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Research Article

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Content and distribution of cyanogenic compounds in cassava roots and leaves in association with physiological age

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Abstract

Background: Cassava roots are widely consumed in tropical regions of Asia, Africa, and Latin America. Although the protein, vitamin, carotenoid, and mineral content in the leaves makes them a nutritionally attractive option, their consumption is limited due to their high levels of cyanogenic compounds (CCs). In this study, the CC content in different parts of the plant (leaves, storage root cortex, and parenchyma) was assessed at harvest for 50 landrace genotypes representative of cassava diversity in Latin America. The changes in CC in leaves at different physiological ages (3, 6, 9, and 11 months after planting) were also investigated.

Results: The average CC was higher in the cortex (804 ppm) and leaves (655 ppm) than in root parenchyma (305 ppm). Genotypes from different regions of Latin America, as identified by seven genetic diversity groups, differed significantly in CC levels. The Andean and Amazon groups had, respectively, the lowest ($P = 0.0008$) and highest ($P < 0.0001$) CC levels in all three parts of the plants. Cyanogenic compound concentrations were higher in leaves from young plants ($P < 0.0001$) and decreased with increasing physiological age.

Conclusion: The results help to guide the selection of parental lines with low CC levels for breeding and to contribute to the expanded use of cassava and its by-products for food and feed. Cassava for fresh consumption, especially, requires varieties with low total CC content, especially in the root cortex and parenchyma. COL1108 (204, 213, and 174 ppm, respectively, in the parenchyma, cortex, and leaves) and PER297 (83, 238, and 299 ppm, respectively, in the parenchyma, cortex, and leaves) can fulfill this requirement.
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Keywords: cassava roots; cassava leaves; cyanide; cyanogenic glycosides; physiological age

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is grown in the tropics and subtropics in Africa, Asia, and the Americas.[1] The plant has storage roots that are rich in carbohydrates (starch) and constitute the main edible part of cassava plants. Depending on the product, the roots can be processed whole, or after manual or mechanical removal of the peel and cortex. The cortex is the 1–5 mm thick layer (generally white but sometimes yellow) between the parenchyma and the outside (usually dark brown but sometimes light-colored) peel. Only the root parenchyma is considered fit for human consumption but the cortex can also be used by itself or as part of starch extraction or whole-root chip processing for animal feed or other uses.[2-4] There is clear genetic variability in the thickness of the peel in the cassava roots.[5] A thin peel may be advantageous for most forms of processing, the thick peel with a high cyanogenic compound (CC) content could be useful to prevent or reduce the damage caused by the burrowing bug *Cyrtomenus bergi* Froeschner[6] or other parasites.

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Cassava leaves are also edible by both humans and animals and offer nutritional benefits, with a high protein content.[5,7] Consumption of cassava leaves as vegetables is reported in at least 60% of countries in sub-Sahara Africa, including Congo, Liberia, Sierra Leone, and Guinea,[8-10] and in some Asian countries such as Indonesia, the Philippines, and Malaysia.[11]

A concern with cassava leaves and root utilization is the presence of CC, mainly glycosides, which hydrolyze during digestion and produce cyanogenic acid.[12] When consumed in large quantities, cassava can cause cyanide poisoning, with symptoms such as nausea, diarrhea, vomiting, a chronic paralytic disorder known as Konzo, and even death. To reduce the total CC content, cassava must be processed by crushing, fermentation (retting), and/or cooking.[10,13,14] Studies on the distribution of CC within the cassava plant (leaves, root parenchyma or root cortex) have reported contradictory results. Some studies reported that the highest concentrations were in the root cortex[15] and others reported that leaves had the highest concentrations.[2,16] Hence the first objective of this research was to characterize the distribution of CC among the leaves, cortex, and parenchyma of a considerable number of genotypes representative of cassava diversity in Latin America, selected from the germplasm collection held at the International Center for Tropical Agriculture (CIAT, Colombia).

Further studies indicate that the physiological age of cassava plants has an important effect on chemical composition, quality of roots, accumulation of starch in the roots, and reduction in antinutritional compounds such as CC and tannins in the vegetative tissues.[17-18] These studies, however, evaluated only one or two genotypes. A second objective of this study was therefore to characterize the changes in the total CC content of leaves with physiological age for more genotypes: eight in total, selected for their contrasting CC content. A third objective was to identify families of genotypes with attractive attributes for increased utilization of cassava leaves.

MATERIALS AND METHODS

Plant materials

Cassava landrace genotypes from the genebank held at CIAT (https://www.genebanks.org/genebanks/ciat/) were grown in Palmira, Colombia (3° 30′ 17″ N, 76° 21′ 24″ W), with an average temperature of 26 °C and relative humidity of 60%. In the first experiment, 50 landrace genotypes were selected across seven Latin American diversity groups. The groups were based on analyses of population genomics and phylogeny using Next-Generation Sequencing Eclipse Plug-in (NGSEP) methodology and Single Nucleotide Polymorphisms (SNPs). These groups correlated well with the geographic information on the origin of each genotype (Table 1).[7] Each genotype was planted in plot of 4 × 4 plants, in clayish loam soil, with irrigation by 30–40 mm water furrows, every 15 days if there was no rain, up to 8 months of age. Six plants (clones) were harvested 11 months after planting (MAP) and pooled together to obtain representative samples. The total CC content of the plants in roots (parenchyma and cortex) and leaves was evaluated 1 h after harvest.

A second experiment assessed the effect of age (3, 6, 9, and 11 MAP) on CC and dry matter content (DMC) in leaves using eight genotypes selected for their contrasting CC content, as observed in previous trials (BRA5, CHN2, COL1468, COL1505, PER183, TAI1, TAI16, and VEN77). For this experiment, three plants per genotype were evaluated individually, out of a total of six plants sowed per genotype. Based on the method described by Munyahali *et al.*[19] and Ospina *et al.*,[7] with modifications, each plant was divided into three levels (upper, middle, and lower) according to height of the plant. For each level, two to three fully expanded leaves were collected, washed with tap water, dried gently, and chopped together using a kitchen knife. This was followed by the determination of total CC content and DMC. Only plants and leaves without symptoms of pests or disease were sampled, as pests and disease can induce variations in cyanide content (usually an increase) when the plant triggers its defense mechanisms.

Cyanogenic compound content in different parts of the cassava plant

Total CC content was determined according to the method described by Essers *et al.*[20] Parenchyma (40 g wet basis (wb)), leaves (4 g wb) and cortex (10 g wb) were evaluated. The samples were homogenized for 2 min in a blender (Osterizer, model 4655, Mexico) at 28 °C in 50 mL of extraction medium (0.1 mol L^{-1}orthophosphoric acid in a mix of 25% v/v ethanol and 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 3226 × *g* and 25 °C. Aliquots of the supernatants (0.1 mL) were added to the tubes containing 0.4 mL phosphate buffer (0.1 mol L^{-1}, pH 7.0). Then, 0.1 mL of linamarase enzyme (prepared as described in Cooke, 1978)[21] was added and the mixture was incubated (Thermo Fisher Scientific model 2870, Marrieta, USA) at 30 °C for 15 min. After incubation, 0.6 mL of 0.2 mol L^{-1} sodium hydroxide was added. After 5 min at room temperature, 2.8 mL of 0.1 mol L^{-1} phosphate buffer (pH 6.0) was added. For spectrophotometer analysis, 0.1 mL of chloramine T was added and incubated at 30 °C 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 total CC content was determined as total hydrogen cyanide (HCN) potential using the following formula:

$$HCN\,(\text{ppm}) = \frac{\frac{m_{HCN}}{V_t} \times (Fd_1) \times (Fd_2) \times V_{\text{sln ext}}}{m_{m\cdot ps}\,(g)} \qquad (1)$$

m_{HCN} : µg HCN read from the calibration curve, using absorbance readings at 605 nm;

V_t : final volume of tube (4.7 mL);

Fd_1 : factor dilutor 1 (4.7 mL/0.1 mL);

Fd_2 : factor dilutor 2 (volume of the diluted sample/volume of the extract);

V_{Slnext} : volume of the solution (50 mL);

$m_{m\cdot ps}$: mass of the sample in dry weight.

All results are presented on a dry weight basis.

Dry matter content

The DMC was measured by drying leaf samples (8 g per repetition, two repetitions per genotype) at 105 °C for 24 h in a hot air oven (Thelco Oven Model 28, Precision Scientific, subsidiary of GCA Corporation, Chicago, USA). In the second experiment, three plants and three levels per plant (upper, middle, lower) were evaluated individually for each genotype. For DMC, two repetitions were taken per level and physiological age (3, 6, 9, 11 months after planting). The data from the three plants were then averaged for each level. Dry matter was expressed as the percentage of dry weight relative to fresh weight.

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Table 1. Cyanogenic compound content (ppm, db) of 50 landrace genotypes evaluated in seven Latin American diversity groups

Diversity groups	Genotype	Parenchyma	Cortex	Leaves	Genotype	Parenchyma	Cortex	Leaves
Amazon	Bra492	830 ± 6	1070 ± 1	738 ± 1	Col2493	411 ± 4	780 ± 1	478 ± 2
	Col2315	827 ± 1	737 ± 9	647 ± 2	Ven25	724 ± 2	424 ± 1	1330 ± 2
	Col2469	403 ± 1	403 ± 6	1235 ± 7				
Averages (st. dev.) for the Amazon group						639 ± 216	683 ± 277	886 ± 375
Andean	Col1108	204 ± 0	213 ± 2	174 ± 6	Per183	188 ± 4	716 ± 9	461 ± 2
	Col2017	324 ± 2	487 ± 1	343 ± 2	Per297	83 ± 1	238 ± 3	300 ± 2
	Ecu72	147 ± 7	633 ± 3	604 ± 8	Per593	163 ± 3	613 ± 1	131 ± 6
Averages (st. dev.) for the Andean group						185 ± 80	483 ± 213	335 ± 177
Dry Atlantic Forest	Arg20	202 ± 9	567 ± 3	726 ± 20	Par38	180 ± 5	460 ± 1	416 ± 2
	Bra12	253 ± 1	669 ± 5	1854 ± 1	Par69	223 ± 2	759 ± 6	178 ± 2
	Bra191	201 ± 4	411 ± 0	976 ± 1	Per239	155 ± 1	796 ± 1	216 ± 6
	Par36	132 ± 3	196 ± 6	789 ± 5				
Averages (st. dev.) for the Dry Atlantic Forest group						192 ± 41	551 ± 213	736 ± 576
Humid Atlantic Forest	Bra117	457 ± 5	547 ± 5	796 ± 1	Bra966	1021 ± 4	1028 ± 3	1009 ± 8
	Bra1173	254 ± 8	457 ± 2	479 ± 1	Col1910	858 ± 1	637 ± 9	789 ± 3
	Bra1282	435 ± 2	850 ± 3	961 ± 4	Mex80	378 ± 3	657 ± 0	561 ± 2
	Bra132	990 ± 0	2017 ± 6	684 ± 2				
Averages (st. dev.) for the Humid Atlantic Forest group						628 ± 318	885 ± 534	754 ± 195
Meso-America Caribbean	Col1722	141 ± 1	413 ± 1	377 ± 1	Col638	184 ± 2	562 ± 4	2243 ± 8
	Col22	154 ± 1	2424 ± 2	1549 ± 7	Cr77	595 ± 2	874 ± 3	865 ± 1
	Col474	148 ± 2	350 ± 0	457 ± 7	Pan90	208 ± 0	736 ± 2	1061 ± 1
Averages (st. dev.) for the Meso-American Caribbean group						239 ± 177	893 ± 775	1092 ± 707
Savanna	Col1292	66 ± 1	505 ± 8	572 ± 4	Cub23	265 ± 1	1256 ± 53	401 ± 2
	Col1505	184 ± 2	386 ± 1	854 ± 1	Cub29	387 ± 2	1273 ± 5	374 ± 2
	Col1823	409 ± 5	798 ± 4	606 ± 4	Ven117B	97 ± 1	511 ± 2	477 ± 6
	Col2246	110 ± 1	647 ± 2	478 ± 4	Ven200	127 ± 4	826 ± 1	442 ± 4
	Cub21	114 ± 4	889 ± 3	397 ± 9	Cub74	97 ± 4	992 ± 1	919 ± 1
Averages (st. dev.) for the Savanna group						195 ± 128	788 ± 317	511 ± 151
South American Rain Forest	Bra255	213 ± 0	855 ± 1	409 ± 1	Pan51	186 ± 2	1019 ± 1	389 ± 1
	Bra5	332 ± 3	469 ± 2	545 ± 2	Per328	131 ± 1	2242 ± 2	409 ± 1
	Bra97	234 ± 2	468 ± 2	290 ± 4	Per489	157 ± 7	1208 ± 2	307 ± 6
	Col1468	276 ± 1	581 ± 1	706 ± 1	Ven77	236 ± 3	1994 ± 1	398 ± 6
	Col2212	157 ± 0	1540 ± 1	350 ± 2				
Averages (st. dev.) for the South American Rain Forest group						202 ± 71	1137 ± 618	472 ± 199
Average (st. dev.) of the 50 genotypes						305 (242)	804 (497)	655 (420)
Least significant difference ($P < 0.05$)						<0.0001	0.1399	0.0112

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Statistical analyses

All the statistical analyses were performed using the software SAS (Version 9.1). A probability of 5% was considered to indicate statistical significance for the analyses. Each trait value was analyzed using ANOVA. Pair means were compared with *t*-tests and Pearson correlation coefficients.

RESULTS

Distribution of CC between different parts of the plant

On average, the total CC content of 50 genotypes from the first experiment (Table 1) was highest in the cassava cortex (804 ppm), followed by leaves (655 ppm) and parenchyma (305 ppm). Nevertheless, some genotypes (COL2315, BRA966, COL1910) contained more CC in the parenchyma than in the leaves or cortex (Table 1). No correlation was observed among CC concentrations in the different parts of the plant (Fig. 1(a),(b)). The Pearson analysis confirmed the absence of correlation between CC in parenchyma and cortex ($r = 0.14$) and leaves and cortex ($r = 0.02$), and the correlation between parenchyma and leaves ($r = 0.22$) was low but positive, indicating that each of these traits can be selected relatively independently.

The levels of CC in different plant parts showed significant differences among some genetic diversity groups (Table 2; Figs 1 and 2(a)). Mesoamerica Caribbean, Amazon, and Dry Atlantic Forest groups were associated with higher levels of CC in leaves (average 1092 ppm, 886 ppm and 736 ppm, respectively), whereas South American Rainforest, Humid Atlantic Forest and Savanna groups were associated with higher levels of CC in the cortex (average 1137 ppm, 885 ppm and 788 ppm, respectively). Two groups, Amazon and Humid Atlantic Forest, had high levels of CC in all parts of the plant. In contrast, the Andean group was associated with low to moderate levels of CC in all parts of the plant (Table 1). The first two principal components had eigenvalues greater than 1 and explained 75.13% of the variation in the data (Fig. 2(b)).

The first principal component represented 42.4% of the total variance. Genotypes from the Amazon and Humid Atlantic Forest genetic groups were associated with higher CC in leaves and root parenchyma. Other groups appeared gathered together towards lower CC, on the left-hand side of the principal component analysis (PCA) plot.

The genotypes COL638, COL22 and BRA966 showed the highest total CC content in leaves (2243 ppm), cortex (2424 ppm), and parenchyma (1021 ppm) respectively. The use of genotypes such as these is not recommended for the production of products for human consumption, in particular in industries where whole roots, i.e. cortex and parenchyma, are processed (as opposed to peeled roots) unless the reduction to safe levels in CC can be guaranteed by retting or fermentation processes, for example. The CC concentrations were lowest in the leaves (131 ppm), cortex (196 ppm), and parenchyma (66 ppm) of PER593, PAR36, and COL1292 genotypes, respectively (Table 1). For breeding cassava for human consumption with the minimal processing, it is therefore essential to select varieties with low cyanogenic potential, in particular in both cortex and parenchyma such as COL1108 (204 ppm in parenchyma, 213 ppm in cortex, 174 ppm in leaves) and PER297 (83 ppm in parenchyma, 238 ppm in cortex, 300 ppm in leaves) (Table 1).

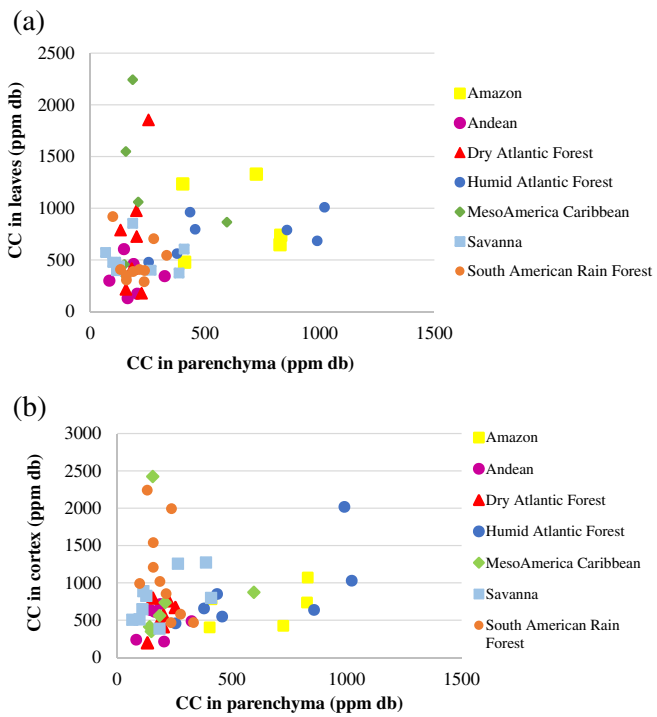

Figure 1. (a) Cyanogenic compounds in the leaves and parenchyma from 50 genotypes representative of the genetic diversity groups of Latin America (number of repetitions for each genotype $n = 2$). (b) Cyanogenic compounds in the cortex and parenchyma from 50 genotypes representative of the genetic diversity groups of Latin America (number of repetitions for each genotype $n = 2$).

Influence of physiological age on CC content and dry matter of the leaves

The average total CC content of leaves of the eight genotypes from the second experiment was highest in young plants (3 MAP). Between 3 and 6 MAP, CC decreased significantly ($P < 0.0001$*, significance level $\alpha = 0.05$), then remained stable through 9 MAP. Between 9 and 11 MAP, the CC again decreased significantly in upper-level leaves, but middle- and lower-level leaves had fallen and were no longer available for analysis. This behavior (with few occasional exceptions) was observed for each of the eight individual genotypes (Fig. 3), even though they were selected for their contrasting total CC content and accordingly exhibited significant variations among themselves, as in the first experiment.

DISCUSSION

The genotypes analyzed (50 in total), selected to represent wide genetic diversity within cassava germplasm, give a good indication of the range of variation that can be expected for total CC content in leaves, cortex, and parenchyma in Latin American cassava germplasm. Significant differences were observed among the seven diversity groups of cassava in Latin America ($P < 0.0001$*, significance level $\alpha = 0.05$). The Amazon and Andean groups, in particular, were associated with high and low total CC content, respectively, in the different parts of the plants that were evaluated (Fig. 4). This may reflect different selection strategies by farmers over centuries in Latin America, with high CC varieties preferred in the Amazon as a deterrent against wild animals, whereas in the Andes such deterrent may have been less necessary. The traditional cultivation systems in the rainforests are conditioned by the selection of varieties with high total CC content because the plantations may be abandoned for long periods (several months) and are sometimes far away from the houses.

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Table 2. Average cyanogenic compounds content and dry matter in the leaves of eight genotypes at 3, 6, 9, and 11 months after planting

	Upper level		Middle level		Lower level	
Months after planting	CC (ppm db)	Dry matter (%)	CC (ppm dm)	Dry matter (%)	CC (ppm dm)	Dry matter (%)
3	1476 ± 478^{A}	24.6 ± 2^{a}	1625 ± 466^{A}	26.7 ± 1^{b}	1358 ± 432^{A}	26.5 ± 1^{ab}
6	948 ± 409^{B}	32.1 ± 1^{c}	784 ± 337^{B}	33.6 ± 2^{cd}	613 ± 253^{B}	33.1 ± 2^{c}
9	814 ± 483^{B}	32.2 ± 2^{c}	615 ± 348^{B}	33.1 ± 2^{c}	593 ± 438^{B}	32.5 ± 2^{c}
11	372 ± 332^{C}	35.4 ± 4^{d}	-	-	-	-

Statistically significant differences across physiological ages and levels ($P < 0.05$) are indicated with different superscript letters, separately for each trait (CC and dry matter).

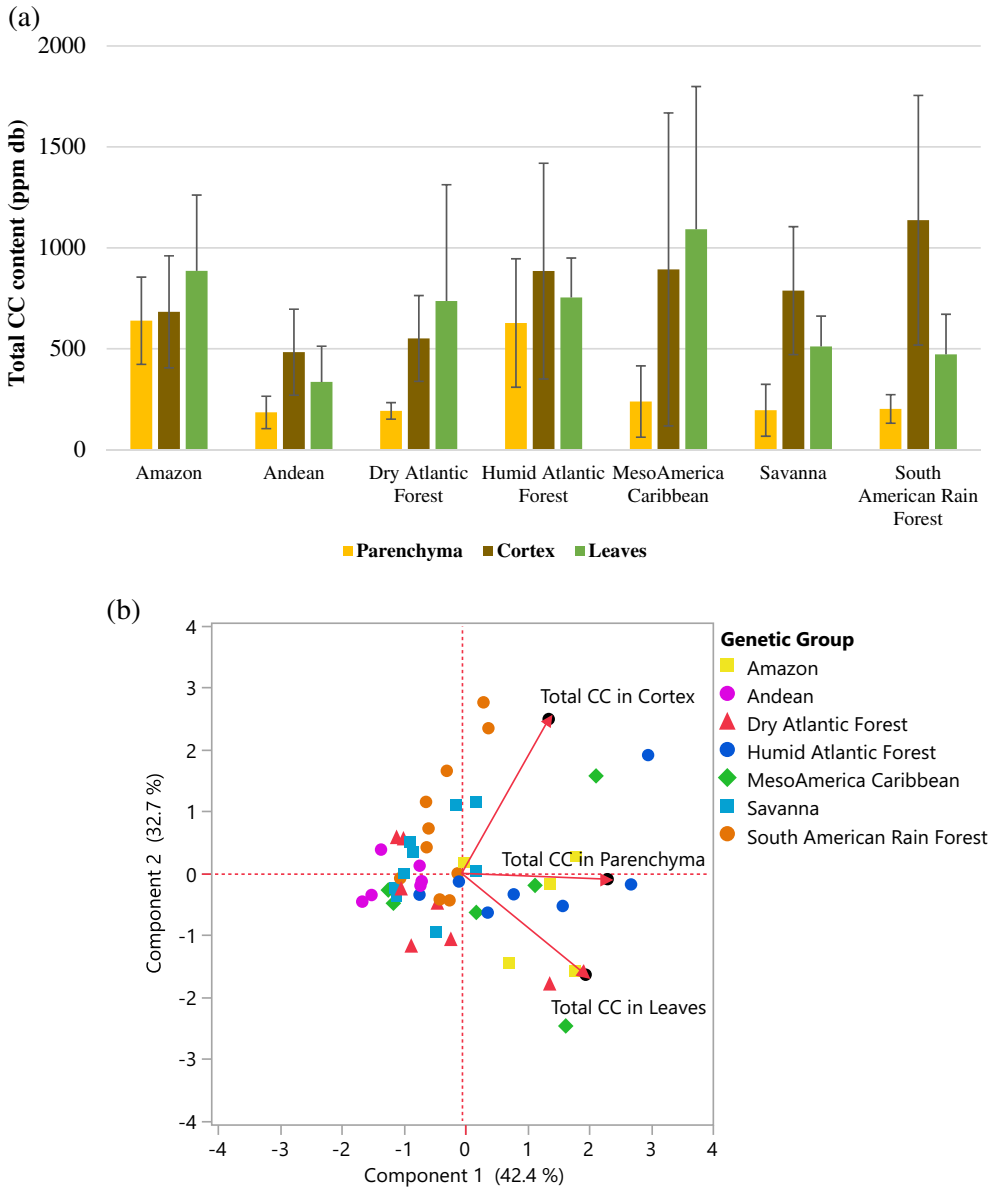

Figure 2. (a) Average cyanogenic compounds content in different parts of cassava plants (leaves, cortex, and parenchyma) in seven genetic diversity groups in Latin America. Vertical bars indicate standard deviations ($n = 5$ to 10 depending on diversity group). (b) Principal component analysis of total CC content in different parts of cassava plants (leaves, cortex, and parenchyma) in seven genetic diversity groups in Latin America ($\alpha = 0.05$, number of repetitions for each genotype $n = 2$).

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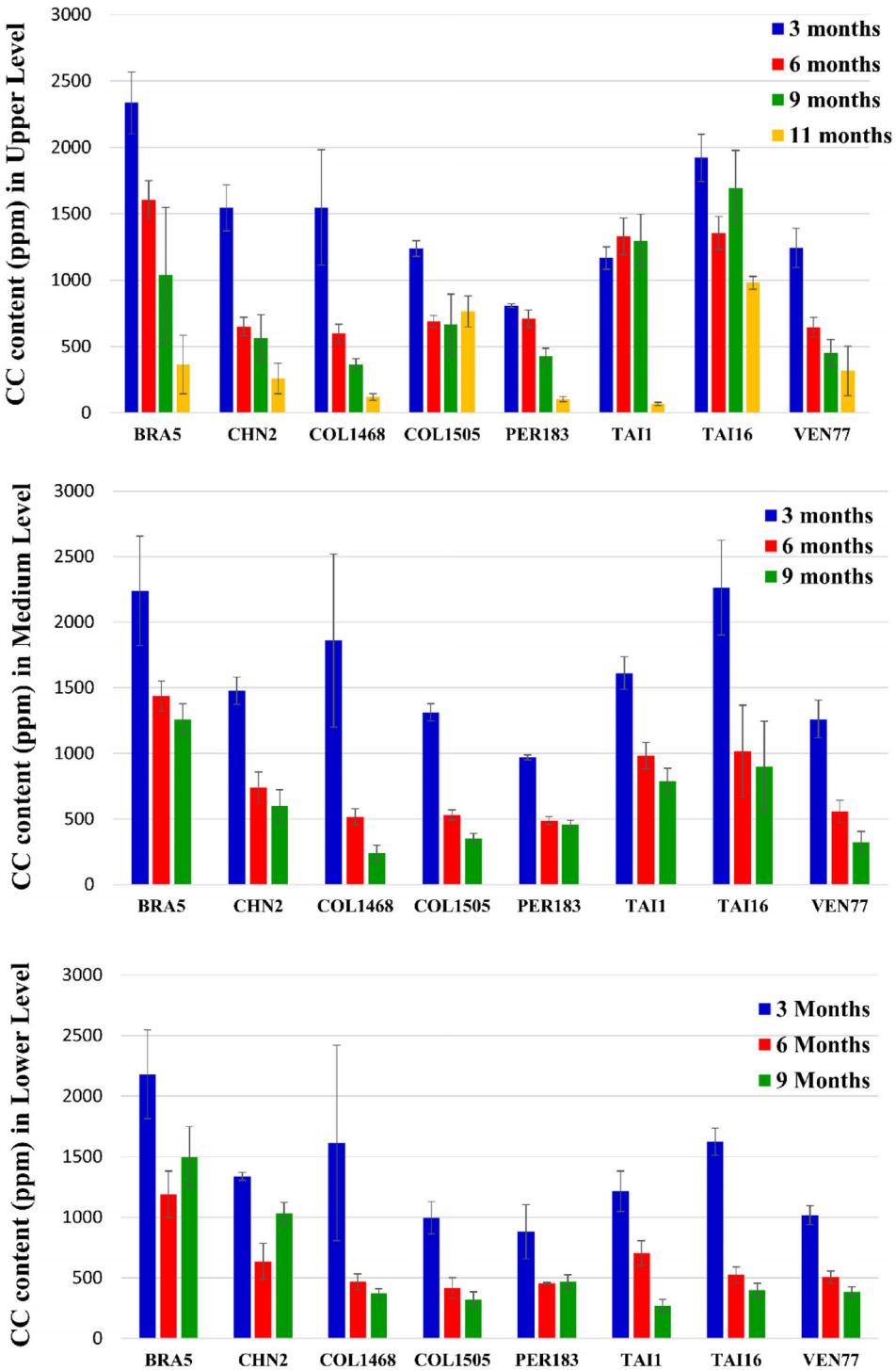

Figure 3. Cyanogenic compounds content in cassava leaves from upper, middle and lower levels of plants at 3, 6, 9 and 11 months physiological age. Vertical bars indicate standard deviations (number of repetitions for each genotype $n = 2$).

Mammalian species such as monkeys, wild pigs, and rodents are major predators of sweet cassava varieties, whereas bitter varieties with high total CC content are more protected as they have an unappealing taste and are not appreciated. Modes of consumption seem adapted accordingly: In the Amazon, lactic acid fermentation of ground or soaked roots is a major operation of processing cassava into farinha (a semolina-like product), whereas in the Andes cassava is commonly boiled and consumed without prior detoxifying treatment. On average, the total CC content was highest in the cassava cortex followed by leaves and parenchyma. This agreed with other researchers who showed higher CC levels in leaves or cortex compared to parenchyma,[15,16,22] and it fits with the hypothesis of CC acting as a defense mechanism, present in higher concentrations in the more exposed parts of the cassava plant. Nevertheless, three genotypes contained more CC in the root parenchyma than in the leaves or cortex. This reflects natural

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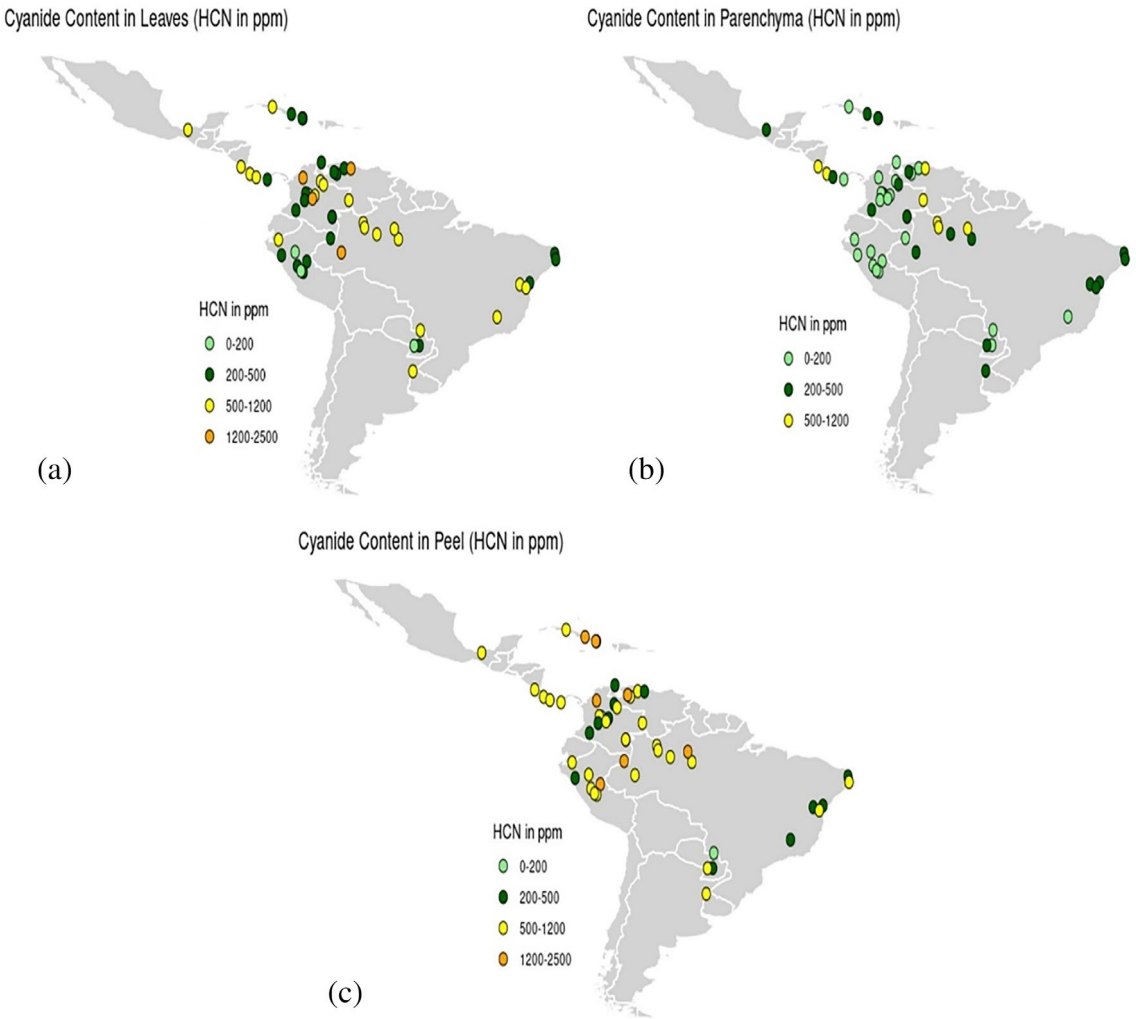

Figure 4. Distribution of CC in different parts of the plant in cassava clones from Latin America: (a) Leaves; (b) parenchyma, (c) peel.

variations among cassava genotypes, and probably has a genetic basis because in previous harvest cycles, over 4 years, these genotypes consistently had high CC content (> 200 ppm) in the parenchyma, on average: BRA966:1032 ppm, COL2315:721 ppm, COL1910:482 ppm db.

Concerning the age of the plant, leaves from young cassava plants (3 months) contained markedly higher levels of CC than more mature plants (6, 9, and 11 MAP). This relationship between the age of the plant and the CC content, as well as dry matter, is vital for consumption: leaves from middle-aged plants (6 months) appeared optimal with less CC and hence less processing needed, and at the same time still relatively soft and palatable. In addition to physiological age, other factors are known to influence CC such as climate conditions and episodes of stress caused by drought and pest attacks.[7] Cyanogenic compounds content of 50 mg/kg or less on a fresh basis can be considered safe for consumption according to FAO/WHO.[23]

The results from this study were in agreement with other studies reporting that CC in young leaves increased until reaching a peak, then decreased as plants aged.[2,3,22,24,25] However, it has been reported that high CC content was present in the medium level leaves at 6 MAP and in the high-level leaves at 12 MAP.[22] Other authors mention that total CC content is related to factors other than age, such as weather, pest attacks, and edapho-climatic conditions.[24] Moreover, high total CC content in young leaves has been attributed to defense mechanisms against herbivore animals[26] and may therefore depend on the pressure from the presence of herbivores around cassava fields because, in response to pressure from herbivores, farmers have selected varieties with higher levels of CC. The general patterns observed throughout the plant, at different ages and across eight genotypes would also support the hypothesis that the cassava plant may use CC as a way to store and/or mobilize N, as suggested by Zidenga *et al.*[27]

These results help to guide the selection of low total CC content parental lines for breeding and contribute to a greater use of cassava by-products such as leaves for food and feed applications. When breeding cassava for fresh consumption or minimal processing, it is important to select varieties with low cyanogenic glycosides in particular in both cortex and parenchyma, such as COL1108 (204, 213 and 174 ppm db in parenchyma, cortex and leaves respectively) and PER297 (83, 238 and 300 ppm db in parenchyma, cortex and leaves respectively). As an alternative approach, a recent study tested mutating (deletion) the CYP79D1 gene using the CRISPR/Cas9 system to generate cassava lines with reduced the levels of cyanogenic glycosides.[28]

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By leveraging the genetic resources of the genebank of the Alliance of Bioversity and the International Center for Tropical Agriculture (CIAT), the present study contributes to an understanding of the diversity of cassava landrace genotypes in terms of cyanogenic content, based on the characterization of 50 genotypes. The study was limited to a single location (CIAT campus at Palmira, Colombia) and a single harvest cycle. As environmental conditions also influence CC in cassava considerably, future studies may assess CC content in different locations and under different climates, as well as over several harvest cycles in the same location.

AUTHOR CONTRIBUTIONS

Maria A. Ospina: Data curation; Formal analysis; Investigation; Methodology; Writing – original draft. Thierry Tran: Formal analysis; Investigation; Methodology; Supervision; Writing – review & editing. Monica Pizarro: Formal analysis; Methodology. Jorge Luna: Formal analysis; Sandra Salazar: Investigation; Methodology. Luis Londoño: Investigation; Methodology. Hernán Ceballos: Writing – review & editing. Luis Augusto Becerra: Formal analysis; Funding acquisition; Investigation; Supervision; Writing – review & editing. Dominique Dufour: Conceptualization; Funding acquisition; Investigation; Project administration; Supervision; Writing – review & editing.

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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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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