

Original article

Cyanogenic, carotenoids and protein composition in leaves and roots across seven diverse population found in the world cassava germplasm collection at CIAT, Colombia

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Summary The objective of this study was to characterise the nutritional potential of leaves and identify a diversity centre with low cyanide and high nutrient content among 178 Latin American cassava genotypes. This field-based collection represents the seven diversity centres, held at The International Center for Tropical Agriculture (CIAT Palmira, Colombia) by the Cassava Program. The cyanide, all-*trans*- β -carotene and lutein concentrations in cassava leaves ranged from 346 to 7484 ppm dry basis (db), from 174–547 $\mu\text{g g}^{-1}$ db and 15–181 $\mu\text{g g}^{-1}$ db, respectively. Cassava leaves also showed significant levels of essential amino acids leucine, lysine, phenylalanine, valine and threonine, and average total protein content of 26.24 g 100 g⁻¹ db. Among seven diversity centres, South American rainforest group showed low cyanide and high carotene content in leaves. In addition, VEN77 and PAN51 genotypes stood out for having low cyanide in leaves and roots and high carotene in leaves. This genetic diversity can be used to select high potential progenitors for breeding purposes.

Keywords Amino acids, carotenoids, cassava leaves, cyanogenic compounds, nutritional value, protein.

Introduction

Cassava is considered the fourth most important crop in the world, playing key roles for food security and income generation. It is a staple food for nearly 600 million people (FAO, 2002). Its global production in 2018 was 277.8 million tons of fresh roots (FAO-STATS, 2020). The roots are the main product from cassava, and represent a major source of carbohydrates, particularly in regions where drought and/or nutrient-poor soils limit the cultivation of other crops (Ceballos *et al.*, 2012a). However, cassava leaves constitute an important source of essential amino acids (i.e. valine and leucine), vitamins and minerals (Ngudi *et al.*, 2003a; Nassar & Marques, 2006; Montagnac *et al.*, 2009), as well as for animal feeding (Nhu Phuc *et al.*, 2000; Balagopalan, 2002; Eruvbetine *et al.*, 2003; Wanapat, 2003; Sokerya *et al.*, 2009; Urribarri *et al.*, 2009; Thang *et al.*, 2010; Oni *et al.*, 2010; Hue

et al., 2012; Lukuyu *et al.*, 2014); including poultry, aquaculture and different mammals (swine, cattle, lamb and goats). Leaves are also used for human consumption, particularly in Africa and South America (Ngudi *et al.*, 2003b; Ufuan Achidi *et al.*, 2005; Kobawila *et al.*, 2005; Latif & Müller, 2015).

Hence, cassava leaves as food are a good source of protein, vitamins and minerals (Nassar & Marques, 2006; Nguyen *et al.*, 2012). Cassava leaves contain significant amounts of carotenoids, especially all-*trans*- β -carotene, a vitamin A precursor (Chávez *et al.*, 2000). Vitamin A deficiency is a public health problem, which can lead to impaired ocular and immune systems, growth and development, epithelial cellular integrity and reproductive functions (Wiseman *et al.*, 2017). Hence, increased use of cassava leaves may contribute to reducing vitamin A deficiency in human populations that rely on cassava as a source of energy in their diets (Ceballos *et al.*, 2013). Another important carotenoid presents in cassava leaves is Lutein,

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which has an antioxidant potential (Ma & Lin, 2010; Zou *et al.*, 2011). Carotenoids can also improve immune system function by stimulating lymphocyte production and have preventive effects against cardiovascular diseases (Rodriguez-Amaya, 2015; Hajizadeh-Sharafabad *et al.*, 2019).

A very important consideration when cassava leaves are used as food is the need to process them to reduce the HCN (Kobawila *et al.*, 2005; Latif & Müller, 2015), high contents of HCN has been linked to the Konzo, a neurone disorder resulting in irreversible paralysis (tetraparesis) of the legs mostly affecting children and young women (Ngudi *et al.*, 2003a; Bradbury *et al.*, 2013; Parmar *et al.*, 2017). Thus, for human consumption, the leaves are often processed by blending with other ingredients or by fermentation (Osseo-Asare, 2005; Kobawila *et al.*, 2005). Despite the potential risks, in Sierra Leone and Liberia, cassava leaves have a reputation to help pregnant women increase breast milk production (Aregheore, 2012). Hence, with appropriate processing techniques and the introduction of selected genotypes for low cyanogenic content, it is possible to achieve food products with permissible cyanide levels without affecting human health and increasing the crop's nutritional value.

For animal nutrition, cassava leaves are dried or undergo silage treatment to reduce the cyanogenic potential and for long-term preservation, in Vietnam, Brazil, Peru, Indonesia, Thailand and Nigeria. Cassava leaves have been used to improve protein to energy ratio in biomass for livestock feeding (Wanapat, 2003; Marie-Magdeleine *et al.*, 2010). Cassava leaves in the diet have beneficial effects in goats, sheep and poultry (Khajarern & Khajarern, 1992; Oni *et al.*, 2010; Sudarman *et al.*, 2016; Morgan & Choct, 2016) and aquaculture (Lukuyu *et al.*, 2014). Furthermore, cassava leaves used as a forage have the potential to reduce by up to one-third enteric methane emission, offering a clear alternative to reduce greenhouse emissions caused by livestock systems (Alvarez, Becerra Lopez-Lavalle and Chirinda, unpublished work). On the other hand, cyanogenic glucosides have been associated with anti-parasitic effects in cassava-based animal feeds (Sokerya *et al.*, 2009; Marie-Magdeleine *et al.*, 2010). Also, cassava leaves can improve the texture of animal feed (Ravindran & Wanasundera, 1993; Marjuki *et al.*, 2008). Cassava leaves constitute a major source of protein and amino acids that can be used as an alternative for manufacturing of animal feed; being particularly important in case of drought or other climatic events that will limit the production of other feeds such as cereals or legumes; making them unavailable or unaffordable.

The uses of cassava leaves are region-specific; thus, it is important to determine the cyanogenic potential (HCN) and nutrient contents of cassava leaves in

different regions for future plant breeding in order to better exploit global use and consumption of cassava leaves. Even more, since a recent fine-scale genome-wide approach undertaken at CIAT's cassava program has revealed seven-ecogeographic populations which explains the domestication and genetic variation of cassava (Becerra Lopez-Lavalle *et al.*, 2015 and Becerra Lopez-Lavalle *et al.*, 2018). The objective of this research is to characterise the hydrogen cyanide gas [cyanogenic potential (HCN)] released from cyanogenic glucosides in leaves and roots, and carotenoid and protein contents in the leaves of 178 genotypes representative of the cassava diversity from the collection held at CIAT in Cali, Colombia. Assessment of the extent of genetic differences and geographic clustering will have important consequences for future plant breeding.

Materials and methods

Cassava materials

Cassava genotypes from the cassava diversity collection at CIAT were planted and harvested in Palmira, Colombia located at 1100 M.A.S.L (3°30'17"N 76°21'24"O). In a first study, 178 genotypes from various regions of Latin America (Table 1) were evaluated for HCN and carotenoids in leaves six months after planting (MAP); ideal time for leaves harvesting as leaf retention may change among cassava genotypes. The same set of genotypes were also evaluated for HCN in roots at eleven MAP, ideal time for root harvesting. In a second study, a set of fifty-three cassava genotypes were chosen because they displayed the most contrasting HCN and β -carotenoid content from the first study. For the second study, the crude protein content and amino acid profiles in the leaves were analysed at six MAP (Table 2). All the materials were planted in a complete randomised block design; each plot (genotype) composed by sixteen clones. For each genotype, roots of three plants (clones) were harvested and pooled together in order to obtain representative samples. For leaves sampling, the youngest leaf at the top of the plant was numbered as 1, and the fully expanded 3rd and 4th leaves were collected for analyses.

Quantification of HCN in cassava leaves and roots

Total HCN (amount released from hydrolysis of cyanogens) was determined according to the method described by Essers *et al.* (1993). Fresh roots [40 g wet basis (wb)] and leaves (4 g wb) were homogenised for 2 min in a blender (Osterizer, model 4655, Mexico) at 28 °C in 50 mL of extraction medium (0.1 M orthophosphoric acid in a mix of 25% v/v ethanol and

Table 1 Summary of 178 genotypes evaluated in the first study

Country	No. of genotypes	Country	No. of genotypes	Country	No. of genotypes
Argentina	4	Dominican Rep.	1	Panamá	4
Bolivia	2	Ecuador	7	Paraguay	10
Brazil	36	Guatemala	6	Perú	11
China	1	Indonesia	9	Puerto Rico	1
Colombia	45	Malaysia	3	Thailand	3
Costa Rica	5	México	7	USA	3
Cuba	9	Nigeria	3	Venezuela	8
Total genotypes	178				

Table 2 Summary of 53 genotypes evaluated in the second study

Country	No. of genotypes	Country	No. of genotypes
Argentina	1	Guatemala	2
Bolivia	2	Indonesia	3
Brazil	7	México	2
China	1	Panamá	3
Colombia	11	Paraguay	1
Costa Rica	4	Perú	3
Cuba	3	Puerto Rico	1
Dominican Rep.	1	Thailand	4
Ecuador	1	Venezuela	3
Total genotypes	53		

75% v/v distilled water). The resulting solutions were transferred to 50 mL Falcon tubes and centrifuged (Eppendorf 5804R, Hamburg, Germany) for 10 min at 6000 revolutions per minute (rpm) and 25 °C. Aliquots of the supernatants (0.1 mL) were added to the tubes containing 0.4 mL phosphate buffer (0.1 M, pH 7.0). Then, 0.1 mL of linamarase enzyme [prepared as described in Cooke (1978)] was added and the mixture was incubated at 30 °C for 15 min. After incubation, 0.6 mL of 0.2 M sodium hydroxide was added. After 5 min at room temperature (23 °C), 2.8 mL of 0.1 M phosphate buffer (pH 6.0) was added. For spectrophotometer analysis, 0.1 mL of chloramine T was added and incubated for 5 min. Then 0.6 mL of the reagent isonicotinate/1,3-dimethyl barbiturate was added and incubated for 10 min. The absorbance of the solution was recorded at 605 nm. The HCN was determined by using the following equation

$$HCN(ppm) = \frac{m_{HCN}}{V_f} \times (Fd_1) \times (Fd_2) \times \frac{V_{sln ext}}{m_{m.ps}(g)}$$

m_{HCN} : µg HCN read from the calibration curve, using absorbance readings at 605 nm; V_f : Final volume of tube (4.7 mL); Fd_1 : Dilution factor 1 (4.7 mL/0.1 mL);

Fd_2 : Dilution factor 2 (Volume of the diluted sample/volume of the extract); $V_{sln ext}$: Volume of the solution (50 mL); $m_{m.ps}$: Mass of the sample in dry weight.

Quantification of carotenoids in cassava leaves

Carotenoid was quantified using the method described by de Sá & Rodríguez-Amaya (2003), with minor modifications. Cassava leaves (1 g wb) were mixed with 5 mL Milli-Q water. Ten mL of cold acetone was added; then, after 10 min 10 mL of petroleum ether was added. The sample was homogenised in an IKA® ULTRA-TURRAX® (Merck KGaA, City Darmstadt, Germany) for 30 s and centrifuged at 1814 *g* for 10 min at 10 °C. The organic phase (supernatant) containing the carotenoids was transferred to a reaction tube. Extraction of carotenoids was repeated thrice. Supernatants were pooled together, mixed with 10 mL of 0.1 M sodium chloride solution and centrifuged at 1814 *g* for 10 min at 10 °C. The ethereal phase containing carotenoids was kept. The volume of extracts was adjusted to 40 mL with petroleum ether (0.1% butylhydroxytoluene). Then, the extract was saponified with 40 mL of 10% potassium hydroxide in methanol by stirring at room temperature for 4 h. After saponification, the extract was washed eight times using 80 mL distilled water each time to remove the methanol phase. The carotenoid content was determined by high performance liquid chromatography (HPLC): Aliquots (15 mL) were dried by nitrogen evaporator (Nevap 112, Organomation Associates, Berlin, MA, USA). Immediately before injection, the dry extract was totally redissolved in 2 mL of (1:1) methanol and methyl tert-butyl ether HPLC-grade. After sonication (10 s) and agitation in a VWR multi-tube vortex (Avantor, New York, NY, USA) (1451 g; 60 s), the extract was filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter. Separation and quantification of carotenoid was achieved using a YMC Carotenoid S-5C30 reversed-phase column (4.6 mm × 150 mm: particle size, 5 µm), with a YMC Carotenoid S-5 guard column (4.0 × 23mm; YMC America Inc.,

Allentown, PA, USA) in an Agilent HPLC system (Agilent Technologies 1200 series, Waldbronn, Germany), using diode array detector (DAD). Peaks were identified by comparing retention time and spectral characteristics against a pure standard (CaroteNature GmbH, Lupsingen, Switzerland). The quantity of each carotenoid was determined by integration of peak area against respective standard curves.

Amino acid profile, protein quantification and nitrogen quantification

For amino acids analysis, proteins were hydrolysed by methane sulphonic acid (4 N) at 150 °C for 2 h, with norleucine added as internal standard (250 nmol mL⁻¹ in 0.2 M sodium citrate buffer, pH 2.2) (Adinsi *et al.*, 2017). The amino acids profile was determined with an amino acid analyser Biochrom 30 (Biochrom Ltd., Cambridge, UK) as mentioned in Loscos *et al.* (2008). Amino acids were quantified by comparing the areas of the peaks with those of a complete standard containing twenty-seven amino acids (Sigma, Saint-Quentin Fallavier, France). We considered that cystine dimers were not hydrolysed by the methane sulphonic acid treatment (Chiou & Wang, 1988). Tryptophan was determined with a separate method (Nurit *et al.*, 2009), because this amino acid is degraded during the acid hydrolysis of proteins.

The protein content of cassava leaves was calculated as the sum of amino acids present in the leaves, with the following precautions: non-proteinogenic amino acids present in the samples were not included in the calculations, such as ornithine and GABA; the molecular weight of free amino acids was reduced by the molecular weight of water (18 g mol⁻¹) to take into account the fact that amino acids were linked together by peptide bonds.

Total nitrogen was quantified by the Dumas method using elementary analyser according to Simonne *et al.* (1997) and nitrogen-to-protein conversion factors were calculated as the ratio (weight by weight, dry basis) between the protein content (sum of all amino acids measured) and the Dumas-determined total nitrogen content (Ceballos *et al.*, 2006).

Statistical analysis

Data obtained were evaluated to establish whether there were any statistically significant differences between the seven-ecogeographic populations that define the cassava tribe at its centre of origin. Statistical analysis was performed with JMP[®] 14.1 software (SAS, Cary, North Carolina, USA) and SAS version 9.1 software (SAS Institute Inc., Cary NC, USA). The 5% probability was considered to indicate statistical significance for the analyses. Each trait value was

analysed using ANOVA. Pair means were compared through the Tukey-Kramer test and by Pearson correlation coefficients.

Results and discussion

HCN in cassava roots

HCN in cassava roots of 178 genotypes ranged from 16 to 1212 ppm db with an average of 198 ppm db and median of 119 ppm db (Fig. 1). The frequency distribution clearly shows a positive skewness, which was in agreement with previous report by Sánchez *et al.* (2009). The roots from 131 genotypes with HCN < 200 ppm can be considered safe for consumption, according to the Codex Alimentarius Commission of the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) of the United Nations (FAO/WHO, 2019, FAO/WHO, 2005). COL2316 and BRA492 of the Amazon group had the highest HCN, while ECU71 and PER484 of the Andean group had the lowest HCN. The log-normal distribution among the genotypes of the current study reflects a selection for lower HCN by farmers during domestication, and/or a selection bias during the establishment of the germplasm collection held at CIAT (genotypes with low cyanide roots were collected preferentially). The genotypes of the Amazon group showed the highest levels of HCN (Table 3 and Fig. 6b). Patterns of cassava processing and

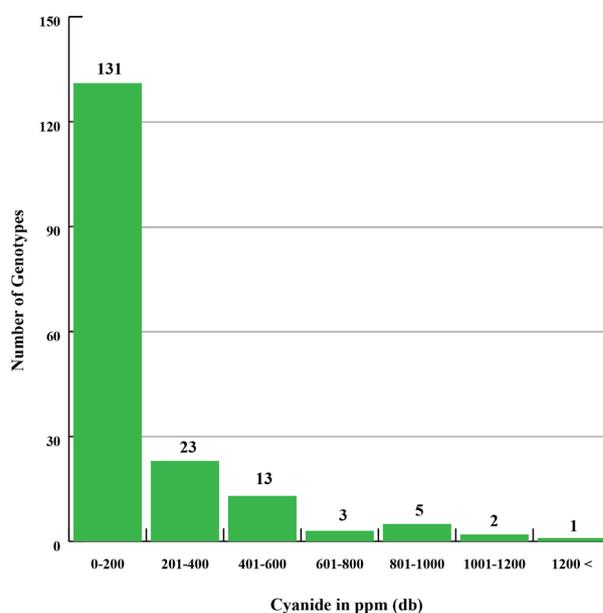


Figure 1 Distribution of HCN content in cassava roots of 178 genotypes from the diversity genetic collection held at CIAT.

Table 3 Results of nutritional evaluation in genetic diversity groups

Genetic diversity group	Genotypes	Country of origin	Cyanide in roots (ppm db)	Cyanide in leaves (ppm db)	All-trans- β -Carotene in leaves ($\mu\text{g g}^{-1}$ db)	Lutein in leaves ($\mu\text{g g}^{-1}$ db)	Protein ($\text{g } 100 \text{ g}^{-1}$ db)
Amazon	BRA325, BRA492 COL2315, COL2316, COL2353, COL2469, COL2493	Brazil Colombia	798 A	4034 A	356 AB	87 A	28 A
Andean	VEN25 BOL3 COL1108, COL2017, COL2019 ECU183, ECU21, ECU72 PER183, PER196, PER297, PER484, PER593	Venezuela Bolivia Colombia Ecuador Peru	88 C	1633 C	328 AB	82 A	26 A
Dry Atlantic Forest	ARG20, ARG55 BOL2 BRA12, BRA191, BRA975 PAR105, PAR36, PAR38, PAR41, PAR57, PAR69, PAR75 PER239	Argentina Bolivia Brazil Paraguay Peru	127 C	1798 C	343 AB	85 A	27 A
Humid Atlantic Forest	BRA110, BRA117, BRA1173, BRA1282, BRA132, BRA222, BRA522, BRA966 COL1495, COL1910 GUA43 MEX80	Brazil Colombia Guatemala Mexico	385 B	3118 AB	321 AB	76 A	29 A
MesoAmerica Caribbean	BRA294 COL1722, COL22, COL474, COL638 CR77 GUA58, GUA6 MEX43, MEX96 PAN100, PAN7, PAN90	Brazil Colombia Costa Rica Guatemala Mexico Panama	109 C	2087 BC	274 B	76 A	25 A
Savanna	COL1292, COL1398, COL1505, COL1823, COL2215, COL2246 CR138 CUB21, CUB23, CUB29, CUB36, CUB56 DOM3 ECU33 GUA66 MEX86 VEN200, VEN204, VEN208, VEN270, VEN309	Colombia Costa Rica Cuba Dominican Rep. Ecuador Guatemala Mexico Venezuela	121 C	1534 C	321 AB	86 A	26 A
South American Rain Forest	BRA1172, BRA125, BRA20, BRA255, BRA73, BRA97, COL1468, COL2212 CUB32, CUB46, CUB74 PAN51 PAR98 PER328, PER489 PTR19 USA5, USA8 VEN77	Brazil Colombia Cuba Panama Paraguay Peru Puerto Rico USA Venezuela	174 C	1662 C	370 A	84 A	26 A
			q 3.14 α 0.05	q 3.14 α 0.05	q 3.14 α 0.05	q 3.01 α 0.05	q 3.13 α 0.05

Groups connected by the same letter are not statistically different (Tukey-Kramer test, $P < 0.05$).

consumption by people in the Amazon allows and even favours high frequency of occurrence of high HCN content cassava varieties (Dufour, 1993; Dufour, 1994; McKey *et al.*, 2010).

Carotenoids and HCN in cassava leaves

The all-*trans*- β -carotene content in cassava leaves of 178 genotypes ranged from 174 to 547 $\mu\text{g g}^{-1}$ db with an average of 339 $\mu\text{g g}^{-1}$ db and median of 340 $\mu\text{g g}^{-1}$ db (Fig. 2). The genotypes with lowest and highest concentrations were identified as VEN309 and VEN77, respectively. The all-*trans*- β -carotene content present in cassava leaves is significantly higher as compared to the other major sources such as sweet potato (*Ipomoea batatas*; 230 $\mu\text{g g}^{-1}$ db), carrot (*Daucus carota*; 65 $\mu\text{g g}^{-1}$ db), pumpkin (*Cucurbita maxima*; 30 $\mu\text{g g}^{-1}$ db), lettuce (*Lactuca sativa*; 52 $\mu\text{g g}^{-1}$ db), and spinach (*Spinacia oleracea*; 56 $\mu\text{g g}^{-1}$ db) (Chung *et al.*, 2019, Bunea *et al.*, 2008, USDA/ARS, 2006, Wang *et al.*, 2010). The lutein content in cassava leaves of 178 genotypes ranged from 15 to 181 $\mu\text{g g}^{-1}$ db with an average of 85 $\mu\text{g g}^{-1}$ db and median of 84 $\mu\text{g g}^{-1}$ db (Fig. 3). The genotypes with lowest and highest concentrations were COL1910 and COL1398, respectively. Thus, the lutein content present in cassava leaves in this study is relatively higher than lutein concentration found in dark green vegetables like spinach (37 $\mu\text{g g}^{-1}$ db), lettuce (44 $\mu\text{g g}^{-1}$ db) and sesame leaf (*Sesamum*

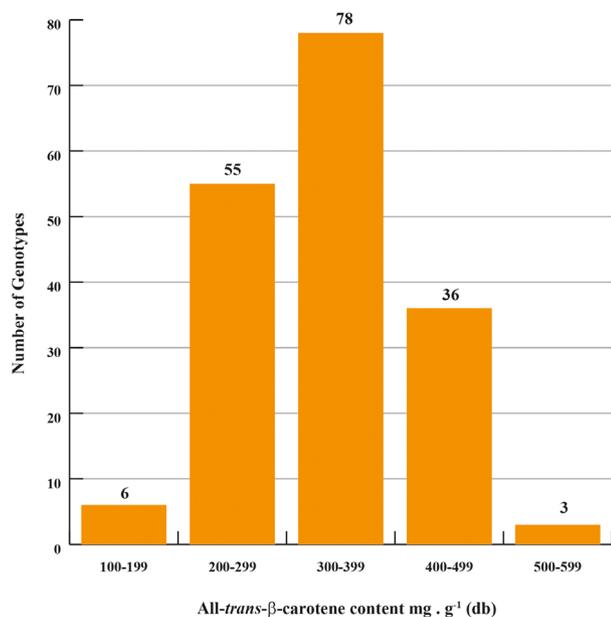


Figure 2 Distribution of all-*trans*- β -carotene content in cassava leaves of 178 genotypes from the diversity genetic collection held at CIAT.

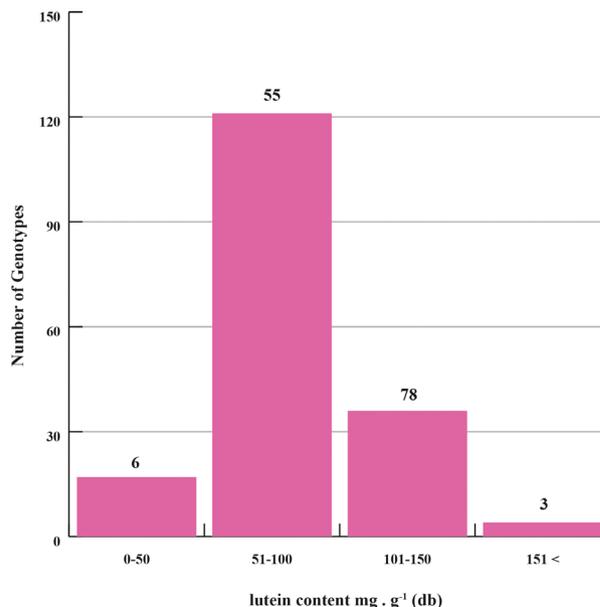


Figure 3 Distribution of lutein content in cassava leaves of 178 genotypes from the diversity genetic collection held at CIAT.

indicum; 44.48 $\mu\text{g g}^{-1}$ db) (Chung *et al.*, 2019, USDA/ARS, 2006, Bunea *et al.*, 2008, Wang *et al.*, 2010).

The HCN in cassava leaves of the 178 genotypes ranged from 346 to 7484 ppm db with an average of 2298 ppm db and median of 2071 ppm db (Fig. 4). COL2316 and VEN25 genotypes of the Amazon group had the highest HCN, while the CUB29 and VEN309 genotypes of the Savanna group had the lowest HCNs. According to the current codex and international standards for sweet cassava, bitter cassava, edible cassava flour and gari (a product obtained from processing cassava tubers), the level of 'total hydrocyanic acid' in the fresh roots, flour and gari must not exceed 50, 10 and 2 mg kg^{-1} , respectively (FAO/WHO, 2019). To reduce the content of cyanogenic compounds, the cassava leaves must be processed by crushing, drying and cooking in water (Ngudi *et al.*, 2003b; Nhassico *et al.*, 2008). Contrary to what is observed in the roots (Fig. 1), the distribution of HCN in cassava leaves followed a normal distribution (Fig. 4), indicating that HCN in leaves has not been under human or natural selection. The moderately large number of genotypes used in this study provides reliable information on the variations that can be expected for cyanide and carotene contents in cassava leaves. Using the data from this study, it is possible to identify cassava genotypes with both favourable levels of carotenoids and low cyanide content, for human and animal consumption, as well as for selecting parental lines for breeding purposes, such as VEN77 and CUB29. The Pearson

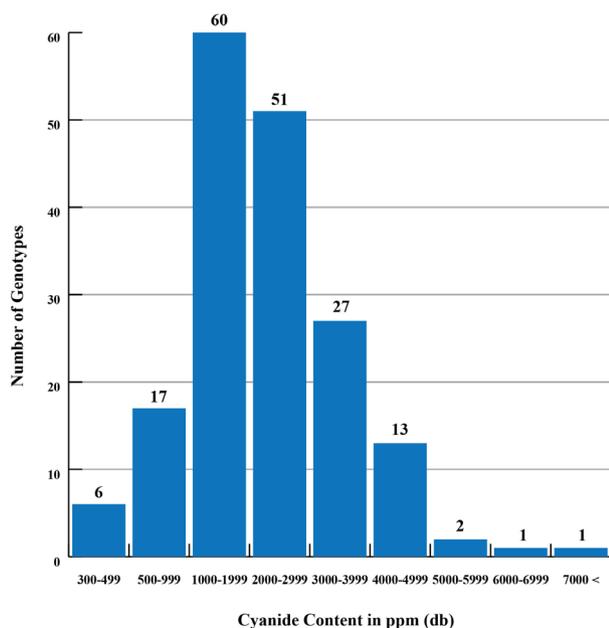
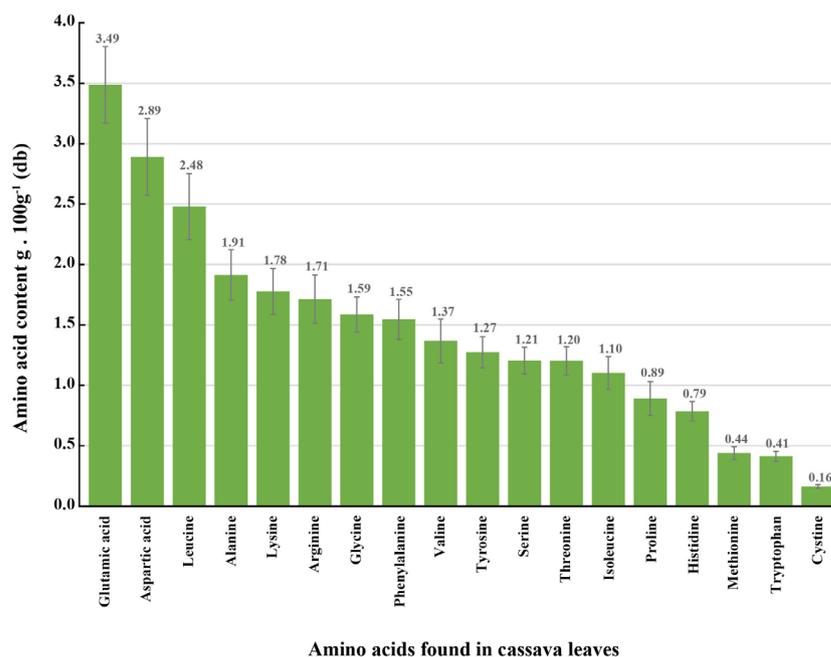


Figure 4 Distribution of HCN content in cassava leaves of 178 genotypes from the diversity genetic collection held at CIAT.

analysis revealed no correlation between HCNs, all-*trans*- β -carotene and lutein ($r = 0.07$ to 0.16), and a weak correlation between HCN in leaves and in roots ($r = 0.45$), indicating that each of these traits can be selected for relatively independently.

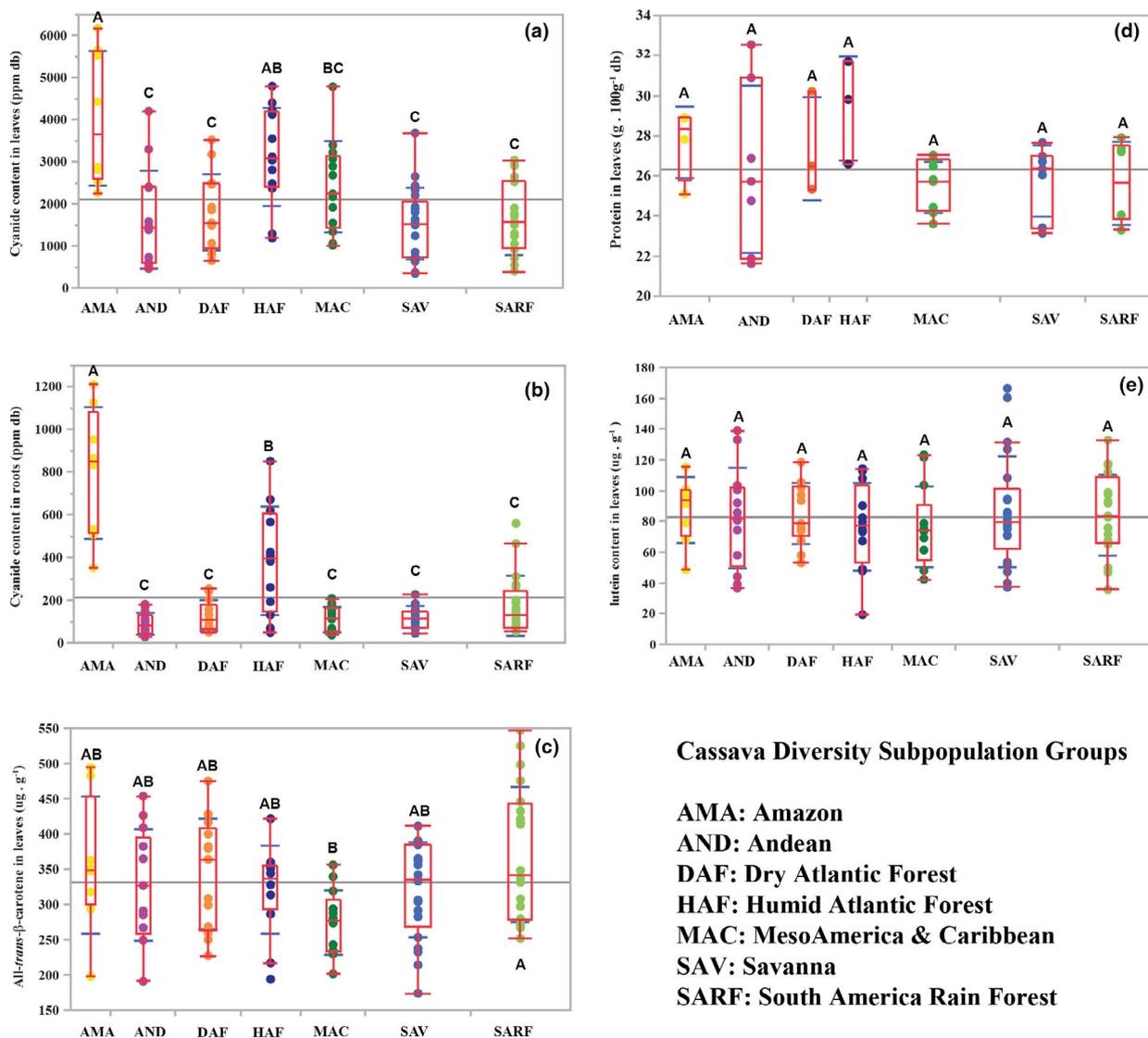
Figure 5 Average amino acids profile in cassava leaves of fifty-three genotypes from the diversity genetic collection held at CIAT.



Regarding the carotene content in cassava roots, the presence of lutein was not detected and the maximum all-*trans*- β -carotene content was $35 \mu\text{g g}^{-1}$ db (Ceballos *et al.*, 2012b); therefore it was not evaluated in this article either.

Protein content and amino acid profile in cassava leaves

In cassava leaves, eighteen of the twenty proteogenic amino acids were detected, including all nine essential amino acids (Fig. 5). Among these, five were present in moderate quantities: leucine, lysine, phenylalanine, valine and threonine (2.5 , 1.8 , 1.6 , 1.4 and $1.2 \text{ g } 100\text{g}^{-1}$ db, respectively), and two were present in lower quantities: isoleucine and histidine (1.1 and $0.8 \text{ g } 100\text{g}^{-1}$ db, respectively), according to the thresholds established by WHO/FAO/UNU (2007). Except for phenylalanine, the observed quantities of all other essential amino acids were higher in the Latin American landraces evaluated here than those previously reported (Ngudi *et al.*, 2003b; Montagnac *et al.*, 2009). Additionally, significant levels of non-essential amino acids were detected such as glutamic acid, aspartic acid, alanine, arginine and glycine (3.5 , 2.9 , 1.9 , 1.7 , and $1.6 \text{ g } 100 \text{ g}^{-1}$ db, respectively). The level of essential amino acid variation observed in the leaves of 178 Latin American landraces opens the opportunity for cassava breeders to further increase these levels; thus, cassava leaves could become a high value vegetable-based protein source for animal feed. Cassava leaf crude protein has more essential amino acids than soybeans (Montagnac *et al.*, 2009); thus, targeting high



Cassava Diversity Subpopulation Groups

AMA: Amazon

AND: Andean

DAF: Dry Atlantic Forest

HAF: Humid Atlantic Forest

MAC: MesoAmerica & Caribbean

SAV: Savanna

SARF: South America Rain Forest

Figure 6 Box plot representation of HCN in leaves (a), HCN in roots (b), all-*trans*-β-carotene content in leaves (c), protein content in leaves (d), lutein content in leaves (e), by genetic diversity groups. Groups connected by the same letter are not statistically different (Tukey-Kramer test, $P < 0.05$). The horizontal line in the central part of each box indicates the median, and the shape of the box indicates the distribution by quartiles. Values are reported on a dry basis.

lysine and methionine levels in the total crude protein content in cassava leaves could improve fat and protein deposition in pigs (Ly *et al.*, 2012).

The total protein content of cassava leaves of fifty-three genotypes ranged from 21.6 to 32.5 g 100 g⁻¹ db with an average of 26.2 g 100 g⁻¹ db. Cassava leaves contain more protein than lettuce (11.2 g 100 g⁻¹), spinach (16 g 100 g⁻¹), wheat (*Triticum aestivum*, 10.7 g 100 g⁻¹), rice (*Oryza sativa*, 4.8 g 100 g⁻¹), sorghum (*Sorghum bicolor*, 10.8 g 100 g⁻¹), and maize

(*Zea mays*, 8.6 g 100 g⁻¹), but not as much as soy bean (*Glycine max*, 37–44 g 100 g⁻¹) (Sosulski & Imafidon, 1990; El-Shemy, 2011; Arowora *et al.*, 2017; Ayalew *et al.*, 2017). The nitrogen-to-protein conversion factors (N:P) ranged from 3.54 to 5.45 for the fifty-three genotypes included in this study, with an average of 4.48. These values are in line with those reported for dicotyledons (Yeoh & Wee, 1994). Yeoh & Truong (1996) reported lower values in cassava roots of fifteen genotypes, ranging from 2.89 to 3.67.

Phenotypic diversity of cyanide, protein and carotenoid contents of leaves between seven genetic diversity groups of Latin America

The 178 genotypes from the first study were previously classified into seven diversity groups of cassava in Latin America (Table 3), based on analyses of population genomics and phylogeny clustering which correlated well with the geographic information on the origin of each genotype using Next-Generation Sequencing Eclipse Plug-in (NGSEP) and SNPs (Becerra Lopez-Lavalle *et al.*, 2015, Becerra Lopez-Lavalle *et al.*, 2018). Analysis of the genetic diversity groups showed that the HCN in cassava leaves was significantly higher in the Amazon group of genotypes, and lower in the Andean, Dry Atlantic forest, Savanna and South American rainforest groups (Fig. 6a, Table 3). This may reflect different selection criteria corresponding to different uses over centuries of cassava domestication. In the Andean region, cassava is commonly consumed as boiled fresh roots; thus varieties with low HCN are important to avoid poisoning. On the other hand, in the Amazon region cassava roots are fermented for several days to prepare *farinha*, a coarse flour. In this case, low cyanide is a less important trait, as fermentation releases cyanide and reduces it to safe levels. Moreover, higher HCN may be desirable in the Amazon as a protection against wild animals (e.g. monkeys, wild pigs) during cassava cultivation. Across diversity groups, the HCNs of the majority of genotypes was below 200 ppm db in roots, that is within the safe limit for consumption according to FAO guidelines (FAO/WHO, 2019, FAO/WHO, 2005). The all-*trans*- β -carotene content in cassava leaves was significantly higher in the South American rainforest group and lower in the Mesoamerica Caribbean group (Fig. 6c, Table 3). Protein content and lutein content in cassava leaves did not show significant differences between the genetic diversity groups (Fig. 6d,e, Table 3). It is possible to identify genotypes with both high nutritional potential and low cyanogenic potential in the South American rainforest group.

Conclusion

The large number of genotypes used in this study informs for the first time the range of variations that can be expected for cyanide, protein, amino acids and carotene contents in cassava leaves along with the HCNs in roots. The higher HCN in cassava leaves than in roots found in this study highlight the importance of careful processing of the leaves to lower the potential of cyanide release before consumption. The leaves also demonstrated significant amounts of proteins, essential amino acids, lutein and

abundant all-*trans*- β -carotene. Thus, consumption or use of cassava leaves in food and feed products may contribute in reducing human malnutrition and is an opportunity for alternative high-quality sources of animal feed. Using the data from this study, it was possible to identify cassava genotypes as parental lines for breeding purposes. Breeding for high carotene content and low HCN in leaves of cassava, VEN77 and PAN51 genotypes from the South American rainforest group can be used; while for low HCN in roots, ECU71 and PER484 genotypes of the Andean group are good candidates. CUB29 and VEN309 genotypes of the Savanna group are promising genotypes for breeding for low cyanogenic potential in leaves.

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Conflict of interest

All the authors declare that they have no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

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Data availability statement

Data available on request from the authors.

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