ARTICLE

Chemical root traits differentiate 'bitter' and 'sweet' cassava accessions from the Amazon

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Abstract: *'Bitter' and 'sweet' cassava are normally distinguished based on the hydrocyanic acid (HCN) content of their roots. Moreover, Brazilian farmers tend to select 'sweet' cassava based on the taste and cooking aspects. The aim of this study was to characterize chemical traits of 'bitter' and 'sweet' cassava roots of the Amazon region and to find genetic relations among accessions based on these traits. Considerable phenotypic variation was detected among the evaluated traits moisture, ashes, total soluble solids, total titratable acidity, pH, total carotenoids, free and total cyanide, crude protein, glucose, fructose, sucrose and starch. Aside from free and total cyanide, the trait means of sugars and moisture differed in 'bitter' and 'sweet' cassava and also differentiated these in different clusters in the dendrogram using the unweighted pair-group method based on arithmetic averages (UPGMA) and in the results of principal component analysis.*

Keywords*: Germplasm characterization, cyanide, genetic variability, Manihot esculenta Crantz, multivariate analysis.*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important source of calories for the world population and is widely grown in tropical and subtropical regions (Lebot 2009), due to its capacity to adapt to unfavorable conditions. Nigeria, Thailand, Indonesia, Brazil and the Democratic Republic of the Congo are the world's main producers of these roots (FAOSTAT 2014). Recent studies have shown that cassava was domesticated along the rim of the Amazonian basin (Léotard et al. 2009) and it is believed that Amazonia is an important source of genetic resources of cassava.

In countries where cassava is widely consumed, the development of nutrient-rich accessions would be advantageous to prevent diseases and improve diets. The characterization of accessions is important for plant breeding programs, in order to identify promising accessions to develop biofortified cultivars (Carvalho et al. 2011, Sánchez et al. 2014). In addition, the quality of food products derived from cassava roots depends on the chemical composition. However, few studies have focused on the chemical characterization of cassava roots that are traditionally grown or maintained in germplasm banks in the Amazon region.

The germplasm bank of cassava from the Eastern Amazon maintains different accessions from the Brazilian Amazon, sampled mostly on traditionally cultivated

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family farms. In the Amazon, farmers tend to differentiate 'bitter' from 'sweet' cassava. Currently, the cassava landraces are classified in two types: 'sweet' cassava, also known as 'aipim', 'macaxera' or table cassava and 'bitter' cassava, also known as cassava for industry (McKey et al. 2010). This classification is related to the capacity of cyanide (HCN) release, a highly toxic substance if ingested. Sweet cassava contains less than 100 mg kg^{-1} of cyanogenic compounds per fresh root, while 'bitter' cassava contains more than 100 mg cyanogenic compounds (McKey et al. 2010). We believe that selection processes for 'sweet' cassava by farmers, based on characteristics of root taste and cooking, may have had some influence on the current chemical composition of 'bitter' and 'sweet' cassava. Thus, the aim of this study was to characterize chemical traits of roots of 'bitter' and 'sweet' cassava from the Amazon and to identify genetic relations among accessions based on these traits.

MATERIAL AND METHODS

Forty-six cassava accessions sampled in different counties of the States of Pará, Amazonas, Amapá and one from Bahia were analyzed, classified as 'bitter' or 'sweet' (Supplementary Table 1), and stored in a germplasm bank. The farmers' classification as 'bitter' or 'sweet' cassava during collection was maintained. The accessions were cultivated in the county of Igarapé Açu (lat 01º 07' S, long 47º 36' W; and alt 54 m asl), State of Pará, Brazil. The climatic conditions of this region are classified as Am, according to Koppen. The mean annual precipitation is 2495 mm and mean annual temperature 26.4 °C.

The accessions were planted in single-row plots, each with nine plants, spaced 1.0 x 1.0 m. The soil was tilled and plowed and fertilizer applied only once, consisting of 40 g $plant^{-1}$ of NPK fertilizer, at a proportion of 10-28-20, at 35 days after planting of the stakes. No irrigation was applied.

The harvest was carried out one year later. Three useful plants per row were evaluated, disregarding the border plants (first and last plant) and the medium values were calculated for each trait. Thereafter, two roots of three plants per accession were chemically analyzed. Roots were mixed to compose one replication. The roots were washed with water, packed in plastic bags and stored in a cold chamber at -18 to -20 °C until analysis. Roots were analyzed for 13 chemical traits. Moisture was analyzed by the gravimetric method nº 920.151, ashes by the gravimetric method nº 940.26, pH by method nº 981.12, total titratable acidity (TTA) by method nº 942.15B and total soluble solids (TSS) by method nº 932.12, as proposed by AOAC (1997). Crude protein concentration was determined by the micro-Kjeldahl technique, based on hydrolysis and later distillation of the sample at factor 6.25 x % N, as suggested by the AOAC (1997).

The cyanogenic compounds (free and total cyanide) were determined as proposed by Essers et al. (1993). For the determination of glycoside cyanide, the enzyme linamarin was used, which was extracted from the cassava root pulp (Essers et al. 1993).

The analysis of total carotenoids (TC) was performed according to the methodology described by Rodriguez-Amaya (2001). Readings for determination of absorbance and quantification of glucose, fructose and sucrose sugar were performed on spectrophotometer for ELISA plates at a wave length of 340 nm, according to Stitt et al. (1989). The starch content in the roots was determined by the method of hydrostatic balance (Grossman and Freitas 1950).

The data of the phenotypic means of the 13 traits of each accession were subjected to analyses of descriptive statistics, where minimum, maximum and medium values and range, coefficient of variation were estimated and the values tested by the Shapiro-Wilk normality test to describe the variability of chemical characters. Moreover, Spearman's correlations among the pairwise traits were computed and the significance was evaluated at 1% probability by the t-test. Descriptive analysis was performed with the package R 3.0.3.

Principal components and clustering methods were used to group the means of the 13 traits for each accession. Data were standardized to calculate the mean Euclidean distances among the 46 accessions. To calculate principal components (PCs), software Minitab 18 (Minitab LCC 2018) was used. The eigenvectors and eigenvalues were obtained from the matrix of genetic dissimilarity. The relative importance of characters, used in the discrimination of clusters, was assessed based on weight variables in eigenvectors. The eigenvectors and eigenvalues were obtained from the correlation matrix of standardized data of original values. Then, the scores for the first principal component were plotted in a graph to detect genetic divergence in the accessions. Based on the matrix of genetic dissimilarities, a dendrogram was generated with the unweighted pair group method with arithmetic means (UPGMA) (Sokal and Michener 1958), using software Genes 5.1 (Cruz 2006). Groups of accessions in the dendrogram were determined based on the NbClust package for R software and Pseudot2 (Charrad et al. 2014). The dissimilarity matrix was estimated by the cophenetic correlation according to Sokal and Rohlf (1962), using Genes 5.1.

RESULTS AND DISCUSSION

The range values were higher than the means for most of the chemical traits, except for ashes, pH, moisture and starch (Table 1). Coefficients of variation varied from 3% for pH to 93% for total cyanide (Table 1) and high values were detected for most characters, with the exception of TTA, TSS, ashes, pH, moisture and starch. Perhaps the values of coefficient of variation were high because the accessions were analyzed in a germplasm bank, using no experimental design. The traits glucose, ashes, moisture and starch had a normal distribution, since the Shapiro-Wilk's test at 5% probability was non-significant (Table 1).

In general, there was great variation for all traits evaluated (Tables 2 and 3). The cyanide contents of the accessions CPATU 516, CPATU 475 and CPATU 375 varied from 50 to 100 mg kg $^{-1}$ of HCN (Table 2), but these accessions were not identified by local farmers as 'sweet' cassava. Observations showed that cyanide contents in an accession can be influenced by the environmental conditions (Burns et al. 2012, Gu et al. 2013). Aside from the low cyanide contents detected for accessions CPATU 475 and CPATU 516, their total carotenoids levels were also moderate (Table 3), i.e., they can be used in genetic breeding programs to generate biofortified cultivars.

For the measurement of total carotenoids, 20 'bitter' and 15 'sweet' cassava accessions were analyzed, based on a visual assessment to indicate the yellow color level of the root pulp of each accession. The highest contents of total carotenoids detected in cassava roots were 18.41, 14.09, 11.34, 11.24 and 10.88 µg g^{-1}, all in accessions of 'bitter' cassava (Table 3). Thus, these accessions can be considered potential parents in genetic breeding programs. 'Sweet' cassava had lower total carotenoid contents (2.08 - 4.38 µg g $^{-1}$) (Table 3). By direct crosses, total carotenoids contents of 25.5 µg g $^{-1}$ were reached in the root of improved cassava clones, which confirmed that classical genetic breeding can gradually increase carotenoid root contents (Sanchéz et al. 2014). All accessions used in this study were landraces grown by traditional farmers in the Amazon and are highly promising for genetic breeding to improve carotenoids in cassava roots.

Protein contents in 'bitter' cassava varied from 0.46 to 2.27% (Table 3) and from 0.18 to 1.10% for 'sweet' cassava, (Table 3). This variation was below that described by Ceballos et al. (2006) for protein contents of 0.95 to 6.42% in accessions from different countries in Asia and America.

Table 1. Descriptive analysis of 13 chemical traits of the roots of 46 cassava (*Manihot esculenta* Crantz) accessions from the Brazilian Amazon and maintained in a germplam bank representing the Eastern Amazon, Brazil. Minimum (Min.), maximum (Max.) and mean values, range, standard deviation, coefficient of variation (CV %), and normality test

Traits[1]	Min.	Max.	Mean	Range	Standard deviation	CV (%)	Normality test
TTA	1.70	4.37	2.43	2.67	0.55	22	0.92**
Glucose	0.04	0.38	0.17	0.35	0.09	52	0.95
Fructose	0.03	0.25	0.08	0.23	0.05	54	0.83**
Sucrose	0.07	0.77	0.38	0.70	0.22	58	0.96**
TSS	1.94	5.60	3.18	3.65	0.73	23	0.94**
TC	1.71	18.41	5.68	16.69	3.88	68	0.87**
Total cyanide	29.80	934.40	228.70	904.60	213.60	93	0.83**
Free cyanide	5.78	183.66	64.68	177.88	53.41	82	0.87**
Ashes	1.35	3.42	2.21	2.06	0.51	23	0.96
pH	5.77	6.78	6.36	1.01	0.21	3	0.94**
Crude protein	0.18	2.27	0.88	2.08	0.48	54	0.90**
Moisture	59.18	79.22	67.67	20.04	5.20	8	0.96
Starch	19.79	34.07	27.26	14.28	3.74	14	0.97

[1] TTA: Total titratable acidity (%); Glucose (%); Fructose (%); Sucrose (%); TSS: total soluble solids (%); TC: Total carotenoids (µg g^{-1}); Total cyanide (mg kg^{-1}); Free cyanide (mg kg^{-1}); Ashes (%); pH; Crude protein (%); Moisture (%); Starch (%). ** P <0.05 by Shapiro-Wilk's test.

Table 2. Non-standardized mean values of chemical traits of the roots of 'bitter' and 'sweet' cassava (*Manihot esculenta* Crantz) accessions from the Brazilian Amazon maintained in the germplasm bank of the Eastern Amazon, Brazil. Root traits were measured on a fresh-weight basis

Accessions	**Moisture** (%)	**Ashes** (%)	**TSS** (%)	**TTA** (%)	**pH**	**Total cyanide** (mg kg^{-1})	**Free cyanide** (mg kg^{-1})
				Bitter cassava			
CPATU 016	63.83	1.99	3.40	1.83	6.21	202.07	70.86
CPATU 507	65.73	2.04	4.27	1.71	6.58	324.03	88.36
CPATU 348	64.44	2.29	3.18	2.44	6.39	227.29	45.88
CPATU 461	72.19	2.28	3.73	2.40	6.23	238.21	56.28
CPATU 160	60.70	2.33	2.77	3.07	6.49	311.63	73.78
CPATU 140	62.68	1.57	3.28	2.37	6.41	123.78	47.51
CPATU 462	72.64	2.14	3.87	1.72	6.23	259.45	97.35
CPATU 026	64.42	3.42	3.84	3.25	6.13	224.18	80.60
CPATU 466	68.62	2.37	3.67	2.41	5.89	226.03	78.56
CPATU 516	65.20	1.75	3.45	2.08	6.38	89.44	75.23
CPATU 513	67.19	2.08	3.87	2.22	6.36	934.37	150.48
CPATU 475	68.78	2.07	3.80	2.29	5.77	85.70	36.18
CPATU 509	64.62	1.66	3.73	3.22	6.30	222.88	43.80
CPATU 458	68.31	2.27	3.87	2.26	6.43	548.94	91.63
CPATU 463	65.79	1.82	3.01	2.52	6.17	234.58	62.13
CPATU 141	66.49	1.69	2.93	2.69	6.31	207.52	66.68
CPATU 514	60.18	1.54	3.73	2.01	6.54	496.34	37.93
CPATU 190	65.57	1.36	3.67	1.76	6.64	453.35	183.66
CPATU 465	71.18	2.45	3.33	2.08	5.85	288.74	70.97
CPATU 375	69.78	2.82	4.00	1.76	6.39	93.43	36.18
CPATU 515	64.00	2.81	4.13	3.26	6.07	539.79	144.31
CPATU 229	62.89	1.51	3.80	2.62	6.50	315.02	66.58
CPATU 530	60.18	1.47	4.26	2.20	6.36	828.47	182.67
CPATU 193	66.70	3.25	5.60	2.47	6.45	535.72	146.99
CPATU 327	66.69	1.86	3.07	1.82	5.95	431.07	176.87
CPATU 092	64.01	1.74	2.93	2.28	6.33	123.27	50.92
CPATU 404	62.91	1.92	2.73	1.91	6.71	101.13	53.58
CPATU 499	60.07	1.87	2.53	1.84	6.35	179.65	88.40
CPATU 500	68.63	2.44	2.53	2.22	6.23	402.61	146.37
CPATU 219	59.18	1.65	3.40	1.70	6.50	479.22	168.82
				Sweet cassava			
CPATU 179 (S)	62.63	3.11	1.94	2.91	6.42	94.28	77.25
CPATU 280 (S)	74.63	2.84	2.53	1.76	6.78	40.20	15.68
CPATU 241 (S)	72.53	2.82	2.93	2.43	6.49	45.45	14.42
CPATU 271 (S)	74.90	2.14	2.48	2.44	6.47	51.60	12.15
CPATU 019 (S)	73.70	2.59	2.82	2.48	6.69	49.24	10.38
CPATU 232 (S)	69.47	3.15	2.49	2.81	6.41	29.81	9.25
CPATU 309 (S)	75.52	2.36	2.92	2.59	6.44	45.87	9.65
CPATU 183 (S)	72.20	1.94	2.43	2.55	6.38	37.17	9.51
CPATU 070 (S)	70.57	2.07	2.45	2.24	6.60	32.74	7.73
CPATU 281 (S)	75.31	2.71	2.44	2.38	6.44	53.67	9.89
CPATU 283 (S)	79.22	2.97	2.46	4.37	6.21	39.94	13.09
CPATU 274 (S)	72.31	2.04	2.48	2.39	6.44	51.35	5.78
CPATU 115 (S)	77.90	2.62	2.45	3.47	6.60	36.38	14.75
CPATU 195 (S)	61.41	1.98	2.01	2.77	6.40	48.06	7.12
CPATU 136 (S)	74.07	2.06	2.99	3.06	6.47	62.97	14.00
CPATU 034 (S)	62.27	2.01	2.07	2.88	6.42	75.65	25.23

TSS: Total soluble solids; TTA: Total titratable acidity.

Table 3. Non-standardized mean values of chemical traits of the roots of 'bitter' and 'sweet' cassava (*Manihot esculenta* Crantz) accessions from the Brazilian Amazon, maintained in the germplasm bank of the Eastern Amazon, Brazil. Root traits were measured on a fresh-weight basis

Accessions	**TC** (μg g^{-1})	**Crude protein** (%)	**Glucose** (%)	**Fructose** (%)	**Sucrose** (%)	**Starch** (%)
			Bitter cassava			
CPATU 016	18.41	0.46	0.19	0.07	0.76	25.20
CPATU 507	14.09	0.64	0.24	0.07	0.51	26.32
CPATU 348	11.34	0.89	0.21	0.05	0.77	28.00
CPATU 461	11.24	1.19	0.24	0.07	0.74	24.63
CPATU 160	10.88	0.86	0.18	0.03	0.26	31.40
CPATU 140	9.44	0.80	0.26	0.07	0.30	24.07
CPATU 462	8.48	1.30	0.38	0.10	0.47	31.77
CPATU 026	8.48	0.63	0.24	0.05	0.63	27.74
CPATU 466	7.71	1.28	0.32	0.14	0.62	30.08
CPATU 516	7.39	0.55	0.28	0.18	0.43	31.12
CPATU 513	7.17	0.83	0.30	0.08	0.44	29.43
CPATU 475	6.00	1.10	0.34	0.26	0.53	25.76
CPATU 509	6.00	2.01	0.16	0.06	0.62	33.09
CPATU 458	5.65	2.27	0.22	0.04	0.48	30.00
CPATU 463	5.41	0.63	0.30	0.12	0.41	24.91
CPATU 141	5.01	1.08	0.11	0.07	0.61	26.61
CPATU 514	3.92	1.00	0.22	0.09	0.49	29.56
CPATU 190	3.84	1.94	0.17	0.12	0.45	27.48
CPATU 465	2.97	0.91	0.35	0.20	0.34	28.86
CPATU 375	1.71	1.82	0.23	0.08	0.39	34.07
CPATU 515	n.d.	0.83	0.26	0.10	0.78	26.22
CPATU 229	n.d.	1.10	0.20	0.05	0.38	32.32
CPATU 530	n.d.	1.09	0.19	0.04	0.46	25.76
CPATU 193	n.d.	1.83	0.17	0.05	0.62	28.02
CPATU 327	n.d.	0.70	0.19	0.06	0.64	33.00
CPATU 092	n.d.	1.36	0.12	0.09	0.39	30.00
CPATU 404	n.d.	0.73	0.12	0.06	0,30	27.73
CPATU 499	n.d.	0.55	0.10	0.03	0.29	32.53
CPATU 500	n.d.	1.09	0.19	0.08	0.77	30.84
CPATU 219	n.d.	0.65	0.17	0.04	0.47	32.25
			Sweet cassava			
CPATU 179 (S)	4.38	0.46	0.08	0.07	0.14	21.19
CPATU 280 (S)	3.87	0.45	0.12	0.13	0.12	28.81
CPATU 241 (S)	3.42	0.55	0.04	0.06	0.09	22.09
CPATU 271 (S)	3.26	0.82	0.05	0.04	0.11	21.53
CPATU 019 (S)	3.03	0.27	0.04	0.06	0.11	19.79
CPATU 232 (S)	2.95	0.18	0.05	0.06	0.08	20.41
CPATU 309 (S)	2.82	0.46	0.12	0.11	0.16	24.07
CPATU 183 (S)	2.78	0.53	0.05	0.05	0.09	24.34
CPATU 070 (S)	2.63	0.45	0.09	0.09	0.10	26.33
CPATU 281 (S)	2.62	0.44	0.11	0.12	0.17	24.00
CPATU 283 (S)	2.61	0.36	0.12	0.11	0.15	23.79
CPATU 274 (S)	2.51	0.48	0.11	0.06	0.16	27.34
CPATU 115 (S)	2.50	0.53	0.05	0.06	0.11	22.38
CPATU 195 (S)	2.30	1.10	0.09	0.05	0.17	21.82
CPATU 136 (S)	2.08	0.64	0.12	0.10	0.14	28.02
CPATU 034 (S)	n.d.	0.92	0.09	0.11	0.28	29.71

TC: Total carotenoids; n.d.: Not determined.

Table 4. Estimates of Spearman's correlation coefficient among 13 chemical root traits of 46 cassava (*Manihot esculenta* Crantz) accessions from the Brazilian Amazon and maintained in a germplasm bank in the Eastern Amazon, Brazil

Traits[1]	TTA	Glucose	Fructose	Sucrose	TSS	TC	Free cyanide	Ashes	pH	Crude protein	Moisture	Starch
Glucose	-0.33											
Fructose	-0.15	0.40**										
Sucrose	-0.23	0.70**	0.06									
TSS	-0.31	0.75**	0.02	0.70**								
TC	-0.04	0.40**	0.14	0.22	0.24							
Free cyanide	-0.40**	0.57**	-0.09	0.70**	0.63**	0.04						
Ashes	0.40**	-0.11	0.15	-0.20	-0.18	0.06	-0.22					
pH	-0.10	-0.53**	-0.25	-0.52**	-0.26	-0.14	-0.28	-0.09				
Crude protein	-0.14	0.47**	-0.04	0.60**	0.60**	-0.01	0.45**	-0.40**	-0.23			
Moisture	0.13	-0.10	0.35	-0.40**	-0.22	0.05	-0.46**	0.52**	0.05	-0.32		
Starch	-0.40**	0.46**	0.01	0.50**	0.42**	-0.13	0.51**	-0.35	-0.15	0.52**	-0.35	
Total cyanide	-0.33	0.65**	-0.14	0.80**	0.73**	0.07	0.90**	-0.32	-0.23	0.61**	-0.50**	0.52**

[1] See code in Table 1. ** Significant by the t test, at 1% probability.

'Bitter' cassava had higher starch and sugar contents than the group of 'sweet' cassava (Table 3). The starch contents were similar to those identified in another study with cassava accessions sampled in Brazil (Vieira et al. 2015, Oliveira et al. 2016).

Spearman correlation values were calculated for the 13 chemical traits (Table 4). Total titratable acidity was negatively correlated with free cyanide and starch (Table 4). Negative correlations of glucose and sucrose with pH confirmed results of Feltran et al. (2004), who concluded that higher levels of pH reduce sugar accumulation. Negative correlations between free cyanide and moisture indicate that an increase in water contents promotes fast solubilization and volatilization of cyanide and higher amounts of water in the root may reduce cyanide concentration as well. A negative correlation

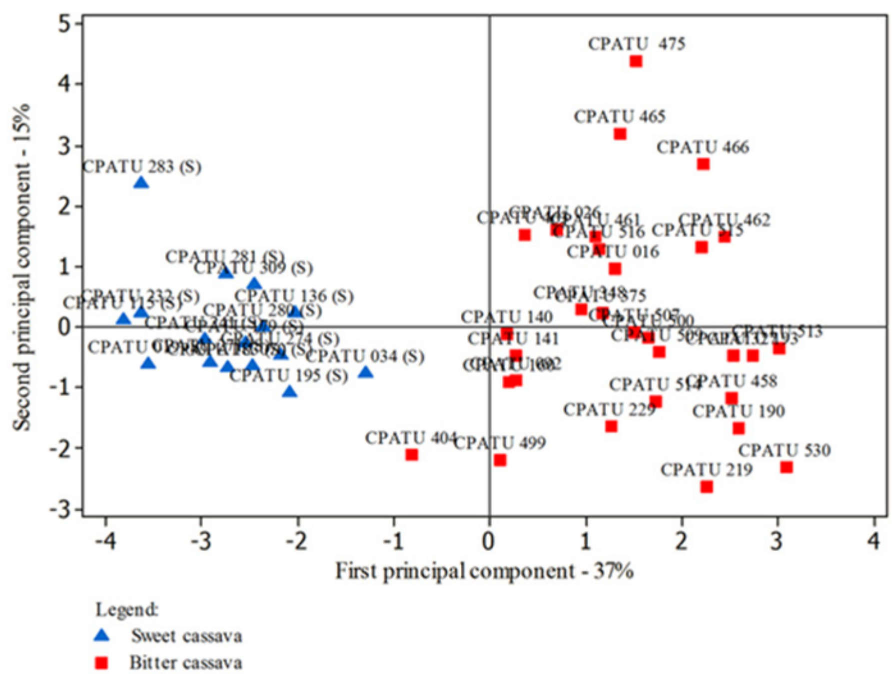

Figure 1. Biplot of 46 cassava (*Manihot esculenta* Crantz) accessions from the Amazon region, according to the analysis of 13 chemical root traits based on the first two principal components.

between cyanide and moisture may be interesting for the manufacturing of products derived from cassava root. Starch was positively correlated with total cyanide (Table 4). The group of 'sweet' cassava had lower starch contents in their roots than that of 'bitter' cassava (Table 3). Further research is needed to investigate if the farmers' selection for genotypes for consumption as 'macaxeiras' i.e., 'sweet' cassava, has influenced starch contents in the roots.

Principal component analysis was used to evaluate the most important descriptors to discriminate between 'sweet' and 'bitter' cassava. The first four principal components (PCs) explained 37, 15, 10 and 9% of the total variation, respectively. The trait with the highest association (eigenvectors) with the first PC is mainly responsible for the phenotypic variation and the trait with the highest association with the last PC contributed least (Jolliffe 1973). The first two PCs accounted for 52% of the variation, and sucrose and fructose showed the highest association with the first and the second components (eigenvectors of 0.38 and -0.51, respectively). Furthermore, the traits total and free cyanide were significantly associated with the first component (both eigenvectors 0.36). Ashes and total titratable acidity (TTA) were highly correlated with the third component, -0.53 and -0.52, respectively, and total carotenoids (TC) was highly correlated with the fourth component (0.81) (Table 4). The graphical dispersion of the 46 accessions based on the first two principal components (Figure 1) showed that the chemical traits were able to separate 'bitter' from 'sweet' accessions. Accessions of 'bitter' cassava were dispersed over three quarters of the graph. Three were located separately at the top of the graph (accessions CPATU 465, CPATU 466 and CPATU 475), with low or moderate TC contents (CPATU 475) and high levels of glucose and fructose. Similarly, Padonou et al. (2005) discriminated accessions of 'bitter' cassava based on total sugar contents. Accessions of 'sweet' cassava were allocated in the left-hand quarter. Accession CPATU 283 (S) was plotted separately in the upper portion of the graph, probably due to its high levels of moisture, ashes and total titratable acidity (TTA) (Tables 2 and 5). Starch was highly associated with the seventh and ninth PCs, which explained a lower portion of genetic variation (5 and 2%, respectively). Shittu et al. (2007) reported the same result. The traits free and total cyanide were highly associated with the 10th and 13th PCs. However, they were more relevant in the first component and are important for the discrimination of the accessions (Table 5). It was already expected that total cyanide would also be responsible for the separation of 'bitter' from 'sweet' cassava (Table 2; Figure 1), because the cyanide content is important for the classification of accessions in 'bitter' and 'sweet'.

The cluster analysis (Figure 2) shows that the 13 chemical root traits of the 46 'bitter' and 'sweet' cassava accessions allowed the classification into two distinct groups that coincide with their differentiation in 'bitter' or 'sweet' cassava, with the exception of CPATU 034 (S). Three subgroups were formed, with 3 to 28 accessions (Figure 2). Members of group

Table 5. Estimates of eigenvalues and eigenvectors associated with the principal components and 13 chemical root traits of 46 cassava (*Manihot esculenta* Crantz) accessions from the Brazilian Amazon and maintained in a germplasm bank in Eastern Amazon, Brazil

Traits[1]	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12	PC 13
TTA	-0.19	-0.17	-0.52	-0.05	-0.26	0.51	0.24	-0.24	0.4	-0.14	-0.13	0.1	-0.09
Glucose	0.35	-0.36	0.13	0.04	0.02	-0.07	-0.01	-0.22	0.22	0.29	0.3	-0.22	-0.67
Fructose	0.04	-0.51	0.38	-0.31	-0.01	-0.15	0.23	-0.36	-0.06	-0.44	-0.2	-0.01	0.25
Sucrose	0.38	-0.13	-0.15	0.2	0.09	0.14	-0.14	0.32	0.21	-0.25	-0.6	-0.4	-0.16
TSS	0.35	-0.08	-0.22	0.03	0.45	0.07	0.11	-0.29	-0.04	0.48	-0.4	0.32	0.15
TC	0.08	-0.25	-0.13	0.81	0.15	0.13	0.02	0.06	0.17	0.22	0.2	0.21	0.25
Free cyanide	0.36	0.16	-0.23	-0.09	-0.18	-0.37	0.05	0.14	0.03	-0.41	0.08	0.6	-0.23
Ashe	-0.18	-0.3	-0.53	-0.18	0.15	-0.23	-0.57	-0.23	-0.24	-0.16	0.21	-0.11	0.08
pH	-0.19	0.49	0.07	0.15	0.46	-0.1	-0.02	-0.38	0.19	-0.4	0.22	-0.16	-0.28
Crude protein	0.29	0.07	-0.06	-0.24	0.44	0.55	0.16	0.06	-0.36	-0.23	0.39	-0.01	0.01
Moisture	-0.25	-0.31	-0.07	-0.22	0.5	-0.14	0.08	0.6	0.38	-0.04	-0.07	0.01	-0.08
Starch	0.3	0.11	0.22	-0.28	0.02	0.26	-0.64	-0.03	0.48	-0.03	-0.04	0.05	0.21
Total cyanide	0.36	0.18	-0.29	-0.02	-0.02	-0.3	0.3	-0.01	0.32	-0.04	0.23	-0.5	0.42
Eigenvalues	4.8	2.07	1.29	1.13	0.87	0.81	0.55	0.39	0.38	0.24	0.23	0.11	0.09
Accumulated eigenvalues	4.8	6.87	8.16	9.29	10.16	10.97	11.52	11.91	12.25	12.53	12.76	12.87	12.96
Variance	0.37	0.15	0.10	0.09	0.07	0.06	0.05	0.03	0.02	0.02	0.02	0.01	0.01
Accumulated variance	0.37	0.52	0.62	0.71	0.78	0.84	0.89	0.92	0.94	0.96	0.98	0.99	1.0

[1] See code in Table 1. Principal components (PCs). Scaling method used: correlation matrix.

I were characterized by lower contents of free and total cyanide, glucose and primarily sucrose when compared with members of groups II and III, with generally higher contents of free and total cyanide. Moreover, accessions allocated in groups II and III also presented higher TC contents, except for CPATU 514, CPATU 190 and CPATU 465. Group II was formed by accessions CPATU 475, CPATU 465 and CPATU 466, which was the most divergent group.

The Pseudo t2 statistic provided a measure of the separation between the groups of accessions and the validity of this hierarchical clustering was then confirmed by of cophenetic correlation coefficient of the dendrogram (0.71), which revealed adequate adjustment between the graphic representation (Figure 2) and genetic dissimilarity matrix (Supplementary Table 2). The result of hierarchical clustering corroborated the graphical dispersion of accessions (Figures 1 and 2), based on the PCs, and highlighted the potential of chemical root traits to separate accessions of 'bitter' from 'sweet' cassava.

Perhaps these results reflected the effects of years of selection of cassava genotypes, performed by farmers in the Amazon region, who selected not only for cyanide contents, but also for traits that affect the final product. Clearly, this selection was based on the farmers' perception, without data means to measure the traits. Therefore, due to the differences in use, specific breeding programs are conducted for 'sweet' and 'bitter' cassava (Ceballos et al. 2004, Vieira et al. 2018).

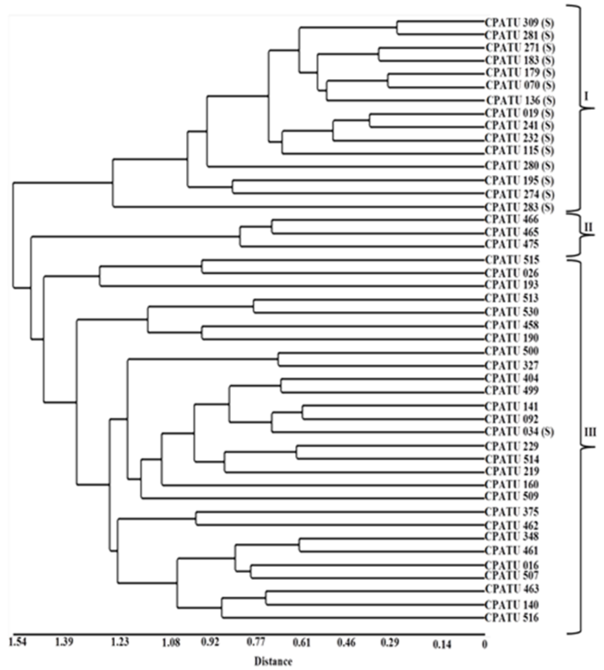

Figure 2. Dendrogram generated by UPGMA based on standardized Euclidean mean distances as a dissimilarity measure, for 46 cassava (*Manihot esculenta* Crantz) accessions from the Amazon regions, according to 13 chemical root traits. Coefficient of cophenetic correlation (r = 0.71). Groups were determined based on the 'NbClust' package for R software using Pseudot2. (S) indicates 'sweet' cassava accessions.

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