forest-savanna transition) and Mokwa (southern Guinea savanna), all maintained by the International Institute of Tropical Agriculture, Nigeria. Mature stem cuttings of the genotypes, including checks, were planted on plots where ridges had been made. They were planted during the rainy season in mid-2005 in an Augmented Completely Randomized Design with three checks (TME 1, 91/02324, and 30572) repeated randomly in each sub-block using a spacing of 1 m × 0.5 m (5 plants per plot). Before planting, the plots had been ploughed and harrowed before being ridged. Ibadan growing locations had soil type-sandy loamy (classified as ferric luvisols), and Mokwa growing locations had soil-type- dystric nitosols (Alamu et al., 2020b). No fertilizers or herbicides were applied throughout the study. Manual weeding was done as necessary. The agroecological characteristics of the growing locations are described as follows:

i. IVS-Ibadan: is an Inland Valley Hydromorphic area situated in the forest-savanna transition zone with an annual rainfall of 1312 mm and an average temperature range of 20.3–33.8 °C
ii. Upland-Ibadan: is a plain level area situated in the same agro-ecological zone as IVS-Ibadan.
iii. Mokwa: is a Southern Guinea savanna zone with an annual rainfall of 1149 mm and an average temperature range of 18.1–37.3 °C. The latitude and longitude for both locations are Ibadan 7° 38′N, 3° 89′E; Mokwa 9° 28′N, 5° 05′E.

2.2. Sample preparation

Mature, succulent leaves from four plants were plucked from their stalks and washed with de-ionized water. They were then chopped before mixing thoroughly. The chopped leaves were divided into quarters, and adjacent sections were mixed. The leaves were wrapped in muslin cloth before being blanched using tepid de-ionized water (60 °C) for 4 min. The leaves were then dried in a convection oven at 40 °C for 48 h using stainless steel trays.

2.3. Determination of mineral profile

The trace and the macro element content were determined using Inductively Coupled Optical Emission Spectrometry (ICP-OES) as presented by Wheal et al. (2011) and replicated in Alamu et al. (2020b), which involved an acid digestion phase before instrumentation. The digestion was done at 125 °C for 2 h using 2.0 mL of HNO_3 and 0.5 mL of

Table 1. Descriptive statistics (mg/kg) for all mineral parameters across three locations.

		Fe	Zn	Mn	B	Cu	Ni	Al	Ti	Ca	Mg	Na	K	P	S
IVS	Minimum	65	42	44	12	5.6	1.4	12	1.5	3800	1760	220	4000	2200	2600
	Maximum	340	124	610	29	13	21	220	56	17600	5900	700	17600	5500	6100
	Mean	119.91	70.09	181.37	19.44	8.42	6.78	51.5	7.42	7429	3316	406.43	10649	3555	4211
	SD	33.85	13.92	86.24	3.3	1.11	3.26	26.3	5.46	1747	519.44	87.34	2513	528.18	702.02
	S.E	1.69	0.69	4.31	0.16	0.06	0.16	1.32	0.27	87.37	25.97	4.37	125.65	26.41	35.10
UPLAND	Minimum	72	38	44	14	5.3	0.8	11	0.14	3800	2100	0.4	12400	2900	2400
	Maximum	660	97	270	30	14	43	280	5.2	12500	4100	650	27000	6400	3900
	Mean	118.15	57.53	128.43	20.35	8.93	3.61	38.72	0.85	7106	2977	139.34	18459	4245	3248
	SD	38.72	8.57	46.97	2.8	1.14	2.7	23.9	0.53	1375	342.82	137.05	2272	603.64	213.09
	S.E	1.94	0.42	2.35	0.14	0.06	0.14	1.19	0.03	68.78	17.14	6.85	113.61	30.18	10.65
MOKWA	Minimum	43	16	16	5.7	0.7	1.1	5.3	0.12	3600	1860	144	3100	1790	1620
	Maximum	280	440	540	35	10	37	630	4.4	15200	6500	720	21000	5500	3600
	Mean	77.36	35.34	251.79	18.76	6.69	4.18	36.85	0.59	8430	3421	348.06	11304	2895	2547
	SD	18.13	33.66	82.07	3.95	1.5	4.12	50.15	0.44	1880	569.6	96.54	3145	576.55	333.89
	S.E	0.91	1.68	4.10	0.19	0.08	0.21	2.51	0.02	94.04	28.48	4.83	157.29	28.83	16.69
All locations	Minimum	43	16	16	5.7	0.7	0.8	5.3	0.12	3600	1760	0.4	3100	1790	1620
	Maximum	660	440	610	35	14	43	630	56	17600	6500	720	27000	6400	6100
	Mean	105.1	54.3	187.2	19.5	8	4.9	42.4	3	7656	3238	298.1	13467	3564	3336
	SD	37.1	26	89.6	3.5	1.6	3.7	36.1	4.5	1774	522.9	158.3	4432	793.1	826.4
	S.E	1.07	0.75	2.59	0.10	0.05	0.11	1.04	0.13	51.22	15.10	4.57	127.95	22.90	23.86

SD- Standard deviation, S.E- Standard error.

H_2O_2 in a 72-position DigiPrep digestion block chamber (SCP Scientific, Baie D'Urf e, Quebec, Canada) and made to the 25 ml level with de-ionized water. Each sample was then run in duplicate. The trace elements identified in the samples investigated were Iron (Fe), Zinc (Zn), Manganese (Mn), Boron (B), Copper (Cu), Nickel (Ni), Aluminum (Al), and Titanium (Ti). The macro elements were Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Phosphorus (P), and Sulphur (S).

2.4. Statistical analysis

The data generated in this study were subjected to descriptive and inferential statistical analysis using the Statistical Analysis System Software Cary, NC, USA (SAS, 2012). Means were separated using Fisher's least significance difference test. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were also utilized.

3. Results and discussion

3.1. Mineral composition of GGA cassava leaves

Table 1 represents the descriptive statistics of trace and macro minerals across all three sampled locations. Across all locations, the iron content ranged from 43 to 660 mg/kg (mean 105.1 ± 37.1 mg/kg), zinc from 16 to 440 mg/kg (mean 54.3 ± 26 mg/kg), Manganese 16–610 mg/kg (mean 187.2 ± 89.6 mg/kg), Boron 5.7–35 mg/kg (mean 19.5 ± 3.5 mg/kg), Copper 0.7–14 mg/kg (mean 8 ± 1.6 mg/kg), Nickel 0.8–43 mg/kg (mean 4.9 ± 3.7 mg/kg), Aluminum 5.3–630 mg/kg (mean 42.4 ± 36.1 mg/kg), Titanium 0.1–56 mg/kg (mean 3 ± 4.5 mg/kg). Among the macro elements were Calcium 3600–17600 mg/kg (mean 7656 ± 1774.4 mg/kg), Magnesium 1760–6500 mg/kg (mean 3238.9 ± 522.9 mg/kg), Sodium 0.4–720 mg/kg (mean 298.1 ± 158.3 mg/kg), Potassium 3100–27,000 mg/kg (mean 13,467.1 ± 4432.4 mg/kg), Phosphorus 1790–6400 mg/kg (mean 3564.9 ± 793.1 mg/kg), Sulphur 1620–6100 mg/kg (mean 3336.2 ± 826.4 mg/kg). When the mineral composition is compared per location, varieties planted in Mokwa had the lowest micro and macro-elements concentrations. The only exception was Nickel (1.1 mg/kg) among microelements, which was the lowest in the Upland planting location. In contrast, magnesium (1760 mg/kg) and sodium (0.4 mg/kg) were the exceptions for macro elements in IVS and Upland locations, respectively.

3.2. Effect of genotypes and growing location on the mineral contents of GGA cassava leaves

Supplementary file 1 and Table 2 present the mean values of trace and macro elements of the sampled 400 leaves by genotype and the analysis of variance based on genotypes and growing locations. There were significant differences among mean values of genotypes except for the microelements Nickel, Aluminum, and Titanium, and one macro element, Sodium. However, there was a significant difference among the genotypes based on growing locations for all elements. This location-specific difference is similar to reported differences in cassava root evaluation as reviewed by Montagnac et al. (2009) and highlighted explicitly by Maroya et al. (2012) and Alamu et al. (2020a). Specifically, the characteristics of the Mokwa agroecological zone may have contributed to comparatively lower compositions found in this study. In general, the results show a wide range of values in which the mean value of each nutrient is higher than the values reported in the literature (Achidi et al., 2008; Montagnac et al., 2009) and also in national and regional food composition tables (NiFCT, 2017; WAFCT 2019). Worthy of mention is the abundance of key physiologically essential minerals like Iron, Zinc, and Calcium in these genotypes, which implies that cassava leaves can be a key food source of these minerals. In comparison with existing literature on the mineral composition of cassava leaves as reviewed by Montagnac et al. (2009) and Latif and Müller (2015), this study's results show that genotypic diversity exists for mineral composition in cassava leaves

Table 2. Mean values of trace and macro minerals by growing location.

Growing locations	Fe	Zn	Mn	B	Cu	Ni	Ti	Ca	Mg	Na	K	P	S	Al
IVS	119.91 a	70.09 a	181.37 b	19.44 b	8.42 b	6.78 a	7.42 a	7429.75 b	3316.90 b	406.43 a	10649.25 c	3555.25 b	4211.75 a	51.50 a
Upland	118.14 a	57.51 b	128.31 c	20.35 a	8.93 a	3.61 c	0.85 b	7102.25 c	2976.25 c	139.00 c	18466.00 a	4245.25 a	3248.50 b	38.75 b
Mokwa	77.36 b	35.33 c	251.79 a	18.76 c	6.69 c	4.18 b	0.59 b	8430.00 a	3421.40 a	348.06 b	11304.75 b	2895.90 c	2547.88 c	36.85 b
Pr > F(Location)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 3. Principal components analysis (PCA) of mineral elements across three locations.

	PC1	PC2	PC3
Fe	0.55	0.49	–
Zn	0.48	0.46	–
Mn	–	–	0.31
B	–	–	0.70
Cu	0.69	–	–
Ni	–	0.49	–
Al	–	0.50	–
Ti	–	0.78	–
Ca	–	–	0.49
Mg	–	0.40	–
Na	–	0.63	–
K	0.68	–	–
P	0.85	–	–
S	0.56	0.60	–
Eigenvalue	3.75	2.90	1.59
Cumulative %	26.81	47.54	58.93

and, therefore, can be exploited for genetic improvement by breeders seeking options to reduce micronutrient deficiencies in sub-Saharan Africa.

Supplementary file 3 shows the goodness of fit statistics for the elemental compositions of genotypes from the three growing locations. The regression model used to analyze variance was predicted by 38–81% of the composition data, which varied based on the dependent variables. The mean square error (MSE) ranged widely from 1.2 to 1,981,516.1, where Copper had the lowest value and Calcium had the highest, respectively. This very high range of MSE (especially for the elements with high concentrations) indicates that the regression models could not adequately predict the variability of the elements considered, as has been applied in a similar study (Alamu et al., 2020b). The elements with extremely high MSE are Calcium and Potassium. Statistically, this may be an unsuitable result, but it presents an advantage for varietal selection since it shows that the compositional spread of the nutrients is vast and thus allows germplasm selection and breeding.

3.3. Principal component analysis of mineral profiling of GGA cassava leaves

The principal component analysis (PCA) results across the genotypes, and growing locations are presented in Table 4. In this current study, PC 1 to 3 were extracted and contributed to 58.9% of the total variation, these three components had eigenvalues of 3.75, 2.9, and 1.59, respectively. The trend found in this study followed a similar statistical approach used by Luchese et al. (2017) and Alamu et al. (2021) to explain the variation. Factor loadings ≥0.3 were selected as a cut-off point to identify important variations and common characteristics in the data. P, K, and Cu were distinctly loaded on PC 1, Ti, Na, Al, Ni, and Mg were loaded on PC 2, and B, Ca, and Mn were loaded on the third component. Sulphur, Iron, and Zinc loaded on both PC 1 and PC 2. The different components reveal similarities in the various elements, which reflect associations that can be valuable for decision-making during breeding. Notable is PC 2, which loads eight elements, five of which distinctly show an association among themselves.

3.4. Cluster analysis of GGA cassava leaf genotypes using the mineral composition

Supplementary file 4 shows a cluster analysis of genotypes based on their similarity to the elements considered in this study. Five independent clusters were discovered and related in a dendrogram (Supplementary file 4). Cluster 1 to 5 contained 187, 147, 60, 2, 4 genotypes respectively. Notably, cluster 2 contained eight varieties (01/0046, 94/0020, 93/0098, 88/112-7(3X), I00/0017, 91/00417, I00/0017, 88/112-7(3X)) that possessed highest mineral compositions in Fe, Al, Ti, Na, K, S, Mn, and B, respectively. Genotypes 93/0681(4X), 92/0430, and 95/0460 in cluster 3 had the highest concentrations of Mg, Na, and Zn, respectively. While 30555(4X) had the highest values of Ca and belonged to cluster 4. Genotype K95/0671 was highest for Ni in cluster 1. Genotypes 96/1630 and M98/0028, belonging to cluster 5, had the highest potassium and lowest calcium concentrations. Genotype 91/00262(3X), which belonged to cluster 1, had the lowest values in Fe and Al, while genotype 98/0406, also in cluster 1, had the lowest values for macronutrients Mg and Na.

3.5. Correlation of mineral content of GGA cassava leaves

Table 4 shows Pearson's correlation coefficients of the elements contained in the genotypes across the growing locations. Generally, the relationships were statistically significant ($p < 0.05$) but had a weak relationship in most cases between the elements. The microelements showed a notable relationship among Iron with Zinc, copper, Aluminum, and titanium ($r = 0.33$, 0.39, 0.48, and 0.56, respectively), Zinc with Nickel, titanium and sulphur ($r = 0.52$, 0.32, and 0.51, respectively), Manganese with calcium, magnesium, sodium, potassium, and

Table 4. Pearson correlation coefficients (PCC)* of mineral composition of cassava leaves across three locations.

Variables	Fe	Zn	Mn	B	Cu	Ni	Al	Ti	Ca	Mg	Na	K	P	S
Fe	**1**													
Zn	0.33	1												
Mn	–0.16	–0.36	1											
B	0.14	–0.05	0.03	1										
Cu	0.39	0.19	–0.27	0.26	1									
Ni	0.17	0.52	–0.01	–0.16	0.04	1								
Al	0.48	0.14	0.08	0.10	0.09	0.06	1							
Ti	0.56	0.32	–0.03	0.01	0.17	0.28	0.04	1						
Ca	–0.08	–0.02	0.45	0.16	–0.21	–0.15	0.11	–0.07	1					
Mg	–0.06	0.02	0.42	0.00	–0.19	0.07	0.09	0.05	0.49	1				
Na	–0.02	0.02	0.32	–0.09	–0.11	0.30	0.14	0.34	0.17	0.31	1			
K	0.19	0.06	–0.43	0.23	0.45	–0.08	–0.02	–0.27	–0.31	–0.40	–0.56	1		
P	0.28	0.27	–0.53	0.21	0.60	0.10	0.00#	0.00	–0.41	–0.36	–0.34	0.77	1	
S	0.44	0.51	–0.20	0.25	0.43	0.29	0.19	0.61	–0.14	–0.08	0.20	–0.02	0.33	1

* significant at $p < 0.05$.
# insignificant at $p > 0.05$.

phosphorus (r = 0.45, 0.42, 0.32,–0.43, and –0.53, respectively). Boron with copper (r = 0.26), Nickel with titanium (r = 0.28). The macro-elements showed relationships between Calcium with Magnesium (r = 0.49), potassium (r = – 0.31), phosphorus (r = –0.41). Magnesium and Sodium negatively correlated with Potassium and phosphorus (r = –0.40, r = –0.36 and r = –0.56, r = –0.34, respectively). The most notable relationship from this study is the correlation between potassium and phosphorus (r = 0.77). This strong correlation of P and K is different from the results presented by Alamu et al. (2020a) when cassava roots were evaluated across multiple harvest times and sampling methods but is consonant with assertions presented by Alamu et al. (2020b) in yellow-fleshed cassava roots. The correlation relationships presented in Table 4 mirror the PCA results in Table 3, where some elements showed similar characteristics of belonging to the same components. Overall, positive relationships will be advantageous from a breeding point of view. In contrast, negative relationships may pose a challenge to the breeders seeking to improve the concentration of select minerals.

4. Conclusion

This study presents data on the compositional information of micro- and macro-elemental composition of a large number of cassava leaves and reports the various statistical interactions using genotype and growing location factors. Generally, the results provide evidence of a large diversity in the composition of elements in cassava leaves. The most abundant elements were Fe & Ca, while Ti & Na were the least abundant micro- and macro-elements. A noticeable location effect was observed on the genotypes, showing that the agroecological zone—Mokwa—did not provide maximum genetic gain compared with other zones. In terms of the specific varieties that could be considered as parental lines for breeding purposes, varieties 01/0046, 94/0020, 93/0098, 88/112-7(3X), I00/0017, 91/00417, I00/0017, 88/112-7(3X)) possessed highest mineral compositions in Fe, Al, Ti, Na, K, S, Mn, and B, respectively and particularly grouped in the cluster analysis. Overall, this study proves that an increase in the utilization of cassava leaves for food can reduce mineral deficiencies (especially in key elements of Fe, Ca, and Zn). The evaluation presented can thus provide breeders with information on varietal selection and trials and inform nutritional choices for improved nutrient intake, as the case may apply.

Declarations

Author contribution statement

Alamu, Emmanuel Oladeji: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dixon, Alfred; Maziya-Dixon, Busie: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Eyinla, Tolu Emma: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in the article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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